

Original Research Article

Genome wide association studies to dissect genetic factors conferring sheath blight resistance in Rice (*Oryza sativa* L.,)

ABSTRACT

Rice being the main staple food of Asia, the farmers have been seeing many challenges while cultivation of the crop due to many biotic and abiotic stresses. One such biotic stress which causes significant losses in places where there is prevalence of hot and humid climate is Rice sheath blight. Sheath blight (ShB) is one of the most serious fungal diseases caused by *Rhizoctonia solani*. Breeding for sheath blight resistance has been ineffective exercise so far, mainly because of lack of good number of reliable sources of resistance in rice germplasm. In this context our studies have indicated that the lines TETEP, JASMINE85 and MTU992 confer resistant to moderately resistant reaction against the pathogen. In order to dissect the genetic factors governing resistance to sheath blight Genome Wide Association Study (GWAS) was done by developing mapping populations by design where in each of the resistant parents were crossed to three to four highly susceptible parents to generate half-sib populations. A total of 1545 Recombinant inbred lines (RILs) derived from eleven crosses were used for the study. During rainy 2020 the F7 RILs were screened for their reaction to ShB in two hot spot locations. The genotyping was done with Illumina platform having 6564 SNP markers. Association mapping was completed with two models Generalized Linear Model (GLM) and Mixed Linear Model (MLM). Results clearly indicate the superiority of MLM over GLM in correcting the population structure. With MLM model for JASMINE85 half-sib populations, five QTLs (Quantitative Trait Loci) were found on Chr1, Chr3, Chr9, Chr10 and Chr11 with $-\log_{10}$ (P-Value) more than 3. For TETEP half-sib populations, seven QTLs were observed on Chr1, Chr2, Chr5, Chr6, Chr7, Chr8, and Chr11 with $-\log_{10}$ (P-Value) more than 4. Whereas for MTU992 half-sib populations, three QTLs were seen on Chr2, Chr6 and Chr11 with $-\log_{10}$ (P-Value) more than 3. Strength of signals in the genomic regions was not that great except few regions in TETEP, presence of several genomic regions conferring resistance to sheath blight in the resistant sources studied clearly indicates,

the inheritance is complex hence genomic selection approach could be rewarding for breeding for sheath blight resistance.

Keywords: Rice, sheath blight, SNPs, GWAS, LD Mapping, Association Mapping

1. INTRODUCTION

Rice (*Oryza sativa* L.) feeds more than half of the world's population and genetic improvement of this food crop can serve as a major component of sustainable food production. Rice sheath blight (ShB) is one of the most devastating fungal diseases of rice, causing significant yield losses in many rice-growing regions of the world. This disease has become popular recently because of intensification of rice-cropping systems with the development of new short stature, high tillering, high yielding cultivars, high plant densities, and an increase in nitrogen fertilization, these morphological and microenvironment situations are very much congenial for the growth and multiplication of the sheath blight fungus, in India it's prevalence is mainly confined to coastal places of India where farmers grow very high yielding varieties and hot humid climate adds to that. These factors promote disease spread by providing a favorable microclimate for the disease agent due to a dense leaf canopy with an increased leaf-to-leaf and leaf-to-sheath contact (Banniza *et al.*, 2007).

The necrotrophic Sheath Blight pathogen possess a broad range of hosts, there are few germplasm lines in Rice which are known to show resistant reaction against this pathogen, most of the breeders are focused on harnessing these resistant sources to breed cultivars which are resistant to tolerant for this disease. Because of lack of authentic and reliable sources of resistance, breeding for sheath blight has been challenging in Rice (Jia *et al.* 2009; Zuo *et al.*

2010; Srinivasachary, Willocquet and Savary 2011). There have been many studies which reported on the existence of sources with diverse levels of resistance in Xiangzaoxian 19 (Che et al. 2003), WSS2 (Sato et al. 2004), Teqing (Li et al. 1995; Pinson et al. 2005), Pecos (Sharma et al. 2009), Tetep (Sha and Zhu, 1989; Channamallikarjuna et al. 2010), Jasmine 85 (Pan et al. 1999; Zou et al. 2000; Liu et al. 2009), Minghui63 (Han et al. 2002), and wild rices *O. rufipogon*, *O. nivara* etc. (Ram et al. 2008; Eizenga et al. 2013).

Upon intensive study it's believed to be controlled by many genomic regions dispersed across the genome (Sha and Zhu 1989; Li et al. 1995; Pinson et al. 2005; Zuo et al. 2013). It is widely believed that quantitative nature of resistance could be advantageous for evolving varieties with durable/horizontal resistance (Young, 1996; Poland et al. 2009).

As of now, around 50 ShB resistance quantitative trait loci (ShBR QTLs) have been mapped to all the 12 rice chromosomes (Jia et al. 2009; Zuo et al. 2010; Xu et al. 2011 and Wang et al. 2012) because of advancement in genotyping technology and availability of genotypic information at cheaper price. Association mapping (AM) or Linkage Disequilibrium (LD) is a great tool for identification of the genomic regions controlling phenotype of interest, it exploits historical recombination events to trace and map trait variations. A major hurdle in AM is controlling false positives and false negatives that can arise from population structure and family relatedness. False positives and negatives can often be controlled by incorporating covariates for structure and kinship in mixed linear models (MLM).

2. MATERIAL AND METHODS

2.1 Parent material and phenotyping of F₇ RILs for ShB

Germplasm lines were screened for identification of lines which are resistant and susceptible for Sheath blight, half sib crosses were created by crossing each resistant lines with three to four agronomically superior susceptible lines to develop RIL populations to dissect out the genetic factors of sheath blight resistance involving JASMINE85, TETEP & MTU9992 as resistant parents and TN1, SWARNASUB1,

II32B, IR54 & IRBB4 as susceptible parents. The RILs were generated by following single seed descent method (SSD) at Rapid Generation Advancement/ Speed breeding facility of Pioneer Hi-Bred Pvt. Ltd. Research Centre at Tunkikalsa village, Medak district, Telangana. The eleven crosses used for the study were grouped into three half-sib hubs, Half-sib hub 1 (JASMINE85XTN1, JASMINE85XSWARNASUB1, JASMINE85XII32B and JASMINE85XIR54), half-sib hub 2 (TETEPXTN1, TETEPXSWARNASUB1, TETEPXII32B and TETEPXIR54) and half-sib hub 3 (MTU9992XTN1, MTU9992XII32B and MTU9992XIRBB4). The 1545 RILs derived from these eleven crosses were phenotyped for sheath blight reaction in two hot spot locations (Seethanagaram and Draksharam) of East Godavari District of Andhra Pradesh state, India (Latitude 16°08' N and Longitude 81°08' E, Latitude 17°10' N and Longitude 81°41' E). The experiments consisting of F₇ progenies along with parental lines were planted in Randomized complete design with two replications. Row length of 1.2 meter with row-to-row distance 15 cm and plant to plant distance 10 cm was considered to ensure dense population which is congenial for the development of disease. TN1 was used as susceptible check and was sown after every two rows as well as all along the border to increase the disease pressure as to serve as spreader rows. In the present study, the virulent local East Godavari isolate of rice sheath blight pathogen was utilized for disease screening. Before the inoculation, the fungus was cultivated in potato dextrose agar medium at optimal temperature for 3–4 days, followed by transferring of disc of medium with mycelia for multiplication. To ensure stringent screening for better disease development, artificial inoculation was done by spraying the mycelia uniformly at the base of plant at maximum tillering stage. The data was recorded at peak milking stage to dough stage by visualizing the relative lesion length to height (%) using 1-9 scale based on development of lesion from the lower to upper part of plant on a scale from 1 (Resistant) to 9 (Susceptible) thereby getting total of five phenotypic categories, where score 1: 0-20%, score 3: 21-30%, score 5: 31-45%, score 7: 46-65%, score 9: 66-100%.

2.2 SNP genotyping

All the RILs used for the study were genotyped using Infinium marker platform which is a fixed plex comprising of 6564 markers, the genotyping was done at marker technology lab of Pioneer Hi-Bred International Limited at Johnston, Iowa State, United States of America.

2.3 Description of association mapping (AM) models

The statistical analysis was done with “TASSEL” application. TASSEL also known as **Trait Analysis by aSSociation, Evolution and Linkage** is a powerful statistical software to conduct association mapping such as General Linear Model (GLM) and Mixed Linear Model (MLM).

In the current study two models were used for statistical analysis, (i) general linear model (GLM) with PCoA (principle coordinate analysis) (Price et al., 2006), (ii) mixed linear model (MLM) with PCoA + K (Kinship matrix for family relatedness estimates) (Yu et al., 2006).

The simplest model is to directly detect the association between a phenotype (y) and markers (S_i) one at a time, where $i=1$ to m , and m is number of markers. In GLM, in order to reduce spurious associations (false positives) while performing AM consideration of population structure (Q) as a cofactor helps in accounting residuals (e) partially and also adjusts some effect that does not belong to the testing markers. The mixed linear model (MLM) applies the same principle by adding individuals' genetic effects as random cofactor effects with variance structure defined by the kinship (K) among individuals. In both Q or $Q+K$ models, Q and K stay the same. Because of inclusion of additional cofactor family relatedness in the model in case of MLM, both false positives and false negatives are taken care.

GLM model equation: $y = S_i + Q + e$

MLM model equation: $y = S_i + Q + K + e$

The analysis was done with both GLM and MLM for all three half-sib hubs separately involving JASMINE85, TETEP and MTU992 to systematically trace the genomic regions governing the phenotype under study.

3. RESULTS AND DISCUSSION

The frequency distribution of 1545 F_7 progenies evaluated showed continuous variation across all half-sib population studied (Figure 1, 2 and 3). The genotypic analysis results were interesting because of usage of large number of markers and excellent distribution of the markers throughout the genome (Table 1), polymorphic markers between parents across population ranged from 1407 to 2849, MTU9992/TN1 and MTU9992/IRBB4 possessed lowest and highest number of informative markers (Table 2).

Association mapping relies mainly on the LD between marker and QTL, the main reason for false positives in AM is Linkage disequilibrium, LD can be observed because of population structure, selection, random drift, familial relatedness. Hence it is important to separate the LD of the marker with QTL from LD due to other reasons. By inclusions of population structure and familial relatedness cofactors in the model, spurious associations were taken care. The power signal detection is determined by several factors including the heritability of trait, population structure, extent of LD in populations, size of the population, pollination mechanism of crop species (Yu et al., 2006).

The results of Principal co-ordinate analysis/multidimensional scaling method clearly indicated that there was enough diversity among the populations present in each half-sib hubs (Figure 4, 5 and 6). With MLM model for JASMINE85 half-sib populations, five QTLs (Quantitative Trait Loci) were found on Chr1, Chr3, Chr9, Chr10 and Chr11 with $-\log_{10}$ (P-Value) more than 3, the results were similar with signals detected on Chr1, Chr3, Chr9, Chr10 and Chr11 with GLM as well (Figure 7 and 8). For TETEP half-sib populations, seven QTLs were observed on Chr1, Chr2, Chr5, Chr6, Chr7, Chr8, and Chr11 with $-\log_{10}$ (P-Value) more than 4, GLM exhibited signals on all chromosomes with $-\log_{10}$ (P-Value) more than 4 (Figure 9 and 10). Whereas for MTU9992 populations, three QTLs were seen on Chr2, Chr6 and Chr11 with $-\log_{10}$ (P-Value) more than 3, the results were similar with signals detected on Chr2, Chr6 and Chr11 in case of GLM (Figure 11 and 12). There have been many studies which reported sheath blight QTLs (***qshb series***) on multiple chromosomes, Jasmine 85 (Pan et al. 1999; Zou et al. 2000; Liu et al. 2009) and Tetep (Sha and Zhu, 1989; Channamallikarjuna et al. 2010). Marker effects generated after genotypic analysis clearly indicated that several loci scattered across the genome are contributing to sheath blight resistance in each resistant parent which demonstrated that the resistance to sheath blight is governed by many genes with additive effect, this was reported by earlier researchers (Sha and Zhu 1989; Li et al. 1995; Pinson et al. 2005; Zuo et al. 2013).

4. CONCLUSION

The MLM model performed better in detecting signals distributed throughout the genome with much more precision by inclusion of familial relatedness cofactor. Strength of signals in the genomic regions across three half-sib hubs was not that great except few regions in TETEP, presence of several factor scattered

across the genome conferring resistance to sheath blight in the resistant sources studied clearly indicates, the inheritance is complex hence genomic selection approach could be rewarding for breeding for sheath blight resistance as genomic selection considers marker effects of all loci dispersed across the genome to provide genomic estimated breeding values which can be used for selection or rejection of breeding lines.

Figure 1: Frequency distribution of ShB phenotypic scores for half-sib families of JASMINE85

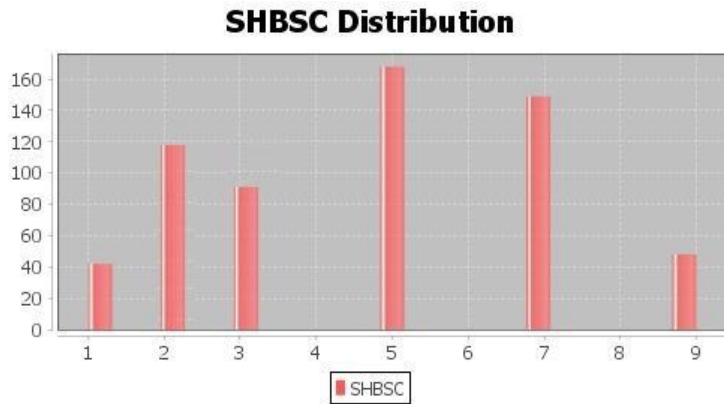


Figure 2: Frequency distribution of ShB phenotypic scores for half-sib families of TETEP

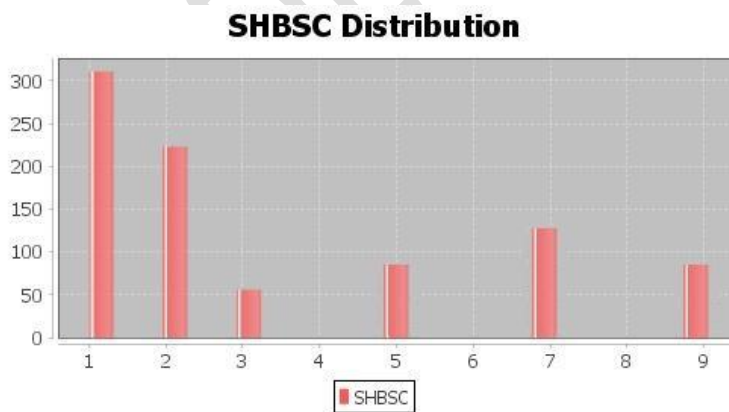


Figure 3: Frequency distribution of ShB phenotypic scores for half-sib families of MTU9992.

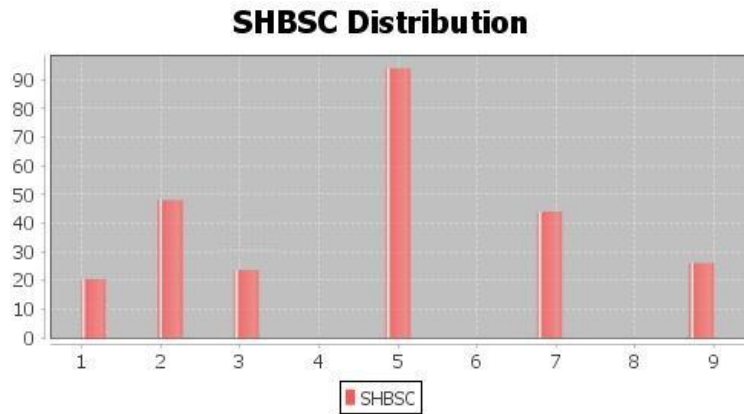


Figure 4: Depiction of principal components analysis results of JASMINE85 half-sib families

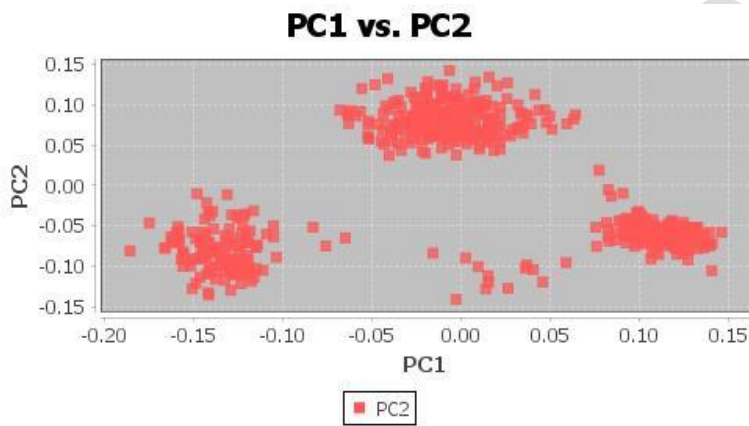


Figure 5: Depiction of principal components analysis results of TETEP half-sib families

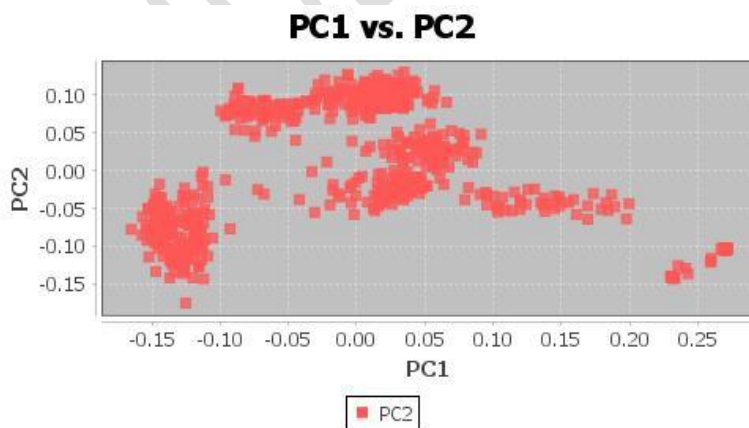


Figure 6: Depiction of principal components analysis results of MTU9992 half-sib families

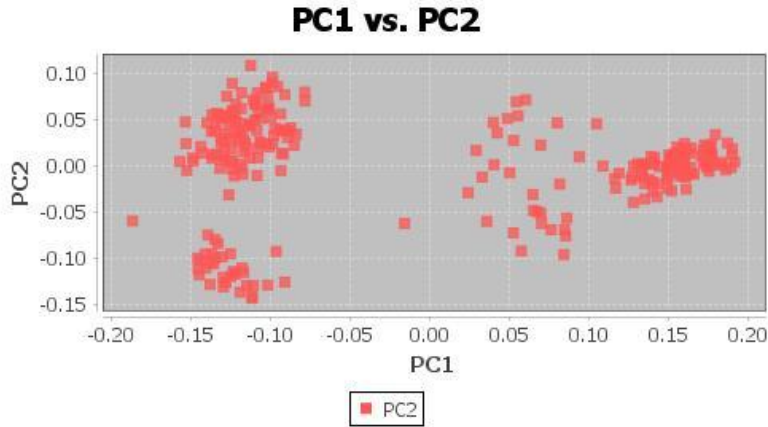


Figure 7: Manhattan plot of genome wide association results for sheath blight trait of **JASMINE85** half-sib populations using generalized linear model (GLM) for analysis.

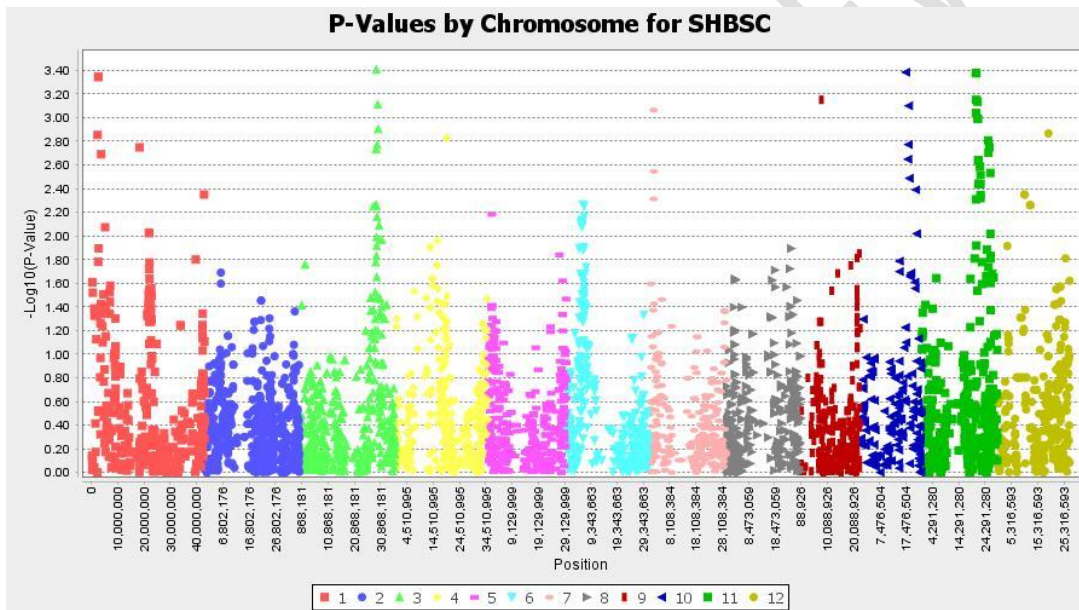


Figure 8: Manhattan plot of genome wide association results for sheath blight trait of **JASMINE85** half-sib populations using mixed linear model (MLM) for analysis

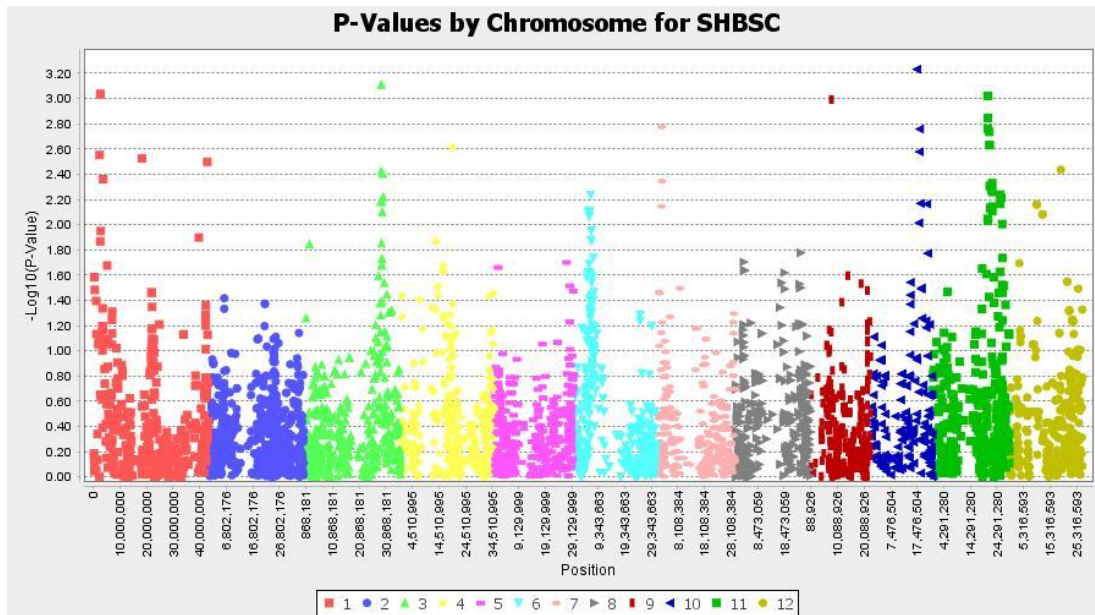


Figure 9: Manhattan plot of genome wide association results for sheath blight trait of **TETEP** half-sib populations using mixed linear model (GLM) for analysis.

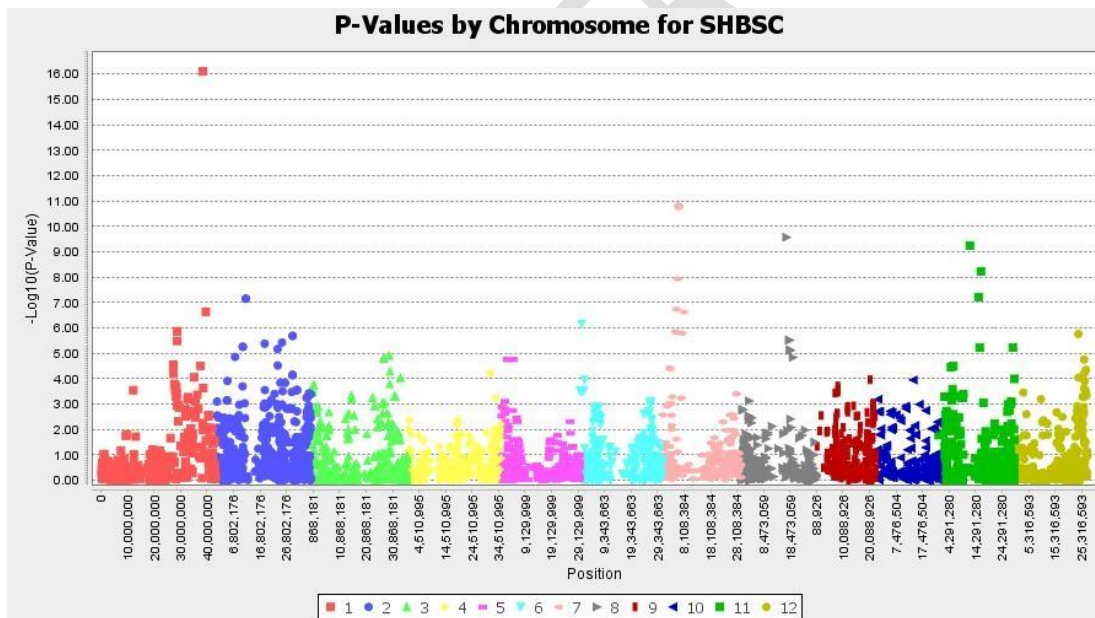


Figure 10: Manhattan plot of genome wide association results for sheath blight trait of **TETEP** half-sib populations using mixed linear model (MLM) for analysis.

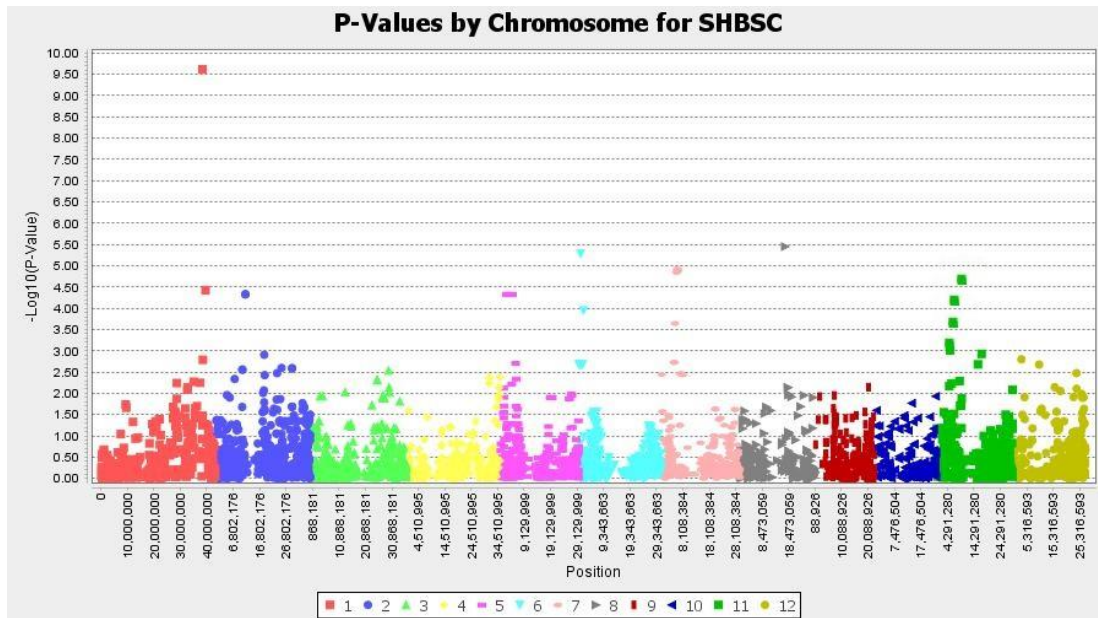


Figure 11: Manhattan plot of genome wide association results for sheath blight trait of **MTU9992** half-sib populations using mixed linear model (GLM) for analysis.

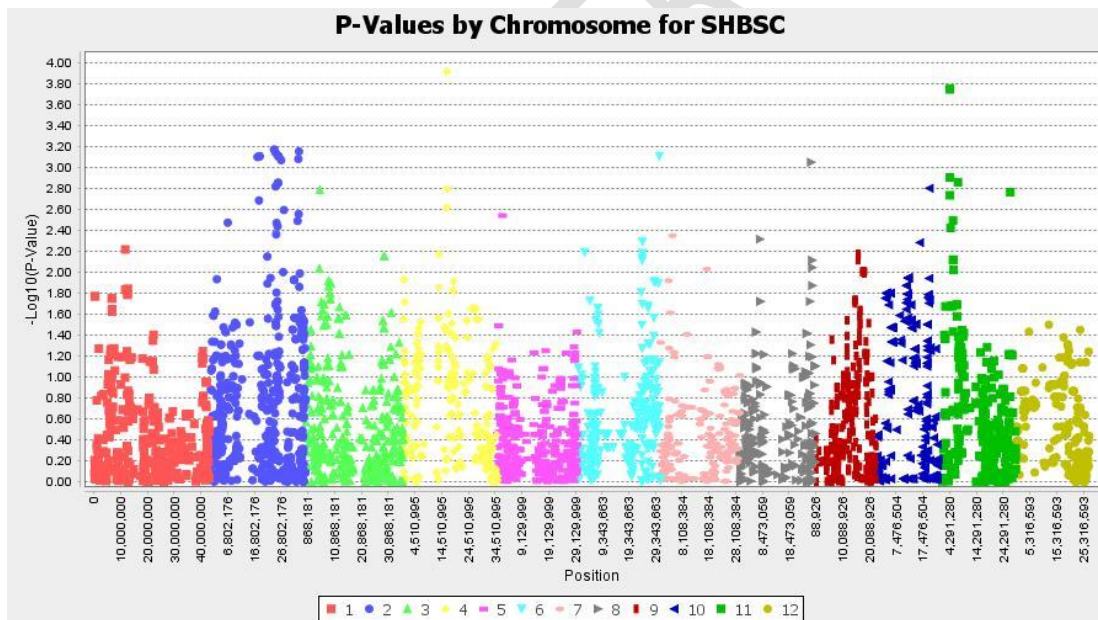


Figure 12: Manhattan plot of genome wide association results for sheath blight trait of **MTU9992** half-sib populations using mixed linear model (MLM) for analysis.

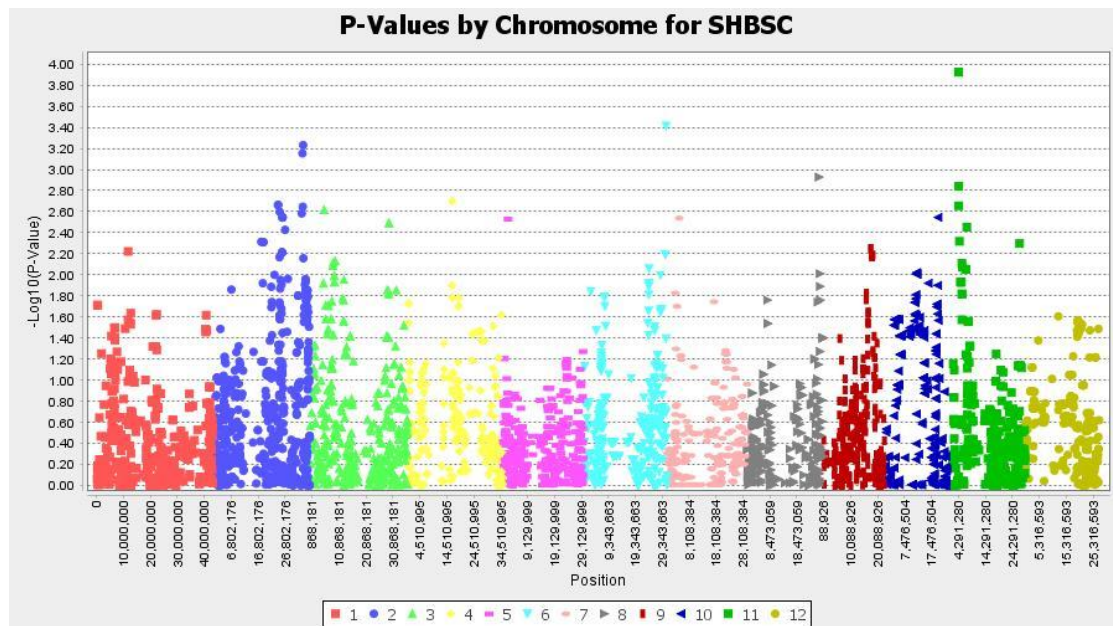


Table 1: Summary of marker data used for analysis and SNPs distribution on each chromosome

Chromosome	SNPs	Length (cM)
Ch1	639	181.8
Ch2	846	162.84
Ch3	598	164.04
Ch4	594	129.6
Ch5	583	128.58
Ch6	577	124.4
Ch7	457	118.6
Ch8	495	121.2
Ch9	427	93
Ch10	324	84.01
Ch11	541	117.9
Ch12	483	109.5
Total	6564	1535.47

Table 2: The informative markers available across the genome for each population used for analysis

Populations	Total Markers	Polymorphic Markers
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JASMINE 85/TN1	6564	2522
JASMINE 85/SWARNA SUB1	6564	2627
JASMINE 85/II32B	6564	2586
JASMINE 85/IR54	6564	2663
TETEP/TN1	6564	2806
TETEP/SWARNA SUB1	6564	2278
TETEP/II32B	6564	2702
TETEP/IR54	6564	2796
MTU9992/TN1	6564	1407
MTU9992/II32B	6564	2314
MTU9992/IRBB4	6564	2849

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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