MOLECULAR MAPPING OF SHEATH BLIGHT QTL(s) FROM THE WILD RICE *Oryza rufipogon* (Griff.) ACCESSION CR100438

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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MASTER OF SCIENCE
in
BIOTECHNOLOGY
(Minor Subject: Plant Breeding and Genetics)

By

Safoora Javed
(L-2021-A-151-M)

School of Agricultural Biotechnology
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CERTIFICATE I

This is to certify that the thesis entitled **"MOLECULAR MAPPING OF SHEATH BLIGHT QTL(s) FROM THE WILD RICE *Oryza rufipogon* (Griff.) ACCESSION CR100438"** submitted for the degree of **M.Sc.** in **Biotechnology** (Minor subject: **Plant Breeding and Genetics**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Safoora Javed (L-2021-A-151-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **“MOLECULAR MAPPING OF SHEATH BLIGHT QTL(s) FROM THE WILD RICE *Oryza rufipogon* (Griff.) ACCESSION CR100438”** submitted by **Safoora Javed (L-2021-A-151-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Biotechnology** (Minor subject: **Plant Breeding and Genetics**) has been approved by the Student’s Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

Sheath blight is the most serious disease of rice (*Oryza sativa* L.), caused by the soil-borne fungus, *Rhizoctonia solani* Kühn (*R. solan*i). Global rice productivity is significantly hampered by this disease, resulting in an annual loss of around 50% in yields. Managing the disease is a formidable task due to factors such as its wide range of host plants, the necrotrophic nature of the fungus, the swift emergence of new pathogen races, and the absence of highly resistant germplasm. Employing resistance or tolerance sources represents an environmentally friendly strategy for managing this disease. Therefore, the present study aimed at mapping of the Quantitative Trait Loci (QTLs) for resistance against sheath blight in rice using wild species of rice, *Oryza rufipogon* acc. CR100438. Seven disease variables *viz*, plant height, lesion height, relative lesion height, number of tillers, number of infected tillers, disease score and heading date were evaluated under artificial inoculation in the BC_2F_5 and BC_2F_6 populations during the *kharif* seasons 2022 and 2023. A total of 26 QTLs were mapped for all the seven traits distributed over chromosome 2, 3, 5, 6, 7, 8, 9, 10, 11 and 12. The major QTLs for lesion height *qLH6*, *qLH11*, *qLH12* and relative lesion height *qRLH6*, *qRLH7* and *qRLH12* were obtained with the contribution to phenotypic variance ranging from 9.10 to 14.26. Moreover, the research identified pre-breeding lines within the advanced backcrossed population that demonstrated enhanced agronomic traits and greater resistance to sheath blight when compared to the recurrent parent. This valuable information can be applied for the precise mapping of sheath blight resistance and the development of linked markers that in turn, facilitates the expedited transfer of disease resistance genes to the elite cultivars through breeding efforts.

Keywords: *Rhizoctonia solani*, Wild species of rice, Sheath blight symptoms, Sheath blight QTLs, Genotyping by Sequencing

Signature of the major advisor

Signature of the student

ਖੋਜ ਪੱਤਰ ਦਾ ਸਿਰਲੇਖ : ਜੰਗਲੀ ਝੋਨੇ *Oryza rufipogon* (Griff.) ਦੇ ਅਕਸੈਸ਼ਨ CR100438 ਤੋਂ ਲਏ ਗਏ ਤਣੇ ਦੁਆਲੇ ਪੱਤੇ ਦੇ ਝੁਲਸ ਰੋਗ ਦੇ QTL(s) ਦੀ ਆਣਵਿਕ ਮੈਪਿੰਗ
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ਸਾਰ ਅੰਸ਼

ਮਿੱਟੀ ਵਿੱਚ ਪਾਈ ਜਾਣ ਵਾਲੀ ਉੱਲੀ *Rhizoctonia solani* Kühn (*R. solan*i) ਕਾਰਨ ਹੋਣ ਵਾਲਾ ਤਣੇ ਦੁਆਲੇ ਤਣੇ ਦਾ ਝੁਲਸ ਰੋਗ, ਝੋਨੇ (*Oryza sativa* L.) ਦਾ ਇੱਕ ਬਹੁਤ ਹੀ ਮਾਰੂ ਰੋਗ ਹੈ। ਇਸ ਰੋਗ ਕਾਰਨ ਸੰਸਾਰ ਭਰ ਵਿੱਚ ਝੋਨੇ ਦੀ ਪੈਦਾਵਾਰ ਉਪਰ ਬਹੁਤ ਮਾੜਾ ਪ੍ਰਭਾਵ ਪੈਂਦਾ ਹੈ ਅਤੇ ਸਿੱਟੇ ਵਜੋਂ ਸਲਾਨਾ ਲਗਭਗ 50% ਝਾੜ ਦਾ ਨੁਕਸਾਨ ਹੁੰਦਾ ਹੈ। ਕਈ ਕਾਰਨਾਂ ਜਿਵੇਂ ਕਿ ਮੇਜ਼ਬਾਨ ਪੌਦਿਆਂ ਦੀ ਵਿਸ਼ਾਲ ਸ਼੍ਰੇਣੀ, ਉੱਲੀ ਦੇ ਨੈਕ੍ਰੋਟ੍ਰੋਫਿਕ ਵਤੀਰੇ, ਨਵੀਂਆਂ ਰੋਗਜਨਕ ਪ੍ਰਜਾਤੀਆਂ ਦੇ ਤੇਜ਼ੀ ਨਾਲ ਵਿਕਾਸ ਹੋਣ ਕਾਰਨ ਅਤੇ ਰੋਗ ਦਾ ਚੰਗੀ ਤਰਹਾਂ ਟਾਕਰਾ ਕਰਨ ਵਾਲੇ ਜਰਮਪਲਾਜ਼ਮਾਂ ਦੀ ਕਮੀ ਕਾਰਨ ਇਸ ਰੋਗ ਦੀ ਰੋਕਥਾਮ ਕਰਨਾ ਬਹੁਤ ਔਖਾ ਹੈ। ਰੋਗ ਪ੍ਰਤੀ ਸਹਿਣਸ਼ੀਲ ਅਤੇ ਸੰਵੇਦਨਸ਼ੀਲ ਸਰੋਤਾਂ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਇਸ ਰੋਗ ਦੀ ਰੋਕਥਾਮ ਲਈ ਵਾਤਾਅਨੁਕੂਲ ਵਿਉਂਤਬੰਦੀ ਕੀਤੀ ਜਾ ਸਕਦੀ ਹੈ। ਇਸ ਲਈ, ਮੌਜੂਦਾ ਅਧਿਐਨ ਦਾ ਉਦੇਸ਼, ਜੰਗਲੀ ਝੋਨੇ *Oryza rufipogon* (Griff.) ਦੇ ਅਕਸੈਸ਼ਨ CR100438 ਤੋਂ ਲਏ ਗਏ ਤਣੇ ਦੁਆਲੇ ਪੱਤੇ ਦੇ ਝੁਲਸ ਰੋਗ ਦੇ QTL(s) ਦੀ ਆਣਵਿਕ ਮੈਪਿੰਗ ਕਰਨਾ ਸੀ। ਸੰਨ 2022 ਅਤੇ 2023 ਦੀ ਸਾਉਣੀ ਰੁੱਤੇ BC_2F_5 ਅਤੇ BC_2F_6 ਜੰਨਸੰਖਿਆਵਾਂ ਵਿੱਚ ਬਨਾਵਟੀ ਇਨੋਕੁਲੇਸ਼ਨ ਅਧੀਨ ਰੋਗ ਦੇ ਸੱਤ ਵੇਰੀਏਬਲਾਂ ਜਿਵੇਂ ਕਿ ਪੌਦੇ ਦੀ ਉਚਾਈ, ਜ਼ਖਮ ਦੀ ਉਚਾਈ, ਵਿਵਹਾਰਿਕ ਜ਼ਖਮ ਦੀ ਉਚਾਈ, ਮੁੰਜਰਾਂ ਦੀ ਗਿਣਤੀ, ਰੋਗ ਗ੍ਰਸਤ ਮੁੰਜਰਾਂ ਦੀ ਗਿਣਤੀ, ਰੋਗ ਅੰਕ ਅਤੇ ਹੈਡਿੰਗ ਡੇਟ ਦਾ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਸਾਰੇ ਦੇ ਸਾਰੇ ਸੱਤ ਗੁਣਾਂ ਲਈ ਗੁਣਸੂਤਰ 2, 3, 5, 6, 7, 8, 9, 10, 11 ਓਤੇ 12 ਉਪਰ ਵਿਤਰਤ ਕੁੱਲ 26 QTLs ਦੀ ਮੈਪਿੰਗ ਕੀਤੀ ਗਈ। 9.10 ਤੋਂ 14.26 ਦਰਮਿਆਨ ਫਿਨੋਟਿਪਿਕ ਵਿਭਿੰਨਤਾ ਵਿੱਚ ਯੋਗਦਾਨ ਨਾਲ, ਜ਼ਖਮ ਦੀ ਉਚਾਈ ਲਈ *qLH6, qLH11, qLH12* ਅਤੇ ਵਿਵਹਾਰਿਕ ਜ਼ਖਮ ਦੀ ਉਚਾਈ ਲਈ *qRLH6, qRLH7* ਅਤੇ *qRLH12*, ਪ੍ਰਮੁੱਖ QTLs ਪ੍ਰਾਪਤ ਹੋਏ। ਇਸ ਤੋਂ ਇਲਾਵਾ, ਅਧਿਐਨ ਦੌਰਾਨ ਵਿਕਸਿਤ ਬੈਕ-ਕਰਾਸ ਜੰਨਸੰਖਿਆ ਵਿੱਚ ਪ੍ਰੀ-ਬ੍ਰੀਡਿੰਗ ਲਾਈਨਾਂ ਦੀ ਪਹਿਚਾਣ ਹੋਈ ਅਤੇ ਜਦੋਂ ਆਵਰਤੀ ਮਾਪੇ ਨਾਲ ਇਹਨਾਂ ਲਈਨਾਂ ਦੀ ਤੁਲਨਾ ਕੀਤੀ ਗਈ ਤਾਂ ਇਹਨਾਂ ਨੇ ਵਧੀਆ ਫ਼ਸਲੀ ਗੁਣਾਂ ਅਤੇ ਤਣੇ ਦੁਆਲੇ ਪੱਤੇ ਦੇ ਝੁਲਸ ਰੋਗ ਦਾ ਟਾਕਰਾ ਕਰਨ ਦੀ ਵਧੀਆ ਸਮਰੱਥਾ ਦਰਸਾਈ। ਇਹ ਬਹੁਮੁੱਲੀ ਜਾਣਕਾਰੀ ਪੱਤੇ ਦੁਆਲੇ ਤਣੇ ਦੇ ਝੁਲਸ ਰੋਗ ਦੀ ਪ੍ਰਤੀਰਰੋਧਕਤਾ ਦੀ ਮੈਪਿੰਗ ਲਈ ਅਤੇ ਲਿੰਗਕ ਮਾਰਕਰਾਂ ਦੇ ਵਿਕਾਸ ਲਈ ਵਰਤੀ ਜਾ ਸਕਦੀ ਹੈ, ਜਿਸ ਨਾਲ, ਬ੍ਰੀਡਿੰਗ ਰਾਹੀਂ ਵਧੀਆ ਕਿਸਮਾਂ ਵਿੱਚ ਰੋਗ ਦਾ ਟਾਕਰਾ ਕਰਨ ਵਾਲੇ ਜੀਨਾਂ ਦਾ ਤੇਜ਼ੀ ਨਾਲ ਸਥਨਾਂਤਰਨ ਕੀਤਾ ਜਾ ਸਕਦਾ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: *Rhizoctonia solani*, ਝੋਨੇ ਦੀਆਂ ਜੰਗਲੀ ਪ੍ਰਜਾਤੀਆਂ, ਤਣੇ ਦੁਆਲੇ ਪੱਤੇ ਦੇ ਝੁਲਸ ਰੋਗ ਦੀਆਂ ਨਿਸ਼ਾਨੀਆਂ, ਤਣੇ ਦੁਆਲੇ ਪੱਤੇ ਦੇ ਝੁਲਸ ਰੋਗ ਨਾਲ ਸਬੰਧਤ QTLs, ਅਨੁਕ੍ਰਮਣ ਰਾਹੀਂ ਜੀਨੋਟਾਈਪਿੰਗ

ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

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CHAPTER – I

INTRODUCTION

Rice, scientifically known as *Oryza sativa*, stands as a fundamental cereal crop worldwide, sustaining over half of the global population. Belonging to the *Poaceae* family, it is predominantly cultivated across tropical and subtropical regions (Ezuka and Kaku 2000). In India, rice cultivation covers an estimated area of 46 million hectares, yielding a production of 130.29 million tonnes, with an average productivity of 2809 kg per hectare (Anonymous 2022). Punjab holds a pivotal role in the agricultural evolution of the nation and is recognized for its highly fertile plains, crucial for sustaining the livelihoods of its inhabitants (Dhillon *et al* 2010). In Punjab, rice is cultivated across 2.79 million hectares, resulting in an output of 12.18 million tonnes and achieving a productivity of 4366 kg per hectare (Anonymous 2023). The anticipated projections suggest a 26% increase in global rice demand over the next 25 years, necessitating an increase in production from 676 million tonnes (mt) to 852 mt during the same period worldwide (Khush 2013). By 2050, the world population is estimated to reach 9.1 billion, posing a significant challenge in feeding this growing population.

The unfavorable conditions such as drought, salinity, rising temperatures, diseases, and insect pests, which constantly evolve, result in substantial losses in rice yield. These challenges arise from rapid alterations in pathogens and strains, necessitating the development of strategies to counteract these harmful biotic and abiotic stresses. Major biotic stresses, including insect pests like brown planthopper (BPH), stem borers, and diseases such as bacterial blight (BB), rice blast, and sheath blight (ShB), cause substantial yield losses (Savary *et al* 1995). Among these, sheath blight, caused by the necrotrophic fungus *Rhizoctonia solani* Kuhn [Telomorph: *Thanatephorus cucumeris* (Frank) Donk], stands out as one of the most critical disease leading to major crop losses worldwide. The ShB disease complex includes three species: *Rhizoctonia solani*, *Rhizoctonia oryzae-sativae*, and *Rhizoctonia oryzae*. In Punjab, *R. solani* is the predominant pathogen (Lore *et al* 2015). This pathogen displays high genetic variability, with 14 different anastomosis groups (AG) assigned to *R. solani* isolates (Carling *et al* 2002b). Symptoms of the disease manifest as circular or irregular greenish-grey patches with brown margins, typically 1-3 cm long, on the leaf sheath during the tillering stage. The affected leaf sheaths, bearing several such patches, often resemble snake skin. Under favorable conditions, the infection rapidly spreads to upper and other plant parts using runner hyphae, eventually leading to the demise of the entire leaf, tiller and plant. Infected plants, observed in a circular pattern, are locally known as "Bird's nests" (Hollier *et al* 2009). This pathogen exhibits significant pathogenic and molecular heterogeneity and has a broad host range. As a soil-borne pathogen, its sclerotia tend to accumulate beneath rice plants at the water-plant interface, initiating infection. These sclerotia can persist for extended periods in temperate rice production areas, accumulating in the soil. Morphological traits such as plant height, lesion height, tiller angle, and leaf size impact rice ShB resistance by altering the plant micro-climate (Srinivasachary *et al* 2011). The heading date is also correlated with ShB resistance, with later-maturing varieties generally exhibiting greater resistance to ShB (Srinivasachary *et al* 2011).

The disease was first recognized by the farmer of Japan in 1910 (Rush and Lee 1992). Its subsequent spread across various oriental countries led to its common reference as "oriental leaf and ShB" (Kozaka 1975). In Japan, it has caused significant yield losses of up to 20%, affecting approximately 120,000−190,000 hectares. In the United States, susceptible cultivars experienced a substantial yield reduction of 50%. Furthermore, ShB has led to staggering yield losses of 60% in tropical Asia, establishing itself as most devastating rice disease (Ou 1985). In India, this disease was first documented by Paracer and Chahal (1963) in Gurdaspur, Punjab. Over years, the prevalence of this disease has been increasing in India and specifically in Punjab. Rice ShB exhibits a broad distribution across various rice-growing areas, spanning tropical, temperate, and subtropical regions (Lee and Rush 1983). Remarkably, this pathogen possesses a broad spectrum of hosts and infects over 200 different plant species, encompassing several crucial crops globally, such as maize, rice, potato, wheat, carrot, soybean, cotton, and tomato (Lehtonen *et al* 2008). ShB is difficult to control due to the low level of inherent resistance of rice against this disease (Khodayari *et al* 2009).

The management of this disease heavily relies on fungicides and cultural practices, since no fully resistant sources have been discovered within the available rice germplasm (Bonman *et al* 1992). Considering the limited effectiveness, environmental impact, and costliness of other management techniques, resistance remains the most desirable method of control. However, the quantitative nature of resistance against ShB, combined with factors such as high genetic variability, extensive host compatibility, and the complex inheritance pattern of this disease, present significant challenges in developing resistant rice genotypes (Jia *et al* 2012). All identified strains responsible for rice ShB belong to the AG1 1A group, despite of variations in their virulence patterns (Pillai and Singh 1994; Singh *et al* 2002). Several genotypes, including Tetep (Channamallikarjuna *et al* 2010), Jasmine 85 (Liu *et al* 2009), and Te-Qing (Li *et al* 1995; Pinson *et al* 2005), have been utilized for QTL mapping for ShB resistance. However, as of now, no rice genotypes have shown complete resistance to ShB, although different cultivars displaying varying levels of tolerance have been identified (Khush 1977; Guo *et al* 1985; Groth and Novick, 1992; Srinivasachary *et al* 2011). Till now, around 50 QTLs associated with ShB resistance have been identified (Zou *et al* 2000; Channamallikarjuna *et al* 2010; Zuo *et al* 2014) and mapped on all twelve rice chromosomes, primarily originating from *indica* rice (Jia *et al* 2009; Zuo *et al* 2010; Xu *et al* 2011; Wang *et al* 2012). However, there is a need to validate and pyramid these QTLs for its utilization in breeding. The sources of resistance in the rice, known so far, in natural population have moderate resistance only and the effectiveness of the QTLs identified needs to be tested and validated. Additionally, there is a need to search for new sources of resistance, which will ultimately pave the way for utilizing host plant resistance in rice improvement through marker assisted pyramiding of QTLs. The vast genomic resources in rice, including molecular markers like simple sequence repeats (SSRs), have facilitated the mapping of QTLs governing traits such as ShB resistance. Marker-assisted selection (MAS) is considered a viable approach for developing ShB-resistant varieties, given the identification of numerous ShB resistance QTLs (Jia *et al* 2009; Zuo *et al* 2010; Eizenga *et al* 2013; Liu *et al* 2013). Breeding and utilization of resistant varieties is still considered the most economic and effective ways for controlling rice ShB.

At PAU, the *Oryza rufipogon* accession CR100438 showed consistent tolerance reaction to ShB disease (Aggarwal *et al* 2022). A set of 162 backcross introgression lines (BILs), were developed from a cross between ShB susceptible, rice cultivar Punjab Rice PR114 and ShB resistant *Oryza rufipogon* accession CR100438. The QTLs/ Gene (s) governing tolerance in *O. rufipogon* is not known. Therefore, the present research study entitled “Molecular mapping of sheath blight QTL(s) from the wild rice *Oryza rufipogon* (Griff.) accession CR100438” was undertaken with the following objectives:

1. Screening of BC_2F_5 and BC_2F_6 population (PR114/ *O. rufipogon* accession CR100438//2* PR114) against sheath blight disease
2. Mapping of QTLs/ genes for sheath blight resistance from *Oryza rufipogon* accessionCR100438

CHAPTER – II

REVIEW OF LITERATURE

Rice is affected by various biotic and abiotic stresses. ShB of rice is a major disease increasing over the year in India (Laha *et al* 2016). The research work done in-relation to rice ShB has been reviewed under this chapter:

2.1 Occurrence and economic impact of ShB (*R. solani*)

2.2 Etiology and symptomatology of ShB

2.3 Methods for evaluating ShB resistance in Rice

2.4 *O. rufipogon* as a source of resistance to biotic and abiotic stresses

2.5 Genetic basis of ShB inheritance

2.6 Molecular mapping of QTL/genes conferring ShB resistance

2.1 Occurrence and economic impact of ShB (*R. solani*)

The occurrence of ShB became more severe with the widespread cultivation of semi-dwarf, high-yielding rice varieties, coupled with a significant increase in the use of nitrogen fertilizers (Savary *et al* 1995). In 1901, ShB disease was initially observed in Japan, as documented by Wei in 1934. However, the first confirmed and reliable report was published by Miyake in 1910, identifying *Sclerotium irregulare* as the causal organism behind the disease. Subsequently, it was established that the ShB fungus is synonymous with Hypochnus sasakii, as observed by Sawada in 1912. Additionally, Reinking (1918) and Palo (1926) independently identified a similar disease affecting rice in the Philippines, which was caused by *Rhizoctonia solani*.The historical records of ShB disease in rice unveil its prevalent manifestation in diverse regions and countries. Park and Bertus (1932) documented the presence of ShB disease in rice from Ceylon (present-day Sri Lanka), identifying the causal organism as *Rhizoctonia solani* Kuhn. In the USA, Ryker and Gooch (1938) reported a similar disease in rice, naming it 'banded sheath spot of rice'. In India, Butler (1918) first observed a disease with symptoms resembling those of banded sclerotial disease in sugarcane, which was later identified as ShB disease. However, it wasn't until Paracer and Chahal (1963) observed and described ShB disease caused by *R. solani* in the Gurdaspur district of Punjab, India. The widespread occurrence of this disease, referred to as oriental leaf and sheath spot of rice, and the identification of its perfect stage, *Corticium sasaki*i (Shirai) Matsumoto, was first reported in Varanasi, Uttar Pradesh, India, by Singh and Pavgi (1969). Additionally, the disease reached epidemic levels in the Srikakulam and Vijayanagaram districts of Andhra Pradesh during 1993 and 1994, as documented by Mathur *et al* (1999).

2.2 Etiology and symptomatology of ShB

R. solani (teleomorph of *Thanatephorus cucumeris*) stands out as significant pathogenic fungi affecting rice (Ou 1985). The epidemic of *R. solani* can be categorized into two distinct phases, as outlined by Savary *et al* (1997). During the initial phase, the soil-borne inoculum typically infects lesions on the lower internodes of the leaf sheaths. These lesions tend to merge and expand, eventually reaching the upper parts of the rice plant. This phase primarily represents the soil-borne stage of the pathogen's life cycle (Savary *et al* 2001). Subsequently, in second phase, pathogen spreads within the canopy's upper structures through mycelial strands. These strands originate from primary or secondary lesions and extend towards healthy tissues of the same or adjacent plants. This extension of mycelia strands leads to the establishment of new infections. These two distinct stages are called as horizontal and vertical spread, respectively (Kozaka 1961). *R. solani* enters plant by utilizing lobate appressoria or infection cushions, or sometimes by employing both methods (Marshall and Rush 1980). These sclerotia, a resting structure produced by the pathogen, can remain dormant in the soil for extended periods. In subsequent crop seasons, they have the potential to cause reinfection in healthy rice plants. This pathogen, exhibits the capability to infect rice plants from seedling to full maturity, thereby resulting in significant yield losses, based on the affected plant part. The symptoms of plant disease include lesions, lodging of the plant, and the presence of unfilled grains. Development of large lesions on the contaminated lower rice leaf sheaths might cause the stem to soften, which in turn can result in stem lodging (Wu *et al* 2012). The pathogen,*R. solani*, demonstrates multiple modes of transmission including being soil borne, seed borne, and water borne. Sclerotia, the resilient structures produced by the pathogen, have the ability to float in fields through irrigation water, leading to the spread of the disease across considerable distances. Surface water run-off, as well as various agricultural practices like transplanting and flood irrigation, aid in the dispersal of these sclerotia, acting as primary sources for infection. Both vertical and horizontal spread of the pathogen have been observed, with horizontal spread potentially reaching up to 20 cm per day (Savary *et al* 1995). Genetically, the pathogen displays substantial variability. Thus far, isolates of *R. solani* have been classified into 14 distinct anastomosis groups (AG). The initial thirteen groups are labeled AG1 to AG13, while the fourteenth group is a bridging isolate identified as AGB1 (Carling *et al* 2002a; Carling *et al* 2002b).

Intensive cropping practices have led to changes in the microclimate of rice crops, resulting in more humid canopies. This alteration in environmental conditions has contributed to an increased incidence of ShB (Kannaiyan and Prasad 1983). Despite extensive efforts, a fully resistant variety of rice against ShB has not yet been identified. This problem arises from the inability to find a sufficient degree of natural resistance within the available rice germplasm. The pathogen responsible for ShB demonstrates a wide host range, infecting nearly 32 taxonomic families of plants (Gangopadhyay and Chakrabarti 1982). The symptoms of ShB in rice are characterized by water-soaked, oval to spherical-shaped, or irregularly elongated discolored lesions. These lesions typically appear greyish to light brownish with brown margins and are observed on the leaf sheath and blades.

2.3 Methods for evaluating ShB resistance in Rice

Various inoculation techniques are employed for field screening to assess ShB resistance in rice. These techniques include straw bit inoculation, spray culture inoculum, stem tape inoculation, and methods involving the incorporation of sclerotium and inoculated rice grain (Dath 1985; Chakraborty *et al* 2006). However, among these methods, a more successful approach involves testing different rice genotypes for resistance to ShB in the field by using grain and rice hull medium between the tillers at the bottom of rice hills. Another effective method is by inserting *R. solani*-colonized toothpicks enters the lower inner leaf sheath of the primary tiller during the maximum tillering stage (Sunder *et al* 2003). Additionally, fungus culture developed on grains of rice, barley, and *Typha angustata* has been utilized for this purpose (Zou *et al* 2000). These techniques enable efficient assessment and evaluation of resistance for ShB in rice genotypes. The association between ShB resistance and various plant morphological characteristics have been studied, revealing dissociation between them in some instances (Zou *et al* 2000; Channamallikarjuna *et al* 2010; Zeng *et al* 2015; Taguchi-Shiobara *et al* 2013). However, previous research has highlighted correlations between certain plant characters, such as compactness of plant and leaf angle, and ShB resistance (Hossain *et al* 2016; Han *et al* 2003). Of particular significance, are two parameters often associated with ShB resistance: vertical sheath colonization and disease severity (Lore *et al* 2013). Interestingly, a cultivar resistant according to one parameter may exhibit susceptibility when assessed based on various parameters (Dath 1985). The scoring system used for ShB heavily relies on symptom height, which is directly linked to plant height, thus indicating how alterations in plant height can influence results. Moreover, by adjusting the heading date, a plant can adapt to varying environmental conditions, which may or may not be conducive to diseases. Plant compactness plays a role in influencing ShB incidence and the proliferation of pathogens by altering the microclimate within the plant canopy. This alteration in microclimate can impact the likelihood of ShB occurrence and the pathogen's ability to thrive. According to Wang *et al* (2009), resistance levels among cultivars showed less variation in greenhouse conditions compared to natural conditions. However, the order of susceptibility among cultivars exhibited similar variations in both environments. Additionally, Singh *et al* (2016) reported a strong positive association (r=0.931) among disease scores observed in the nursery and those in grown in field.

Various inoculation approaches have been employed for assessing ShB resistance in rice. These techniques include distributing fungal inoculum replicated on rice hull and grain media across the soil surface surrounding the plant, detached leaf technique, detached tiller method, micro-chamber screening method, mist-chamber screening method, and syringe inoculation (Araujo *et al* 2007). These diverse methods help researchers evaluate the resistance levels of rice cultivars under different conditions and facilitate a comprehensive understanding of ShB resistance. According to Kumar *et al* (2015), the field-based parental line screening was carried out at the ADAC & RI experimental farm in Trichy during the thaladi season of 2012–2013. Six lines *viz.* IW Ponni, BPT 5204, IR 64, RNR 57979, FL 478 and TETEP—were planted alongside the susceptible check, TN 1, using spacing of 20 x 15 cm and entries were represented by a single seedling planted in five rows, each measuring 3 meters long. This planting setup was replicated three times. To facilitate interaction with the ShB isolate, pieces of paddy straw containing the fungus mycelia were inserted between the tillers of each rice hill, just above the water level, and securely tied. At the maximal tillering stage, the ShB isolate was introduced into the rice seedlings (Bhaktavatchalam *et al* 1978) to create a conducive environment for disease development, high humidity levels (90–95%) were maintained by consistently maintaining a water level of 5–10 cm. Standard agronomic practices were followed to support crop growth. The tied tillers were untied once typical lesions began to appear, which usually occurred three days after the initial inoculation. These lesions, observed on the lower leaf sheaths and leaves, appeared as greyish-green patches that expanded and merged over time.

2.4 *O. rufipogon* as a source of resistance to biotic and abiotic stresses

IRRI maintain a collection of over 4,000 accessions of wild *Oryza* species, alongside 1,500 accessions of cultivated African rice (*O. glaberrima*). These wild species serve as donor of numerous valuable genes, especially those related to major biotic and abiotic stress resistance. The genetic diversity within the wild *Oryza* species can be instrumental in enhancing rice productivity. The *Oryza* genus includes two domesticated species (*O. sativa* and *O. glaberrima*) and 22 wild species, representing between 15 and 25 million years of evolutionary diversification (Vaughan 1994). Although these wild *Oryza* species might exhibit traits that are agriculturally inferior, such as low grain yield, undesirable plant characteristics, and natural shattering tendencies, they contain remarkable diversity in morphological traits. These traits include growth habits, plant height, flowering, tillering, panicle and leaf structure, culm properties, seed characteristics, and adaptations to various environmental conditions. The genus *Oryza* consists of 24 species and 9 recognized genome sets, providing an extensive gene pool for enhancing rice cultivars genetically. The method of transferring genes from wild species into rice relies on the specific trait targeted within the wild species and any barriers that might exist due to genetic incompatibility. There are no barriers among species possessing the AA genome, facilitating their use in crosses with *O. sativa* and establishing them as the primary pool for hybridization. The utilization of *O.*

rufipogon in breeding with *O. sativa* has been involved in conferring resistance to various diseases in rice. McKenzie *et al* (1994) demonstrated its effectiveness in providing resistance against stem rot disease, while Cabunagan *et al* (1998) reported its resistance to rice tungro virus. Additionally, studies have highlighted its resistance to other critical diseases like rice blast and bacterial leaf blight caused by *Xanthomonas oryzae* pv. o*ryzae* (Zhang *et al* 1998). Martinez *et al* (1998) observed that the introduction of specific genes from *O. rufipogon* contributed to enhancing the yield of rice cultivars. Specific alleles from *O. rufipogon* are associated with increased yield. Xiao *et al* (1996) reported that alleles of *O. rufipogon* identified at marker RM5 on chromosome 1 and RM256 on chromosome 2 were linked to enhanced yield. Lines carrying *O. rufipogon* alleles at these loci exhibited a phenotypic advantage of 1.2 and 1.1 t/ha, respectively. Moreover, Zhang *et al* (1998) identified the bacterial blight-resistant gene *Xa23* sourced from *O. rufipogon,* which was mapped on chromosome 11. These findings underscore the significant potential of *O. rufipogon* in contributing valuable disease resistance traits and improving the yield potential of cultivated rice varieties through genetic introgression and breeding programs.

2.5 Genetic basis of ShB inheritance

The identification of ShB QTLs began with Li *et al* (1995) using RFLP markers under unproctected conditions. Approximately 50 ShB QTLs have been detected across all 12 rice chromosomes, encompassing cultivated varieties, deep water varieties, and wild species (Huang *et al* 2012). The majority of identified ShB resistance QTLs have originated from *indica* rice, while relatively fewer have been traced to *japonica* rice (Zuo *et al* 2010; Jia *et al* 2012). ShB resistance is controlled by multiple genes, with minor effects (Sha and Zhu, 1990; Li *et al* 1995; Pinson *et al* 2005). Some studies (Xie *et al* 1992; Pan *et al* 1999) have proposed that in certain rice accessions, ShB resistance could be governed by a limited number of major genes. This diversity in genetic control highlights the complexity of ShB resistance in rice and underscores the need for continued research to fully understand the underlying genetic mechanisms involved in combatting this damaging disease. The observations made by Pan *et al* (1999) and Xie (1990) highlight the diverse genetic mechanisms governing resistance for ShB in rice cultivars. Pan *et al* (1999) reported the non-allelic dominant major resistant genes in the cultivars Teqing and Jasmine 85, each with ten independent genes segregated separately. This finding suggested the potential of combining major genes that confer high degrees of partial resistance to ShB in order to achieve near-complete resistance. Similarly, in a long-grain rice cultivar, SC 8620001-33, two independent recessive genes were identified to control resistance, while SC 8620001-5 was found to possess one independent recessive gene for resistance (Xie 1990). Additionally, some studies have indicated that resistance may act as a dominant character, as evidenced by most F_2 populations resulting from crosses between resistant and susceptible parents displaying resistance (Dath 1990). However, despite these observations pointing to the presence of major genes and dominant or recessive genetic control in certain cultivars, the majority of studies lean towards ShB resistance being a complex polygenic trait regulated by a large number of polygenes. This complexity underscores the multifaceted nature of genetic control over resistance for ShB in rice.

Table 2.1: Reports on the nature of inheritance of sheath blight resistance

S. No	Nature of inheritance	Reference
1	Complementary genes with epistatic interaction	Goita 1985
2	Recessive genes	Xie 1990
3	Dominant gene	Dath 1990

2.6 Molecular mapping of QTL/genes conferring ShB resistance

Despite extensive efforts worldwide to discover highly resistant sources against ShB, adequate levels of resistance have not yet been identified (Nadarajah *et al* 2014; Lore *et al* 2015). It is commonly understood that multiple genes control the quantitative trait for resistance to ShB in rice (Zuo *et al* 2014). Consequently, mapping, and detailed characterization of QTL responsible for ShB resistance is of utmost importance in the development to resistant to ShB (Yadav *et al* 2015; Molla *et al* 2020). Phenotyping diseases, greatly influenced by factors such as canopy density, plant height and weather conditions, remains the most challenging aspect of mapping loci resistant to ShB. Several studies have indicated association between factors like Plant Height, Heading Date, and ShB resistance (Fu *et al* 2011; Eizenga *et al* 2013; Kunihiro *et al* 2002; Liu *et al* 2014; Nelson *et al* 2012; Pan *et al* 1999; Pinson *et al* 2005; Sato *et al* 2004; Sharma *et al* 2009; Li *et al* 1995). While some varieties, such as Swarnadhan, Radha, Pankaj, and Vikramarya, have been found to possess moderate resistance, traditional rice cultivars like Tetep, Jasmine85, Tequing, Bhasamanik, Lalsatkara, ARC 15762, ARC 18119, ARC 18275, D-256, MTU 1010, and YSBR 1 exhibit moderate resistance to ShB (Wang *et al* 2009; Srinivasachary *et al* 2011; Lore *et al* 2015). Certain rice lines, including LSBR 33 (Xie *et al* 1992) and YSBR 1 in China (Zuo *et al* 2009), BPL 7-12 and BPL 27-1 in India (Dubey *et al* 2014a; Dubey *et al* 2014b), as well as Pecos in Malaysia and the United States of America (Sharma *et al* 2009; Willocquet *et al* 2011), have been reported to demonstrate a high level of resistance.

The localization of *qSB9-TQ* was accurately mapped within a 146-Kb region, linked with flanking markers CY85 and Y86 (Zuo *et al* 2014a). Zuo *et al* (2002) identified an additional 12 potential genes within the *qSB9-TQ* region. Within the *qSBR11-1* region, approximately 154 genes were located, among which 26 were found to be responsive to the disease (Channamallikarjuna *et al* 2010). An intriguing discovery was the presence of eleven class III chitinase genes, which are renowned for their antifungal properties, arranged in a tandem array (Channamallikarjuna *et al* 2010). Furthermore, a chitinase gene (LOC-Os11g47510) expressed in the ShB susceptible cultivar, Taipei 309 exhibited varying degrees of resistance, as confirmed by detached leaf bioassays (Richa *et al* 2017). Despite these extensive studies, there has been no success thus far in identifying novel candidate genes within the identified ShB QTLs that could be applicable in breeding or genetic engineering programs. Aggarwal *et al* (2022) conducted an assessment of 405 *O. rufipogon* accessions over the years 2015, 2016, and 2017 focusing on four traits associated with ShB: plant height, relative lesion length, and disease score. Employing a genome-wide association study and leveraging 44,109 high-quality SNP markers across 301 accessions, the researchers identified significant marker-trait associations. Across all rice chromosomes except for 10 and 12, the GWAS analysis detected a total of 22 noteworthy SNPs associated with ShB-related traits. Specifically, eleven SNPs were associated with RLH, seven with PH, one with LH, and one with DS. Traits linked to these SNPs were notably observed on chromosomes 3 and 9. Moreover, seven ShB resistance loci were identified to co-localize with previously identified ShB resistance genes which offer crucial insights into the significant SNPs related with ShB resistance.

Zou *et al* (2000) discovered six QTLs governing resistance to ShB, namely *qSB-2, qSB-7*, *qSB-3*, *qSB-9-2,* and *qSB-11*, located on chromosomes 2, 3, 7, 9, and 11, respectively. The alleles related with *qSB-2*, *qSB-7*, and *qSB-9-2* from Jasmine 85 explained 21.2%, 26.5%, 22.2%, and 10.1% of the total phenotypic variation, respectively. On the other hand, Che *et al* (2003) utilized a population generated between '4011' (resistant to ShB) and 'Xiangzaoxian19' (susceptible variety) to find markers associated to the resistant trait. They identified three RFLP markers converted from RAPD and AFLP markers, along with two SSR markers, that were associated with resistance to ShB. This dominant resistant gene was named *Rsb 1* and was mapped on rice chromosome 5. The linkage distances between the markers and *Rsb 1* were 1.6, 9.9, 1.6, 15.2, and 1.6 cM, respectively. In the review conducted by Srinivasachary *et al* (2011) on resistance to ShB QTL from various articles, they identified 12 ShB-QTL studies, with experiments focusing on *indica* rice cultivars and involving a *japonica* cultivar (Sharma *et al* 2009). Across all 12 rice chromosomes, 33 ShB-QTL were discovered and among these, only 16 QTL were consistent. To map these QTL, they utilized the rice genetic map initially by Temnykh *et al* (2001) and generated maps using MapChart (Voorrips 2002). SSR markers were predominantly used as anchor points to denote ShB-QTL locations. Interestingly, six PH-QTL (on chromosomes 1, 2, 3, 7, 8, and 9) from different studies were mapped in the same regions as ShB-QTL. In many instances, the peak LOD score, which indicates statistical significance, for PH-QTL coincided with the peak LOD for ShB-QTL.

In the rice variety Tetep, (Channamallikarjuna *et al* 2010) mapped the QTL *qSBR11-1* on chromosome 11, within the genomic region spanning from RM1233 to RM224. Another ShB QTL, *qSB-9TQ*, has been found from an *indica* cultivar named Teqing (Pinson *et al* 2005; Tan *et al* 2005). Pinson *et al* (2008) utilized markers closely linked to the ShB-QTL and marker-assisted selection was done to incorporate resistant alleles from Teqing (PI 536047) into three rice germplasm lines. Fang *et al* (2015) conducted QTL mapping for disease scale, relative lesion height, and plant height utilizing Introgression Lines. These ILs were selected from crosses between two varieties, IR64 and Teqing, and two tall varieties, Tarom Molaii and Binam, known for high grain quality and ShB resistance, acting as donors. They observed a significant positive correlation between DS and RH, while both DS and RH exhibited significant negative correlations with PH. Despite relatively small introgression populations (ranging from 28 to 60), DS, RH, and PH displayed considerable variations. Even some semi-dwarf plants showed segregation patterns aligning with the parent's SBR traits. Using one-way ANOVA analysis, they identified ten, eight, eight, and six QTLs for the three traits in the IR64/Tarom Molaii, Teqing/Tarom Molaii, IR64/Binam, and Teqing/Binam populations, respectively. Most QTLs associated with donor alleles decreased DS and RH while increasing PH. Interestingly, no common QTL for three traits was found in the populations of the identical donor in different backgrounds, exhibiting genetic background effect on the expression of SBR- and PH-QTLs. They found that QTLs linked to the same marker loci between PH and DS and PH and RH, accounted for 38% and 52% of the total QTLs for the respective traits. This suggested a close relationship between SBR and PH, indicating that both traits shared certain related loci.

Yadav *et al* (2015) in their study, found nine QTLs, exhibiting phenotypic variance ranging from 8.40% to 21.76%. They utilized backcross mapping populations derived from the cross between BPT-5204 and ARC10531. Among these QTLs, two major ones, named*qshb7.3* and *qshb9.2* located on chromosomes 7 and 9 respectively, displayed substantial phenotypic variance (R2) of 21.76% and 19.81% respectively. The markers RM205 and RM336 related with these QTLs, were verified for their linkage with moderately resistant disease reaction in the backcrosspopulation. Nelson *et al* (2012) developed Double Haploid lines using *japonica*, resulting from a cross between MCR10277 (resistant) and Cocodrie (susceptible) varieties. They identified four ShB Resistance QTLs originating from MCR10277, collectively explaining 47% of the genetic variation observed in the field. Among these QTLs, the most pronounced effect in all trials was attributed to a QTL on chromosome 9, designated as *qsbr_9.1*. However, some QTLs showed variations concerning

U.S. and Colombian *R. solani* isolates, indicating specific responses to different pathogen strains. Notably, while several height or heading time QTLs were identified, only a couple of them coincided with SBR QTLs. Furthermore, when tested with U.S. isolates in a micro chamber greenhouse assay, the same QTLs identified through field inoculation were confirmed, reinforcing the relevance of these QTLs in controlling resistance to ShB. Fu *et al* (2011) conducted a study utilizing a Recombinant Inbred Line population to identify 28 QTL associated with five traits related to ShB resistance: disease rating, lesion length, lesion height, relative lesion length, and relative lesion height. These QTLs were found distributed across nine chromosomes, and many of them exhibited specificity to particular environments. Notably, a major QTL named *qSBR1* on chromosome 1 was identified, contributing to 12.7% and 42.6% of the variation in disease rating at Shanghai and Hainan, respectively. This QTL coincided with a QTL for plant height (PH), suggesting a linkage between ShB resistance and plant height. The allele from *RSB03*, the resistant parent, enhanced ShB resistance and increased plant height at this locus. Furthermore, another QTL for disease rating on chromosome 7 was found adjacent to QTLs associated with heading date (HD) and four other disease traits. *RSB03* also carried the resistant allele at this locus, leading to a shortened heading date. Conversely, the susceptible parent, HH1B, provided the resistance allele at the locus *qSBR8*. Their mapping results demonstrated that most QTLs for lesion length, lesion height, relative lesion length, and relative lesion height coincided with QTLs for disease rating. Additionally, three QTLs for disease rating were independent of heading date, plant height, and four other disease traits, while four QTLs were closely linked to heading date and plant height. This study indicated that lesion height, relative lesion length, and relative lesion height might serve as crucial indices, alongside disease rating, for estimating the level of resistance to ShB based on correlation analysis and QTL mapping results.

Indeed, the genetic control of resistance to ShB in rice involves multiple genes, and it is considered a quantitative trait, influenced by various numerous genetic factors. To date, among the reported 8,646 QTLs in rice, 38 QTLs have been specifically identified for resistance to ShB. Over the past decade, extensive efforts have been made in screening rice germplasms for mapping QTLs associated with ShB (ShB) resistance, employing various approaches, as outlined by Channamallikarjuna *et al* (2010). Despite continuous efforts, major QTL to ShB has not yet been achieved in any rice variety currently deployed in Asia. This indicates the complexity of the trait and highlights the ongoing challenges in developing rice varieties with robust resistance to ShB. The multifaceted nature of genetic control underscores the need for continued research and innovative strategies to ShB resistance in rice varieties for future agricultural advancements. The characterization of ShB resistance QTLs in rice have been explored by various studies, each highlighting specific loci associated with resistance.

Liu *et al* (2009) pinpointed a major ShB-QTL, *qShB9-2*, using a population of 256 Lemont/Jasmine 85 RILs and 199 SSR markers across the 12 rice chromosomes. This QTL, validated by both mist- and micro-chamber assays, was flanked by RM245 and RM215 on chromosome 9, showing high LOD values of 19.9 and 17.3, respectively. Similarly, Zou *et al* (2000) conducted an experiment utilizing an F_2 clonal population derived from Jasmine 85/Lemont and identified six QTLs, namely *qaSB-11*, *qSB-9-1*, *qSB-9-2*, *qSB-7*, *qSB-3*, and *qSB-2*, which confer resistance to ShB. These QTLs were located on chromosomes 11, 9, 7, 3, and 2, respectively. The alleles from Lemont for *qSB-11* and *qSB-9-1* explained 31.2% and 9.8% of the total phenotypic variation, while alleles from Jasmine 85 for *qSB-9-2*, *qSB-7*, *qSB-3*, and *qSB-2* explained 10.1%, 22.2%, 26.5%, and 21.2% of the total phenotypic variation, respectively. Moreover, Pinson *et al* (2005) evaluated ShB resistance QTLs in a 'Lemont' X 'Teqing' RIL population ($F_{2:10}$) and confirmed the location and effect of six ShB-QTLs: *qSB 3-1* (displaying the highest LOD score), *qSB-9*, *qSB-8-1*, *qSB-4-2*, *qSB-3-2,* and *qSB-2*. Field conditions can indeed pose challenges in efficiently phenotyping ShB due to varying humidity and temperature, which might affect the accuracy of assessments. Therefore, accounting for these environmental factors is crucial when evaluating resistance to ShB in field conditions.

In a study by Eizenga *et al* (2013), they utilized *Oryza nivara* accessions IRGC100898 and IRGC104705 to create two distinct backcross populations referred to as Wild-1 and Wild-2, respectively. These populations, consisting of 252 Wild-1 and 253 Wild-2 BC_2F_2 families, were assessed for their response to ShB disease in field conditions. The researchers identified that the resistance present in *O. nivara* was associated with a specific QTL named *qShB6*, which consistently appeared as the most significant ShB-QTL across all field trials. Additionally, although not consistently linked to the same parental source, *qShB1* and *qShB3* were consistently identified in every study conducted. It was noted that these ShB-QTLs might sometimes be masked or influenced by other traits within the genome. For instance, the *qShB6* QTL was located in the same genomic region as the DH-QTL (quantitative trait locus for heading date) named *qDH6*. Similarly, the *qShB1* QTL coincided with a large QTL for plant height, known as *qPH1*. This observation suggests that the genetic regions associated with ShB resistance (*qShB6*, *qShB1*, and *qShB3*) may share common regions with other important agronomic or developmental traits in rice, potentially leading to interactions or trade-offs between resistance to ShB and these other traits. In their research, Zuo *et al* (2010) investigated the breeding implications of a resistant QTL, *qSB-11*, and a resistant allele, *qSB-11LE*, associated with rice ShB located on chromosome 11, utilizing molecular markers Z286 and Z405. They studied the impact of these markers in the Lemont variety. Furthermore, the addition of *qSB-9Tq* to *Japanese* rice cultivars demonstrated an enhancement in resistance against ShB, resulting in lowered ShB scores, which averaged 1.0 (ranging from 0.5 to 1.3). This finding suggested that *qSB-9Tq* possessed considerable potential to strengthen *Japanese* rice's resilience against ShB, as it contributed to a reduction in disease severity. It's important to note that *O. sativa* subspecies *indica* harbors a QTL, *qSB-9Tq*, situated on chromosome 9, which imparts some degree of resistance against ShB, as highlighted in the study by Zuo *et al* (2008). The studies conducted by Liu *et al* (2013), Shiobara *et al* (2013), and Dubey *et al* (2014) collectively shed light on the identification and evaluation of several significant QTLs associated with ShB resistance in rice.

Liu *et al* (2013) evaluated resistance using 216 LJ RILs (Lemont × Jasmine Rice Introgression Lines) in field conditions and identified new QTLs on chromosomes 2, 11, and 7. Notably, *qShB2-1-AR* on chromosome 2 and *qShB11-2-TX* and *qShB11-1-TX* on chromosome 11 were among the identified QTLs. Additionally, reliable markers RM215 and RM245 were found to be closely linked to *qShB9-2*. Shiobara *et al* (2013) reported eight QTLs in backcrossed inbred lines (BILs) derived from the parents Jadan and Koshihikari. Notably, they found a stable QTL on chromosome 9, which consistently co-segregated with markers NagO8KK18184 and NagO8KK18871 across three years and various locations. Dubey *et al* (2014) tested 100 rice genotypes inoculated with the Kapurthala *R. solani* isolate under field conditions. They identified only four genotypes—BPL 7-12, BML 27-1, BML 21-1, and Kajarahwa—with high levels of resistance to ShB, exhibiting low Relative Lesion Height (RLH) percentages after 25 Days After Inoculation (DAI). Molecular validation confirmed the presence of the major ShB QTL, *qSBR11-1*, in all these four tolerant entries. These studies collectively contribute to the identification and validation of various QTLs associated with ShB resistance in rice, providing insights into markers linked to resistance traits and highlighting specific genotypes with enhanced resistance against this disease. Yadav *et al* (2015), employed BC_1F_2 mapping populations derived from cross between BPT-5204 and ARC10531 to investigate ShB resistance in rice. Through their research, they identified two markers, RM336 and RM205, that were associated with two major QTLs linked to moderate resistance against ShB. These QTLs were named *qshb9.2* and *qshb7.3* and were located on chromosomes 9 and 7, respectively. The phenotypic variance attributed to these QTLs was reported as 19.81% for qshb9.2 and 21.76% for *qshb7.3*. This finding underscores the importance of these genomic regions in conferring moderate resistance to ShB and highlights the potential significance of markers RM336 and RM205 as indicators or tools for identifying and selecting resistance to ShB in rice breeding programs.

Chen *et al* (2014) emphasized the challenge of developing rice varieties resistant to ShB through conventional breeding methods due to the complex nature of ShB resistance, which is considered a typical quantitative trait in rice. To address these challenges, a potential approach involves utilizing SSRs and SNPs. These markers offer valuable tools in the context of backcrossing strategies, enabling more efficient and precise breeding processes. The incorporation of molecular markers facilitates the backcrossing process by aiding in the selection of specific genomic regions associated with the desired trait, thereby reducing the number of backcrosses required to recover the desired phenotype. This technology enables breeders to more effectively introduce and retain the targeted traits, such as ShB resistance, from a donor parent into a recurrent parent, leading to the development of improved rice varieties with enhanced resistance to ShB. This approach enhances the efficiency and accuracy of the breeding process compared to traditional methods, offering a promising avenue for the development of ShB-resistant rice varieties. Advancements in molecular markers and quantitative trait mapping have led to the identification of numerous QTLs related to resistance against ShB in rice. Interestingly, many of these reported QTLs have been observed to correlate with increased plant height, as documented by Li *et al* in 1995. Moreover, besides the QTLs, several host genes associated with various biochemical pathways have been recognized for their contributions to resistance against this disease. Studies conducted by Venu *et al*(2007), identified specific host genes that play crucial roles in different biochemical pathways, thereby influencing resistance mechanisms against ShB in rice. These genes are involved in intricate pathways within the plant's biological systems, contributing to the overall defense mechanisms against the disease. This collective information underscores the multifaceted nature of ShB resistance in rice, highlighting the intricate interplay between genetic factors, QTLs, plant physiology, and biochemical pathways that collectively contribute to the plant's ability to resist this particular disease.

The identification of several QTLs linked with resistance to ShB in rice have been carried out using various populations derived from Teqing and Lemont crosses across different studies. Li *et al* (1995) identified two QTLs, *Qsbr3a* and *Qsbr9a*, which together accounted for 37.1% of the phenotypic variance. *Qsbr3a* was situated on chromosome 9, flanked by markers RG348–RG944. This discovery stemmed from an F4 bulk derived from Teqing/Lemont. Pinson *et al* (2005) used RIL population from the Lemont/Teqing cross to map two QTLs, *qSB-1* and *qSB-9*, located on chromosomes 1 and 3, respectively. Xie *et al* (2008) identified two QTLs, *Qsh8a* and *Qsh8b*, on chromosome 8. *Qsh8a*, flanked by markers RM25–RM126, accounted for 14.36% of the phenotypic variance, while *Qsh8b*, flanked by RM210–OSR7, explained 7.39% of the phenotypic variance. This discovery was made using an RIL population derived from the Lemont/Teqing cross. Zuo *et al* (2014) utilized Chromosome Segment Substitution Lines developed from the Lemont/Teqing cross to map a QTL, *qSB-9Tq*, located on chromosome 9 and flanked by markers RM201–RM6971. These studies collectively highlight the utilization of different populations from crosses between

Teqing and Lemont to identify and map various QTLs linked with resistance to ShB, providing information about the genetic basis of resistance against this particular disease. The studies conducted by Zou *et al* (2000), Li *et al* (2009) and Liu *et al* (2009, 2013) focused on identifying several QTLs associated with ShB resistance in rice derived from crosses involving Jasmine 85 and Lemont. Zou *et al* (2000) discovered six QTLs, namely *qSB-2*, *qSB-3*, *qSB-7*, *qSB-9-1*, *qSB-9-2*, and *qSB-11*, located on chromosomes 2, 3, 7, 9, and 11, respectively from an F2 population derived from the Jasmine 85/Lemont cross. These QTLs were found to individually explain varying percentages of phenotypic variance, ranging from 9.8% to 31.2%. Li *et al* (2009) identified the *QRh1* QTL on chromosome 1 using RILs obtained from Jasmine 85 as the resistant parent and Lemont as the susceptible parent. This QTL was associated with marker RM265 and explained 24.3% of the phenotypic variance related to ShB resistance. Liu *et al* (2009, 2013) reported a QTL, *qShB9-2* situated on chromosome 9, which was linked to marker RM245. This QTL accounted for 27.2% of the total phenotypic variance and was identified using a RIL population derived from the Jasmine 85/Lemont cross. These studies collectively contribute valuable insights into the genetic factors governing ShB resistance in rice, specifically highlighting the identification and characterization of multiple QTLs associated with resistance against this particular disease, derived from crosses involving Jasmine 85 and Lemont rice varieties.

Zuo *et al* (2010) employed a different approach by utilizing Near Isogenic Lines (NILs) derived from Lemont (resistance parent) and Yangdao (susceptible parent) to identify a specific QTL associated with resistance to ShB in rice. Through their research, they successfully mapped a QTL named *qSB-11LE*, situated on chromosome 11, flanked by markers RM167-RM202. This particular QTL was found to govern resistance against ShB in rice. The utilization of NILs allowed researchers to isolate and study the specific genomic region on chromosome 11 that contributes to ShB resistance. This approach provides a clear understanding of the genetic factors involved in resistance mechanisms against the disease, offering valuable insights for further studies and potential applications in rice breeding programs aimed at enhancing ShB resistance in rice varieties. The studies conducted by Kunihiro *et al* (2002), Che *et al* (2003), Sato *et al* (2004), and Xiang *et al* (2007) each identified specific (QTLs) associated with resistance to ShB in rice, derived from distinct crosses and populations. Kunihiro *et al* (2002) utilized a Doubled Haploid (DH) population resulting from the Jingxi 17 (resistant parent) and Zhaiyeqing 8 (susceptible parent) cross. They identified *qSBR-2* on chromosome 11, flanked by markers RG171–G243A, which explained 11.2% of the phenotypic variance related to ShB resistance. Che *et al* (2003) reported another QTL, *Rsb1*, located on chromosome 2. This QTL, identified from the F2 population derived from the cross between 4011 (resistance donor) and XZX19, explained

11.2% of the phenotypic variance associated with ShB resistance. Sato *et al* (2004) mapped *qSB-3* on chromosome 5, linked with marker RM3856, using a BC_1F_1 population derived from the resistance donor WSS2 and the susceptible cultivar Hinohikari. This QTL accounted for 19.4% of the phenotypic variance related to ShB resistance. Xiang *et al* (2007) identified *Rsb-2(t)* on chromosome 9, linked to marker RM218, through a cross between a mutant displaying resistance to ShB and Shuhui 881. These studies collectively highlight the diversity of genetic sources contributing to ShB resistance in rice and illustrate various QTLs distributed across different chromosomes, each playing a role in conferring resistance against this particular disease.

Sharma *et al* (2009) identified a significant (QTL) associated with ShB resistance in rice, specifically located on chromosome 1 and linked to marker RM1339. This QTL was found using an F2 population derived from the cross between Rosemont and Pecos, where Pecos served as the resistant parent. The QTL associated to RM1339 on chromosome 1 was discovered to account for a significant amount of total phenotypic diversity, approximately 35%. Channamallikarjuna *et al* (2010) derived RIL population from the cross between Tetep (resistant parent) and HP2216 (susceptible parent) to identify several (QTLs) associated with ShB resistance across different chromosomes in rice. Among the eight identified QTLs, are distributed over four out of the 12 rice chromosomes: Two QTLs, *qSBR1-1* and *qSBR1-2,* were positioned on chromosome 2, flanked by Hvssr68-RM306 and RM1232-Hvssr68-RM306 markers, respectively. They explained 15.01% and 8.13% of the phenotypic variance. On chromosome 7, two QTLs, *qSBR7-1* (flanked by RM3691-RM336) and *qSBR7-2* (flanked by RM5481-RM3691), accounted for 10.02% and 26.05% of the phenotypic variance, respectively. Another QTL, *qSBR8-1*, was situated on chromosome 8 and was flanked by the markers RM210-Hvssr47, explaining 8.37% of the total phenotypic variance. Three QTLs were found on chromosome 11, collectively contributing to 43.39% of the total phenotypic variance: *qSBR11-1* (flanked by Sbq1–RM224, Sbq11–RM224) contributed 13.99% to the total phenotypic variance, *qSBR11-2* (flanked by RM3428–RM209) explained 7.81% of the total phenotypic variance and *qSBR11-3* (flanked by RM536–RM20) contributed 21.59% to the total phenotypic variance. These findings signify the genetic complexity underlying ShB resistance in rice, with multiple genomic regions across various chromosomes playing significant roles in providing resistance to this particular disease. Shiobara *et al* (2013) utilized BC_2F_3 population derived from a cross between Jarjan (resistant parent) and Koshihikari (susceptible parent) and mapped a QTL, *qSBR-9*, which was found to be flanked by markers Nag08KK18184-Nag08KK18871 on chromosome 9. This QTL was associated with ShB resistance in rice. On the other hand, Yadav *et al* (2015) conducted a study using a BC_1F_2 population derived from the cross between ARC10531 (resistant parent) and BPT5204 (susceptible parent). They identified and reported two significant QTLs, *qshb7.3* and *qshb9.2*, located on chromosomes 7 and 9, respectively. These QTLs explained approximately 21.76% and 19.81% of the phenotypic variance associated with resistance to ShB in rice. Both studies utilized different mapping populations and identified important QTLs associated with ShB resistance in rice, providing valuable insights into the genetic factors contributing to resistance against ShB disease in different rice species.

The challenges in achieving robust ShB resistance in rice varieties are multifaceted. Most available sources only offer moderate resistance, and the inconsistency in their resistant reactions complicates result interpretation. The presence of desirable genetic alleles scattered across populations necessitates their identification through molecular markers for effective combinations in breeding programs (Lande and Thompson, 1990). The quantitative nature of resistance further adds to the complexity of breeding efforts (Sharma *et al* 2009). Additionally, there are differing perspectives on the mode of ShB resistance, underscoring the importance of understanding the inheritance patterns within the studied population. It becomes crucial to discern the mode of inheritance in the specific population under investigation before mapping genes governing resistance to ShB, particularly in resistant sources derived from *O. rufipogon*. This understanding can significantly inform targeted breeding strategies aimed at enhancing ShB resistance in rice varieties.

CHAPTER – III

MATERIAL AND METHODS

The present experiment was undertaken to phenotype and genotype the BC_2F_5 and BC_2F_6 populations derived from a cross between sheath blight susceptible rice cultivar PR114 and resistant wild species of rice *rufipogon* accession CR100438. This study was carried out at the experimental field area and the molecular biology lab of the School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. The materials used and methods employed in the present study are discussed below under following sub-headings representing different experiments conducted in this research work.

3.1 Screening of the BC_2F_5 and BC_2F_6 populations against sheath blight disease

3.1.1 Plant material

At PAU, Ludhiana, the wild rice *Oryza rufipogon* accession CR100438 showed consistent tolerant reaction to ShB for the last five years. The recipient cultivar is PR114, is high yielding rice cultivar but susceptible to the sheath blight disease. The F_1 was developed by crossing rice cultivar PR114 and wild species *O. rufipogon* acc. CR100438. The BC_2F_5 population was developed by two backcross with the recipient parent (PR114/ *O. rufipogon* accession CR100438//2* PR114) and further selfing using single seed decent method till BC_2F_5 generation. The plants were sown in row to plant spacing of 20 x 15 cm during the two *kharif* crop seasons 2022 and 2023. The standard agronomic practices were followed.

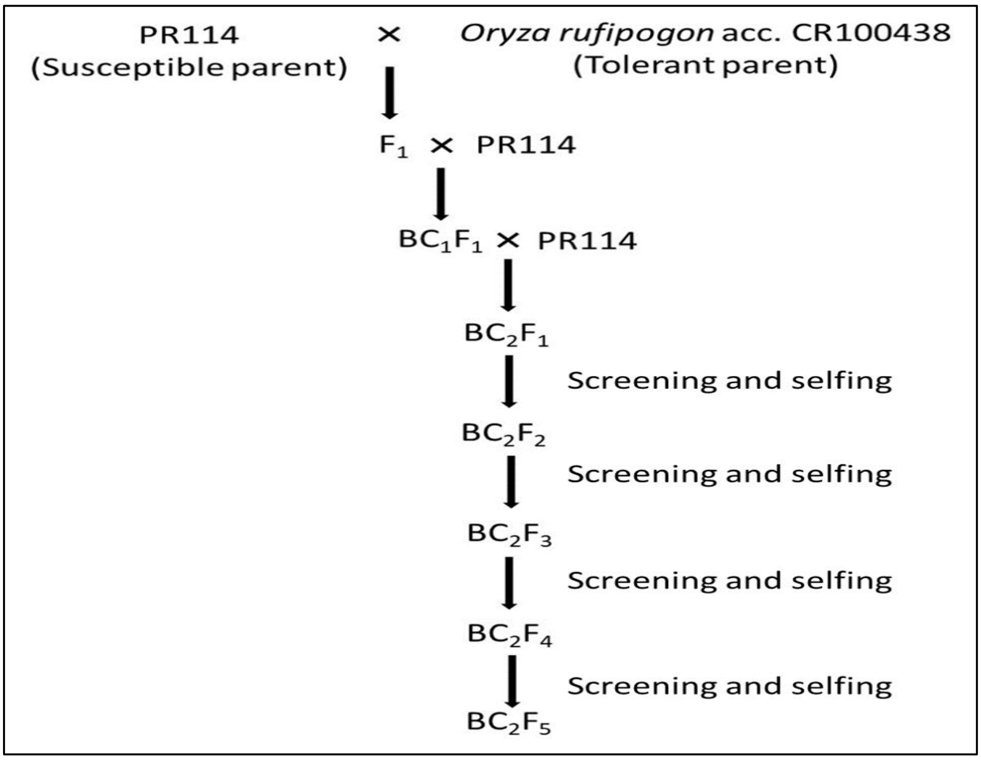

Fig. 3.1: Generation advancement for BC_2F_5

3.1.2 Multiplication of pathogen or isolation, purification and preservation of *R. solani* pathogen

R. solani was isolated from sheath blight infected rice plants showing clear symptoms of the disease. Collected leaf sheaths were washed in tap water and sliced into one cm pieces. The obtained slices were surface sterilized using 0.1 percent solution of mercuric chloride for few minutes followed by multiple washings with autoclaved distilled water. The sterilized slices were alienated to sterilized petri plates which contained potato dextrose agar medium (PDA) and incubated at 26±2^{o}C. The in-vitro growth of *R. solani* in the PDA medium was observed in 5 to 7 days of incubation. The sclerotia or hyphal tip were sub cultured into sterilized test tubes on same medium. Inoculated slants were transferred into BOD incubator at 26±2^{0}C for 7 days. Pure mature sclerotia cultures were transferred to refrigerator and stored at 4^{o}C for further studies.

3.1.3 Inoculation

The BC_2F_5 and BC_2F_6 populations were artificially inoculated using *R. solani* isolate belonging to AG-1 IA anastomosis group of the fungus. Mass multiplication of the *R. solani* isolate was carried out on maize meal-sand (1:3 w/w) medium enriched with 20g sucrose and autoclaved at 121^{o}C (15psi) for 30 minutes for three consecutive days (Lore *et al* 2012). After ten days mature hyphal growth of *R. solani* was visible in the inoculum which is desirable for use. The plants were inoculated at peak of tillering stage, 40 days after transplanting, by transferring 5g of the inoculum to the center of hill of each plant to be phenotyped. A total of 1030 plants consisting of five plants from each line of BC_2F_5 and three plants from each line of BC_2F_6 populations were inoculated during *kharif* 2022 and 2023, respectively (Fig. 3.2).

3.1.4. Disease assessment

Disease assessment was carried out 21 days after inoculation of the plants with *R. solani* under field conditions. PR114 was used as the susceptible check. Seven parameters were evaluated during the assessment of the BC_2F_5 and BC_2F_6 populations. The mean of the data from inoculated plants of each line were subjected to analysis. Mean, standard deviation, kurtosis and skewness of the disease index of BC_2F_5 and BC_2F_6 populations were calculated.

Following disease variables were evaluated for the BC_2F_5 and BC_2F_6 genotypes during *kharif* 2022 and 2023.

A. Plant Height (PH)

Using a centimeter’s scale, the height of the plants was measured from the plant's base to the tip of the highest panicle (Fig. 3.3).

B. Lesion Height (LH)

The total lesion height was recorded by measuring the height of the tallest lesion on the sheath of stem from the base of the plant using a centimeter scale (Fig. 3.4 and 3.5).

Fig. 3.2: Inoculum placed in the center of rice plant

Fig. 3.3: Recording plant height disease parameter in the field

Fig. 3.4: Progression of lesion towards tillers in young rice seedlings

Fig. 3.5: Susceptible parent PR114

C. Number of Tillers

The total number of tillers produced by each plant was calculated in each inoculated plant of the BC_2F_5 and BC_2F_6 populations.

D. No. of Infected Tillers

The total number of infected tillers in each inoculated plant was calculated in BC_2F_5 and BC_2F_6 populations.

E. Heading Date (Number of Days to flowering)

The date of heading of each line in the BC_2F_5 and BC_2F_6 populations was recorded.

F. Relative Lesion Height (RLH per cent)

Relative Lesion Height (RLH) was calculated from the observed values of plant height and lesion height of the plants using the standard formula by Sharma *et al* (1990).

$$\text{RLH (per cent)} = \frac{\text{Lesion height (LH)}}{\text{Plant height (PH)}} \times 100$$

G. Disease Scoring

The disease scoring was done on the basis of Relative Lesion Height (RLH). RLH is the average vertical height of the uppermost lesion on leaf or sheath expressed as percentage of the average plant height. Disease severity will be calculated using scale (0-9) as mentioned in table (SES, IRRI, 2013).

Table 3.1: Standard Evaluation System (SES) for sheath blight of rice using 0-9 scale (IRRI 2013)

Disease Score	Disease reaction	Description (based on relative lesion height-RLH per cent)
0	Immune	No infection
1	Resistant	Vertical spread of lesion up to 20 per cent of plant height
3	Moderately resistant	Vertical spread of lesion up to 21-30 per cent of plant height
5	Moderately susceptible	Vertical spread of lesion up to 31-45 per cent of plant height
7	Susceptible	Vertical spread of lesion up to 46-65 per cent of plant height
9	Highly susceptible	Vertical spread of lesion up to 66-100 per cent of plant height

3.2 Mapping of the QTLs/Genes for sheath blight resistance from *Oryza rufipogon* CR100438

3.2.1. DNA extraction

DNA of the BC_2F_5 population along with the parents i.e., PR114 and *O. rufipogon* accession CR100438 was extracted using small scale standard CTAB method of DNA extraction (Saghai Maroof *et al* 1984). The methodology is described below:

I. Leaf samples were collected from young, disease free leaves of 45 days old plants. Eight to ten leaves were collected from each plant.

II. The collected samples were crushed in liquid nitrogen using pestle and mortar to form a fine powder. The crushed tissue was collected in pre labelled 2mL eppendorf.

III. 800μl of CTAB buffer was added to the eppendorf, which were then subjected to heating at 65^{o}C in water bath for one hour.

IV. After one hour of hot water bath, 800 μl of chloroform: isoamyl in the ratio of 24:1 was added to the mixture in the eppendorf. Later were placed on a shaker which was set at 70 rpm for 40-50 min.

V. The mixture was subjected to centrifugation after removing the eppendorf from the shaker using a eppendorf centrifuge. The centrifugation was carried out at 12000 rpm for 15 min.

VI. After centrifugation three layers were visible in the eppendorf and the organic components settled down in the bottom layer and DNA was separated in the top aqueous layer. Supernatant was carefully collected and transferred to fresh 1.5mL eppendorfs.

VII. The volume of the solution was doubled by the adding chilled isopropanol solution in equal amounts. The newly formed solution was gently inverted few times and then transferred to -20^{o}C for 20-30 min.

VIII. After incubation, the solution was centrifuged at 5000 rpm for 15 min. A DNA thread was visible at the bottom of each eppendorf which was then collected and transferred to fresh 1.5 mL eppendorf tubes.

IX. The 1.5mL eppendorf were centrifuged at 10,000 rpm for 10 min to form the pellet.

X. The solution was discarded carefully and the pellet was processed with centrifugation in 70% ethanol for washing followed by air drying for 50-60 min.

XI. After proper drying, 100μL of TE buffer was added to the pellets for dissolving and storage.

XII. After proper dissolving, RNase A treatment by adding 10 μL RNase A to each sample followed by incubation at 37^{o}C for 45 min.

XIII. The samples were maintained at -20^{o}C for long term storage in the freezer.

Table 3.2: Composition of 2X CTAB Extraction Buffer

Component	Concentration
CTAB	200ml
TrisHCL	20 ml
NaCl	16.36g
EDTA	8 ml
Mercapto-ethanol	2% (4 ml)

3.2.2. Quantification and estimation of quality of DNA

The quantity and the quality of the extracted DNA samples were determined using three methods.

A. Nano drop method

Thermo Sceintific NanoDropTM8000 spectrophotometer USA was the instrument used to evaluate the quantity and quality of the DNA samples, operated via PC software. The instrument measured the quantity of DNA, RNA and proteins in 2 µL of each sample placed on the pedestal.

B. Agarose gel electrophoresis

0.8% agarose gel was used to assess the quality and quantity of the extracted DNA samples followed by electrophoresis. The samples were prepared by adding 6 X loading dye (Sucrose 4 gm, bromophenol blue 0.025g, xylene cyanol 0.025g, final volume 10ml) in the ratio of 2:8. 0.8% agarose gel was prepared by dissolving 0.8g agarose (Hi-Media, India) in 100mL of 0.5X TBE buffer. The mixture was heated in a microwave till the solution became transparent. The clear gel was cooled down to 60^{o}C by manual stirring at room temperature. 5µL stock of ethidium bromide (10mg/ml) was added at final concentration 5µg/mL to 100 mL gel. The gel was poured in already prepared casting tray with combs and left for solidification at room temperature for 45 min. The solidified gel was placed in electrophoresis tank having 0.5X TBE buffer and the combs were taken out. This was followed by loading of prepared DNA samples in the wells. The electrophoresis tank was switched on and run at a voltage of 5V/cm for a duration of 45-55 min. The DNA samples were observed by placing the gel in a gel documentation system (SynGENE). The system photographed the gel under UV light and the picture was analysed for the quality and quantity of the DNA. Single band indicated good quality DNA while a smear indicated poor quality. Similarly the quantity was assessed as per the density and brightness of the band where lighter bands indicated low quantity and highly bright bands indicated higher quantity of DNA in the sample.

C. Spectrophotometer method

Measurement of the amount of ultraviolet radiation absorbed by nucleotides offers an accurate measure to quantify DNA concentration in a sample. The DNA samples were diluted in 96 well PCR plate mixing 5µl of DNA with 95µlof double-distilled water (ddH_20). The dilution was mixed well by vertexing the PCR plate. The settings of the spectrophotometer machine were validated beforehand. The dilution factor was 1:20. The ddH_20 used to dilute the DNA was used as blank. The samples were loaded in the cuvette one by one and the readings were recorded.

Table 3.3: Composition and preparation of important buffers and solutions

Reagents	Composition	Concentration
Extraction Buffer2X	Tris HCL (pH8.0)	20 ml
	EDTA	8 ml
	Sodium chloride	16.36g
	CTAB	200ml
	*Mercapto ethanol	1%/2%
Wash Solutions	Ethanol	70%
	Sodium acetate	0.2mM
RNase A (10mg/ml)	Tris (pH8.0)	10mM
	Sodium chloride	15mM
	RNase A	10mg
TE Buffer (200ml)	1MTris	2ml
	0.5MEDTA	400µl

3.2.3. Genotyping by analysis (GBS)

The genotyping of the BC_2F_5 population and parents was done by outsourcing to AgriGenome using double-digest restriction site-associated DNA sequencing (ddRAD-seq). For genotyping, DNA samples were digested with two restriction enzymes *SphI* and *MlucI* (Non-methylation sensitive) used for ddRAD sequencing. The sequencing was done using the Illumina HiSeq 2000/2500 sequencing platform. Single nucleotide polymorphisms (SNPs) were identified by aligning the separate fastq files of reads with the Nipponbare reference IRGSP 1.0 SNPs using Bowtie2 (Langmead and Salzberg 2012). The alignments were transformed to the SAM (sequence alignment map) format and combined into a single BAM (binary file of SAM) using SAMtools 0.1.18. SNPs were called using the genome analysis toolkit (GATK) using the BAM file. Raw SNP data were filtered using the threshold for SNP call rate ≥ 0.7 and minor allele frequency (MAF) ≥ 0.05.

SNP markers with <10% missing data were used for the construction of a genetic map. Segregation distortion in marker data was assessed through the Chi-square test. The SNP markers with a Chi-square value >10 were considered distorted and excluded from the linkage analysis. RECORD (REcombination Counting and ORDering) software was used for ordering the markers on the linkage groups (LGs) (Van Os *et al* 2005). The ordering of markers was performed using default functions with the Kosambi mapping function and a 30% recombination rate. In the case of multiple SNPs showing similar segregation patterns, all the markers were placed in the same genetic bin and only one representative marker was used for the final map construction. The linkage map was drawn using MapChart 2.0 software (Voorrips 2002).

3.2.5 QTL mapping

The QTL analysis was performed for ShB and related traits using a composite interval mapping (CIM) function with a window size of 10 cM in Window QTL cartographer software (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The threshold logarithm of the odds (LOD) scores was determined using 1000 permutations (Churchill and Doerge 1994). The CIM model was selected based on markers used as cofactors by forward and backward regression analyses with a walking speed of 10 cM. The observed phenotypic variance (R^2) exhibited by the QTL was estimated by the CIM model.

CHAPTER – IV

RESULTS AND DISCUSSION

The present study was conducted to identify the QTLs/genes responsible for tolerance against sheath blight from a backcross population derived from a cross between susceptible parent PR114 and donor parent *Oryza rufipogon* accession CR100438. The detailed results are presented below.

4.1 Screening of BC_2F_5 population for sheath blight resistance.

The screening of 162 BC_2F_5 progenies was conducted at the experimental research field area of School of Agricultural Biotechnology, PAU, Ludhiana during *Kharif* 2022 along with the parents, PR114 and *Oryza rufipogon* accession CR100438. The progenies were raised with a standard row to row spacing of 20cm and plant to plant spacing of 15cm. Five plants from each line were inoculated with *R. solani* AG-IA isolate supplied by the rice pathology lab of Department of Plant Breeding and Genetics, PAU, Ludhiana. Seven disease variables *viz* plant height (PH), lesion height (LH), relative lesion height (RLH), disease score (DS), number of tillers (NT), number of infected tillers (NOIT) and heading date (HD) were evaluated. The phenotypic screening revealed segregation of progenies for all the concerned traits responsive for sheath blight reaction, displaying continuous frequency distribution. The evaluation of BC_2F_5 population revealed that out of 162 individuals, 134 individuals (82.71%) were moderately resistant, 24 individuals (14.81%) were moderately susceptible and only 1 individual (0.61%) showed resistant reaction towards ShB (Table 4.1).

Table 4.1: Disease reactions of BC_2F_5 and BC_2F_6 progenies against sheath blight

Type of population	**No. of Genotypes**	**RLH(%)**			
		Resistant	**Moderately Resistant**	**Moderately susceptible**	**Susceptible**
BC_2F_5	162	1	134	24	0
BC_2F_6	110	1	80	28	0

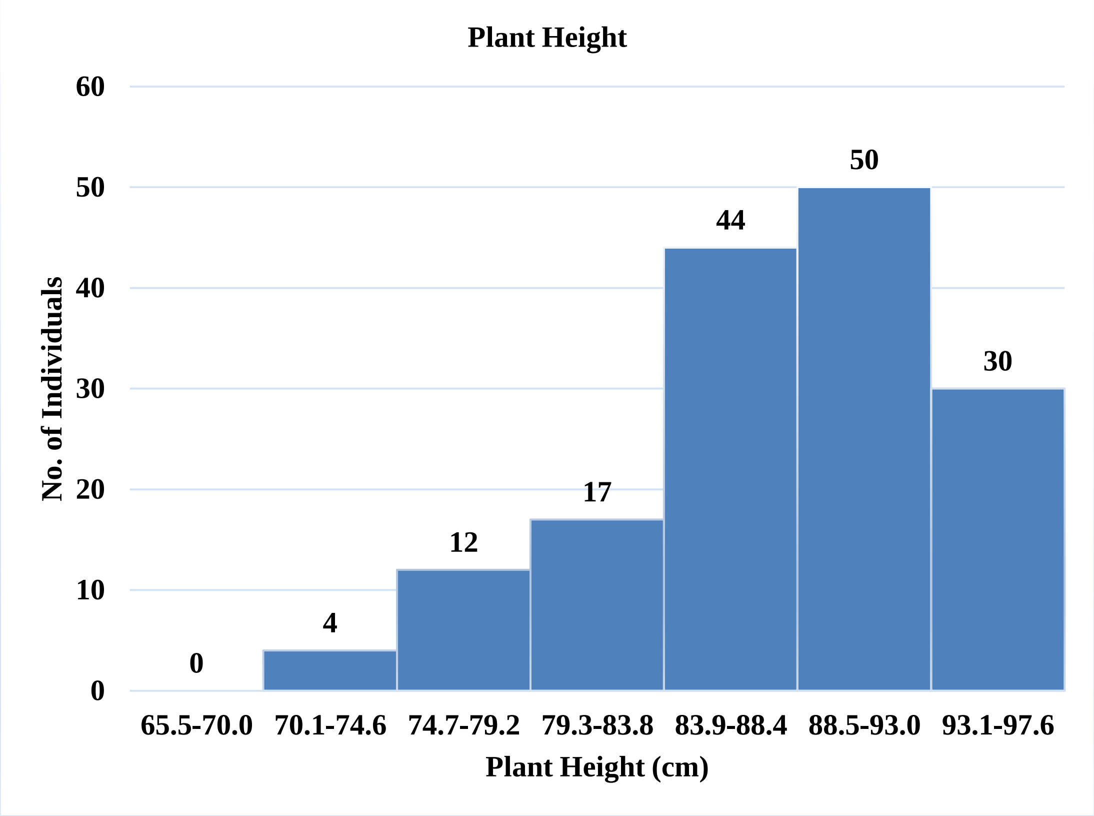

Fig. 4.1: Frequency distribution of plant height of BC_2F_5 population under field conditions

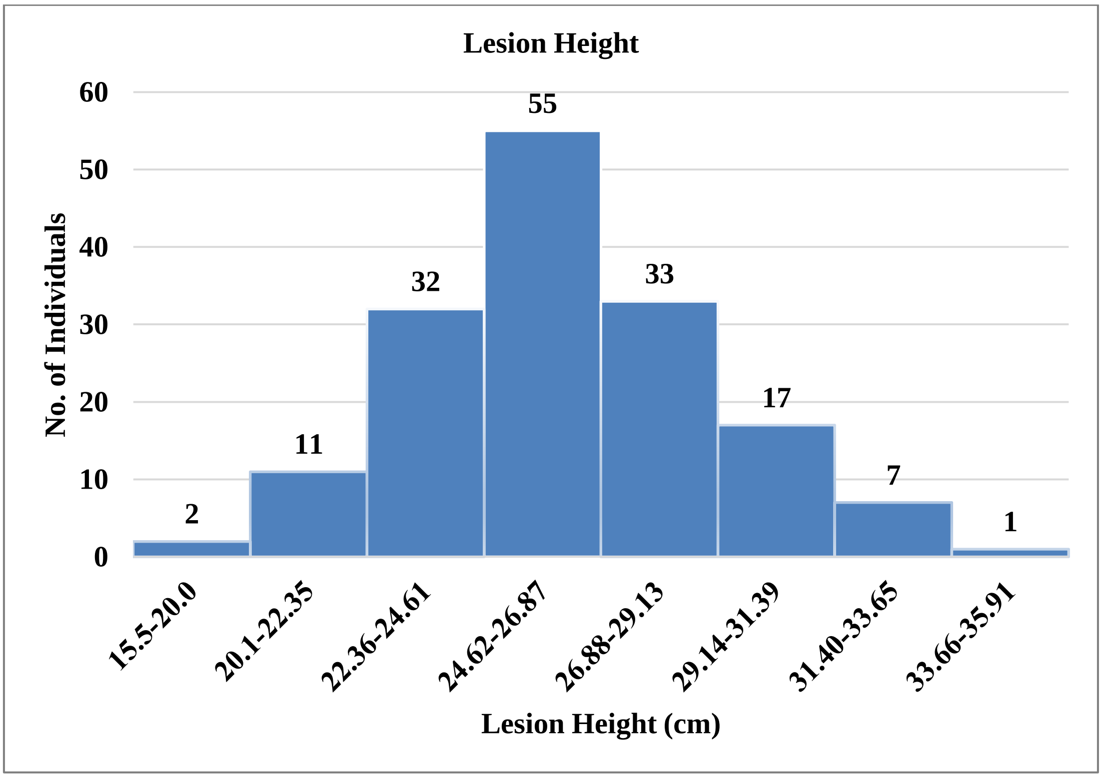

Fig. 4.2: Frequency distribution of lesion height of BC_2F_5 population under field conditions

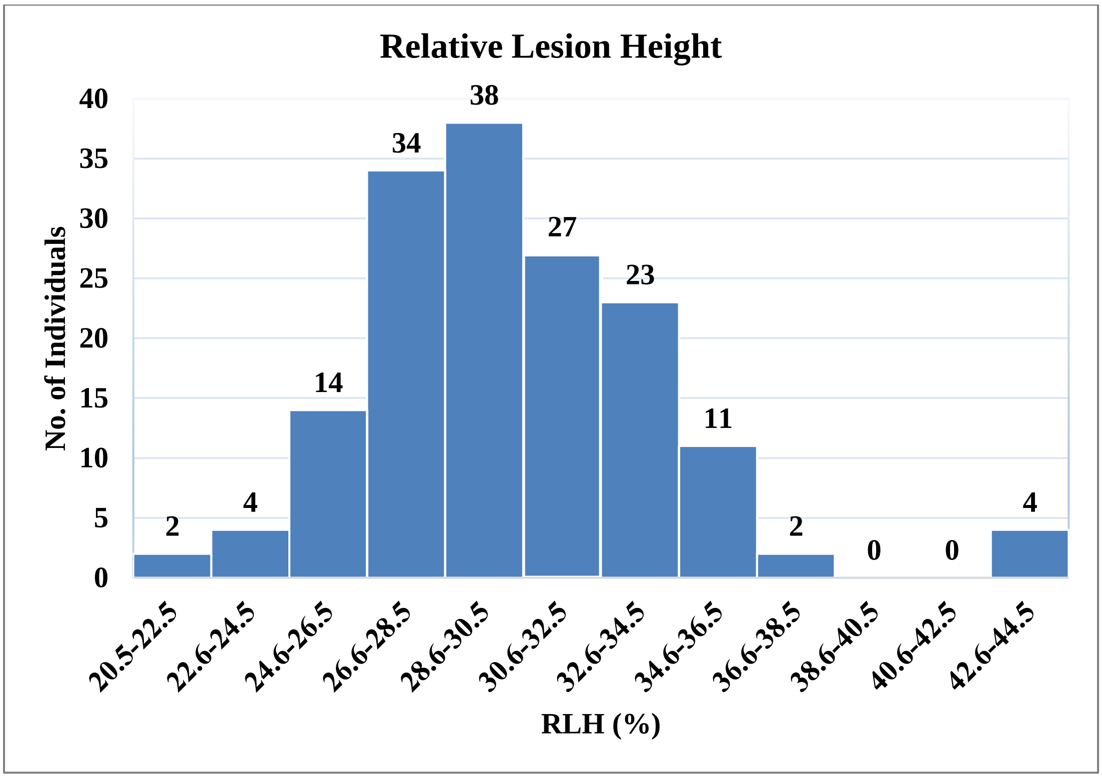

Fig. 4.3: **Frequency distribution of relative lesion height of BC_2F_5 population under field conditions**

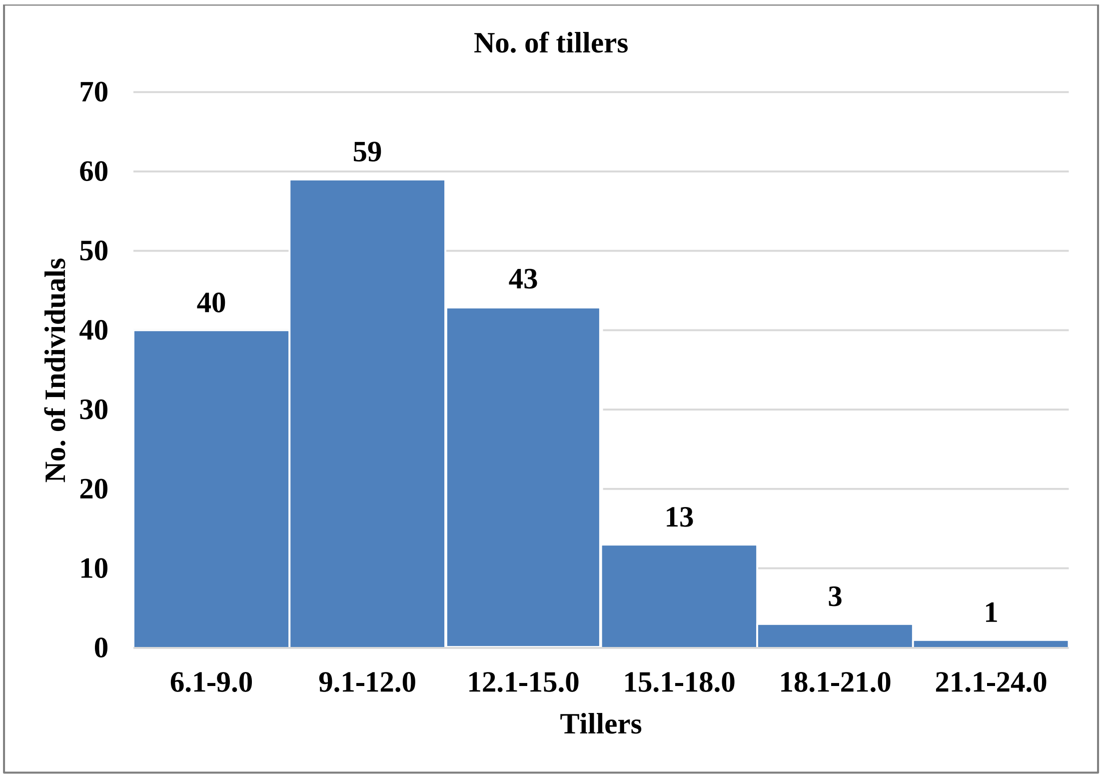

Fig. 4.4: **Frequency distribution of number of tillers of BC_2F_5 population under field conditions**

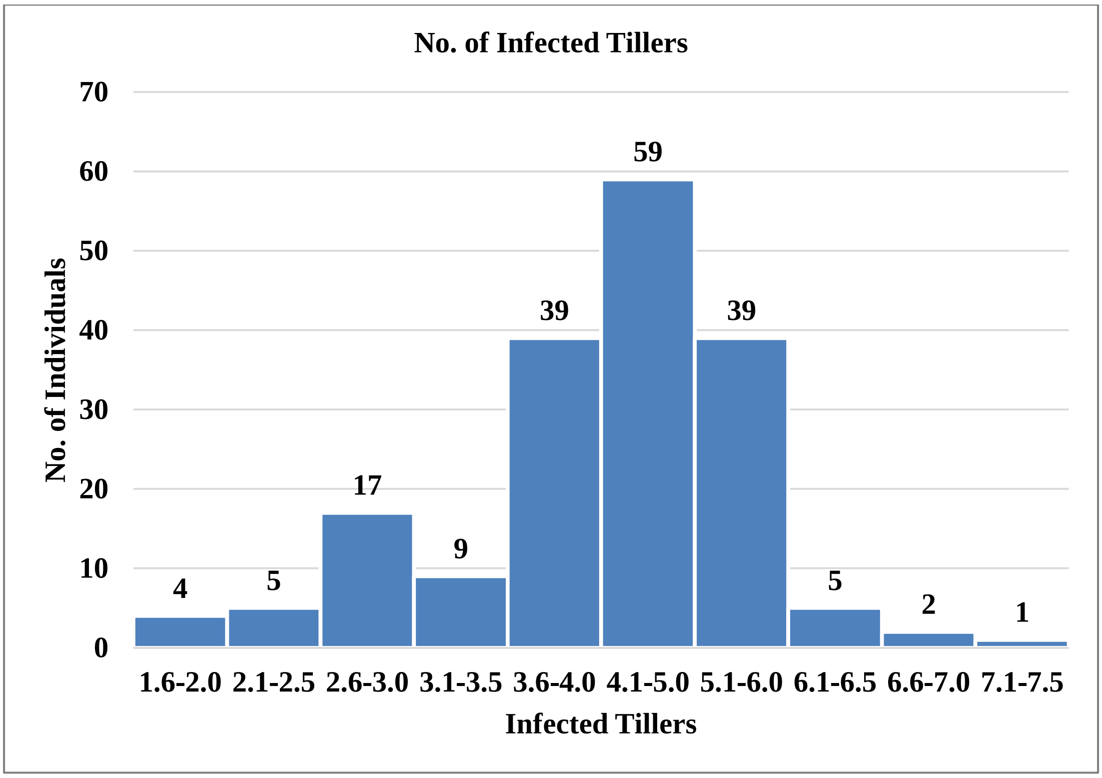

Fig. 4.5: **Frequency distribution of number of infected tillers of BC_2F_5 population under field conditions**

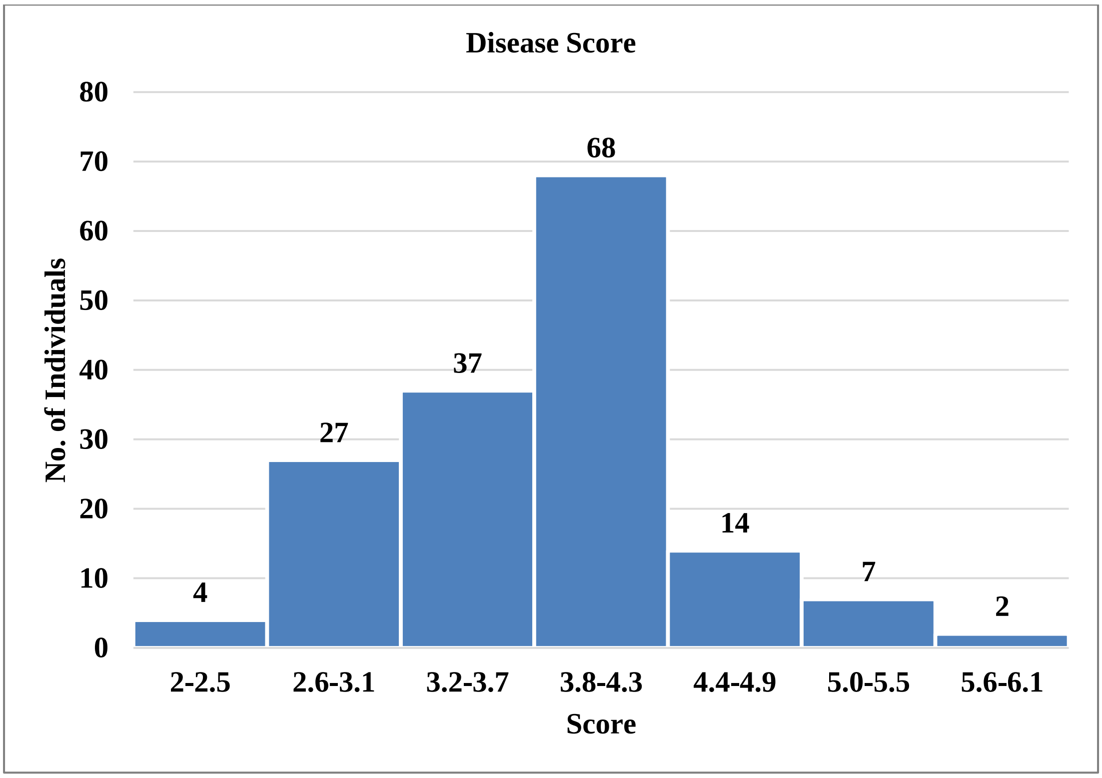

Fig. 4.6: **Frequency distribution of disease score of BC_2F_5population under field conditions**

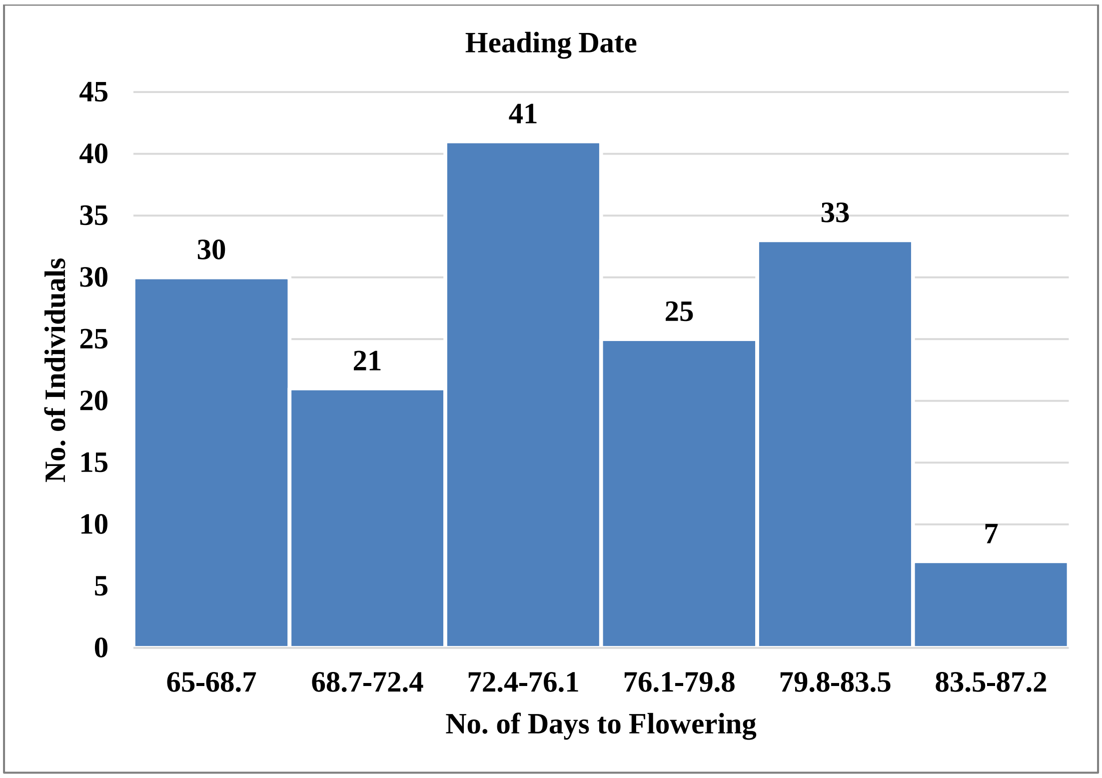

Fig. 4.7: Frequency distribution of heading date of BC_2F_5 population under field conditions

4.2 Descriptive statistical analysis for disease variables against rice sheath blight in BC_2F_5 population during *Kharif* 2022

The statistical analysis of the progenies was done using the SAS. A total of 162 individuals were analyzed for seven traits. The plant height showed a minimum of 63 cm and a maximum of 131cm owing to the mean value of 90.84. The skewness and kurtosis for plant height were observed to be 2.3 and 2.9, respectively. Similarly, for lesion height a maximum and minimum was observed at 33.75 cm and 18.5 cm, respectively. The observed values for range, mean, standard deviation, skewness and kurtosis for lesion height were 15.25,27.1, 2.4765, 0.98 and 1.58, respectively. For RLH the observed values of minimum, maximum, range, mean, standard deviation, skewness and kurtosis were 20.41,39.36,18.95, 27.25,3.0, 1.0 and 1.7, respectively. The disease score showed the following values of the seven statistical parameters 2.0, 5, 3, 3.3, 0.6, 1.2 and 1.1. Similarly, the no of tillers and no of infected tillers were also analyzed and results revealed the following values 5.5, 21.3, 15.8, 11.2, 3.0, 0.8 and 0.8 for no. of tillers and 2.0, 9.0, 7.0, 4.2, 1.2, 1.3 and 0.9 for no. of infected tillers. The heading date showed the following values 65, 84, 19, 75.3, 5.7, -1.1 and -0.3 (Table 4.2). For traits *viz* lesion height, relative lesion height, no. of tillers, no. of infected tillers, heading date and score, the kurtosis value showed a normal univariate distribution. The skewness value for major characters showed values near zero which confirms their symmetric distribution. Similar results were also obtained by Aggarwal *et al* (2019) during screening of 67 accessions of *Oryza nivara* using the susceptible check PR114. They also obtained univariate symmetric distribution for plant height (81 to 158 cm), lesion height (12 to 72 cm), relative lesion height (8.33 to 67.28%) and disease score (1 to 9). The frequency distribution graphs of the population shows a continuous distribution of values thereby confirming the quantitative nature of the traits.

Table 4.2: Differential statistical parameters of BC_2F_5 population screening against sheath blight during *kharif* 2022

Disease Variables	**Statistical parameter**						
	Minimum	**Maximum**	**Range**	**Mean**	**Standard Deviation**	**Kurtosis**	**Skewness**
Plant Height (cm)	64.3	131	66.7	90.8	5	2.9	2.3
Lesion Height (cm)	18.5	33.75	15.25	24.71	2.47	1.58	0.98
Relative Lesion Height (%)	20.4	39.4	18.9	27.3	3	1.7	1
No. of Tillers	5.5	21.3	15.8	11.2	3	0.8	0.8
No. of Infected Tillers	2	9	7	4.2	1.2	1.3	0.9
Heading Date	65	84	19	75.3	5.7	-1.1	-0.3
Score	2	5	3	3.3	0.6	1.2	1.1

4.3 Disease variables and their correlation

The disease variables were analyzed for Pearson's coefficient of correlation in the BC_2F_5 progenies and were found to be significantly correlated ($P<0.05$) (Fig. 4.8). A significant positive correlation was found for relative lesion height with disease score (0.89), lesion height with disease score (0.81) and RLH (0.83) while no. of tillers showed a significant positive correlation with no. of infected tillers (0.72). The positive correlation between relative lesion height and disease score may suggest that as the relative lesion height increases, the severity of sheath blight also increases. This could indicate that the disease progresses in a way that is visually reflected in the height of the lesions (Liu *et al* 2014). Also, disease score displayed a positive correlation with heading date (0.17) while heading date was found to be positively correlated with no. of infected tillers (0.21) as well as with no. of tillers (0.24). In addition to this, plant height showed a negative correlation with relative lesion height (-0.38) and a positive correlation with lesion height (0.19).

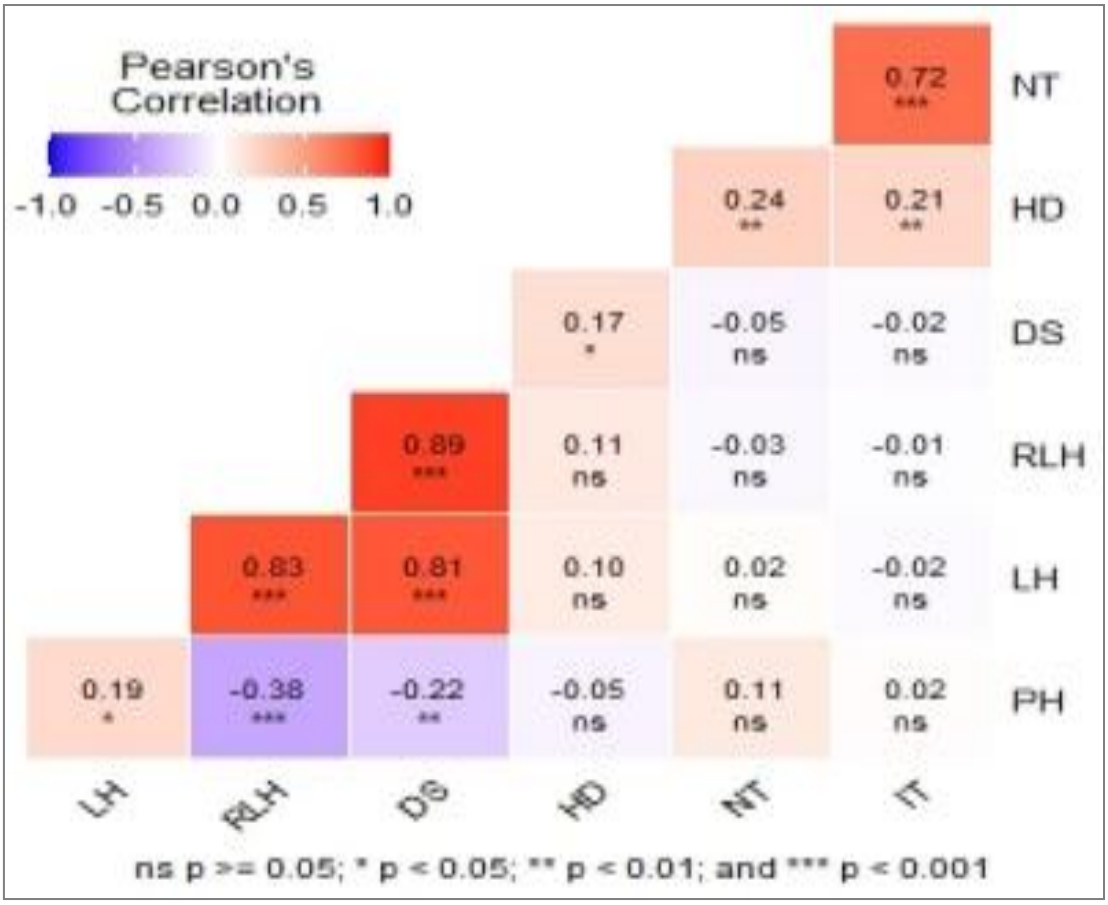

Fig. 4.8: Pearson's correlation coefficients between different disease variables of rice sheath blight of BC_2F_5 progeny

4.4 Screening of BC_2F_6 population for sheath blight resistance

The BC_2F_6 progeny was screened for seven disease variables during *kharif* 2023. The progenies were raised with a standard row to row spacing of 20cm and plant to plant spacing of 15 cm. The seven disease variables previously taken under study were plant height (PH), lesion height (LH), relative lesion height (RLH), disease score (DS), no of tillers (NT), no of infected tillers (NOIT), and heading date (HD). The response of the BC_2F_6 progeny was also normally distributed with slight deviations in skewness and kurtosis. Out of 110 individuals, 80 individuals (72.72%) showed moderate resistance, 28 individuals (25.45%) showed moderate susceptibility and 1 individual (0.9%) showed resistance disease reaction towards sheath blight.

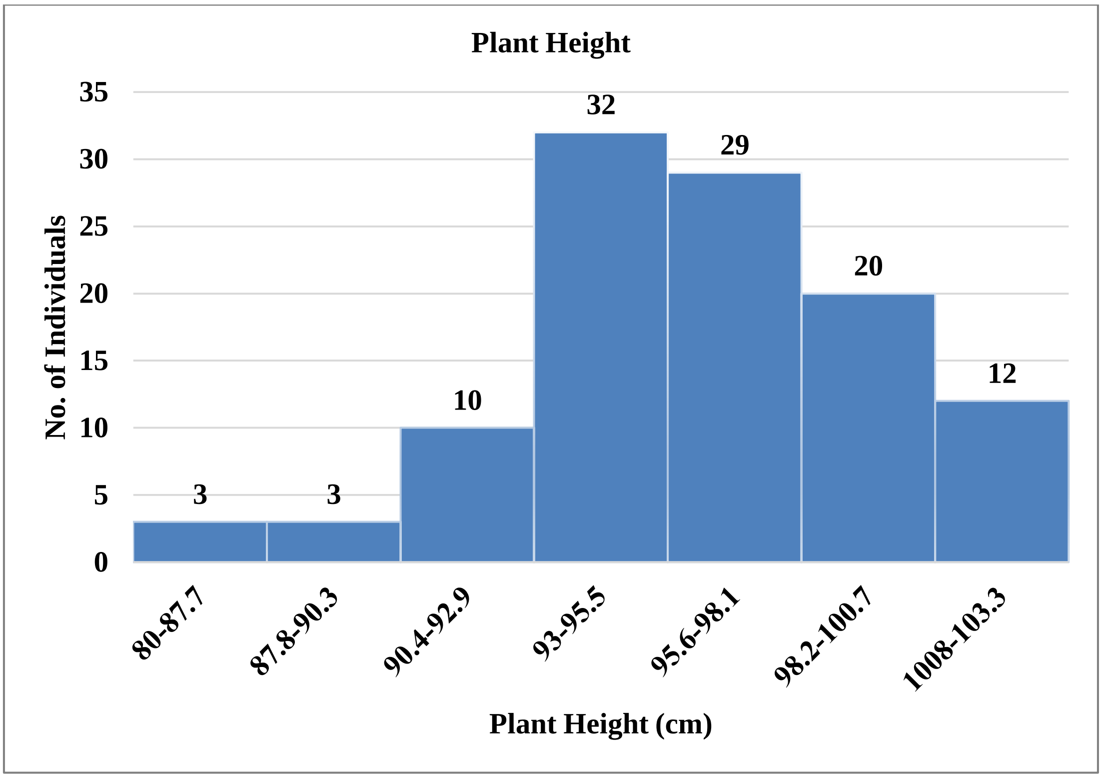

Fig. 4.9: Frequency distribution of plant height of BC_2F_6 population under field conditions

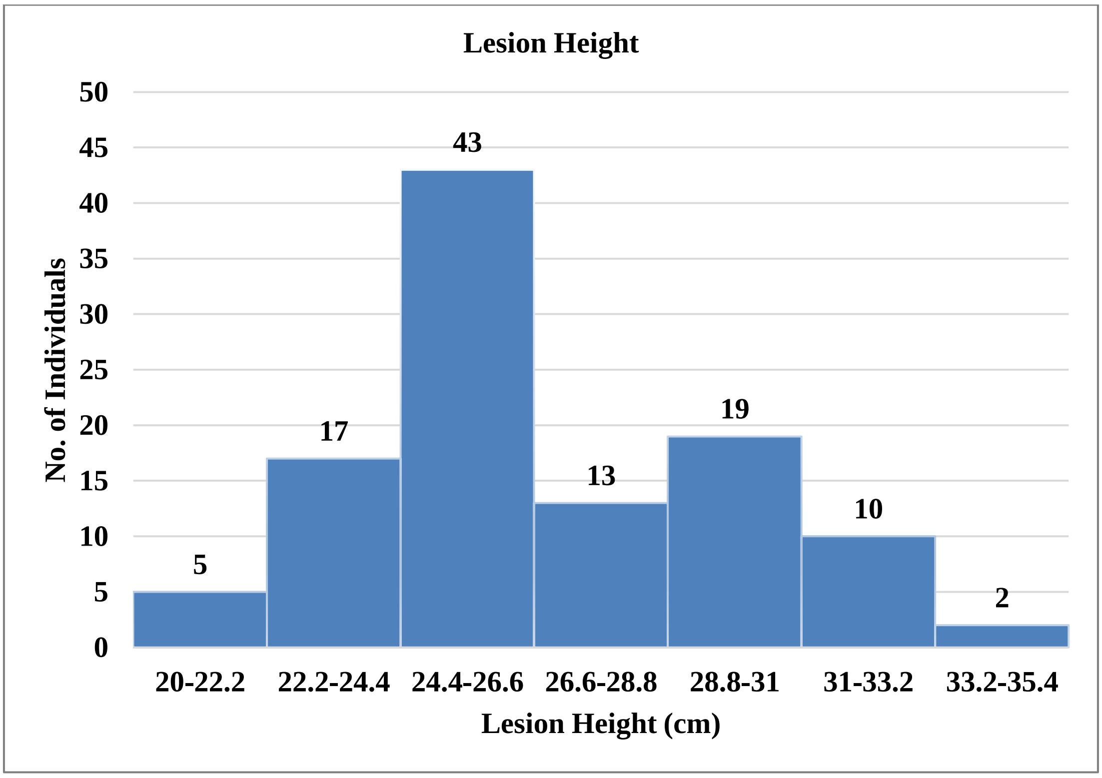

Fig. 4.10: Frequency distribution of lesion height of BC_2F_6 population under field conditions

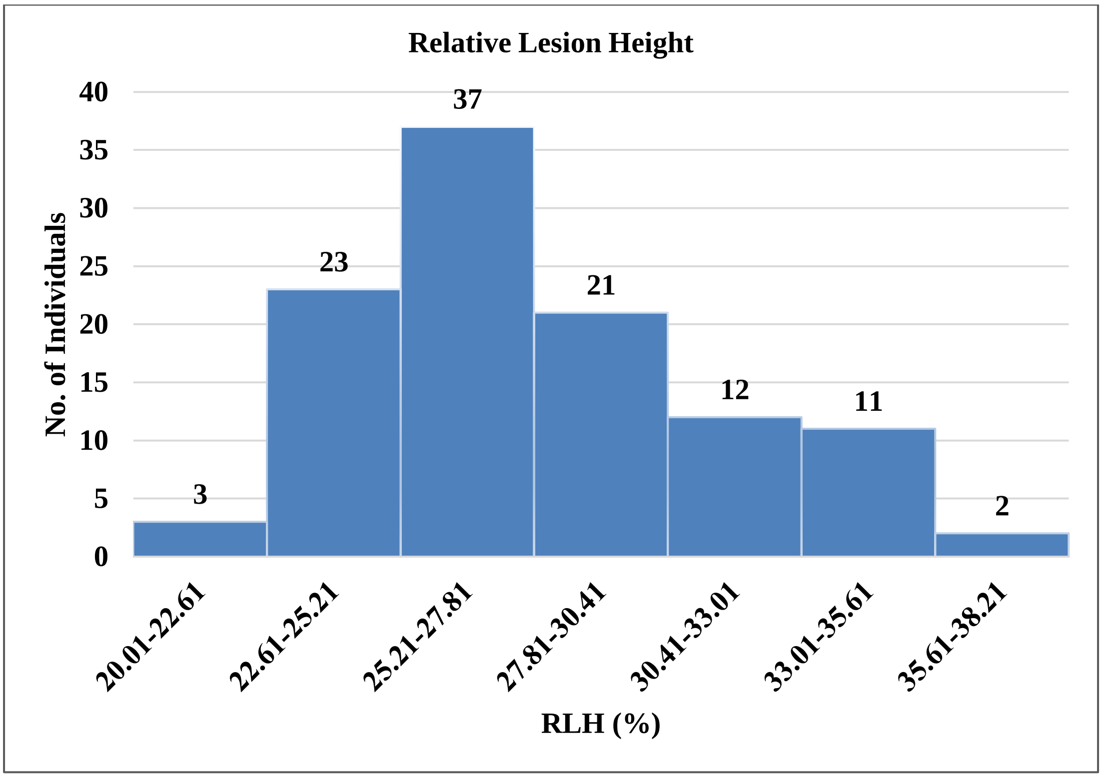

Fig. 4.11: Frequency distribution of relative lesion height of BC_2F_6 population under field conditions

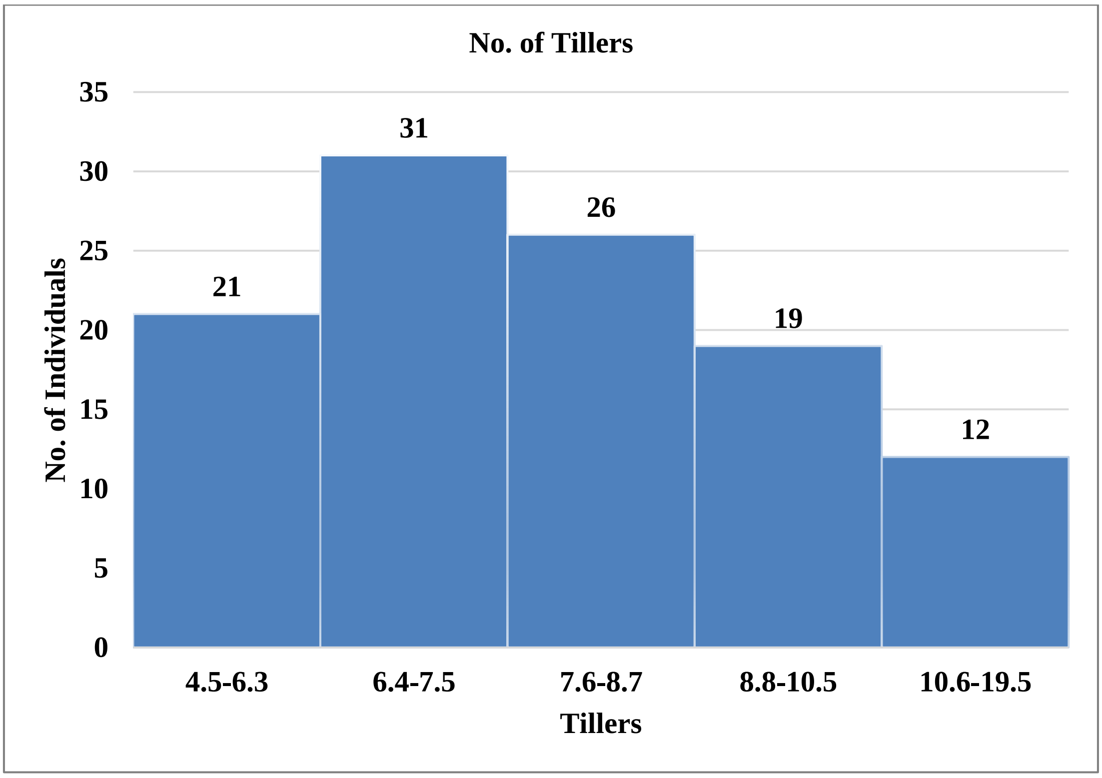

Fig. 4.12: Frequency distribution of no. of tillers of BC_2F_6 population under field conditions

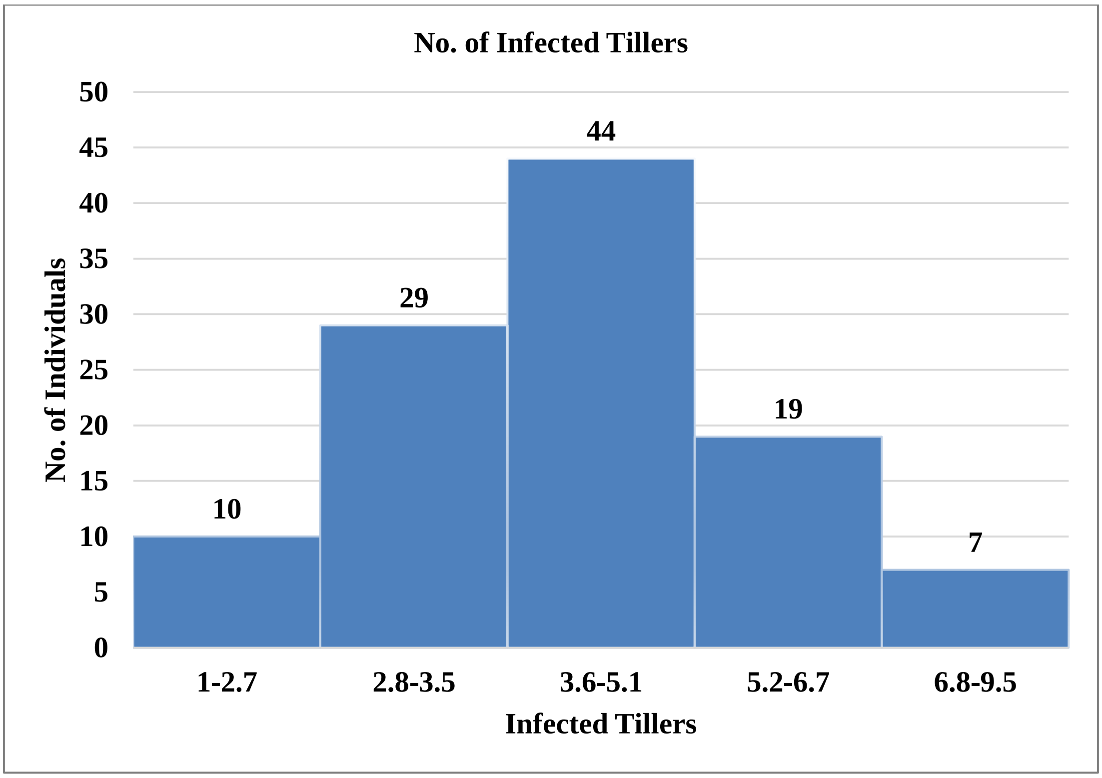

Fig. 4.13: **Frequency distribution of no of infected tillers of BC_2F_6 population under field conditions**

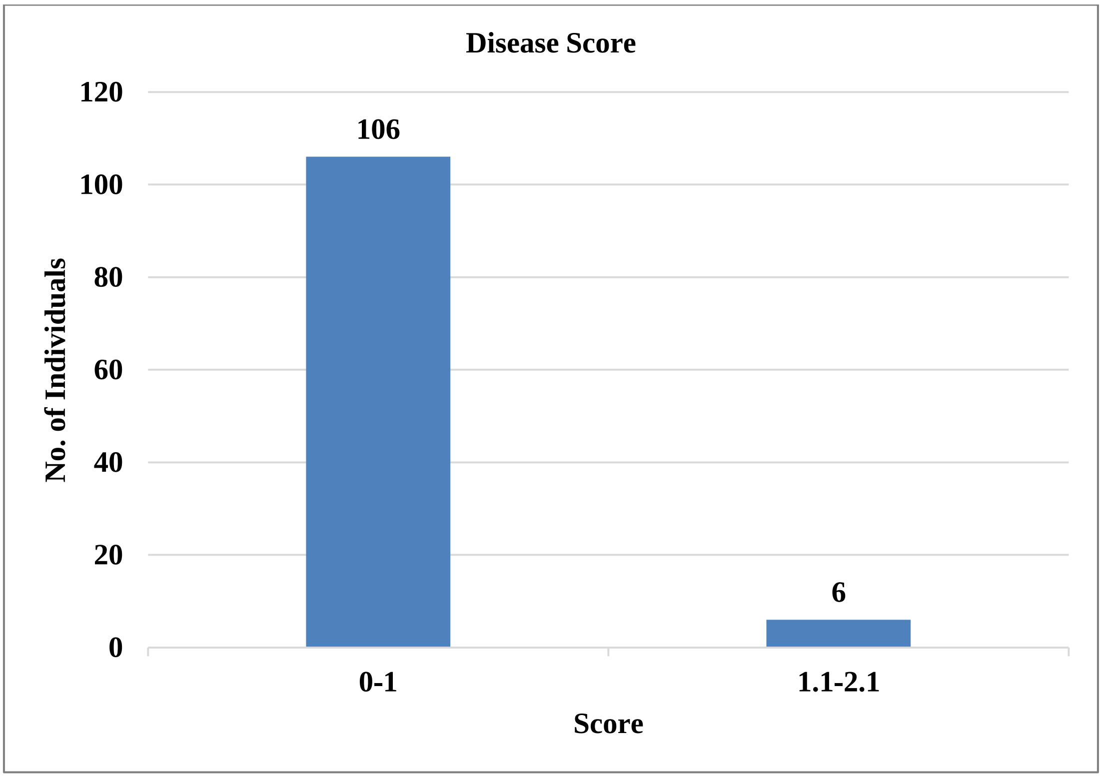

Fig. 4.14: **Frequency distribution of disease score of BC_2F_6 population under field conditions**

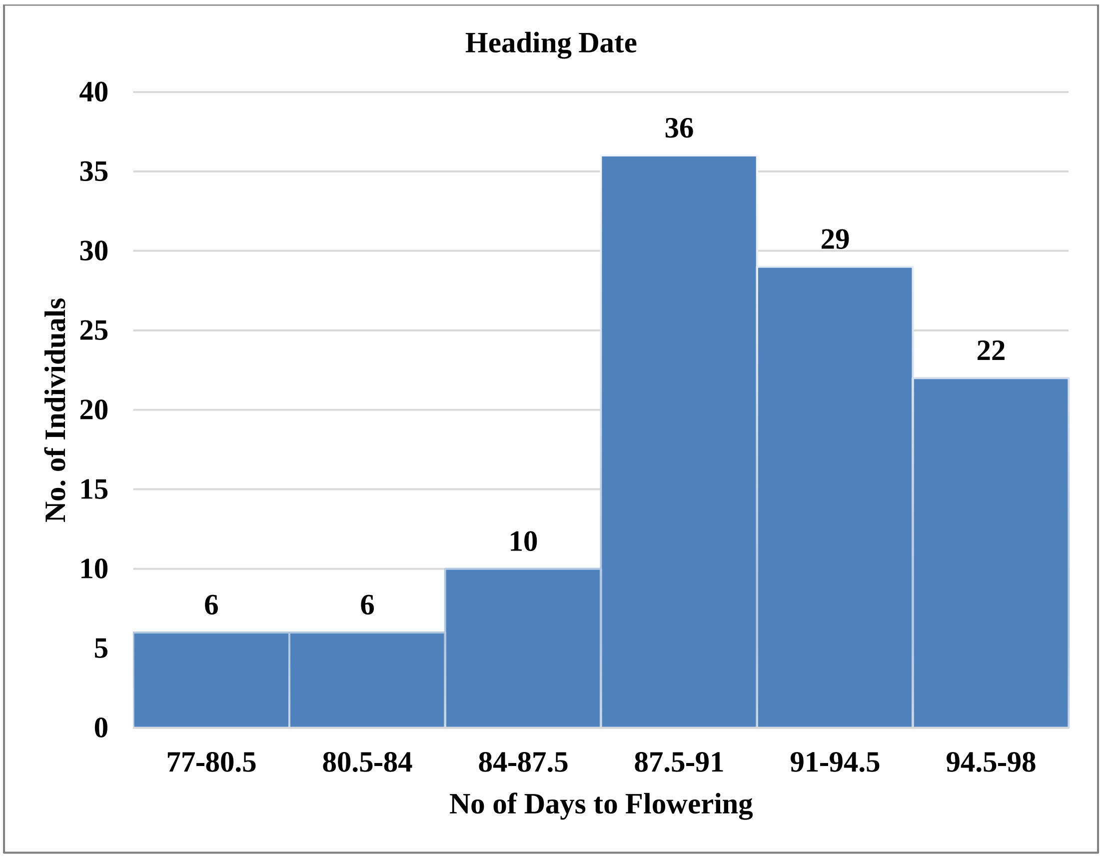

Fig. 4.15: Frequency distribution of heading date of BC_2F_6 population under field conditions

4.5 Descriptive statistical analysis for disease variables against rice sheath blight in BC_2F_6 population during *Kharif* 2023

The statistical analysis of the progenies was done using the same tool as BC_2F_5 i.e., SAS. The observed minimum and maximum values of PH, LH, RLH, NT and NOIT are 82.5, 20.0, 20.01, 4 and 1.5 and 103.5, 34.5, 36.4,19.5 and 9.5 respectively. The observed mean values were 96.2, 26.7, 27.82, 8.15 and 4.42. The observed value for range for LH was 14.5. For RLH the observed value for range was 16.39. The observed value for range for PH was 21, for number of tillers, the range was 15.5 and for number of infected tillers, the range was 8 (Table 4.3).

Table 4.3: Differential statistical parameters of BC_2F_6 population screening against sheath blight during *kharif* 2023

Disease Variables	Statistical parameter						
	Minimum	Maximum	Range	Mean	Standard Deviation	Kurtosis	Skewness
Plant Height (cm)	82.5	103.5	21	96.2	3.7	5.0	-0.9
Lesion Height (cm)	20	34.5	14.5	26.7	3.1	-0.6	0.4
Relative Lesion Height (%)	20.01	36.4	16.39	27.82	3.54	-0.4	0.5
No. of Tillers	4	19.5	15.5	8.15	2.47	6.6	2.06
No. of Infected Tillers	1.5	9.5	8	4.42	1.46	1.92	1.15
Heading Date	77	97	20	90.27	4.8	0.47	-0.9
Score	2	5	3	3.48	0.81	-0.3	0.99

4.6 Disease variables and their correlation

The disease variables were analyzed for Pearson's coefficient of correlation in the BC_2F_6 progeny showing significant correlation ($P<0.05$) (Fig. 4.16). A negative correlation was found for relative lesion height with plant height (-0.44) and a negative non-significant correlation with heading date (-0.19). No. of tillers displayed a significant positive correlation with no. of infected tillers (0.84) Plant height showed a positive non-significant correlation with no. of infected tillers (0.17) and with no. of tillers (0.10).

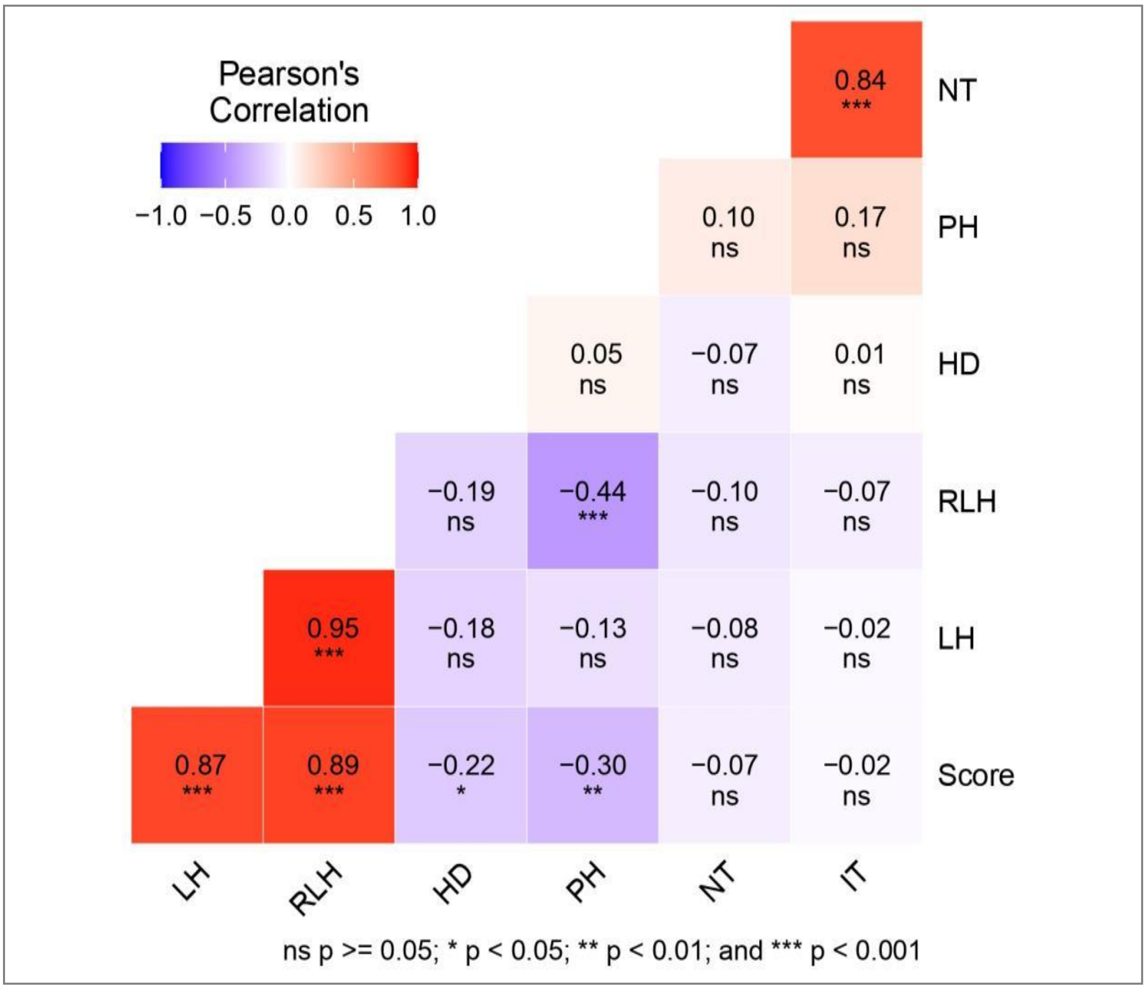

Fig. 4.16: Pearson's correlation coefficients between different disease variables of rice sheath blight of BC_2F_6 progeny

Lesion height showed a significant positive correlation with relative lesion height (0.95) and a negative non-significant correlation with heading date (-0.18). Heading date showed a negative non-significant correlation with no. of tillers (-0.07). Disease score showed a positive significant correlation with relative lesion height (0.89) and also with lesion height (0.87).

4.7 Identification of promising genotypes from BC_2F_5 and BC_2F_6 populations during *kharif* 2022 and 2023

Based on the screening of BC_2F_5 and BC_2F_6 progenies, the mean values for all the disease variables were evaluated. On the basis of the seven disease variables *viz,* mean plant height, lesion height, relative lesion height, disease score, no. of tillers, no. of infected tillers and heading date values, 75 resistant genotypes were selected. Among these genotypes, 35 genotypes with consistent phenotypic variables were selected which could be used as pre-breeding lines for the transfer of ShB resistance to the elite cultivars in future (Table 4.4 and Table 4.5).

Table 4.4: Promising genotypes of BC_2F_5 for sheath blight resistance during *kharif* 2022

Genotype	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	PH (cm)	LH (cm)	RLH (%)	NT	NOIT	DS	HD
7174	92.2	30.4	33.0	10.7	3.7	4.6	83.0
7176	94.8	26.4	27.8	16.6	4.6	3.0	79.0
7177	91.2	23.8	26.0	9.6	2.3	3.0	82.0
7178	87.8	27.4	31.2	13.3	4.3	3.8	76.0
7179	90.6	32.0	35.3	19.3	6.6	5.0	79.0
7180	89.6	25.2	28.1	19.5	7.5	3.0	79.0
7181	92.4	27.0	29.2	11.7	5.5	3.8	76.0
7182	87.6	23.0	26.3	13.0	5.0	3.0	81.0
7183	89.2	26.4	29.6	9.2	3.7	3.8	79.0
7184	90.2	28.8	32.0	13.0	4.2	4.6	79.0
7185	88.5	25.7	29.1	12.5	5.5	3.0	76.0
7186	91.6	26.2	28.5	9.6	5.3	3.8	81.0
7187	86.0	24.4	28.3	9.0	3.3	3.4	83.0
7188	90.0	24.0	26.6	12.5	6.5	3.4	81.0
7189	88.8	21.6	24.3	11.6	3.5	3.0	83.0
7190	81.6	23.0	28.1	7.0	2.0	3.0	76.0
7191	86.2	22.0	25.5	11.7	4.5	3.4	76.0
7192	87.6	23.8	27.2	12.2	4.0	3.4	76.0
***7193**	89.2	20.2	22.6	15.0	6.0	2.6	81.0
7194	87.2	22.8	26.0	8.2	3.5	3.0	81.0
7195	90.2	23.8	26.4	12.7	4.5	3.4	74.0
7196	92.4	24.8	26.8	14.3	5.6	3.0	74.0
7197	89.0	21.8	24.5	9.6	4.0	3.0	82.0
7198	94.2	23.0	24.4	15.0	5.6	2.6	81.0
7199	93.8	22.6	24.0	19.0	7.0	3.0	76.0
7200	97.0	24.0	24.7	13.6	3.6	3.0	76.0
7201	94.8	24.6	25.8	9.0	3.0	3.0	76.0
7202	89.2	23.0	25.9	13.0	4.0	3.0	76.0
7203	94.8	24.2	25.5	16.0	6.5	3.0	76.0
7204	90.6	24.4	27.0	11.3	5.3	3.4	76.0
***7205**	91.8	21.0	22.7	10.5	3.2	2.2	74.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
7206	89.8	29.0	32.4	19.5	6.0	4.2	79.0
7207	89.6	21.6	24.0	10.5	3.2	3.0	79.0
7208	92.2	27.4	29.7	13.7	6.2	4.2	76.0
7209	91.8	30.4	33.0	14.0	4.0	5.0	76.0
7210	91.0	24.0	26.3	8.7	3.7	3.4	81.0
7211	88.4	24.4	27.6	8.0	3.5	3.0	79.0
7212	91.0	22.5	24.6	12.6	4.6	3.0	79.0
7213	91.7	28.2	30.8	17.0	2.6	4.0	76.0
7214	94.8	25.0	26.3	11.0	4.0	3.0	76.0
7215	94.4	24.4	25.8	10.6	3.6	3.0	82.0
7216	93.6	23.6	25.2	21.3	7.3	3.0	79.0
7218	90.8	28.6	31.5	6.7	3.7	3.8	79.0
***7219**	95.6	21.8	22.8	11.0	5.0	3.0	76.0
7220	92.2	24.0	26.0	13.0	4.2	3.0	76.0
7221	93.2	25.6	27.5	13.6	4.3	3.4	82.0
7222	96.6	23.8	24.6	11.0	4.6	3.0	82.0
***7223**	98.6	23.0	23.2	10.0	3.6	2.6	79.0
7224	93.4	25.2	27.0	15.0	4.0	2.6	76.0
7225	95.8	22.8	23.8	16.0	5.0	2.6	81.0
7226	95.8	25.6	26.7	9.3	3.3	3.0	79.0
7227	93.0	25.0	26.9	14.0	3.5	3.0	81.0
7228	94.8	26.4	27.8	13.0	4.5	3.0	81.0
7229	89.4	21.6	24.1	12.33	5.3	2.6	79.0
7230	92.2	23.0	24.9	12.6	4.3	2.6	81.0
7231	92.8	24.0	25.8	7.0	3.0	3.0	81.0
7232	91.8	23.8	25.9	12.5	4.5	3.4	81.0
7233	93.4	25.4	27.2	8.5	3.7	3.4	76.0
7234	87.6	22.8	25.9	11.2	4.2	3.0	76.0
7235	91.8	23.0	25.0	10.6	3.3	3.0	76.0
***7236**	93.6	21.8	23.2	14.0	5.2	3.0	76.0
7237	91.5	23.2	25.4	10.0	5.6	3.0	79.0
7238	93.7	24.0	25.5	9.6	4.0	3.0	79.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
7239	95.2	23.0	24.1	15.0	4.6	3.0	81.0
7240	93.0	23.8	25.6	13.5	4.0	3.0	81.0
***7241**	91.4	20.6	22.5	15.3	5.0	2.6	76.0
7242	91.4	22.8	24.9	11.0	4.0	3.0	74.0
7243	92.2	25.0	27.1	10.5	2.5	3.5	74.0
7244	91.7	24.5	26.7	14.0	4.0	3.0	74.0
7245	89.4	24.0	26.9	10.6	3.3	3.4	79.0
7246	89.6	23.0	25.6	13.5	2.5	3.0	74.0
7247	89.6	30.2	33.7	6.6	2.3	4.6	74.0
7248	90.6	24.0	26.5	11.0	3.3	3.0	74.0
7249	89.8	24.2	26.9	6.7	3.2	3.4	72.0
7250	87.0	21.4	24.6	7.2	3.6	3.0	72.0
7251	85.2	18.5	21.6	13.6	3.6	2.0	74.0
7252	86.0	24.6	28.5	12.2	3.5	3.4	70.0
7253	88.2	23.6	26.7	10.0	3.0	3.0	70.0
7254	89.4	24.0	26.8	13.0	6.3	3.0	70.0
***7255**	91.4	20.6	22.5	11.3	3.6	2.2	70.0
7256	86.4	25.0	28.9	8.5	3.5	3.4	70.0
7257	89.8	22.4	24.9	13.6	6.0	3.0	71.0
7258	88.0	23.0	26.1	7.2	3.7	3.0	69.0
7259	89.5	25.0	28.0	15.5	5.5	3.5	68.0
7260	87.4	23.6	27.0	11.0	3.6	3.0	68.0
7261	92.2	24.0	26.0	15.3	4.6	3.0	68.0
7262	91.8	22.2	24.1	9.5	3.5	3.0	70.0
7264	93.2	25.8	27.6	11.0	4.2	3.0	72.0
7265	91.6	23.6	25.8	13.6	5.6	3.0	70.0
***7266**	93.3	22.3	23.9	7.0	3.5	3.0	76.0
7267	93.8	23.2	24.7	7.3	4.0	3.4	70.0
7268	93.0	25.6	27.5	11.0	4.5	3.4	68.0
7269	95.5	25.2	26.4	9.6	4.3	3.0	65.0
7270	93.6	26.4	28.1	8.6	3.6	3.8	67.0
***7271**	95.2	22.8	23.9	7.6	3.6	2.6	68.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
7272	95.2	25.2	26.5	7.5	3.2	3.0	68.0
***7273**	96.4	22.6	23.4	12.3	5.6	3.0	67.0
***7274**	96.0	23.8	24.7	8.6	4.0	3.0	69.0
7275	90.0	23.2	25.7	10.2	4.5	3.0	68.0
7276	97.5	23.7	24.4	14.0	6.0	3.0	68.0
***7277**	96.2	21.0	21.8	11.6	5.0	2.2	67.0
***7278**	95.2	23.2	24.3	9.0	2.7	2.6	69.0
***7279**	94.6	22.2	23.5	12.0	5.0	2.6	67.0
7280	94.8	27.6	29.1	13.7	6.2	3.8	69.0
7281	94.6	28.6	30.3	11.8	5.0	3.8	65.0
7282	94.2	24.6	26.0	10.0	3.7	3.0	65.0
7283	95.6	24.0	25.1	6.5	2.2	3.0	67.0
***7284**	96.4	23.6	24.5	10.6	3.0	2.6	65.0
7285	90.2	23.8	26.4	11.7	4.0	3.0	67.0
7286	89.0	20.5	23.0	9.0	2.5	3.0	65.0
7287	89.8	23.8	26.4	7.7	2.7	3.0	72.0
7288	89.0	29.0	32.5	7.5	3.2	4.6	72.0
7289	92.4	24.0	25.9	11.8	4.0	3.0	67.0
7290	87.4	25.4	29.1	7.6	2.8	3.4	67.0
7291	89.4	24.6	27.5	12.2	4.2	3.4	65.0
7292	92.2	24.2	26.2	10.2	3.0	3.4	67.0
7293	87.2	27.0	30.9	8.7	3.7	4.0	67.0
7294	86.8	23.8	27.3	7.2	2.0	3.0	67.0
7295	87.2	25.6	29.3	8.8	3.6	3.0	65.0
7296	87.0	24.0	27.5	7.0	3.0	3.0	65.0
7297	91.6	24.8	27.1	6.5	2.0	3.0	67.0
7298	88.2	32.8	37.3	5.5	2.5	5.0	67.0
7299	89.0	26.0	29.1	7.6	3.0	3.8	69.0
7300	82.7	25.7	31.0	11.5	3.7	4.5	79.0
7301	93.0	25.2	27.1	10.6	5.0	3.4	79.0
7302	83.0	23.7	28.6	9.0	5.0	3.5	69.0
7303	85.0	22.6	26.5	9.0	4.7	3.4	72.0
7304	86.0	23.2	26.9	9.5	3.5	3.0	65.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
7305	88.5	30.7	34.8	10.2	3.0	5.0	67.0
7306	83.6	24.0	28.6	9.2	4.0	3.4	76.0
7307	64.3	25.3	39.3	9.0	5.0	5.0	72.0
7308	85.3	23.6	27.8	14.3	6.0	3.0	79.0
7309	88.6	24.8	27.9	11.4	5.4	3.4	82.0
7310	91.0	25.0	27.4	16.3	9.0	3.0	81.0
7311	88.6	23.2	26.2	9.5	5.0	3.0	82.0
7312	86.4	29.8	34.4	10.2	4.4	5.0	82.0
7313	87.2	28.8	33.2	11.4	5.0	4.6	82.0
7314	86.8	22.6	26.0	13.0	4.2	3.4	82.0
7315	87.0	24.4	28.0	9.8	3.8	3.4	84.0
7316	88.4	27.0	30.8	9.2	3.2	4.2	84.0
7317	90.6	24.8	27.4	10.6	4.6	3.4	84.0
7318	89.8	25.4	28.2	10.2	4.2	3.4	84.0
7319	86.5	25.2	29.2	8.2	4.0	4.0	81.0
7320	87.8	25.2	28.7	12.5	4.7	3.4	84.0
7321	95.0	25.2	26.7	12.6	4.3	3.5	74.0
7322	86.4	24.4	28.3	9.2	3.7	3.8	74.0
7323	90.8	25.2	27.7	9.2	3.0	3.0	76.0
7324	91.8	29.4	31.9	13.0	4.0	4.6	82.0
7325	89.7	29.2	32.5	19.0	8.0	4.0	79.0
7326	92.5	25.7	27.9	9.0	2.3	3.5	79.0
7327	88.5	26.0	29.4	9.3	3.6	3.5	79.0
7328	93.2	28.7	30.8	9.6	3.0	4.5	82.0
7329	87.2	24.6	28.2	13.4	5.4	3.4	84.0
7330	86.4	25.8	30.1	11.3	5.0	3.8	82.0
7331	86.8	22.8	26.2	8.3	3.3	3.4	84.0
7332	91.8	27.0	29.4	10.5	3.5	3.8	76.0
7333	89.8	28.6	31.8	11.5	4.7	4.2	76.0
7334	88.8	26.4	29.7	8.3	2.6	3.8	78.0
***7335**	131.0	33.7	20.4	10.0	2.7	3.0	78.0

* Asterisk indicates the lines showing moderate resistant reaction towards sheath blight

*Mean indicates average data of three plants after inoculation with *Rhizoctonia solani*

Table 4.5: Promising genotypes of BC_2F_6 for sheath blight resistance during *kharif*2023

Genotype	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	PH (cm)	LH (cm)	RLH (%)	NT	NOIT	DS	HD
2440	100.0	28.5	28.4	12.0	7.0	3.0	86.0
***2442**	97.5	24.0	24.6	8.0	5.5	3.0	90.0
2443	95.0	31.0	32.8	7.5	5.0	4.0	94.0
2444	96.5	29.0	30.0	9.0	6.5	4.0	93.0
***2445**	99.0	23.5	23.7	8.0	5.0	3.0	90.0
2446	98.5	29.0	29.4	9.0	4.0	4.0	90.0
2447	97.5	25.5	26.1	4.5	2.5	3.0	95.0
2448	101.5	26.5	26.0	7.0	3.5	3.0	93.0
2449	97.5	28.0	28.7	19.0	9.5	3.0	93.0
***2450**	102.5	24.5	23.9	19.5	9.5	3.0	88.0
2451	98.0	26.0	26.5	8.0	3.5	3.0	95.0
2452	98.5	25.0	25.3	6.0	4.0	3.0	95.0
2453	96.0	25.5	26.5	8.0	4.5	3.0	82.0
2454	103.5	30.0	29.0	8.0	4.5	4.0	82.0
2455	92.0	26.0	28.2	5.5	3.0	3.0	90.0
2456	95.5	26.0	27.1	6.5	4.0	3.0	84.0
2457	94.5	25.0	26.4	9.0	4.0	3.0	97.0
2458	97.0	29.0	29.8	8.0	5.5	4.0	95.0
2459	98.5	25.5	25.8	6.0	3.5	3.0	95.0
2460	102.0	26.5	25.9	8.0	5.0	3.0	95.0
2461	97.5	25.5	26.1	7.5	4.0	3.0	90.0
2462	94.0	25.5	27.1	5.5	2.5	3.0	93.0
2463	96.5	27.5	28.5	6.5	4.5	4.0	88.0
***2464**	100.0	20.0	20.0	8.0	4.5	2.0	88.0
2465	97.0	23.0	23.7	6.5	4.0	3.0	95.0
***2466**	99.0	22.0	22.2	11.0	6.0	3.0	93.0
2467	94.5	24.5	25.9	5.5	2.5	3.0	90.0
2468	98.5	27.0	27.4	5.5	2.5	3.0	95.0
2469	97.5	23.5	24.0	8.0	3.5	3.0	97.0
2470	98.0	26.5	27.0	11.5	7.0	3.0	97.0
***2471**	92.5	21.5	23.2	11.0	6.5	3.0	93.0
2472	90.5	29.5	32.5	7.5	3.5	5.0	86.0
2473	94.5	23.5	24.8	15.0	4.0	3.0	88.0
2474	98.5	30.0	30.4	10.5	4.0	4.0	95.0
2475	95.0	24.5	25.8	7.5	4.0	3.0	90.0
***2476**	94.0	22.5	24.0	10.5	5.5	3.0	97.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
2477	94.5	24.5	25.9	8.5	5.5	3.0	94.0
2478	98.5	27.0	27.4	7.0	3.5	3.0	86.0
2479	90.5	32.0	35.3	5.0	2.5	5.0	79.0
2480	92.0	32.0	34.7	7.5	4.5	5.0	90.0
2481	94.5	24.0	25.4	11.5	5.0	3.0	77.0
***2483**	94.5	23.0	24.3	15.0	9.0	3.0	77.0
2484	96.0	27.0	28.1	10.5	6.0	3.0	94.0
2485	97.5	30.5	31.3	9.0	4.0	4.0	81.0
***2486**	101.0	25.0	24.7	8.0	4.0	3.0	90.0
2487	101.0	30.0	29.7	6.0	3.0	3.0	90.0
2488	96.0	23.5	24.4	6.5	3.5	3.0	93.0
2489	101.0	26.0	25.8	11.5	6.0	3.0	78.0
2490	91.0	30.5	33.5	6.0	3.5	5.0	80.0
2491	98.0	25.5	26.0	7.0	3.5	3.0	88.0
2492	95.5	32.0	33.4	10.0	6.5	5.0	95.0
2493	101.5	28.0	27.6	7.0	4.5	3.0	95.0
2494	96.0	33.5	34.8	8.5	4.5	5.0	91.0
2495	95.0	31.0	32.6	4.0	2.5	5.0	89.0
2496	95.5	29.0	30.3	8.0	5.0	4.0	90.0
2497	99.5	31.5	31.6	8.5	6.0	5.0	90.0
2498	97.0	28.0	28.8	6.5	3.0	4.0	81.0
2499	100.0	25.0	25.0	6.0	3.5	3.0	94.0
***2500**	96.5	23.5	24.3	6.5	2.5	3.0	95.0
***2501**	95.5	23.0	24.2	10.0	6.0	3.0	90.0
***2502**	101.0	24.5	24.2	9.0	5.5	3.0	93.0
2503	92.5	29.5	31.8	8.0	3.5	5.0	93.0
2504	98.0	25.5	26.0	7.0	4.0	3.0	78.0
2505	98.5	25.0	25.3	7.5	4.0	3.0	97.0
2506	82.5	27.0	33.2	8.0	4.0	4.0	88.0
***2507**	96.0	21.0	21.8	6.5	3.5	2.0	95.0
***2508**	98.5	24.0	24.3	8.5	5.5	3.0	89.0
***2509**	103.0	24.5	23.8	9.5	6.0	3.0	89.0
2510	90.0	25.5	28.3	8.5	4.5	3.0	93.0
2511	100.0	26.0	26.0	7.5	4.0	3.0	96.0
2512	95.0	26.0	27.4	7.0	4.0	3.0	88.0
2513	97.5	31.5	32.2	11.5	7.0	5.0	88.0
2514	93.0	26.0	27.9	6.0	3.0	3.0	96.0

Genotype	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	PH (cm)	**LH (cm)**	**RLH (%)**	**NT**	**NOIT**	**DS**	**HD**
2515	100.5	24.5	24.3	9.0	5.0	3.0	93.0
2516	95.5	30.0	31.4	6.0	3.0	5.0	89.0
2517	96.0	27.0	28.1	6.0	4.0	3.0	93.0
2518	97.0	23.0	23.7	8.0	4.5	3.0	85.0
2519	90.0	25.0	27.7	6.5	2.5	3.0	85.0
2520	94.0	31.5	33.4	6.5	4.0	5.0	86.0
2521	94.5	26.0	27.6	8.5	3.5	3.0	86.0
2522	93.5	31.0	33.1	7.5	3.5	5.0	86.0
2523	96.0	25.5	26.5	5.0	1.5	3.0	89.0
2524	95.0	25.0	26.4	10.5	6.5	3.0	93.0
2525	100.0	30.0	30.0	9.0	4.5	4.0	93.0
2526	95.0	24.5	25.8	9.0	6.5	3.0	97.0
2527	95.5	25.5	26.7	6.0	3.0	3.0	94.0
2528	93.0	27.0	29.0	9.5	5.0	4.0	88.0
2529	99.5	25.5	25.6	8.5	6.0	3.0	93.0
2530	91.5	26.5	28.9	6.0	3.0	3.0	93.0
2531	96.0	30.5	31.7	9.0	4.0	5.0	87.0
2532	91.0	29.5	32.4	9.5	5.0	5.0	94.0
2533	87.0	31.5	36.1	12.0	7.5	5.0	94.0
2534	95.5	28.0	29.3	9.0	5.5	3.0	89.0
2535	83.0	24.5	29.5	8.5	3.5	3.0	93.0
***2536**	93.0	23.0	24.7	7.0	4.0	3.0	93.0
2537	95.0	32.0	33.6	6.5	4.0	5.0	81.0
2538	101.0	25.5	25.2	8.0	3.5	3.0	86.0
2539	99.5	28.0	28.4	6.0	3.5	4.0	90.0
***2540**	101.5	24.0	23.6	6.0	2.5	3.0	90.0
2541	95.5	25.0	26.1	8.5	4.5	3.0	93.0
***2542**	97.0	22.0	22.6	7.0	3.5	3.0	89.0
2543	92.5	24.0	25.9	6.0	3.5	3.0	89.0
2544	96.0	25.5	26.5	7.0	4.0	3.0	96.0
2545	95.0	30.5	32.1	8.5	4.5	4.0	88.0
***2546**	98.0	23.0	23.5	8.5	4.5	3.0	94.0
2547	88.0	32.0	36.4	7.0	3.0	5.0	88.0
2548	93.0	25.0	26.9	7.0	3.5	3.0	93.0
2549	95.0	31.5	33.1	7.0	3.0	5.0	93.0
2550	100.0	34.5	34.5	7.0	5.0	5.0	90.0

* Asterisk indicates the lines showing moderate resistant reaction towards sheath blight

*Mean indicates average data of three plants after inoculation with *Rhizoctonia solani*

4.8 Genotypic Analysis

QTL mapping was performed using composite interval mapping (ICIM) functions implemented in the QTL-ICIMapping software v4.2. A total of 118 individuals and seven traits *viz.*, plant height, lesion height, relative lesion height, disease score, no. of tillers, no. of infected tillers and heading date were used for the genotypic analysis. After alignment, calling and precise SNP filtration criteria, total 3531 polymorphic SNPs were detected in the genotypes (Table 4.6).

Table 4.6: SNP marker generation using variant calling

Total Variants [SNPs + Indels]	2187892
Total SNPs	1986376
Biallelic SNPs	1978661
Biallelic Chromosome Only SNPs	1973111
SNPs after Parental filtering:	269460
this filtering was done as follows	
(a) all heterozygous SNP were removed between the parents.	
(b) all missing Parental SNPs were removed	
(c) monomorphic SNPs between parents were discarded	
SNPs left after discarding markers	**3531**
Total no. of markers grouped into 12 linkage groups	**3504**

Table 4.7: Linkage maps spanned by different chromosomes

Chromosome No.	**Number of markers**	**Map length (cM)**	**Average marker distance (cM)**
1	319	55.914	0.18
2	296	58.475	0.2
3	302	79.099	0.26
4	345	54.339	0.16
5	214	61.088	0.29
6	328	56.813	0.17
7	330	61.69	0.19
8	278	55.186	0.2
9	278	54.1	0.19
10	200	53.081	0.27
11	360	53.485	0.15
12	254	60.905	0.24
Total Map Length (cM) 704.175			
Total Number of Markers 3504			

4.8.1 QTLs Mapping

A total of 26 QTLs were mapped for all the seven traits distributed over chromosome 2, 3, 5, 6, 7, 8, 9, 10, 11 and 12. The list of QTLs along with their position associated with individual traits is given in table 4.8. The QTLs for plant height (PH) *qPH2*,*qPH7* and *qPH8* were mapped on three chromosomes viz 2, 7 and 8. The *qPH2* showed marker position of 1636 with a LOD score of 10.59 and was explaining 8.0472% of phenotypic variation. Another QTL, *qPH7* was mapped on chromosome 7 with marker position of 2003 having LOD score 6.01 and 5.56% of phenotypic variation. The third QTLs for PH i.e., *qPH8* was mapped on chromosome 8 having marker position of 286 with LOD score 10.66 and explaining a phenotypic variation of 8.34%. Few ShBR QTLs have been established on chromosome 8 earlier (Dayanand, 2021 and Zhang *et al* 2021). Also, *qPH7* and *qPH8* co-localized with the QTLs of number of tillers (NT) *qNT7* and *qNT8* with LOD scores of 2.82 and 2.73 and explaining 7.73% and 5.14% phenotypic variation respectively.

Five QTLs were mapped for the trait lesion height (LH) *qLH5*, *qLH6*, *qLH10*, *qLH11* and *qLH12.* These QTLs were present on chromosome 5, 6, 10, 11 and 12 with LOD scores of 3.16, 6.52, 5.16, 9.32 and 3.02 and explaining a phenotypic variation of 6.02%, 12.6%, 5.16%, 10.4% and 5.18% respectively. Eizenga *et al* (2013), used wild type of mapping population consisting of 252 Wild-1 and 253 Wild-2 BC$_2$F$_2$ population derived from the cross (*Oryza nivara* X Bengal^{J} (*O. sativa*). They identified the QTLs *qShB6* (Wild-1), *qShB6* (Wild-2) on chromosome 6 for the traits controlling lesion height, relative lesion height, disease score and heading date. The colocalization of *qShB6* with *qLH6*, *qRLH6*, *qDS6* and *qHD6* indicates authenticity of the identified QTLs in this study.

Further, a total of seven QTLs were mapped for relative lesion height (RLH) *qRLH3*, *qRLH5*, *qRLH6*, *qRLH7*, *qRLH10*, *qRLH11* and *qRLH12,* on chromosome 3, 5, 6, 7, 10, 11 and 12. The LOD scores for these QTLs were 3.7, 5.54, 2.5, 2.76, 7.75, 5.7 and 6.7. The phenotypic variation explained by these QTLs were 10.83%, 8.00%, 9.1%, 6.41%, 14.26%, 8.22% and 9.96% respectively. The *qRLH6* colocalized with a QTL of disease score (DS) *qDS6*. Fu *et al* (2011) identified the QTLs on chromosome 5 and chromosome 7 for the trait of relative lesion height. The QTLs, *qSBR5-2* and *qSBR7,* colocalized with the QTLs of *qRLH5*, *qRLH7* and *qLH5*. Through their study; they confirmed that the sheath blight resistance is correlated with relative lesion height and lesion height.

Table 4.8: Details of Sheath blight resistance contributing QTLs mapped for plant height, lesion height, relative lesion height, number of tillers, number of infected tillers, disease score and heading date

Trait Name	LGs	QTLs	Position	Left Marker	Right Marker	LOD Value	PVE (%)
Plant Height	2	*qPH2*	1636	2:9780219	2:11255113	10.5943	8.04
	7	*qPH7*	2607	7:5273211	7:26501958	6.0115	5.56
	8	*qPH8*	286	8:13638337	8:9711042	10.6634	8.34
Lesion Height	5	*qLH5*	357	5:1603843	5:1603843	3.1688	6.02
	6	*qLH6*	668	6:9877591	6:22497899	6.529	12.60
	10	*qLH10*	1596	10:21258620	10:17555834	5.1647	5.16
	11	*qLH11*	1908	11:10371515	11:10371344	9.3293	10.48
	12	*qLH12*	1607	12:66331	12:663254	7.6432	10.89
Relative Lesion Height	3	*qRLH3*	949	3:13462926	3:26029188	3.7403	10.83
	5	*qRLH5*	2275	5:11950775	5:21774564	5.5488	8.00
	6	*qRLH6*	2232	6:24827970	6:15689215	2.5645	9.10
	7	*qRLH7*	529	7:16499080	7:2343927	2.768	6.41
	10	*qRLH10*	1723	10:12989601	10:8516188	7.7592	14.26
	11	*qRLH11*	825	11:17982161	11:15667654	5.7018	8.22
	12	*qRLH12*	355	12:22869469	12:3990596	6.7658	9.96
No. of Tillers	7	*qNT7*	383	7:11926466	7:8677651	2.8234	7.73
	8	*qNT8*	473	8:7182240	8:12259527	2.7346	5.14
	9	*qNT9*	49	9:7929974	9:7835155	2.8839	8.06
	11	*qNT11*	297	11:25677271	11:25677253	4.5644	5.82
No. of Infected Tillers	9	*qNOIT9*	2551	9:4914338	9:12696996	3.4003	6.60
	10	*qNOIT10*	861	10:2318348	10:12737888	4.4235	7.12
Heading date	6	*qHD6*	1117	6:21274055	6:10660354	3.4716	8.16
	11	*qHD11*	3045	11:18765388	11:28122332	4.52	5.49
Score	2	*qDS2*	2540	2:32339218	2:30105	3.2985	5.51
	6	*qDS6*	215	6:14872222	6:25478495	3.0361	5.06
	10	*qDS10*	1037	10:15253044	10:15704671	3.5474	5.85

Four QTLs, *qNT7*, *qNT8*, *qNT9* and *qNT11* were mapped for the trait of number of tillers (NT) over chromosome 7, 8, 9 and 11. The respective LOD scores were 2.82, 2.73, 2.88 and 4.56. These QTLs explained a phenotypic variation of 7.73%, 5.14%, 8.06% and 5.82% respectively. *qNT7* and *qNT8*, colocalized with the *qPH7* and *qPH8*. For the number of infected tillers (NOIT) two QTLs i.e., *qNOIT9* and *qNOIT10* were mapped with an LOD score of 3.4 and 4.42 and explaining 6.6% and 7.12% phenotypic variation respectively. Taguchi-Shiobara *et a*l (2013), while working with a backcross inbred line derived from a cross (Jarjan^{I} X Koshihikari^{J}), identified a QTL, *qRTL9* on chromosome 9 for the trait of no. of infected tillers. This identified QTL colocalized with the QTL of *qNOIT9*,revealing the relationship between no. of infected tillers with sheath blight resistance.

Three QTLs were mapped for the trait disease score (DS) *qDS2*, *qDS6* and *qDS10*, present on chromosome 2, 6 and 10. The respective LOD scores were 3.29, 3.03 and 3.54. These QTLs explained a phenotypic variation of 5.15%, 5.06% and 5.85% respectively. The QTLs, *qDS6* and *qDS10* colocalized with the QTLs of *qLH6* and *qLH10* and *qRLH6* and *qRLH10* respectively. Liu *et al* (2014), identified a QTL qSB-11^{LE} on chromosome 11. The QTL, *qSB-11*^{LE} co localized with the QTL of disease score, *qDS11* and confirmed that the sheath blight resistance is directly related to the disease scoring.

For heading date (HD), two QTLs *qHD6* and *qHD11* were mapped on chromosome 6 and chromosome 11. The *qHD6* was having marker position of 1867 with LOD score of 3.47, explained 8.16% of phenotypic variation. Another *qHD11* mapped on chromosome 11 was having marker position 1534, LOD score 3.54 and explaining 5.22% of phenotypic variation.

Conclusion

In conclusion, this in-depth analysis offers valuable insights into the genetic foundation of sheath blight resistance in the examined rice populations. Rice chromosomes 5, 6, 10, 11 and 12 showed the consistent QTLs for lesion height and relative lesion height which indicates simultaneous improvement for both traits could be achieved. Furthermore, the investigation has pinpointed pre-breeding lines within the advanced backcrossed population exhibiting superior agronomic traits and heightened resistance to sheath blight compared to the recurrent parent. These promising lines present a significant opportunity to enhance sheath blight resistance in elite rice cultivars through focused improvement initiatives.

CHAPTER V

SUMMARY

Rice serves as the primary food for over half of the global population, meeting the dietary needs of more than 90% of people in Asia. However, rice cultivation faces significant challenges due to numerous pests and diseases. Among them, sheath blight (ShB) caused by necrotrophic fungus *Rhizoctonia solani* Kuhn [(Telomorph: *Thanatephorus cucumeris* (Frank)Donk)], is one of the most prevalent disease of rice worldwide. Several researchers have made an effort to identify sources of sheath blight resistance through screening of local accessions, advanced breeding lines, landraces, cultivars and released varieties. In the absence of true genetic resistance as disease is controlled by polygenes or no resistance gene (R gene) has been identified against sheath blight in rice to date. Biological controls are still ineffective in the field while fungicides used on a regular basis are causing significant concerns about ecology and difficult to manage the disease, if not applied on time. Therefore, management of the disease through host-plant resistance is efficient and ecofriendly method. Thus, the present study was conducted to map the QTLs for resistance against sheath blight in rice utilizing the wild species of rice *O. rufipogon* acc. CR100438. The BC_2F_5 and BC_2F_6 populations along with their parental genotypes were grown at the experimental field area of School of Agricultural Biotechnology, PAU, Ludhiana during *Kharif* 2022 and 2023. Inoculations were carried out at maximum tillering stage of rice using the artificial inoculation method, while at maturity, phenotypic data was recorded and the inoculated plants were harvested. DNA was extracted from the aforementioned populations for further molecular investigations.

Seven disease variables *viz,* Plant height (PH), Lesion height (LH), Relative lesion height (RLH), No. of tillers (NT), No. of infected tillers (NOIT), Disease score (DS) and Heading date (HD) were evaluated in the BC_2F_5 and BC_2F_6 populations during the *kharif* season 2022 and 2023. The screening of BC_2F_5 population revealed that out of 162 genotypes, 134 (82.71%) of the genotypes were moderately resistant, 24 genotypes (14.81%) were moderately susceptible and 1 genotype (0.61%) showed resistant reaction towards ShB. In BC_2F_6 population, out of 110 genotypes, 80 (72.72%) genotypes showed moderate resistance, 28 (25.45%) genotypes showed moderate susceptibility and 1 genotype (0.9%) showed resistance disease reaction towards sheath blight. The frequency distribution graphs of the populations showed a continuous distribution of values thereby confirming the quantitative nature of the traits. Parental lines showed significant difference in their resistance level in all the experiments.

The disease variables were also analyzed for Pearson's coefficient of correlation. The disease variables were significantly correlated ($P<0.001$) for both populations. In BC_2F_5

population, relative lesion height showed strong positive correlation with disease score (0.89), lesion height with disease score (0.81) while as no. of tillers also showed a positive correlation with no. of infected tillers (0.72). Lesion height also showed a positive correlation with relative lesion height (0.83). Disease score showed a positive correlation with heading date (0.17). Heading date showed a moderate correlation with no. of infected tillers (0.21) as well as with no. of tillers (0.24). In addition, disease score showed a very weak correlation with heading date (0.17). Plant height showed a negative correlation with relative lesion height (-0.38) and also it showed moderate correlation with lesion height (0.19).Similarly, in the BC_2F_6 genotypes, relative lesion height showed a negative correlation with plant height (-0.44) and a negative correlation with heading date (-0.19). No. of tillers showed a strong positive correlation with no. of infected tillers (0.84). Plant height showed a moderate positive non-significant correlation with no. of infected tillers (0.17) and with no. of tillers (0.10). Lesion height showed a strong positive correlation with relative lesion height (0.95) and a negative non-significant correlation with heading date (-0.18). Disease score showed a strong positive significant correlation with relative lesion height (0.89) and also with lesion height (0.87). Heading date showed a negative correlation with no. of tillers (-0.07).

A set of 118 lines from the backcross population BC_2F_5 underwent genotyping through the use of SNP markers. In the present study, after alignment, calling and precise SNP filtration criteria, a total of 3531 polymorphic SNPs were detected. A genetic linkage map was constructed using the QTL-ICIMapping software v4.2. A total of 118 individuals and the mean of year 2022 and 2023 for the seven traits *viz,* plant height, lesion height, relative lesion height, disease score, no. of tillers, no. of infected tillers and heading date were used to map the QTLs. A total of 26 QTLs were mapped for all the seven traits distributed over chromosome 2, 3, 5, 6, 7, 8, 9, 10, 11 and 12. All the major QTLs attained a satisfactory LOD score of ≥ 2.5 and P ≤ 0.001 during the composite interval mapping analysis. Three QTLs were mapped for plant height among which *qPH7* and *qPH8* co localized with the QTLs of number of tillers (NT) *qNT7* and *qNT8*. The *qPH8* contained genes for resistance against brown spot disease in rice. Five QTLs were mapped for the trait lesion height (LH) *qLH5*, *qLH6*, *qLH10*, *qLH11* and *qLH12*,present on chromosome 5, 6, 10, 11 and 12. This region also contains the gene for rust resistance in rice. Seven QTLs were mapped for relative lesion height (RLH)*qRLH3*, *qRLH5*, *qRLH6*, *qRLH7*, *qRLH10*, *qRLH11* and *qRLH12* over chromosome 3, 5, 6, 7, 10, 11 and 12. The positions of the QTLs on chromosome 5,6,10, 11 and 12 are consistent over the years for LH and RLH. One RLH QTL, *qRLH10* colocalized with a QTL of disease score *qDS10*and another covered the genomic region containing autophagy related protein coding genes. Further, four QTLs were mapped for no. of tillers *qNT7*, *qNT8*, *qNT9* and *qNT11*, two QTLs, *qNOIT9* and *qNOIT10* were mapped for no. of infected tillers over chromosome 9 and 10, three QTLs were mapped for the trait disease score *qDS2*, *qDS6* and *qDS10*, and also two QTLs, *qHD6* and *qHD11* were mapped for the heading date on chromosome 6 and 11.

In summary, this comprehensive analysis provides valuable insights into the genetic basis of sheath blight resistance in the studied rice populations. The study also identified the QTLs that contribute to the understanding of the complex genetic architecture underlying the evaluated traits, paving the way for targeted breeding efforts to enhance sheath blight resistance in rice cultivars. Moreover, the investigation pinpointed pre-breeding lines within the advanced backcrossed population that demonstrated superior agronomic traits and greater resistance to sheath blight in comparison to the recurrent parent. These promising lines represent a substantial opportunity to boost sheath blight resistance in elite rice cultivars through focused improvement initiatives.

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