GENETIC ANALYSIS OF INDICA RICE (*Oryza sativa* L.) GENOTYPES FOR HYBRID SEED PRODUCTION TRAITS AND BROWN PLANT HOPPER (BPH) RESISTANCE TO IDENTIFY PARENTAL LINES

A Dissertation

by

KOTILINGAM KONDA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Amir M.H. Ibrahim
Co-Chair of Committee,	Yog Raj
Committee Members,	Seth C Murray
	Hongbin Zhang
	Wayne Smith
Head of Department,	David Baltensperger

December 2018

Major Subject: Plant Breeding

ABSTRACT

Hybrid rice cultivation has expanded slowly in India and other countries in Southeast Asian because of the high cost of F_1 seed production, poor grain quality, yield heterosis, and biotic and abiotic stresses.

This study aims to determine the genetic association between QTLs that regulate brown plant hopper (BPH) resistance (for protecting the rice (*Oryza sative* L.) crop from "hopper burn"), photoperiod response of heading date, and pollen number (for enhancing seed set in female cytoplasmic male sterile parents). This study also seeks to identify high heterotic restorer lines in a recombinant inbred line (RIL) population derived from Bayer restorer germplasms BRGB02489 and BRGB04267.

A genetic association between the host plant and insect was revealed upon BPH nymph infestation with the identification of a major additive (A) effect QTL on chromosome 4 (*qBP4*), while significant differences were found for antixenosis and antibiosis modes of insect resistance in genotypes carrying *qBP4*-resistant alleles.

The heading date data of the RIL population was evaluated in seven wet- and dry-season environments at latitudes 17.4°N (Chandippa) and 29.9°N (Dhantori). Out of 21 putative QTLs mapped across chromosomes 3, 4, 6a, 6b, 7 and 11, the major A effect QTL located between 0.0 cM and 26.7 cM on chromosome 6b was associated with early and late flowering behavior independent of photoperiod changes, while a photoperiod-sensitivity QTL was identified on chromosome 6a with degree of photoperiod sensitivity data. The results of a genetic analysis study based on heading date data suggest that it is possible to breed for stable and early

flowering restorer lines by introgression of the BRGB04267 allele for the QTL on chromosome 6a and the BRGB02489 allele for the QTL on chromosome 6b. The least mean square data of pollen number (pollen load) of the RIL population indicated that significant difference among genotypes, and the major additive effect QTL associated with high pollen load was mapped on chromosome 9 (*qPL9*).

A linear mixed model analysis was conducted with ASReml in order to derive the best linear unbiased predictor (BLUP) value, and the RILs BYRIL117, BYRIL026, BYRIL055, BYRIL060, BYRIL073, BYRIL140, BYRIL228, BYRIL072, and BYRIL020 were identified as providing notable yield advantage in hybrids derived from testers BRGB07288A and BRGB06355A.

The present study is an example of an exploration of multiple traits from a single donor. Development of functional single nucleotide polymorphisms (SNPs) associated with BPH resistance, heading date, photoperiod sensitivity and pollen load could facilitate marker-assisted breeding in order to improve germplasms for stable flowering behavior, high pollen load and BPH resistance.

DEDICATION

To the farmer whose hard work and seamless efforts creating a hunger free world.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my committee chair, Dr. Amir M.H. Ibrahim and co-chair Dr. Yog Raj (Bayer CropScience) for their excellent support throughout my research study. I am also grateful to my committee members, Dr. Seth C Murray, Dr. Hongbin Zhang and Dr. Wayne Smith for their support and guidance. My sincere appreciations go to Ms. LeAnn Hague and Ms. Amanda Ray for their excellent support starting from the enrollment in the distance Ph.D. program to the final submission of my dissertation.

If the distance Ph.D. program by Texas A&M wasn't offered or Bayer hadn't given me the support to pursue my interest, my Ph.D. education would not have become the success that it is today. I would like to thank Dr. Steve Calhoun for his great support; it would not have been possible for me to fulfill my dream without his support. I extend my gratitude to Mr. Ramakrishna Reddy, Dr. Mohan Kumar Varma, Mr. T. Praveen Kumar, Dr. Satish Chitgupekar, Ms. Jie Chen, Dr. Bay Duy Nguyen, Dr. Ninh Thuan Nguyen and Bayer CropScience colleagues in Rice Breeding, Crop Genetics, Crop Analytics and, Biometrics functions for their unconditional support.

It is impossible for me to reach this day without support of my beloved wife Usha and son Charan. Lastly, thanks go to my parents and in-laws. I am blessed to be a part of their thoughts and prayers that have given me the strength and courage to pursue my graduate education.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Dr. Amir M.H. Ibrahim (Advisor), Dr. Seth C Murray, Dr. Hongbin Zhang, and Dr. Wayne Smith of the Department of Soil and Crop Sciences and Dr. Yog Raj (Co-Advisor, Bayer CropScience).

The genotyping data for Chapter V was generated at Bayer CropScince Innovation Center, Gent, Belgium by Dr. Stephen Rae.

All other work conducted for the dissertation was completed by the student independently.

Funding Sources

My graduate study was supported by plant breeding graduate student support from Bayer CropScience.

NOMENCLATURE

BLUE	Best Linear Unbiased Estimator
BLUP	Best Linear Unbiased Predictor
BPH	Brown Plant Hopper
CHP	Chandippa, India
CMS	Cytoplasmic Male Sterile
DHA	Dhantori, India
DS	Dry Season
GCA	General Combining Ability
HAAS	Hunan Academy of Agriculture Sciences
HI	Heat Index
IRRI	International Rice Research Institute
QTL	Quantitative Trait Loci
RILs	Recombinant Inbred Lines
SCA	Specific Combining Ability
SNP	Single Nucleotide Polymorphism
SSD	Single Seed Descent
TQ	Thermal Quotient
WA	Wild Abortive
WS	Wet Season

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

1.1 Introduction

The world demand for rice is increasing while the area of the world under rice cultivation is decreasing. The International Rice Research Institute (IRRI) has estimated that an additional 8-10 million tons of rice will need to be produced each year to meet the growing demand. Possible approaches that have been suggested to accomplish this include increasing rice production in a sustainable manner through commercial planting of hybrid rice and protecting crops against biotic and abiotic stresses through the development of resistant cultivars. The proportion of rice fields planted with hybrid rice is estimated to be around 63% in China (Li, Xin, and Yuan 2009) and around 10% in other Asian countries (Doberman, International Rice Research Center, pers. comm., 2011). The expansion of hybrid rice cultivation has proceeded at a slower pace than expected in India and other Asian countries because of limitations such as yield heterosis, grain quality, biotic and abiotic stresses and unstable hybrid seed production (David J. Spielman et al., 2012).

As rice is a self-pollinating crop, the efficient and economic commercial production of hybrid seed plays an especially important role in the successful implementation of hybrid varieties (Wen Gui Yan et al., 2009). Since rice is a short-day plant, it can be highly sensitive to photoperiod, and different rice cultivars vary widely in their degrees of sensitivity (IRRI, 1985) and flowering behaviors. Changing

environmental conditions further influence production of first-generation (F_1) hybrid seeds. Outcrossing traits such as pollen load, stigma exertion, stigma receptivity and spikelet opening are other factors that play a vital role in F_1 hybrid seed production (Virmani SS, and Athwal DS., 1974). Day/night temperature fluctuations can also have detrimental effects, restricting anther dehiscence and reducing the quality and viability of pollen (A.R. Mohammed et al., 2010). Poor anther dehiscence and low pollen production lead to sterility in fertile plants, resulting in low numbers of germinating pollen grains on the plants' stigmas (Matsui et al., 2000, 2001; Prasad et al., 2006). Exploration of genetic variability in hybrid rice breeding for pollen load and spikelet fertility under high temperatures presents opportunities for improving restorer lines.

Among the large number of insect pests hosted by rice, the brown plant hopper (BPH), *Nilaparvata lugens* (Stål), is one of the most harmful to rice in Asia. The damage that BPHs cause to rice plants is called "hopper burn" and is produced by BPHs sucking phloem sap from the rice and transmitting viral diseases, such as rice grassy stunt virus (RGSV), *Tenuivirus,* and rice ragged stunt virus (RRSV), *Oryzavirus*. In recent years, BPHs have caused devastating damage in counties such as China, Vietnam, Japan, Korea and India. In 2005 and 2008, China reported a combined yield loss of 2.7 million tons of rice due to direct damage from BPHs, while Vietnam reported 0.4 million tons of yield loss mainly due to the viral diseases RGSV and RRSV (Brar et al., 2010). The BPH is a very dynamic insect that continually evolves and changes its behavior, making it difficult to control. Improving host-plant resistance is the most effective and

environmentally friendly means of controlling the damage caused by insects and increasing yield potential of cereal crops (Jena et al., 2006).

The proposed research project will contribute to the genetic analysis and mapping of BPH resistance genes and marker validation in varied genetic backgrounds. The study will enable understanding of the genetic basis of the different traits that influence outcrossing in rice hybrid seed production, namely photoperiod sensitivity, pollen production and sensitivity to environmental conditions. It will also allow the identification of new commercially viable restorer lines of hybrid rice with BPH resistance and high pollen load. The specific objectives of the study are as follows: 1) to phenotype BPH resistance in a mapping population; 2) to phenotype rice restorer mapping populations for photoperiod sensitivity and pollen load related traits in varied environmental conditions in India; 3) to genotype mapping populations and identify quantitative trait loci (QTLs) for BPH resistance, photoperiod sensitivity and pollen load traits; and 4) to identify promising restorer genotypes from the populations with good combining ability for photoperiod insensitivity, BPH resistance and yield heterosis.

The findings of this project can facilitate positive selection for traits such as photoperiod insensitivity, pollen load and BPH resistance through the use of molecular markers, as well as contribute to the development of rice hybridization by addressing issues related to hybrid seed production and BPH resistance.

CHAPTER II

REVIEW OF LITERATURE

2.1 Hybrid Rice Technology

The possibility of rice hybridization was first documented in 1954 by S. Sampath and H.K. Mohanty at the Central Rice Research Institute of Cuttack in the Indian state of Orissa (Janaiah, 2002). Rice hybrids often have yields between 15% and 20% higher than high-yielding inbred cultivars (Virmani et al., 2003). At present, three different breeding methods (one-line, two-line and three-line) have been proposed for to the exploration of heterosis in rice (Yuan, 2002).

The one-line method of hybrid seed production involves apomictic systems. Apomixis is asexual reproduction without genetic segregation; this breeding method allows farmers to use harvests of hybrid crops as seed for subsequent hybrid crops (Virmani, 1994). To enhance the need for the hybrid rice seed production, Yuan (1987) proposed introducing apomixis in rice. Apomixis is common in grasses and in several polyploid plant species, but no clear evidence has been found of apomixis in rice (Virmani et al., 1996).

The two-line method of heterosis breeding, which has become popular in the development of rice hybrids in China (Wang et al., 1995), makes use of two techniques, namely chemical emasculation and environmentally-sensitive genic male sterility (EGMS). Photoperiod-sensitive genic male sterile (PGMS) lines and thermo-sensitive genic male sterile (TGMS) lines are two major types of EGMS germplasm resources that

are widely used for the breeding of two-line hybrid rice. The application of EGMS in two-line hybrid rice breeding offers many advantages, including a wide range of germplasm resources that may be used as breeding parents, higher yields and simple procedures for breeding and hybrid production (Virmani et al., 2003; Zhou et al., 2012). Beginning with the discovery of the PGMS line Nongken 58S (NK58S) in rice (Shi 1985), considerable progress has been made in the use of two-line hybrid rice breeding in agriculture. The 'NK58S' line retains complete male sterility during anther development when the day length (photoperiod) is longer than 13.75 hours and converts to partial or complete male fertility when the day length is shorter than 13.5 hours. However, the male sterility–fertility transformation of 'PA64S' and of other *indica* lines derived from NK58S is controlled mainly by temperature rather than by day length. The PA64S line, for example, exhibits male sterility at temperatures higher than 23.5°C during anther development, but converts to male fertility when the temperature is between approximately 21ºC and 23°C (Lu et al., 2007; Xu et al., 1999). To date, a number of loci that control PGMS or TGMS have been mapped to distinct chromosomes: photoperiod-sensitive genic male sterile genes *pms1, pms2,* and *pms3*; reverse photoperiod-sensitive genic male sterile genes *rpms1* and *rpms2*; thermo-sensitive genic male sterile genes *tms1, tms2, tms3, tms4, tms5, tms6,* and *tms6(t)*; photoperiod-thermo-sensitive genic male sterile genes *ptgms2-1* and *pms1(t)*; and the reverse thermo-sensitive genic male-sterile gene *rtms1* (Hai et al., 2012). Understanding is still limited, however, as to how environmental factors affect male sterility–fertility in

EGMS lines (Namaky et al., 2017), which determines the behavior of parental germplasm in two-line hybrids and impacts the quality of F_1 seed.

The three-line system is the most popular and successful method for exploring heterosis in rice and accounts for most of the rice hybrids that are developed worldwide (Li and Xin, 2000), including 90% of the rice hybrids produced in China and 100% of those developed outside China (Sattari et al., 2007). With the development of wild abortive (WA) cytoplasmic male sterility (CMS) in 1970, a rice researcher in the team of Longping Yuan identified the critical rice germplasm for the three-line hybrid rice line wild abortive cytoplasmic male sterile rice, which provided new opportunities for the exploration of rice heterosis (Li 1997). Since the discovery of the WA-CMS line, more than sixty CMS lines, including Dissi, Gambodia, Indonesia rice, Dian I, Honglian (HL), Boro-II (BT) and Maxie, have been developed from interspecies, inter sub-species and inter-variety crosses, such as that of the *Oryza* species with AA genomes (Zhu et al., 2000, Li and Yuan, 2000). The CMS system is divided into the categories WA-CMS, HL-CMS and BT-CMS based on evidence gathered in genetic and cytological studies (Rao 1988, Li and Yuan 2000). Fertility in CMS lines can be restored by *Rf* genes. For instance, in the WA-CMS line, pollen abortion caused by the *WA352* gene is rescued by nuclear genes *Rf3* and *Rf4*, which are located on chromosomes 1 and 10, respectively (Luo et al., 2013). To date, more than 17 *Rf* genes, distributed across all chromosomes except for chromosome 9, have been identified for the rescue of various CMS types (Biao-lin et al., 2016). Of these, seven *Rf* genes have been functionally characterized: *Rf1* (*Rf1a* and *Rf1b*) for BT-type CMS (Komori et al., 2004 and Wang et al., 2006), *Rf2*

for Lead rice (LD)-type CMS (Itabashi et al., 2011), *Rf4* for WA-type CMS (Kazama et al., 2014 and Tang et al., 2014), *Rf5* and *Rf6* for HL-type CMS (Hu et al., 2012 and Huang et al., 2015) and *Rf17* for Chinese wild rice (CW)-type CMS (Fujii et al., 2009).

The three-line system involves the cytoplasmic male sterile line (A), the maintainer line (B) and the restorer line (R). The cytoplasmic male sterile line A is a CMS line that is unable to produce functional pollen during microspore formation, and it is used as a female line in hybrid seed production. The maintainer line B is an isogenic line for A with cytoplasmic fertility. The maintainer line can, therefore, produce viable pollen grain and set normal seed. This line is used as a pollinator to maintain male sterility. The restorer line R possesses dominant fertility restorer (*Rf*) genes and can restore fertility in derived F_1 hybrids when crossed with the CMS line. A diagrammatic representation of the three-line system of hybrid seed production is given in Figure 1.1.

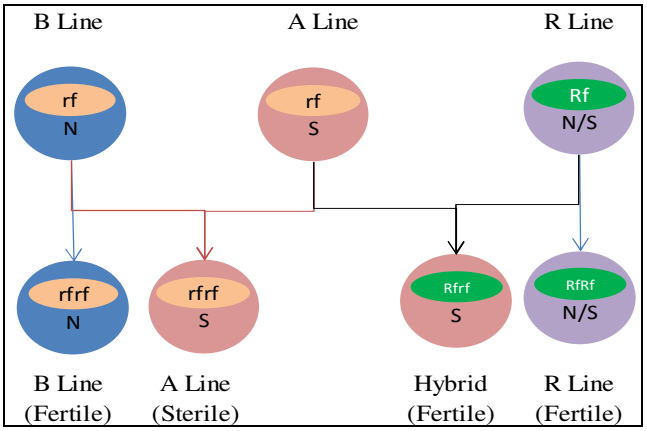


Figure 1.1: The diagrammatic representation of three line system of hybrid rice production.

2.2 Hybrid Rice Opportunities and Challenges

Hybrid rice technology presents a viable possibility for meeting increasing demand for rice as population grows. China, for instance, was able to raise its national

average rice production from 3.5 to 6.2 tons per hectare by adopting hybrid rice technology over a period of a four decades (FAO, 2004), and hybrid rice now accounts for an estimated 63% of all area under rice cultivation in the country (Li, Xin and Yuan, 2009). The increase in rice yields attributed to hybrid rice has improved food security in China and helped feed an estimated 60 million additional people per year (Li et al., 2010). The superior performance of hybrid rice in saline conditions has been recorded, with hybrid rice producing 16–22% higher yields than tolerant check rice varieties (Zayed et al., 2013). Because of its yield advantage, hybrid rice technology is highly important for food security in rice-consuming countries where amounts of arable land are decreasing, populations are increasing, and labor remains inexpensive (FAO, 2004).

In the Asian countries of India, Bangladesh, Indonesia, Vietnam, and the Philippines, hybrid rice accounts for less than 10% of the total area under rice cultivation (Spielman et al., 2013). One of the reasons for the slow and sporadic adoption of hybrid rice technology in these countries is that the narrow germplasm availability of female lines causes conversion to male sterile systems, resulting in grain quality issues and poor levels of abiotic and biotic stress tolerance (Janaiah, 2002; Janaiah and Hossain, 2003). The successful commercialization of hybrid rice is clearly related to the development of hybrid seed production technology (Virmani et al., 1993); however, the sensitivity of parental lines to environmental conditions poses challenges to the production of hybrid seed of suitable quality and sufficient quantity. The environmental factors that influence outcrossing in rice include temperature, relative humidity, light intensity and wind velocity (Virmani 1996). In China, the conditions favorable to good outcrossing in rice

have been identified as a daily temperature of 24–28^{o}C, a relative humidity of 70–80%, a diurnal difference in temperature of 8–10^{o}C, sunny weather and breeze (Xu and Li 1988). The floral traits that influence outcrossing in rice include stigma size, style length, stigma exertion (in seed parents), anther length, filament length and pollen number per anther (in pollen parents). Finally, the flowering behavior traits that influence outcrossing in rice are number of days of blooming, time of blooming, duration of blooming, duration of floret opening, angle of floret opening and male and female flowering synchrony (Virmani, 1996).

2.3 Brown Plant Hopper (BPH) Resistance

The brown plant hopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is a migratory monophagous rice herbivore that causes a type of damage known as "hopper burn" by sucking phloem sap, feeding by phloem abstraction (Watanabe and Kitagawa, 200; Liu et al., 2008), and transmitting viral diseases such as grassy stunt virus (RGSV) and ragged stunt virus (RRSV) (Ling et al., 1978). A rice plant suffers 40–70% yield loss if attacked by 100–200 first instar nymphs of BPH at 25 days after transplanting of the rice seed (Bae and Pathak, 1970). The application of pesticides is the most common method for controlling BPH damage, but the BPH has already developed high to very high levels of resistance against almost all insecticides (Krishnaiah, 2016). The insects avoid the toxic effects of the chemicals as they develop the ability to resist the penetration of the insecticide through their integuments (Krishnaiah, 2015). It is important to develop a sustainable pest management system by balancing breeding and management strategies in order to reduce the ecological fitness

of BPHs and keep the pest below the economic threshold level (Bosque-Perez and Buddenhagen, 1992). For these purposes, improving host-plant resistance is the most effective and environmentally friendly approach to controlling the damage caused by BPHs (Jena et al., 2006).

The mechanisms that effect host-plants' resistance to BPHs can be divided into the categories of antixenosis, antibiosis and tolerance (Alam and Cohen, 1998; Painter, 1951). Antibiosis is the most commonly studied of these mechanisms, and the host preference, feeding and hatching behaviors of BPHs are most clearly impacted in those varieties that develop resistance through antibiosis (Cohen et al., 1997; Du et al, 2009; Qiu et al., 2010). Rice plants also activate their own defensive stress responses in cases of BPH infestation by increasing secretion of insect-toxic compounds, activating metabolic inhibitors and forming physical barriers (by increasing cuticle thickness and callose deposition) to prevent BPH feeding (Cheng et al., 2013). The first BPH resistance was identified in 1967 (Pathak et al., 1969). Since then, the resistant genes *BPh1*, *bph2*, *Bph3* and *bph4* have been identified through genetic analysis of various donors (Lakshminarayana and Khush, 1971; Khush et al., 1985) and used extensively in breeding programs in Southeast Asia. As of now, 29 BPH resistance genes (shown in Table 1.1) have been identified from the subspecies *indica* and its wild relatives (Ali and Chowdhury, 2014; Wang et al., 2015), and more than 10 of these genes have been fine-mapped to regions of less than 200 kilobases (kb) in size. Introgression lines derived from crosses of *O. sativa* and wild species have been used to map many of the BPH resistance genes (Jene and Khush, 1990; Brara and Khush, 1997). Thus far, 11 resistance

genes have been identified in wild rice, including *Bph11* and *Bph15* from *O. officinalis*, *Bph10* and *Bph18* from *O. australiensis*, *Bph20* and *Bph21* from *O. minuta*, Bph27, and *bph29* from *O. rufipogan* (Jie et al., 2016). With several resistance genes available, it is important to identify the resistance of new genes to new biotypes and pyramid major genes in order to provide durable resistance to BPH (Kshirod et al., 2010).

Table 1.1: Summary of identified brown plant hopper (BPH) resistance genes, donors, chromosome location and its position.

Gene/QTL	chr	Position(Mbp)	Donor	References
Bph1	12	13.10–13.28	Mudgo, TKM-6	Kim et Sohn, 2005
	12L	22.81–22.93	Mudgo	Cha et al., 2008
	12L	24.00–25.00	Nori-PL3	Sharma et al., 2002
bph2	12	22.13–23.18	IR1154-243	Murai et al., 2001
	12L	13.21–22.13	ASD7	Sun et al., 2006
Bph26/bph2	12	22.87–22.88	ADR52	Tamura et al., 2014
bph7	12L	19.95–20.87	T12	Qiu et al., 2014
Bph9	12L	19.11–22.13	Kaharamana	Su et al., 2006
	12L	19.00–22.50	Pokkali	Murata et al., 2001
Bph10(t)	12L	19.00–23.00	IR65482-4-136, *O. australiensis*	Ishii et al.,1994
Bph18(t)	12L	22.25–23.48	IR65482-7-216, *O. australiensis*	Jena et al., 2006
Bph21(t)	12L	23.28–24.41	IR71033-121-15, *O.minuta*	Rahman et al., 2009
Bph12	4S	5.21–5.66	*O. latifolia*	Qiu et al., 2012
Bph15	4S	6.68–6.90	*O. officinalis*	Lv et al., 2014
QBph4.1	4S	6.70–6.90	*O. officinalis*	Hu et al., 2015a
QBph4.2	4S	6.58–6.89	*O. australiensis*	Hu et al., 2015b
Bph17	4S	6.93–6.97	Heenati	Sun et al., 2005
Bph20(t)	4S	8.20–9.60	*O. minuta*	Rahman et al., 2009
Bph6	4L	21.36–21.39	Swarnalata	Qiu et al., 2010
Bph27	4L	19.12–19.20	GX2183, *O. rufipogon*	Huang et al., 2013
Bph27(t)	4L	20.79–21.33	Balamawee	He et al., 2013
bph12(t)	4L	20.20–21.20	*O. officinalis*	Hirabayashi et al.,1999
bph11(t)	3L	35.60–35.80	*O. officinalis*	Hirabayashi et al.,1998
Bph14	3L	35.70–35.72	B5, *O. officinalis*	Du et al., 2009
QBph3	3L	35.63–35.67	IR02W101, *O. officinalis*	Hu et al., 2015a
Bph13	3S	5.18–5.70	*O. officinalis*	Renganayaki et al., 2002
bph19	3S	7.18–7.24	AS20-1	Chen et al., 2006
qBph3	3	18.27–20.25	Rathu Heenati	Kumari et al., 2010
Bph3	6S	1.21–1.40	Rathu Heenati	Jairin et al., 2007
bph4	6S	1.20–1.76	Babawee	Kawaguchi et al., 2001
Bph25	6S	0.20–1.71	ADR52	Myint et al., 2012
bph29	6S	0.48–0.49	*O. rufipogon*	Wang et al., 2015
Bph6	11	17.23–18.27	*O. officinalis*	Jena et al., 2003
Bph28(t)	11	16.90–16.96	DV85	Wu et al., 2014

2.4 Flowering and Photoperiod Sensitivity

Flowering is an important transition from the vegetative to reproduction phase of a plant's development and is the end result of many complex physiological and biochemical processes. These processes are regulated by several genes within the organism and are also influenced by environmental stimuli (Murfet, 1977). For instance, a plant's endogenous circadian clock mechanism, which is responsible for day-length measurement, allows the plant to modulate its development to maximize adaptation to periodic changes in day length and temperature (Jarillo et al., 2008). The importance of day length to flowering behavior was first demonstrated in studies of soybeans and tobacco conducted in controlled photoperiod conditions (Garner and Allard, 1920). Based on their day-length responses, flowering plants are classified as long-day (LD), short-day (SD) or day-neutral (DN) plants. Flowering is promoted in LD plants by periods of daylight that are longer than a critical day length and in SD plants by periods of daylight below this threshold, while DN plants flower at the same time irrespective of photoperiod conditions. Several studies concerning the effects of photoperiods on flowering have identified molecular components of the mechanisms responsible for day length discrimination (Searle and Coupland, 2004; Corbesier and Coupland, 2005; Baurle and Dean, 2006; Imaizumi and Kay, 2006; Jarillo and Pineiro, 2006). One discovery that significantly contributed to the understanding of photoperiod regulation was the identification of leaves as the site where day-length perception occurs, activating florigen, a universal flowering-inducing signal that evokes the stem terminal meristematic tissue (Chailakyan, 1936a, b, 1937).

Rice is a facultative SD plant that flowers earlier in short-day conditions than long-day conditions. To allow vegetative growth, flowering in rice is inhibited during early growth stages. A distinction is, therefore, drawn between the flowering duration of rice in its basic vegetative phase (BVP) and photoperiod-sensitive phase (PSP) (Chang et.al, 1969). Reproductive development is induced when the rice plant reaches a certain growth stage by the protein heading date 3a (*Hd3a*), which is also considered a florigen protein (Yano et al., 1997). The protein *Hd3a* was initially identified by QTL mapping that used a population derived from a cross between the photoperiod-sensitive cultivar 'Nipponbare' and the photoperiod-insensitive cultivar 'Kasalatha' (Lin et al., 1995). Another protein that functions as a florigen, *RFT1*, is adjacent to *Hd3a* on Chromosome 6; the two proteins are separated by only 11.5kb, suggesting that they developed through tandem duplication (Chardon et al., 2005; Komiya et al., 2009; Hagiwara, 2009). Overexpression studies of *Hd3a* and *RFT1* during the callus induction stage illustrate the importance of these two proteins in the flowering of rice (Monna et al., 2002; Hori et al., 2013; Tsuji et al., 2008). The protein *Hd3a* is controlled by *Hd1*, a QTL that binds to the *Hd3a* promoter and is thought to have a major role in the control of photoperiod sensitivity (Inoue et al., 1992). Depending on light conditions, *Hd1* can act either as an activator or repressor of flowing, as nonfunctional *Hd1* causes late flowering in SD conditions but early flowering in LD conditions (Lin et al., 2000). The protein *Ehd1*, a QTL identified in the mapping population derived from the cross between 'Taichung 65' and *Oryza glaberrima*, also controls the expression of *Hd3a*, while the introgression of the *Ehd 1* allele from *O. glaberrima* causes early flowering in Taichung 65 under both

SD and LD conditions. Heading date 7 (*Ghd7*), an LD-specific grain yield repressor identified in the mapping population developed from the cross of 'Minghui 63' and 'Zhenshan 97', occasions pleotropic phenotypes such as late flowering and increased height. The functional allele of the QTL *Hd5*, located on chromosome 8, can cause late flowering under LD conditions by suppressing the expression of *Ehd1*, *Hd3a* and *RFT1*, while under SD conditions the expression of these genes are not affected by *Hd5*. The protein *Hd16* is another flowering repressor gene that was identified from a cross between Nipponbare and Koshihikari. The deficient allele of *Hd16* from Koshihikari weakens photoperiodic sensitivity and increases expression of the floral activators *Ehd1*, *Hd3a* and *RFT1* under LD conditions (Hori et al., 2013). Fourteen QTLs that control flowering time have been identified by QTL analyses for heading date performed on several populations derived from the cross between Nipponbare and Kasaltha mentioned earlier. The five QTLs *Hd1* through *Hd5* have been mapped based on analysis of the F_2 population of the cross (Yano et al., 1997); *Hd7*, *Hd8* and *Hd11* have been detected using backcross (BC) lines BC_1F_5 (Lin et al., 1998); *Hd6*, *Hd9*, *Hd10*, *Hd12*, *Hd13* and *Hd14* have been detected using backcross progenies BC_3F_2 and BC_4F_2 (Yamamoto et al., 2000). The QTLs *Hd1*, *Hd2*, *Hd3*, *Hd5* and *Hd6* have been found to confer photoperiod sensitivity, while epistatic interaction between *Hd1* and *Hd3* has been clarified by the study of interaction effects between Kasalatha and Nipponbare alleles in LD and SD conditions (Lin et al., 2000). However, while several flowering genes and their regulatory mechanisms have been identified, better understanding is still required of

their relationships and interactions with environmental stimuli such as temperature extremes, nutrient deficiencies and various other stresses (Lee et al., 2015).

2.5 Outcrossing Traits

The commercial exploitation of hybrid vigor offers significant possibilities for the solution of food shortages caused by an increasing global population (Duvik, 1999; Virmani, 2003). The success of hybrid maize (*Zea Mays L.*) has motivated breeders of other crops to develop hybrids as well; however, progress in the hybridization of self-pollinating crops such as rice and wheat (*Triticum aestivum* L.) has been limited due to the difficulty of controlling reproduction (cross-pollination) in these crops. Although hybrid rice has been commercialized on a large scale, possibilities for further expansion of hybrid rice cultivation in South Asian countries are limited due to the high cost and low production rate of F_1 hybrid rice seeds. As rice is a self-pollinating crop, it has a natural outcrossing rate of lower than 4% (Hayes et al., 1955). However, higher outcrossing rates are observed in tropical than in subtropical climates (Sahadevan et al., 1963), and natural outcrossing in male sterile rice lines has been observed to vary widely, ranging from 0% to 44% (Salgotra et al., 2009; Sheeba et al., 2006; Sidharthan et al., 2007). This variability in male sterile lines can be attributed to variations in the plants' flowering behavior, the floral characters of male sterile and pollen parents and environmental factors (Virmani 1994). Important genetic factors that influence natural outcrossing include time intervals between flowering and pollen dispersal, stigma length and style and number of pollen grains per anther. A high percentage of stigma protrusion in the plant's seed parents and large amount of residual pollen in its pollen parent

generally results in a higher natural outcrossing rate (Kato and Namai, 1987a). Important non-genetic factors that influence high outcrossing include low temperature (Baechell et al., 1938), high humidity (Ramaiah 1953), low wind velocity, and high amounts of airborn pollen (Kato and Namai, 1987b).

The genetics of outcrossing traits have been studied using mapping populations developed from the cross of *O. sativa* and *O. rufipogon*. To map and detect the genomic regions that influence floral traits, cultivated germplasm accessions and various mapping populations have been used, including F_2s, BCs, recombinant inbred lines (RILs), and doubled haploid lines (DHLs) (Cai and Morishima, 2002; Hu et al., 2009; Li et al., 2001; Miyata et al., 2007; Yu et al., 2006). The major QTL *qES3*, which was identified on chromosome 3 using an F_2 population derived from Koshikari and a breeding line, has been shown to increase rates of stigma exertion by about 20% (Miyata et al., 2007). Using eight different mapping populations, 26 QTLs that influence stigma length in rice have been identified, distributed through all chromosome except chromosome 11 (Uga et al., 2010; Yan et al., 2009). Meanwhile, 291 mature anther-preferential expression genes (*OsSTA*) have been identified in analyses of transcriptome profiling and gene co-expression based on Affymetrix microarray data and the functioning of *OsSTA* genes in male fertility, pollen germination and anther dehiscence (Ling et al., 2015). Further developments in this area that are needed to increase hybrid seed production in rice include the exploration of the molecular basis of genetic variation in outcrossing traits and the mapping of those traits, and functional validation and marker-assisted transfer of QTLs from wild species that influence outcrossing traits (Marathi and Jena, 2015).

2.6 Linkage Maps and QTL Mapping

A linkage map may be thought of as a "road map" of the chromosomes derived from two separate parents (Paterson, 1996). Linkage maps indicate the positions of markers along chromosomes and the relative distances between them, similar to signs or landmarks along a highway. Their most important function is to identify chromosomal locations that contain genes and QTLs that are associated with traits of interest; maps produced for these purposes may be referred to as "QTL" (or "genetic") maps. Quantitative trait locus mapping is based on the principle that genes and markers segregate via chromosome recombination (or "crossing-over") during the meiotic phase of cell division, thus allowing genes' and markers' locations to be analyzed in the progeny (Paterson, 1996).

Quantitative trait locus analysis is a statistical method that links phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an attempt to explain the genetic basis of variation in complex traits (Falconer & Mackay, 1996; Kearsey, 1998).

To perform a QTL analysis, one first needs to select two genotypes that differ genetically with regard to the trait of interest. Second, one must identify genetic markers that distinguish between these genotypes. Molecular markers are preferred for genotyping, because they are unlikely to affect the trait of interest. Several types of markers are commonly used, including single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs, or microsatellites). Next, the QTL analysis is carried out using populations such as F_2s, BC_2F_2s, RILs, and DHLs. Finally, the phenotypes and

genotypes of the derived (F_2) population are scored. Markers that are genetically linked to a QTL that influence the trait of interest will segregate more frequently with trait values, whereas markers that are not linked to a QTL will not show significant associations with those values. For traits that are controlled by tens or hundreds of genes, the parental lines need not actually differ for the phenotype in question because some degree of transgressive segregation is expected. Instead, they must simply contain different alleles, which are then re-assorted by recombination in the derived population to produce a range of phenotypic values.

QTL analysis is based on the detection of associations between phenotypes and genotypes of markers. Markers are used to partition a mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993; Young, 1996). Depending on the marker system and type of population employed, a significant difference between the phenotypic means of the groups can indicate that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait.

Quantitative trait loci and markers are generally both inherited in the progeny, and the means of a group with a tightly linked marker are significantly different ($P < 0.05$) from the means of a group without a marker. When a marker is loosely linked or not linked to a QTL, the marker and QTL segregate independently. The presence or absence of the loosely linked marker has no significance for the means of the genotype groups, and as unlinked markers located far from the QTL or on a different chromosome

are randomly inherited with the QTL, in this case there are also no significant differences to detect between the means of the genotype groups. Three widely used methods for detecting QTLs are single-marker analysis, simple interval mapping (SIM) and composite interval mapping (Liu, 1998; Tanksley, 1993).

The statistical methods used for single-marker analysis include t-tests, analysis of variance (ANOVA) and linear regression. QGene and MapManager QTX are commonly used computer programs for performing single-marker analysis (Manly et al., 2001; Nelson, 1997). Instead of analyzing single markers, the SIM method of QTL detection uses linkage maps and simultaneously analyzes intervals between adjacent pairs of linked markers along chromosomes (Lander & Botstein, 1989). Many studies have used the computer programs MapMaker/QTL (Lincoln et al., 1993b) and QGene (Nelson, 1997) to conduct interval mapping and composite interval mapping. Composite interval mapping (CIM) is a popular method for mapping QTL that combines interval mapping with linear regression and includes additional genetic markers in its statistical model and adjacent pairs of linked markers for interval mapping (Jansen, 1993; Jansen & Stam, 1994; Zeng, 1993, 1994). The main advantage of CIM is that it is more precise and more powerful at mapping QTLs than single-point analysis and interval mapping, especially when linked QTLs are involved. However, it is not necessarily more accurate and substantial numbers of false positive results can still occur. Many researchers have used the programs QTL Cartographer (Basten et al., 1994, 2001), MapManager QTX (Manly et al., 2001) and PLABQTL (Utz & Melchinger, 1996) to perform CIM. None of these tools can simultaneously analyze epistasis and QTL environmental interactions (Yang et

al., 2008). The QTL Network 2.0 software, however, is based on a mixed linear model and has been developed for the purpose of mapping QTL with additive and epistatic effects and the interactions between them (Yang et al., 2008; Wang et al., 1999). This method has been used in QTL mapping studies of heading date in rice (Liu et al., 2007), and both plant height (Zhang et al., 2008) and flour color (Zhang et al., 2009) in wheat.

2.7 Heterosis and Combining Ability

The term "heterosis" was coined by Shull (1914) and refers to the superiority of an F_1 hybrid's traits compared to the mean parental value (mid-parent heterosis), the better parent's value (heterobeltiosis or high-parent heterosis) or the value of the best commercial variety (standard or commercial heterosis) for those traits. Heterosis in rice was first reported by Jones in 1926, who in comparing an F_1 rice hybrid to its parents observed a marked increase in culm number and grain yield. Heterotic combinations of parents can be selected to improve heterosis through investigation of a plant's combining ability. The concept of combining ability was introduced by Sparague and Tatum (1942) and refers to the capacity of a genotype to transmit its superior performance to its crosses. One can distinguish between general combining ability (GCA), which is attributed to additive gene effects and additive-by-additive epistasis, and specific combining ability (SCA), which may be attributed to dominance or epistasis or both (Cockerham, 1961; Pradhan et al, 2006). Several scientists have studied heterosis and combining ability for different productivity traits in rice; many of their findings can be found in Table 1.2 and Table 1.3 below (Veeresha et al., 2015).

Table 1.2: Summary of heterosis reported for yield and productivity traits in rice.

Character	Heterosis		Reference
	Standard heterosis	Heterobeltiosis	
Days to 50% flowering	Negative		Malini et al. 2006, Veeresha et al.2013
	Positive		Bisne et al. 2008, Malvizi et al. 2009
		-23.60 to 4.07	Nadali Bagheri 2010
	-4.22 to - 16.57	-16.57 to 7.27	Tiwari et al. 2011
		-8.36 to 2.88	Sunil Kumar et al. 2012
Plant height		3.25 to 42.99	Saleem et al. 2008
		-32.2o to 3.41	Nadali Baghart 2010
	-8.30 to 60.90		Rahimi et al. 2010
	-19.62 to 0.18	-16.99 to 8.29	Tiwari et al. 2011
		-15.97 to 15.09	Patil et al. 2011
Number of panicles/plant	Positive		Anand and Singh 2002, Veeresha et al.2013
		-37.50 to 11.40	Faiz et al. 2006
	-16.00 to 34.00		Saravana 2008
	-8.330 to 66.67	-34.00 to 39.53	Tiwari et al. 2011
		-30.56 to 22.22	Sunil Kumar et al. 2012
Panicle length	Positive		Anand and Singh 2002, Khoyumthem et al. 2005
		Positive	Bisne et al. 2008
		-13.30 to 15.59	Nadal Bagheri 2010
	-14.90 to 6.90		Rahimi et al. 2010
	-40.63 to 23.20	-39.26 to 48.30	Tiwari et al. 2011
Spikelet fertility		-57.62 to 66.11	Sarker et al. 2002
	Positive		Ganasekaran 2006
		64.45	Manojkumar 2008
	-0.81 to 16.31	-6.89 to 46.40	Tiwari et al. 2011
		-36.24 to 10.89	Sunil Kumar et al. 2012
Number of spikelets / panicle	-20.00 to 60.00		Chao et al. 1994
		Positive	Lingaraju et al. 1999, Munnisonnappa et al. 2007
	Positive		Anand and Singh 2002
		-40.38 to 36.98	Faiz et al. 2006
	-33.56 to 12.08	-40.44 to 8.56	Tiwari et al. 2011
Grain Yield	Positive		Rajesh Singh 2000, Bisne et al. 2008
	Positive	Positive	Narasimman et al. 2007, Manoj Kumar 2008
	-73.70 to 129.16	-75.71 to 219.75	Malini et al. 2006
	18.00 to 40.00		Malarvizhi et al. 2003
		-39.59 to 6.04	Sunil Kumar et al. 2012

Table 1.3: Summary of gene action and combining ability reported in rice.

Character	Additive (GCA)	Non-additive (SCA)	Additive and Non-additive
Days to 50% flowering	Swamy et al. 2003		Faiz et al. 2006
Plant height	Senguttuvel and Kannan 2007	Akash and Pathak 2008, Pradhan 2006	Zhao 2008
Number of panicles/plant	Veeresha et al. 2013	Akash and Pathak 2008, Pradhan 2006	Rahimi 2010
Panicle length	Veeresha et al. 2013	Pradeep Kumar and Reddy 2011, Hariprasanna et al. 2006	Sawant 2006
Spiklet fertility	Vani and rani 2003	Pradeep Kumar and Reddy 2011, Nadali Bagheri et al. 2010	
Number of spikelets / panicle	Vani and rani 2003	Narasimman et al. 2007	Swamy et al. 2003
Grain Yield	Sengutuvel and Kannan 2007	Pradeep Kumar and Reddy 2011, Hariprasanna et al. 2006	Faiz et al. 2006, Sawnt 2006

2.8 Multi-Environmental Trials and Data Analysis

Multi-environmental trials (METs) are often used in plant breeding to evaluate entries into a diverse target region under varied environmental conditions (Smith et al., 2001; Piepho et al., 2008; Burgueno et al., 2011). Multi-environmental trials help researchers to select the most suitable genotypes for an environment and better understand genotype-by-environment (G×E) interactions (Smith et al., 2005). Genotype-by-environment interactions are the differential responses of genotypes across a range of environments (Kang, 2004); investigations of interactions that are repeatable are the most useful for developing breeding strategies (Baker, 1988). Muir et al. (1992) have offered methods for partitioning G×E interactions into those caused by heterogeneous variances and those caused by lack of correlation. For the quantitative analysis of G×E interactions, Yang and Baker (1991) applied multivariate analysis of variance (MANOVA) and proposed a significance test based on assumptions concerning the

sampling distributions of estimated variance and covariance components of G×E interactions; the significance test results in non-positive definite estimates of genetic variance–covariance matrices. On this basis, Yang (2002) has also applied a restricted maximum likelihood (REML) approach to estimate genetic parameters and test the significance of different sources of G×E interaction. Restricted maximum likelihood approaches are frequently used to estimate variance parameters in mixed-model analyses of multi-environment trial data (Smith et al., 2001). In addition, the development of statistical packages such as ASREML (Gilmour et al., 1999) allows REML estimations of a range of mixed models and enables the fitting of more informative and complex models to accommodate different forms of G×E data.

Best linear unbiased prediction (BLUP) is a standard method for estimating the random effects of a mixed model. One major property of BLUP is shrinkage toward the mean, which is a desirable statistical property because the accuracy of the method increases as the bias incurred from shrinkage is balanced by the reduction in variance, thus leading to a lower mean squared error (MSE) (Rosenberger 1985). The BLUP method maximizes the correlation between true genotypic values and predicted genotypic values (Searle et al., 1992), which significantly improves one's ability to make efficient breeding decision. Analyses of metric data from plant breeding and varietal trials are based on the mixed linear model $y = X\beta + Zu + e$, where y is the vector of observations, β and u are vectors of fixed and random effects respectively, X and Z represent the design matrix associated with the model, and e is a random residual vector. The fixed effects can be estimated by calculating the best linear unbiased estimate

(BLUE), while random effects can be estimated by calculating the BLUP. Both BLUE and BLUP are computed by solving the mixed model equation (MME) given by Henderson (1986) and Searle et al. (1992).

Cullis et al. (1989) and Stroup and Mulitze (1991) have shown that BLUPs of genotype values in single trials can be enhanced through use of a special model in which a large number of new entries are tested without replication. In early-generation evaluations of hybrids in augmented field designs, replicated check hybrids can be utilized as controls for local error estimates, as well as for adjusting estimates for un-replicated test hybrids and genotype and environmental effects nested within an environment (Federer 1998). Cullis et al. (2006) suggested that genotype effects be estimated using BLUPs when a special model for early-generation varietal testing is used as the basis of an analysis. Hill and Rosenberger (1985) found that BLUPs outperform BLUEs when genotype main effects in G×E data are considered. The additive main effects multiplicative interactions (AMMI) model proposed by Gauch (1988) is a fixed model for estimating G×E interaction effects that is more accurate than least squares estimates based on a usual two-way ANOVA. A study conducted by Piepho (1994) comparing the shrinkage properties of the AMMI and BLUP models has indicated that the BLUP model is more accurate than the fixed-effect AMMI model. Finally, combinations of single-trial information based on special models with flexible variance–covariance structure for G×E effects have been suggested by Freshman et al. (1997), Cullis et al. (1998) and Smith et al. (2001); these models are routinely used in the analysis of crop variety evaluation data (Smith et al., 2001, 2005).

CHAPTER III

PHENOTYPING BROWN PLANT HOPPER RESISTANCE IN A RICE RECOMBINANT INBRED LINE MAPPING POPULATION

3.1 Introduction

Rice (*Oryza sativa* L.) has been extensively cultivated in diverse ecosystems of the tropical and subtropical regions of the world. Therefore, it is frequently exposed to various biotic and abiotic influence that can cause significant yield reductions. Among the biotic stresses, insect pests are of prime importance (Heong and Hardy, 2009). Over 100 species of insects have been reported as pests of this crop, including the brown plant hopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), which emerged as a major pest through its devastation of rice production in Asia (Chen and Cheng, 1978). Both the nymph and adult BPH suck sap from the lower portion of the plant, which results in yellow leaves, reduced tiller numbers and plant height, and increased numbers of unfilled grains. During severe infestations, BPH feeds on all succulent tissues of the plant, including panicles. Being fed upon by BPH also causes reduction in chlorophyll and protein content of leaves and lowers the rate of photosynthesis; in the case of a severe attack, extensive plant mortality, known as 'hopper burn,' can occur (Watanabe and Kitagawa, 2000; Liu et al., 2008; Horgan, 2009). These BPH also act as a vector for transmitting viral diseases like rice grassy stunt (*Tenuivirus)*, and rice ragged stunt (*Oryzavirus*).

Among all pest management strategies, using insecticides is the most common method to control BPH, although the BPH has now developed a resistance to most of the insecticides used on rice. Consequently, it is important to develop integrated pest management strategies, which comprise host plant resistance, crop management, and restricted insecticide usage. These tactics can reduce the ecological fitness of BPH and increase predator population to provide adequate resistance against infestation while protecting the environment from chemical pollution.

Antixenosis, antibiosis, and tolerance (Alam and Cohen, 1998; Painter, 1951) are three different mechanisms used to combat BPH through host plant resistance by affecting insect behavior for host preference, feeding, fecundity, and survival. The International Rice Research Institute (IRRI) in the Philippines identified 573 BPH-resistant cultivars in mass screening evaluations since the 1970s. These cultivars showed resistance against at least one biotype, whereas 80 of them showed resistance against all three BPH biotypes (Hu et al., 2016). Since the first identification of BPH resistance germplasm in 1967 (Pathak et al., 1969), 29 BPH resistance genes have been reported on five different chromosomes from *indica* and wild relatives (Wang et al., 2015). The *Bph1*, *bph2*, *Bph3* and *Bph4* genes were extensively used in breeding programs in Southeast Asia, but some of the varieties carrying these genes have lost their effectiveness against BPH due to emergence of new biotypes (Hu et al., 2016).

The main objectives of this study were to 1) develop a recombinant inbred line (RIL) mapping population from a BPH-resistant source available in rice germplasm at Bayer and another non-resistant source; 2) generate phenotypic data for BPH resistance

in RIL populations for quantitative trait loci (QTL) mapping; and 3) evaluate the selected subset of RILs for insect behaviors of feeding, fecundity, and preference.

3.2 Materials and Methods

3.2.1 Plant Material

An elite line, BRGB04267, has been identified as a BPH-resistant line in rice germplasm based on BPH greenhouse screening studies. This elite line was crossed with a susceptible restorer line (BRGB02489) to develop the RIL population consisting of 260 lines for the current study. The $F_{2:7}$ RILs, the parents, resistant check 'PTB33', tolerant check 'MTU1010', and susceptible check 'TN1' were used in the screening experiments that were conducted to gauge tolerance (degree of damage), antixenosis (adult preference and feeding rate), and antibiosis (fecundity and egg mortality) in a greenhouse at the multi-crop breeding station, Bayer BioScience, Chandippa, Hyderabad, India.

3.2.2 Insect Population

The source BPH population was collected from BPH-infested fields at Bayer's breeding farms in Andhra Pradesh, India. Insects were collected during 2012 and continuously reared under greenhouse conditions on 30-day-old TN1 rice plants at the breeding facility of Bayer by maintaining the conditions with a temperature of 28±2 ^{0}C, 75±5% relative humidity, and photoperiods of 14 hours of light with 10 hours of dark, according to Heinrichs et al. (1985).

3.2.3 Tolerance – Degree of Damage

The modified seed box test proposed by Panda and Khush (1995) has been recognized as a standard method of evaluating the degree of damage and was adapted to evaluate RILs to generate data for tolerance. In this experiment, pre-germinated seeds of 260 RILs, parents, and checks were sown in rows 5 cm apart in random order in trays (100 cm x 50 cm x 10 cm); with each row containing 10 seeds. The experimental design was an augmented design with two replications; each tray was divided into three parts to restrict the movement of nymphs across the trays (Figure 3.1), and the susceptible control TN1 was sown in two border rows within each block. Test entries were infested with second- and third-instar BPH nymphs with 10-15 nymphs per plant at the 2-3 leaf stage of plant growth (~15 days after sowing at Zadocks' growth stage 14). Damage scores were assigned using a 0-9 scale as defined by IRRI (2002) based on leaf yellowing, plant withering, and dwarfing (Table 3.1). Observations of degrees of damage were recorded ~20 days after infestation when susceptible parent and susceptible check were completely dead (score of 9; Figure 3.2).

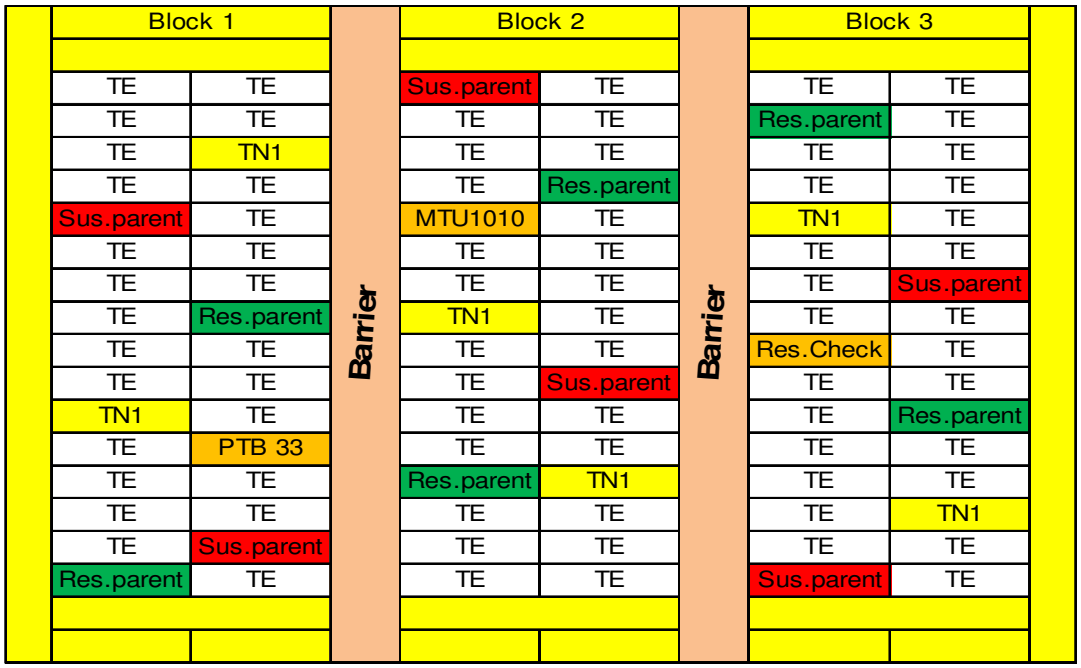


Figure 3.1: Design of the layout adopted for brown plant hopper phenotyping experiment for RIL population, its parents and checks.

Table 3.1: Brown plant hopper damage score scale in greenhouse experiments.

Scale	Damage symptom
0	No damage
1	Very slight damage
3	First and 2nd leaves of most plants partially yellow
5	Pronounced yellowing and stunting or about 10 to 25% of the plants wilting or dead and remaining plants severely stunted or dying
7	More than half of the plants dead
9	All plants dead

Figure 3.2: Damage symptoms due to brown plant hopper nymph, observed 15 and 20 days after infestation.

3.2.4 Antixenosis – Adult Preference

An adult preference experiment was conducted under field culture at a multi-crop breeding station of Bayer BioScience, Chandippa, Hyderabad, India, with a subset of 22 randomly selected RILs, and resistant and susceptible checks (Table 3.2). The experimental method for the adult preference study was adopted from the adult settlement behavior study conducted by Sarao et al. (2016). Test entries were sown in the field, and 25-day-old plants were transplanted into a greenhouse and transplanted in a randomized complete block design (RCBD) with two replications, three plants per genotype, and 20 cm by 20 cm spacing between plants. In total, 10 pairs (female and male) of macropterous adults were released on each plant 40 days after transplanting under the free choice test. The pairs were observed for establishment on each plant by counting the number of female and male insects 48 hours later.

3.2.5 Antixenosis – Feeding Rate

The quantification of honeydew excretion of BPH is utilized as an indirect method to estimate the feeding rate of insects on a host plant (Paguia et al, 1980). Individual test genotypes of selected 22 RIL (Table 3.2) were grown in plastic pots in three replications. When the lants were 6 weeks old, the plant base was enclosed by a feeding chamber (an inverted plastic cup), and bromocresol green-treated filter paper (Whatman No.1) was placed at the bottom of the feeding chamber (Figure 3.3). Next, 10 brachypterous insects were starved for two hours and then released in each feeding chamber and allowed to feed on each plant for 24 hours. Bromocresol green-treated Whatman filter paper was stained in blue color honeydew excreted by BPH. The feeding rate was recorded using a scale from 1 to 5 based on the percentage of the blue-stained area on the filter paper (1 = <10%; 2 = 11-20 %; 3 = 21-30%; 4 = 31-40%; 5 > 40%).

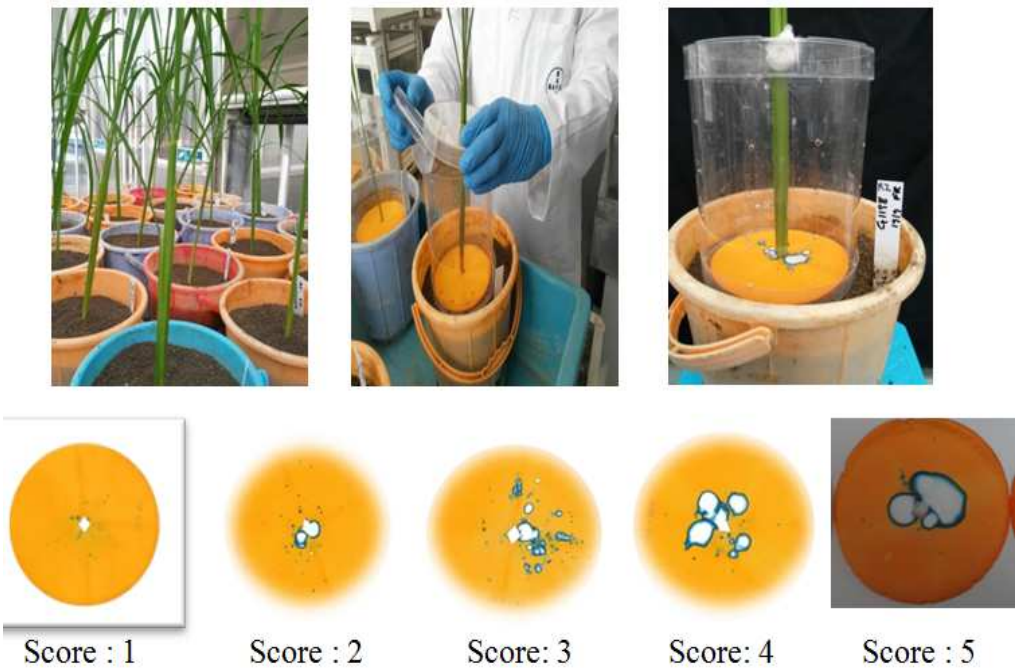


Figure 3.3: Experimental set up and observation scale for honeydew excretion study to assess feeding rate of brown plant hopper on test genotype.

Table 3.2: List of recombinant inbred line test entries (BYRIL) included in the anixenosis and antibiosis experiments conducted with adult brown plant hopper infestation.

Genotype	Entry Type
BYRIL007	RIL
BYRIL031	RIL
BYRIL033	RIL
BYRIL043	RIL
BYRIL065	RIL
BYRIL066	RIL
BYRIL084	RIL
BYRIL093	RIL
BYRIL094	RIL
BYRIL096	RIL
BYRIL131	RIL
BYRIL133	RIL
BYRIL156	RIL
BYRIL171	RIL
BYRIL174	RIL
BYRIL177	RIL
BYRIL187	RIL
BYRIL196	RIL
BYRIL228	RIL
BYRIL242	RIL
BYRIL250	RIL
BYRIL279	RIL
BRGB02489	Susceptible Parent
BRGB04267	Resistent Parent
BRGB07253	Resistent Check
MTU1010	Tolerant Check
TN1	Susceptible Check

3.2.6 Antibiosis – Fecundity

Fecundity studies were undertaken to assess the effect of host plant resistance on insect biology, especially on egg laying, egg hatching, and egg mortality, and performed according to the method described by Khan and Saxena (1985). Six plants from each of the 22 test genotype were grown in individual pots for 50 days, and a single tiller in each plant was maintained for adult BPH infestation (Table 3.2). The bottommost 10-cm portion of each plant was covered with a perforated polyethylene cylinder (feeding chamber). One pair of newly emerged BPH adults was released into a feeding chamber, and the cylinders were plugged with cotton to prevent the adult insects from escaping (Figure 3.4). A subset of three plants were removed from each pot seven days after the insect release and eggs laid under the leaf sheath were counted with the help of a stage microscope. The remaining three plants were used to count the number of nymphs hatched. The hatching percentage was derived based on the observations of the number of eggs hatched in the first subset of plants in the three replications. The number of nymphs from the second subset of plants in the three replications was determined by deriving the following formulae:

$$\text{Hatching \%} = \frac{\text{No.of Nymphs observed}}{\text{No.of eggs laid}} \text{ X } 100$$

$$\text{Egg Mortality \%} = \frac{(\text{No.of eggs laid} - \text{No.of Nymphs observed})}{\text{No.of eggs laid}} \text{ X } 100$$

Figure 3.4: Fecundity experiment setup and microscopic observation of egg masses and newly hatched nymphs.

3.2.7 Statistical Analysis

An analysis of variance (ANOVA) was performed using the JMP Pro 12.0.1 (SAS Institute Inc., 2015) software. The different treatment means were separated by the F-protected least significant difference (LSD) with a level of significance at 0.05 (Gomez and Gomez, 1984).

3.3 Results and Discussion

3.3.1 Tolerance – Degree of Damage

The degree of damage data recorded from BPH screening experiment consisted of 260 RILs and seven controls, and the greenhouse screening trial was conducted in two

replications and with eight blocks. Damage score data between blocks and replicates or block x replicate interactions were not significant ($P<0.31$, $P<0.17$, $P<0.37$)), which indicates uniform damage due to BPH nymph infestation. Significance was found ($P<0.001$) in the difference among the test entries, demonstrating genotypic differences for BPH tolerance (Table 3.3).

Among the check genotypes that were screened, resistant parent BRGB04267 and resistant check PTB 33 outperformed susceptible check TN1 and susceptible parent BRGB02489, with a mean damage score of 2.29 and 3.24, respectively, while TN1 average 8.98 (Table 3.4). The superior performance of PTB 33 over TN 1 confirms the previous results from international screening nurseries conducted by the IRRI (Seshu, D.V. and H.E. Kauffman. 1980). The mean performance of RILs ranged from 1.60 to 9.00 with a LSD of 1.50 between genotypes. Within the data subset reported in Table 3.5, genotypes BYRIL-007, BYRIL-084, BYRIL-094, BYRIL-131, BYRIL-156, BYRIL-177, and BYRIL-196 were observed with degree of damage score for tolerance between 2.16 and 3.56, and it was found to be non-significant with the performance of resistant parent BRGB04267.

The degree of damage data of RILs from greenhouse screening trials can be further analyzed for genetic analysis studies to map QTL(s) associated with BPH resistance.

Table 3.3: Analysis of variance, coefficient of determination, coefficient of variation, heritability, root mean square error and grand mean of brown plant hopper degree of damage screening trial with nymphal infestation for tolerance study.

Source	df	SS	MS	F Ratio	Prob > F
Model	281	4306.3	15.32**	26.18	<.0001
Genotype	266	4213.2	15.83**	27.06	<.0001
Block	7	4.8	0.69	1.18	0.3083
Rep.	1	1.1	1.11	1.90	0.1681
Block*Rep.	7	4.4	0.64	1.09	0.3650
Residual	293	171.4	0.58		
R^2	0.96				
CV%	34.83				
H^2	0.99				
Root MSE	0.76				
Grand Mean	4.82				

** Significance at the 0.01 probability level

Table 3.4: Performance of checks and parents in brown plant hopper degree of damage screening trial with nymphal infestation for tolerance study.

Genotype	Entry Type	Mean	Lower 95%	Upper 95%
BRGB04267	Resistant Parent	2.29	1.117	3.476
BRGB02489	Susceptible Parent	8.67	7.497	9.856
PTB 33	Resistant Check 1	3.24	2.064	4.423
BRGB07253	Resistant Check 2	1.99	0.814	3.174
MTU1010	Tolerant Check	7.82	6.643	9.003
BRGB07288	Susceptible Check 1	8.70	7.527	9.886
TN1	Susceptible Check 2	8.98	7.809	10.169

Table 3.5: Recombinant inbred lines (BYRIL) selected for Antixenosis and Antibiosis studies and its performance in brown plant hopper degree of damage screening trial with nymphal infestation for tolerance study.

Genotype	Entry Type	Mean Score for BPH	Lower 95%	Upper 95%
BYRIL065	RIL	9.00	7.08	10.91
BYRIL093	RIL	9.00	7.08	10.91
BYRIL133	RIL	9.00	6.29	11.71
BYRIL228	RIL	9.00	7.08	10.91
BYRIL242	RIL	9.00	6.29	11.71
BYRIL250	RIL	9.00	7.08	10.91
BYRIL279	RIL	9.00	7.08	10.91
BRGB02489	Susceptible Parent	8.67	7.99	9.35
BYRIL187	RIL	6.66	3.58	7.41
BYRIL033	RIL	5.91	3.99	7.83
BYRIL174	RIL	5.91	4.00	7.83
BYRIL096	RIL	4.83	2.91	6.75
BYRIL043	RIL	4.77	3.89	7.73
BYRIL171	RIL	4.64	2.72	6.56
BYRIL066	RIL	4.01	2.54	6.37
BYRIL084	RIL	**3.56**	1.64	5.47
BYRIL131	RIL	**3.04**	1.12	4.95
BYRIL007	RIL	**2.94**	1.02	4.86
BRGB04267	Resistant parent	**2.30**	1.61	2.97
BYRIL094	RIL	**2.29**	0.37	4.21
BYRIL177	RIL	**2.26**	0.34	4.17
BYRIL156	RIL	**2.17**	0.25	4.08
BYRIL196	RIL	**2.16**	0.24	4.08
LSD		1.5		

Mean value in bold: Promising genotypes with low degree of damage score.

3.3.2 Antixenosis – Adult Preference and Feeding Rate

A subset of 22 RILs (Table 3.2) along with resistant, tolerant, and susceptible checks were evaluated for adult preference and feeding rate studies of antixenosis in field and greenhouse experiments.

The highest number of adult BPH (female + male) settled on BYRIL093, followed by the susceptible parent BRGB02489 and susceptible check TN1 (Table 3.6). Significantly low level of adult (female + male) settlements were observed on resistant parent BRGB04267 and resistant check BRGB07253, with a mean number of 6.00 and 5.00 adults (female + male), respectively. The mean BPH settlement numbers on RILs ranged from 0.66 to 7.33 adult male, 1.66 to 26.66 adult female, and 2.33 to 34.00 adult female + male, with LSDs of 1.91, 5.69, and 7.05, respectively. Whereas, highest honeydew secretion by BPH feeding was found on susceptible check TN1, with a feeding rate of 4.00, and followed by BYRIL279 and susceptible parent BRGB02489 with feeding rates of 3.33 and 2.66, respectively (Table 3.6). Feeding rate variation of adult BPH observed among the RILs tested ranged from 1.00 to 3.33 with an LSD of 0.64, which indicates that <10% to 30% of the bromocresol green-treated filter paper was stained blue with honeydew secretion.

The ANOVA of the antixenosis study of macropterous BPH adult male and female preference and adult feeding rate study found significant differences between genotypes and replications (Table 3.7, Table 3.8, Table 3.9 and Table 3.10). Correlation studies between degree of damage, male preference, female preference, female + male preference and feeding rate were identified with positive significant correlation of 0.65

(P<0.001) to 0.69 (P<0.001) between degree of damage to adult BPH preference, and positive significant correlation of 0.62 (P<0.001) between degree of damage and feeding rate (Figure 3.5).

The preference behavior and feeding rate of adult BPH on selected subset of 22 RILs differed significantly among the genotypes evaluated in the field and green house screening experiments, which confirms the genotype response to the antixenosis modes of BPH resistance.

Host choice test is an indicator of the antixenosis factor. The different preferential behavior of BPH adults between genotypes confirms past reports and suggests a high number in BPH settlements on susceptible genotypes compared to resistant ones (Samal and Mishra, 1990; Qiu et al, 2012; He et al, 2013). Variation in the settlement of adults on RILs indicates that the BPH response to host genotype elucidates the genetic basis of the BPH's preference.

Feeding rate variations among genotypes determined the insect's food intake due to its probing response, introduction of stylets into the food source, and duration of feeding. In the present study, a high feeding rate is greater than 3.00, a moderate feeding rate is between 2.00 and 3.00, while a low feeding rate is 1.00 (Table 3.6). The breakdown of feeding rates among genotypes suggests that test plants presented some mechanical barrier to penetration for probing or that the plant sap was not palatable to the insects (Preetinder Singh Sarao and Jagadish Sanmallappa Bentur, 2016). These differences are attributable to genotypes, and the results are also further supported by the studies of Heinrichs and Rapusas, 1983; Shukla, 1984, Bhattal, 1992 and Du et al., 2009.

Table 3.6: Performance of recombinant inbred lines (BYRIL) for antixenosis (adult brown plant hopper preference behavior and feeding rate).

Genotype	Entry type	BPH Adult preference			Feeding rate
		Male	Female	Male + Female	
BRGB02489	Susceptible Parent	5.66	22.66	28.66	2.66
BYRIL093	RIL	7.33	26.66	34.00	2.33
BYRIL279	RIL	6.33	18.66	24.66	3.33
TN1	Susceptible Check	5.66	22.66	28.66	4.00
BYRIL228	RIL	5.66	20.00	25.33	1.66
BYRIL242	RIL	4.66	13.00	17.66	2.33
BYRIL043	RIL	4.00	13.66	18.00	3.33
BYRIL171	RIL	3.33	8.66	12.00	1.00
BYRIL065	RIL	3.00	9.33	11.66	3.00
MTU1010	Tolerant Check	3.00	9.44	12.54	1.66
BYRIL156	RIL	3.00	9.66	13.00	1.00
BYRIL174	RIL	3.00	8.66	11.66	1.33
BYRIL177	RIL	2.66	8.33	11.00	1.00
BYRIL187	RIL	2.66	10.33	13.66	2.66
BYRIL196	RIL	2.33	6.66	8.66	1.00
BYRIL250	RIL	2.33	5.66	8.00	2.33
BYRIL066	RIL	2.00	7.66	9.66	1.00
BYRIL133	RIL	2.00	4.33	6.33	1.00
BRGB04267	Resistant Parent	1.66	4.33	6.00	1.00
BYRIL007	RIL	1.66	5.00	6.33	2.00
BYRIL033	RIL	1.66	5.00	6.66	2.00
BRGB07253	Resistant Check	1.33	4.00	5.00	1.00
BYRIL094	RIL	1.33	3.00	4.33	1.00
BYRIL084	RIL	1.00	4.00	5.33	1.66
BYRIL096	RIL	1.00	2.66	3.66	1.00
BYRIL131	RIL	1.00	2.66	3.66	1.00
BYRIL031	RIL	0.66	1.66	2.33	2.00
H^2		0.89	0.93	0.93	0.98
LSD		1.91	5.69	7.05	0.64

Table 3.7: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antoixenosis study of adult preference for brown plant hopper male establishment after 48 hrs of insect release.

Source	df	SS	MS	F Ratio	Prob > F
Model	28	294.4	10.51**	7.65	<.0001
Genotype	26	277.1	10.66**	7.77	<.0001
Rep.	2	17.2	8.64**	6.29	0.0036
Residual	52	71.3	1.37		
R^2	0.80				
CV%	38.42				
Root MSE	1.17				
Grand Mean	3.04				

** Significance at the 0.01 probability level

Table 3.8: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antoixenosis study of adult preference for brown plant hopper female establishment after 48 hrs of insect release.

Source	df	SS	MS	F Ratio	Prob > F
Model	28	3651.6	130.41**	10.82	<.0001
Genotype	26	3449.4	132.67**	11.01	<.0001
Rep.	2	202.2	101.12**	8.39	0.0007
Residual	52	626.4	12.04		
R^2	0.85				
CV%	36.37				
Root MSE	3.47				
Grand Mean	9.54				

** Significance at the 0.01 probability level

Table 3.9: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antoixenosis study of adult preference for brown plant hopper female and male establishment after 48 hrs of insect release.

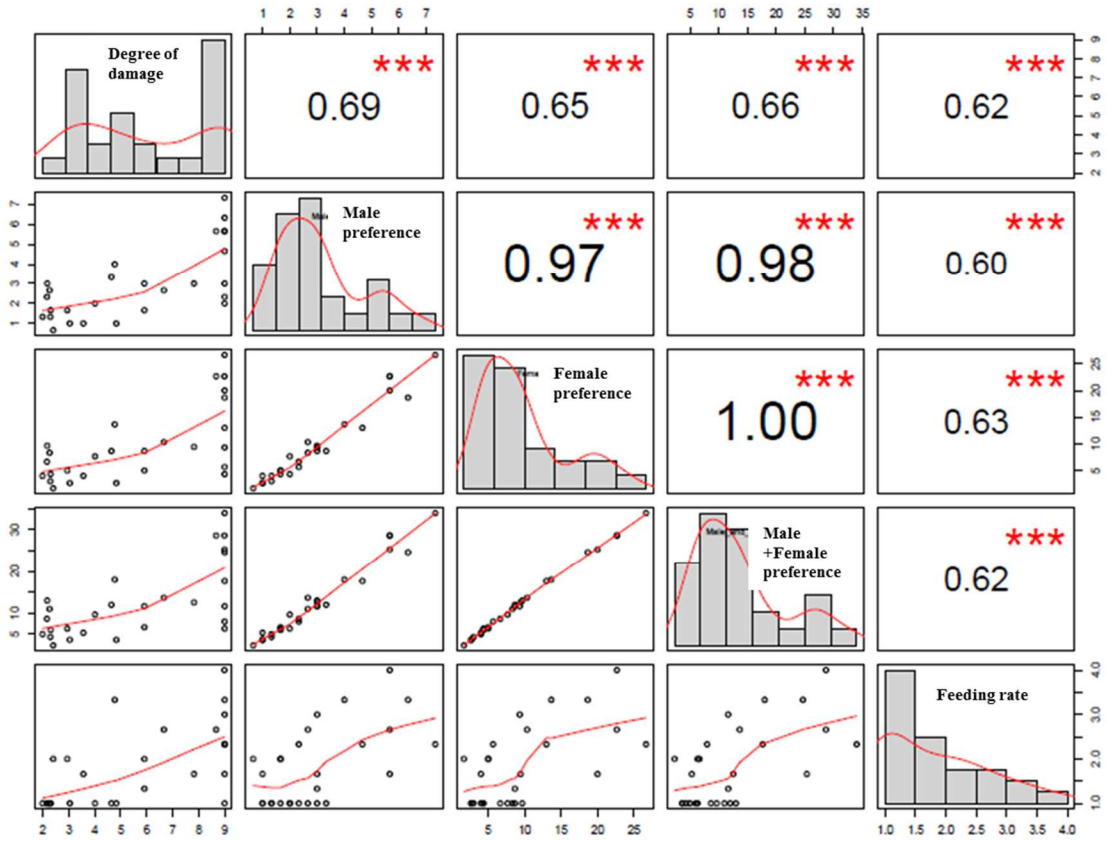
Source	df	SS	MS	F Ratio	Prob > F
Model	28	5957.6	212.77**	11.48	<.0001
Genotype	26	5651.5	217.36**	11.72	<.0001
Rep.	2	306.0	153.03**	8.25	0.0008
Residual	52	963.9	18.53		
R^2	0.86				
CV%	36.37				
Root MSE	4.30				
Grand Mean	12.59				

** Significance at the 0.01 probability level

Table 3.10: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antoixenosis study of feeding rate of brown plant hopper adults on test genotypes.

Source	Df	SS	MS	F Ratio	Prob > F
Model	28	62.5	2.27**	16.57	<.0001
Genotype	26	62.2	2.39**	17.75	<.0001
Rep.	2	0.3	0.16**	1.19	0.3123
Residual	52	7.0	0.15		
R^2	0.84				
CV%	21.53				
Root MSE	0.37				
Grand Mean	1.83				

** Significance at the 0.01 probability level



***: significance at 0.001 probability; red line: line of best fit.

Figure 3.5: Scatterplot, histogram and correlation between degree of damage and antixenosis modes of resistance (male preference, female preference, male and female preference and feeding rate).

3.3.3 Antibiosis – Fecundity

A subset of RILs along with parents and checks (Table 3.2) was evaluated for fecundity in the greenhouse screening experiment.

The greatest number of eggs were laid (147 and 140) on susceptible check TN1 and susceptible parent BRGB02489, and these types also had high numbers of nymphs (88 and 77, respectively) and low egg mortality percentage (27 and 33.66, respectively) compared to the other tested genotypes. An antibiosis study for fecundity on RILs was conducted, and the average recorded number of eggs laid on each genotype ranged from 57 to 147, with an LSD of 10.56. The mean number of nymphs hatched was between 21 and 88 with an LSD of 8.14, and the hatching and mortality percentages ranged from 32 to 82.6 (LSD = 13.56) and 14 to 40 (LSD = 7.16), respectively. On the other hand, the resistant parent was observed with mean values per plant recorded as 61 laid eggs, 26 hatched nymphs, 43% egg hatching, and 35.33% egg mortality (Table 3.11). Among the RILs evaluated for the fecundity study, genotypes BYRIL156, BYRIL171, BYRIL174, BYRIL177, and BYRIL196 were found to be promising, with performances on par with resistant parent BYGB04267.

The ANOVA of the antibiosis study for egg laying, egg hatching, egg hatching percentage and egg mortality percentage revealed significance between the genotypes tested (Table 3.12, Table 3.13, Table 3.14, and Table 3.15). Correlation coefficients among degree of damage, egg laying, number of nymphs, egg mortality rate, and egg hatching percentage revealed a positive and highly significant correlation of 0. 83 ($P <$ 0.001) between egg laying and number of nymph, and a highly significant negative

correlation of -0.99 ($P < 0.001$) between egg mortality percentage and egg hatching percentage (. A non-significant correlation was observed for the relation between egg laying, egg mortality percentage, and egg hatching percentage. A significant negative correlation of -0.45 ($P <0.05$) was found between the number of nymphs and egg mortality percentage, and a significant positive correlation of 0.43 ($P < 0.05$) between the number of nymphs hatched and egg hatching percentage was observed. A significant positive correlation of 0.67 ($P<0.001$) between degree of damage and egg laying, and positive correlation of 0.53 ($P<0.05$) between degree of damage and egg hatching was identified (Figure 3.6). The correlation studies signify the relationship among variables tested in the antibiosis study by providing evidence for genetic relations among test genotypes for the observed mechanism in BPH resistance.

The observed deviation in the number of eggs laid on RILs (57 to 113) was much higher than the other parameters, namely the number of nymphs (21 to 55), egg hatching percentage (32 to 82), and egg mortality percentage (26 to 40). A significant and high positive correlation between the number of eggs laid and number of nymphs 7 days after release of BPH adults exists. These results further support the notion that the antixenosis mechanism of egg-laying resistance may be due to the result of BPH preference for feeding, palatability, and physical barrier. Moreover, this hypothesis further clarifies the findings of Preetinder Singh Sarao and Jagadish Sanmallappa Bentur (2016), who found a significant difference among genotypes with lower fecundity in resistant genotypes like PTB33, Rathu Heenathi in comparison with the susceptible genotype TN1.

Although there was a significant difference between egg mortality among RILs, results do not provide enough evidence, as fewer differences were observed in egg mortality percentage (26 to 40) among tested genotypes, including susceptible and resistant parents. As ovicidal response is highly expressed between the tillering to heading stages (Suzuki *et al.* 1996), the selected subset of RILs must be further evaluated at maximum tillering stage to understand the genetic association with the egg mortality trait.

Table 3.11: Performance of recombinant inbred lines (BYRIL) for antibiosis mode of resistance for female brown plant hopper fecundity (egg laying, egg hatching and egg mortality).

Genotype	Entry type	Egg Laying	Number of Nymph	Hatching %	Mortality %
TN1	Susceptible Check	147	88	59.6	27.0
BRGB02489	Susceptible Parent	140	77	54.0	30.6
BYRIL279	RIL	113	47	43.0	35.6
MTU1010	Tolerant Check	93	50	52.3	30.3
BYRIL065	RIL	92	56	62.3	26.6
BYRIL250	RIL	84	40	49.0	33.3
BYRIL177	RIL	80	34	42.6	36.3
BYRIL156	RIL	76	30	40.6	37.3
BYRIL094	RIL	73	40	55.3	30.3
BYRIL093	RIL	71	45	64.0	25.3
BYRIL242	RIL	70	40	57.0	29.6
BYRIL196	RIL	68	31	47.0	34.0
BYRIL131	RIL	67	39	58.6	28.3
BYRIL031	RIL	66	44	67.3	24.0
BYRIL084	RIL	66	47	71.0	22.3
BYRIL174	RIL	65	20	32.0	40.0
BYRIL007	RIL	63	38	62.0	28.6
BYRIL096	RIL	62	34	55.6	30.3
BYRIL066	RIL	62	35	58.3	29.0
BYRIL187	RIL	62	43	70.6	21.6
BYRIL133	RIL	62	37	61.0	28.0
BYRIL043	RIL	61	45	62.6	26.0
BRGB04267	Resistant Parent	60	26	43.0	35.3
BYRIL033	RIL	60	35	60.3	27.6
BYRIL171	RIL	60	28	47.3	34.0
BRGB07253	Resistant Check	59	23	37.3	38.3
BYRIL228	RIL	57	39	69.3	23.0
H^2		1.0	1.0	0.8	0.7
LSD		10.6	8.4	13.5	7.6

Table 3.12: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antibiosis study of egg laying behavior of gravid female brown plant hopper on test genotypes.

Source	df	SS	MS	F Ratio	Prob > F
Model	28	43540.6	1555.02**	37.36	<.0001
Genotype	26	43006.5	1654.09**	39.75	<.0001
Rep.	2	534.0	267.04**	6.41	0.0032
Residual	52	2163.9	41.61		
R^2	0.95				
CV%	8.51				
Root MSE	6.45				
Grand Mean	75.76				

** Significance at the 0.01 probability level

Table 3.13: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antibiosis study of egg hatching on test genotypes.

Source	df	SS	MS	F Ratio	Prob > F
Model	28	16515.3	589.83**	23.88	<.0001
Genotype	26	16489.8	634.22**	25.68	<.0001
Rep.	2	25.5	12.75	0.52	0.5998
Residual	52	1284.4	24.70		
R^2	0.92				
CV%	11.99				
Root MSE	4.97				
Grand Mean	41.43				

** Significance at the 0.01 probability level

Table 3.14: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antibiosis study of egg hatching percent on test genotypes.

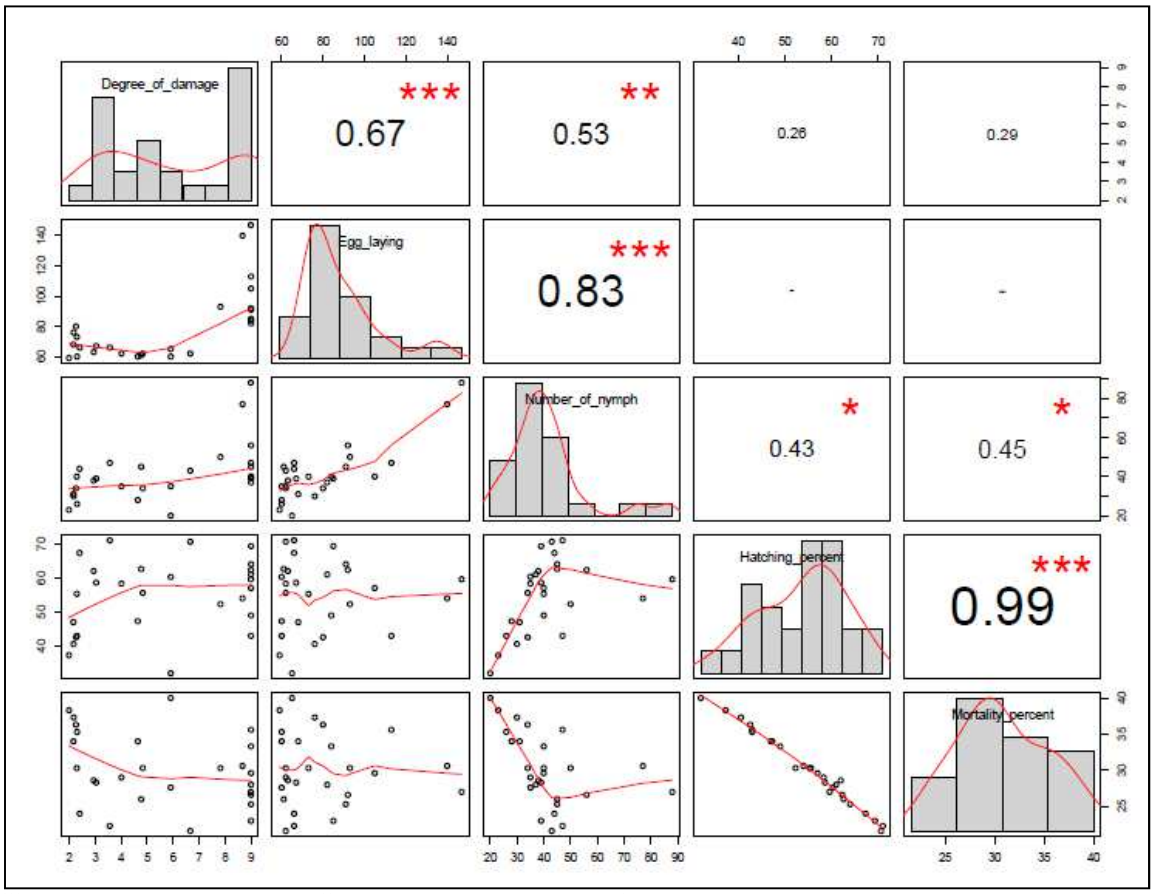
Source	df	SS	MS	F Ratio	Prob > F
Model	28	10877.8	388.49**	5.66	<.0001
Genotype	26	10692.6	411.25**	5.99	<.0001
Rep.	2	185.2	92.61	1.35	0.268
Residual	52	3565.4	68.57		
R^2	0.75				
CV%	14.87				
Root MSE	8.28				
Grand Mean	55.69				

** Significance at the 0.01 probability level

Table 3.15: Analysis of variance, coefficient of determination, coefficient of variation, root mean square error and grand mean of antibiosis study of egg mortality percent on test genotypes.

Source	df	SS	MS	F Ratio	Prob > F
Model	28	2746.4	98.09**	5.12	<.0001
Genotype	26	2673.6	102.83**	5.37	<.0001
Rep.	2	72.7	36.38	1.90	0.160
Residual	52	995.2	19.13		
R^2	0.73				
CV%	14.47				
Root MSE	4.37				
Grand Mean	29.67				

** Significance at the 0.01 probability level



***: significance at 0.001 probability; **: significance at 0.01 probability; *: significance at 0.05 probability;
red line: line of best fit.

Figure 3.6: Scatterplot, histogram and correlation between degree of damage and antibiosis modes of resistance (egg laying, number of nymph, hatching % and mortality %).

3.4 Conclusions

Host plant resistance is an essential pest management system because it is specific to targeting pests and has no adverse effect on a non-targeted organism. It is a core method of maintaining the ecological balance of the pest under integrated pest management. Therefore, understanding and ultimately pyramiding modes of insect resistance (tolerance, antixenosis, and antibiosis) is essential for the genetic analysis of BPH resistance.

Uniform BPH nymph damage in the modified seed box method of tolerance screening is a vital aspect in the assessment of genotypic differences, which was statistically supported with a non-significant difference between blocks for damage score data recorded in the BPH tolerance trial. The degree of damage data of RILs from the greenhouse screening trial can be further exploited to map BPH-resistant QTL for the development of a BPH-resistant germplasm through marker-assisted breeding.

A positive and significant correlation between degree of damage and adult preference (r=0.66, P>0.001), feeding rate (r=0.62, P>0.001), and egg laying (r=0.67, P>0.001) shows the effect of BPH preference on multiple modes of resistance. These results further support the antixenosis studies of Preetinder Singh Sarao and Jagadish Sanmallappa Bentur (2016) on the effect of BPH preference on fecundity. However, variability in egg mortality data among RILs and parents did not present enough evidence to conclude genotype differences. As suggested by Suzuki et al. (1996), ovicidal response is highly expressed between the tillering to heading stages. For this reason, the present studies were carried out before the tillering stage, though it is

recommended to evaluate RIL at the maximum tillering stage to reveal the genotype differences of the trait.

CHAPTER IV

PHENOTYPING A RICE RESTORER MAPPING POPULATION FOR PHOTOPERIOD SENSITIVITY AND POLLEN LOAD RELATED TRAITS IN VARIED ENVIRONMENTAL CONDITIONS IN INDIA

4.1 Introduction

Hybrid rice technology has opened new avenues in modern agriculture to fulfill growing demands of food security and to feed an additional 10 to 18 million people each year. The discovery of a wild-abortive cytoplasmic male sterile (WA-CMS) rice mutant in 1973 resulted in the immense success of three-line hybrid rice breeding in China. Although hybrid rice has been commercialized on a large scale with its proven heterosis over open pollinated varieties, South and Southeast Asian countries face major constraints to develop it further, particularly because of the high cost of its seeds and the need for farmers to purchase seeds every year (Xie, 2009).

Cultivated rice is predominantly self-pollinating because of the morphology of its flower, which is perfect, consisting of six short anthers and a stigma. The outcrossing potential of hybrid seed production depends on the floral characteristics of cytoplasmic male sterile (CMS) female and fertile male parents, where the anther dehisces shortly before the florets open (Oka, 1988). The extent of outcrossing in the seed parent is logically influenced by floral traits such as stigma size (length and breadth), length of style, and stigma exertion, in addition to the stigma morphology, angle, and duration of the glume opening. In the pollen parent, on the other hand, it is logically influenced by

anther size, number of pollen grains per anther, percent fertility, filament length, and duration of spikelet blooming (Virmani, 1994). Apart from the floral characteristics, the stable flowering behavior of parental lines across environments also influences the flowering synchrony to facilitate the timely availability of pollen from pollen parent to seed parent. Furthermore, it is important to breed for parental lines with similar flowering responses to photoperiod changes for enhanced outcrossing potential of the seed parent.

Flowering is a complex phenological trait influenced by numerous physiological and biochemical processes within the plant, all of which are regulated by interactions with environmental stimuli (Murfet, 1977). Rice is a short-day plant with rapid progress towards flowering and reproduction when the day length shortens. Several genetic studies on flowering time (heading date) have demonstrated the role of various photoperiod sensitivity genes' response in flowering behavior with photoperiod changes.

The advent of CMS in rice spurred scientists to investigate the regulatory mechanism of pollen development and to understand restorability of male sterility due to wild-abortive cytoplasm (Ouyang, 2010). Rice anther development initiates stamen primordia formation, followed by the primordia differentiation to form the anther wall and pollen mother cell; the subsequent meiotic and mitotic division results in the development of tri-cellular pollen grains, with accumulated starch and lipids, which are released during anther dehiscence. It has been reported that environmental stresses, mainly high temperatures during flowering, cause a decline in pollen development, pollen viability, and pollen germination or retardation in pollen tube growth. In hybrid

seed production, the number of pollen grains and their fertility, viability, and germination play a significant role, along with flowering synchrony and environmental conditions.

The present study has focused on flowering synchrony in an RIL population to identify lines with stable flowering behavior, pollen morphology, and high pollen load (number of fertile pollen). A recombinant inbred line (RIL) population that was developed at the Multicrop Breeding Station, Bayer Bioscience, Chandippa, Hyderabad, India by crossing parents with differential responses to photoperiods for heading date and pollen load was used in a study with the following objectives:

1. Study flowering behavior under different photoperiod conditions in a set of RILs population and its derived hybrids across multiple environments at different latitudes in India.
2. Generate fertile pollen count data from experiments conducted for flowering behavior trials and study pollen load variability.

4.2 Materials and Methods

4.2.1 Plant Material

An $F_{2:7}$ RIL population was derived from a cross between 'BRGB04267' (with less photoperiod sensitivity and low pollen load) and 'BRGB02489' (with high photoperiod sensitivity and high pollen load) elite restorer lines, which were developed at Bayer. Hybrids were created by crossing the RILs with female testers BRGB07288A and BRGB06355A. BRGB04267 and BRGB02489 were used as checks in the flowering behavior studies conducted at two locations: one at Multicrop Breeding Station, Bayer Bioscience, Chandippa, Hyderabad, India and other at Bayer Bioscience Product Evaluation Center, Dhantori, Haryana, India. The same $F_{2:7}$ RIL population was evaluated for pollen load at one Bayer Bioscience location, at Chandippa, Hyderabad, India.

4.2.2 Experimental Design

The RIL population of 274 genotypes and 475 hybrids derived from RILs and parents as checks were evaluated for heading date at the Chandippa (latitude 17.4^{o}N, longitude 78.1^{o}E) and Dhantori (latitude 29.9^{o}N, longitude 76.8^{o}E) locations. The experiment was conducted in wet and dry seasons by creating seven test environments, which was done by staggered sowings of test entries at different dates within each season at both the Chandippa and Dhantori locations, as detailed in Table 4.1. Each trial was created in an augmented design with 12 blocks and repeated checks in each block. Test entries were sown initially in nursery beds, and each genotype was transplanted 25-30 days after sowing into the main field in two-row plots spaced 15 cm apart.

4.2.3 Weather Data

Temperature (^{0}C), relative humidity (%), and solar radiation (w m^{-2}) data were collected at 10-minute intervals from the weather stations installed at the trial locations. Further data were processed to derive daily maximum temperatures, minimum temperatures, mean temperatures, mean relative humidity, and accumulated solar radiation each day. The photoperiod (day length) was calculated based on the model proposed by Forsythe et al. (1995) as a function of the latitude and day of the year. Heat index data were computed based on the multiple regression equation proposed by Rothfusz (1990), which uses the following formula: HI = -42.379 + 2.04901523*T + 10.14333127*RH - .22475541*T*RH - .00683783*T*T - .05481717*RH*RH + .00122874*T*T*RH + .00085282*T*RH*RH - .00000199*T*T*RH*RH, where T is temperature in ^{0}C, RH is relative humidity in percent, and HI is the heat index. The photothermal quotient was calculated as PQ = Rs/(Tmed-Tb), where Rs is solar radiation, Tmed is the mean daily temperature, Tb is base temperature (considered 12 ^{0}C as base temperature), and PQ is the photothermal quotient in KW m^{-2} day^{-1} ^{0}C (Villalobos and Ritchie, 1992).

4.2.4 Heading Date

Heading date notes were taken in all test entries from flowering behavior trials conducted in the Chandippa and Dhantori test locations. Heading dates were recorded as the number of days from sowing until 50% of the plants' panicles were fully emerged from the boots.

4.2.5 Pollen Observations Trial

The pollen observation data were collected from 247 RILs and their parents (BRGB04267 and BRGB02489) from the flowering behavior trial conducted in environments 5, 6, and 7 at the Multicrop Breeding Station, Bayer Bioscience in Chandippa, Hyderabad, India (Table 4.1).

4.2.6 Sample Preparation, Data Recording and Analysis

Spikelets for pollen studies, which were collected from the field and samples, were stored at ~5^{O}C for further processing in a lab at the Multicrop Breeding Station, Bayer Bioscience, Chandippa, Hyderabad, India. Sample preparation and data observation were conducted by following these steps below (Figure 4.1):

Spikelets were collected before anthesis from 10 different plants within a line, and three representative spikelets from each plant were collected in a sampling vial filled with 60% (v/v) alcohol.

Sample solution was prepared in 8 replications by taking 18 anthers from 3 spikelets in 300µl alcohol and crushing the anthers to extract pollen; the sample solution was homogenized in preparation for the observation slide.

A subsample of 2µl of the homogenized pollen solution mix was placed on the observation slide and treated with 1% iodized potassium iodide (IKI) solution to observe the dark blue color stains of the fertile pollen (Prasad et. al., 2006).

The IKI-treated observation slides were studied under a stage microscope at 2.5X magnification, and a picture was captured using ProgRess CapturePro v2.8.8 software and Jenoptik ProgRes C5 camera.

Captured pictures were further studied to count the number of stained pollen, measure the pollen size, and evaluate the staining intensity of observed pollen with the help of the Image-Pro Premier 9.2 software.

Finally, image analysis was performed with the help of macro images developed within the tool by setting the rules for pollen shape, pollen size, roundness, color intensity, and aspect ratio to minimize error in reading the pollen for the set parameters.

4.2.6.1 Pollen Data Curation

The data output from Image-Pro Premier 9.2 contained individual pollen grain measurements with pollen grain numbers, pollen size (μm^2), and staining intensity (absorbance units). The total number of fertile pollen, average pollen size, and average stain intensity data for each genotype was derived from Image-Pro Premier 9.2 output for further data analysis.

4.2.6.2 Statistical Analysis

Correlations of weather data and flowering data were calculated using the multivariate analysis method in JMP Pro 12.0.1 (SAS Institute Inc., 2015). An ANOVA for flowering behavior and pollen data was performed using JMP to estimate the standard error in order to compare among RILs and between RILs and parents by the least significance interval (LSI) with a 0.05 level of significance (Gomez and Gomez, 1984).

Table 4.1: Test environments of flowering behavior trial conducted with staggered sowing of recombinant inbred lines (RIL) and Hybrid (HYB) at Chandippa (CHP) and Dhnatori (DHA) locations.

Environment Number	Trial Name	Population	Test Location	Season	Sowing date
Environment 1	CHP-1RIL	RIL	Chandippa	Wet Season	11th Jun.2015
Environment 1	CHP-1HYB	Hybrid			11th Jun.2015
Environment 2	CHP-2RIL	RIL			1st Jul.2015
Environment 3	DHA-1RIL	RIL	Dhantori		15th Jun.2015
Environment 3	DHA-1HYB	Hybrid			29th Jun.2015
Environment 4	DHA-2RIL	RIL			6th Jul.2015
Environment 5	CHP-3RIL	RIL	Chandippa	Dry Season	6th Dec.2015
Environment 6	CHP-4RIL	RIL			15th Dec.2015
Environment 7	CHP-5RIL	RIL			25th Dec.2015

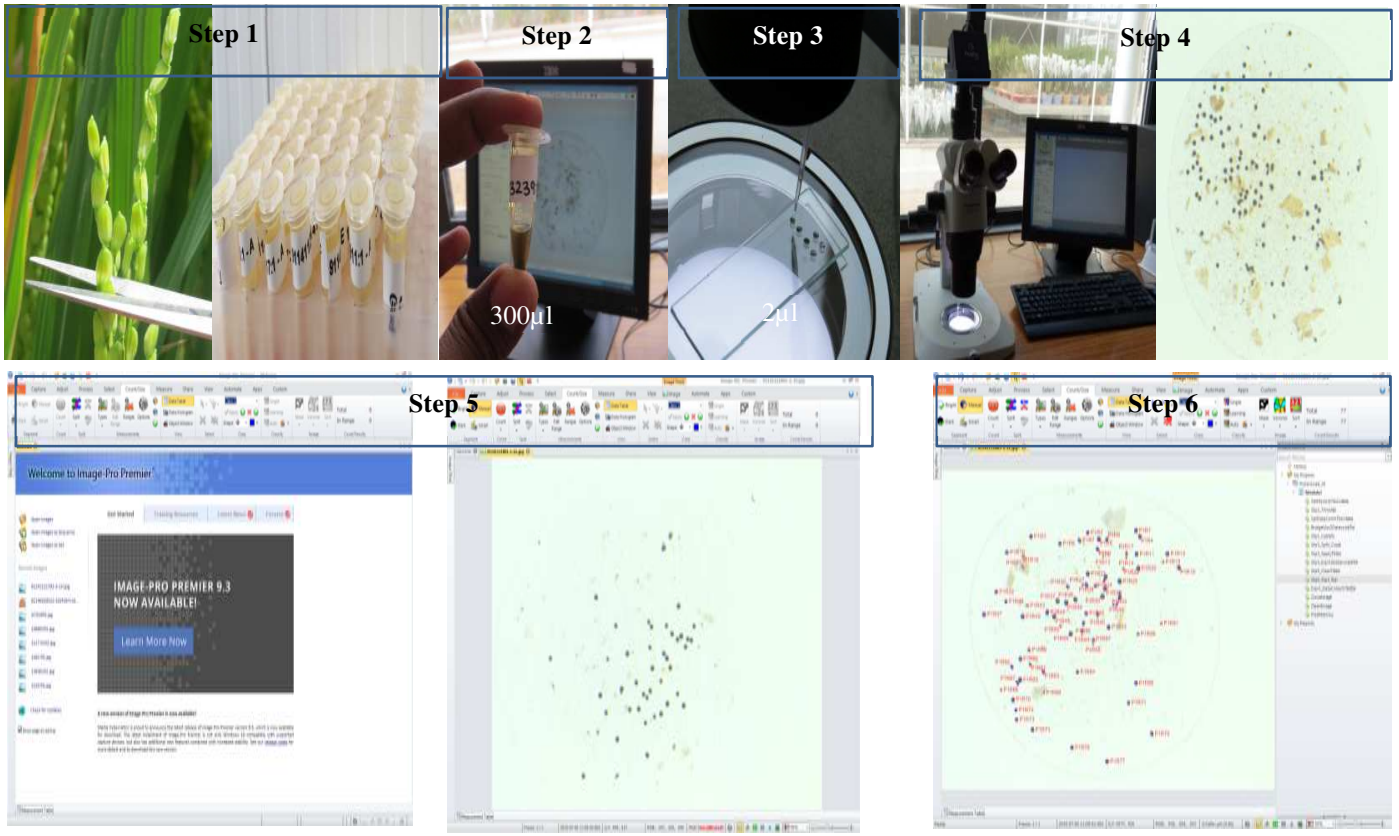


Figure 4.1: Sample preparation procedure for pollen load study and image analysis using image-pro premier 9.3.

4.3 Results and Discussion

4.3.1 Test Environments

Chandippa (CHP) and Dhantori (DHA) have been classified as semi-arid climate zones and are located at 17.4^0N of southern plateau and hills and 29.9^0N of trans-gangetic plains of India, respectively. The annual (May 15, 2015 to May 15, 2016) weather pattern with photoperiod (hours), accumulated solar radiation (KW m^{-2} day^{-1}), and heat index (HI) data for the Chandippa and Dhantori locations are shown in Figure 4.2. An interdependency of weather parameters with strong positive correlations was observed in Chandippa between the mean temperature (^{o}C), mean photoperiod (hrs), and mean heat index with coefficients of 0.87, 0.89, and 0.85, respectively. The relationship between the photoperiod and accumulated solar radiation produced a low correlation coefficient, which signifies that these weather parameters are not especially influenced by one another (Table 4.2).

Vergara and Chang (1985) have described the effect of the growing environment on the interval between sowing and flowering in rice. The current study with the RIL population was evaluated against a range of weather conditions with a focus on photoperiod and temperature by sowing the RIL population at different time intervals within the wet season and dry season at the Chandippa and Dhantori locations.

Accordingly, the wet and dry season climate created variability for photoperiod (hrs), solar radiation accumulation per day (KW m^{-2} day^{-1}), and heat index to assess flowering behavior of recombinant inbred lines across environments. High mean photoperiods of 12.33 hours and 12.53 hours were recorded between the sowing to

flowering period of the RIL population grown in Chandippa and Dhantori's 2015 wet season environments, respectively, and followed by Chandippa's 2016 dry season environments with a mean photoperiod of 11.83 hours. While the mean accumulated solar radiation between sowing and flowering was recorded with a high of 17.07 KW m^{-2} day^{-1} in the 2015 wet season environments of Chandippa, a low mean of 3.73 KW m^{-2} day^{-1} was observed in the 2016 dry season environments of Chandippa. Heat index did not show any significant variation across environments during the vegetative period of crop growth in both the wet and dry season environments of Chandippa and Dhantori (Table 4.3).

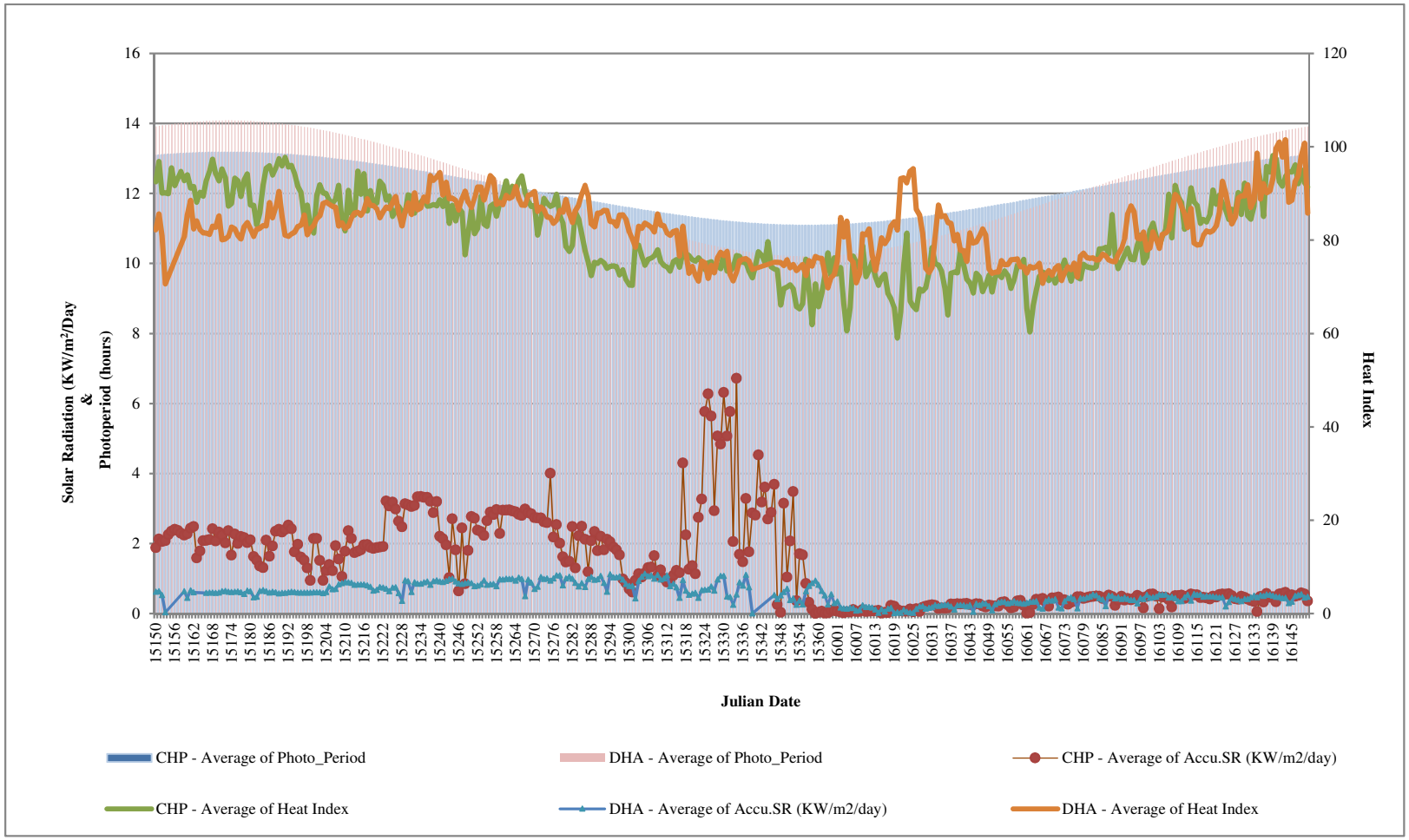


Figure 4.2: Chart showing the observations recorded for Photoperiod, Solar Radiation and Heat Index data from 30th May 2015 to 30th May 2016 at test locations Chandippa (CHP) (latitude = 17.4°N, longitude = 78.1°E) and Dhantori (DHA) (latitude = 29.9°N, longitude = 76.8°E), India.

Table 4.2: Correlation coefficient for photo-thermal quotient (KW m^{-2} day^{-1} ^{O}C), mean temperature (^{O}C), mean humidity (%), heat index (HI) and photoperiod (hrs.) at Chandippa (CHP) and Dhantori (DHA), India.

	Location	Photo-thermal Quotient (KW m^{-2} day^{-1} ^{O}C)	Mean Temperature (^{O}C)	Mean Humidity (%)	Heat Index (HI)	Photo Period (hrs.)
Solar Radiation (KW/m2/day)	Chandippa	-0.08*	0.27**	-0.10*	0.32**	0.10^{NS}
	Dhantori	0.14**	0.69**	-0.69**	0.33**	0.19**
Photo-thermal Quotient (KW/m2/day ^{O}C)	Chandippa		0.13*	-0.10*	0.08^{NS}	0.09^{NS}
	Dhantori		0.10*	-0.07^{NS}	-0.10*	0.01^{NS}
Mean Temp (^{0}C)	Chandippa			-0.63**	**0.87****	**0.89****
	Dhantori			**-0.84****	0.59**	0.69**
Mean Humidity (%)	Chandippa				-0.51**	-0.46**
	Dhantori				-0.51**	-0.51**
Heat Index	Chandippa					**0.85****
	Dhantori					0.49**

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level and ^{NS} Not significance at the 0.01 probability level
KW m^{-2} day^{-1}: kilowatt per square meter per day; ^{o}C: degree Celsius; hrs.: hours

Table 4.3: Range, mean and standard deviation of photoperiod (hours), solar radiation (KW/m^2/day) and heat index (HI) during the crop period from sowing to heading in flowering behavior trials conducted in different environments at Chandippa (CHP) and Dhantori (DHA), India.

Season	Location	Latitude	Longitude	Environment	Range (Minimum – Maximum)	Mean	Srd. Dev.
Photoperiod (hours)							
Wet Season – 2015	Chandippa	17.4°N	78.1°E	Environment 1 Environment 2	11.17 - 13.17	12.33	0.67
	Dhantori	29.9°N	76.8°E	Environment 3 Environment 4	10.38 - 14.08	12.53	1.24
Dry Season – 2016	Chandippa	17.4°N	78.1°E	Environment 5 Environment 6 Environment 7	11.08 - 13.05	11.89	0.67
Solar Radiation (KW m^{-2} day^{-1})							
Wet Season – 2015	Chandippa	17.4°N	78.1°E	Environment 1 Environment 2	4.59 - 50.38	17.07	8.09
	Dhantori	29.9°N	76.8°E	Environment 3 Environment 4	1.83 - 8.51	5.88	1.39
Dry Season – 2016	Chandippa	17.4°N	78.1°E	Environment 5 Environment 6 Environment 7	0.02 - 34.00	3.73	5.47
Heat Index (HI)							
Wet Season – 2015	Chandippa	17.4°N	78.1°E	Environment 1 Environment 2	70.35 - 97.76	84.74	7.19
	Dhantori	29.9°N	76.8°E	Environment 3 Environment 4	71.19 - 94.49	84.56	4.77
Dry Season – 2016	Chandippa	17.4°N	78.1°E	Environment 5 Environment 6 Environment 7	59.03 - 98.13	76.73	8.55

4.3.2 Flowering Behavior of Recombinant Inbred Lines and Derived Hybrids

The inbred flowering behavior trial consisted of 274 RILs across seven environments, whereas the hybrid flowering trial was evaluated in two wet season environments with 475 hybrids derived from RILs crossed with two testers. Days to heading data distribution of RIL and the hybrid genotypes evaluated across test environments are represented in the boxplot in Figure 4.3.

A multi-environment ANOVA was performed to determine if genotype by environment interactions were significant. The recombinant inbred lines showed the main effect of genotype and environment, and their interactions were significant ($P <$ 0.0001) for heading date (Table 4.4). Similar results were also observed for heading date of RIL-derived hybrids (Table 4.5). Therefore, the genetic architecture of the RIL population showed differential behavior for heading with respect to the prevailing environmental conditions. The differences in the flowering patterns across environments further supports the idea of Murfet (1977), who has suggested that environmental stimuli influence complex physiological and biochemical processes and flowering phenomena.

The mean days to heading, accumulated solar radiation from sowing to heading, and photoperiod at the time of heading of the RIL population parents are presented in Table 4.6. Both parents, BRGB02489 and BRGB04267, flowered in all environments with a range of 81 to 118 days and 108 to 128 days, respectively. The BRGB02489 parent flowered earlier in wet season environments (environments 1 to 4) than dry season environments (environments 5 to 7). However, BRGB04267 flowered late, with a

range of 108 to 128 days across all environments. Thus, variation in flowering behavior of parents was observed in wet and dry season environments with response to photoperiod changes. In the wet season environment, BRGB02489 flowered in 81 to 97 days when the day length attained 11.94 to 12.65 hours, and BRGB04267 took 108 to 128 days to flower when the photoperiod was 11.30 to 11.75 hours. It was also observed that both parents BRGB02489 and BRGB 04267 behaved similarly to the photoperiod in dry season, with flowering initiation at 12.35 to 12.63 hours of day length. Therefore, the flowering response of parents to photoperiod stimuli across environments represents more stable flowering behavior of the parent BRGB04267 compared to the other parent BRGB02489. The mean heading date of the RILs also followed the same trend to day length response in both wet and dry season environments, as observed in the parents (Figure 4.4), while the influence of the accumulated solar radiation on parents for flowering behavior was found independent of the test environments (Figure 4.5). These results further support the low correlation observed between accumulated solar radiation and photoperiod recorded across the test locations (Table 4.2).

Based on the observations from flowering behavior trials of the RIL population, wet season trials were found to be significant between test environments, unlike dry season environments (Table 4.7). The mean performance of the RIL population heading date across all wet season environments ranged from 104 to 118 days. Dry season environments at Chandippa had delayed flowering with mean days to flowering of 124 to 126 days. The early mean flowering behavior of RILs in the wet season compared to dry season environments provide evidence that progressive short days in these

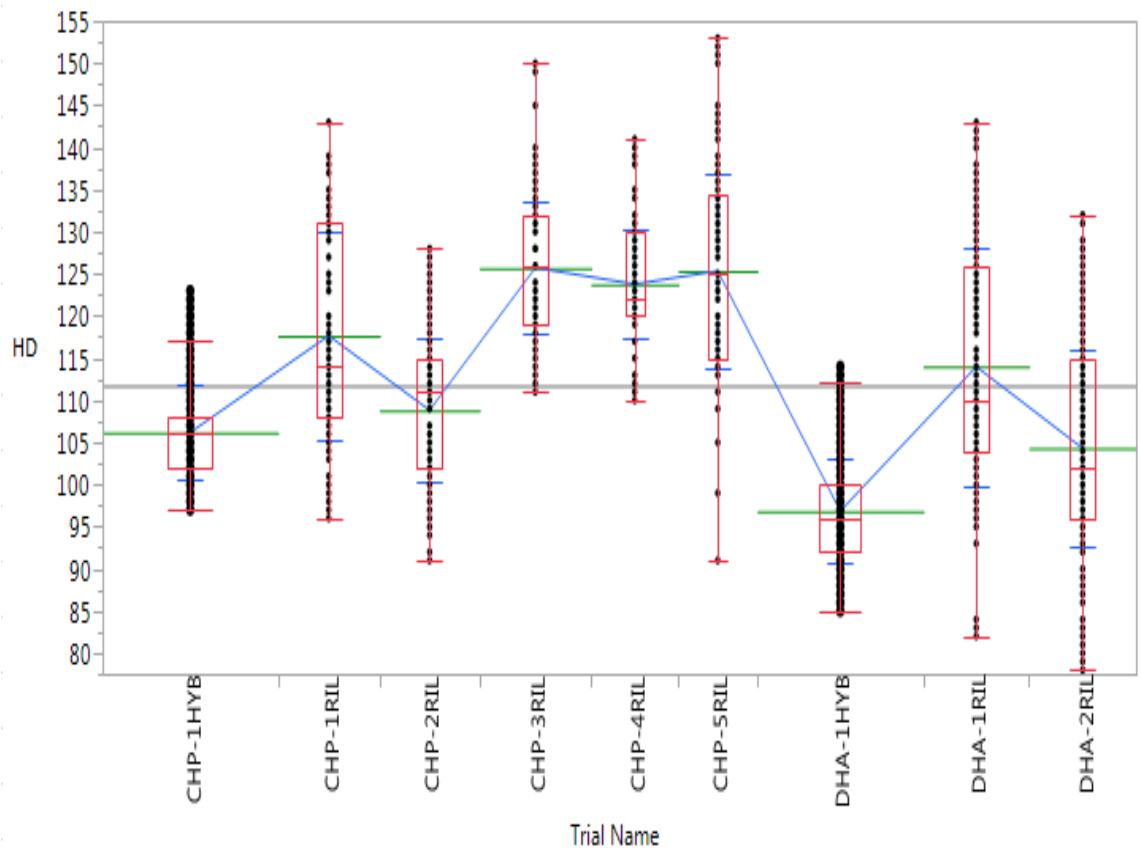
environments accelerate flowering stimuli, since this phenomenon was observed in short-day plants like rice (Maheswaran M, 1999).

The magnitude of heading date variation due to different weather parameters estimated with residual maximum likelihood (REML) model was found significant for photoperiod with estimated variance of 1299.29 at Z.ratio, 2.09 (Table 4.8). The correlation coefficients of the heading date in each test environment with photoperiod, accumulated solar radiation, mean temperature, mean humidity, photo-thermal quotient, and mean heat index are presented in Table 4.9. Chandippa and Dhantori wet season environments exemplified a high negative significant correlation coefficient of -0.999 to -1.000 at $P < 0.0001$ between heading date and photoperiod. However, dry season environments demonstrated high positive significant correlation of 0.988 to 1.000 ($P < 0.0001$) between heading date and photoperiod at Chandippa. Therefore, the correlation studies suggest that the day length influences accelerated flowering and reproduction when day hours are shortening, and the high coefficient values indicate the genetic response due to photoperiod on early and delayed flowering behavior of RIL population. A flowering response study by Maheswaran (1994) on 47 rice varieties with different photoperiods revealed that, under a specific temperature, each variety has its own optimum day length under which it flowers, and the time to flowering is delayed according to the sensitivity of the variety to the photoperiod. Ogiso et al. (2010) identified QTL that influence flowering behavior from cultivars grown in different geographical locations. The authors defined the role of casein kinase II in flowering time

regulation and its diversification during evolution as an adaptive mechanism to photoperiod response.

Figure 4.6 shows days to heading and standard deviation of the RIL population tested for flowering behavior across seven wet and dry season environments. Among the 275 RILs evaluated in both Chandippa and Dhantori locations, 207 were observed with heading when the photoperiod was between 11.97 to 12.15 hours, while 203 flowered upon accumulation of solar radiation between 970 and 1194 KW m^{-2} day^{-1} (Figure 4.7).

The RIL mapping population studied across environments was identified with early and late flowering lines along with stable and variable flowering behavior. The subset of selected stable-performing RILs are reported in Table 4.10 with mean days to heading from 110 days to 133 days with a standard deviation ranging from 5.26 to 7.00 days, which is a similar behavior as the parent BRGB04267 across all test environments. Table 4.11 reports the heading data of stable-performing RIL-derived hybrids with two tester combination crosses. The tester BRGB07288A derived hybrids flowered in 88 to 100 days and 100 to111 days in Dhantori and Chandippa locations, respectively. On the other hand, RIL X BRGB06355A cross-derived hybrids flowered in 104 to 112 days at Chandippa and 100 to 111 days at Dhnatori. These results indicate that early flowering behavior of hybrids is influenced by the tester genotype compared with their corresponding RILs studied at same test environment. Moreover, these findings are in line with Falconer's (1981) description of environmental influence on qualitative traits to show the varied degree of genotype and environment interaction for better adaptation to the changing environment.



Green line: experimental mean; connecting blue line: connecting mean heading date across trials;
CHP-1HYD: Hybrid trail at environment 1; DHA-1HYD: Hybrid trail at environment 3;
CHP-1RIL: RIL trial at environment 1; CHP-2RIL: RIL trial at environment 2; CHP-3RIL: RIL trial at environment 3:
CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5:
DHA-1RIL: RIL trail at environment 3; DHA-2RIL: RIL trail at environment 4.

Figure 4.3: Box and whisker plot of days to heading (HD) observed in recombinant inbred lines (RIL) and its derived hybrids (HYB) evaluated across trials at Chandippa(CHP) and Dhantori (DHA), India.

Table 4.4: Analysis of variance of heading date of recombinant inbred lines tested across environments at Chandippa (CHP) and Dhantori (DHA), India.

Source	DF	SS	MS	F value	P value
Model	1831	346113	189.0**	19.8	<.0001
Genotype	274	145782.4	532.1**	55.7	<.0001
Environment	6	114205.9	19034.3**	1993.4	<.0001
Genotype x Environment	1540	75483.2	49.0**	5.1	<.0001
Block	11	10641.2	967.4**	101.3	<.0001
Residual	139	1327.2	9.5		
R^2	0.78				
H^2	0.96				
Root MSE	6.76				
Grand Mean	116.7				

** Significance at the 0.01 probability level;

Table 4.5: Analysis of variance of heading date of recombinant inbred lines derived hybrids tested at Chandippa (CHP) and Dhantori (DHA), India.

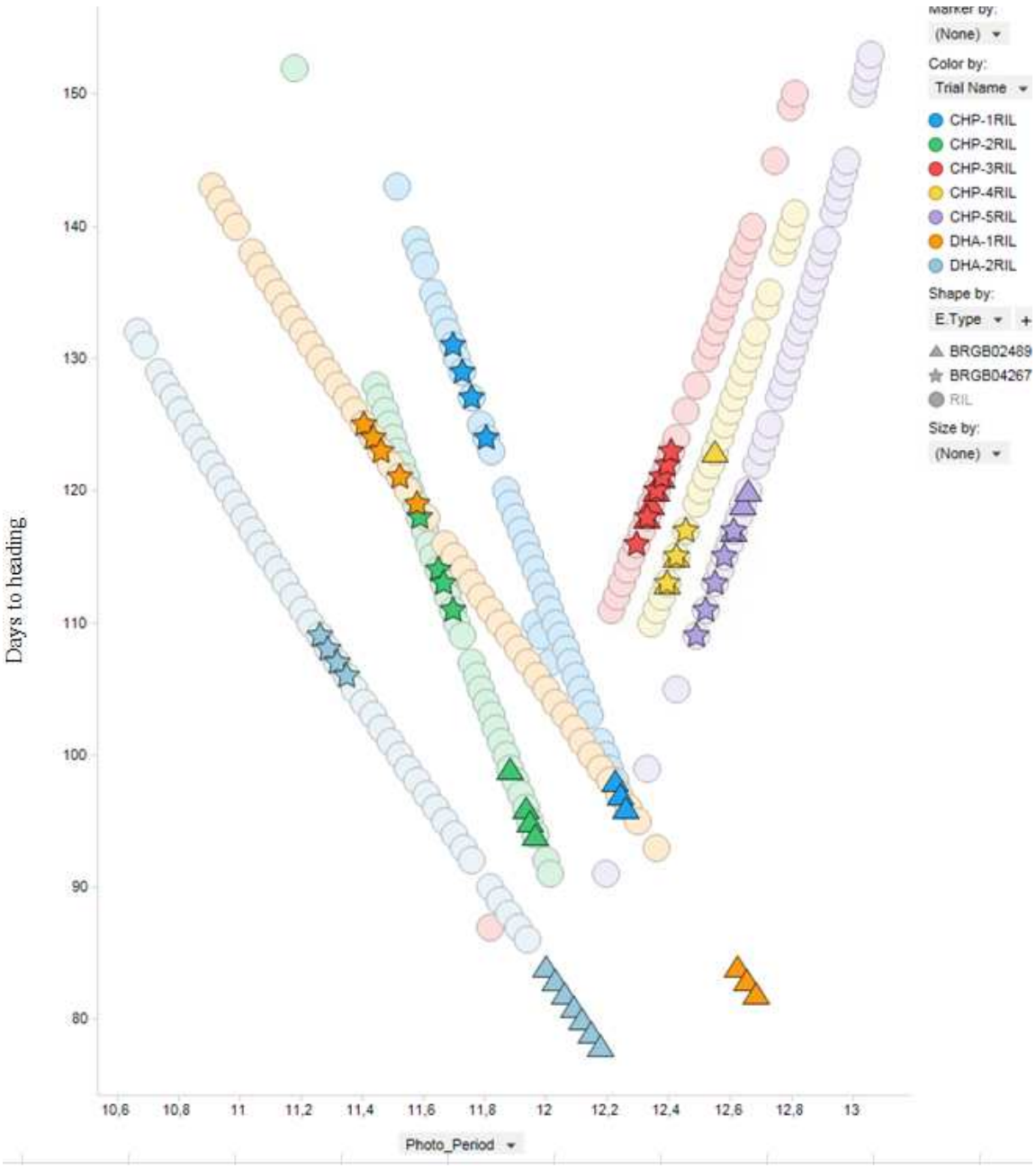
Source	DF	SS	MS	F value	P value
Model	971	55126.5	56.773**	18.6	<.0001
Genotype	475	29705.5	62.5**	20.5	<.0001
Environment	1	19444.2	19444.1**	6375.2	<.0001
Genotype x environment	443	3439.25	7.7**	2.5	<.0002
Block	11	2412.55	219.3**	71.9	<.0001
Residual	41	125.0483	3.04		
R^2	0.93				
H^2	0.91				
Root MSE	2.71				
Grand Mean	101.64				

** Significance at the 0.01 probability level

Table 4.6: Flowering behavior of parental lines (BRGB02489 and BRGB04267) of recombinant inbred lines (RIL) population in response to solar radiation (KW m^{-2}) and photoperiod (Hrs) across test environments at Chandippa(CHP) and Dhantori (DHA), India.

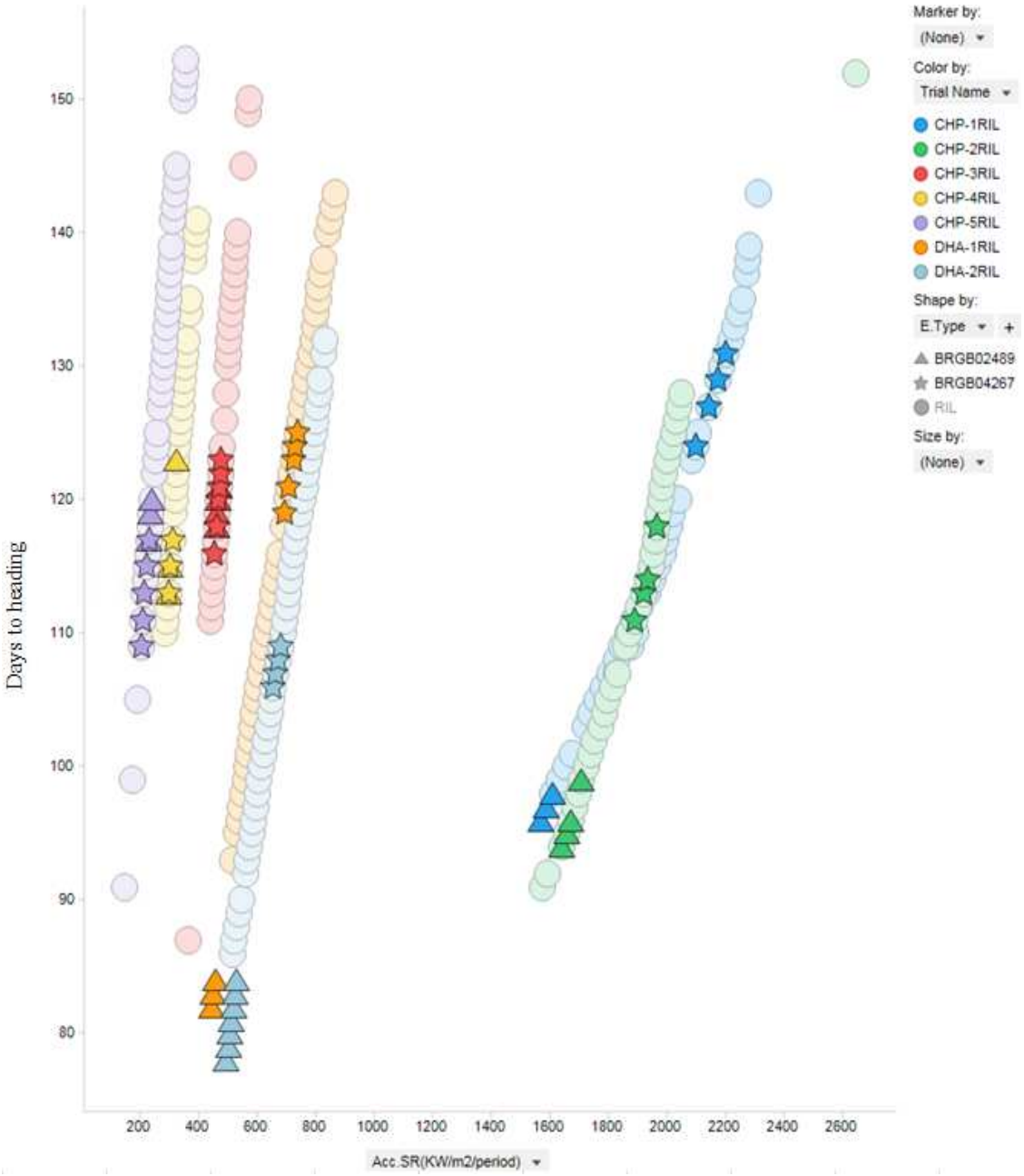
Season	Environment Number	Trial Name	BRGB02489			BRGB04267		
			Mean HD±SE	Solar radiation (KW m^{-2})	Photoperiod (Hrs)	Mean HD±SE	Solar radiation (KW m^{-2})	Photoperiod (Hrs)
Wet Season	**Environment 1**	CHP-1RIL	97±4.18	1766.59	12.09	128±5.28	2149.60	11.75
	Environment 2	CHP-2RIL	96±2.88	1746.68	11.94	113±3.33	1917.14	11.66
	Environment 3	DHA-1RIL	83±2.99	450.13	12.65	121±2.99	711.86	11.51
	Environment 4	DHA-2RIL	81±2.60	512.43	12.09	118±2.60	667.75	11.30
Dry Season	**Environment 5**	CHP-3RIL	119±2.71	466.19	12.35	120±2.55	466.91	12.36
	Environment 6	CHP-4RIL	116±3.20	305.46	12.44	115±3.20	333.10	12.56
	Environment 7	CHP-5RIL	118±4.58	235.36	12.63	113±4.58	215.68	12.54

HD: heading date; SE: standard error; KW m^{-2}: accumulated solar radiation in kilowatt per square meter from sowing to heading; Hrs : photoperiod in hours in the day of heading.
CHP-1RIL: RIL trial at environment 1; CHP-2RIL: RIL trial at environment 2; CHP-3RIL: RIL trial at environment 3;
CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5; DHA-1RIL: RIL trail at environment 3; DHA-2RIL: RIL trail at environment 4.



CHP-1RIL: Wet Season environment 1; CHP-2RIL: Wet Season environment 2; DHA-1RIL: Wet Season environment 3
DHA-2RIL: Wet Season environment 4; CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6;
CHP-5RIL: Dry Season environment 7.
Negative slope: Wet season environments with advancing shorter days;
Positive slope: Dry season environments with advancing longer days.

Figure 4.4: Flowering behavior of recombinant inbred lines (RIL) and their parents (BRGB02489 and BRGB04267) in response to photoperiod (hours) across test environments at Chandippa (CHP) and Dhantori (DHA), India.



CHP-1RIL: Wet Season environment 1; CHP-2RIL: Wet Season environment 2; DHA-1RIL: Wet Season environment 3 DHA-2RIL: Wet Season environment 4; CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6; CHP-5RIL: Dry Season environment 7.

Figure 4.5: Flowering behavior of recombinant inbred lines (RIL) and their parents (BRGB02489 and BRGB04267) in response to accumulated solar radiation (KW/m^2/period) across test environments at Chandippa (CHP) and Dhantori (DHA), India.

Table 4.7: Flowering behavior trial mean, range, standard error, coefficient of determination (R^2), coefficient of variability (CV%) and heritability (H^2) of recombinant inbred lines (RIL) and hybrids (HYB) tested across environments at Chandippa (CHP) and Dhantori (DHA), India.

Season	Environment	Trial Name	Test Entries	Mean Heading date	Range	Standard Error	R^2	CV (%)	H^2
Wet Season	**Environment 1**	CHP-1RIL	290	118[C]	96-143	0.553	0.99	2.35	0.96
	Environment 1	CHP-1HYB	531	106[F]	91-132	0.409	0.98	2.25	0.87
	Environment 2	CHP-2RIL	284	109[E]	91- 128	0.559	0.99	1.91	0.94
	Environment 3	DHA-1RIL	301	114[D]	82-143	0.543	0.99	1.21	0.99
	Environment 3	DHA-1HYB	497	97[F]	70-114	0.423	0.99	1.60	0.94
	Environment 4	DHA-2RIL	305	104[G]	78-132	0.54	0.99	1.24	0.98
Dry Season	**Environment 5**	CHP-3RIL	318	126[A]	111-150	0.529	0.91	5.43	0.66
	Environment 6	CHP-4RIL	248	124[B]	110-141	0.599	0.99	3.42	0.76
	Environment 7	CHP-5RIL	225	125[AB]	91-153	0.628	0.99	2.20	0.96

[A] – Means within column followed by same letter are not significantly different at the 0.01 probability level
CHP-1HYD: Hybrid trail at environment 1; DHA-1HYD: Hybrid trail at environment 3; CHP-1RIL: RIL trial at environment 1; CHP-2RIL: RIL trial at environment 2; CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5; DHA-1RIL: RIL trail at environment 3; DHA-2RIL: RIL trail at environment 4.

Table 4.8: Estimated components of variance for heading date of recombinant inbred line (RIL) population tested across test environments at Chandippa (CHP) and Dhantori (DHA), India.

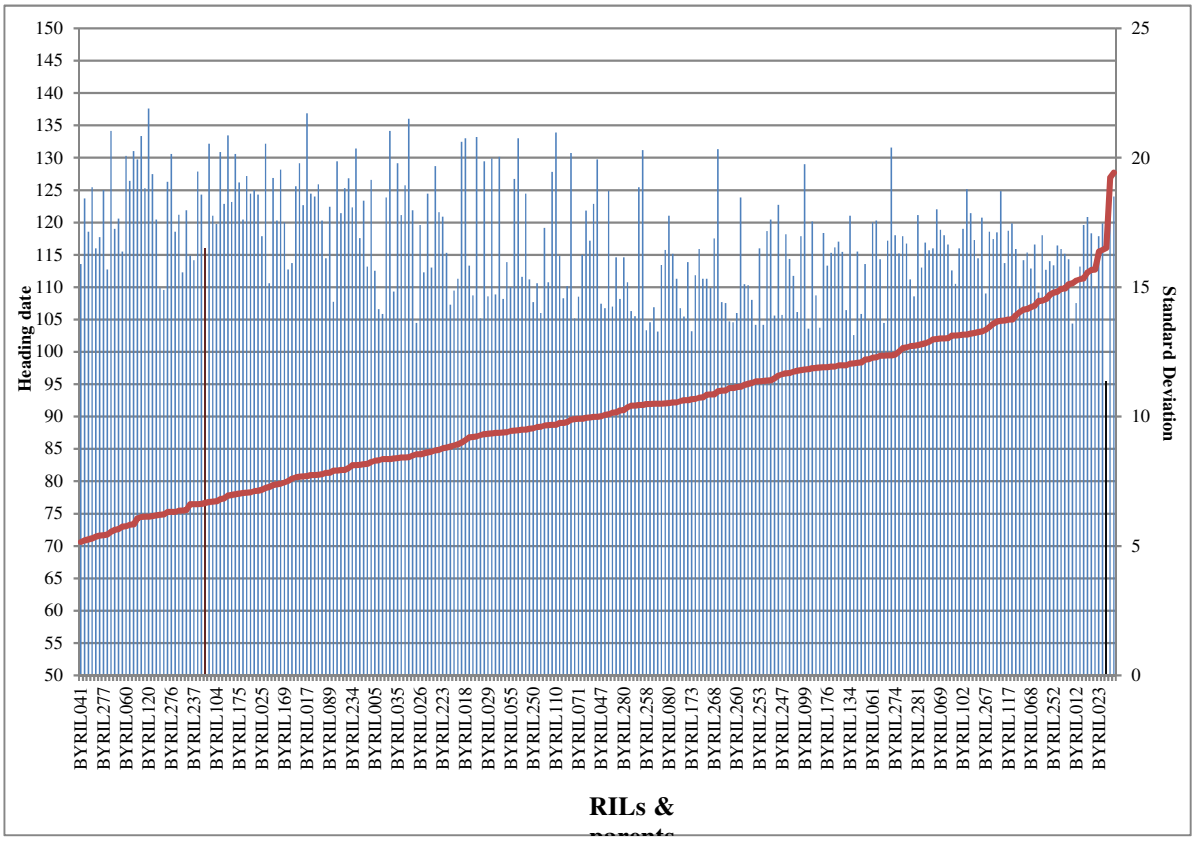
Source	Gamma	Variance	SE	z.ratio	Constraint
Photo Period (hrs.)	66180.57	1299.29	621.78	2.09*	Positive
Photo-thermal Quotient (KW m-2 day-1 OC)	53756.25	1055.37	576.17	1.83	Positive
Mean Temp (^{0}C)	1526.00	29.96	6.22	4.82*	Positive
Heat Index	477.60	9.38	4.60	2.04*	Positive
Genotype	0.89	0.02	0.00	7.42*	Positive
Solar Radiation (KW m^{-2} day^{-1})	0.39	0.01	0.00	1.85	Positive
Residual	1.00	0.02	0.00	9.20	Positive

*: Significant at Z.ratio >2.00 ; SE: standard error; hrs. : hours; KW m^{-2} day^{-1}: kilowatt per square meter per day; ^{o}C: degree Celsius ;

Table 4.9: Correlation coefficient of heading date of recombinant inbred lines (RIL) and hybrids (HYB) with photoperiod (Hrs), solar radiation (KW/m^2), mean temperature (^0C), mean humidity (%), photo-thermal quotient and mean heat index across test environments at Chandippa (CHP) and Dhantori (DHA), India.

Season	Environment Number	Trial Name	Photo Period (Hrs)	Solar Radiation (KW m^{-2})	Mean Temp. (^oC)	Mean Humidity (%)	Photo-thermal Quotient (KW m^{-2} day^{-1} ^oC)	Mean Heat Index
Wet Season	**Environment 1**	CHP-1HYB	-1.000**	0.997**	-0.705**	-0.313**	0.952**	-0.646**
	Environment 1	CHP-1RIL	-0.999**	0.994**	-0.937**	-0.665**	0.897**	-0.940**
	Environment 2	CHP-2RIL	-1.000**	0.992**	-0.988**	-0.879**	0.216**	-0.993**
	Environment 3	DHA-1HYB	-1.000**	1.000**	0.781**	-0.874**	0.758**	-0.238**
	Environment 3	DHA-1RIL	-1.000**	1.000**	0.627**	-0.769**	0.889**	0.336**
	Environment 4	DHA-2RIL	-1.000**	0.997**	-0.667**	0.319**	0.944**	-0.916**
Dry Season	**Environment 5**	CHP-3RIL	1.000**	0.999**	0.997**	-0.985**	-0.996**	0.980**
	Environment 6	CHP-4RIL	1.000**	0.999**	0.998**	-0.993**	-0.985**	0.992**
	Environment 7	CHP-5RIL	0.998**	0.999**	0.999**	-0.993**	-0.937**	0.998**

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level KW m^{-2}: accumulated solar radiation in kilowatt per square meter from sowing to heading; Hrs : photoperiod in hours on the day of heading; KW m^{-2} day^{-1}: kilowatt per square meter per day; ^oC: degree Celsius ;



Brown (BRGB04267) and black (BRGB02489) bars are parents of the recombinant inbred line population.
Bars represent heading date and line represent standard deviation of heading date observed across test environments.

Figure 4.6: Mean heading date standard deviation of recombinant inbred lines tested across environments.

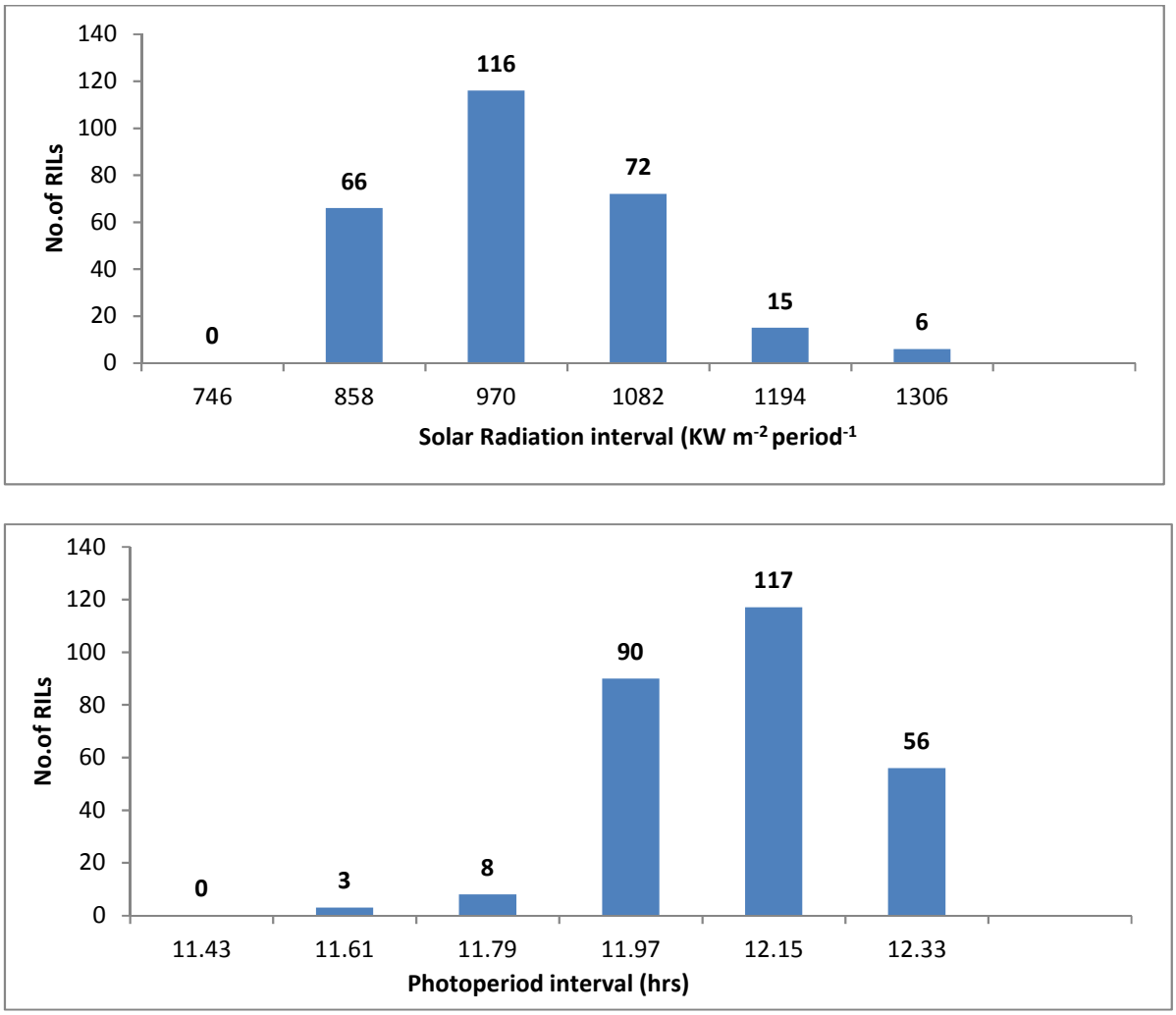


Figure 4.7: Frequency distribution of recombinant inbred lines flowered at different ranges of photoperiod and solar radiation across test environments at Chandippa (CHP) and Dhantori (DHA), India.

Table 4.10: Stable flowering recombinant inbred lines (BYRIL) with environment wise days to heading data, overall mean (Mean), standard deviation (SD), mean photoperiod (hrs) and mean accumulated solar radiation (KW m^{-2}).

Genotype	Days to heading								Photoperiod (hrs)	Solar radiation (KW/m^2)
	[1]CHP-1 RIL	[1]CHP-2 RIL	[2]CHP-3 RIL	[2]CHP-4 RIL	[2]CHP-5 RIL	[3]DHA-1 RIL	[3]DHA-2 RIL	Mean±SD		
BYRIL284	109	103	117	113	115	110	100	110±6.2	12.07	834
BYRIL063	112	109	119	117	118	110	101	112±6.4	12.07	860
BYRIL043	112	111	121	115	115	112	103	113±5.4	12.04	867
BYRIL237	116	104	118	121	111	121	108	114±6.6	11.99	878
BYRIL038	127	114	119	113	118	124	115	119±5.2	11.90	930
BYRIL116	129	113	126	119	117	123	117	121±5.7	11.92	939
BYRIL115	133	115	126	119	117	125	118	122±6.4	11.90	955
BYRIL052	124	112	128	125	132	123	116	123±6.8	11.99	937
BYRIL036	129	117	126	120	127	129	118	124±5.2	11.91	958
BYRIL277	134	120	122	122	125	131	121	125±5.4	11.86	974
BYRIL042	133	119	130	119	132	130	121	126±6.3	11.89	976
BYRIL046	134	118	128	127	123	136	126	127±6.1	11.85	986
BYRIL112	135	118	130	121	134	132	125	128±6.6	11.88	988
BYRIL059	135	119	131	127	135	135	126	130±6.0	11.88	996
BYRIL060	135	120	131	128	135	136	127	130±5.7	11.87	998.
BYRIL276	133	119	134	130	138	134	126	131±6.3	11.90	995
BYRIL096	135	119	137	130	134	136	123	131±7.0	11.90	997
BYRIL095	135	119	138	132	133	135	124	131±6.8	11.91	999
BYRIL119	135	122	138	139	138	137	125	133±6.9	11.91	1011
BRGB02489*	97	96	120	116	118	83	81	95±16.5	12.33	746
BRGB04267*	128	113	120	115	113	121	108	116±6.7	11.95	923

1: Wet Season trials at Chandippa CHP-1RIL (environment 1) and CHP-2RIL (environment 2); 2: Dry season trials at Chandippa CHP-3RIL (environment 5), CHP-4RIL (environment 6) and CHP-5RIL (environment 5); 3: Wet season trials at Dhantori DHA-1RIL (environment 3) and Dhantori DHA-2RIL (environment 4). * Parents of recombinant inbred line (RIL) population; KW m^{-2}: accumulated solar radiation in kilowatt per square meter from sowing to heading; Hrs : photoperiod in hours in the day of heading: Dark green cells: Observation with earliest flowering; Dark red cell: Observation with late flowering.

Table 4.11: Flowering behavior of selected set of recombinant inbred lines (BYRIL) and its derived hybrids evaluated in 2015 wet season at Chandippa (CHP) and Dhantori (DHA), India.

Genotype	Days to heading in Environment 1 @ Chandippa (CHP)			Days to heading in Environment 2 @ Dhantori (DHA)		
	RIL	[1]BRGB06355A X RIL	[2]BRGB07288A X RIL	RIL	[1]BRGB06355A X RIL	[2]BRGB07288A X RIL
BYRIL284	109	104	108	110	98	90
BYRIL063	112	114	107	110	97	92
BYRIL043	112	108	110	112	103	88
BYRIL237	116	107	100	121	105	92
BYRIL038	127	110	102	124	105	92
BYRIL116	129	113	103	123	106	93
BYRIL115	133	122	106	125	105	96
BYRIL052	124	114	105	123	107	93
BYRIL036	129	108	101	129	108	93
BYRIL277	134	118	101	131	109	91
BYRIL042	133	115	109	130	109	98
BYRIL151	135	112	103	132	105	90
BYRIL046	134	120	103	136	109	93
BYRIL112	135	122	108	132	108	96
BYRIL059	135	112	104	135	109	97
BYRIL060	135	113	103	136	112	97
BYRIL276	133	117	107	134	109	100
BYRIL096	135	122	102	136	106	97
BYRIL095	135	114	111	135	109	95
BYRIL119	135	114	108	137	111	99
BRGB02489*	97	106	103	83	99	93
BRGB04267*	128	107	103	121	102	96

* Parents of recombinant inbred line (RIL) population;
1: Hybrid made from cytoplasmic male sterile tester BRGB06355A and recombinant inbred line (RIL);
2: Hybrid made from cytoplasmic male sterile tester BRGB07288A and recombinant inbred line (RIL);
Dark green cells: Observation with earlY flowering; Dark red cell: Observation with late flowering.

4.3.3 Pollen Characteristics of Recombinant Inbred Lines

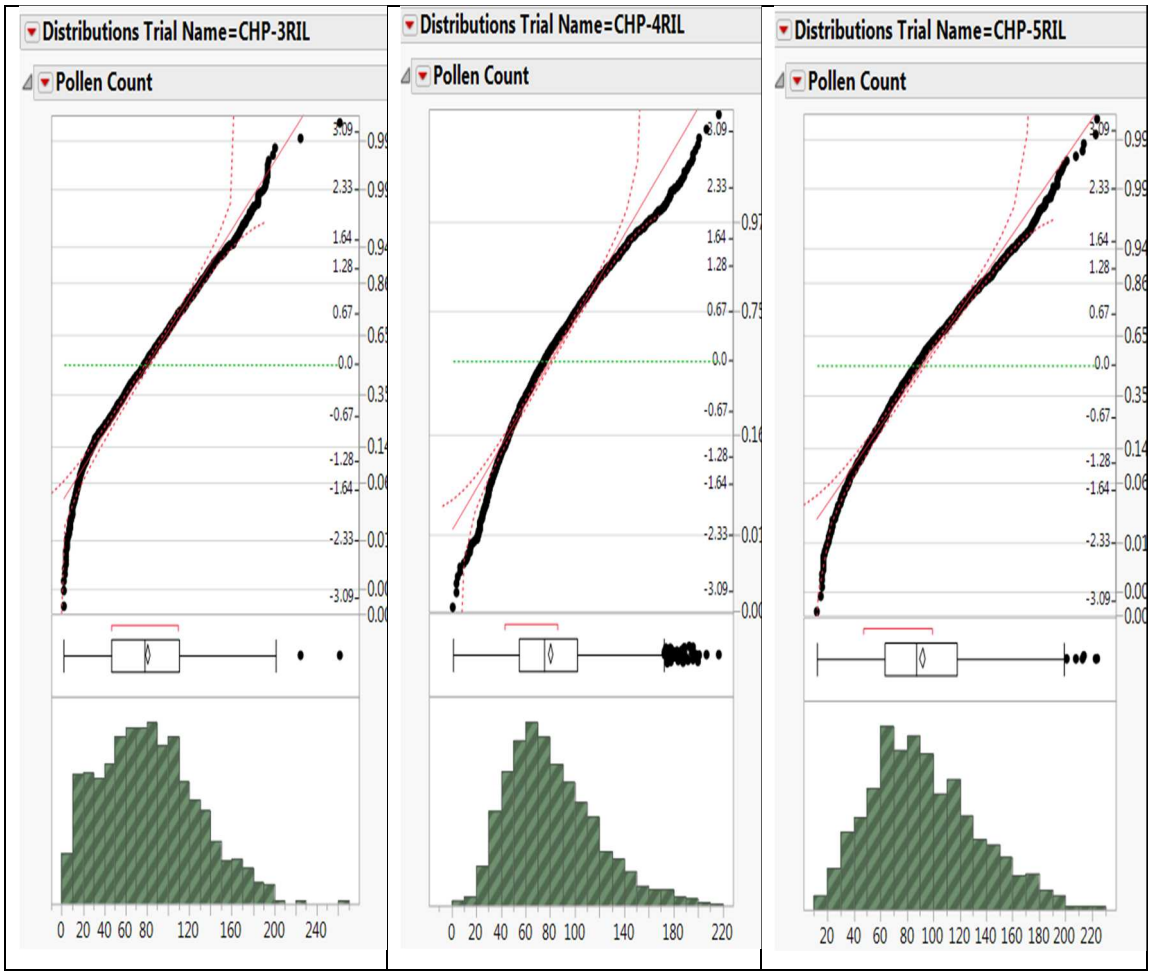
The microscopic studies for pollen morphology and pollen fertility were conducted in the RIL mapping population grown in the 2015 dry season environments at Chandippa, India (Table 4.1). Mature anthers from 272 RILs and parents were collected from environments 5 (CHP-3RIL), 6 (CHP-4RIL), and 7 (CHP-5RIL) to record microscopic observations of fertile pollen number (pollen load), pollen size, and pollen stain intensity. The recorded data were further cleaned by removing outliers from each environment, and data of 177, 268, and 210 RILs from environments 5, 6, and 7, respectively, were processed for the final data analysis.

Figure 4.8 shows the normal quantile plot, boxplot, and normal distribution plot of pollen count data collected from the RIL populations evaluated across the three test environments. Combined ANOVAs of genotype with environment interaction were found significant ($P < 0.001$) for pollen count (Table 4.12), pollen size (Table 4.13), and pollen stain intensity (Table 4.14). The ANOVA of pollen count for the RIL mapping population in individual environments was also significant at $P < 0.001$ (Table 4.15). Parent BRGB02489, with high pollen load, was distinguished with a fertile pollen count of 74 to 126 pollen grains in 2 µl of pollen solution and had 20% higher pollen grains compared to parent BRGB04267(Table 4.16).

Correlation studies between pollen count, pollen size, stain intensity, and heat index were performed for individual test environments, and data are presented in Table 4.17. The significant negative correlation (r= -0.77 to -0.66; $P < 0.001$) between pollen size and staining intensity across environments suggests that the differences in starch

accumulation are related to pollen grain size. However, a significant positive correlation between pollen count and pollen size (r=0.54; $P < 0.001$) and significant negative correlation between pollen count and staining intensity (r=-0.72; $P < 0.001$) were observed in only environment 4 (CHP-3RIL).

Aloni et al. (2001) and Karni and Aloni (2002) have reported that decreased pollen germination at high temperatures has been linked to pollen morphology and failure of metabolic processes such as rehydration, reduced sugar activity by increased sucrose, and starch accumulation. In contrast, at low temperatures, the decline in pollen germination has been associated with decreased availability of sucrose (Rosenfeld and Pressman, 2004). Consequently, pollen size and staining intensity data in the current study do not act as evidence for pollen fertility and pollen germination. Jagadish et al. (2010), Fang et.al. (2010), and Prased et al. (2006) have testified to the importance of pollen production, pollen number on the stigma, pollen viability, and pollen germination as contributory factors to spikelet fertility. Thus, the availability of the number of engorged pollen grains could contribute to the degree of pollen grains to fall on stigma for fertilization. In the present experiment, the RIL population studied across dry season environments was identified with genotypes similar to parent BRGB02489 for high pollen load, and the subset of high-performing RILs are shown in Table 4.18.



Dry season environments: CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6; CHP-5RIL: Dry Season environment 3.

Figure 4.8: Normal quantile plot, boxplot & normal distribution plot of pollen count data observed across recombinant inbred line (RIL) evaluation in dry season 2015 environments at Chandippa (CHP), India.

Table 4.12: Analysis of variance of pollen count observed in recombinant inbred lines (RIL) evaluated in dry season 2015 environments at Chandippa (CHP), India.

Source	DF	SS	MS	F Ratio	Prob > F
Model	684	5726796	8372.5**	6.26	<.0001
Genotype (G)	274	2460081	8978.3**	6.71	<.0001
Environment (E)	2	111204.9	55602.4**	41.61	<.0001
Genotype X Environment	402	3119045	7778.1**	5.82	<.0001
Residual	4700	6279796	1336.1		
R^2	0.60				
H^2	0.14				
Root MSE	24.34				
Grand Mean	84.64				

** Significance at the 0.01 probability level; Dry season environments: CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6; CHP-5RIL: Dry Season environment 3

Table 4.13: Analysis of variance of pollen size (µm) observed in recombinant inbred lines (RIL) evaluated in dry season 2015 environments at Chandippa (CHP), India.

Source	DF	SS	MS	F Ratio	Prob > F
Model	685	101642172	148382.7**	8.13	<.0001
Genotype (G)	274	44963892	164101.8**	8.99	<.0001
Environment (E)	2	3237031	1618516**	88.73	<.0001
Genotype X Environment	402	53119859	132139**	7.24	<.0001
Residual	4702	85761574	18239.3		
R^2	0.48				
H^2	0.58				
Root MSE	121.3				
Grand Mean	908.7				

** Significance at the 0.01 probability level; Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

Table 4.14: Analysis of variance of pollen stain intensity observed in recombinant inbred lines (RIL) evaluated in dry season 2015 environments at Chandippa (CHP), India.

Source	DF	SS	MS	F Ratio	Prob > F
Model	685	262655.4	383.4**	20.82	<.0001
Genotype (G)	274	85725.9	420.8**	16.99	<.0001
Environment (E)	2	31795.9	15897.9**	863.61	<.0001
Genotype X Environment	402	144503.2	359.4**	19.52	<.0001
Residual	4702	86557.6	18.4		
R^2	0.42				
H^2	0.14				
Root MSE	4.52				
Grand Mean	146.6				

** Significance at the 0.01 probability level; Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

Table 4.15: Analysis of variance, coefficient of variation (CV %) and heritability estimates (H^2) of recombinant inbred lines evaluated for pollen count in dry season 2015 environments at Chandippa (CHP), India.

Source	Df	SS	MS	F Ratio	Prob > F	CV %	H^2
Environment 5 - CHP-3RIL							
Model	184	1612047	8761.13**	9.0047	<.0001	38.53	0.74
Genotype	177	1591584	8991.99**	9.242	<.0001		
Rep.	7	21064.3	3009.18**	3.0928	0.0031		
Residual	1232	1198672	972.95				
Environment 6 - CHP-4RIL							
Model	275	853987.2	3105.41**	3.2111	<.0001	38.56	0.50
Genotype	268	832770.2	3107.35**	3.2131	<.0001		
Rep.	7	21292.09	3041.73**	3.1452	0.0026		
Residual	1875	1813308	967.098				
Environment 7 - CHP-5RIL							
Model	217	943051.9	4345.86**	3.7794	<.0001	36.84	0.792
Genotype	210	914685.4	4355.64**	3.7879	<.0001		
Rep.	7	28366.52	4052.36**	3.5242	0.0009		
Residual	1470	1690310	1149.87				

*,** Significant at p < .05 and .01, respectively; NS not significant at p<.05;
Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

Table 4.16: Comparative performance of pollen count data observed in parents (BRGB02489 and BRGB04267) of recombinant inbred line (RIL) population across dry season 2015 environments at Chandippa (CHP), India.

Trial Name	BRGB02489				BRGB04267			
	Mean	Std. Dev	Min	Max	Mean	Std. Dev	Min	Max
CHP-3RIL	81^{A}	6.99	68	87	60^{B}	7.07	48	70
CHP-4RIL	74^{A}	6.25	63	81	58^{B}	8.07	46	67
CHP-5RIL	126^{A}	11.65	111	144	101^{B}	15.48	76	121

^{A,B} – Means within column followed by same letter are not significantly different at P<0.05
Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

Table 4.17: Correlation coefficient for pollen count, pollen size, stain intensity and heat index in recombinant inbred lines (RIL) evaluated in dry season 2015 environments at Chandippa (CHP), India.

	Pollen count	Pollen size	Stain intensity	Heat index
Environment - 4 (CHP-3RIL)				
Pollen count	1.000**	**0.542****	**-0.722****	0.054NS
Pollen size		1.000**	**-0.654****	0.108NS
Stain intensity			1.000**	-0.165NS
Heat index				1.000**
Environment – 5 (CHP-4RIL)				
Pollen count	1.000**	-0.081NS	-0.100NS	-0.053NS
Pollen size		1.000**	**-0.719****	0.021NS
Stain intensity			1.000**	-0.055NS
Heat index				1.000**
Environment - 6 (CHP-5RIL)				
Pollen count	1.000**	-0.106NS	0.027NS	0.200**
Pollen size		1.000**	**-0.773****	-0.246**
Stain intensity			1.000**	0.114NS
Heat index				1.000**

*,** Significant at $p < 0.05$ and 0.01, respectively; NS not significant at $p < 0.05$;
Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

Table 4.18: Overall mean and environment wise performance of recombinant inbred lines (BYRIL) evaluated for pollen count across dry season 2015 environments at Chandippa (CHP), India.

Genotype ID	Overall		CHP-3RIL		CHP-4RIL		CHP-5RIL	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
BYRIL154	134	36.38	145	27.16	130	43.11	126	38.81
BYRIL115	117	48.55	78	39.78	142	33.77	130	48.45
BYRIL144	115	42.72	142	41.49	94	33.73	110	41.70
BYRIL136	115	42.47	135	35.67	104	43.83	105	44.95
BYRIL194	114	36.40	101	24.95	92	14.99	148	38.53
BYRIL150	113	35.45	131	26.41	91	22.44	118	44.83
BYRIL118	109	39.45	125	40.39	102	28.49	101	47.42
BYRIL074	109	40.88	95	37.14	99	30.74	134	45.77
BYRIL202	108	34.64	97	48.07	92	13.99	134	14.52
BYRIL198	106	38.53	97	40.93	113	31.13	108	45.68
BYRIL243	105	52.30	119	25.52	58	15.71	137	64.40
BYRIL009	102	48.05	110	50.21	81	59.73	113	28.21
BYRIL232	101	37.40	115	34.08	77	19.53	112	44.97
BYRIL084	101	36.38	94	24.63	98	37.61	109	46.82
BYRIL070	101	37.78	101	37.92	92	34.49	109	43.65
BYRIL100	100	42.83	110	47.76	88	27.44	102	52.11
BYRIL025	100	22.28	113	15.56	87	17.74	100	26.02
BYRIL022	99	41.74	120	40.89	73	38.16	105	35.73
BYRIL078	99	39.91	92	38.80	78	37.51	127	28.84
BYRIL092	97	42.67	97	47.03	85	37.95	109	44.70
BYRIL161	97	39.73	89	24.29	80	27.48	121	52.99
BRGB02489*	93	25.16	80	6.99	73	6.25	126	11.66
BRGB04267*	73	22.72	60	7.07	58	8.07	101	15.48

*: Parents of recombinant inbred line (RIL) mapping population; Dry season 2015 environments: CHP-3RIL: RIL trial at environment 3; CHP-4RIL: RIL trial at environment 4; CHP-5RIL: RIL trial at environment 5.

4.4 Conclusions

Hybrid rice technology has potential given the relatively high heterosis, but the expansion of technology adoption has been further challenged by several constraints, including high costs of hybrid seed (F_1) due to low and inconsistent hybrid seed production. Among various traits that influence outcrossing rate, stable flowering behavior of parental lines and pollen load of the male parent play a significant role in improving the seed set on male sterile parent in hybrid seed production (Virmani 1994). Therefore, it has become important to understand the genetic variability of outcrossing traits and their molecular genetic basis of variation to help improve hybrid seed (F_1) yield (B.Marathi & K.K.Jena, 2015).

In the present study, wet and dry season environments of Chandippa and Dhantori locations were evaluated for their effectiveness for RIL populations with regard to the appearance of variability in flowering behavior of genotypes in the population in response to photoperiod changes. However, solar radiation and heading date were found to be significantly correlated within environment though independent of photoperiod changes.

In the flowering behavior study, genotype and environmental interactions were found significant in both inbred and hybrid trials. These findings are in accordance with the study of Maheswaran (1999), who observed that progressive short-day conditions in wet season environments accelerated the flowering in short-day plants. Overall, some of the RILs were identified as stable, and varied flowering behaviors across environments in response to photoperiod changes were observed. Therefore, the evaluation of breeding

populations in different environments can allow breeders to identify stable flowering parental lines for hybrid seed production.

The studies conducted at Chandippa in dry season environments for pollen number (pollen load), pollen size, and staining intensity were found to be significantly different ($P<0.0001$) for genotype and for genotype-environment interaction. Among the test environments, a significant and negative correlation was observed between pollen size and staining intensity. However, the results of the spikelet fertility studies by Rosenfeld and Pressman (2004) do not substantiate the role of stain intensity towards high pollen fertility. Thus, genotypes with high pollen number (pollen load) allow a greater number of stigmas to fertilize for improving outcrossing rate of male sterile parent (Fang et. al., 2010).

Further evaluation of RILs having stable flowering behavior and high pollen load is required to validate for its stable and high out-crossing rate through seed production experiments at multiple locations. In addition to phenotypic selection, mapping QTL for photoperiod response and pollen load can help breeders, as they can use these identified RIL genotypes as a source to develop lines for stable flowering and high pollen load in hybrid rice.

CHAPTER V

GENOTYPING MAPPING POPULATION AND IDENTIFYING QUANTITATIVE TRAIT LOCI FOR BROWN PLANT HOPPER RESISTANCE, PHOTOPERIOD SENSITIVITY, AND POLLEN LOAD TRAITS

5.1 Introduction

5.1.1 Rice

Rice (*Oryza sativa* L.) is staple food for a large segment of the world's population that comprises more than 3.5 billion people (Rice Almanac, 2013). Possibly the oldest domesticated grain (~10,000 years) and grown in an area covering more than 9% of the earth's arable land, rice provides 21% of global human energy per capita and 15% of the protein per capita (IRRI, 2002). Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake (IRRI, 2001). As expected, Asia accounts for over 90% of the world's production of rice, with China, India, and Indonesia as the leading producers (IRRI, Rice Web).

5.1.2 Genetic Mapping for BPH Resistance

The brown plant hopper (BPH), *Nilaparvata lugens* (Stal), is one of the most serious and destructive pests of the rice plant. Due to its high damage to crops and frequent outbreaks, researchers were prompted to seek BPH-resistant germplasm from various sources and utilize the resistant genes to improve rice crops. Wild relatives of rice species offer a rich source of resistance to BPH. So far, 10 major resistance genes have been identified from the wild rice species: *Bph10* was identified on the long arm of

chromosome 12 in *O. australiensis* (Ishii et al., 1994); the *bph11(t)* gene was mapped on the long arm of chromosome 3 in *O. officinalis* (Hirabayashi et al., 1998); a dominant gene *Bph12* – formerly *Bph12(t)* – was detected on the short arm of chromosome 4, flanked by RM16459 and RM1305 in *O. latifolia* (Yang et al., 2002; Qiu et al., 2012); the recessive resistance gene, bph16 – formerly *bph12(t)* – was mapped to the long arm of chromosome 4 and flanked by two RFLP markers, G271 and R93 (Hirabayashi et al., 1998); two dominant genes, both named *Bph13(t)*, were found on the long arm of chromosome 2 of *O. eichingeri* (Liu et al., 2001) and on the short arm of chromosome 3 of *O. officinalis* (Renganayaki et al., 2002); in *O. officinalis*, two more dominant resistance genes, *Bph14* and *Bph15*, were mapped to the long arm of chromosome 3 and the short arm of chromosome 4, respectively (Huang et al., 2001); *Bph18(t)* was mapped to the long arm of chromosome 12 in *O. australiensis* (Jena et al., 2006); and, finally, two newly identified genes from *O. minuta*, *Bph20(t)* and *Bph21(t)*, were mapped to the short arm of chromosome 4 and to the long arm of chromosome 12, respectively (Rahman et al., 2009). These studies of BPH resistance genes have led to rice and BPH becoming an ideal model system for the study of interactions between plants and sucking herbivorous insects.

5.1.3 Photoperiod Sensitivity

Rice is a short-day plant that exhibits robust photoperiod sensitivity. Generally, its flowering is delayed when days are long, and nights are short, but flowering accelerates when days get shorter. Cultivars with reduced photoperiod sensitivity response are characterized by early flowering and were developed for growing at higher

temperate latitudes (Gao et al., 2013; Wu et al., 2013). On the other hand, cultivars with enhanced photoperiod sensitivity (late flowering) have been developed for increased grain yield in most rice-planting regions (Xue et al., 2008, Wei et al., 2010). Thus, deciphering the molecular genetic mechanisms that underlie the flowering time control and regional adaptability has been a major goal of rice breeders and plant biologists. Several research groups have identified the many QTL that affect the heading date in rice (Li et al., 1995; Xiao et al., 1996; Lu et al., 1997). Five QTLs that control heading date (*Hd1* to *Hd5*) have been found in an F_2 population derived from a cross between a japonica variety, 'Nipponbare', and an indica variety, 'Kasalath' (Yano et al., 1997). Among them, two major QTLs, *Hd1* and *Hd2*, exist in the middle of chromosome 6 and at the end of chromosome 7, respectively (Yano et al., 1997). Kasalath alleles on both loci greatly reduced the number of days to heading. Furthermore, *Hd3*, *Hd4*, and *Hd5* were detected on chromosome 6, 7, and 8, respectively, and Nipponbare alleles also reduced days to heading (Yano et al., 1997).

5.1.4 Outcrossing Traits

Hybrid rice exhibits a yield increase of 15 to 20% more than the best traditional varieties in a large-scale production worldwide (Xu, 2003; FAO 2004). As a self-pollinated crop, improving commercial production of hybrid seeds plays a key role in the successful implementation of hybrid rice. Anther dehiscence or pollen production and spikelet flowering in rice occur more or less simultaneously, so male sterility has to be adapted to the female parents to prevent self-pollination and secure cross-pollination (Virmani, 1994). However, in spite of male sterility barriers such as incomplete exertion

of the panicle, which prevents access to about 20% of the spikelets and the failure of about 20% of spikelets to open at all, selfing can be encountered (Yan and Li, 1987; Tian, 1991). Thus, up to 40% of spikelets may not be available for pollination and subsequent seed production if gibberellin (GA3) technology is not adapted (Yuan, 1981; Yuan and Fu, 1995). The asynchronous flowering results in many spikelets being unavailable for cross-pollination. Among these factors, pollen load is important for increasing the seed production.

Spikelet opening triggers rapid pollen swelling, which leads to anther dehiscence and pollen shedding from the anther's apical and basal pores (Matsui et al., 1999). Increased basal pore length in a dehisced anther has been found to contribute significantly to successful pollination (Matsui and Kagata, 2003), likely because of its proximity to the stigmatic surface; longer stigmas may also be important for the same reason. Genotypic differences in pollen number and germinating pollen on the stigma (Matsui et al., 1997a) and spikelet fertility (Matsui and Omasa, 2002; Prasad et al., 2006) in rice have also been studied.

The present study was carried out to develop new photoperiod-insensitive and BPH-resistant restorer lines capable of producing higher load of pollen with enhanced outcrossing potential. This objective can be achieved by mapping the QTL(s) responsible for BPH resistance, photoperiod sensitivity, and high pollen load traits from donors like BRGB04267 and BRGB02489. For this study, a RIL population consisting of 273 lines was developed from the parents, 'BRGB02489' and 'BRGB04267',

possessing contrasting response to BPH resistance, photoperiod sensitivity, and pollen load. This RIL population was evaluated to address the following objectives:

1. Genotype and phenotype a RIL population to map QTLs for BPH resistance, heading behavior, and pollen count or load.
2. Identify lines that carry favorable QTLs for BPH resistance, stable flowering, and pollen load for further use as elite donor in marker-assisted breeding.

5.2 Materials and Methods

5.2.1 Plant Material

The RIL population consisting of 272 lines was derived from the cross between the BRGB02489 and BRGB04267, both elite Bayer restorer lines, which exhibit contrasting behavior for BPH resistance, photoperiod sensitivity, and pollen load (Table 5.1). The mapping population was developed using the single seed descent method, and genotyping and phenotyping of the population was done at the $F_{2:7}$ generation, as outlined in Figure 5.1.

Table 5.1: Characteristics of parents BRGB02489 and BRGB04267 for brown plant hopper (BPH) resistance, photoperiod sensitivity and pollen load.

Characteristics	BRGB02489	BRGB04267
BPH Resistance	Susceptible	Resistant
Photoperiod sensitivity	High	Less sensitive
Pollen Load	High	Moderate

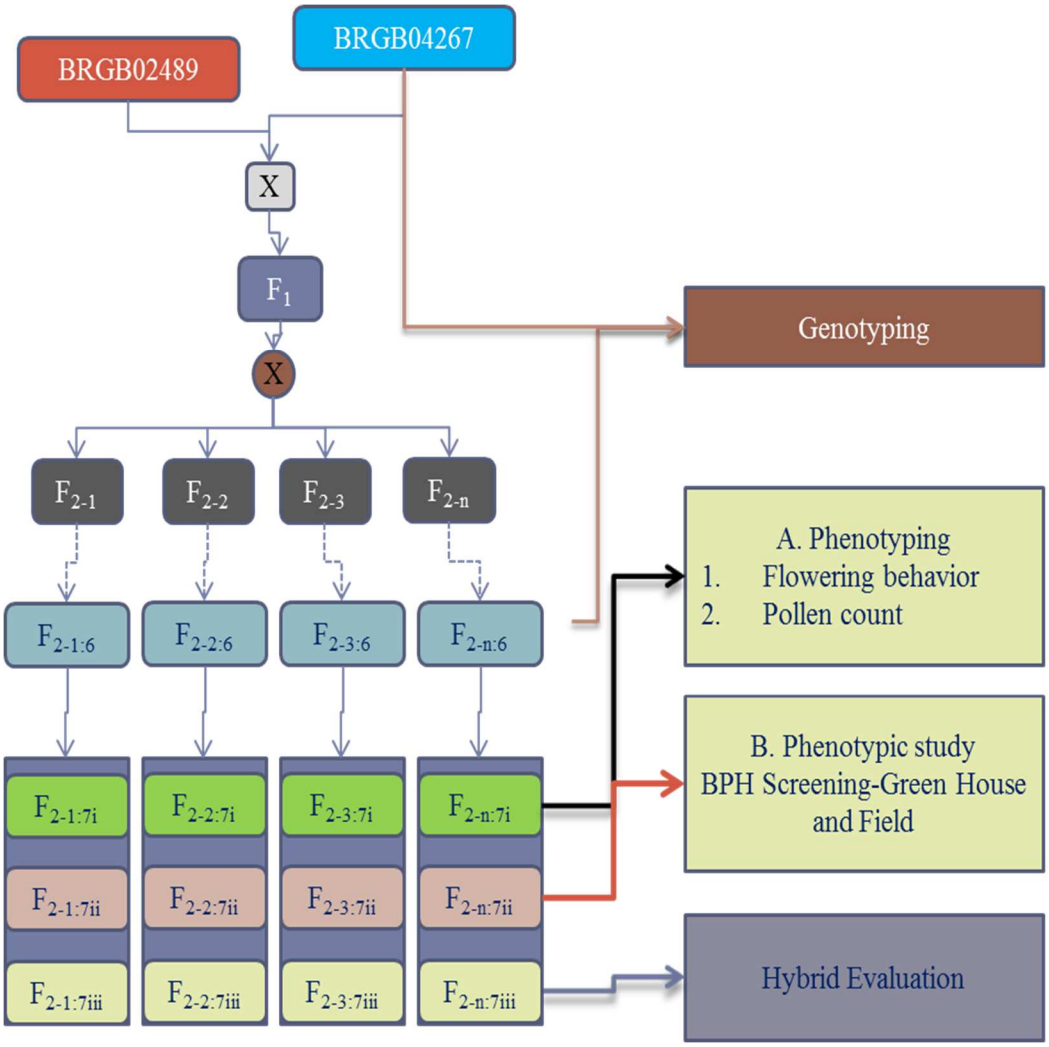


Figure 5.1: Schematic representation of recombinant inbred line (RIL) mapping population development for quantitative trait loci (QTL) mapping of brown plant hopper (BPH) resistance, photoperiod sensitivity and pollen load traits.

5.2.2 Phenotyping

5.2.2.1 Brown Plant Hopper Resistance Screening

Phenotyping data of 260 out of 272 RILs for the degree of insect damage (tolerance levels) was generated from a trial laid out in an augmented design with two replications using a modified version of the seed box method. The modified seed box test was developed at the IRRI by Panda and Khush (1995) and was recognized as a standard method for evaluating the degree of damage. Individual RIL genotypes were infested in chosen conditions with BPH second-instar nymphs at the 2-3 leaf stage of plant growth (~15 days after sowing at Zadoks growth stage of 14 (Zadoks et al., 1974)). The damage score was assessed with a 0-9 scale, and the same data were used for QTL analysis. The QTL effect on insect behavior was further assessed by studying the subset of RILs (Table 3.2) for antixenosis (adult preference and feeding rate) and antibiosis (fecundity). Experiments on the degree of BPH damage, antibiosis, and antixenosis were conducted in greenhouse and field screening facilities at Bayer BioScience, Multicrop Breeding Station, Chandippa, India.

5.2.2.2 Flowering Behavior Experiment

The RIL population with 272 genotypes and its parents were evaluated for heading date at the Multicrop Breeding Station in Chandippa, Hyderabad, India (latitude 17.4^{o}N, longitude 78.1^{o}E) and at the Bayer BioScience Product Evaluation Center in Dhantori, Haryana, India (latitude 29.9^{o}N, longitude 76.8^{o}E) locations. Experiments were conducted for the wet season (WS) and dry season (DS) by creating seven test environments (trials) via sowing test entries at different dates within each season at both

the Chandippa (CHP) and Dhantori (DHA) locations. Each trial was created in an augmented design with 12 blocks and repeated checks in each block to estimate error variance. Heading data recorded in each environment were used for QTL mapping, and results were interpreted along with weather data recorded for solar radiation and photoperiod.

5.2.2.3 Pollen Load Study Experiment

The pollen count was recorded from 272 RILs and their parents (BRGB04267 and BRGB02489) and evaluated in three dry season environments, CHP-3RIL (Environment 5), CHP-4RIL (Environment 6), and CHP-5RIL (Environment 7) at Bayer Bioscience, Multicrop Breeding Station, Chandippa, Hyderabad, India. Mature anthers from three random spikelets were collected from each genotype in eight replications and prepared in a standard volume (2 µl) of iodized potassium iodide (IKI) stained pollen solution. The observation slide with 2 µl of the stained pollen solution from each replicate was studied under a stage microscope, and pictures were captured for further image analysis with the help of Image-Pro Premier 9.2 software. The least mean square data of the fertile pollen count from all three environments were used for QTL mapping.

5.2.3 Genotyping and Data Analysis

5.2.3.1 DNA extraction

The extraction of DNA was done from young leaf tissue after crushing in genome grinder using a Tris/SDS extraction buffer (100 mM Tris–HCl pH 8, 50 mM EDTA pH 8, 500 mM NaCl, 1.25% SDS, and 0.38% sodium bisulfate) and chloroform extraction followed by ethanol precipitation. The DNA samples were quantified using a

NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to 50 ng/lL DNA concentration.

5.2.3.2 Infinium SNP Genotyping

For each GoldenGate VeraCode oligo pool assay (OPA) run, a plate of 96 samples with 5 lL of unamplified genomic DNA normalized to 50 ng/lL was genotyped using the GoldenGate Genotyping Assay for VeraCode Manual Protocol (Illumina Part # 11275211) following the manufacturer's instructions. An Allegra 25R (Beckman Coulter, Brea, CA, USA) was used for the plate centrifuge steps, and a GS1 thermal cycler (G-Storm, Surrey, UK) was used for the universal polymerase chain reaction amplification step. Each microbead was also coated with oligonucleotides that contain a unique address that hybridizes to the labeled products (Lin et al., 2009). During scanning on the BeadXpress Reader, the bead codes and cy3/cy5 signal intensities were measured across replicated sets of beads to assign the single nucleotide polymorphic (SNP) alleles.

5.2.3.3 SNP allele calling and data analysis

The SNP data from each plate were analyzed using the Genotyping module (v1.6.3) of the Illumina GenomeStudio (v2010.1) software.

5.2.3.4 Segregation Distortion

In the absence of selection pressure, the expected genotypic ratio in the RIL population would be 1:1 or 50% of the recurrent parent alleles to 50% donor alleles. Segregation ratios for the two genotypic classes were compared with expected Mendelian ratios (1:1) based on χ^2 test ($P < 0.01$) and employing One Map package using R-studio. The uninformative SNP markers, such as SNPs which are monomorphic

between the parents; SNPs which deviate from the expected ratio; and the SNPs, which have more than 25% of the missing genotype calls, were all cleaned using the R Script in the R-studio (Table 5.2).

Table 5.2: Genotype data cleaning procedure followed for linkage map construction.

Criteria	Removed SNPs / Plants
Monomorphic markers	7287 SNP
Missing data >25%	260 SNP
Heterozygous markers	212 SNP
Distorted markers @ p 0.0001	1218 SNP
Plants with Missing data	7 Plants

5.2.3.5 Linkage Map Construction

A total of 1299 polymorphic SNP-based marker data from 265 plants were used to construct the linkage map utilizing the OneMap in R-Studio (Margarido et al., 2007) following the Kosambi Function (Kosambi, 1944). Markers were allocated to linkage groups with a minimum threshold LOD score of 3.0 and a maximum recombination fraction $\theta = 0.5$ using the "group" command. The order of linkage groups was determined using the "compare," "try," and "first order" commands. Linkage groups were assigned to the respective chromosomes based on the rice physical map developed from OryzaSNP Project at MSU (http://oryzasnp.plantbiology.msu.edu/).

5.2.3.6 QTL Analysis

The QTL analysis for the BPH resistance, heading date, photoperiod sensitivity, and pollen load was performed with an analytical approach in a mixed linear composite interval mapping using software QTLNetwork 2.0 based on a mixed linear model

(Yang.J et al., 2008; Wang DL et al., 1999). Testing window, work speed, and filtration window were set at 10cM, 2cM, and 10cM, respectively. Significance testing was based on the F-test using Henderson's method III, and 10,000 permutation tests were used to calculate the critical F-value to control the genome wise type-I error (Doerge and Churchill, 1996). Finally, all of the detected QTL and epistasis QTL were fit by a full-QTL model to estimate the main effect of QTL and epistasis and their interaction effects by the Markov Chain Monte Carlo (MCMA) algorithm (Wang et al., 1994). The QTL detection was undertaken for each trait separately for BPH resistance and pollen load. For heading date, QTL analyses were performed on single environment basis because mapped QTL were expected to have mixed effects of additive (A) and additive epistatic interaction effects (AA). The QTL effects and their environment interactions were analyzed separately for heading date by ANOVA to test the significance of individual QTL allele effects on flowering.

5.2.3.7 QTL nomenclature

Nomenclature for QTLs was followed, as described by McCouch et al. (1997). Two or three letters were abbreviated from the trait name and followed by the chromosome number of rice where the QTL was found; a terminal suffix, separated by a period, gives a unique identifier to distinguish multiple QTLs on a single chromosome.

5.3 Results and Discussion

5.3.1 Genetic Linkage Map Construction

A total of 10,276 SNP markers were genotyped and scored through 265 $F_{2:7}$ individuals, which resulted in a map covering a distance of 2,115 cM over 13 linkage groups with an average of 2.21 cM between adjacent marker loci (Figure 5.2). Of the total loci genotyped, 2% were found to be heterozygous, which would be expected in an F_7 and especially in crosses involving diverse parents. Markers that were monomorphic (70.1%), distorted (11.8%), and un-amplified (2.5%) were excluded from the map construction. Chromosome 6 was grouped into two linkage groups after assigning markers based on the physical map developed from OryzaSNP Project @MSU.

5.3.2 QTL Mapping for Brown Plant Hopper Resistance

5.3.2.1 Phenotypic Analysis

The degree of damage score data of 260 RILs infested with BPH nymphs in modified seed box test was found to be significant ($P < 0.001$) between genotypes. The tolerance (degree of damage) scores of RILs were distributed from susceptible (score 7 to 9) to resistant (score 0 to 3) groups showing a normal distribution, which indicates the qualitative nature of inheritance of the trait under study (Figure 5.3). These findings were similar to the genetic analysis studies of BPH resistance conducted by Alam and Cohen, 1998; Soundararajan et al., 2004; Liu et al., 2009; and Ali and Chowdhury, 2014. However, the phenotypic variance of population for degree of damage is independent from the virulence of the biotype used for screening (Sogawa, 1978).

5.3.2.2 QTL Analysis

Mapping of QTL based on the degree of insect damage scores in the RIL population from the greenhouse experiment led to the identification of a QTL with additive (A) and additive X additive (AA) epistatic interaction for BPH resistance. The mixed linear composite interval mapping analysis revealed three putative QTLs (Figure 5.4) associated with BPH resistance with a significant ($P < 0.001$) A effect on chromosome 4 (*qBP4*) and with AA epistatic interaction effect between chromosome 6b (*qBP6i*) and chromosome 11 (*qBP11i*). The total phenotypic variance explained by all three detected QTL was 10.6%. The QTL (*qBP6i* and *qBP11i*) with AA epistatic interaction did not have an independent A effect but elucidated a low level of phenotypic variance (3.2%) when both QTL were inherited together in the population. On the other hand, the A effect of QTL-*qBP4* alone contributed 7.3% of phenotypic variance, with a QTL peak at 11.6 cM between the marker interval of 8.0 cM to 24.4 cM (Table 5.3). Therefore, marker interval interaction analysis with mixed linear composite interval mapping partitioned a major A effect of QTL with minor QTL having an epistatic interaction. As of now, 29 BPH resistance genes have been identified from ssp. *indica* and wild relatives (Ali and Chowdhury, 2014). The major QTL on chromosome 4 found in the present study is in congruence with several other independent researchers' studies, which have identified QTL for BPH resistance using different BPH-resistant sources, namely, *Bph12* (Qiu et al., 2012), *QBph4.1* (Hu et al., 2015a), *QBph4.2* (Hu et al., 2015a), *Bph15* (Lv et al., 2014), *Bph17* (Sun et al., 2005), *Bph20* (Rahman et al., 2009), *Bph6* (Qiu et al., 2010), and *Bph27* (Huang et al., 2013); these QTL were clustered in a

region of 5 to 22Mb on chromosome 4. Meanwhile, QTL identified in Rathu Heenati (*Bph3*, Jairin et al., 2007b), Babawee (*bph4*, Kawaguchi et al., 2001), and ADR52 (*Bph25*, Myint et al., 2012) were clustered on chromosome 6 and, *Bph 6* (Jena et al., 2003) and *Bph28(t)* (Wu et al., 2014) were mapped on chromosome 11. Therefore, QTL regions identified on chromosomes 4, 6 and 11 from this study and the previous studies reveal significantly important gene clusters collocated in these regions that contribute to BPH resistance in rice.

The results of antibiosis (fecundity, egg hatching, egg mortality, and feeding rate) and antixenosis (male preference and female preference) studies done in RIL population are presented in Table 5.4. The RIL carrying QTL *qBP4* were found to be significantly associated with insect fecundity, egg hatching, and feeding rate traits in the antibiosis mode of action. However, the differences in egg mortality percentages were not as significant as observed in resistant and susceptible parents. Additionally, an adult male BPH preference towards parents was observed in the antixenosis study. Similarly, in the antixenosis study, the RIL with *qBP4* QTL and resistant parent BRGB04267 were less preferred by female BPH insects, which indicates that the major A effects of this QTL (*qBP4*) on chromosome 4 may possibly affect different stages of BPH life cycle to disrupt the insect's companionship with host plant. The current findings are in agreement with the independent studies conducted by Qiu et al. (2010) and P.S. Sarao et al. (2016), and they demonstrated that the same gene can mediate different mechanisms of resistance or show different responses to different biotypes.

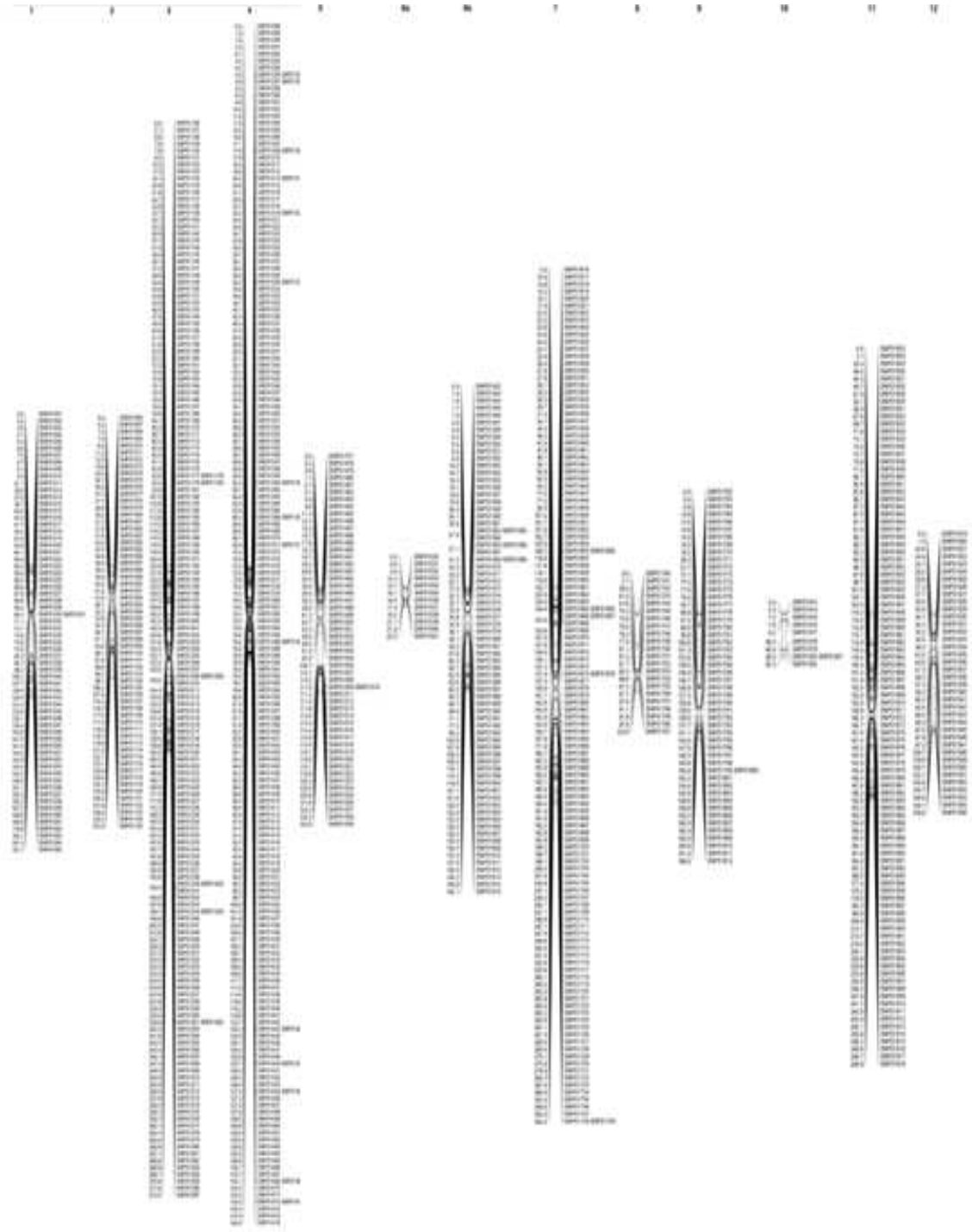


Figure 5.2: Genetic linkage map of recombinant inbred line population derived from BRGB02489 X BRGB04267.

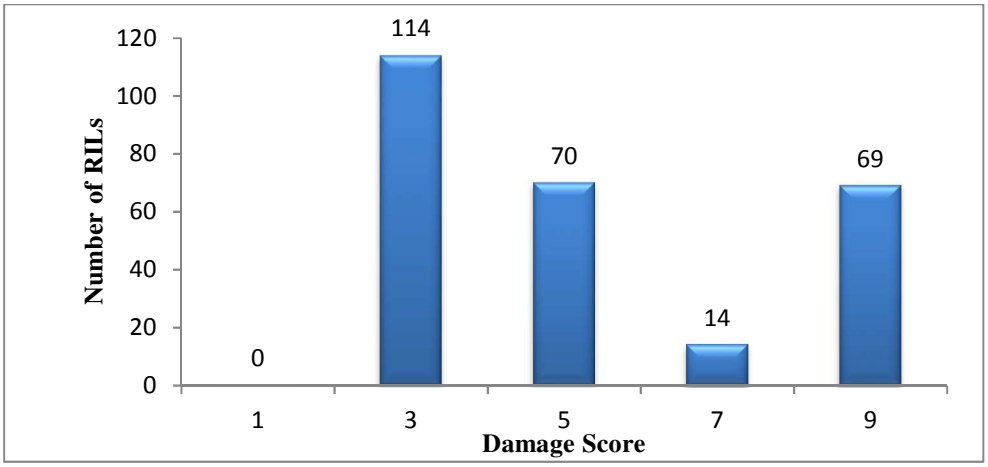


Figure 5.3: Frequency distribution of degree of damage score of recombinant inbred lines screened with brown plant hopper nymph.

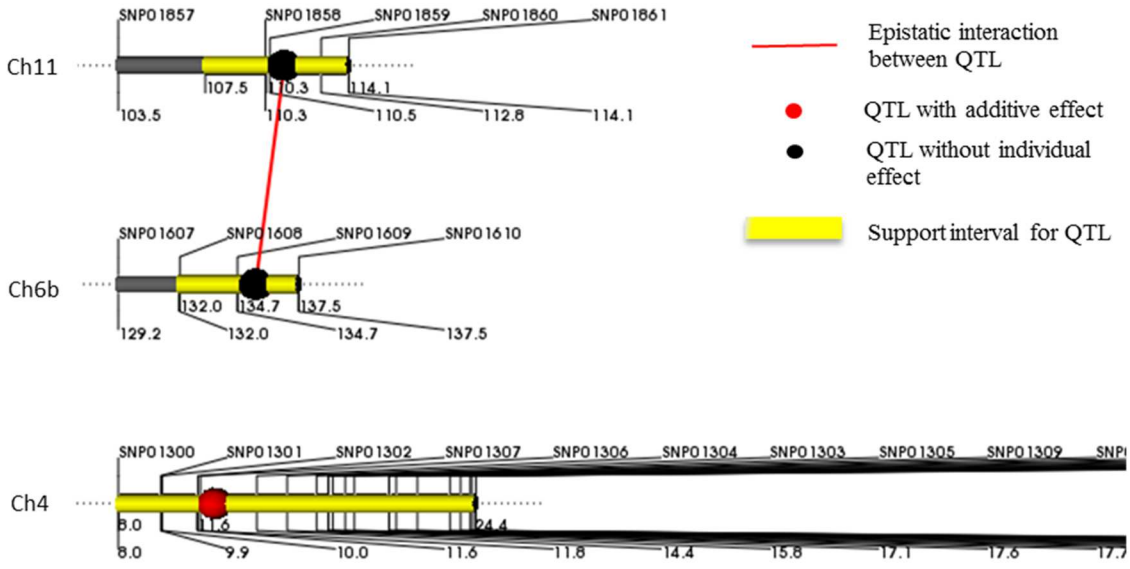


Figure 5.4: Predicted genetic architecture of brown plant hopper resistance quantitative trait loci (QTL) mapped with degree of damage score data.

Table 5.3: Quantitative trait loci (QTL) detected with additive (A) and additive interaction (AA), standard error (SE), F-value, P-value and variance (h^2) for brown plant hopper (BPH) resistance in recombinant inbred line population.

	Additive Effect	**Epistatic Interaction**	
Chromosome Number	4	6b	11
QTL Name	*qBP4*	*qBP6i*	*qBP11i*
Position	11.6	134.7	110.3
Support interval	8.0 - 24.4	132.0 - 137.5	107.5 - 114.1
A (additive main)	0.7801	-	
AA (additive interaction)	-	0.470	
Standard Error	0.1511	0.154	
P-Value	0.000	0.0022	
h^2(A)	0.073	-	
h^2(AA)	-	0.032	

Table 5.4: Performance of recombinant inbred lines (BYRIL) carrying brown plant hopper resistance additive effect quantitative trait locus (QTL) for tolerance (damage score), antibiosis (fecundity, egg hatching, egg mortality percent and feeding rate) and antixenosis (male and female preference) mechanism of resistance compared to susceptible (BRGB02489) and resistant parents (BRGB04267).

Genotype	QTL	Tolerance	Antibiosis				Antixenosis	
		Damage Score	Fecundity	Egg Hatching	Egg Mortality (%)	Feeding rate	Male Preference	Female Preference
BRGB02489	Susceptible parent	8.67 ± 0.15	140 ± 2.03	75 ± 2.49	30 ± 1.04	3.57 ± 0.13	4.77 ± 0.50	13.00 ± 1.67
BRGB04267	Resistant parent	2.29 ± 0.15	60 ± 1.99	25 ± 2.44	35 ± 1.02	1.13 ± 0.13	1.65 ± 0.22	4.34 ± 0.75
BYRIL007	RIL with BPH resistant QTL: qBP4	2.94 ± 0.42	64 ± 5.94	38 ± 7.26	27 ± 3.05	2.00 ± 0.29	1.91 ± 0.43	5.08 ± 1.44
BYRIL084		3.56 ± 0.42	66 ± 5.14	46 ± 6.29	22 ± 2.64	1.66 ± 0.29	1.11 ± 0.50	4.11 ± 1.67
BYRIL094		2.29 ± 0.42	73 ± 4.85	39 ± 5.93	30 ± 2.49	1.00 ± 0.29	1.22 ± 0.50	3.00 ± 1.67
BYRIL096		4.83 ± 0.42	62 ± 4.85	34 ± 5.93	30 ± 2.49	1.00 ± 0.29	1.00 ± 0.50	2.55 ± 1.67
BYRIL131		3.04 ± 0.42	67 ± 4.85	39 ± 5.93	28 ± 2.49	1.00 ± 0.29	1.11 ± 0.50	2.55 ± 1.67
BYRIL156		2.17 ± 0.42	76 ± 4.85	30 ± 5.93	37 ± 2.49	1.00 ± 0.29	3.11 ± 0.50	9.66 ± 1.67
BYRIL171		4.64 ± 0.42	60 ± 4.85	28 ± 5.93	34 ± 2.49	1.00 ± 0.29	3.37 ± 0.53	9.37 ± 1.77
BYRIL177		2.26 ± 0.42	80 ± 4.85	34 ± 5.93	36 ± 2.49	1.00 ± 0.29	2.66 ± 0.50	8.33 ± 1.67
BYRIL066		4.46 ± 0.42	62 ± 4.85	35 ± 5.93	28 ± 2.49	1.00 ± 0.29	2.00 ± 0.50	7.77 ± 1.67
BYRIL174		5.92 ± 0.42	65 ± 5.14	20 ± 6.29	40 ± 2.64	1.33 ± 0.29	2.53 ± 0.39	7.33 ± 1.29
BYRIL196		2.165 ± 0.42	68 ± 4.85	31 ± 5.93	34 ± 2.49	1.00 ± 0.29	2.33 ± 0.50	6.66 ± 1.67
	LSD	1.50	10.56	8.14	7.16	0.72	2.89	9.35
	H^2	0.96	0.98	0.96	0.89	0.95	0.94	0.93

5.3.3 QTL Mapping for Heading Date & Photoperiod Sensitivity

5.3.3.1 Phenotypic Analysis

The heading date data recorded in the RIL mapping population at seven environments was significant with a high mean performance in dry season environments (124 to 126 days) compared to the wet season (105 to 115 days). Table 5.5 includes the range, mean, and standard deviation of the heading date along with the photoperiod and accumulated solar radiation recorded across environments in the Chandippa (CHP) and Dhantori (DHA) locations in India. The latitude differences coupled with staggered sowing dates created test environment differences for photoperiod that influenced the significant flowering variation of the RIL population with high heritability ranging from 0.40 to 0.99 (Table 5.6); the RIL population in wet season environments flowered in shorter day lengths (11.37 to 11.90 hours) compared to dry season environments (12.45 to 12.74 hours). These findings are in agreement with the observations made by Maheswaran et al. (1999), which reconfirmed the acceleration of flowering stimulus in short day conditions as an observed flowering phenomenon in short-day plants like rice.

The mean number of days to heading for the parents are shown in Table 5.7. Both parents, BRGB02489 and BRGB04267, flowered in the CHP and DHA locations with response to photoperiod changes across test environments. The photoperiod sensitive parent BRGB02489 flowered 20 to 36 days earlier in the wet season environments of CHP and DHA compared to the dry season environments of CHP. Meanwhile, parent BRGB04267 was observed to have a stable heading response in both wet and dry season environments with a flowering difference of 1 to 4

days between seasons. The mean heading date of the RILs followed the same trend in response through changes in day length in different environments (Figure 5.5 and Figure 5.6).

The degree of photoperiod sensitivity was derived from the mean difference of the heading date of the RILs and parents between wet season and dry season environments of the same latitude (CHP) and between latitudes (DHA and CHP). The delay in flowering indicated each genotype's degree of sensitivity to the photoperiod. The delay of BRGB02489 was longer than BRGB04267, which confirmed the higher degree of photoperiod sensitivity of the former genotype than the latter. The frequency distribution of the degree of sensitivity of the RILs is reported in Figure 5.7. The heading date of some RILs fell outside the range of the flowering window of parents across environments, which indicates a transgressive variation for photoperiod sensitivity among the progeny.

5.3.3.2 QTL Analysis

QTL mapping based on heading date estimated in individual wet and dry season trials led to the identification of a QTL with A and AA epistatic interaction effect for the flowering time of RILs evaluated at two different latitudes. Twenty-one putative QTLs were identified on chromosome 3, 4, 6 (6a and 6b), 7, and 11 across the wet and dry season environments (Figure 5.8 and Figure 5.9). Of these, co-localization between QTL was observed only for QTL *qFD6.1E1* and *qFD6.1E3* on chromosome 6a, although some QTL did show a similar genetic location within their respective environments.

The genetic analysis of flowering data from wet season environments of CHP (Latitude 17.4^{o}N) and DHA (Latitude 29.9^{o}N) were identified with nine QTL. Among those, seven A effect QTL were mapped on both chromosome 6a and chromosome 6b. The QTLs identified on

chromosome 6b in environment 1 (*qFD6.2E1*), 2 (*qFD6.2E2*), 3 (*qFD6.2E3*), and 4 (*qFD6.2E4*) were observed with phenotypic variance ranging from 12.3% to 18.6%. Whereas, *qFD6.1E1* (environment 1), *qFD6.1E3* (environment 3), and *qFD6.1E4* (environment 4) were mapped on chromosome 6a with an A phenotypic variance of 7.9%, 8.2%, and 8.5%, respectively (Table 5.8), and the AA epistatic interaction of *qFD3E4i* (chromosome 3) and *qFD4E4i* (chromosome 4) was observed in environment 4 (DHA-2RIL) with a phenotypic variance of 5.3% (Table 5.9).

In the Chandippa dry season, 12 QTL with A (5.9% to 13.3% variance) and AA epistatic interaction (2.0% to 8.1% variance) effects were identified in the RIL population evaluated through long day length conditions (Table 5.10 and Table 5.11). A major A effect of QTL was observed on chromosome 6b with a contribution of 11.3% (*qFD6.2E5*), 8.8% (*qFD6.2E6*), and 13.3% (*qFD6.2E7*) phenotypic variance in environments 5, 6, and 7, respectively. Additive effect QTLs (*qFD3E5, qFD6.2E5,* and *qFD7E5*) identified in environment 5 were also observed with epistatic interaction effect contributing 4.3% to heading date variation.

The QTL identified on chromosome 6a overlapped between 8.8cM and 21.8cM for heading date in wet season environments. Similarly, on chromosome 6b, QTLs were mapped within the interval of 3.8cM to 16.7cM and 9.8cM to 26.7cM in both wet and dry season environments, respectively. This overlap of QTL interval indicates that this may be a major QTL-governing flowering behavior with respect to photoperiod changes. As the values that were recorded for heading date across environments may have been affected due to the other crop management factors, variance in data may have caused this single QTL to appear as separate loci. The interaction between QTL and the environment were not found to be significant in both short (wet season) and long day (dry season) conditions (Table 5.12). Conversely, the QTL

identified in wet season environments significantly different at $P < 0.05$, which further confirms that the differential flowering response of RIL in short day environments were more pronounced than in long day conditions. Consequently, RILs carrying the allele from the photoperiod-sensitive parent BRGB02489 for the QTL identified on chromosome 6a (*qFD6.1E1, qFD6.1E3,* and *qFD6.1E4*) and chromosome 6b (*qFD6.2E4, qFD6.2E3, qFD6.2E1,* and *qFD6.2E2*) flowered two weeks earlier in short days than in long-day environments (Table 5.13). Current findings are similar to the independent studies made by Monna et al. (2002) and Lin et al. (2000), which have demonstrated the functional differences of genes mapped on chromosome 6 (*Hd3a*, *Hd3b,* and *Hd1)* with early flowering in short days and delayed late flowering in long day conditions.

The major A effect of the putative QTL for the degree of heading date sensitivity to photoperiod were mapped on chromosome 6a at the QTL interval of 15.7cM to 21.8cM (*qPP6.1C*) and 3.8cM to 14.4cM (*qPP6.1D*) with a phenotypic variance of 8.4% and 7.8%, respectively (Figure 5.10 and Table 5.14). Colocalization of QTL on chromosome 6a is an indicator of QTL consistency in conferring the major effect on the heading date response to photoperiod changes. The genetic analysis studies of heading date by Mackill DJ et al. (1993), Yokoo. M and Okuno. K (1993), Sono.Y (1983), and Maheswaram M (2000) have also identified the photoperiod-sensitive QTL on chromosome 6. Allelic distribution of A effect QTL in the selected subset of RILs with flowering variation is reported in Table 5.15.

Table 5.5: Mean, range and standard deviation (SD) of heading date and, photoperiod and accumulated solar radiation during the flowering period of recombinant inbred lines (RIL) evaluated across environments at Chandippa (CHP) and Dhantori (DHA), India.

Season	Location (Latitude & Longitude)	Environment (Trial Name)	Sowing date	Heading date (days)		Photoperiod (hrs)		Accumulated Solar Radiation (KWm^{-2})	
				Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD
Wet Season	Chandippa (17.4^0N & 78.1^0E)	Environment 1 (CHP-1RIL)	11th Jun.2015	97-143	118^B±12	11.51 - 12.25	11.90±0.19	1568 - 2311	1989±199
		Environment 2 (CHP-2RIL)	1st Jul.2015	91-152	109^D±9	11.17 – 12.01	11.72±0.13	1572 - 2642	1852±121
	Dhantori (29.9^0N & 76.8^0E)	Environment 3 (DHA-1RIL)	15th Jun.2015	83-143	115^C±13	10.90 – 12.68	11.69±0.37	443 - 865	669±90
		Environment 4 (DHA-2RIL)	6th Jul.201515	81-132	105^E±11	10.66 – 12.17	11.37±0.31	494 - 830	652±77
Dry Season	Chandippa (17.4^0N & 78.1^0E)	Environment 5 (CHP-3RIL)	6th Dec.2015	87-150	125^A±8	11.81 – 12.81	12.45±0.13	366 - 571	485±25
		Environment 6 (CHP-4RIL)	15th Dec.2015	110-141	124^A±6	12.34 – 12.81	12.56±0.10	286 - 393	332±22
		Environment 7 (CHP-5RIL)	25th Dec.2015	91-153	126^A±11	12.19 – 13.05	12.73±0.16	147 - 357	262±41

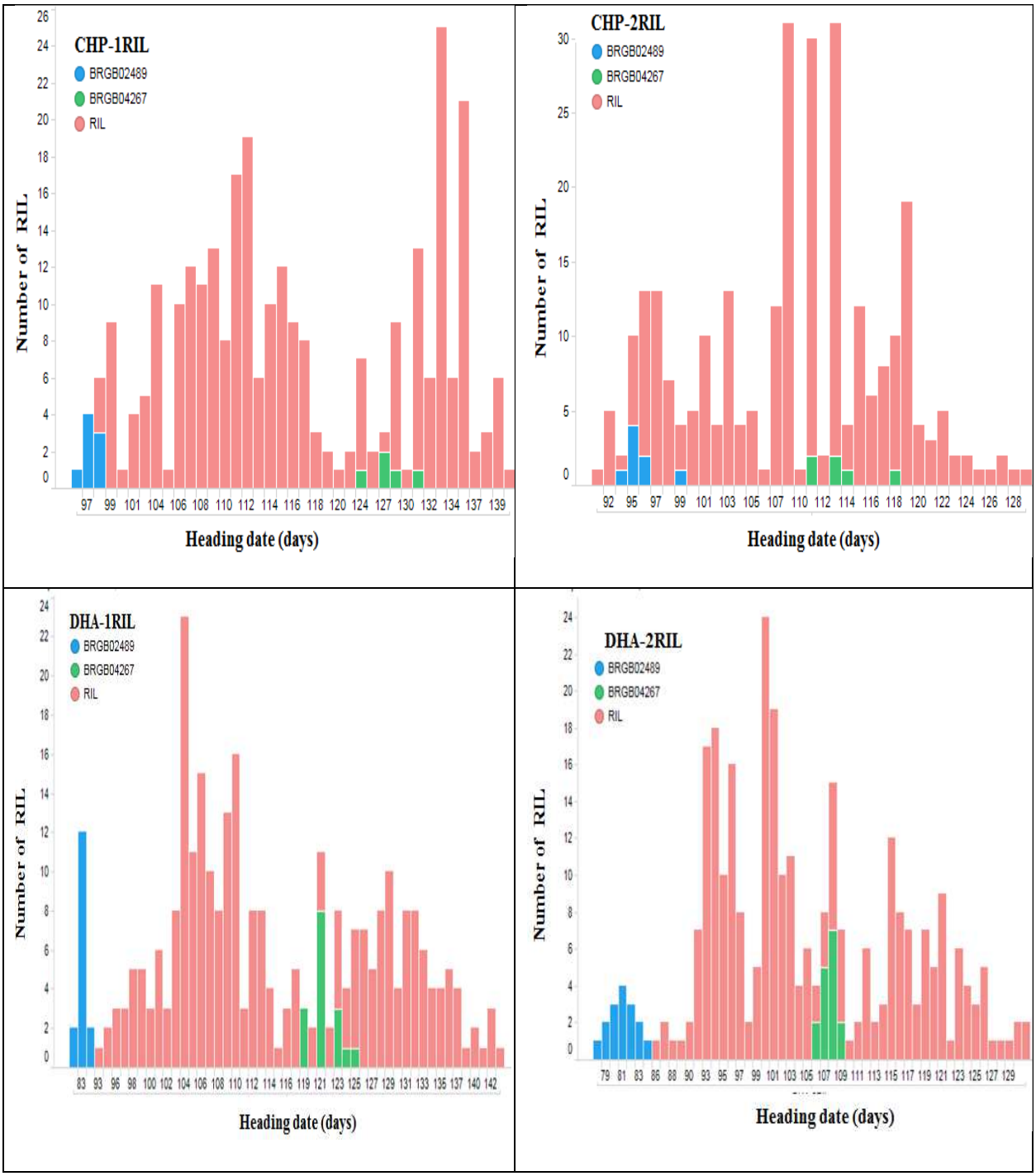
hrs: hours, KW m^{-2}: kilo watt / square meter; ^{0}N: degree north; ^{0}S: degree south

^{A} – Means within column followed by same letter are not significantly different at the 0.01 probability level

Table 5.6: Estimated phenotypic variance and heritability (H^2) of recombinant inbred line (RIL) genotype, photoperiod (hrs), accumulated solar radiation (KW m^{-2}) and photo-thermal quotient (KW m^{-2} day^{-1} ^oC) across test environments at Chandippa (CHP) and Dhantori (DHA), India.

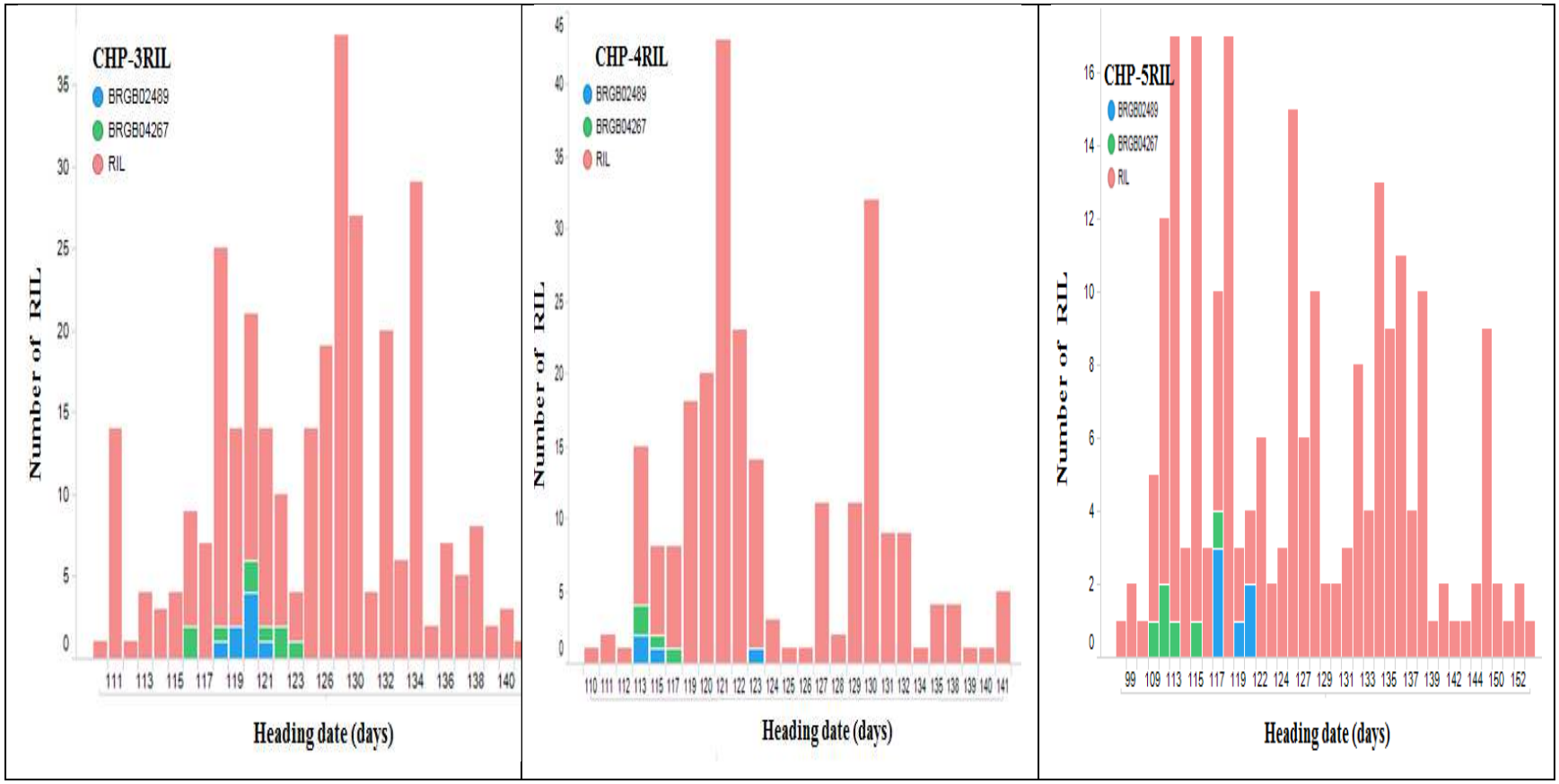
Season	Location (Latitude & Longitude)	Environment (Trial Name)	Sowing date	Genotype		Photoperiod (hrs)		Accumulated Solar Radiation (KW m^{-2})		Photo-thermal Quotient (KW m^{-2} day^{-1} ^oC)	
				Variance	H^2	Variance	H^2	Variance	H^2	Variance	H^2
Wet Season	Chandippa (17.4^0N & 78.1^0E)	Environment 1 (CHP-1RIL)	11th Jun.2015	0.04	0.00	1897.94	0.40	0.02	0.00	2764.98	0.59
		Environment 2 (CHP-2RIL)	1st Jul.2015	0.02	0.00	1245.15	0.66	0.00	0.00	618.89	0.32
	Dhantori (29.9^0N & 76.8^0E)	Environment 3 (DHA-1RIL)	15th Jun.2015	0.01	0.00	886.42	0.97	0.00	0.00	16.52	0.01
		Environment 4 (DHA-2RIL)	6th Jul.201515	0.01	0.00	491.22	0.99	0.00	0.00	0.00	0.00
Dry Season	Chandippa (17.4^0N & 78.1^0E)	Environment 5 (CHP-3RIL)	6th Dec.2015	0.01	0.00	645.78	0.87	0.00	0.00	0.00	0.00
		Environment 6 (CHP-4RIL)	15th Dec.2015	0.08	0.00	1055.10	0.99	0.01	0.00	0.00	0.00
		Environment 7 (CHP-5RIL)	25th Dec.2015	0.01	0.00	1690.36	0.42	0.03	0.00	226.78	0.56

^{0}N: degree north; ^{0}S: degree south;hrs. hours; KW m^{-2} day^{-1} ^oC: kilowatt per square meter per day; ^oC: degree Celsius ; hrs. : hours



Short day environments: CHP-1RIL: Wet Season environment 1; CHP-2RIL: Wet Season environment 2;
DHA-1RIL: Wet Season environment 3; DHA-2RIL: Wet Season environment 4.

Figure 5.5: Frequency distribution of heading date (HD) of recombinant inbred lines (RIL) evaluated across wet season environments at Chandippa (CHP) and Dhantori (DHA), India.



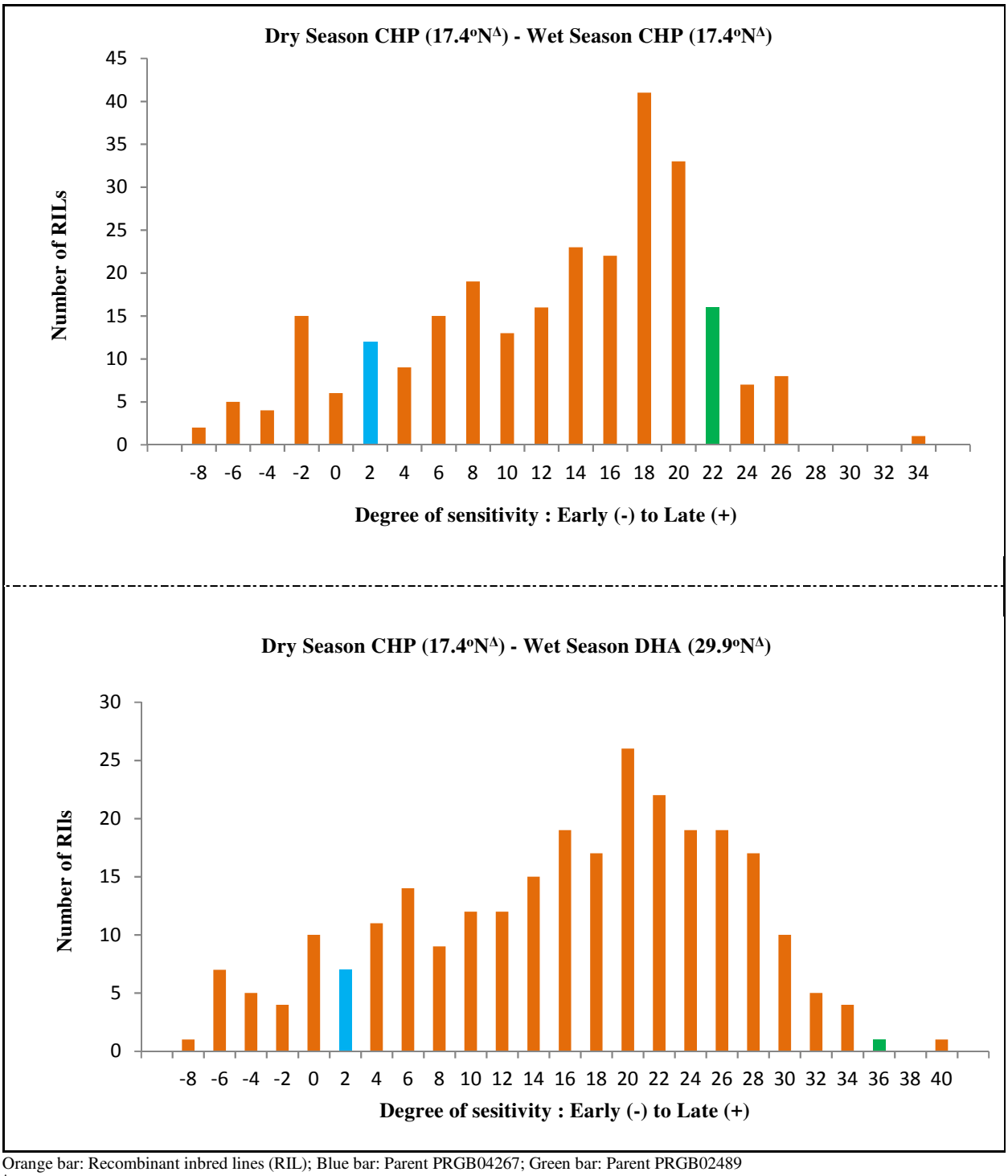
Long day environments: CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6; CHP-5RIL: Dry Season environment 7.

Figure 5.6: Frequency distribution of heading date (HD) of recombinant inbred lines (RIL) evaluated in dry season environments at Chandippa (CHP), India.

Table 5.7: Range, mean and standard deviation (SD) of heading date of recombinant inbred line parents evaluated across environments at Chandippa (CHP) and Dhantori (DHA), India.

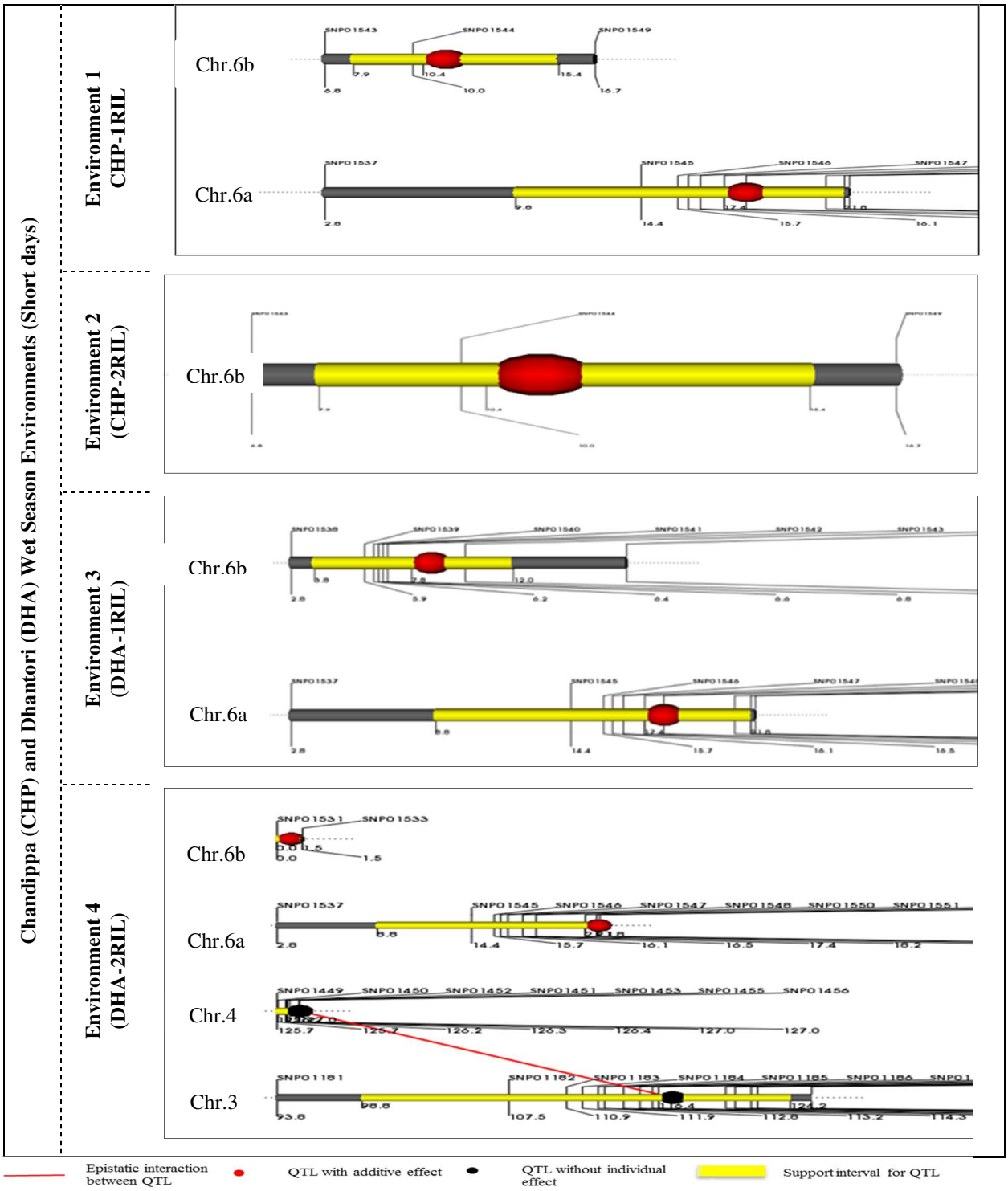
Season	Location (Latitude & Longitude)	Environment (Trial Name)	BRGB02489*			BRGB04267**		
			Heading date (Days)		Photoperiod (Hrs)	Heading date (Days)		Photoperiod (Hrs)
			Range	Mean ±SD	Mean ±SD	Range	Mean ±SD	Mean ±SD
Wet Season	Chandippa (17.4^0N & 78.1^0E)	Environment 1 (CHP-1RIL) Environment 2 (CHP-2RIL)	94-99	96±1.41	12.09±0.16	111-131	118±4.85	11.70±0.06
	Dhantori (29.9^0N & 76.8^0E)	Environment 3 (DHA-1RIL) Environment 4 (DHA-2RIL)	78-84	82±1.57	12.37±0.29	106-125	115±4.18	11.40±0.11
Dry Season	Chandippa (17.4^0N & 78.1^0E)	Environment 5 (CHP-3RIL) Environment 6 (CHP-4RIL) Environment 7 (CHP-5RIL)	113-123	118±2.66	12.47±0.29	109-123	120±4.14	12.48±0.13

* – Photoperiod sensitive parent of recombinant inbred line population;
* *– Less photoperiod sensitive parent of recombinant inbred line population.
Short day environments: CHP-1RIL: Wet Season environment 1; CHP-2RIL: Wet Season environment 2; DHA-1RIL: Wet Season environment 3; DHA-2RIL: Wet Season environment 4.
Long day environments: CHP-3RIL: Dry Season environment 5; CHP-4RIL: Dry Season environment 6; CHP-5RIL: Dry Season environment 7.



Orange bar: Recombinant inbred lines (RIL); Blue bar: Parent PRGB04267; Green bar: Parent PRGB02489
[A]: Latitude; CHP : Chandippa; DHA: Dhantori.

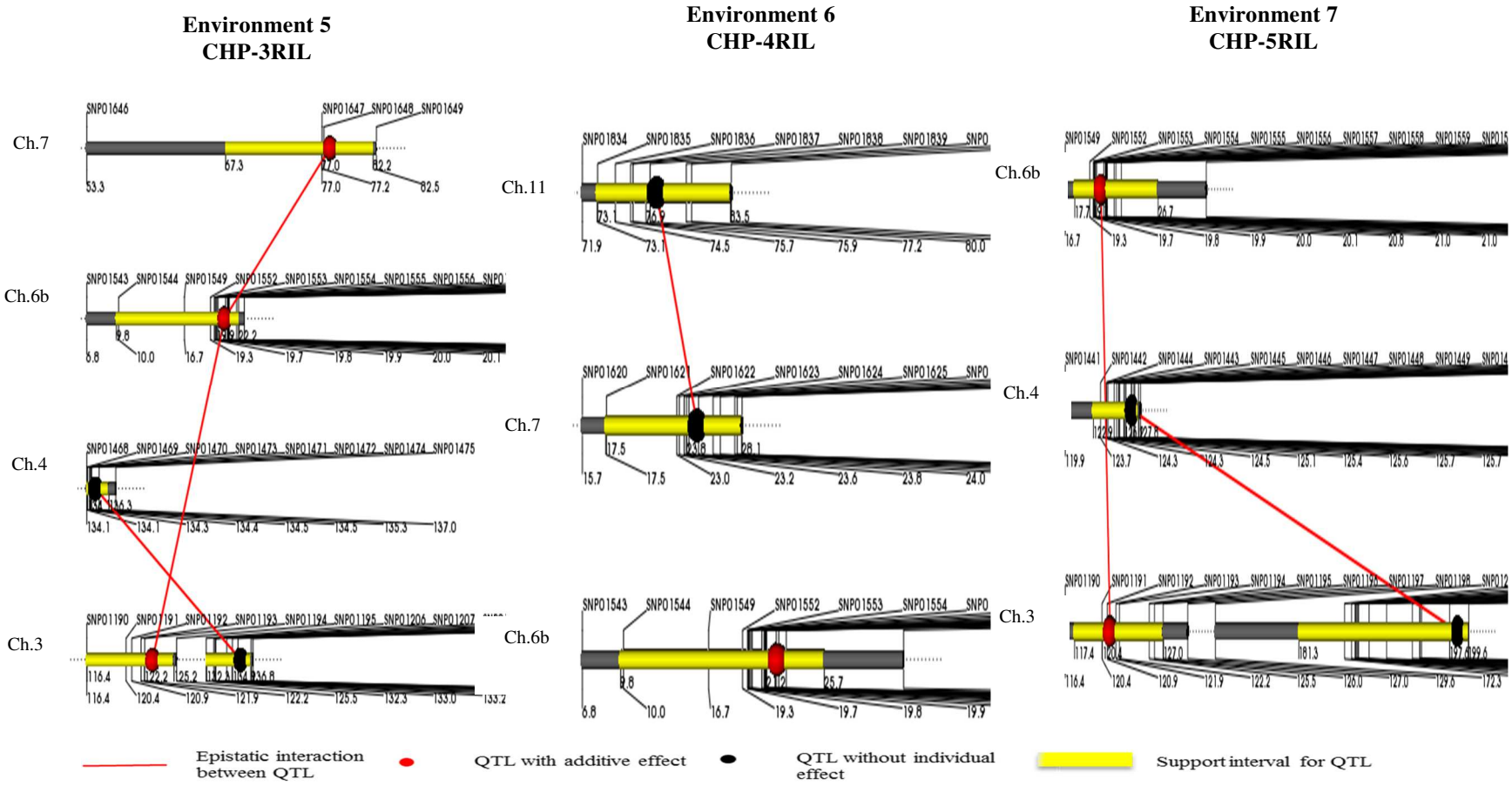
Figure 5.7: Frequency distribution of photoperiod response (degree of sensitivity) derived from the difference in heading date of each recombinant inbred line between wet and dry season environment data recorded in Chanippa(CHP) and Dhantori (DHA) locations in India.



Putative QTL Size: proportionate to the phenotypic variance explained by the QTL: QTL without individual effect: only epistatic interaction between QTL without additive effect.

Figure 5.8: Predicted genetic architecture of putative heading date quantitative trait loci (QTL) mapped in recombinant inbred line (RIL) population evaluated in wet season environments at Chandippa (CHP) and Dhantori (DHA), India.

Chandippa (CHP) Dry Season Environments (Long days)



Putative QTL Size: proportionate to the phenotypic variance explained by the QTL: QTL without individual effect: only epistatic interaction between QTL without additive effect.

Figure 5.9: Predicted genetic architecture of putative heading date quantitative trait loci (QTL) mapped in recombinant inbred line (RIL) population evaluated in dry season environments at Chandippa (CHP), India.

Table 5.8: Quantitative trait loci (QTL) identified with additive effect (A), standard error (SE), F-value, P-value and variance (h^2) for heading date of recombinant inbred line population (RIL) evaluated across wet season environments at Chandippa (CHP) and Dhantori (DHA), India.

Environment (Trial Name)	QTL Name	Chromosome	Peak marker Position (cM)	QTL Interval (cM)	A[a]	SE	F Value	P-Value	h^2(a)[b]
Environment.1 (CHP-1RIL)	*qFD6.1E1*	6a	17.4	9.8-21.8	-3.202	0.655	18.48	0.000001	0.079
Environment.1 (CHP-1RIL)	*qFD6.2E1*	6b	8.8	5.9-14.0	-5.264	0.666	61.79	0.000001	0.186
Environment.2 (CHP-2RIL)	*qFD6.2E2*	6b	12	9.8-16.7	-3.843	0.525	52.75	0.000	0.170
Environment.3 (DHA-1RIL)	*qFD6.1E3*	6a	17.4	8.8-21.8	-3.436	0.714	20.2	0.000001	0.082
Environment.3 (DHA-1RIL)	*qFD6.2E3*	6b	7.8	3.8-12.0	-4.394	0.726	35.68	0.000	0.123
Environment.4 (DHA-2RIL)	*qFD6.1E4*	6a	21.1	8.8-21.8	-2.142	0.599	16.33	0.00035	0.085
Environment.4 (DHA-2RIL)	*qFD6.2E4*	6b	0	0.0-1.5	-4.363	0.598	40.15	0.000	0.170

[a]: Additive effect: positive values indicated that parent 1 allele for an increase in the trait, negative value indicated that parent 2 allele contributed an increase in the trait value.
[b]: Contribution explained by putative main-effect QTL.

Table 5.9: Quantitative trait loci (QTL) identified with additive interaction (AA), standard error (SE), F-value, P-value and variance (h^2) for heading date of recombinant inbred line population (RIL) evaluated across wet season environments at Chandippa (CHP) and Dhantori (DHA), India.

Environment (Trial Name)	QTL_i				QTL_j							
	QTL	Chr.	Position (cM)	Interval (cM)	QTL	Chr.	Position (cM)	Interval (cM)	AA[a]	SE	P-Value	h^2(aa)[b]
Environment. 4 (DHA-2RIL)	*qFD3E4i*	3	116.4	98.8-124.2	*qFD4E4i*	4	126.2	125.7-127.0	2.706	0.605	0.000008	0.053

* QTL having both additive and epistasis effect, cM: centi morgan

[a]: Epistatic effect: positive values indicated that parent 1 allele interaction for an increase in the trait, negative value indicated that parent 2 allele interaction contributed an increase in the trait value.

[b]: Contribution explained by putative epistatic additive x additive interaction -effect QTL.

Table 5.10: Quantitative trait loci (QTL) identified with additive effect (A), standard error (SE), F-value, P-value and variance (h^2) for heading date of recombinant inbred line population (RIL) evaluated across dry season environments of Chandippa (CHP), India.

Environment (Trial Name)	QTL Name	Chromosome	Peak marker Position (cM)	QTL Interval (cM)	A[a]	SE	F Value	P-Value	h^2(a)[b]
Environment.5 (CHP-3RIL)	*qFD3E5*[*]	3	122.2	116.4-125.2	1.649	0.438	16.73	0.000168	0.059
Environment.5 (CHP-3RIL)	*qFD6.2E5*[*]	6b	19.9	9.8-22.2	-2.789	0.439	30.51	0.000	0.113
Environment.5 (CHP-3RIL)	*qFD7E5*[*]	7	77	67.3-82.2	-1.705	0.438	15.26	0.000099	0.065
Environment.6 (CHP-4RIL)	*qFD6.2E6*	6b	21.2	9.8-25.7	-1.727	0.392	24.65	0.000011	0.088
Environment.7 (CHP-5RIL)	*qFD3E7*	3	120.4	117.4-127.0	2.342	0.682	15.68	0.000596	0.062
Environment.7 (CHP-5RIL)	*qFD6.2E7*[*]	6b	19.7	17.7-26.7	-4.123	0.685	31.04	0.000	0.133

* QTL with both additive and epistasis interaction effect, cM: centi morgan

[a]: Additive effect: positive values indicated that parent 1 allele for an increase in the trait, negative value indicated that parent 2 allele contributed an increase in the trait value.

[b]: Contribution explained by putative main-effect QTL.

Table 5.11: Quantitative trait loci (QTL) identified with additive interaction (AA), standard error (SE), F-value, P-value and variance (h^2) for heading date of recombinant inbred line population (RIL) evaluated across dry season environments of Chandippa (CHP), India.

	QTL_i				QTL_j							
Environment (Trial Name)	**QTL**	**Chr.**	**Position (cM)**	**Interval (cM)**	**QTL**	**Chr.**	**Position (cM)**	**Interval (cM)**	**AA[a]**	**SE**	**P-Value**	**h^2(aa)[b]**
Environment. 5 (CHP-3RIL)	*qFD3E5**	3	122.2	116.4-125.2	*qFD6.2E5**	6b	19.9	9.8-22.2	0.91	0.442	0.039718	0.02
Environment. 5 (CHP-3RIL)	*qFD3E5i*	3	134.9	132.3-136.8	*qFD4E5i*	4	134.1	134.1-136.3	1.803	0.438	0.000039	0.045
Environment. 5 (CHP-3RIL)	*qFD6.2E5**	6b	19.9	9.8-22.2	*qFD7E5**	7	77	67.3-82.2	-1.303	0.444	0.003359	0.023
Environment. 6 (CHP-4RIL)	*qFD7E6i*	7	23.8	17.5-28.1	*qFD11E6i*	11	76.9	73.1-83.5	1.774	0.394	0.000007	0.065
Environment. 7 (CHP-5RIL)	*qFD3E7i*	3	120.4	117.4-127.0	*qFD6.2E7**	6b	19.7	17.7-26.7	1.987	0.689	0.003926	0.026
Environment.7 (CHP-5RIL)	*qFD3E7i*	3	197.6	181.3-199.6	*qFD4E7i*	4	126.2	122.9-127.8	-3.49	0.701	0.000001	0.081

* QTL having both additive and epistasis effect, cM: centi morgan

[a]: Epistatic effect: positive values indicated that parent 1 allele interaction for an increase in the trait, negative value indicated that parent 2 allele interaction contributed an increase in the trait value.

[b]: Contribution explained by putative epistatic additive x additive interaction -effect QTL.

Table 5.12: Analysis of variance of heading date quantitative trait loci (QTL) mapped across wet season and dry season environments at Chandippa (CHP) and Dhantori(DHA), India.

Wet Season Environments

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	3	222224.7	74074.88	618.73**	<.0001
QTL	8	2203.01	220.301	1.840*	0.0487
Environment x QTL	24	1.55	0.051667	0.0004NS	1
Allele	1	20933.38	20933.38	174.852**	<.0001
QTL x Allele	8	22631.06	2263.106	18.903**	<.0001

Dry Season Environments

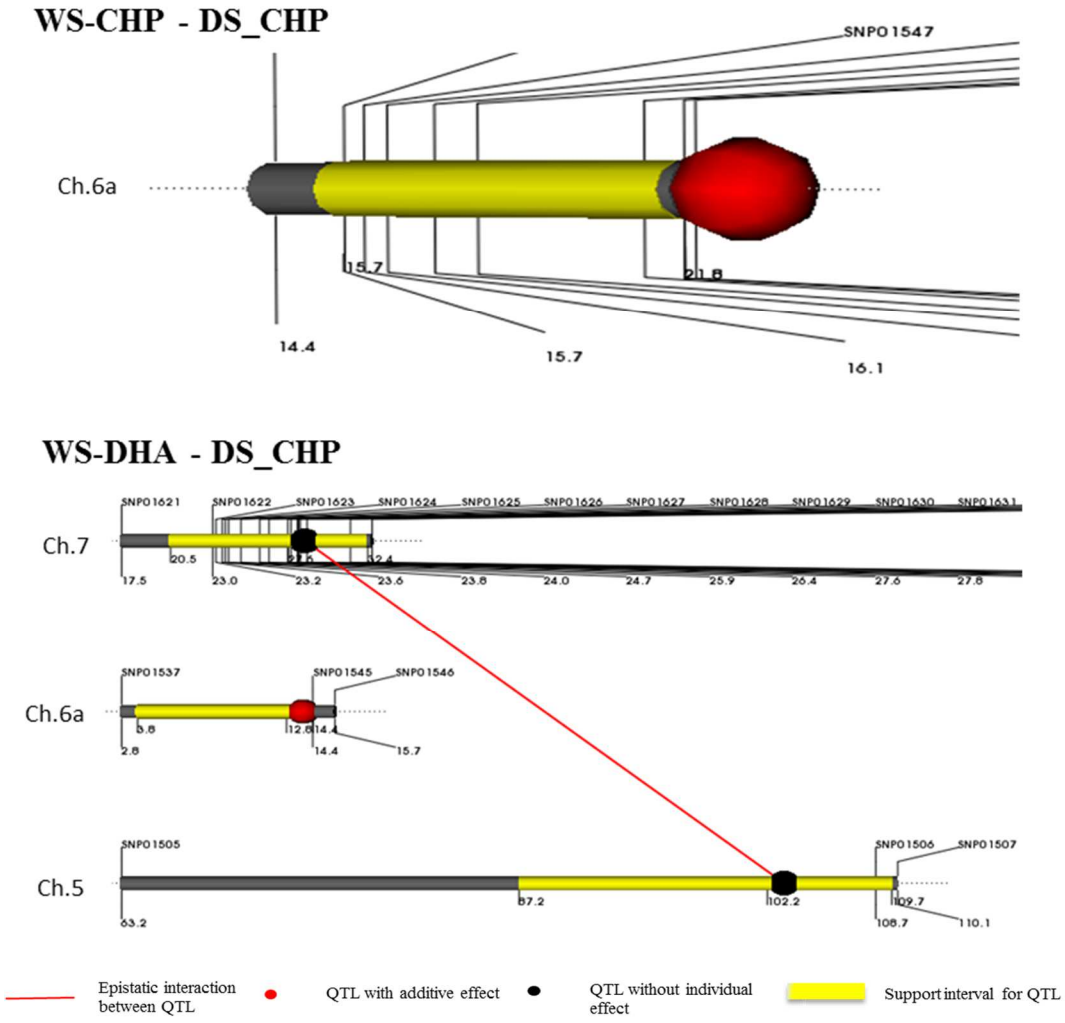
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	2	4835.6	2417.8	16.032**	<.0001
QTL	11	2751.4	250.1	0.3317NS	0.9729
Environment x QTL	22	128.3	6.417	0.0043NS	1
Allele	1	4004.645	4004.645	53.1088**	<.0001
QTL x Allele	11	143801.6	13072.87	17.337**	<.0001

*,** Significant at 0.05 and 0.01 probability level respectively; NS not significant at probability level 0.05

Table 5.13: Quantitative trait loci (QTL) and their effect of BRGB02489 (photoperiod sensitive parent) and BRGB04267 (less photoperiod sensitive parent) allele on mean heading date (HD) of recombinant inbred lines evaluated across short day (SD) and long day (LD) environments at Chandippa (CHP) and Dhantori (DHA) locations, India.

Chromosome No.	QTL	Peak marker position cM	QTL Interval cM	Mean HD in SD environments		Mean HD in LD environments	
				BRGB02489	BRGB04267	BRGB02489	BRGB04267
3	*qFD3E4i***	116.3	98.8 – 124.2	112	111	126	123
3	*qFD3E7*	120.3	117.4 – 127.0	112	111	126	123
3	*qFD3E5**	122.2	116.4 – 125.2	112	111	126	123
3	*qFD3E5i***	134.9	132.3 – 136.8	112	111	126	123
3	*qFD3E7i***	19.7	17.7 – 26.7	111	112	125	124
4	*qFD4E4i***	126.1	125.7-127.0	112	112	-	-
4	*qFD4E7i***	126.2	122.9 – 127.8	112	112	125	124
4	*qFD4E5i***	134.1	134.1 – 136.3	111	112	125	124
6a	*qFD6.1E1*	17.4	9.8 – 21.8	108	115	124	125
6a	*qFD6.1E3*	17.4	8.8 – 21.8	108	115	124	125
6a	*qFD6.1E4*	21.1	8.8 – 21.8	108	115	124	125
6b	*qFD6.2E4*	0.0	0.0 – 1.5	107	116	122	128
6b	*qFD6.2E3*	7.8	3.8 – 12.0	108	116	122	128
6b	*qFD6.2E1*	8.8	5.9 – 14.0	108	116	122	128
6b	*qFD6.2E2*	12.0	9.8 – 16.7	108	116	122	128
6b	*qFD6.2E7**	19.7	17.7 – 26.7	108	118	122	128
6b	*qFD6.2E5**	19.8	9.8 – 22.2	108	116	122	128
6b	*qFD6.2E6*	21.2	9.8 – 25.7	89	112	118	125
7	*qFD7E6i***	23.8	17.5 – 28.1	112	112	126	124
7	*qFD7E5**	77.0	67.3 – 82.2	110	113	123	127
11	*qFD11E6i*	76.9	73.1 – 83.5	112	112	125	124

*:QTL having both additive and epistatic interaction effect, cM: centi morgan,
**: QTL having epistatic interaction effect.



Putative QTL Size: proportionate to the phenotypic variance explained by the QTL: QTL without individual effect: only epistatic interaction between QTL without additive effect.

Figure 5.10: Predicted genetic architecture for photoperiod sensitivity on heading days of recombinant inbred lines evaluated in wet season (WS) and dry season (DS) environments in Chandippa (CHP) and Dhantori (DHA) locations, India.

Table 5.14: Quantitative trait loci (QTL) identified with additive effect (A) and additive interaction effects (AA), standard error (SE), F-value, P-value and variance (h^2) for photoperiod sensitivity to heading date with the differential flowering of recombinant inbred lines evaluated in wet season (WS) and dry season (DS) environment at Chandippa (CHP) and Dhantori (DHA), India.

Source	WS_CHP-DS_CHP	WS_DHA-DS_CHP		
	Additive Effect	Additive Effect	Epistatic interaction	
Chromosome	6a	6a	5	7
QTL	*qPP6.1C*	*qPP6.1D*	*qPP5Di*	*qPP7Di*
Position	21.8	12.8	102.2	27.6
Support interval	15.7-21.8	3.8-14.4	87.2-109.7	20.5-32.4
A[a]	-2.4312	-3.1292	-	
AA[b]	-	-	-3.8178	
Standard error	0.4928	0.6064	0.7382	
P-Value	0.000	0.000	0.000	
h^2(a)[c]	0.0846	0.078	-	
h^2(aa)[d]	-	-	0.0851	

* QTL having both additive and epistasis effect, cM: centi morgan

[a]: Additive effect: positive values indicated that parent 1 allele for an increase in the trait, negative value indicated that parent 2 allele contributed an increase in the trait value.

[b]: Epistatic effect: positive values indicated that parent 1 allele interaction for an increase in the trait, negative value indicated that parent 2 allele interaction contributed an increase in the trait value.

[c]: Contribution explained by putative main-effect QTL.

[d]: Contribution explained by putative epistatic additive x additive interaction -effect QTL.

Table 5.15: Recombinant inbred lines (BYRIL) with different combinations of major heading date and photoperiod sensitivity quantitative trait loci (QTL) and its allelic effect on flowering behavior.

Genotype	Photoperiod QTL		Photoperiod QTL						Heading date (Days)		
	qPP6.1D	*qPP6.1C*	*qFD6.2E4*	*qFD6.2E1 & qFD6.2E3*	*qFD6.2E2*	*qFD6.2E7*	*qFD6.2E5*	*qFD6.2E6*	Range	Average	SD
BRGB02489	+	+	+	+	+	+	+	+	81-120	102	16.52
BRGB04267	-	-	-	-	-	-	-	-	108-128	117	6.67
BYRIL133	+	+	+	+	+	+	+	+	94-138	109	14.46
BYRIL267	+	+	+	+	+	+	+	+	96-128	109	13.35
BYRIL270	+	+	+	+	+	+	+	+	90-122	103	11.89
BYRIL206	+	+	+	+	+	+	+	+	92-119	104	11.36
BYRIL244	+	+	+	+	+	+	+	+	93-121	104	11.10
BYRIL023	+	+	-	-	-	-	-	-	93-132	117	15.76
BYRIL022	+	+	-	-	-	-	-	-	94-132	115	15.12
BYRIL295	+	+	-	-	-	-	-	-	97-136	116	14.81
BYRIL252	+	+	-	-	-	-	-	-	99-133	113	14.78
BYRIL086	+	+	-	-	-	-	-	-	94-130	112	14.54
BYRIL203	-	-	+	+	+	+	+	+	94-121	109	8.88
BYRIL001	-	-	+	+	+	+	+	+	95-118	109	8.92
BYRIL029	-	-	+	+	+	+	+	+	94-120	108	9.29
BYRIL204	-	-	+	+	+	+	+	+	94-122	108	9.37
BYRIL004	-	-	+	+	+	+	+	+	102-124	111	9.40
BYRIL198	-	-	-	-	-	-	-	-	121-138	131	5.83
BYRIL059	-	-	-	-	-	-	-	-	119-135	129	6.07
BYRIL006	-	-	-	-	-	-	-	-	128-142	134	6.12
BYRIL120	-	-	-	-	-	-	-	-	127-145	137	6.13
BYRIL095	-	-	-	-	-	-	-	-	119-138	130	6.81

"+" BRGB02489 allele & " – " BRGB04267 allele ; PP: Photoperiod QTL ; FD: Flowering time QTL

5.3.4 QTL Mapping for Pollen Count (Pollen Load)

5.3.4.1 Phenotypic Analysis

The pollen load data recorded in the dry season environments of Chandippa (CHP) were found to be significant between the RIL genotypes with mean numbers of fertile pollen of 80.0, 80.1, and 90.6 in environment 5, 6, and 7, respectively. The pollen count of BRGB02489 (high pollen load parent) was 19.8% to 25.4% higher compared to BRGB04267 (low pollen load parent). Among the environments tested, environment 7 was significantly different from the other two test environments with a highest experimental mean of 92.5 (Table 5.16). The frequency distribution of the least mean square data was normally distributed (Figure 5.11) with an overall mean of 80.73 fertile pollen across environments.

5.3.4.2 QTL Analysis

The mixed linear composite interval mapping study of the RIL population for fertile pollen load revealed a major significant A effect QTL (*qPL9*) ($P < 0.00001$) (Figure 5.12). The QTL and *qPL9* were mapped on chromosome 9 within a QTL interval of 132cM to 143.1cM, which explained the phenotypic variance of 8.7% (Table 5.17). The subset of RILs carrying the BRGB02489 and BRGB04267 alleles for *qPL9* are presented in Table 5.18. While the QTL identified has a major A effect and its relative contribution to the variance of the total phenotype was moderate. No relevant literature was found to support the present findings on fertile pollen number. However, the heat tolerance mapping study for spikelet fertility conducted by Shanmugavadivel et al. (2017) found significant QTL on chromosome 9 associated with spikelet sterility. Their finding indicates that this region controls high pollen production even under heat stress conditions. Furthermore, Sheng Ling et al. (2015) identified a mature anther-preferential

expressed gene (*OsSTA*) on chromosome 9 that showed 10 - 100 times higher expression in mature anthers than in other tissues. Thus, *qPL9* on chromosome 9 has shown indirect relevance with other findings of pollen fertility-related studies.

Table 5.16: Mean, range, standard deviation (SD) and heritability (H^2) of pollen count data observed in recombinant inbred line (RIL) population and parents (BRGB02489 & BRGB04267) studied in dry season environments at Chandippa (CHP), India.

Genotype	Source	Environment 5 CHP-3RIL	Environment 6 CHP-4RIL	Environment 7 CHP-5RIL
BRGB02489	Range	68 - 87	63 - 81	111 - 114
	Mean	80.5	73.8	126.1
	SD	6.99	6.25	11.65
BRGB04267	Range	48 - 70	46 - 67	76 - 121
	Mean	60.0	58.4	101.1
	SD	7.07	8.07	15.48
RIL	Range	2 - 190	1 - 189	12 - 190
	Mean	80.0	80.1	90.6
	SD	43.28	34.36	38.12
Experimental Mean ± Standard Error		80.7^{B} ±2.05	81.2^{B} ±1.51	92.5^{A} ±1.83
H^2		0.74	0.50	0.79

[A]: Same letter in a row indicate no significant difference between environments at P<0.05.

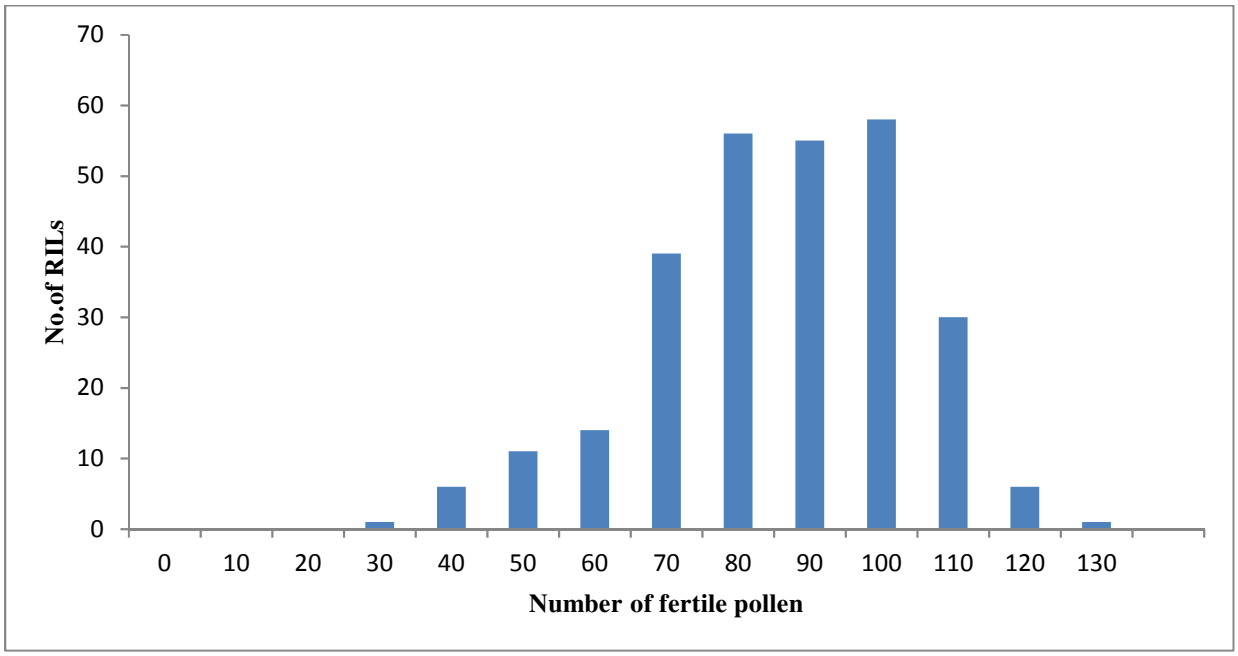


Figure 5.11: Frequency distribution of fertile pollen count data observed in recombinant inbred lines (RIL) evaluated in dry season environments at Chandippa (CHP), India.

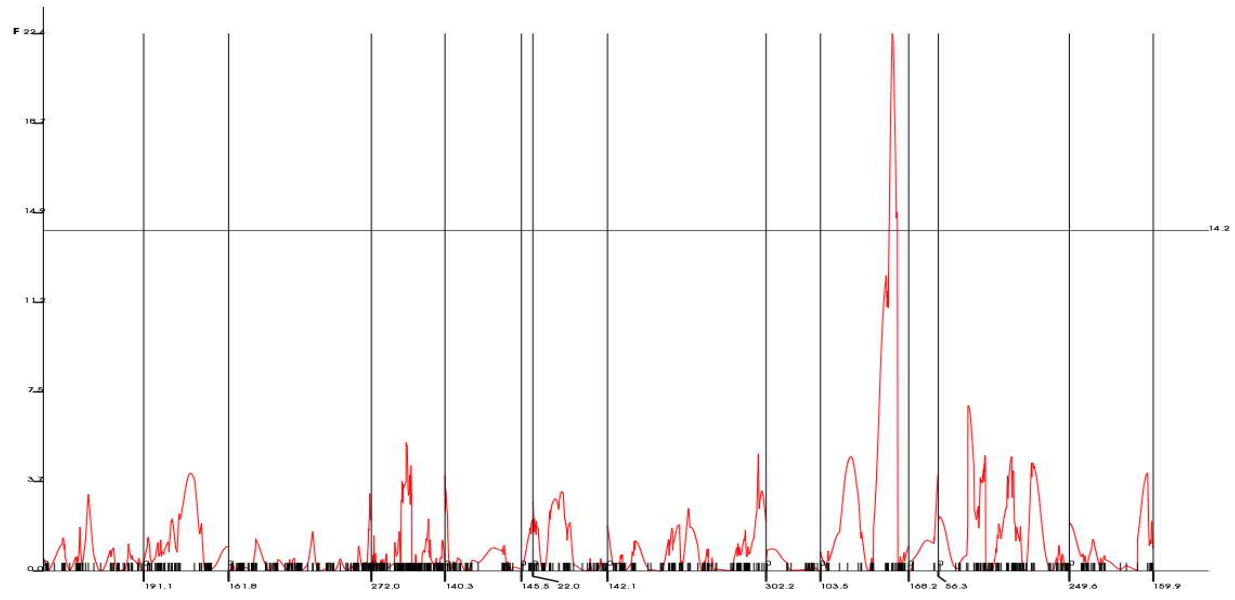


Figure 5.12: Quantitative trait loci (QTL) plot of pollen count in recombinant inbred line mapping population evaluated at Chandippa (CHP), India.

Table 5.17: Quantitative trait locus (QTL) identified with additive effect (A),), standard error (SE), F-value, P-value and variance (h^2) for pollen count in a recombinant inbred line (RIL) mapping population studied in dry season 2015 at Chandippa (CHP), India.

QTL	Chromosome	Position (cM)	Interval (cM)	A[a]	SE	F Value	P-Value	h^2(a)[b]
qPL9	9	136.9	132 - 143.1	5.4460	1.0874	22.4	0.00001	0.087

[a]: Additive effect: positive values indicated that parent 1 allele for an increase in the trait, negative value indicated that parent 2 allele contributed an increase in the trait value.
[b]: Contribution explained by putative main-effect QTL.

Table 5.18: Performance of recombinant inbred line genotypes (BYRIL) for pollen count with and without presence of pollen load quantitative trait locus (*qPL9*) identified in recombinant inbred line mapping population studied in dry season 2015 at Chandippa (CHP), India.

Genotype	*qPL9* (+)	Genotype	*qPL9* (-)
	No. of pollen		No. of pollen
BRGB02489	74±6.30	BRGB04267	58±8.15
BYRIL241	116±21.36	BYRIL039	50±15.10
BYRIL016	105±22.65	BYRIL103	50±18.50
BYRIL241	116±21.36	BYRIL138	61±19.25
BYRIL016	105±22.65	BYRIL138	61±19.25
BYRIL102	98±24.91	BYRIL021	55±19.31
BYRIL248	96±20.22	BYRIL042	54±19.52
BYRIL194	93±14.99	BYRIL085	53±19.85
BYRIL194	93±14.99	BYRIL197	63±20.38
BYRIL226	90±21.19	BYRIL146	65±20.41
BYRIL229	88±22.28	BYRIL146	65±20.41

"+" BRGB02489 allele & " –" BRGB04267 allele

5.4 Conclusions

The genetic analysis in the BPH resistance study deciphered the effect of QTL on multiple modes of host-plant resistance. The major additive effect QTL identified on chromosome 4 was co-localized with a cluster of different resistance genes and QTL, namely *Bph6, Bph12, Bph15, Bph17, Bph20,* and *Bph27* (Jena et al., 2006; Jie Hu et al., 2016). Although the QTLs mapped in the current population explained the total phenotypic variance of 10.6%, they could also be pyramided with other BPH genes and tested for efficacy in conferring resistance to new evolving biotypes of BPH. In order to achieve stable broad-spectrum resistance to BPH, pyramiding major genes or QTL may provide durable resistance and improve yield potential of cultivars or hybrids.

As expected, the flowering behavior of RILs responded to photoperiod changes in the wet and dry season environments at latitudes 17.4^0N (Chandippa) and 29.9^0N (Dhantori). Similar to the parents, long day conditions in dry season environments decreased the ability to discern phenotypic differences among genotypes. Contrary to long day environments, short days in the Chandippa and Dhantori wet season exhibited phenotypic variation with photoperiod response. Despite the reduced variation of parents in long days at Chandippa, it was possible to detect a major additive effect QTL on chromosome 6b with 8.8% to 11.3% phenotypic variance. While in the short-day environments of Chandippa and Dhantori, QTL mapped on the same chromosome 6b explained the phenotypic variance of 12.3% to 18.6%. Interestingly, QTLs identified on chromosome 6b were mapped between 0.0 cM to 26.7 cM in both short and long day

environments. This finding indicates that, a major heading date QTL is associated with early and late flowering behavior of RILs despite the photoperiod changes.

The photoperiod sensitivity QTL identified on chromosome 6a with a degree of photoperiod sensitivity data was collinear with the heading date QTL that was identified in short day environments of Chandippa and Dhantori. It is evident from this study that phenotypic variation observed with decreased day lengths in wet season environments improved the ability to detect the QTL. Genetic diversity within the population was present in the long and short-day environments and is confirmed by our identification of photoperiod-sensitive QTL with the degree of photoperiod sensitivity in the data. Although QTLs identified in the present study were not unique from previous studies (Jarillo J.A. 2008), by analyzing more photoperiod-sensitive populations in environments with varying day lengths provides a higher level of validation for QTL if they are present in multiple populations. This confidence can support practical use in breeding as well as justify gene cloning. It can be further concluded from heading date mapping results that RILs carrying the BRGB04267 allele for QTL mapped on chromosome 6a and the BRGB02489 allele of QTL on chromosome 6b were found to be stable and flowered earlier than their reciprocal combination.

The mapping study of the pollen load was characterized with a significant additive effect QTL on chromosome 9 associated with the fertile pollen number variation in the present RIL population. However, RILs carrying the QTL allele for high pollen parent explained 8.7% of the phenotypic variance. Gene expression studies conducted by Sheng Ling et al. (2015) identified mature anther-preferentially expressed

genes (*OsSTA*) on chromosome 9. The feasibility of the pollen load QTL identification in the RIL population can be validated through association studies by identifying similar traits in individuals with more diverse pollen loads. The QTL identified in this study can also be validated through similar studies using diverse RIL populations or environments.

The present study is an example of an exploration of multiple traits from a single donor source. The identification of the promising lines for BPH resistance, flowering behavior, and pollen load from the mapping population can be used in forward breeding to develop new restorer germplasm with trait. The development of functional SNPs associated with BPH resistance, heading date, photoperiod sensitivity, and pollen load could provide additional genomic tools to enable marker-assisted breeding for germplasm improvement for stable flowering behavior, high pollen load, and BPH resistance.

CHAPTER VI

IDENTIFYING PROMISING RESTORER GENOTYPES FROM A POPULATION WITH GOOD COMBINING ABILITY FOR PHOTOPERIOD INSENSITIVITY, BROWN PLANT HOPPER RESISTANCE, AND YIELD HETEROSIS

6.1 Introduction

Rice (*Oryza Sativa* L.) is a staple food for more than half of the global population. However, it is essential to enhance rice yield potential to achieve the global rice requirement for year 2025 of 900 million tons (Hossain, 1995). Hybrid rice breeding technology is a practical, feasible, and sustainable approach to enhance genetic yield potential. Breeding strategies based on the selection of hybrids require an expected level of heterosis as well as a specific combining ability and adequate floral characteristics. The performance of parental lines does not guarantee adequate combining ability per se. Combining ability analysis is a powerful tool available to aid the selection of desirable parents for exploiting heterosis (Sarker et al., 2002; Rashid et al., 2007), though this tool requires knowledge of gene effects and their roles in combining ability. General combining ability (GCA) is attributed to additive gene effects and additive x additive epistasis, and it is fixable. On the other hand, specific combining ability (SCA) attributed to non-additive gene action may be due to dominance, epistasis, or both, and it is non-fixable. The presence of non-additive genetic variance is the primary justification for initiating a hybrid program (Cockerham, 1961; Pradhan et al., 2006). There is a need to

study various morphological traits associated with hybrid performance to have a stronger understanding of inheritance and to select or identify superior genotypes for the hybrid crossing blocks. Heritability values have varied depending upon the genetic nature of genotypes for different morphological characters (Mahto et al., 2003; Swati and Ramesh, 2004). Heterosis estimates were attributed to both additive and high dominance variance, epistatic interactions, or both for one or more morphological traits.

The present study was conducted to evaluate the hybrids derived from restorer lines (RILs of BRGB04267 X BRGB02489) and CMS female testers BRGB07288A and BRGB06355A. Hybrid evaluation trials with test genotypes and checks were conducted at three locations (Chandippa, Dhantori, and Faizabad) with the following objectives:

1. Identify high heterotic restorer RILs with good GCA and SCA by BLUP estimates.
2. Identify GCA and SCA restorer RILs with stable flowering behavior, high pollen load, and BPH resistance.
3. Classify high heterotic lines that carry QTL for increased pollen load, lack of photoperiod response, and increased BPH resistance.

6.2 Materials and Methods

6.2.1 Plant Material

6.2.1.1 Hybrid Production

The test hybrid seed was produced in 250 isolations in the 2014 dry season at the Multicrop Breeding Station, Bayer Bioscience, Chandippa, Hyderabad, India (Figure 6.1). Each isolation comprised 10 plants of individual RIL ($F_{2:7}$) that were planted twice at 10-day intervals and 5 plants each of 2 CMS female testers BRGB07288A and BRGB06355A. Our team harvested 472 hybrids derived from the two testers based on the outcrossing rate for conducting a hybrid evaluation trial in the 2015 wet season.

Figure 6.1: Hybrid seed production in cages at the Multicrop Breeding Station, Bayer Bioscience, Chandippa (CHP), Hyderabad, India.

6.2.1.2 Test Genotypes

The yield evaluation trial was carried out with 472 hybrids derived from the cross BRGB07288A X RILs and BRGB06355A X RILs as test entries. Hybrids derived from parents BRGB04267 and BRGB02489 with the same female testers (BRGB07288A and BRGB06355A) and Arize 6444 Gold (commercial hybrid) were used as checks.

6.2.2 Experimental Design

The hybrid yield performance trial was conducted in the wet season 2015 at three locations namely the Multicrop Breeding Station, Bayer Bioscience, Chandippa, Hyderabad, India; the Bayer Bioscience hybrid testing location, Raipur, Chhattisgarh, India; and the Bayer Bioscience hybrid testing location, Faizabad, Uttar Pradesh, India. Each trial was constructed in a modified augmented design in a single replication with replicated spatial checks to estimate the error mean square and block effects. The number of test entries in each location varied because of available seed quantities. Therefore, the blocks and number of check replications were adjusted according to the trial size to achieve the required error degrees of freedom (Table 6.1).

Table 6.1: Experimental details of yield performance trial conducted at Chandippa (CHP), Raipur (RP) and Faizabad (FZB) locations in India.

Parameter	Chandippa	Raipur	Faizabad
Latitude	17.4°N	21.2°N	26.7°N
Longitude	78.1°E	81.6°E	82.1°E
Number of Test Entries	472	421	345
Number of Checks (C)	5	5	5
Number of Blocks (B)	5	5	4
Number of check repeats in Block (R)	3	3	4

6.2.3 Trait Evaluation

6.2.3.1 Heading Date (HD)

Heading date notes were taken for all trial entries as the number of days from sowing until 50% of plants' panicles fully emerged from the boot.

6.2.3.2 Pant Height (PH)

Plant height was measured in centimeters from the base of plants to the panicle tip at the stage of physiological maturity.

6.2.3.3 Panicle Bearing Tillers (PBT)

Panicle-bearing tillers were counted from five random plants in each hybrid to derive the average number of panicle-bearing tillers.

6.2.3.4 Spikelet Fertility % (SF)

The main panicles from five random plants were collected within each test entry at physiological maturity. Based on counting the filled and unfilled spikelet from each panicle, the spikelet fertility percentage was derived using the following formula.

$$\text{Spikelet fertility \% (SF)} = \frac{Number\ of\ filled\ spiklets}{(Number\ of\ filled\ spkelets + Number\ of\ unfilled\ spikelets)}$$

6.2.3.5 Panicle length (PL)

Panicle length was measured in centimeters from the node of the panicle joining to the tiller to the tip of panicle.

6.2.3.6 Yield Per Hectare (YLDH)

The plot was harvested at physiological maturity by excluding border rows. The plot yield was measured in kilograms, and grain moisture was evaluated in percentage. Plot yield was further adjusted to 13% moisture, and the derived yield per hectare data in tons was calculated using the following formula:

$$\text{Adjusted plot yield @ 13\% moisture} = \left(\frac{\text{Plot Yield (kg)}}{1000}\right) \text{x} \left(\frac{100-\text{MCPlotGY}}{87}\right)$$

$$\text{Yield per hectare (Kg)} = \left(\frac{\text{Adjusted plot yiled @ 13 \% moisture}}{\text{plot size (m2)}}\right) \text{x } 10000$$

6.2.4 Statistical Analysis

The data were analyzed using JMP to fit a model for ancillary characters including the heading date, plant height, number of panicle-bearing tillers per plant, spikelet fertility percentage, and panicle length. Yield analysis was performed by using the model suggested by Walter T. Federer (1994) for special correction to estimate the error mean square and block effects with the following model expression:

$$y_{ijk} = (\mu+\tau_i+\delta_j+(\tau\delta)_{ij}+(\delta)_{jk}+\varepsilon_{ijk})\eta_{ijk},$$

where y_{ijk} is the measured response (yield) of the *k*-th block of *i*-th genotype in the *j*-th environment, μ is the overall mean, τ_i is the effect of genotype, δ_j is the effect of environment, $(\tau\delta)_{ij}$ interaction effect of *i*-th genotype with the *j*-th environment, $(\delta)_{jk}$

effect of *k*-th block in the *j*-th environment, η_{ijk} is 1 if *i*-th genotype occur in *k*-th block with the *j*-th environment and zero otherwise, and ε_{ijk} is a random error.

We calculated the error variance for each location using the check performance across the blocks to test homogeneity of error variance in each trial before combining the data from all three locations, as suggested by Gomez and Gomez (1984). Hartley's test for homogeneity of variance was performed across locations, and the results were found to be significant when all locations were combined, which indicated that data could not be combined across all environments for analysis.

The best linear unbiased estimator (BLUE) for fixed effects and best linear unbiased predictor (BLUP) for random effects were calculated by using the R-Asreml package. Locations and checks were considered as fixed effects, and RILs and interaction effects between location X RILs, location X RILs X tester, location X block, location X row, and location X range were considered as random effects, as indicated in model below.

"model <- asreml(fixed=YLDH~ LOC + LOC:CHECK - 1, random=~ diag(LOC):MALE + at(LOC):MALE:FEMALE + at(LOC):BLOCK,rcov=~at(LOC):units, data=data)"

The BLUP value for an individual RIL-derived hybrid was compared with the check hybrid performance to identify high heterotic recombinant inbred restorer lines. Furthermore, these RILs were referred back with their QTL profiles and phenotype results for heading date, photoperiod sensitivity, pollen load, and BPH resistance traits.

6.3 Results and Discussion

The hybrid performance trial, which consisted of up to 472 hybrids derived from crosses between $F_{2:7}$ RILs and testers, was planted across three locations. With available seed quantities, an augmented design was laid out, including replicated checks and test hybrids at Chandippa, Faizabad, and Raipur.

The mean performance of hybrids for heading date, plant height, panicle-bearing tillers, panicle length, spikelet fertility percentage, and yield per hectare data are presented in Table 6.2. Hybrids derived from tester BRGB07288A generally exhibited early flowering across test locations. The hybrids evaluated in Faizabad and Raipur were taller than hybrids in Chandippa. Spikelet fertility % recorded in Faizabad were the lowest (57% to 59%) across locations, while the lowest yield was observed at Raipur with a mean of 3.81 and 4.52 tons per hectare for the progenies of testers BRGB07288A and BRGB06355A, respectively.

The multi-location analysis showed significant differences among genotypes for all observed ancillary traits, whereas genotype-by-environment interaction for panicle-bearing tillers and spikelet fertility percent was significant ($P < 0.01$, Table 6.3). Although the RIL-derived hybrids showed significant differences across locations for all observed characters, significant differences between testers was observed only for spikelet fertility % at Raipur and Faizabad (Table 6.4, Table 6.5, Table 6.6, Table 6.7, and Table 6.8). Therefore, the multi-location testing indicated the importance of genetic variability when selecting high-performing hybrids that possess desirable maturity, plant height, and spikelet fertility. Significant differences among various traits have been

observed in earlier research conducted by Surek and Korkut (2002), Swati and Ramesh (2004), and Nadali Bagheri (2010).

The ANOVA of the yield performance of checks was performed to estimate the local error since the test entries were not replicated (Federer, 2001), and they were found to be significant with moderate heritability values ranging between 0.43 and 0.63 across locations (Table 6.9). Line (RIL) X tester interaction was not detected at any location, which implies that the testers' performance was consistent across experimental lines without SCA effect (Table 6.10).

Cullis et al. (1989) and Stroup and Mulitze (1991) showed the BLUPs of genotypic values in a special model that can be enhanced with replicated checks such that a large number of new entries were tested without replication. In the present study, a linear mixed model analysis using ASReml package was performed to derive the BLUP of each RIL. Santosh et al. (2002) showed through simulation that BLUPs with a known variance component were superior to the BLUEs for estimating genotype variance in an unbalanced data set. The RILs with high predicted BLUP values that were superior to the high-performing restorer check (BRGB07186) are presented in Table 6.11. Although the tester effects were not statistically significant, meaning they performed equally on all RILs, hybrids derived from BYRIL117, BYRIL026, BYRIL055, BYRIL060, and BYRIL073 RILs crossed with tester BRGB07288A exhibited numerical yield superiority over hybrids derived from the BRGB06355A tester. Similarly, hybrids of tester BRGB06355A and BYRIL140, BYRIL228, BYRIL072, and BYRIL020 RILs produced a higher yield than the hybrids of the BRGB07288A tester (Table 6.12).

The distribution of QTL for pollen load, BPH resistance, photoperiod sensitivity, and heading date that were detected in the present study in high heterotic restorer lines were presented in Table 6.13. Lines BYRIL001, BYRIL116, and BYRIL175 were observed to have high pollen load, resistance to BPH, and stable heading date across environments.

Table 6.2: Range, mean, standard error, coefficient of variability (CV%) of traits evaluated for heading date (HD), pant height (PH), number of panicle bearing tillers per plant (PBT), panicle length (PL), spikelet fertility % (SF), and yield per hectare of hybrid yield trials conducted at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Location	Female Tester	HD (days)			PH (cm)			PBT		
		Range	Mean ± SE	CV%	Range	Mean ± SE	CV%	Range	Mean +-SE	CV%
Chandippa	BRGB06355A	100-132	110 ± 0.38	5.28	81-142.6	118 ± 0.74	9.67	5.8-10	8 ± 0.04	9.17
	BRGB07288A	91-114	102 ± 0.20	3.46	92.8-144.3	118 ± 0.56	8.24	6-9.2	7 ± 0.03	9.05
Faizabad	BRGB06355A	92-131	112 ± 0.74	8.1	12.6-159	130 ± 1.09	10.3	4-10.6	6 ± 0.08	17.93
	BRGB07288A	68-109	99 ± 0.40	6.12	107.6-154.6	135 ± 0.62	7.04	4-9.6	6 ± 0.06	16.98
Raipur	BRGB06355A	81-122	106 ± 0.55	7.4	100-146.3	124 ± 0.60	6.77	4.3-11	7 ± 0.08	16.66
	BRGB07288A	95-119	100 ± 0.19	3.15	93.3-152.6	124 ± 0.57	7.65	4.6-11	7 ± 0.06	15.43

Location	Female Tester	PL (cm)			SF %			Yield (tons/ha)		
		Range	Mean +-SE	CV%	Range	Mean +-SE	CV%	Range	Mean +-SE	CV%
Chandippa	BRGB06355A	20-29.8	23.8 ± 0.13	8.39	7.8-93.3	69 ± 0.73	16.11	2.79-12.74	7.77 ± 0.14	27.25
	BRGB07288A	17.8-38.4	24.1 ± 0.12	8.94	30.1-89	69 ± 0.51	12.72	4.00-12.93	8.50 ± 0.09	18.41
Faizabad	BRGB06355A	-†	-†	-†	51.8-66.8	57 ± 0.23	5.07	1.29-12.32	5.94 ± 0.13	28.32
	BRGB07288A	-†	-†	-†	52.4-87.3	59 ± 0.24	6.40	1.77-12.92	6.31 ± 0.10	23.46
Raipur	BRGB06355A	-†	-†	-†	5.00-92.0	78 ± 0.67	12.22	0.39-9.86	4.51 ± 0.10	31.22
	BRGB07288A	-†	-†	-†	5.00-89.0	64 ± 0.90	23.6	0.39-8.03	3.81 ± 0.08	35.39

† Panicle length data not available

Table 6.3: Analysis of variance, coefficient of determination, coefficient of variation, and heritability estimates of heading date (HD), plant height (PH), number of panicle bearing tillers per plant (PBT), panicle length (PL) and fertile spikelet percent (SF) in combined analysis of test hybrids evaluated across Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	HD (days)	HT (cm)	PBT	PL (cm)	SF (%)
Genotype	75.2**	306.8**	1.1**	6.06**	140.4**
Environment	765.4**	27398.9**	184.5**	-†	16020.7**
Genotype X Environment	28.6 NS	55.3NS	1.0**	-†	138.2**
Residual	26.9	0.05	0.0	0.09	104.8
F Ratio	6.12	8.74	3.13	2.42	2.84
R^2	0.60	0.68	0.43	0.72	0.41
CV%	4.95	6.03	13.40	6.61	15.40
H^2	0.73	0.84	0.57	0.70	0.57

*,** Significant at 0.05 probability level and 0.01 probability level respectively
NS Not significant at 0.05 probability level.
† Panicle length data was collected from single location.

Table 6.4: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of heading date (HD) of recombinant inbred line (RIL) derived test hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	DF	SS	MS	F Ratio	Prob > F
Chandippa					
Model	275	15013.4	54.5**	3.885	<.0001
Genotype	274	8065.8	29.4**	2.094	<.0001
Tester	1	4698.6	4698.6**	334.38	<.0001
Residual	256	256	3597.2	14.051	
R^2	0.86				
H^2	0.68				
CV%	3.53				
Root MSE	3.74				
Grand Mean	106				
Raipur					
Model	261	14574.3	55.9**	2.389	<.0001
Genotype	260	10321.1	39.7**	1.698	<.0001
Tester	1	2979.9	2979.9**	127.527	<.0001
Residual	215	5023.9	23.4		
R^2	0.74				
H^2	0.62				
CV%	4.67				
Root MSE	4.83				
Grand Mean	103				
Faizabad					
Model	234	30622.5	130.8**	3.509	<.0001
Genotype	233	15519.1	66.6**	1.786	<.0001
Tester	1	9482.1	9482.1**	254.298	<.0001
Residual	147	5481.2	37.2		
R^2	0.85				
H^2	0.64				
CV%	5.82				
Root MSE	6.10				
Grand Mean	105				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level

Table 6.5: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of plant height (cm) of recombinant inbred line (RIL) derived test hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	df	SS	MS	F Ratio	Prob > F
Chandippa					
Model	275	42494.1	154.5**	2.509	<.0001
Genotype	274	42482.6	155.1**	2.517	<.0001
Tester	1	56.8	56.8NS	0.922	0.3378
Residual	256	15766.5	61.6		
R^2	0.73				
H^2	0.72				
CV%	6.66				
Root MSE	7.85				
Grand Mean	118				
Raipur					
Model	263	32392.1	123.1**	3.973	<.0001
Genotype	262	32389.2	123.6**	3.988	<.0001
Tester	1	43.7	43.8 NS	1.412	0.236
Residual	210	6509.1	31.0		
R^2	0.83				
H^2	0.8				
CV%	4.47				
Root MSE	5.57				
Grand Mean	124				
Faizabad					
Model	234	38576.7	164.9**	2.162	<.0001
Genotype	233	36507.7	156.7**	2.055	<.0001
Tester	1	1417.1	1417.2**	18.587	<.0001
Residual	147	11207.8	76.2		
R^2	0.77				
H^2	0.67				
CV%	6.56				
Root MSE	8.73				
Grand Mean	133				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level, NS Not significance at the 0.05 probability level.

Table 6.6: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of number of panicle bearing tillers of recombinant inbred line (RIL) derived test hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	df	SS	MS	F Ratio	Prob > F
Chandippa					
Model	275	158.3	0.5**	1.584	0.0001
Genotype	274	150.3	0.5**	1.509	0.0004
Tester	1	6.3	6.3**	17.401	<.0001
Residual	256	93.0	0.3		
R^2	0.63				
H^2	0.6				
CV%	8.11				
Root MSE	0.6				
Grand Mean	7.44				
Raipur					
Model	263	393.7	1.4**	1.586	0.0003
Genotype	262	393.6	1.5**	1.592	0.0002
Tester	1	0.1	0.1NS	0.089	0.7646
Residual	210	198.1	0.9		
R^2	0.67				
H^2	0.61				
CV%	13.83				
Root MSE	0.97				
Grand Mean	7.02				
Faizabad					
Model	234	287.8	1.2*	1.3244	0.0321
Genotype	233	283.0	1.2*	1.3079	0.0385
Tester	1	4.0	4.0*	4.3073	0.0397
Residual	147	136.5	0.9		
R^2	0.68				
H^2	0.57				
CV%	15.91				
Root MSE	0.96				
Grand Mean	6.06				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level, NS Not significance at the 0.05 probability level.

Table 6.7: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of spikelet fertility percent of recombinant inbred line derived test hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	df	SS	MS	F Ratio	Prob > F
Chandippa					
Model	275	32514.1	118.2**	1.547	0.0002
RIL	274	32511.9	118.6**	1.553	0.0002
Tester	1	82.9	82.9 NS	1.085	0.2986
Residual	251	19177.5	76.4		
R^2	0.63				
H^2	0.6				
CV%	12.61				
Root MSE	8.74				
Grand Mean	69.28				
Raipur					
Model	263	89321.8	339.6**	4.605	<.0001
RIL	262	65709.7	250.8**	3.400	<.0001
Tester	1	16276.9	16276.9**	220.71	<.0001
Residual	216	15929.4	73.7		
R^2	0.85				
H^2	0.77				
CV%	12.26				
Root MSE	8.59				
Grand Mean	70				
Faizabad					
Model	233	2712.5	11.6NS	0.877	0.8131
RIL	232	2552.1	11.0NS	0.829	0.8981
Tester	1	156.7	156.8**	11.814	0.0008
Residual	145	1924.1	13.3		
R^2	0.59				
H^2	0.45				
CV%	6.27				
Root MSE	3.64				
Grand Mean	58.05				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level, NS Not significance at the 0.05 probability level.

Table 6.8: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of panicle length (cm) of recombinant inbred line derived test hybrids evaluated at Chandippa (CHP), India.

Source	df	SS	MS	F Ratio	Prob > F
Model	274	1659.3	6.1**	2.425	<.0001
RIL	273	1654.6	6.1**	2.425	<.0001
Tester	1	0.1	0.1 NS	0.055	0.8148
Residual	256	639.2	2.5		
R^2	0.72				
H^2	0.7				
CV%	6.61				
Root MSE	1.58				
Grand Mean	23.9				

Table 6.9: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of checks yield (tons/ha) performance in recombinant inbred derived hybrids evaluated in augmented trial design with replicated checks at test locations Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	df	SS	MS	F Ratio	Prob > F
Chandippa					
Model	8	35.2	4.4*	2.459	0.0214
Checks	4	26.3	6.6**	3.674	0.0092
Block	4	8.9	2.2*	1.243	0.3011
Residual	66	118.0	1.8		
R^2	0.229				
H^2	0.438				
CV%	16.7				
Root MSE	1.337				
Grand Mean	7.97				
Raipur					
Model	8	121.5	15.2**	15.031	<.0001
Checks	4	117.5	29.4**	29.085	<.0001
Block	4	3.9	0.9*	0.968	0.4304
Residual	67	67.7	1.0		
R^2	0.25				
H^2	0.63				
CV%	28.38				
Root MSE	1.00				
Grand Mean	3.54				
Faizabad					
Model	6	25.6	4.3*	2.734	0.0233
Checks	4	24.4	6.1**	3.894	0.0083
Block	2	1.3	0.7NS	0.415	0.6618
Residual	47	73.6	1.6		
R^2	0.163				
H^2	0.48				
CV%	19.52				
Root MSE	1.25				
Grand Mean	6.41				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level, NS Not significance at the 0.05 probability level.

Table 6.10: Analysis of variance, coefficient of determination, coefficient of variation, heritability estimates, root error mean square, and grand mean of yield (tons/ha) data of recombinant inbred line (RIL) derived test hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Source	df	SS	MS	F Ratio	Prob > F
Chandippa					
Line (RIL)	274	1689.55	6.1^{NS}	1.12	0.308
Tester	1	61.77	61.7^{**}	11.29	0.001
Line x Tester	193	1023.8	5.3^{NS}	0.97	0.571
Residual	51	278.84	5.4		
R^2	0.56				
H^2	0.53				
CV%	21.1				
Root MSE	1.72				
Grand Mean	8.18				
Raipur					
Line (RIL)	262	621.4	2.3^{**}	2.53	<.0001
Tester	1	60.67	60.6^{**}	64.79	<.0001
Line x Tester	164	206.2	1.2^{NS}	1.34	0.1240
Residual	45	42.1	0.9		
R^2	0.72				
H^2	0.64				
CV%	26.53				
Root MSE	1.09				
Grand Mean	4.10				
Faizabad					
Line (RIL)	233	850.0	3.6^{*}	1.67	0.0458
Tester	1	22.7	22.7^{**}	10.46	0.0029
Line (RIL) x Tester	114	143.8	1.2^{NS}	0.57	0.9784
Residual	30	65.2	2.1		
R^2	0.76				
H^2	0.68				
CV%	19.33				
Root MSE	1.19				
Grand Mean	6.16				

** Significance at the 0.01 probability level, * Significance at the 0.05 probability level, ^{NS} Not significance at the 0.05 probability level.

Table 6.11: Location-wise and overall best linear unbiased predictor (BLUP) and standard error (SE) values for yield of the promising recombinant inbred line (BYRIL) derived hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Genotype	Mean Yield ± SE (Kg/ha)	BLUP ± SE			
		Chandippa	Faizabad	Raipur	Overall
BYRIL026	7.99 ± 3.03	8.14 ± 0.61	6.73 ± 0.65	4.17 ± 0.66	6.35 ± 0.37
BYRIL140	7.45 ± 2.71	8.20 ± 0.61	6.27 ± 0.65	4.40 ± 0.66	6.29 ± 0.37
BYRIL010	7.01 ± 1.48	8.05 ± 0.61	6.67 ± 0.64	4.06 ± 0.66	6.26 ± 0.37
BYRIL117	7.98 ± 3.28	8.19 ± 0.61	6.61 ± 0.65	3.91 ± 0.66	6.23 ± 0.37
BYRIL055	9.52 ± 3.23	8.34 ± 0.61	6.57 ± 0.65	3.77 ± 0.69	6.23 ± 0.37
BYRIL001	6.08 ± 1.30	8.18 ± 0.62	6.33 ± 0.64	4.17 ± 0.66	6.23 ± 0.37
BYRIL228	7.18 ± 2.44	8.13 ± 0.61	6.36 ± 0.64	4.17 ± 0.66	6.22 ± 0.37
BYRIL072	7.11 ± 2.94	8.20 ± 0.61	6.58 ± 0.64	3.85 ± 0.66	6.21 ± 0.37
BYRIL197	6.49 ± 1.20	8.20 ± 0.61	6.19 ± 0.64	4.22 ± 0.66	6.20 ± 0.37
BYRIL060	6.83 ± 1.05	8.15 ± 0.61	6.20 ± 0.64	4.24 ± 0.66	6.20 ± 0.37
BYRIL175	6.15 ± 1.75	8.06 ± 0.61	6.09 ± 0.64	4.44 ± 0.66	6.19 ± 0.37
BYRIL090	7.70 ± 1.33	8.19 ± 0.62	6.20 ± 0.65	4.17 ± 0.69	6.19 ± 0.38
BYRIL073	6.70 ± 1.74	8.18 ± 0.61	6.49 ± 0.64	3.89 ± 0.66	6.19 ± 0.37
BYRIL035	7.78 ± 3.22	8.25 ± 0.61	6.20 ± 0.66	4.10 ± 0.66	6.18 ± 0.37
BYRIL116	6.79 ± 0.57	8.06 ± 0.61	6.19 ± 0.64	4.30 ± 0.66	6.18 ± 0.37
BYRIL234	6.32 ± 1.38	8.16 ± 0.61	6.16 ± 0.64	4.23 ± 0.66	6.18 ± 0.37
BYRIL273	8.32 ± 3.13	8.36 ± 0.61	6.20 ± 0.66	3.97 ± 0.67	6.18 ± 0.37
BYRIL137	6.53 ± 1.26	8.18 ± 0.61	6.15 ± 0.64	4.20 ± 0.66	6.18 ± 0.37
BYRIL295	8.50 ± 2.52	8.18 ± 0.62	6.20 ± 0.66	4.14 ± 0.69	6.17 ± 0.38
BYRIL020	7.58 ± 3.39	8.15 ± 0.61	6.61 ± 0.65	3.75 ± 0.66	6.17 ± 0.37
BRGB04267*	5.92 ± 2.63	8.21 ± 0.54	6.20 ± 0.57	3.67 ± 0.57	6.03 ± 0.32
BRGB02489*	5.40 ± 2.36	8.07 ± 0.54	6.20 ± 0.57	3.67 ± 0.57	5.98 ± 0.32
BRGB07186†	6.97 ± 1.68	8.14 ± 0.59	6.20 ± 0.62	3.67 ± 0.65	6.01 ± 0.36

* Hybrids derived from RIL parents, † Commercial check hybrid parent

Table 6.12: Tester-wise and overall best linear unbiased predictor (BLUP) and standard error (SE) values for yield performance of best performing recombinant inbred line (BYRIL) derived hybrids evaluated at Chandippa (CHP), Raipur (RP) and Faizabad (FZB), India.

Genotype	BLUP ± SE		
	BRGB07288A	**BRGB06355A**	**Overall**
BYRIL117**	7.71 ± 0.45	5.92 ± 0.55	6.23 ± 7.71
BYRIL026**	7.81 ± 0.45	6.56 ± 0.55	6.35 ± 7.81
BYRIL055**	7.18 ± 0.45	6.28 ± 0.62	6.23 ± 7.18
BYRIL060**	6.77 ± 0.45	6.17 ± 0.45	6.20 ± 6.77
BYRIL073**	6.90 ± 0.45	6.35 ±0.45	6.19 ± 6.90
BYRIL090	6.69 ± 0.45	6.19 ± 0.62	6.19 ± 6.69
BYRIL234	6.48 ±0.45	6.33 ± 0.45	6.18 ± 6.48
BYRIL137	6.42 ± 0.45	6.34 ± 0.45	6.18 ± 6.42
BYRIL001	6.65 ± 0.45	6.58 ± 0.45	6.23 ± 6.65
BYRIL197	6.48 ± 0.45	6.47 ± 0.45	6.20 ± 6.48
BYRIL035	6.36 ± 0.55	6.46 ± 0.55	6.18 ± 6.36
BYRIL273	6.30 ± 0.55	6.44 ± 0.55	6.18 ± 6.30
BYRIL116	6.33 ± 0.45	6.57 ± 0.45	6.18 ± 6.33
BYRIL175	6.26 ± 0.45	6.58 ± 0.45	6.19 ± 6.26
BYRIL010	6.74 ± 0.45	7.17 ± 0.45	6.26 ± 6.74
BYRIL295	6.17 ± 0.63	6.64 ± 0.55	6.17 ± 6.17
BYRIL140††	6.17 ± 0.45	7.29 ± 0.55	6.29 ± 6.17
BYRIL228††	5.91 ± 0.45	7.36 ±0.45	6.22 ± 5.91
BYRIL072††	5.95 ± 0.45	7.50 ± 0.45	6.21 ± 5.95
BYRIL020††	5.83 ± 0.55	7.52 ± 0.45	6.17 ± 5.83
BRGB04267*	6.03 ± 0.52	6.06 ± 0.52	6.03 ± 6.03
BRGB07186†	6.01 ± 0.54	6.01 ± 0.61	6.01 ± 6.01
BRGB02489*	5.95 ± 0.51	5.98 ± 0.52	5.98 ± 5.95

* Hybrids derived from RIL parents BRGB02489 and BRGB04267, † Commercial check hybrid parent
** High heterotic RILs with tester BRGB07288A, ††: High heterotic RILs with tester BRGB06355A;
Bold: Superior hybrids with both BRGB07288A and BRGB06344A tester.

Table 6.13: Summary of pollen load (PL) brown plant hopper resistance (BPH), photoperiod sensitivity (PP) and heading date (HD) quantitative trait loci (QTL) in high heterotic recombinant inbred lines (BYRIL) identified in BLUP analysis.

Genotype	[1]qPL9	[2]qBP4	[3]qPP6.1D	[3]qPP6.1C	[4]qFD6.2E4	[4]qFD6.2E13	[4]qFD6.2E2	[4]qFD6.2E7	[4]qFD6.2E5	Pollen Load	BPH Score	HD Mean ±SD[!]
BYRIL001[¥]	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**81.5**	**2.89**	**110±8.9**
BYRIL020[††]	-	+	+	+	-	-	-	-	-	74.6	2.84	130±9.1
BYRIL035[¥]	-	-	-	-	-	-	+	+	+	47.6	9.00	129±11.4
BYRIL055[**]	+	+	-	-	+	+	+	+	+	87.6	3.44	110±12.5
BYRIL060[**]	-	+	-	-	-	-	-	-	-	49.8	6.14	130±15.8
BYRIL072[††]	+	+	+	+	-	-	-	-	-	80.7	3.56	117±8.9
BYRIL073[**]	+	+	+	+	-	-	-	-	-	96.8	3.27	119±9.7
BYRIL090[¥]	-	+	-	-	-	-	+	+	+	69.8	2.87	111±11
BYRIL295[**]	+	+	-	-	-	-	-	-	-	92.2	2.67	116±15
BYRIL116[¥]	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**85.6**	**2.79**	**121±5.7**
BYRIL117[**]	+	+	-	-	-	-	-	-	-	93.3	3.65	119±14
BYRIL137[¥]	-	+	+	+	+	+	-	-	-	67	2.50	114±7.6
BYRIL140[††]	-	+	-	-	+	+	+	+	+	65	3.22	118±12
BYRIL175[¥]	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**111.1**	**3.00**	**126±7**
BYRIL197[¥]	-	+	-	-	+	+	+	+	+	62.8	4.44	117±13
BYRIL228[††]	+	+	+	+	-	-	-	-	-	92.8	3.50	120±7.4
BRGB02489[*]	+	-	-	-	+	+	+	+	+	73.8	8.67	102±17
BRGB04267[†]	-	+	+	+	-	-	-	-	-	58.4	2.29	117±6.7

[1]: Pollen QTL ("+" favorable QTL for high pollen load); [2]: Brown plant hopper resistance QTL ("+" favorable QTL for BPH resistance) ; [3]: Photoperiod sensitive QTL ("+" photoperiod insensitive QTL, "-"photoperiod sensitive QTL); [4]: Flowering time QTL ("+" early flowering QTL, "-" late flowering QTL), [!]: Standard deviation of heading date of RILs tested across seven wet and dry environments at Chandippa(Latitude:17.4^{0}N) and Dhantori (Latitude:29.9^{0}N), India. **Bold font**: RILs with high pollen load, BPH resistance and stable flowering behavior; [*]: BPH susceptible, high photoperiod sensitive and high pollen load parent; [†]: BPH resistant, less photoperiod sensitive and low pollen load parent;
[**]: High heterotic RILs with tester BRGB07288A; [††]: High heterotic RILs with tester BRGB06355A; [¥]: RILs with superior heterosis with both BRGB07288A and BRGB06344A tester

6.4 Conclusions

In summary, the unbalanced yield performance trials conducted at the Chandippa, Faizabad, and Raipur locations in India have provided insights into identifying hybrid candidates with high heterosis for advanced levels of hybrid performance tests. In this study, no significant difference was found for genotype and environment interaction for the heading date of test entries. For this reason, selection based on flowering differences was not advantageous from the multi-location testing of the current set of hybrids, whereas significant RIL X tester interaction has allowed for the selecting of lines with desired level of fertility restoration at Faizabad and Raipur locations. Virmani et al. (1981) concluded in their studies that heterosis in hybrid yields was primarily due to increased numbers of fertile spikelets per plant.

Ever since an augmented trial design was proposed by Federer (1998), it has been predominantly implemented in experiments in which replicated design for checks is augmented by a large number of un-replicated test entries. Furthermore, linear mixed model analyses with ASReml led to smaller mean square errors of unbalanced data sets for deriving the predicted BLUP value for individual RILs. Searle et al. (1992) suggested from their studies that, under a general assumption, BLUPs maximize the correlation of true genotypic value and predicts genotype value, which is the primary aim of breeders. The non-significance of the RIL X tester was indicated as the general combining ability of RILs with testers under study. However, BLUP for BYRIL117, BYRIL026, BYRIL055, BYRIL060, BYRIL073, BYRIL140, BYRIL228, BYRIL072, and BYRIL020 RILs were observed with notable yield advantages between hybrids derived from testers BRGB07288A and BRGB06355A. A confirmation of combining ability

lends to validity; by using testers that provide large tests, cross variance could help in the discrimination of experimental lines with GCA and SCA effects.

Overall, BYRIL001, BYRIL116, and BYRIL175 RILs had high heterosis, high pollen load, resistance to BPH, and stable flowering behavior across environments. The present findings provide insights into the feasibility of breeding for BPH resistance and high pollen-producing restorers by recombining QTL among high-performing restorer lines.

CHAPTER VII

SUMMARY

Hybrid rice technology has precipitated increases in rice (*Oryza sativa* L.) productivity in China since the discovery of wild abortive cytoplasmic male sterility (WA-CMS) in 1970 (Li, 1997). However, hybrid rice accounts for less than 10% of all rice cultivated in India, Bangladesh, Indonesia, the Philippines and Vietnam. A variety of technical challenges, market failures and policy constraints have hindered the adoption of hybrid rice in South Asian and Southeast Asian countries (David J. Spielman et al., 2012). Research on the genetic basis of seed production traits and brown plant hopper (BPH) resistance can help remove technical barriers to breeding and cultivating high heterotic hybrid rice and thus benefit Asian farmers.

In this study, a recombinant inbred line (RIL) population derived using Bayer restorer germplasms BRGB02489 and BRGB04267 was studied and tested for BPH resistance against different modes of action, heading date in multiple environments, pollen number (pollen load) and combining ability. The study also aimed to determine the association between quantitative trait loci (QTLs) regulating BPH resistance, heading date response to photoperiod changes and pollen load.

Significant genetic association was found between BPH resistance in the host plant and tolerance (degree of damage), antixenosis (adult preference and feeding rate) and antibiosis (fecundity). The major additive (A) effect QTL (*qBP4*) on chromosome 4 was co-localized with a cluster of resistant genes (Jena et al., 2006; Jie Hu et al., 2016); further stacking of major resistant genes or QTLs was shown to provide stable broad-spectrum resistance to BPH.

The flowering behavior of the RIL population responded to photoperiod changes in wet- and dry-season environments at latitude 17.4°N (Chandippa) and 29.9°N (Dhantori). The test genotypes exhibited phenotypic variance for heading date with the beginning of short day conditions in wet-season environments, contrary to their behavior in increasing day length conditions in dry-season environments. A mixed linear composite interval mapping study revealed major additive (A) effect QTLs on chromosome 6b (*qFD6.2E1, qFD6.2E2, qFD6.2E3* and *qFD6.2E4*) associated with early and late flowering behavior with photoperiod changes. In addition, QTLs identified on chromosome 6a (*qPP6.1C* and *qPP6.1D*) associated with a degree of photoperiod sensitivity were collinear with the heading date QTLs (*qFD6.1E1, qFD6.1E3* and *qFD6.1E4*) detected in short-day conditions. The results of a genetic analysis study based on heading date data suggested that it is possible to breed for stable and early flowering restorer lines by introgression of the BRGB04267 allele for the QTLs on chromosome 6a and the BRGB02489 allele for the QTLs on chromosome 6b. Meanwhile, the high pollen parent allele of QTL (*qPL9*) mapped on chromosome 9 explained 8.7% of the phenotypic variance for pollen number (pollen load).

Unbalanced yield performance trials were conducted using an augmented trial design at locations in Chandippa, Faizabad and Raipur in India, and found significant difference for hybrid yield and spikelet fertility. However, the non-significance of the RIL X tester interaction indicated that further validation is required to determine the line's GCA and SCA effects.

REFERENCES

Akarsh P., Pathak A. R. (2008) Heterosis for various quantitative traits in rice. Oryza 45:181-187.

Alam S.N., Cohen M.B. (1998) Detection and analysis of QTLs for resistance to the brown plant hopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theoritical and Applied Genetics 97:1370–1379.

Ali M.P., Chowdhury T.R. (2014) Tagging and mapping of genes and QTLs of *Nilaparvata lugens* resistance in rice. Euphytica 195:1–30.

Anand K., Singh N. K. (2002) Standard heterosis of rice hybrids for yield and yield components. Journal of Applied Biology 12:20-22.

Bae S.H., Pathak M. D. (1970) Life history of *Nilaparvata lugens* (Homoptera: Delphacidae) and susceptibility of rice varieties to its attacks. Annals of Entomological Society of America 63:149–155.

Baker R.J. (1988) Analysis of genotype-environmental interactions in crops. ISI Atlas of Science: Animal and Plant Sciences 1:1–4.

Balram Marathi., Kshirod K. Jena. (2015) Floral traits to enhance outcrossing for higher hybrid seed production in rice: present status and future prospects. Euphytica 201:1–14.

Beachell H.M., Adair C.R., Jodon N.E., Davis L.L., Jones J.W. (1938) Extent of natural crossing in rice. Journal of the American Society of Agronomy 30:743–753.

Bhattal J. S. (1992) Patterns of insect-plant relationship determining resistance in paddy to *Sogatella furcifera* (Horvath). Punjab Agricultural University, Ludhiana. pp.1–61.

Biao-lin Hu., Jian-kun Xie., Yong Wan., Jin-wei Zhang., Fan-tao Zhang., Xia Li. (2016) Mapping QTLs for Fertility Restoration of Different Cytoplasmic Male Sterility Types in Rice Using Two *Oryza sativa* × *O. rufipogon* Backcross Inbred Line Populations. BioMed Research International 2016: ID:9236573. http://dx.doi.org/10.1155/2016/9236573 .

Bisne R., Motiramani N.K., Sarawgi A.K. (2008) Evaluation of standard heterosis in hybrid rice. Advances in Plant Science 21(1):155-156.

Bosque-Perez NA., Buddenhagen I.W. (1992) The development of host-plant resistance to insect pests: outlook for the tropics. In: Menken SBJ, Visser JH, Harrewijn P (eds) Proc 8th Int Symp insect-plant relationships. Kluwer, Dordrecht. 235:249.

Burgueno J., Crossa J., Cotes JM., Vicente FS., Das B. (2011) Prediction assessment of linear mixed models for multi-environment trials. Crop Science 51:944–954.

Cai H.W., Morishima H. (2002) QTL clusters reflect character associations in wild and cultivated rice. Theoritical and Applied Genetics 104:1217–1228.

Cha YS., Ji H., Yun D.W. (2008) Fine mapping of the rice Bph1 gene, which confers resistance to the brown plant hopper (*Nilaparvata lugens*) and development of STS markers for markerassisted Selection. Molecules and Cells 26:146–151.

Chailakhyan M.K. (1936a) [About the mechanism of the photoperiodic response.]. Dokl Akad Nauk SSSR (Academy of Sciences of the USSR) 1:85-89.

Chailakhyan M.K. (1936b) [New facts supporting the hormonal theory of plant development.]. Dokl Akad NaukSSSR (Academy of Sciences of the USSR) 4:77-81.

Chailakhyan M.K. (1937) Gormonal'naya teoriya razvitiya rastenii [Hormonal theory of plant development]. Akademii Nauk SSSR (Academy of Sciences of the USSR), Moscow.

Chang T.T., Li C.C., Vergara B.S. (1969) Component analysis of duration from seeding to heading in rice by the basic vegetative phase and the photoperiod sensitive phase. Euphytica 18:79–91.

Chardon F., Damerval C. (2005) Phylogenomic analysis of the PEBP gene family in cereals. Journal of Molecular Evolution 61:579-590.

Chaudhary R.C. (1984) Introduction to Plant Breeding. New Delhi, Oxford and IBH.

Chen C.N., Cheng C.C. (1978) The population levels of *Nilaparvata lugens* (Stal.) in relation to the yield loss of rice. International Plant Protection Bulletin 20:197–209.

Chen J.W., Wang L., Pang F. (2006) Genetic analysis and fine mapping of a rice brown plant hopper (*Nilaparvata lugens* Stål) resistance gene bph19(t). Molecular Genetics and Genomics 275:321–329.

Cheng X.Y., Zhu L.L., He G.C. (2013) Towards understanding of molecular interactions between rice and the brown plant hopper. Molecular Plant 6:621–634.

Clark Cockerham.C. (1961) Implications of Genetic Variances in a Hybrid Breeding Program. Crop Science 1:53-55.

Cockerham C.C. (1961) Implication of genetic variances in a hybrid breeding programme. Crop Science 8:720-722.

Cohen M.B,, Alam S.N,, Medina E.B. (1997) Brown plant hopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful N.lugens management in Central Luzon, Philippines. Entomologia Experimantalis et Applicata 85:221–229.

Cullis B.R., Gogel B.J., Verbyla A.P., Thompson R. (1998) Spatial analysis of multi-environment early generation trials. Biometrics 54:1–18.

Cullis B.R., Smith A., Coombes N. (2006) On the design of early generation variety trials with correlated data. Journal of Agricultural, Biological, and Environmental Statistics 11:381–393.

David J. Spielman., Deepthi Kolady., Anthony J. Cavalieri. (2012) The Economics of Hybrid Rice in South Asia. International Association of Agricultural Economists (IAAE) Triennial Conference, Foz do Iguaçu, Brazil, August 18-24.

David J. Spielman., Deepthi E. Kolady., Patrick S. Ward. (2013) The prospects for hybrid rice in India. Food Security 5:651-665.

Doerge R.W., Churchill G.A. (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294.

Du B., Zhang W.L., Liu B.F., Hu J., Wei Z., Shi Z Y., He R.F., Zhu L.L., Chen R.Z., Han B., He G.C. (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown plant hopper in rice. Proceedings of the National Academy of Sciences of the United States of America 106:22163–22168.

Duvick D.N. (1999) Heterosis: feeding people and protecting natural resources. In: genetics and exploitation of heterosis in crops American society of agronomy, Inc, crop science society of America, Inc, soil science society of America, Inc Madison, Wisconsin, USA.

Faiz F. A., Sabar M., Awan T.H., Ijaz M., Manzoor, M. (2006) Heterosis and Combining ability analysis in Basmati rice hybrids. Journal of Animal and Plant Sciences 16(1-2):56-59.

Falconer D.S. (1981) Introduction to quantitative genetics, 2nd edition Burnt Mill.London and New York.

Fang X., Turner N.C., Tan G., Li F., Siddique K.H.M. (2010) Flower numbers, pod production, pollen viability and pistil function are reduced and flower and pod abortion increased in chickpea under terminal drought. Journal of Experimental Botany 61:335-345.

FAO. (2004) Hybrid rice for food security- International year of rice, Rice is Life.

Federer W.T. (1956) Augmented (or hoonuiaku) designs. Hawaii Plant Rec 55:191–208.

Federer W.T. (1998) Recovery of interblock, intergradient, and intervarietal information in incomplete block and lattice rectangle designed experiments. Biometrics 54:471–481.

Federer W.T., Rehnolds M., Crossa J. (2001) Combining results from augmented designs over sites. Agronomy Journal 93:389-395.

Frensham A.B., Cullis B.R., Verbyla A.P. (1997) Genotype by environment variance heterogeneity in a two-stage analysis. Biometrics 53:1373–1383.

Fujii S., Toriyama K. (2009) Suppressed expression of retrograde-regulated male sterility restores pollen fertility in cytoplasmic male sterile rice plants. Proceedings of the National Academy of Sciences of the United States of America 106(23):9513–9518.

Garner W.W., Allard H.A., (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Journal of Agricultural Research 18:553-606.

Gauch H.G. (1988) Model selection and validation for yield trials with interaction. Biometrics 44:705–715.

Gilmour A.R., Cullis B.R., Wielham S.J., Thomson R. (1999) ASREMLreference manual. NSW Agriculture Biometric Bulletin 3.

Gnansekaran M., Vivekanandan P., Muthuramu S. (2006) Combining ability and heterosis for yield and grain quality in two-line rice (*Oryza sativa* L.) hybrids. Indian Journal of Genetics and Plant Breeding 66(1):6-9.

Gomez K.A., Gomez A.A. (1984) Statistical Procedures for Agricultural Research. New York: Wiley – Interscience.

GRiSP (Global Rice Science Partnership) (2013) Rice almanac, 4th edition. Los Baños (Philippines): International Rice Research Institute. 283 p.

Hagiwara W.E., Uwatoko A., Sasaki A., Matsubara K., Nagano H., Onishi K., Sano Y. (2009) Diversification in flowering time due to tandem FT-like gene duplication, generating novel Mendelian factors in wild and cultivated rice. Molecular Ecology 18:1537-1549.

Hai Zhou., Qinjian Liu., Jing Li., Dagang Jiang., Lingyan Zhou., Ping Wu., Sen Lu., Feng Li., Liya Zhu., Zhenlan Liu., Letian Chen., Yao-Guang Liu1., Chuxiong Zhuang. (2012) Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. Cell Research 22:649–660.

Hariprasanna K., Zaman F.U., Singh A.K., Tomar S.M.S., (2006) Analysis of combining ability status among parents and hybrids in rice (*Oryza sativa* L.). Indian Journal of Genetics and Plant Breeding 66(1): 28-30.

Hayes H.K., Immer F.R., Smith D.C. (1955) Methods of plant breeding. McGraw Hill Book Co, New York.

He J., Liu Y.Q., Liu Y.L., Jiang L., Wu H., Kang H., Liu S., Chen L., Liu X., Cheng X., Wan J. (2013) High-resolution mapping of brown plant hopper (BPH) resistance gene Bph27(t) in rice (*Oryza sativa* L.). Molecular Breeding 31:549–557

Heinrichs E.A., Rapusas H.R. (1983) Levels of resistance to the whitebacked plant hopper, Sogatella furcifera (Homoptera: Delphacidae) in rice varieties with different resistance genes. *Environmental Entomology* 12(6):1793–1797.

Heinrichs E.A., Medrano F.G., Rapusas H.R. (1985) Genetic Evaluation for Insect Resistance in Rice. Los Baños, the Philippines: International Rice Research Institute: 1–355.

Henderson C.R. (1984) Application of linear models in animal breeding values. Biometrics 32:69–83.

Heong K.L., Hardy B. (2009) Plant hoppers: New threats to the sustainability of intensive rice production systems in Asia. Los Baños, the Philippines: International Rice Research Institute:1–470.

Hill R.R Jr., Rosenberger J.L. (1985) Methods for combining data from germplasm evaluation trials. Crop Science 25:467– 470.

Hirabayashi H., Angeles ER., Kaji R., Ogawa T., Brara D.S., Khush G.S. (1998) Identification of brown plant hopper resistance gene derived from O. officinalis using molecular markers in rice. Breed Sci 48(Suppl 1):82. (in Japanese with English abstract).

Hirabayashi H., Angeles ER., Kaji R., Ogawa T., Brara D.S., Khush G.S. (1999) RFLP analysis of a new gene for resistance to brown plant hopper derived from *O. officinalis* on rice chromosome 4. Breed Sci 48:48–53 (in Japanese with English abstract).

Horgan F (2009) Mechanisms of resistance: a major gap in understanding plantohopper–rice interactions. In Plant hoppers: new threats to the sustainability of intensive rice production systems in Asia (eds Heong KL, Hardy B, editors.), Los Baños, Philippines: International Rice Research Institute pp. 281–302.

Hori K., Ogiso-Tanaka E., Matsubara K., Yamanouchi U., Ebana K., Yano M. (2013) Hd16, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. The Plant Journal 76: 36-46.

Hossain M., Fischer K.S. (1995) Rice research for food security and sustainable development in Asia: Achievement and future challenges. Geo Journal 35(3): 286–298.

Hu J., Wang.K., Huang W., Liu G., Gao Y., Wang J., Huang Q., Qil X., Wan L., Zhu R., Li S., Yang D., Zhu Y. (2012) The rice pentatricopeptide repeat protein RF5 restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein GRP162. The Plant Cell 24(1):109–122.

Hu J., Xiao C., Cheng M.X., Gao G., Zhang Q., He Y (2015a) Fine mapping and pyramiding of brown plant hopper resistance genes QBph3 and QBph4 in an introgression line from wild rice O. officinalis. Molecular Breeding 35:3. doi:10.1007/s11032-015-0228-2.

Hu J., Xiao C., Cheng M.X., Gao G., Zhang Q., He Y (2015b) A new finely mapped *Oryza australiensis* derived QTL in rice confers resistance to brown plant hopper. Gene 561:132–137.

Hu S., Zhou Y., Zhang L., Zhu X., Wang Z., Li L., Luo L., Zhou Q. (2009) QTL analysis of floral traits of rice (*Oryza sativa* L.) under well-watered and drought stress conditions. Genes & Genomics 31(2):173–181.

Huang D., Qiu Y., Zhang Y., Huang F., Meng J., Wei S., Li R., Chen B. (2013) Fine mapping and characterization of BPH27, a brown plant hopper resistance gene from wild rice (*Oryza rufipogon* Griff.). Theoritical and Applied Genetics 126:219–229.

Huang W., Yu C., Hu J., Wang L., Dan Z., Zhou W., He C., Zeng Y., Yao G., Qi J., Zhang Z., Zhu R., Chen X., Zhu Y. (2015) Pentatricopeptide-repeat family protein RF6 functions with hexokinase 6 to rescue rice cytoplasmic male sterility. Proceedings of the National Academy of Sciences of the United States of America, 112 (48):1 4984–14989.

Inoue H., Tanisaka T., Okumoto Y., Yamagata H. (1992) An early-heading mutant gene of a mutant line HS66 of rice (in Japanese with English summary). Rep Soc Crop Sci Breed Kinki 37:47-52.

IRRI. (2002) Standard Evaluation System for Rice (SES). Rice knowledge bank. pp.20.

Ishii T., Brar D.S., Multani D.S., Kush G.S. (1994) Molecular tagging of genes for brown plant hopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice *Oryza sativa*. Genome 37:217–221.

Itabashi. N.E., Iwata S. Fujii., Kazama.T., Toriyama.K (2011) The fertility restorer gene, Rf2, for Lead Rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. The Plant Journal 65(3):359–367.

Jagadish S.V.K., Muthurajan R., Oane R., Wheeler TR., Heuer S., Bennett J., Craufurd P.Q. (2010) Physiological and proteomic approaches to address heat tolerance during anthesis in rice. Journal of Experimental Botany 61: 143-156.

Jairin, Jirapong & Teangdeerith, Sanguan & Leelagud, Phikul & Phengrat, Kittiphong & Vanavichit, Apichart & Toojinda, Theerayut. (2007). Detection of Brown Plant hopper Resistance Genes from Different Rice Mapping Populations in the Same Genomic Location. ScienceAsia. 33. 347-352. 10.2306/scienceasia1513-1874.2007.33.347.

Janaiah. A. (2002) Hybrid rice for Indian farmers: Myths and realities. Economic and Political Weekly 37 (42):4319–4328.

Janaiah A., Hossain M. (2003) Can hybrid rice technology help pro-ductivity growth in Asian tropics? Farmers' experiences. Economical and Political Weekly 38(25):2492-2501.

Jarillo J.A., del Olmo I., Gómez-Zambrano A., Lázaro A., López-González L., Miguel E., Narro-Diego L., Sáez D., Piñeiro M. (2008) Review. Photoperiodic control of flowering time. Spanish Journal of Agricultural Research 6:221-244.

Jena K.K., Pasalu I.C., Rao Y.K., Varalaxmi Y., Krishnaiah K., Khush G.S., Kochert G. (2003) Molecular tagging of a gene for resistance to brown plant hopper in rice (*Oryza sativa* L.). Euphytica 129:81–88.

Jena K.K., Jeung J.U., Lee J.H., Choi H.C., Brar D.S. (2006) High-resolution mapping of a new brown plant hopper (BPH) resistance gene, Bph18(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theoritical and Applied Genetics 97:112:288.

Jie Hu., Cong Xiao., Yuqing He. (2016) Recent progress on the genetics and molecular breeding of brown plant hopper resistance in rice. Rice 9:30.

Kang M.S. (2004) Breeding: genotype-by-environment interaction. Goodman, R.M. (Ed.) "Encyclopedia of Plant and Crop Science". New York, Basel pp. 218–221.

Karni L., Aloni B. (2002) Fructokinase and hexokinase from pollen grains of bell pepper (*Capsicum annuum* L.): Possible role in pollen germination under conditions of high temperature and CO2 enrichment. Annals of Botany 90, 607-612.

Kato H., Namai H. (1987a) Intervarietal variations of floral characteristics with special reference to F1 seed production in Japonica rice (*Oryza sativa* L.). Japanese Journal of Breeding 37:75–87.

Kato H., Namai H. (1987b) Floral characteristics and environmental factors for increasing natural outcrossing rate for F1 hybrid seed production of rice (*Oryza sativa* L). Japanese Journal of Breeding 37:318–330.

Kawaguchi M., Murata K., Ishii T., Takumi S., Mori N., Nakamura C. (2001) Assigment of a brown plant hopper (*Nilaparvata lugens* Stål) resistance gene bph4 to the rice chromosome 6. Breeding Science 51:13–18.

Kazama T., Toriyama K. (2014) A fertility restorer gene, Rf4, widely used for hybrid rice breeding encodes a pentatricopeptide repeat protein. Rice 7(1):1–5.

Khan Z.R., Saxena R.C. (1985) Behavioural and physiological responses of *Sogatella furcifera* (Horvath) (Delphacidae: Hemiptera) to selected resistant and susceptible rice cultivars. Journal of Economic Entomology 78(6):1280–1286.

Khoyumthem P., Sharma P. R., Singh N. B., Singh M. R. K., (2005) Heterosis for grain yield and its component characters in rice (*Oryza sativa* L.). Environment and Ecology 23 (4): 687-691.

Khush G.S. (1971) Rice breeding for disease and insect resistance at IRRI. Oryza 8:111–119.

Khush G.S., Karim A.R., Angeles E.R. (1985) Genetics of resistance of rice cultivar ARC 10550 to Bangladesh brown plant hopper biotype. Journal of Genetics 64:121–125.

Kim S.M., Sohn J.K. (2005) Identification of rice gene (Bph1) conferring resistance to brown plant hopper (*Nilaparvata lugens* Stål) using STS markers. Molecules and Cells 20:30–34.

Komiya R., Yokoi S., Shimamoto K. (2009) A gene network for long-day flowering activates RFT1 encoding a mobile flowering signal in rice. Development 136: 3443-3450.

Komori T., Ohta S., Murai N., Takakura Y., Kuraya Y., Suzuki S., Hiei Y., Imaseki H., Nitta N. (2004) Map-based cloning of a fertility restorer gene, Rf-1, in rice (*Oryza sativa* L.). The Plant Journal 37(3):315–325.

Kosambi D.D. (1944) The estimation of map distance from recombination values, *Annals of Eugenics*, Vol.12, pp.172–175.

Krishnaiah N.V. (2015) Rice Brown Plant hopper-A Global Scenario, M/S Sophia Publishers, British Columbia, Canada.

Krishnaiah N. V. (2016) Varietal resistance breaking ability and insecticide resistance developing ability of BPH- Is there any relation between the two? Molecular Entomology 7(1):1-9.

Kshirod.K.Jena., Suk-Man Kim. (2010) Current Status of Brown Plant hopper (BPH) resistance and Genetics. Rice 3:161-171.

Kumari S., Sheba J.M., Marappan M., Ponnuswamy S., Seetharaman S., Pothi N., Subbarayalu M., Natesan S. (2010) Screening of IR50 x Rathu Heenati F7 RILs and identification of SSR markers linked to brown plant hopper (*Nilaparvata lugens* Stål) resistance in rice (*Oryza sativa* L.). Molecular Biotechnol 46:63–71.

Lakashminarayana A., Khush G.S. (1977) New genes for resistance to the brown plant hopper in rice. Crop Science 17:96–100.

Lans P., Rothfusz. (1990) The computation of the heat index is a refinement of a result obtained by multiple regression analysis described in National Weather Service (NWS) Technical Attachment SR: 90-23.

Lee Y.S., An G. (2015) Complex Regulatory Networks of Flowering Time in Rice. Journal of Rice Research 3:141.

Li C., Sun C.Q., Mu P., Chen L., Wang X.K., (2001) QTL analysis of anther length and ratio of stigma exsertion, two key traits of classification for cultivated rice (*Oryza sativa* L.) and common wild rice (O. rufipogon Griff.). Acta Genetica Sinica 28(8):746–751.

Li J., Xin Y. (2000) Dedication: Longping Yuan—Rice breeder and world hunger fighter. Plant Breeding Reviews 17: 1–15.

Li J., Xin Y., Yuan L. (2009) Hybrid rice technology development: Ensuring China's food security. IFPRI discussion paper no. 00918. Washington, D.C.: IFPRI.

Li, J., Xin, Y., & Yuan, L. (2010). Hybrid rice technology development:Ensuring China's food security. In D. J. Spielman & R. Pandya-Lorch (Eds.), Proven successes in agricultural development: Atechnical compendium to millions fed. Washington, DC:International Food Policy Research Institute.

Li J.M., Yuan, L.P. (2000) Hybrid rice: genetics, breeding, and seed production. Plant Breed. Rev. 17: 15–158.

Li P.H. (1977) How we studied hybrid rice. *Acta Botanica Sinica* 19:7–10.

Lin H.X., Yamamoto T., Sasaki T., Yano M. (2000) Characterization and detection of epistatic interactions of three QTLs, Hd1, Hd2 and Hd3, controlling heading date in rice using nearly isogenic lines. Theoritical and Applied Genetics 101: 1021-1028.

Lin S. C., Yuan L.P., (1980) Hybrid rice breeding in China. In: Innovative approaches to rice breeding. Selected papers from the 1979 International Rice Research Conference. Manila, Philippines: International Rice Research Institute.

Lin S.Y, Sasaki T, Yano M (1998) Theoritical and Applied Genetics 96: 997–1003.

Ling K.C., Tiongco E.R., Aguiero V.M. (1978) Rice ragged stunt, a new virus disease. Plant Disease Rep, 62(8):701–705.

Liu G.F., Yang J., Xu H.M., Zhu J. (2007) Influence of Epistasis and QTL6Environment Interaction on Heading Date of Rice (*Oryza sativa* L.). Journal of Genetics and Genomics 34: 608–615.

Liu J.L., Yu J.F., Wu J.C., Yin J.L., Gu H.N. (2008) Physiological responses to *Nilaparvata lugens* in susceptible and resistant rice varieties: allocation of assimilates between shoots and roots. Journal of Economic Entomology 101(2): 384–390.

Liu Y., Su C., Jiang L., He J., Wu H., Peng C., Wan J. (2009) The distribution and identification of brown plant hopper resistance genes in rice. Hereditas 146:67–73.

Lu C.G., Zou J.S., Hu N., Yao K.M. (2007) Plant temperature for sterile alteration of a temperature-sensitive genic male sterile rice, Pei'ai64S. Sci Agric Sin 6:1283–1290.

Luo D., Xu H., Liu Z., Guo J., Li H., Chen L., Fang C., Zhang Q., Bai M., Yao N., Wu H., Ji C., Zheng H., Chen Y., Ye S., Li X., Zhao X., Li R., Liu Y.G. (2013) A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. Nature Genetics 45(5):573–577.

Lv W.T., Du B., Shangguan X.X., Zhao Y., Pan Y., Zhu L., He Y., He G. (2014) BAC and RNA sequencing reveal the brown plant hopper resistance gene BPH15 in a recombination cold spot that mediates a unique defense mechanism. BMC Genomics 15:674–589.

Mackill D.J., Salam M.A., Wang Z.Y., Tanksley S.D. (1993) A major photoperiod sensitivity gene tagged with RFLP and isozyme markers in rice. Theoretical and Applied Genetics 85: 536–540.

Maheswaran M. (1994) Identification of quantitative trait loci for days to flowering and photoperiod sensitivity in rice (*Oryza sativa* L.). Ph. D thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India.

Mahto R. N., Yadava M. S., Mohan K. S. (2003). Genetic variation, character association and path analysis in rainfed upland rice. Indian Journal of Dryland Agriculture Research and Development 18(2): 196-198.

Malarvizhi D., Thiyagarajan K., Manonmani S., Deepa S.P. (2003) Fertility restoration behaviour of promising CMS lines in rice (*Oryza sativa* L.). Indian Journal of Agricultural Research 37(4):259 – 263.

Malini, N., Sundaram, T., Hari S. R. and Saravanan, S., 2006, Genetic Interpretation of Yield Related Traits in Rice (*Oryza sativa* L.). Research Journal of Agricultural and Biological Sciences 2(4):153-155.

Manoj Kumar, Verma, G. P. and Yadav, H. C., 2008. Heterosis for yield and associated traits in rice (*Oryza sativa* L.) using CMS lines under saline-alkali soil. Plant Archievs, 8(1): 255-258.

Margarido G.R.A., Souza A.P., Garcia A.A.F. (2007) OneMap: software for genetic mapping in outcrossing species. Hereditas 144: 78-79.

McCouch S.R., Cho Y.G., Yano M., Paul E., Blinstrub M., (1997) Report on QTL nomenclature. Rice Genet Newsl 14: 11–13.

Miyata M, Yamamoto T, Komori T, Nitta N (2007) Markerassisted selection and evaluation of the QTL for stigma exsertion under japonica rice genetic background. Theoretical and Applied Genetics 114:539–548.

Monna L, Lin X, Kojima S, Sasaki T, Yano M (2002) Genetic dissection of a genomic region for a quantitative trait locus, Hd3, into two loci, Hd3a and Hd3b, controlling heading date in rice. Theoretical and Applied Genetics 104: 772-778.

Muhammad R., Akbar A.C., Muhammad A. (2007) Line X Tester analysis in basmati rice. Pakistan Journal of Botany 39(6): 2035-2042.

Muir W., Nyquist W.E., Xu S. (1992) Alternative partitioning of the genotype-by-environment interaction. Theoretical and Applied Genetics 84: 193–200.

Munisonnappa S., Vidyachandra B. (2007) Standard heterosis in newly developed rice hybrids. Karnataka Journal of Agricultural Sciences 20(2): 379- 380.

Murai H., Hashimoto Z., Sharma P., Shimizu T., Murata K., Takumi S., Mori S., Nakamura C. (2001) Construction of a high-resolution linkage map of a rice brown plant hopper (*Nilaparvata lugens* Stål) resistance gene bph2. Theoretical and Applied Genetics 103:526–532.

Murata K., Fujiwara M., Murai H., Koji., Takumi., Shigeo., Mori., Nakamura., Chiharu. (2001) Mapping of a brown plant hopper (*Nilaparvata lugens* Stål) resistance gene Bph9 on the long arm of rice chromosome 12. Cereal Research Communications. 29. 245-250.

Murfet I.C. (1977) Environmental interaction and the genetics of flowering. Annual Review of Plant Physiology 28: 253–278.

Myint K, Fujita D, Matsumura M et al (2012) Mapping and pyramiding of two major genes for resistance to the brown plant hopper (*Nilaparvata lugens* [Stål]) in the ricecultivar ADR52. Theor Appl Genet 124:495–504.

Nadali B. (2010) Heterosis and Combining Ability Analysis for Yield and Related-Yield Traits in Hybrid Rice. International Journal of Biology 2(2):222-231.

Namaky R.El., Oort P.A.J. (2017) Phenology, sterility and inheritance of two environment genetic male sterility (EGMS) lines for hybrid rice. Rice (N Y).10:31.

Narasimman R., Kumar S. T., Eswarn R., Kumar C.R.S., Anandan A. (2007) combining ability and its components characters in rice (*Oryza sativa* L.) Crop Improvement 34 (1):16-18.

Ogiso E., Takahashi Y., Sasaki T., Yano M., Izawa T. (2010) The role of casein kinase II in flowering time regulation has diversified during evolution. Plant Physiology 152: 808-820.

Ouyang Y., Liu Y.G., Zhang Q. (2010) Hybrid sterility in plant: stories from rice. Current Opinion in Plant Biology 13:186–92.

Paguia P., Pathak M.D., Heinrichs E.A. (1980). Honeydew Excretion Measurement Techniques for Determining Differential Feeding Activity of Biotypes of *Nilaparvata lugens* on Rice Varieties. Journal of Economic Entomology 73:35-40.

Painter R.H. (1951) Insect resistance in crop plants. Macmillan Co., New York

Panda N., Khush G.S. (1995) Host Plant Resistance to Insects. Wallingford (UK): CAB International: 1–431.

Pathak M.D., Cheng C.H., Furtono M.E. (1969) Resistance to *Nephotettix cincticeps* and *Nilaparvata lugens* in varieties of rice. Nature 223:502–504.

Patil P.P., Vashi R.D., Shinde D.A., Lodam V. A. (2011) Nature and magnitude of heterosis for grain yield and yield attributing traits in rice (*Oryza sativa* L.) Plant Archives, 11(1):423-427.

Piepho H.P. (1994) Best linear unbiased prediction (BLUP) for regional yield trials: A comparison to additive main effects multiplicative interaction (AMMI) analysis. Theoretical and Applied Genetics 89:647–654.

Piepho H-P., Möhring J., Melchinger A.E., Büchse A. (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161:209–228.

Pradeep Kumar, V. and Reddy, C. V. C. M., (2011) Study of specific combining ability for yield characters in hybrid rice Plant Archives, 11(1): 449-452.

Pradhan, S. K., Boss, L. K., & Meher, J. (2006). Studies on gene action and combining ability analysis in Basmati rice. Journal of Centeral European Agriculture 7(2):267-272.

Prasad P.V.V., Boote K.J., Allen J.L.H., Sheehy J.E., Thomas J.M.G. (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crop Research 95: 398-411.

Preetinder Singh Sarao, Jagadaish Sanmallappa Bentur. 2016. Antixenosis and Tolerance of Rice Genotypes Against Brown Plant hopper. Rice Science, 23(2): 96−103.

Qiu Y., Guo J., Jing S., Zhu L., He G. (2010) High-resolution mapping of the brown plant hopper resistance gene Bph6 in rice and characterizing its resistance in the 93–11 and Nipponbare near isogenic backgrounds. Theoretical and Applied Genetics 121:1601–1611.

Qiu Y., Guo J., Jing S., Zhu L., He G. (2012) Development and characterization of japonica rice lines carrying the brown plant hopper-resistance genes BPH12 and BPH6. Theoretical and Applied Genetics 124:485–494.

Qiu Y.F., Cheng L., Zhou P., Liu F., Li R.B. (2012) Identification of antixenosis and antibiosis in two newly explored brown plant hopper resistance rice lines. Advance Journal of Food Science and Technology 4(5): 299–303.

Qiu Y.F., Guo J.P., Jing S.L. (2014) Fine mapping of the rice brown plant hopper resistance gene BPH7 and characterization of its resistance in the 93-11background. Euphytica 198:369–379.

Rahimi M., Rabiei B., Samizadeh H., Kafighasemi A. (2010) Combining ability and heterosis in rice (*Oryza sativa* L.) cultivars. Journal of Agriculture Science and Technology 12: 223-231.

Rahman M.L., Jiang W., Chu S.H. (2009) High-resolution mapping of two rice brown plant hopper resistance genes, Bph20(t) and Bph21(t), originating from *Oryza minuta*. Theoretical and Applied Genetics 119:1237–1246.

Raj D., Singh D. N., Madhuri A., Singh P. (2007) Heterosis in rainfed transplanted rice. Oryza, 44(3):264-267.

Rajesh Singh. (2000) Heterosis studies in rice using "WA" based CMS system for developing hybrids for eastern Uttar Pradesh. Annals of Agricultural Research 21(1): 79-83.

Ramaiah K. (1953) Rice breeding and genetics. Sci Monograph No 19, Indian Council of Agricultural Research, New Delhi.

Rao Y.S. (1998) Cytohistology of cytoplasmic male sterile lines in hybrid rice -In: Smith, W.H., Bostian, L.R., Cervantes, E.P. (ed.): Hybrid Rice. IRRI, Manila Pp. 115–128.

Rashid M., Cheema A. A., Ashraf M. (2007). Line x tester analysis in basmati rice. Pakistan Journal of Botany 39 (6):2035-2042.

Renganayaki K., Fritz A.K., Sadasivam S. (2002) Mapping and progress toward map-based cloning of brown plant hopper biotype-4 resistance gene introgressed from Oryza officinalis into cultivated rice. O. sativa. Crop Science 42:2112–2117.

Roberts E.H., Summeffield R.J., Ellis R.H., Qi A. (1993) Adaptation of flowering in crops to climate. Outlook on Agriculture 22:1115 110.

Sahadevan P.C., Namboodiri K.M.N. (1963) Natural crossing in rice. Proceedings of the Indian Academy of Science, Section B 58:176–185.

Saleem M.Y., Mirza J. I., Haq M.A. (2008) Heritability, genetic advance and Heterosis. Journal of Agriculture Research 46: 15-27.

Salgotra R.K., Gupta B.B., Singh S. (2009) Evaluation of various floral traits in some rice CMS lines that influence seed setting under subtropical conditions. SABRAO Journal of Breeding and Genetics 41(2):115–122.

Samal P., Misra B.C. (1990) Antibiosis and preference for shelter of rice varieties to the brown plant hopper, *Nilaparvata lugens* (Stål.). Oryza 27: 358–359.

Sano Y. (1983) A new gene controlling sterility in F1 hybrids of two cultivated rice species: its association with photoperiod sensitivity. Journal of Heredity 74: 435–439.

Santos A.H., Bearoti E., Ferreira D.F., da Silva Filho J.L. (2002) Simulation of mixed models in augmented block design. Scientia Agricola 59:483–489.

Saravanan K., Sabesan T., Kumar S.T. (2008) Heterosis for yield and yield components in rice (*Oryza sativa* L.). Advances in Plant Sciences 21(1):119-121.

Sarial A. K., Singh V.P., Ram K. (2006) Heterotic potential of basmati fertility restorers for grain yield and its components in rice (*Oryza sativa* L.). Indian Journal of Genetics and Plant Breeding 66(4): 293-298.

Sarker U., Biswas P.S., Prasad B., Khaleque Mian M.A. (2002) Heterosis and genetic analysis in rice hybrid. Pakistan Journal of Biological Sciences, 5 (1): 1-5.

Sattari M., Kathiresan A., Gregorio G.B., Hernandez J.E., Nas T.M., Virmani S.S. (2007) Development and use of a two-gene marker-aided selection system for fertility restorer genes in rice. Euphytica 153:35–42.

Sawant D. S., Shetye V. N., Desai S. S. (2006) Combining ability studies in rice involving diverse cytosteriles. International Journal of Plant Sciences 1(2): 193-196.

Searle S.R., Gasella G., McCulloch C.E. (1992) Variance Components. John Wiley & Sons, New York.

Senguttuvel P., Kannan B.J.R. (2007) Combining ability analysis for yield and yield components in Rice. Advances in Plant Sciences. 20(1): 59-62.

Seshu D.V., Kauffman H.K. (1980) Differential reaction of rice verities to the brown plant hopper in International Screening Trials. International Rice Research Institute, Philippines. IRRI Res. Paper Series. 52:13.

Shaked R., Rosenfeld K., Pressman E. (2004) The effect of low night temperatures on carbohydrates metabolism in developing pollen grains of pepper in relation to their number and functioning. Scientia Horticulturae 102, 29-36.

Shanmugavadivel P.S., Amitha Mithra S.V., Chandra Prakash., Ramkumar MK., Ratan Tiwari., Trilochan Mohapatra., Nagendra Kumar Singh. (2017) High Resolution Mapping of QTLs for Heat Tolerance in Rice Using a 5K SNP Array. Rice 10:28.

Sharma P.N., Ketipearachchi Y., Murata K., Torri., Takumi., Nakamura C. (2002) RFLP/AFLP mapping of a brown plant hopper (*Nilaparvata lugens* Stål) resistance gene Bph1 in rice. Euphytica 129:109–117. 10.1023/A:1021514829783.

Sheeba A., Vivekanandan P., Ibrahim S.M. (2006) Genetic variability for floral traits influencing outcrossing in the CMS lines of rice. Indian Journal of Agriculture Research 40(4):272–276.

Sheng Ling., Caisheng Chen., Yang Wang., Xiaocong Sun., Zhanhua Lu., Yidan Ouyang., Jialing Yao. (2015) The mature anther-preferentially expressed genes are associated with pollen fertility, pollen germination and anther dehiscence in rice. BMC Genomics 16:101.

Shukla K.K. (1984) Mechanisms of resistance in rice to whitebacked plant hopper, *Sogatella furcifera* (Horvath) (Delphacidae: Hemiptera). Ludhiana: Punjab Agricultural University Newsletter. 2:15-18.

Sidharthan B., Thiyagarajan K., Manonmani S. (2007) Cytoplasmic male sterile lines for hybrid rice production. Journal of Applied Sciences Research 3(10):935–937.

Smith A.B., Cullis B.R., Gilmour A.R. (2001) Analyzing variety by environment trials using multiplicative mixed models and adjustments for spatial field trend. Biometrics 57:1138–1147.

Smith A.B., Cullis B.R., Thompson R. (2005) The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. The Journal of Agriculture Science 143:449–462.

Sogawa K. (1978) Quantitative morphological variations among biotypes of the brown plant hopper. Rice Research Newsletter 3:9–10.

Soundararajan R.P., Kadirvel P., Gunathilagaraj K., Maheswaran M. (2004) Mapping of quantitative trait loci associated with resistance to brown plant hopper in rice by means of a doubled haploid population. Crop Science 44:2214–2220.

Stroup W.W., Mulitze D.K. (1991) Nearest neighbor adjusted best linear unbiased prediction. The American Statistician 45:194–200.

Su CC., Zhai H.Q., Wang C.M., Sun L.H., Wan J.M. (2006) SSR mapping of brown plant hopper resistance gene Bph9 in Kaharamana, an indica rice (*Oryza sativa* L.). Acta Genetica Sinica 33:262–268.

Sun L., Su C., Wang C., Zai H., Wan J. (2005) Mapping of a major resistance gene to brown plant hopper in the rice cultivar Rathu Heenati. Breeding Science 55:391–396.

Sun L.H., Wang C.M., Su C.C. Liu Y.Q., Zhai H.Q., Was J.M. (2006) Mapping and marker-assisted selection of a brown plant hopper resistance gene bph2 in rice (*Oryza sativa* L.). Acta Genetica Sinica 33(8):717–723.

Sunil Kumar., Satish K., Narendra P., Dwivedi D.K. (2012) Estimation of heterosis for grain yield and yield attributing traits in rice (*Oryza sativa* L.) Plant Archives 12 (1): 159-164.

Surek H., Korkut K.Z. (2002) Heterosis for yield and its components under temperate conditions. Proc. Euro Rice-2001 Symp. Krasnodar, Russia, Sep. 3-8 p: 1-10.

Suzuki Y., Sogawa K., Seino Y. (1996) Ovicidal reaction of rice plants against the whitebacked plant hopper, Sogatella furcifera HORVATH (Homoptera: Delphacidae). Applied Entomolgy and Zoology 31:111-118.

Swamy M.H., Gururaja Rao M.R., Vidyachandra B. (2003) Studies on combining ability in rice hybrids involving new CMS lines. Karnataka Journal of Agrilcultural Sciences. 16 (2): 228-233.

Swati P.G., Ramesh B.R. (2004) The nature and divergence in relation to yield traits in rice germplasm. Annals of Agricultural Research 25(4): 598-602.

Tang H.W., Luo D.P., Zhou D.G., Zang Q., Tian D., Zheng X., Chen L., Liu Y.G. (2014) The rice restorer Rf4 for wild-abortive cytoplasmic male sterility encodes a mitochondrial-localized PPR protein that functions in reduction of WA352 transcripts. Molecular Plant, vol. 7, no. 9, pp. 1497–1500, 2014.

Tiwary D.K., Pandey P., Giri S.P., Dwivedi J.L. (2011) Heterosis studies for yield and its components in rice hybrids using CMS system. Asian Journal of Plant Sciences 10(1): 29-42.

Tsuji H., Tamaki S., Komiya R., Shimamoto K. (2008) Florigen and the photoperiodic control of flowering in rice. Rice 1: 25-35.

Uga Y., Siangliw M., Nagamine T., Ohsawa R., Fujimura T., Fukuta Y. (2010) Comparative mapping of QTLs determining glume, pistil and stamen sizes in cultivated rice (*Oryza sativa* L.). Plant Breed 129:657–669.

Veeresha B.A., Hanamaratti N.G., Salimath P.M., Chetti M.B. (2013) Heterosis for yield and yield traits in hybrid rice (*Oryza sativa* L.) under aerobic condition. Bioinfolet. 10 (2A): 521-529.

Veni B.K., Rani N.S. (2003) Heterosis and combining ability for yield and yield components in rice. Journal of Research 31(3) : 44-51.

Vergara B.S., Chang T.T. (1985) The flowering response of the rice plant to phomperiod, 4th Ed., IRRI, Los Bafios, Philippines, Pp:61.

Villalobos F.J., Ritchie J.T. (1992) The effect of temperature on leaf emergence rate of sunflower genotypes. Field Crop Res. 29:37-46.

Virmani S.S., Chaudhary R.C., Khush G.S. (1981) Current outlook on hybrid rice. Oryza, 18: 67-84.

Virmani S.S., Sharma H.L., (1993) Manual for Hybrid Rice Seed Production. International Rice Research Institute, Los Banos, Laguna, Philippines.

Virmani S.S. (1994) Heterosis and Hybrid Rice Breeding. Monographs on Theoretical and Applied Genetics Vol. 22.

Virmani S.S. (1994) Heterosis and hybrid rice breeding, In: Frankel R, Grossman BM, Linskens WHF, Maliga NP, Riley PR (ed) Monographs on theoretical and applied genetics, Springer-Verlag, Berlin, Heidelberg, Germany Vol. 22.

Virmani S.S. (1996) Hybrid Rice. Advances in Agronomy 57: 378-462.

Virmani S.S., Siddiq E.A., Muralidharan K. (1998) Advances in hybrid rice technology. Proceedings of the 3rd International Symposium on Hybrid Rice, 14-16 November 1996. Hyderabad, India. Manila (Philippines): International Rice Research Institute p: 443.

Virmani S.S., Sun Z.X., Mou T.M., Jauhar A.A., Mao C.X. (2003) Two-line hybrid rice breeding manual. Los Baños: International Rice Research Institute p: 88.

Virmani S.S. (2003) Advances in hybrid rice research and development in the tropics. In: Virmani SS, Mao CX, Hardy B (eds) Hybrid rice for food security, poverty alleviation,

and environmental protection International Rice Research Institute. Los Ban˜os, Philippines Pp:7–20.

Wang B., Wang J.Z., Wu W., Zheng H.G., Yang Z.Y., Xu W.W., Ray J.P., Nguyen H.T. (1995) Tagging and mapping the thermosensitive genic male sterile gene in rice (*Oryza sativa* L.) with molecular markers. Theoretical and Applied Genetics 91(6-7):1111-1114.

Wang C.S., Rutledge J.J., Gianola D. (1994) Bayesian analysis of mixed linear models via Gibbs sampling with an application to litter size in Iberian pigs. Genetics Selection Evolution 26:91–115.

Wang D.L., Zhu J., Li Z.K., Paterson A.H. (1999) Mapping QTLs with epistatic effects and QTL6environment interactions by mixed linear model approaches. Theoretical and Applied Genetics 99:1255–1264.

Wang S., Basten C.J., Gaffney P., Zeng Z.B. (2005) Windows QTL Cartographer 2.5 user manual. Bioinformatics Research Center, North Carolina State Univ., Raleigh.

Wang Y., Cao L.M., Zhang Y.X., Cao C.X., Liu F., Huang F., Qiu Y., Li R., Lou X. (2015) Map-based cloning and characterization of BPH29, a B3 domain-containing recessive gene conferring brown plant hopper resistance in rice. Journal of Experimental Botany 66:6035–6045.

Wang Z.H., Zou Y.J., Li X., Zhang Q., Chen L., Wu H., Su D., Chen Y., Guo J., Luo D., Long Y., Zhong Y., Liy Y.G. (2006) Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. The Plant Cell 18 (3):676–687.

Watanabe T., Kitagawa H. (2000) Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the plant hopper *Nilaparvata lugens* (Homoptera: Delphacidae). Journal of Economic Entomology 93: 1192–1198.

William C., Forsythe., Edward J., Rykiel Jr., Randal S. Stahl., Hsin-i., Rober M. Schoolfield. (1995) A model comparison for daylength as a function of latitude and day of year. Ecological Modelling 80(1): 87-95.

Wu H., Liu Y.Q., He J., Liu Y., Jian L., Liu L., Wang C., Cheng X., Was J. (2014) Fine mapping of brown plant hopper (*Nilaparvata lugens* Stål) resistance gene Bph28(t) in rice (*Oryza sativa* L.). Molecular Breeding 33:909–918.

Xie F. (2009) Priorities of IRRI hybrid rice breeding. In: Xie F, Hardy B (eds) Accelerating hybrid rice development. International Rice Research Institute, Los Ban˜os, Pp:49–62.

Xu M.L., Zhou G.Q., Chen L.B. (1999) Response of fertility of Pei'ai 64S to temperature and photoperiod in rice. Acta Agronomica Sinica 25:772–776.

Xu S.J., Li B. (1988) Managing hybrid rice seed production. In: Hybrid Rice. International Rice Research Institute, Los Banos, Laguna, Philippines, Pp: 157-163.

Yang J., Hu C.C., Hu H., Yu R.D., Xia Z., Ye X., Zhu J. (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24: 721–723.

Yang J., Zhu J. (2005) Methods for predicting superior genotypes under multiple environments based on QTL effects. Theoretical and Applied Genetics 110:1268–1274.

Yano M., Sasaki T. (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Molecular Biology 35: 145-153.

Yano M., Harushima Y., Nagamura Y., Kurata N., Minobe Y., Sasaki T. (1997) Theoretical and Applied Genetics 95: 1025–1032.

Yang R.C. Baker R.J. (1991) Genotype-environment interactions in two wheat crosses. Crop Science 31: 83–87.

Yang R.C. (2002) Likelihood-based analysis of genotype-environment interaction. Crop Science 42:1434–1440.

Yamamoto T., Lin H.X., Sasaki T., Yano M. (2000) Genetics 154: 885–891.

Yokoo M., Okuno K. (1993) Genetic analysis of earliness mutations induced in the rice cultivar Norin 8. Japanese Journal of Breeding 43: 1–11 (1993).

Yu X.Q., Mei H.W., Luo L.J., Liu G.L., Zou G.H., Hu S.P., Li M.S., Wu J.H. (2006) Dissection of additive, epistatic and Q X E interaction of quantitative trait loci influencing stigma exsertion under water stress in rice. Acta Genetica Sinica 33(6):542–550.

Yuan L.P. (1966) A preliminary report on male sterility in rice. Science Bulletin 4: 32–34.

Yuan L.P. (1987) Strategy for hybrid rice breeding. Hybrid Rice 1: 1–3.

Zadocks J.C., Chang T.T., Konzak C.F (1974) A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Zayed B.A., EI-Namaky R.A., Seedek S.E.M., EI – Mowafi H.F. (2013) Exploration hybrid rice under saline soil conditions in Egypt. Journal of Plant Production, Mansoura University 4(1):1-13.

Zhao Q.Y., Zhu Z., Zhang Y., Zhao L., Zhang Q., Xu L., Wang C. (2008) Combining ability and Heterosis of quality characters in japonica hybrid rice. Jiangsu Journal of Agrcultural Sciences 24(4): 387-393.

Zhang K.P., Tian J.C., Zhao L., Wang S.S. (2008) Mapping QTLs with epistatic effects and QTL6environment interactions for plant height using a doubled haploid population in cultivated wheat. Journal of Genetics and Genomics 35: 119–127.

Zhang K.P., Chen G.F., Zhao L., Liu B., Xu X.B., Tian J.C. (2009) Molecular genetic analysis of flour color using a doubled haploid population in bread wheat (Triticum aestivum L.). Euphytica 165: 471–484-484. 10.1007/s10681-008-9756-8.

Zhou G., Chen Y., Yao W., Zhang C., Xie W., Hua J., Xing Y., Xiao J., Zhang Q. (2012) Genetic composition of yield heterosis in an elite rice hybrid. Proceedings of National Academy of Sciences U S A. 2012;109(39):15847–15852.

Zhu Y.G. (2000) Studies on rice male sterility and its restoration. In: Zhu, Y.G., Li, Y.Q., Wang, M.Q. (ed.): Biology of Male Sterility in Rice. Wuhan University Press, Wuhan Pp: 398–438.

APPENDIX

Appendix 5.1: Brown plant hopper (BPH) resistance screening data of recombinant inbred lines evaluated for degree of damage.

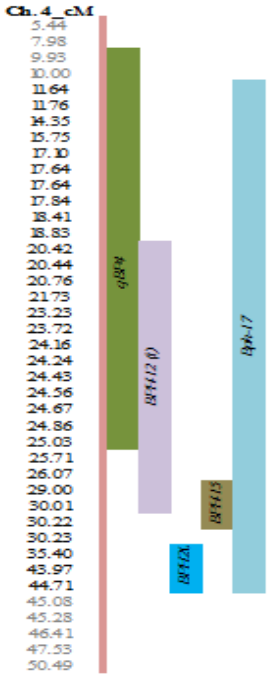
Genotype	Mean Damage Score	Genotype	Mean Damage Score	Genotype	Mean Damage Score
BYRIL001	2.90	BYRIL041	3.37	BYRIL080	3.65
BYRIL002	2.50	BYRIL042	9.00	BYRIL081	4.04
BYRIL003	2.50	BYRIL043	9.00	BYRIL082	5.44
BYRIL004	8.75	BYRIL046	3.50	BYRIL084	3.56
BYRIL005	8.75	BYRIL047	3.84	BYRIL085	3.67
BYRIL006	2.75	BYRIL048	2.75	BYRIL086	6.48
BYRIL007	2.95	BYRIL049	2.67	BYRIL089	2.61
BYRIL008	2.90	BYRIL050	3.13	BYRIL090	2.88
BYRIL009	2.90	BYRIL051	2.89	BYRIL091	9.00
BYRIL010	2.75	BYRIL052	2.75	BYRIL092	4.04
BYRIL011	9.00	BYRIL053	3.15	BYRIL093	9.00
BYRIL012	9.00	BYRIL054	2.88	BYRIL094	2.30
BYRIL013	8.50	BYRIL055	3.44	BYRIL095	2.79
BYRIL014	3.09	BYRIL056	2.86	BYRIL096	4.84
BYRIL015	2.84	BYRIL057	2.88	BYRIL098	2.50
BYRIL016	2.63	BYRIL058	3.40	BYRIL099	2.56
BYRIL017	3.03	BYRIL059	4.76	BYRIL100	3.33
BYRIL018	9.00	BYRIL060	6.15	BYRIL101	2.89
BYRIL019	2.88	BYRIL061	3.57	BYRIL103	4.34
BYRIL020	2.84	BYRIL062	3.23	BYRIL104	2.75
BYRIL021	2.67	BYRIL063	3.15	BYRIL105	9.00
BYRIL022	2.53	BYRIL064	2.07	BYRIL106	4.71
BYRIL024	2.88	BYRIL065	9.00	BYRIL107	3.33
BYRIL025	2.92	BYRIL066	4.46	BYRIL108	8.75
BYRIL026	2.63	BYRIL067	2.75	BYRIL109	8.54
BYRIL027	3.00	BYRIL068	2.67	BYRIL110	9.00
BYRIL028	2.88	BYRIL069	3.11	BYRIL111	9.00
BYRIL029	3.04	BYRIL070	2.79	BYRIL112	4.68
BYRIL030	3.02	BYRIL071	3.22	BYRIL113	2.88
BYRIL032	2.80	BYRIL072	3.57	BYRIL114	2.50
BYRIL033	9.00	BYRIL073	3.27	BYRIL115	2.50
BYRIL035	9.00	BYRIL074	4.46	BYRIL116	2.79
BYRIL036	9.00	BYRIL075	6.55	BYRIL117	3.65

Appendix 5.1: Continued

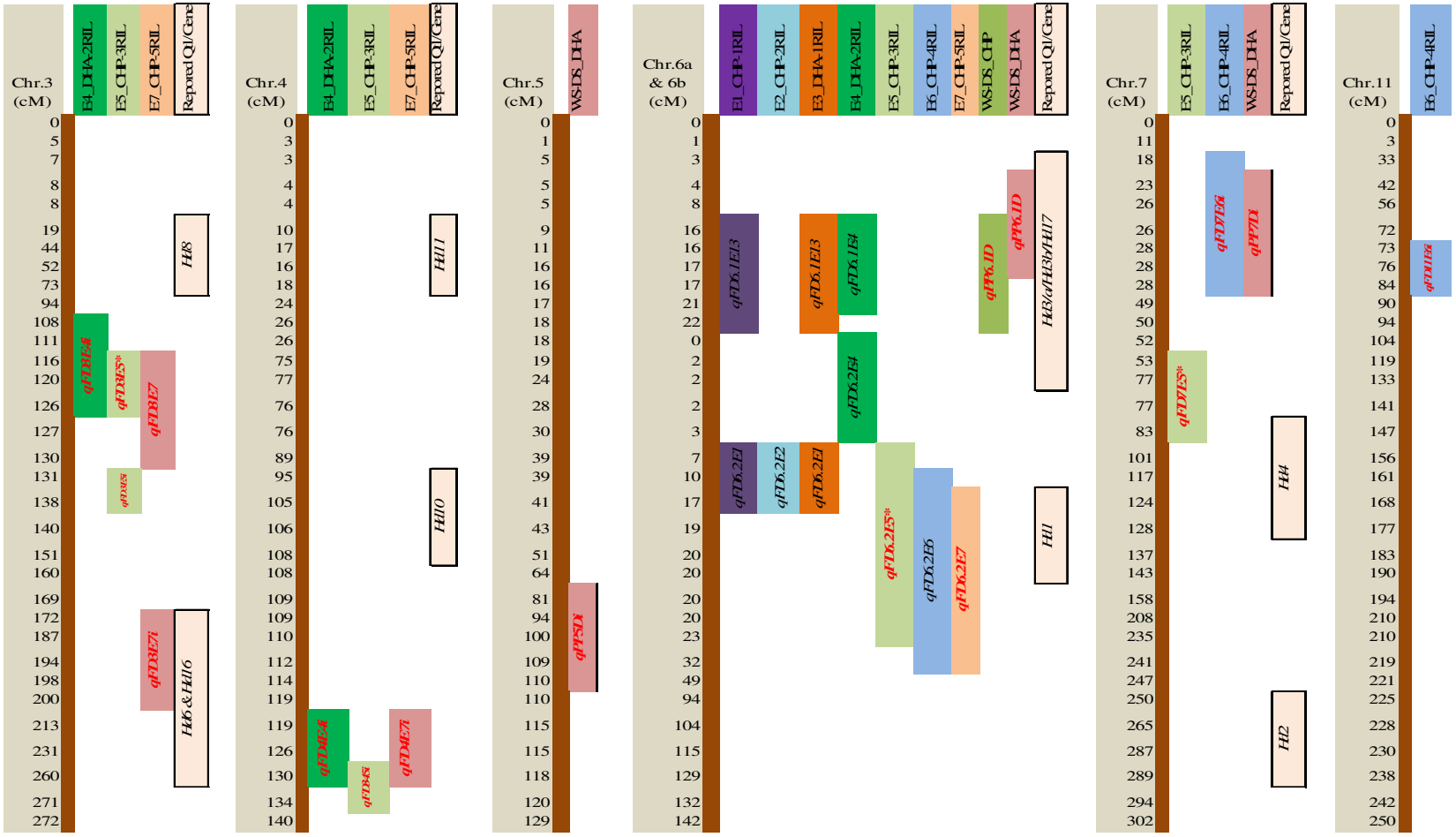
Genotype	Mean Damage Score	Genotype	Mean Damage Score	Genotype	Mean Damage Score
BYRIL037	9.00	BYRIL076	3.96	BYRIL118	2.41
BYRIL038	9.00	BYRIL077	4.50	BYRIL119	3.25
BYRIL039	9.00	BYRIL078	5.01	BYRIL120	2.45
BYRIL040	2.96	BYRIL079	5.81	BYRIL121	2.17
BYRIL122	2.68	BYRIL167	2.38	BYRIL210	2.17
BYRIL123	9.00	BYRIL168	3.65	BYRIL211	1.76
BYRIL125	8.25	BYRIL169	2.13	BYRIL212	2.00
BYRIL126	3.60	BYRIL170	5.01	BYRIL213	9.00
BYRIL127	2.58	BYRIL171	4.65	BYRIL214	1.88
BYRIL128	9.00	BYRIL173	2.38	BYRIL215	7.00
BYRIL129	1.61	BYRIL174	5.92	BYRIL216	8.75
BYRIL130	8.50	BYRIL175	3.00	BYRIL217	8.75
BYRIL131	3.04	BYRIL176	9.00	BYRIL218	1.88
BYRIL132	2.97	BYRIL177	2.26	BYRIL219	1.92
BYRIL133	9.00	BYRIL178	9.00	BYRIL220	1.94
BYRIL134	3.75	BYRIL179	9.00	BYRIL221	8.50
BYRIL135	2.28	BYRIL180	9.00	BYRIL223	9.00
BYRIL136	2.93	BYRIL181	9.00	BYRIL224	8.25
BYRIL137	2.50	BYRIL182	4.69	BYRIL225	8.75
BYRIL138	3.39	BYRIL184	9.00	BYRIL226	1.83
BYRIL140	3.23	BYRIL185	2.13	BYRIL227	1.82
BYRIL142	5.50	BYRIL186	9.00	BYRIL228	9.00
BYRIL143	4.19	BYRIL187	9.00	BYRIL229	9.00
BYRIL144	5.11	BYRIL190	3.00	BYRIL230	8.75
BYRIL146	3.82	BYRIL191	3.38	BYRIL231	9.00
BYRIL148	2.01	BYRIL192	9.00	BYRIL232	2.92
BYRIL149	3.18	BYRIL193	3.40	BYRIL233	2.06
BYRIL150	2.33	BYRIL194	4.06	BYRIL234	2.44
BYRIL151	2.90	BYRIL196	2.17	BYRIL235	2.07
BYRIL152	4.90	BYRIL197	4.44	BYRIL236	9.00
BYRIL153	5.23	BYRIL198	2.13	BYRIL237	2.61
BYRIL155	9.00	BYRIL199	1.96	BYRIL238	3.84
BYRIL156	2.17	BYRIL200	2.38	BYRIL241	1.92
BYRIL157	4.13	BYRIL201	3.00	BYRIL242	9.00
BYRIL158	3.25	BYRIL202	3.71	BYRIL243	9.00
BYRIL161	5.50	BYRIL203	2.61	BYRIL244	4.32
BYRIL162	2.91	BYRIL204	2.42	BYRIL247	7.32
BYRIL163	2.46	BYRIL206	2.25	BYRIL248	9.00
BYRIL164	2.25	BYRIL207	1.94	BYRIL249	9.00

Appendix 5.1: Continued

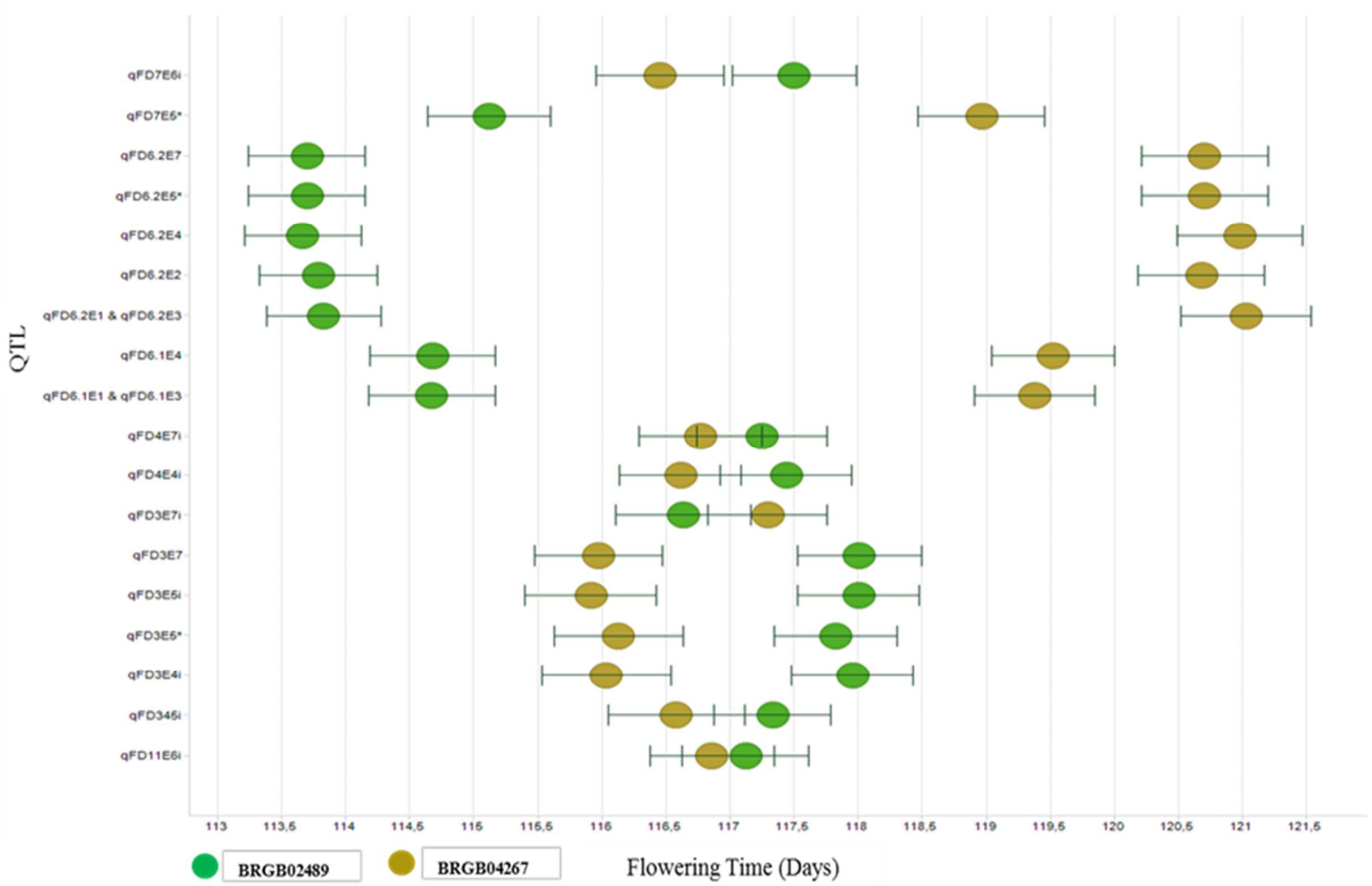
Genotype	Mean Damage Score	Genotype	Mean Damage Score	Genotype	Mean Damage Score
BYRIL165	2.02	BYRIL208	3.29	BYRIL250	9.00
BYRIL166	2.13	BYRIL209	2.63	BYRIL251	9.00
BYRIL252	2.36	BYRIL268	2.98	BYRIL285	2.11
BYRIL253	4.19	BYRIL269	2.69	BYRIL286	1.94
BYRIL254	2.65	BYRIL270	3.23	BYRIL287	9.00
BYRIL255	2.67	BYRIL271	9.00	BYRIL288	8.50
BYRIL256	2.75	BYRIL272	2.84	BYRIL289	2.07
BYRIL257	3.09	BYRIL273	2.75	BYRIL290	2.20
BYRIL258	3.21	BYRIL274	8.75	BYRIL292	2.04
BYRIL259	9.00	BYRIL277	2.71	BYRIL293	2.00
BYRIL260	3.21	BYRIL278	9.00	BRGB02489	8.68
BYRIL261	3.05	BYRIL279	9.00	BRGB04267	2.30
BYRIL262	2.58	BYRIL280	9.00	BRGB07253	1.99
BYRIL263	2.46	BYRIL281	5.75	BRGB07288	8.71
BYRIL265	9.00	BYRIL282	3.23	MTU1010	7.82
BYRIL266	3.58	BYRIL283	3.92	PTB 33	3.24
BYRIL267	3.04	BYRIL284	3.77	TN1	8.99



Appendix 5.2: Genetic position of identified putative effect quantitative trait loci (QTL) for brown plant hopper resistance with other reported brown plant hopper resistance genes/ QTL in different studies.



Appendix 5.3: Putative quantitative trait loci (QTL) identified in recombinant inbred line population studied for heading date and photoperiod sensitivity at Chandippa (CHP) and Dhantori (DHA) environments (E1, E2, E3, E4, E5, E6 & E7) in wet season (WS) and dry season (DS) along with flowering genes reported in other studies.



Appendix 5.4: Quantitative trait loci (QTL) effect of BRGB02489 (Parent 1) & BRGB04267 (Parent 2) allele on mean flowering time of recombinant inbred lines evaluated across wet season and dry season environments at Chandippa and Dhantori locations, India.

Appendix 5.5: Parental alleles (BRGB02489 and BRGB04267) frequency of heading date (HD) and photoperiod sensitive (PP) quantitative trait loci (QTL) in early duration group (Group1), late duration group (Group 2), stable flowering group with standard deviation <7.5 days and (Group 3) and unstable flowering group with standard deviation > 14.5 days (Group 4).

		Heading date (Days)				Standard deviation of heading date across environments			
		Group 1 : 102-110 (days)		Group 2 : 131-137 (days)		Group 3: <7.5 (days)		Group 4: >14.5	
No. of RILs		56		20		54		13	
Parent Genotypes		BRGB02489	BRGB04267	BRGB02489	BRGB04267	BRGB02489	BRGB04267	BRGB02489	BRGB04267
Photoperiod	***qPP5Di***	0.643	0.357	0.450	0.550	0.537	0.463	0.615	0.385
	qPP6.1D	0.426	0.574	0.250	0.750	0.519	0.481	**1.000**	0.000
	qPP6.1C	0.473	0.527	0.250	0.750	0.527	0.473	**1.000**	0.000
	qPP7Di	0.571	0.429	0.412	0.588	0.518	0.482	0.462	0.538
Heading date QTL	***qFD3E4i***	0.364	0.636	0.550	0.450	0.473	0.527	0.714	0.286
	qFD3E7	0.364	0.636	0.550	0.450	0.393	0.607	0.643	0.357
	qFD3E5	0.382	0.618	0.550	0.450	0.429	0.571	0.714	0.286
	qFD3E5i	0.382	0.618	0.500	0.500	0.536	0.464	0.692	0.308
	qFD3E7i	0.446	0.554	0.400	0.600	0.357	0.643	0.500	0.500
	qFD4E7i	0.482	0.518	0.444	0.556	0.259	0.741	0.462	0.538
	qFD4E4i	0.464	0.536	0.444	0.556	0.222	0.778	0.462	0.538
	qFD345i	0.536	0.464	0.550	0.450	0.393	0.607	0.643	0.357
	qFD6.1E13	0.691	0.309	0.400	0.600	0.429	0.571	0.615	0.385
	qFD6.1E4	0.709	0.291	0.400	0.600	0.444	*0.556*	0.615	0.385
	qFD6.2E4	**0.830**	0.170	0.105	**0.895**	0.250	0.750	0.462	0.538
	qFD6.2E13	**0.833**	0.167	0.105	**0.895**	0.357	0.643	0.500	0.500
	qFD6.2E2	**0.855**	0.145	0.105	**0.895**	0.393	0.607	0.500	0.500
	qFD6.2E7	**0.836**	0.164	0.105	**0.895**	0.393	0.607	0.500	0.500
	qFD6.2E5	**0.836**	0.164	0.105	**0.895**	0.393	0.607	0.500	0.500
	qFD6.2E6	**0.833**	0.167	0.105	**0.895**	0.393	0.607	0.462	0.538
	qFD7E6i	0.482	0.518	0.550	0.450	0.481	0.519	0.286	0.714
	qFD7E5	0.661	0.339	0.350	0.650	0.423	0.577	0.214	0.786
	qFD11E6i	0.589	0.411	0.750	0.250	0.630	0.370	0.571	0.429

Bold font: frequency of favorable allele