Study on Sheath Blight Resistance and Yielding Capacity by Phenotype and QTL Analysis in Rice Line 32R

イネ系統32Rにおける表現型とQTL解析による紋枯病抵抗性と多収性改善に関する研究

GAIHRE YUBA RAJ

2015

Table of Contents

Abbreviations

BC	Back cross
CTAB	Cetyl trimethyl ammonium bromide
CIA	Chrlorophorm isoamyl alcohal
CsCl	Cesium chloride
DH	Doubled haploids
DNA	Deoxyribonucleic acid
DMA	Dry mass accumulation
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
F_1	First filial generation
F_2	Second filial generation
Ka	Kasalath
NSC	Nonstructural carbohydrate
PCR	Polymerization chain reaction
RILs	Recombinant inbred lines
SSR	Simple sequence repeats
TBE	Tris/Borate/EDTA
TEMED	Tetramethylethylenediamine
MAS	Marker assisted Selection
Nb	Nipponbare
QTL	Quantitative trait loci
UV	Ultra violet
32R	$2F_{18}$-7-32 (Resistance line)
29S	$2F_{21}$-21-29 (Susceptible line)

CHAPTER 1

General Introduction

General literature of rice

The issue of food security is always challenging problem in developing country and need to be resolved firstly in the world. Similarly, producing the quality food is also the challenging problem in the developed country. Among the cereals, rice is an important food grain and is a staple food for nearly one-half of the world's population. To meet increasing global demand and consumption, rice productivity must be enhanced. In 2014, the crop was grown on 163.1 million hectares of land, and production amounted to 744.4 million metric tons of grain (paddy, rough rice) (www.fao.org). Although rice is grown in 112 countries, spanning an area from 53° latitude north to 35° south, about 95 percent of the crop is grown and consumed mainly in Asia. Rice provides fully 60 percent of the food intake in Southeast Asia and about 35 percent in East Asia and South Asia (www.irri.org). Rice is a monocotyledonous annual grass belongs to the family *Gramineae* and the genus *Oryza*. The genus *Oryza* includes 20 wild species and 2 cultivated species (cultigens): *Oryza sativa* and *Oryza glaberrima*. The wild species are widely distributed in the humid tropics and subtropics of Africa, Asia, Central and South America, and Australia (Chang 1985). Of the two cultivated species, African rice (*O. glaberrima* Steud.) is confined to West Africa, whereas common or Asian rice (*O. sativa* L.) is now commercially grown in 112 countries, covering all continents (Bertin *et al*., 1971). Rice grain is a composite of carbohydrate, protein, fat, fiber, and other significant nutritive constituents (Torres-Escribano *et al*., 2008; Qian *et al*., 2010). Harvested rice is commonly known as rough or paddy rice. Milling is a process where

the outer layers of the grain is removed, exposing the white kernel inside. Rice comes in several sizes, colors, fragrances, and textures.

On the other hand, in some high-income Asian countries there were factors that caused a decline in total demand for rice and other food grains. Firstly, there was increasing evidence of declining population growth rates. Secondly, and more importantly, the rapid rise per capita incomes in countries such as Japan, Korea, and Taiwan, resulted in diversified food habits, with consumers moving away from rice and cereals to other foods. In these countries, the demand elasticity for rice had become very low, was declining, and (in a few cases) had even become negative. The economic prosperity in middle and high-income countries caused smaller increases (for some countries even a decline) per capita rice consumption (http://www.fao.org/wairdocs/tac/x5801e/x5801e08.htm#chapter 1 rice in the world). There was much greater diversity in diet pattern, and willingness to pay higher prices for better quality rice. This tendency is likely to become even stronger in the future as more countries reach high-income status. For the example of consumption trend of rice, Japan can be the model in future. Rice is staple food of Japan. The rice harvested in Japan is nearly all *Japonica* rice, which features a natural sweetness, mild fragrance and plump moistness (www.maff.go.jp). Being simple and mild, it makes a tasty food on its own and complements a variety of side dishes. Rice plays a pivotal role in a healthy Japanese diet. Now a days daily intake of cooked rice in Japan has been decreasing but people are practiced to take rice in the different forms such as Sake (rice wine), sweets, senbei (rice cracker), rice vinegar, nuka (rice bran), rice cake (mochi), rice bread (Kome pan), rice milk etc. For rice, this could mean that the demand pattern may change more from table rice as a source of staple carbohydrates to processed rice.

Rice production may increase by one of several means or a combination such as enhancing production area and increasing crop yields using chemicals, fertilizers, biological controls, and improved management of soil and water (Fernando, 2006). Currently, rice production throughout the world heavily depends on the fertilizers, pesticides and other inputs, which are proved less environmentally friendly and economically effective. Concerning about the harmful effects caused by the intensive employment of agrochemicals and chemical fertilizers, the integrated biotechnology is recommended and utilized into cultivation practice in many countries.

Sheath blight disease in rice

Rice crop is subjected to attack of 50 diseases that including 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Webster and Gunnel 1992). However, major diseases are rice blast, brown spot, bacterial leaf blight and leaf streak, sheath blight, sheath rot, *Fusarium* wilt or *Bakanae*, stem rot, Tungro virus, false smut and post-harvest diseases. These diseases either attack at any growth stage of rice plant or infect rice grains after harvest, causing considerable losses in both quality and quantity of the produce. It is estimated that about 14-18% these diseases worldwide caused yield reduction.

Rice sheath blight disease, caused by the fungal pathogen *Rhizoctonia solani* Kuhn is one of the major production constraints in rice growing countries of the world and ranks next to blast in causing economical loss (Marshall and Rush 1980). The causal agent of rice sheath blight is *R. solani* Kuhn, a fungus that survives either as sclerotia or mycelia in plant debris, floats to the surface of floodwater and by germinating, infects the rice plants (Marchetti, 1983). Mycelial growth and sclerotia formation are at its higher at 25 to 30°C and 80 to 95% relative humidity are optimal for disease development. Soil type may also influence disease development. Disease

severity is higher in sandy clay loam than in clayey or sandy soils (Tiwari and Chaure, 1997). Mycelia growth and sclerotia formation are optimum at pH 6.0 - 7.0, and no growth at pH 3.0 and 9.0. Crop losses generally vary from 0 to 50% depending on the severity of the disease and the stage at which the crop is infected and environmental conditions (Merchetti *et al*., 1991). Apart from rice, the pathogen also infects many other plant species, including barley, lettuce, tomato, sorghum, maize etc. (Zhang *et al*., 2009). *R. solani* has limited movement due to lack of spores and survives in unfavorable conditions by forming sclerotia or dormant mycelia (Sumner, 1996; Anees *et al*., 2010). Sclerotia in soil can survive for 2 years, and are spread during field preparation and flooding the field for irrigation (Webster and Gunnell, 1992; Brooks, 2007). The inspection of field, where the fungus inoculation study was done shows that the disease appears every year in rice although pesticides has been used. The appearance of sheath blight disease in the field where the sheath blight disease severity was studied after inoculation in rice has been shown in Figure 1.1. During permanent flooding, the sclerotia may float and move within the field or to bordering fields through continuous flood irrigation. Sclerotia or hyphae attach to the plant, infecting and causing sheath blight disease, and the pathogen spreads under conditions favorable to disease development. Soil borne pathogens normally are dormant and immobile in the field, therefore, the host plant typically grows towards the stationary pathogen (Anees *et al*., 2010). With pathogen and plant contact, mature sclerotia cause sheath blight disease infection. Initial symptoms occur on leaf sheaths near the water line as water-soaked lesions. Secondary infections are caused by hyphae growing upward towards uninfected plant parts, producing additional lesions and sclerotia on leaf sheaths to complete the disease cycle (Webster and Gunnell, 1992; Brooks, 2007). The disease peaks during heading to flowering period when the rice canopy is most dense, forming a microclimate favorable to pathogen growth and spread (Brooks, 2007). *R. solani* can infect seed

to fully mature plant, causing moderate to significant yield losses depending on the plant part affected. Visible plant disease symptoms include formation of lesions, plant lodging, and presence of empty grains. Large lesions formed on infected sheaths of lower rice leaves may lead to softness of the stem thereby initiating stem lodging (Wu *et al*., 2012).

Sheath blight disease presence during flowering or panicle initiation causes a reduction of total seed weight due to a lower percentage of filled grain and results in significant yield losses (Cu *et al*., 1996; Nagarajkumar *et al*., 2004). During rice sheath blight epidemics, severe lodging may occur, which obstructs the transportation of water, nutrients, and carbohydrate assimilates through the xylem and phloem channels, affecting grain filling (Wu *et al*., 2012). Disease spread and intensity is dependent on the amount of infectious inoculum present in planting material and residues of previous crop remaining in the field or in the top soil where rice is grown. Other impact factors for sheath blight disease severity are rice development stage at infection, ecological surroundings, cultivar resistance, and cultural and seasonal crop practices (Groth *et al*., 1992). The presence of one or many factors may enhance the severity of sheath blight disease beyond economic threshold levels, thereby incurring low to high yield losses.

Host plant resistance is a very efficient, pro-poor technology to manage crop diseases, and represents a potentially important tool to improve sheath blight management. Improving phenotyping methods for resistance to sheath blight is expected to provide one means to improve resistance to sheath blight through more efficient breeding approaches. The combined use of phenotyping methods, appropriate analytical methods, and molecular tools should provide a sound basis to contribute identifying the genetic basis of partial resistance to sheath blight disease.

Figure 1.1. Symptom of sheath blight disease appeared in the field in 2014, where the field study of sheath blight disease was done by inoculating the fungus in 2010.

Breeding strategies of sheath blight disease resistance

Resistance breeding programs are emphatically carried out worldwide to develop high-level resistant cultivars, which are generally accepted to have the potential of significantly relieving the pressure imposed on rice yield by sheath blight without, or with less employment of fungicides (Groth and Bond 2007). However, no high-level resistance but several moderately resistant cultivars has been established (Lee and Rush 1983).

To achieve sustainability of rice production, we need a rice production system built upon effective resistant varieties with broad resilience to a range of diseases and insect pests. Broad-spectrum resistance at the genotypic level and sustainability at the cropping systems level are therefore complementary approaches in managing rice diseases. Although considerable progress has been made over the past decades, much more can be done to integrate these two approaches to achieve results in the field. The challenge ahead is to develop broad-spectrum resistance and production systems that suppress a multitude of biotic stresses. To meet such a challenge, research on host plant resistance must evolve not only to respond to the emerging problems but also to capture advances in new science to enable better use of genetic resources in managing diseases (Leung *et al*., 2003).

R. solani is considered a complex species because it contains related but genetically distinct sub specific groups that have been identified traditionally based on hyphal anastomosis reactions (Carling *et al*., 2002). There are at least 13 hyphal anastomosis groups (AGs) of *R. solani* with distinct ecological and host adaptations and sensitivities to fungicides (Carling *et al*., 2002; Gonzalez Garcıa *et al*., 2006 and Martin *et al*., 1984). Absolute resistance to *R. solani* is not available in any of the rice germplasm grown worldwide. However, substantial differences in the levels of susceptibility to the sheath blight pathogen among rice cultivars have been observed

under field conditions (Marchetti *et al*., 1991 and Jia *et al*., 2007). Physiological resistance and morphological resistance are the two mechanisms of resistance of sheath blight disease in rice (Lore *et al*., 2012). The resistance to *R. solani* is a typical quantitative trait controlled by polygene in rice (Li *et al*., 1995 and Zou *et al*., 2000). In the rice breeding, development polygenic resistance mechanism is thought be better than major genic resistance mechanism because in the disease resistant variety, breakdown of host resistance can occurs within a few years after the release of a resistant variety owing to the appearance of a virulent strain of fungus or bacterium. Furthermore the variety of polygenic resistance is able to continue to be stable and lasting resistance in open field because it shows the same levels of resistance to any strains because the field resistance can be attained by accumulating the polygenes governing the resistance for a disease (Wasano and Dhanapala 1982).

The main strategies for breeding disease resistance rice combinations with wide adaptability, high yielding potential, good rice quality and multiple disease and insect resistances are to combine the ideal plant type with physiological vigor and to harmonize all the growth traits, by improving the selective pressure based on crosses from the super parents (Chen *et al*., 2007). Wild relatives of rice are an important source of novel resistance genes for rice improvement. Some rice lines such as Tetep, Tadukan, Teqing, Saza, Marsi, Tauli, Brimful, Jasmine 85, ZYQ8, Minghui 63, LSBR-5 and LSBR-33 in which a high degree of quantitative resistance is available against this pathogen under field conditions (Khush 1977; Groth and Nowick, 1992; Li *et al*., 1995; Wasano 1988; Pan *et al*., 1999 and Sato *et al*., 2004). Among them Tetep offers, excellent protection against the pathogen under the field condition and in this variety resistance is expressed as fewer and smaller lesions, reduced number of infection cushions and production of oxidized phenolic compound, which slows the spread of *R. solani* within the plant, suggesting the presence

of physiological mechanisms of resistance (Wasano et al., 1983; Groth and Nowick, 1992 and Lore *et al*., 2012). Tetep, a primitive cultivar from Vietnam, has been identified as one of the cultivars most resistance to sheath blight disease. However, the plant has weak tall culms, and flowers late in the season (Wasano *et al*., 1983; Groth and Nowick 1992 and Lore *et al*., 2013). Based on these facts, Wasano *et al.* (1985) developed materials that showed field resistance through classical crossbreeding. The lines identified by Wasano *et al.* (1985) were obtained by crossing Tetep and CN_4-4-2. The cross of Chugoku 45 and Nipponbare, a susceptible *Japonica* variety, developed the rice line CN_4-4-2. Wasano and Hirota (1986) reported that the resistant rice line showed more resistance than the parent, Tetep and denoted as 32R. Another rice line 29S also developed with same parent in similar selection method with 32R. The breeding scheme for developing the plant material for this study is shown in Figure 1.2. The tendency towards lodging and spikelets falling in Tetep had been improved in 32R and 29S but other agronomic characters especially fertility and yield, remains extremely poor.

In this study, parent cultivar was selected based on the phenotypic performance regarding sheath blight disease resistance yield related characteristics. Rice line 32R, 29S, Nipponbare and Kasalath was selected for parent material. The rice line 32R is resistance to sheath blight, having short and thick grain and suitable height for the resistance for sheath blight resistance. The rice 29S is susceptible to sheath blight, having short and thick grain, shorter flowering days and short culm length. The rice cultivar Nipponbare well genotyped model *Japonica* cultivar available whole genomic sequence (International Rice Genome Sequencing Project 2005), high yielding, susceptible to sheath blight and earlier parent of 32R and 29S. Another rice cultivar Kasalath is an *Indica* rice, susceptible to the sheath blight, having longer culm length and thin, shorter flowering days and thin and long grain.

The rice line 32R and 29S were crossed with Nipponbare and Kasalath. The detail of crossing is shown in Figure 1.3. The progeny developed after cross were evaluated based on the physio-morphological traits including plant architecture, sheath blight symptom after inoculation and yield performance (Data was not shown). The progeny developed with cross Kasalath gave high sterility and did not give resistance to sheath blight. Similarly, the progeny of 29S and Kasalath also did not give good cross combination. Similarly, 29S and Nipponbare showed poor combining ability and the plant height was not suitable for the resistance of sheath blight. In this study, the progeny developed from 29S and 32R with Kasalath, were not found suitable for the study because of the high percentage of lines with poor performance and poor plant type. The rice line 32R and Nipponbare gave the specific combining ability. Rice line 32R and Nipponbare has distinct traits related yield and sheath blight resistance. Because of above reason, the rice line 32R and Nipponbare was selected as parent cultivar.

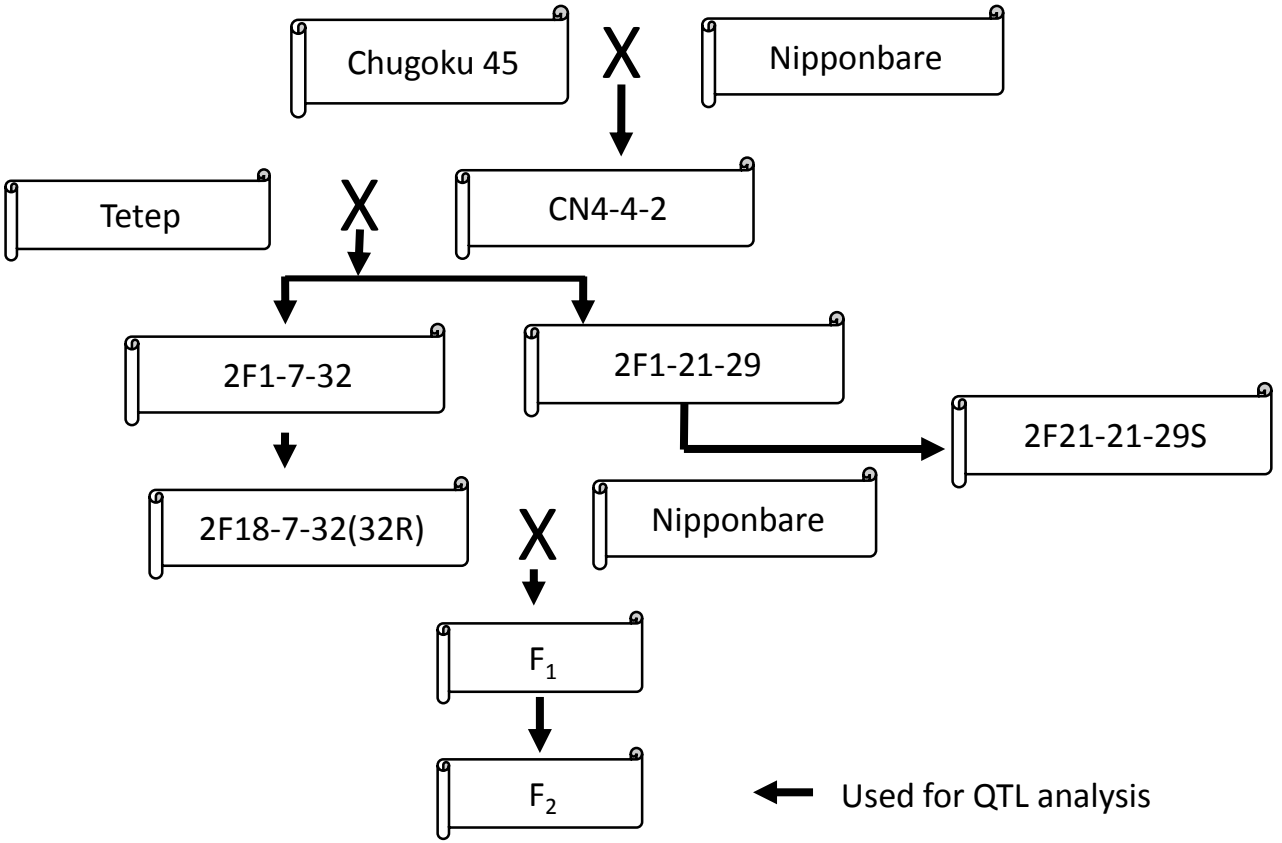

Figure 1.2. Pedigree chart of the experimental materials.

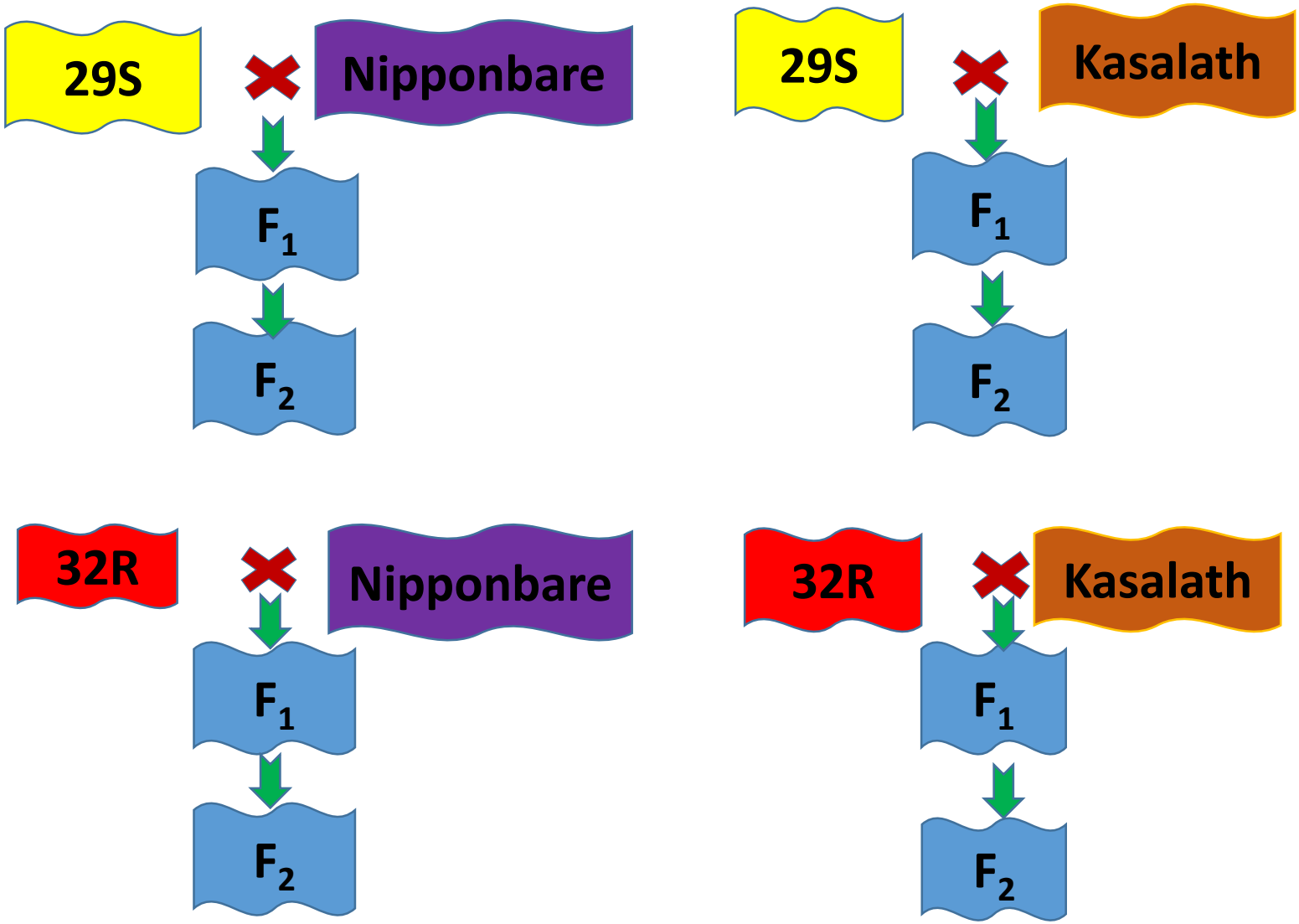

Figure 1.3. Crossing of rice line for the parent selection.

The objective and the summary

The rice line combined with very high yielding capacity and outstanding disease resistance is the requirements for the sustainable rice cultivar. The rice line 32R was continuously screened for sheath blight disease resistance over 15 years, along with another sheath blight susceptible rice line 29S. Study on different aspects of sheath blight pathogen including disease symptoms in field, metabolic pathways and proteomics analysis after *R. solani* infection showed that 32R has strong resistance capacity against sheath blight disease pathogen (Wasano *et al*., 1985; Wasano and Hirota 1986; Danson, 2000; Miyagi *et al*., 2006; Mutuku and Nose 2010; Gaihre and Nose 2011; Mutuku and Nose 2012). Furthermore, the study of temperature effect showed that 32R has strong growing capacity under different temperature regime (Kiet and Nose 2011). These all facts indicate that 32R has high degree of quantitative resistance and also has important agronomic traits for the development of sustainable rice. However, the yielding capacity of 32R has not been studied and attempt of improving yielding capacity of this important rice line was not done. So, to develop high yielding sheath blight resistance rice line by improving the yielding capacity of 32R, it is crossed with one of the high yielding earlier parent cultivar Nipponbare. The cross combination of 32R and Nipponbare led to attribute 12.5 metric ton yield per hectare. The physio-morphological parameter in relation with the disease resistance and yielding capacity were studied. The combination of *Indica* and *Japonica* cultivar enhance the capacity of source and sink size. Similarly, genotyping of rice line 32R to identify QTLs related to resistance of sheath blight for the marker assisted breeding is also hindered. Based on the above facts, present study is focused on the high yielding capabilities and QTL analysis of resistance to sheath blight in rice.

The objective of this study was to develop sustainable sheath blight disease resistance rice varieties that will produce acceptable yields under favorable environments by the marker-assisted

breeding. Many traits are known to contribute to improving yield under sheath blight disease, but the anatomical, physiological, morphological and molecular pathways controlling them are not still clear. There is now a concerted effort to understand the physiological and genetic basis of sheath blight resistance in rice. A better understanding of the genetic basis of sheath blight disease resistance will probably be achieved by using more diverse mapping populations and by precisely identifying the genes affecting variation in sheath blight disease resistance through fine-mapping, gene regulating mechanism. Mapping of QTL to identify chromosomal regions improving sheath blight resistance under humid conditions is hampered by genetic and environment effects, QTL and genetic background interactions, the genes affecting sheath blight resistance. Characterization of such genes and their anatomical, physiological, and molecular genetic effects, will be key factors in the application of molecular marker technology to the development of more sheath blight disease resistance rice varieties.

This study is mainly divided in three Chapters. In the first Chapter, the yielding capacity of rice in relation with sheath blight disease will be discussed in the anatomical, physiological and molecular pathways by utilizing the physio-morphological characteristics. In the second Chapter, the method of identification of polymorphic marker and relation of parent cultivar will be discussed. In the third Chapter, QTL mapping of rice line 32R will be presented in the field condition. In the General Discussion, the characteristics of F_1 progeny developed from 32R and Nipponbare and QTLs of sheath blight disease resistance will be discussed and summarized. Furthermore, I will discuss further step required for marker-assisted breeding sheath blight disease resistance high yielding cultivar from this rice material.

CHAPTER 2

High yielding capabilities and genetic variation in crossing of sheath blight disease resistant rice line

Introduction

Rapid population growth demands more food production, while agricultural land is gradually reducing with urbanization (Timsina and Connor 2001). Rice (*Oryza sativa* L.) is one of the most important food crops of the world. Demand for rice is increasing every year. Yield losses caused by diseases represent a major threat to fulfill the future rice demand. Sheath blight is a rice fungal disease which can cause yield loss up to 50% (Marchetti *et al*., 1991). The epidemic area of sheath blight disease in Japan is increasing every year because of global warming (Iizumi and Yokozawa 2008). Nowadays, sheath blight disease is mainly controlled by the use of harmful fungicides (Gorth and Bond 2007). This method of control is incompatible with sustainable crop management. Rice sheath blight disease is caused by the fungal pathogen *Rhizoctonia solani* Kuhn. The pathogen can survive both in soil and water. Moreover, it produces a phytotoxin (RS toxin), which can reproduce most of the symptoms of the disease (Vidhyasekaran *et al*., 1997). Environmental factors, in particular temperature and humidity, affect both infection and development of the fungus (Han *et al*., 2003). Due to the semi-saprophytic nature and low specificity of the pathogenicity mechanisms in *R. solani*, the fungus infects a large number of plant species such as maize, tomato, cabbage, Chinese cabbage and so on (Zeng *et al*., 2011). Despite extensive efforts to identify sources of resistance in rice germ-plasms, major genes which provide complete resistances to the fungus have not been identified (Wasano 1988; Jia *et al*., 2012).

Screening for combinations of physio-morphological characteristics and resistance for sheath blight using markers derived by QTL analysis is a potentially interesting approach for the development of high yielding cultivar with high degree of disease resistance. It is apparent that information of morphological and physiological aspects of rice is also key feature to plan a resourceful breeding program (Peng *et al*., 2008). Sink and source functions, and their relationships including nonstructural carbohydrates (NSC), Rubisco content, leaf area, panicle length and number of filled grain per panicle are fundamental physio-morphological basis of biomass production and yield in rice. Thus, the development of plant architecture suitable for disease resistance and high yielding capacity by the utilization of information of morphological and physiological traits is required for developing sustainable high yielding crop varieties. Yield traits are quantitatively inherited and affected by different biotic and abiotic factors (Kreye *et al*., 2009). Hence it is also necessary to detect QTLs associated with yield along with disease resistance using reliable populations in order to understand their genetic bases well and for that selection of best parent is very important. Study of QTL analysis has been found QTLs of sheath blight disease resistance in chromosome 1, 3, 5, 7, 8 and 9 on rice line 32R (Chapter 4).

The objective of this chapter was to identify the yielding capacity of sheath blight resistance line and to quantify the different physio-morphological traits contributing to the high grain yield. For the evaluation of yield capacity, plant characteristics and its combining effort for photosynthesis, growth, and grain production based on knowledge of plant and crop physio-morphology is used. This work is the process of constructing reliable genetic resources by utilizing the sheath blight disease resistance characteristics of 32R and yield oriented characteristics of Nipponbare for the marker assisted breeding.

Materials and Methods

Plant materials

The development of F_1 progeny was done in 2007 and 2008. Parent lines used for the experiments were Nipponbare *(Japonica* variety) as a male parent and 2F18-7-32 (32R) as a female parent. The later cultivar was developed from a crossing Tetep (*Indica* variety) as the female parent and CN_4-4-2 (*Japonica* variety) the male parent. CN_4-4-2 resulted from a crossing Chugoku 45 with Nipponbare resulted in the development of CN_4-4-2 (Wasano *et al*., 1985). F_1 rice hybrid progeny was obtained by artificial emasculation and pollination at the flowering stage of 32R with Nipponbare by the modified method of rice crossing (Sarkarung, 1991).

Plant cultivation

The experiment was conducted at the agronomy field of Saga University, Saga, Japan (33^o 16" N and 130^o 18" E) during April 2007 to October 2009 in a heavy clay soil. Seeds of parent cultivars and F_1 were treated with a systemic insecticide and fungicide. 0.1% of Sumichion, an insecticide (Yashima Chemicals Industry Co. LTD) and 0.5% Tekurido C, a fungicide (Kumiai Chemicals Industry Co. LTD) for 24 h and then washed by tap water and incubated at 28^{o}C for 48 h for germination. Pre-germinated seeds were sown on seedling trays. A common procedure was followed in rising of seedling in bed. Seedlings of 30 days old plant were transplanted in the well-puddled experimental plots as single plant per hill with a spacing of 30 x 25 cm. Nitrogen, phosphorus and potash were applied at 50, 33 and 33 kg/ha respectively just before transplanting.

Measurement of physio-morphological properties

The physio-morphological parameters were measured at the physiological maturity stage taking 20 plants from each cultivar. Based on the identification of effective improvement of sheath blight disease resistance and yielding capacity in F_1 progeny in 2008, to identify the relation of sheath blight disease resistance, yielding capacity and physio-morphological traits, the physio-morphological parameter and sheath blight disease resistance of each plant was measured in 2009 by increasing the measurement of physiological parameter than in 2008.

Fresh weight of total biomass was measured at mature stage and the dry weight of each sample was also determined after oven drying at 80^{o}C to constant weight. Harvesting index was estimated by the total dry weight of filled grain to the dry weight of whole plant. Data were analyzed on single plant basis. Tiller and leaf angles are important traits associated with the morphology of ideal plant type. Specially, erect leaves and relatively small tillering angle, allowing a high leaf area are desired characteristics for rice breeding. Leaf area, leaf sheath area and lesion area were measured by using LIA 32 scanner software (Yamamoto, 2004). Second leaf was selected for the comparative study of leaf angle and leaf area as it is reasonable for sheath blight resistance breeding to select the erect second leaf (Han et al., 2003). It is almost exposed to sun and it is less affected by the grain weight during grain filling, also it is similar to third leaf. Leaf angle was measured from the ventral part of the leaf to vertical stem. Productive tiller of plant were counted to determine the total number of tiller in each plant. Tillering angle of tiller was measured from the vertical line from where the tiller attached to the soil surface. The length, width and thickness of seed determine the grain quality and grain shape. The length, width and thickness of seed were measured with bran by using Absolute Digimetric Caliper (Mitutoyo, Japan). First heading date of each cultivar was determined when any one plant of the cultivar have shown ear emergence starting from the day of showing. Culm length was measured from soil surface to

panicle base in centimeters. Panicle length was measured from panicle base to tip in centimeters. The 1000 grains weight of each cultivar was measured after harvesting. Harvest index (HI) was calculated for each cross as HI = (dry weight of grain yield / dry weight of whole plant) x 100 (%).

Total nonstructural carbohydrate measurement

The leaf sheaths were dried at 80^{o}C for 3 days. Nonstructural carbohydrate (NSC) accumulated in the leaf sheath was measured according to the method of Tsukaguchi *et al*. (1996) with some modifications: milled samples (0.5 g) were added into 30 ml of water and autoclaved at 120^{o}C for 20 minute to extract NSC. After cooling, 1.5 mg *a*-amylase (A0521, Sigma-Aldrich) and 0.5 mg amyloglucosidase (A9228, Sigma-Aldrich) was added in 20 ml buffer made by 88.7 mM KH_2PO_4 and 11.1 mM Na_2HPO_4-$12H_2O$ and incubated at 40^{o}C for 24 h to disbranch NSC into monosaccharides. After filtrating incubated samples, residuals were dried at 80^{o}C for 24 h and weighed. NSC was calculated from the weight difference between the initial sample and residual.

Determination of Ribulose-1, 5-bisphosphate carboxylase oxygenase content

Ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco) content was determined with some modification Kanbe *et al*. (2009). Leaf-disks without midribs were sampled from flag leaf blade sample on the main stem during heading time, and then homogenized first by using liquid nitrogen. Powder leaf sample was again homogenized with quartz sand in a buffer solution (pH 7.9) containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.5 mM EDTA-2Na, 5 M DTT, 0.2% (w/v) PVPP and 0.1% (w/v) Triton X-100. After the homogenate was centrifuged at 19,000 x g for 20 min, the supernatant was treated with 0.625 M Tris-HCl (pH 6.8), 2.0% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) BPB and 5.0% (v/v) 2-mercaptoethanol at 100^{o}C for 3 min. The Rubisco content was determined by SDS-PAGE gel documentation system (ATTO, Tokyo, Japan) after

CBB-R250-staining. The SDS-PAGE image of Rubisco bands was captured with a scanner (Canon MP640). The intensity of the band corresponding to the large subunit of Rubisco was measured with ImageJ software (ImageJ 1.43u; http://rsbweb.nih.gov/ij). A calibration was made with bovine serum albumin (BSA).

Evaluation of sheath blight resistance

The *R. solani* isolate C-154, No. 305229 maintained in the Agricultural Resource Gene Bank, Tsukuba, Japan was used for the inoculation. Culture and inoculations of the mycelium were based on the syringe inoculation method Wasano *et al*. (1983). The potato sucrose agar (PSA) was made containing 300 g potato, 20 g sucrose and 15 g agar. *R solani* fungus was grown at 28^{o}C for about 4 days. The fungus which is ready to inoculate in rice is shown in Figure 2.1. It was chopped into small pieces that it would fit in syringe and then it was homogenized by syringe. Then, 0.2 ml of prepared inoculums was injected to the third leaf sheath from the flag leaf using plastic syringe during the heading date. The fungus was inoculated in 20 plants of each cultivar selecting 5 tillers per hill. Disease system was scored based on ratio of lesion area to leaf sheath area one month after inoculation, according to the method of Wasano *et al*. (1983).

Data analysis

Data were analyzed following Student T Test (SPASS 16.0 SPSS Inc. 2007). The F_1 progeny and parental inbreed were compared at the 0.05 probability level. Multiple regression analysis was done to identify the contribution of physio-morphological traits on yield and sheath blight traits in F_1 progeny by taking yield and sheath blight disease score as dependent variable, respectively.

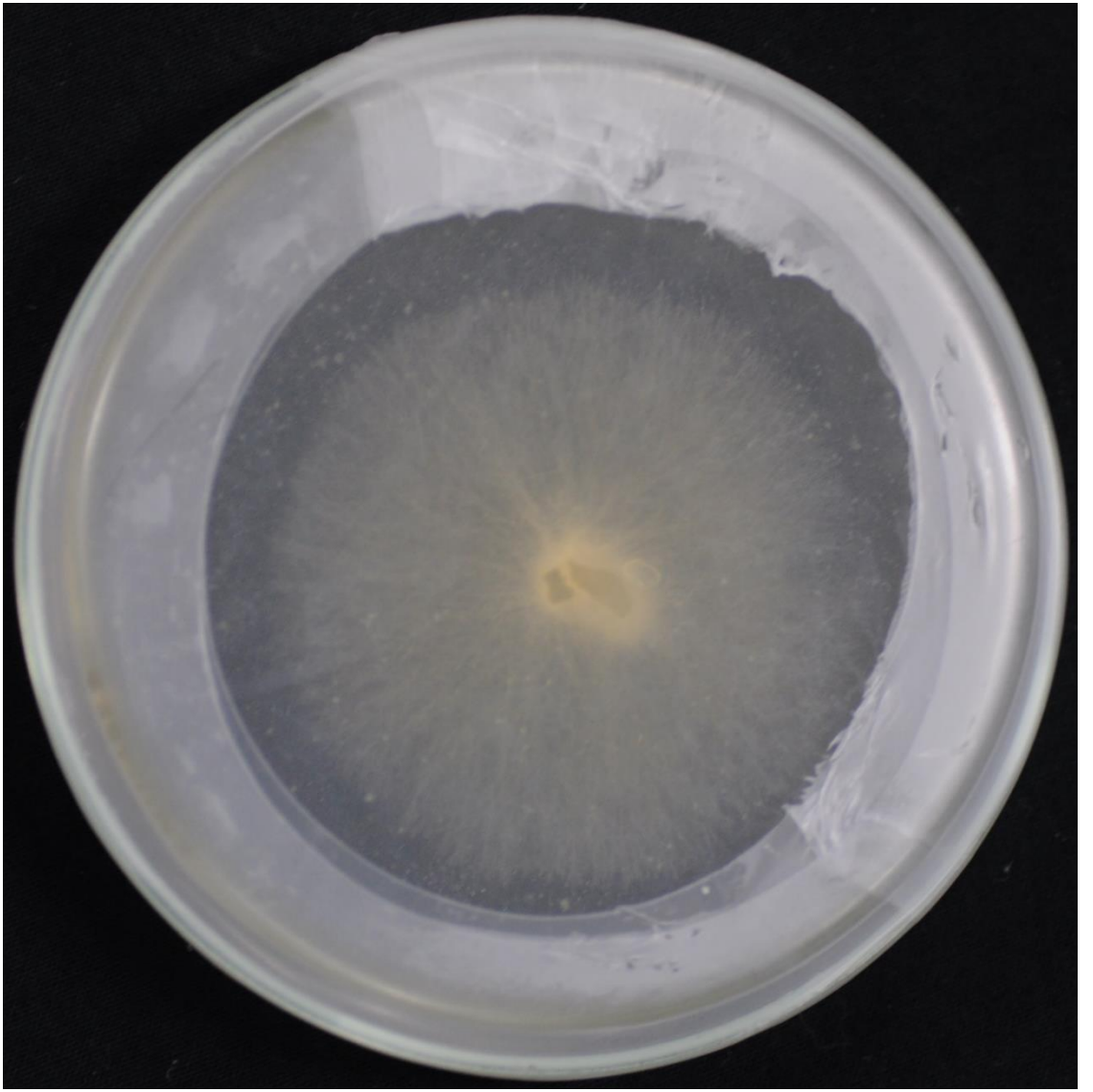

Figure 2.1. *R. solani* grown in PSA (Potato Sucrose Agar) medium.

Chemicals

CBB-R-250, Glycerol and TritonX-100 were purchased from Wako Chemicals Inc. and other chemicals used in this study were purchased from Sigma-Aldrich Inc.

Results

The rice line 32R grain yield was 7.94 t/ha, which is less than Nipponbare, a high-yielding modern leading cultivar in Japan, by 17.5% (9.63 t/ha; Table 2.1). The cross of 32R and Nipponbare, F_1 progeny (F_1), performed better than better yielding parent Nipponbare. F_1 performed 12.5 t/ha yielding capacity. Dry matter accumulation (DMA) of F_1 rice line was greater than that of both parents. DMA per tiller of F_1 was 5.51 g greater than 4.12 g of 32R and 4.11 g of Nipponbare. Similarly, dry seed weight per tiller of F_1 was 2.47 g greater than 2.36 and 1.56 g of Nipponbare and 32R, respectively and also coefficient of variation of F_1 was 4.45% comparatively less than 12.7 and 5.76% of Nipponbare and 32R, respectively. Harvest index was 44.8% in F_1, 57.6% in Nipponbare and 37.9% in 32R, respectively. The panicle length of F_1 progeny was significantly different with both male and female parents (Table 2.2). 32R and Nipponbare were not significantly different with each other in the case of panicle length. The panicle length of F_1 was 22.3 cm longer than 20.2 cm of 32R and Nipponbare. The number of filled grain per panicle of F_1 was significantly different to both parents. F_1 had 118 filled grains per panicle greater than both parents. The number of grain has been studied by separating, the empty grain and filled grain. The empty grain is mentioned as unfilled grain in this study. F_1 had more unfilled grains than that of parents. F_1 had 167 total grains including filled and unfilled grains greater than 120 and 113 grains of Nipponbare and 32R, respectively. This indicates that F_1 has large sink size and high yield potential.

Table 2.1. Yield and harvest index of rice

Rice line	DMA per tiller (g)	CV of DMA per tiller (%)	Dry seed weight per tiller (g)	CV of dry seed weight per tiller (%)	Harvest index per plant (%)	Yield per hector (metric ton)
32R	4.12 ± 0.63 a	15.3	1.56 ± 0.09 a	5.76	37.9	7.94
Nipponbare	4.11 ± 0.72 a	17.5	2.36 ± 0.30 b	12.7	57.6	9.63
F_1 (32R x Nb)	5.51 ± 1.12 b	20.3	2.47 ± 0.11 c	4.45	44.8	12.5

CV, coefficient of variation; DMA, dry mass accumulation; DMA is for above ground parts during mature stage. Tiller of rice plant was randomly selected including main culm, primary tiller, secondary tiller and tertiary tiller. Harvest index was calculated by the total dry weight of filled grain to the dry weight of whole plant. Means followed by different letters in a column are significantly different at 5% level by student t test.

Table 2.2. Characteristics of grain and yielding capacity of rice

Rice line	Width of seed (mm)	Thickness of seed (mm)	Length of seed (mm)	Panicle length (cm)	1000 grain weight (g)	Total unfilled grain per panicle	Total filled grain per panicle
32R	2.68 ± 0.11 a	1.74 ± 0.09 a	7.40 ± 0.25 a	20.2 ± 1.96 a	18.6 ± 1.18 a	14.4 ± 6.01 a	97.9 ± 22.4 a
Nb	3.11 ± 0.15 c	2.26 ± 0.13 c	7.26 ± 0.52 a	20.2 ± 1.31 a	26.3 ± 2.83 c	17.0 ± 7.03 a	103 ± 20.8 a
F_1 (32R x Nb)	3.00 ± 0.10 b	2.06 ± 0.09 b	7.39 ± 0.19 a	22.3 ± 1.14 b	24.7 ± 1.13 b	49.3 ± 7.49 b	118 ± 14.7 b

Length width and thickness of seed are taken as morphological indicator of grain quality. Means followed by different letters in a column are significantly different at 5% level by student t test.

Table 2.3. First heading date of rice line

Rice line	First heading date (days)
32R	92
Nipponbare	91
F_1 (32R x Nb)	97

Nb, Nipponbare; F_1 (32R x Nb) is first filial generation of rice line 32R and Nipponbare. First heading date was determined by counting days from the seedling to first flower seen on that particular rice line.

Table 2.4. Culm and internode lengths of parents and hybrid rice line

Rice line	1st internode from top (cm)	2nd internode from top (cm)	3rd internode from top (cm)	4th internode from top (cm)	Total culm length (cm)	CV of culm length (%)
32R	32.5 ± 1.52 a	20.6 ± 1.55 a	16.1 ± 1.38 b	7.36 ± 3.14 b	76.9 ± 4.31 a	5.60
Nipponbare	35.2 ± 1.87 b	21.2 ± 2.13 b	15.5 ± 1.95 a	5.15 ± 2.24 a	79.1 ± 9.49 b	12.0
F_1 (32R x Nb)	35.1 ± 1.57 b	24.7 ±1.61 c	21.2 ± 1.68 c	13.4 ± 5.37 c	93.6 ± 15.7 c	16.8

CV, coefficient of variation; 1st intenode is the upper internode and then 2^{nd}, 3^{rd} and 4^{th} from the top. Means followed by different letters in a column are significantly different at the 5% level by student t test.

Table 2.5. Physio-morphological characters of parents and F_1 progeny

Rice line	Leaf area (cm^2)	Leaf angle (degree)	Number of tiller per hill	Tillering angle (degree)
32R	26.8 ± 5.20 a	17.0 ± 4.00 a	33.0 ± 5.00 a	19.5 ± 5.87 a
Nipponbare	36.0 ± 6.00 b	22.4 ± 4.86 b	27.0 ± 5.00 b	9.60 ± 6.27 b
F_1(32R x Nb)	37.6 ± 5.00 b	19.9 ± 4.19 b	30.0 ± 8.00 ab	15.8 ± 5.15 a

Second leaf from the flag was selected for the leaf area and leaf angle measurement. Leaf angle was measured from the ventral part of the leaf to the vertical stem. Tillering angle of each tiller was from the vertical. Means followed by same letters are not significantly different at the 5% level by student t test.

The grain shape of F_1 was similar to that of 32R but it was comparatively thicker than 32R (Table 2.2). The grain length of parent cultivar and progeny was not significantly different. The grain of F_1 was slightly longer than Nipponbare but less than 32R. Seed width and seed thickness was significantly different to each other. Grain weight of Nipponbare was higher than F_1 progeny. 1000 grains weight of Nipponbare is 26.3 g which is higher than F_1, 24.7 g. 1000 grains weight of 32R is comparatively less than F_1.

The days to first heading was different in parent cultivar and F_1 (Table 2.3). The first heading day of 32R and Nipponbare was 92 and 91 days, respectively but in F_1 was 97 days, almost one week delayed than that of parents. Parent cultivar took few days for the complete flowering from the first heading date. However, F_1 took 15 days for the complete flowering. Culm length of F_1 progeny was significantly different with parents (Table 2.4). F_1 was taller than both parents. There was interesting results in internode length. First internode of F_1 from the upper node is slightly shorter than Nipponbare and significantly longer than 32R. Second internode of F_1 was 1.19 fold longer than 32R and 1.16 fold longer than Nipponbare. Third internode of F_1 was 1.31 fold longer than 32R and 1.36 fold longer than Nipponbare. Fourth internode of F_1 was 1.82 and 2.60 fold longer than 32R and Nipponbare, respectively.

The leaf area of second leaf counting from the flag leaf in the F_1 was significantly different with 32R but it was not significantly different with Nipponbare (Table 2.5). That is, leaf area of second leaf of F_1 was 37.6 cm^2 greater than 36.0 cm^2 of Nipponbare and 26.8 cm^2 of 32R. Leaf angle of second leaf of parent cultivar was significantly different with each other. Leaf angle of F_1, 19.9^o was significantly greater than 32R but slightly less than Nipponbare. F_1 had great variation in tillering capacity, ranged 21 to 47 number of tiller per hill. Number of tiller of F_1 was

slightly greater than both the parents. The tillering angle of F_1 was significantly greater than Nipponbare and it was slightly less than 32R. The tillering angle of F_1 progeny was 15.8°.

The Rubisco content in flag leaf of F_1, 2.19 mg/g FW was significantly higher than those of 32R and Nipponbare (Table 2.6). Although there was no significant difference in non-structural carbohydrate (NSC) content of leaf sheath during heading, F_1 progeny has larger average NSC accumulation in leaf sheath. NSC accumulated in leaf sheath might contribute to grain filling during heading in F_1 progeny.

A significant difference in sheath blight disease resistance was found between 32R and Nipponbare. The average disease scores for the two cross parent cultivars were 12.0 in 32R and 38.0 for the Nipponbare (Fig. 2.2). Only one third of inoculated F_1 showed disease symptom. The disease score of F_1 was 22.2 ± 17.0 in the diseased plants.

Multiple regression analysis in F_1 was done to identify the contribution of physio-morphological traits of F_1 to the 12.5 metric ton per hectare yielding capacity. Parameters estimated by multiple regression analysis are presented in Table 2.7. The strong and significant coefficient of determination (R^2) attributed for the yield was 0.906. Multiple linear regression analysis showed number of filled grain per panicle, panicle length and leaf area of second leaf had statistically significant positive effects and seed thickness has a significant negative effect. Other physio-morphological traits including culm length, leaf angle, number of tiller, tillering angle, Rubisco content, DMA and NSC did not have significant effect for grain yield in F_1 progeny. Number of filled grain per panicle, panicle length and leaf area contributed its 83.0, 28.4 and 29.9% effort to the yield, respectively. In addition, the coefficient of determination (R^2) attributed for the number of filled grain per panicle by the physio-morphological traits was 0.798. Furthermore, this

study shows the strong dependency of number of filled grain per panicle to the total number filled and unfilled grain per panicle. The multiple regression analysis of yield on parent cultivar was also done (Table 2.8). Number of tiller, leaf area of second leaf, seed length, seed width and number of grain per panicle had significant effect on yield in rice line 32R. In the rice line Nipponbare, number of tiller and number of filled grain per panicle had significant effect on yield. The yield potential of F_1 progeny and parent had significantly depended on number of filled grain per panicle. Consequently, the conclusion can be derived that yield potential depends on source capacity based on leaf area and sink size based on number of filled grain per panicle and panicle length.

Multiple regression analysis was done by keeping disease score a dependent variable with the morphological traits which play major role for disease build up before heading during the disease inoculated period suggested that there is significant dependency between some traits and disease score (Table 2.9). The significant negative estimated value was found with culm length, leaf area and tillering angle in the F_1 progeny. In rice line 32R, only tillering angle had significant negative effect on sheath blight disease score and in rice line Nipponbare leaf area had positive significant effect on sheath blight disease score.

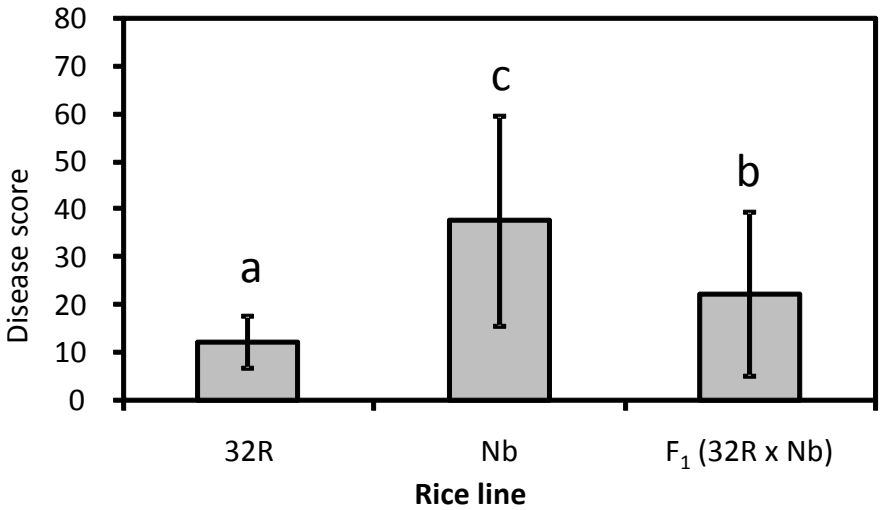

Figure 2.2. Disease score of sheath blight disease in rice. Disease score were measured based on the ratio of lesion area to the leaf sheath area. Means followed by different letters are significantly different at the 5% level by student t test. Vertical bars represent standard deviation.

Table 2.6. Rubisco content and NSC accumulation in parents and F_1 progeny

Rice line	Rubisco content (mg/g FW)	NSC content (mg/g DW)
32R	1.19 ± 0.14 a	92.0 ± 22.8 a
Nipponbare	1.94 ± 0.16 b	95.0 ± 9.78 a
F_1(32R x Nb)	2.19 ± 0.15 c	113.6 ± 20.6 a

Flag leaf at the early stage of heading was used for the Rubisco content measurement. NSC (nonstructural carbohydrate) accumulated was measured from leaf sheath during heading. Means followed by same letters are not significantly different at the 5% level by student t test.

Discussion

This study is related to develop sheath blight resistance rice line having high yield capacity. To elucidate the factors contributing to high grain yield and sheath blight resistance in rice, comparative study of physio-morphological character of parents and F_1 progeny related to grain yield and sheath blight resistance was done. The important result of this study is that F_1 progeny achieved 12.5 t/ha yielding potential with sheath blight disease resistance. Multiple regression analysis of physio-morphological traits of F_1 progeny suggests that the high yield potential of F_1 is due to number of filled grain, panicle length and second leaf area and the comparative study of physio-morphological traits of F_1 progeny with parents cultivar shows that higher Rubisco content, suitable culm length and higher NSC accumulation during heading has also contribution for high yield potential in F_1 progeny ((Table 2.5 and Table 2.6).

F_1 progeny had 167 grains per panicle including filled and unfilled grains. F_1 progeny had high yield potential due to higher number of rough grains and also has chance to increase yield by field crop management and marker assisted breeding in future. The unfilled grains in F_1 progeny are comparatively high (Table 2.2). It can be due to delayed heading (Virmani *et al*., 1982). But the delayed heading can contribute decrease of disease infection in the actual field by avoiding the attack of the pathogen because of decreasing temperature and humidity in rice growing areas (Wasano *et al*., 1983). Long culm type cultivars with a superior lodging resistance are important mainly for animal forage and biofuel production (Ookawa *et al*., 2010). The culm length of F_1 progeny was 93.6 cm (Table 2.4). The actual plant height of F_1 progeny including culm length and upper part from the panicle base is more than 110 cm. The rice line having plant height 110-120 cm rice can favors biomass accumulation and lodging resistance when attacked by typhoon accompanied with heavy rain (Jiang-Shi and Chuan-Gen 2005). Additionally, tall plants might

result in better air ventilation, reduced relative humidity and increased light transmission inside the canopy, which are not favorable for sheath blight development (Han *et al*., 2003). The large erect leaf with high number of tiller in F_1 progeny attributed large source. The photosynthesis is greater when large erect leaf is exposed to light on both sides (Kush *et al*., 1995). F_1 progeny has large number of panicle per unit area because of high tillering ability (Table 2.5). Panicle number per unit area depends on tillering ability (Counce and Wells, 1990; Miller *et al*., 1991; Li *et al*., 2003 and Ao *et al*., 2010). Thus, these all facts suggest that canopy architecture is a major target in rice breeding for the improvement of disease resistance and yield.

F_1 progeny has comparatively large amount of Rubisco content, NSC and DMA (Table 2.1, Table 2.2 and Table 2.6). Rubisco accumulated to a certain level excess of photosynthetic requirements, serving as stored nitrogen for grain filling (Murchie *et al*., 2002). NSC that accumulates in leaf sheaths and culms before heading plays important role in compensating for the source supply after heading for the grain formation, grain filling and grain quality (Takai *et al*., 2006). *Indica* cultivars accumulate large amount of NSC before heading and the rate of utilization for the grain filling after heading is also higher than that of *Japonica* cultivar (Nagata *et al*., 2002 and Yoshinaga *et al*., 2011). In addition, the high amount of NSC accumulation mediates heat tolerance and contributes to the ripening stability under adverse climate conditions (Morita and Nakano 2011). For the high yielding variety which contains large number of grains, the amount of NSC accumulation and utilization rate should be high to achieve large grain.

Table 2.7. Parameter estimated by multiple linear regression analysis for the dependent variable yield and number of filled grain per panicle in F_1 progeny with several traits

Independent variables	Dependent variable			
	Yield		Number of filled grain per panicle	
	Parameter estimated	Sig.	Parameter estimated	Sig.
Culm length	-0.161	<0.106	0.141	<0.331
Panicle length	0.284	<0.009*	-0.238	<0.123
Leaf area (second leaf)	0.299	<0.011*	-0.159	<0.303
Number of tiller	-0.133	<0.195	0.163	<0.317
Tillering angle	0.048	<0.664	-0.142	<0.418
Leaf angle (second leaf)	0.009	<0.924	-0.119	<0.417
Disease score	0.175	<0.111	-0.111	<0.489
Rubisco	-0.071	<0.542	-0.076	<0.663
NSC	0.161	<0.227	-0.191	<0.355
Dry weight of plant	-0.202	<0.090	0.297	<0.137
Seed length	0.127	<0.171	0.028	<0.833
Seed thickness	-0.353	<0.026*	0.001	<0.998
Seed width	-0.156	<0.231	0.171	<0.372
Rough grains	-	-	1.045	<0.000*
Number of filled grain	0.830	<0.000*	-	-
R^2	0.906	<0.000*	0.798	<0.001*

R^2: coefficient of determination and < symbol in table denotes significantly different less than the indicated level. Rough grains include total grains including filled grains and unfilled grains. * in a column denotes statistically significant less than 0.05 levels.

Table 2.8. Parameter estimated by multiple linear regression analysis for the dependent variable yield in parent cultivar with several traits

Independent variables	Dependent variable yield			
	32R		Nipponbare	
	Parameter estimated	Sig.	Parameter estimated	Sig.
Culm length	0.014	<0.786	-0.132	<0.270
Panicle length	0.012	<0.802	-0.103	<0.353
Leaf area (second leaf)	0.034	<0.367	-0.095	<0.431
Number of tiller	0.385	<0.000*	0.659	<0.000*
Tillering angle	0.024	<0.572	-0.274	<0.051
Leaf angle (second leaf)	-0.138	<0.002*	-0.073	<0.524
Disease score	0.047	<0.304	-0.144	<0.241
Rubisco	0.004	<0.900	-0.070	<0.514
NSC	-0.100	<0.016	0.077	<0.460
Dry weight of plant	0.073	<0.051	0.146	<0.120
Seed length	0.148	<0.012*	0.044	<0.672
Seed thickness	0.081	<0.152	-0.073	<0.592
Seed width	0.109	<0.010*	-0.119	<0.419
Number of filled grain	0.792	<0.000*	0.843	0.000*
R^2	0.997	<0.000*	0.944	<0.000*

Notes: see Table 2.8 for descriptions.

Table 2.9. Parameter estimated by multiple linear regression analysis for the dependent variable sheath blight disease score in F_1 progeny and parent cultivar with sheath blight disease related traits

Independent variables	Dependent variable					
	F_1 progeny		32R		Nipponbare	
	Parameter estimated	Sig.	Parameter estimated	Sig.	Parameter estimated	Sig.
Culm length	-0.379	<0.045*	0.028	<0.882	-0.370	<0.076
Leaf area (second leaf)	-0.402	<0.040*	0.280	<0.161	0.344	<0.083
Number of tiller	0.122	<0.529	-0.383	<0.062	0.012	<0.950
Tillering angle	-0.526	<0.019*	-0.508	<0.012*	0.097	<0.632
Leaf angle (second leaf)	0.256	<0.166	-0.001	<0.997	0.427	<0.038*
R^2	0.461	<0.027*	0.421	<0.049*	0.399	<0.065

Note: see Table 2.8 for descriptions.

By the study of physio-morphological traits of F_1 progeny, the conclusion can be derived that the high yielding capacity attributed in F_1 progeny might be due to the induced characteristics of NSC accumulation and utilization of earlier *Indica* based parent Tetep. Although, there were no significant different but high average NSC accumulation was observed in F_1 progeny. The DMA from elongation to heading is also increase grain filling rate (San-oh *et al*., 2004 and Katsura *et al*., 2007). High yield can be achieved either by increasing the biomass production or harvest index or both. Large amount of NSC and DMA increase the source strength and the large amount of filled grain can be obtained. Large sink size with higher source capacity is basic requirement for the development high yielding varieties. The number of filled grain per panicle can contribute 83.0% of its effort to the yield (Table 2.7). Harvest index and plant height has negative relationship but plant height and biomass production has positive relationship (Kuroda *et al*., 1989 and Yoshida, 1981).

For stability evaluations, the coefficient of variation (CV) is used to estimate the yield variation associated with each variety. A low CV suggests consistency of rice yield on that environment. The wider CV of morphological character is common in rice, because of the short duration of vegetative and reproductive periods of the late-initiated tiller. Specially, there is short duration of vegetative and reproductive periods in the events like tiller emergence, booting, delayed anthesis and synchronized maturity date of whole tiller of hill. In this study also, CV of DMA was 15.3, 17.5 and 20.3% in rice line 32R, Nipponbare and F_1 progeny, respectively (Table 2.1). However, it is interesting to note that the CV of dry seed weight in F_1 was 4.45% which is less than 5.76% of 32R and 12.7% of Nipponbare. The CV of filled grain per panicle was 22.8, 20.9 and 12.4 % in rice line 32R, Nipponbare and F_1 progeny (Table 2.2). Particularly, the CV of 4th internode length was comparatively higher 42.6, 43.5 and 40.1% in rice line 32R, Nipponbare

and F_1 progeny, respectively (Table 2.4). But the culm length measurement was quite uniform. The CV of culm length in F_1 progeny was 16.8% higher than 12.0 and 5.60% of Nipponbare and 32R, respectively. In evaluations of both grain yield and stability, F_1 progeny were more desirable because of high grain yields and low CV. A small coefficient of variation is expected for the morphological properties of seed to obtain uniform seed size and good farm yield in rice.

To determine an effective improvement of physio-morphological character in F_1 progeny, heterosis effects were analyzed. F_1 progeny showed positive heterosis on most of the physio-morphological character such as culm length, panicle length, number of grains per panicle, leaf area, number of tiller per hill and DMA. In some character, F_1 progeny showed negative better parent heterosis such as tillering angle with 32R, and grain size and harvest index with Nipponbare. Result shows 42.3% mid parent heterosis and 29.8% better parent heterosis for the yield having 12.5 t/ha yielding capacity. In addition, the important phenotypic character of sheath blight resistant line, long culm length, late heading date, high tillering angle and erect leaf were attributed in F_1 progeny.

Multiple regression analysis shows that number of filled grain per panicle has significant contribution in yield in all cultivar and other variable depends on the types of cultivar (Table 2.7 and Table 2.8). Leaf area of second leaf, panicle length and number of filled grain per panicle had significant role for the yield performance of F_1 progeny. Multiple regression analysis on sheath blight disease denotes that higher leaf angle is favored for sheath blight disease transformation and build up in rice line Nipponbare and higher tillering angle has reduced sheath blight transformation by creating less humidity inside the hill in the rice line 32R. On the other hand, culm length, leaf area and tillering angle have significant effect on sheath blight disease with the poor predictor value in the F_1 progeny. The negative significant relation with cum length, leaf area and tillering

angle with sheath blight disease score in this study suggest that plant height, large leaf area and big tillering angle are not favorable for the disease transformation and build up. Furthermore, lower predictor value suggest that plant morphological character are not the major factors for the sheath blight disease resistance and individually does not affect resistance capacity. Plant traits affects sheath blight development by changing canopy density rather than changing sheath blight susceptibility of individual plants. Canopy density was different among the varieties due to the differences in plant height, tillering angle and leaf area. Varieties with more tillers, big tillering angle, higher leaf area with higher leaf angle, and shorter plant height usually have dense canopy. Canopy density affects sheath blight development and transformation by altering micro environmental conditions (Han *et al*., 2003 and Tang *et al*., 2007).

One of the improved characteristic of the F_1 progeny is high sink capacity determined by large sink size (Table 2.7). The sink size depends on total number of grains including filled and unfilled grains and panicle length. The sink size of F_1 progeny indicates that the possibility of increasing yielding capacity more than 12.5 metric ton per hectare by improving grain filling percentage. The desired percentage of grain filling of rice plant is more than 80% for high yielding ideotype plant (Peng *et al*., 2008). The grain filling percentage of F_1 progeny was 70.5%. To overcome the problem of the poor grain filling percentage, attempt should be given to increase the source and sink regulation as rice yield potential is determined by the balance between sink size and source capacity (Oshumi *et al*., 2011). Source capacity is determined by the NSC, DMA, and Rubisco collected in plant, which is higher in F_1 progeny than that of recurrent parent but not sufficient to fulfill the requirements of its large sink size.

This study elucidated that the high yield potential in F_1 progeny was due to polygenic characters; especially the dominance characteristics isolated in some important agronomic traits

and sink size. The comparative study of physio-morphological traits of F_1 progeny and parent cultivars indicates that there is effective improvement of number of filled grain per panicle, Rubisco, NSC, panicle length, culm length, number of tiller, seed weight, leaf area and DMA in F_1 progeny. The high yield of F_1 progeny than that of parent cultivar is due to the effective improvement of these all physio-morphological traits. Although, the multiple regression analysis of physio-morphological traits of F_1 progeny only revealed that panicle length, second leaf area and number of filled grain per panicle had significant contribution for the 12.5 t/ha yield potential compare to other traits in F_1 progeny. The source capacity and sink size related traits especially number of filled grain per panicle, panicle length and leaf area can be utilized for marker assisted breeding followed by the QTL analysis for the development of sustainable high yield rice. Furthermore, F_1 progeny has induced sheath blight resistance character of 32R along with ideotype plant structure. These characteristics lead us to do QTL analysis of sheath blight disease by developing F_2 population. The QTL analysis done by using total 1338 SSR markers identify some important dominant sheath blight gene in chromosomes 1, 3, 4, 5, 7, 8 and 9 (Chapter 4). Therefore, we may use this rice line for marker assisted breeding for the development in high yielding sheath blight disease resistance rice in future.

The cross combination of sheath blight resistance rice line 32R with Nipponbare, developed 12.5 t/ha yielding capacity in the F_1 progeny with ideotype plant structure for sheath blight disease resistance and high yield. Furthermore, from this cross combination, super yielding rice with sheath blight resistant can be developed by general selection. Study of yielding capabilities suggests that yield potential depends on source capacity based on leaf area and sink size based on number of filled grain per panicle and panicle length.

Summary

Rice breeding has achieved significant progress towards enhancement effects of genetic improvement for the yield but the development of high yielding cultivar with fungal disease resistance is still an important step needed to fulfill the future demand of food for growing population. Rice line 32R is a well-documented source of durable and broad spectrum resistance to sheath blight disease. The objective of this study was to determine the genetic component of yielding capabilities of rice line 32R and quantifying physio-morphological characteristics related to high yielding capacity. Characteristics of sink and source were studied in relation to grain yield and disease resistant. This study revealed that 12.5 metric ton per hectare yielding capacity in F_1 progeny was attributed because of improved performance culm length, panicle length, number of tiller, tillering angle, Rubisco content in leaf, nonstructural carbohydrate (NSC), dry matter accumulation (DMA), leaf area and number of filled grain per panicle. Especially, multiple regressions showed that number of filled grain per panicle, panicle length and leaf area had contributed 83.0, 28.4 and 29.9 % of its effort to the grain yield, respectively in F_1 progeny.

CHAPTER 3

Detection of genetic diversity using SSR markers in rice 32R and Nipponbare

Introduction

Identification of genetic diversity and relationships among breeding lines is great important to facilitate parent selection in rice breeding programs. Ever since the domestication of crop plants, human activities has been improving them giving selection emphasis to traits that suits agro-ecological and socioeconomic needs. In rice, like many other crops, selection preference has been for improvement of yield enhancing traits like compact panicle with more filled grains per panicle, large seed size, non-shattering habit etc. The selection process continued for centuries result in cultivars far different from the wild/weedy progenitor species in their habit and potential (Choudary *et al*., 2013). Unlike physio-morphological traits used earlier to estimate genetic variability, molecular markers have become quite handy in precisely understanding the extent of genetic divergence among varieties being chosen these days as parental sources in breeding programs. Although there are more than 40,000 rice varieties reported worldwide, a small fraction of these have been used in practical breeding. Therefore, better understanding of the genetic makeup of underused rice germplasm is an important issue for rice breeding. Recent advanced molecular and computational tools enables the estimation of genetic diversity and population structure of rice germplasm easily (Vannirajan *et al*., 2012). Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. DNA fingerprinting is used to describe the combined use of several single locus detection systems and is being used as versatile tools for investigating various aspects of plant genomes including characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant

breeding. Molecular data would provide a basis for better management and conservation of the collection and could be used as reference for its enhanced use in breeding programs.

SSR markers are polymorphic loci present in nuclear and organelle DNA that consist of repeating units of 1 - 6 base pairs in length. They are typically neutral, co-dominant and are used as molecular markers which have wide-ranging applications in the field of genetics, including population studies (Morgante *et al*., 2002). Microsatellites can also be used to study gene dosage (looking for duplications or deletions of a particular genetic region). Microsatellites can be amplified for identification by the polymerase chain reaction (PCR) process, using the unique sequences of flanking regions as primers. DNA is repeatedly denatured at a high temperature to separate the double strand, and then cooled to allow annealing of primers and the extension of nucleotide sequences through the microsatellite. This process results in production of enough DNA to be visible on agars or polyacrylamide gels; only small amounts of DNA are needed for amplification as thermo cycling in this manner creates an exponential increase in the replicated segment. Evaluations under controlled conditions done at later breeding stages are very expensive and MAS can reduce costs to less than one tenth. Furthermore, the technique requires less capital expenditure, is very rapid, highly reliable, and can be used simultaneously for other traits the ability to distinguish between closely related individuals is particularly important for many crop species, which tend to have a narrow genetic base. To date, an evaluation of the amount of diversity detected with microsatellites has revealed more polymorphism compared with other assay procedures (Powel *et al*., 1996).

In order to map QTLs of sheath blight resistance of rice, the cultivars were first being screened with DNA markers to establish parental polymorphism between them. For this purpose six possible varieties were selected. Cultivars selected to study parental polymorphism are

resistance to sheath blight disease, susceptibility to sheath blight disease, high yielding characteristics as well as having complete genotyping information. The cultivar selected to study parental polymorphism are Tetep, Chugoku 45, CN_4-4-2, 29S and 32R. In the present study, SSR markers were used to study the parental polymorphism between the selected rice varieties. About 1338 RM markers located on 12 rice chromosomes, based on the reported distribution with covering whole genome were listed.

The objective of this study was to identify the polymorphic SSR marker in between rice line 32R and Nipponbare to determine the QTLs of sheath blight disease. The propose of including Tetep , Chugoku 45, CN_4-4-2 and 29S in this study is to determine the genetic relation of these rice line with 32R .

Materials and Methods

Plant material and plant cultivation

The plant was grown at the agronomy field of Saga University, Saga, Japan (33^o 16" N and 130^o 18" E) in 2010 in a heavy clay soil. The rice lines evaluated in this study are Tetep, Nipponbare, Chugoku 45, CN_4-4-2, 29S and 32R. Seeds of all cultivars were treated with a systemic insecticide and fungicide. 0.1% of Sumichion, an insecticide (Yashima Chemicals Industry Co. LTD) and 0.5% Tekurido C, a fungicide (Kumiai Chemicals Industry Co. LTD) for 24 h and then washed by tap water and incubated at 28^{o}C for 48 h for germination. Pre-germinated seeds were sown on seedling trays. A common procedure was followed in rising of seedling in bed. Seedlings of 30 days old plant were transplanted in the well-puddled experimental plots as single plant per hill with a spacing of 30 x 25 cm. Nitrogen, phosphorus and potash were applied at 50, 33 and 33 kg/ha respectively just before transplanting. Only rice leaf has been used for this

study. The flag leaf and second leaf at the early heading stage were sampled in August of 2010. Rice leaves of each rice lines were first collected in aluminum foil and then put in liquid nitrogen. After that all sample were grinded and kept in -80^{o}C.

DNA isolation

Total genomic DNA was extracted from the leaves of rice according to the modified CTAB method described by Murray et al. (1995). 0.1- 0.2 g grinded leaf powder was put in a 1.5 ml tube and 300 µl 2% CTAB and incubated more than 30 min. in the water of 65^{o}C. After that 300 µl CIA was added and then incubated 5 min. To separate the content material centrifuging was done at 25^{o}C for 15 min. in 12,000 rpm. After centrifuging, the upper layers was separated on the next tube and incubated 5 min. after adding 300 µl CIA. After the incubation, centrifuged at 25^{o}C for 15 min. in 12,000 rpm. After that upper part of aqueous were transferred to next tube. 1.5 volume of 1% CTAB is added on transferred aqueous of new tube and incubated 1 hour. After one hour the solution was centrifuged 10 min. in 8,000 rpm at 25^{o}C. The upper part of aqueous was thrown out, after completion of centrifuge. For the dilution of remained DNA, 400 µl of 1M CsCl was added and then for clarification of impurities, 800 µl isopropanol was added. The solution was then incubated in -20^{o}C more than 20 min and centrifuged 5 min. in 12,000 rpm at 25^{o}C. Then upper solution was thrown out carefully keeping DNA on the bottom of the tube as a white pellet. That pellet DNA was washed by 70% ethanol. 30 µl SPW was added on clear DNA pellet and then 1.6 µl ribonuclease was added to clear impurities of RNA. The DNA was kept 10 min. in heat block. After this, the quality and quantity of DNA was determined.

PCR and visualization

The amount of DNA template used for PCR was determined by checking the image of PCR amplification by the 5, 10, 20, 50 ng. PCR reaction for the SSR markers was performed in 15 µl buffer reactions containing 2 pmol of primer 1.5 µl, 10x PCR dye 1.5 µl, 10x PCR buffer 1.5 µl, 25 mM $MgCl_2$ 0.9 µl, 10mM dNTP 0.3 µl, Taq polymerase 0.15 µl and diluted template of DNA. Taq DNA Polymerase has been used of Takara Co. limited Japan. 10x PCR buffer was maintained by 500 mM KCI and 100 mM Tris-HCl pH. 9.0 at 25^{o}C. 10xPCR dye was maintained 0.15 bromophenolblue (BPB) and 30% glycerol. Temperature cycles for the PCR parameters were maintained according to Chen et al. (1997). The reaction consisted of 95^{o}C as initial denaturation for 5 min, denaturation at 95^{o}C for 30 sec., annealing at 55^{o}C for 30 sec., extension at 72^{o}C for 30 sec. repeated for 35 cycles. The final extension was at 72^{o}C for 5 min. The final hold was set at 4^{o}C indefinitely. PCR products were detected on 4% agarose gel. The parental relationship of 32R and genotype of 32R was detected by the analysis PCR amplification image of all cultivar,

Statistical analysis

SSR fingerprint patterns were transformed into a binary character matrix with 1 for presence or 0 for absence of a band at a particular position in a lane. Monomorphic bands were removed. Cluster analysis based on SSR markers data from all the markers were used to estimate the similarity on the basis of the number of shared bands. Cluster analysis of all quantitative and genetics data was constructed based on Bray and Cruits method and performed with data analysis package StatistiXL 1.5. (StatistiXL Ltd).

Results

An example of PCR amplification is shown in Figure 3.1. The selected cultivar belongs to two subspecies and their progeny. The rice line 32R and 29S are the cultivar developed from *Indica*

and *Japonica* species. The detail of pedigree of development 32R and 29S are shown in Chapter 1 (Fig. 1.2). The genetic relation of Tetep, 32R, 29S, CN_4-4-2, Chugoku 45 and Nipponbare was determined by Bray and Curtis cluster analysis (1957) and presented in Figure 3.2. The two subspecies *Indica* and *Japonica* showed a distinguished difference in SSR diversity. Higher genetic diversity was noted in varieties Tetep and Nipponbare. Similarly, 29S, CN_4-4-2 and Chugoku 45 has genetic diversity with Tetep. 32R has 45% similarity with Tetep and 29S has 97% similarity with Chugoku 45. The rice line Chugoku 45 and 29S has 94% similarity with CN_4-4-2. The rice line Nipponbare, Chugoku 45, CN4-4-2 and 29S has 91% similarity. By the cluster analysis, six rice line are categorized into two big cluster *Indica* dominant group and *Japonica* dominant group. 32R and Tetep belongs to *Indica* dominant group and Nipponbare, Chugoku 45, CN_4-4-2 and 29S is in *Japonica* dominant group. The *Japonica* dominant group and *Indica* dominant group has 41% similarity. The *Japonica* dominant group also divided into three subgroup. Chugoku 45 and 29S in first subgroup, Chugoku 45 and 29S with CN_4-4-2 into second subgroup and Chugoku 45, 29S and CN_4-4-2 with Nipponbare is third subgroup.

The genetic relation of 32R and Nipponbare was taken priority to further study because these two rice cultivar has distinct disease resistance capacity and agronomic traits. Out of 1338 SSR markers tested 91 produced polymorphic bands between 32R and Nipponbare. Only 85 primers amplified clear and scorable bands in between 32R and Nipponbare. The details of polymorphic markers are listed in Table 3.1. There were a few polymorphic markers detected on chromosome 2, 6, 11 and 12. This homomorphism may reflect the genetic similarity between 32R and Nipponbare on chromosome 2, 6, 11 and 12. This homomorphism may be the induced characteristics of Nipponbare on 32R because Nipponbare is one of the earlier parents of 32R. However, two cultivar 32R and Nipponbare showed high genetic variability on chromosomes 1,

3, 4, 5, 7, 8, 9 and 10. There were only a few markers derived from Chugoku 45 on rice line 32R polymorphic with Nipponbare in chromosome 3 and 7.

Discussion

Simple sequence repeat (SSR) marker polymorphism is an important source of genetic diversity, providing support for map-based cloning and molecular breeding. As comparing with allozyme analysis, it is clear that SSR could produce good polymorphism of rice germplasm. The genetic similarity and genetic diversity among selected six cultivar Tetep, 32R, 29S, CN_4-4-2, Chugoku 45 and Nipponbare were determined by selecting 1338 SSR markers. The polymorphic markers among all six cultivar were detected.

The genetic relation of six cultivar was determined by the Cluster analysis of all quantitative and genetics data based on Bray and Cruits method. Bray and Curtis calculates standard correlation coefficients between all the traits analyzed among the isolates evaluated (Castillo-Munera *et al*., 2013). The dendrogram obtained shows the relationship between the rice lines according to the markers used, but it did not show phylogenetic relationships. Higher genetic distance was obtained in *Indica* dominant rice and *Japonica* dominant rice. There were two clear group, Tetep and 32R in one group and 29S, CN_4-4-2, Chugoku 45 and Nipponbare were in another group. The two *Japonica* dominant group and *Indica* dominant group had 41% similarity (Fig. 3.2). The *Japonica* group had 91% similarity among each other. The rice line resistance to sheath blight 32R and Tetep had 45% similarity.

The rice line 32R and 29S were developed from the same parent but they had different characteristics. Sheath blight resistance and agronomical traits were different in this two cultivar. The rice line 29S was more homomorphism with Nipponbare than that of 32R. Similarly, the rice

line 32R had more polymorphism with Nipponbare than that of 29S. Rice line 32R had more induced character of Tetep and 29S had more induced characteristic of Chugoku 45. The rice line 32R and 29S had difference in disease resistance capacity as well as agronomical traits (data were not shown). Thus the difference in phenotypic and genotypic traits of the rice line 32R and 29S are due to selection of plant type for the special purpose during the classical breeding process. The *Japonica* cultivar had high rate of homomorphism. Large number of specially designed markers are required to get polymorphism in same types of rice cultivar.

There were genetic variability on chromosomes 1, 3, 4, 5, 7, 8, 9 and 10 and there were a few polymorphic markers detected on chromosome 2, 6, 11 and 12 in between 32R and Nipponbare. It may be due to the marker utilized here are mainly designed for *Japonica* variety and also used in *Japonica* variety. In addition, the rice line Nipponbare and Chugoku 45 has less polymorphism because both of them are *Japonica* cultivar. The polymorphic marker in between 32R and Nipponbare are comparatively less as 1338 SSR marker has been used in this study because of the Nipponbare and Chugoku 45 are earlier parent of 32R. Although there were difficult to get polymorphic marker in this type of rice cultivar, we became able to get sufficient polymorphic marker in 32R derived from Tetep with Nipponbare to do QTL analysis.

In this study, the larger range of similarity values for cultivars and diversity in between 32R and Nipponbare in some location revealed by SSR markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs of sheath blight disease resistance as well as yield potential.

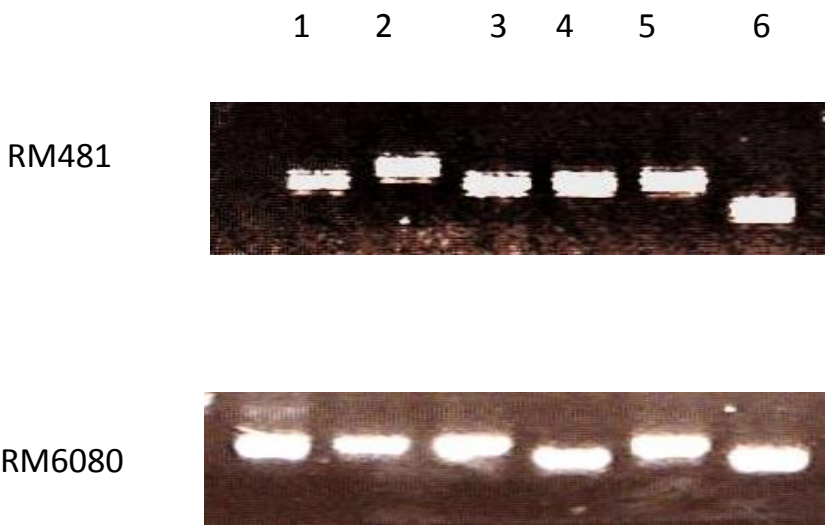

Figure 3.1. PCR image of RM481 and RM6080 used to identify polymorphism. The number above the PCR image denotes the rice line; 1, Chugoku 45; 2, Nipponbare; 3, CN_4-4-2; 4, 32R; 5, 29S and 6, Tetep.

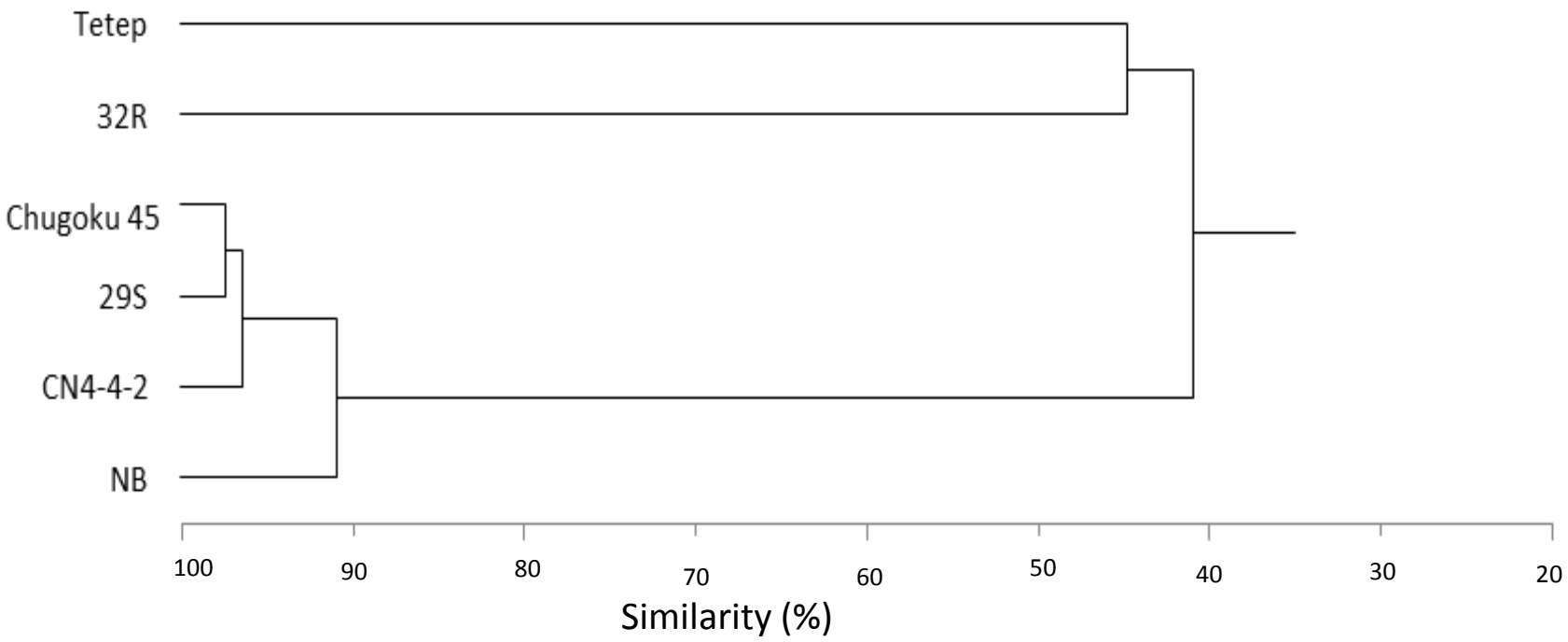

Figure 3.2. Genetic similarity of the rice lines Tetep, 32R, Chugoku 45, 29S, CN_4-4-2 and Nipponbare determined by the cluster analysis of genetic information obtained from the SSR markers according to Bray and Curtis (1957).

Table 3.1 Polymorphic marker identified in between 32R and Nipponbare

Numuber	Locus_ID	Chr	Forward Primer	Reverse Primer	Expected Marker Length	SSR start	SSR end
1	RM10285	1	GGCATGAGAGTCTGTGATGTTGG	TAGTACTGCTCCATCTGCCTTGG	116	4885944	4885983
2	RM10305	1	CAGGAACCAACCTTCTTCTTGACC	GTCAGACTCCGATCTGGGATGG	167	5139380	5139405
3	RM10336	1	TGCCACAGTGGAGAGAGAAAGG	CAGTGCTTGCTGTGTGTAGTATGC	171	5566582	5566605
4	RM10348	1	ACATTGGTGGAGTTCTGGATGG	TGACACCTACACTGGCGTCTACC	189	5826605	5826631
5	RM10451	1	CGAAGGTGTAGACGCCCATGACG	ATCTCCACTCGCCTCGCAAGC	93	7279176	7279202
6	RM10453	1	CACCGACTCCCACTATGTCTGC	AGCTCCCAACTGACAAACTCTGG	300	7281142	7281173
7	RM10481	1	TGGAGAGAAGACAGAGCATATACTGG	CAGGGATGGAGCATTGGTAGG	179	7662542	7662563
8	RM10487	1	CCCATGTGATCGGAATATACGC	CGCCATGCTTTCTAACATCATCC	268	7735440	7735459
9	RM10502	1	GAGAACGGCAGCGTCTCAGG	CGTCACTTAAGCAGGTGGAAGACC	97	7926455	7926481
10	RM10505	1	CCGCCGATATCTGTTCATCTACC	TTCTGAAACACCAACCCAGATCC	89	7973817	7973842
11	RM10587	1	AACACGCACACTTCGCTTCC	GCACGAGGGATCGATACAGACC	305	9369805	9369825
12	RM10623	1	AGTACGATTTCTGTCAGCGTTGC	TGAAAGGAGTAGCCAGAGAAAGC	350	9938655	9938710
13	RM10624	1	AGTACGATTTCTGTCAGCGTTGC	TGAAAGGAGTAGCCAGAGAAAGC	350	9938716	9938747
14	RM10446	1	TTCGTATCCTGATACCGTTACGC	GGCCTTGATGAACTTGATTTCG	180	7246143	7246194
15	RM10588	1	TTCAGGGATAACCAAGAAGTGC	ACCGCTGTCCCTCAGATAGC	220	9390310	9390341
16	RM11815	1	GCGCCCAATGCATGTAAATTCC	TGCCGATACCTGTGATCAAGTCC	289	34895399	34895434
17	RM11370	1	CTCAACCCGGCTTTCCATCTCG	GCTGCAGAGTCTCGCACGTTCC	187	25067420	25067465
18	RM11759	1	TTCCAGCTTTGCATGAACAACG	TGTACGCGACCCTGATGTCC	225	33470285	33470308
19	RM11819	1	CTGCCTCCTCCATTTCCCACTCC	CGAACGACTGCTCCCTCTTCAGC	166	34990000	34990033
20	RM11849	1	CCGAGTTGTATTACTGGTGCAAGG	CTTTGCTCCAAACCTCAGTCTCC	225	35831791	35831818
21	RM11871	1	TTTCCGACTGAACAAGTACACAGC	GAGTGAGCAAGAACTGTCATCACC	164	36376256	36376285
22	RM11874	1	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC	193	36464545	36464590
23	RM12086	1	GTTAGGTTAACGGGATCTTGTTCG	ATGCAGTCTCCATCATCGAAGC	198	40136356	40136397
24	RM12086	1	GTTAGGTTAACGGGATCTTGTTCG	ATGCAGTCTCCATCATCGAAGC	198	40136356	40136397
25	RM10001	1	CAATCACCCTCACCCTCTTATATGC	CCGCTGTGAACAACAATCATGC	386	26895	26974

26	RM13416	2	TGAGTGTTTGCGGGAGGAGAGC	CAGGGTGAGAGGTTCCAGTCAGG	284	20996965	20996988
27	RM13506	2	NA	NA	NA	22420221	22420250
28	RM13416	2	TGAGTGTTTGCGGGAGGAGAGC	CAGGGTGAGAGGTTCCAGTCAGG	284	20996965	20996988
29	RM13506	2	NA	NA	NA	22420221	22420250
30	RM13743	2	CGTGTATTGCATGGATTGTCTGG	CGGAACCCACAATTTCTTTCTGC	269	27172500	27172539
31	RM14637	3	CGCGTTCGATCGATCTCTCTCC	GGCCAGAGTACGTGCCAGATGC	142	6902135	6902170
32	RM14281	3	CATGCATGCAACTCTGCTAAACG	ATCAGGATCCAGGAAATCGAACC	187	514907	514934
33	RM14413	3	TTGAGCATCAGGGTACTTTCTCC	GGAGTATTGTGCTAGTCACGTTTGG	275	3162643	3162662
34	RM14323	3	CAATTTGCTATGGACGGTTGTTCG	AATGTGGTCCGGGAGAACAATGC	138	1376915	1376970
35	RM14904	3	CTTACCTTCTGAAATGGAGGTAGC	CAAGGTGAAAGACGAAGAATGC	382	12547408	12547509
36	RM15027	3	AGAGGAGAGATACAGATTGAGACG	GACCCTTGATGTGAGTAGTTGG	308	14327686	14327721
37	RM14324	3	CAAAGAATCCAAGGAGGCCAAAGC	ATGCCGATGAGCACCCAGAGG	249	1437206	1437237
38	RM14563	3	CTGCTGCTCCGCTACACAAAGC	TTCGGACTCGTGCTCCAAACG	129	5617583	5617616
39	RM17503	4	CCAGATCATCCAGGCATAACATCACC	CGGCGCTGGTAAACTCCATTCC	100	31717625	31717670
40	RM16965	4	TCGCGTCGTAGCTGAACACTGC	TAGCTTGCTCTCCCTCTGCTTGC	169	20889864	20889899
41	RM17681	4	ACCATTAAGCCTTGTACTCAGC	TAACCAGAGATCGATTGGTAGC	126	35147621	35147644
42	RM16661	4	TGTTGAAGGAGATACACCGAAACC	TAGGATGTTAAAGCGCCACTTGC	262	13825526	13825569
43	RM17445	4	GGAAGGGAAGAAGATAGGAGATGG	CTAGCATAGGCCTCCAACAAACC	180	30397705	30397758
44	RM17457	4	ACAGGTCGATCTCGATGAACTCC	CTCTTCTCCGTCTCGACTCTTCC	198	30715761	30715786
45	RM17496	4	GGGCCCTACTAGTACAGCTAAAGG	AATTGGGAAGGAAGGAACAGG	243	31517587	31517616
46	RM17508	4	CGGAATCACCAATTTCTCTCTCAGC	CGCAAGAAACGGAAACGAAACC	282	31845534	31845577
47	RM17472	4	GAGGAGGAGGAGGAGAGATCAGG	AACGAAACCGCTCAGTTCAACC	119	30991473	30991510
48	RM17556	4	TTGCCAGATGGAGGTTCTAATGC	TTATCAACATGAGACTGCGGAGTAGG	277	32875499	32875520
49	RM17716	5	CTCTCAGGTGTGTTGTACATTGTTCC	CTGCTCAAACAAGCAGCTAATGG	260	62666	62695
50	RM18676	5	AAGACCGAAATTAGACGGATGC	GGGTCTTATTACCTTCGTTTCAGG	193	20179718	20179765
51	RM18111	5	CCATCAAGAGATAGTGCTCCAACC	ACACAGCCTATGTTTAGGGTTTCC	162	6941334	6941383
52	RM17802	5	AGGCGATGGAACATGAAGTGTGC	AGAGCCGGAATGTTCTCCTTTGC	100	1138400	1138423
53	RM19494	6	ACCCAATATGGTGCAATAGAGACC	CACCTCCACCAACTATTGACAGG	397	4315069	4315114
54	RM19370	6	CCATTGCAACTTGGCGAAAGC	CCAGATCCGGATGGCAGTGG	195	2364986	2365069
56	RM22144	7	GCAGAAGCAGGAGCCCAATATCC	CTGTCTGTCCAGCACCAACACC	276	28871504	28871553
57	RM21719	7	CTCCTCAGTCCTGAGTCTCCTGTCC	CCCAGAGAGATTACACAGAGCAAGC	198	20053345	20053378

58	RM21883	7	GCACAGTGAATGAGCTAAGAACACG	TCCAATACGATAAGTGGCTGATGG	143	23607662	23607695
59	RM20864	7	CAAGTAGGCCCAGTCCCAATACG	CGGGAAATGGAACCGTAGAAGC	482	1164814	1164857
60	RM20960	7	NA	NA	NA	2600025	2600063
61	RM20932	7	ACAAATACAGTGGAAGCGTGTCG	GAACACGTCTGGGAGCACTACG	153	2111971	2111994
62	RM21024	7	ATTAAGCTACTGTCTGCCTCCTTCG	GCTTCGTTTCAGGTGGTCAGG	289	3345016	3345063
63	RM21105	7	CACCACGATATCCACCTCTAGC	CCTAGGATGAACACTGATGATGG	94	4692609	4692632
64	RM21423	7	GAACATGCTTTCAACCATCAGG	GATCCTCTCAGTTCAGTGCAAGC	400	12730966	12731029
65	RM21502	7	CGCATGGATCAATCAATAGTGG	CAAGTGCTGCTACTCTGTCTCTTGG	195	15335246	15335281
66	RM21760	7	AGTCTCCGCAACACAGAGTCAGC	TGAAGAGAAGTGCGTGATTCTTCC	153	20754864	20754905
67	RM21998	7	CCATACGACTCCACAACACACTGC	AATCGCAAGCGGATCGAAAGC	239	25878064	25878095
68	RM22146	7	AGTCTCAAAGGCATACAGTACACACC	GCCAATTACCTTCCCGTACATAGC	141	28894398	28894433
69	RM21423	7	GAACATGCTTTCAACCATCAGG	GATCCTCTCAGTTCAGTGCAAGC	400	12730966	12731029
70	RM22828	8	TGGTACGGGAGAACTGGTACGC	AATCGAGCCAGCCTAGCAAGC	360	11842678	11842749
71	RM22882	8	NA	NA	NA	14329414	14329507
72	RM22387	8	GATTCCCTAATCTGCAAGCAAGG	TTGTCGCTAGCTTGACCCTTACC	264	2953486	2953545
73	RM23164	8	ATTTCAGGGTTACAGCCCTACC	AAATGCCACATCTATCCCTACC	381	20971707	20971736
74	RM22406	8	ATGCATGTGTGATGACTGACAGG	GGTACTCTTGCCAAATGGTCTCC	176	3162555	3162574
75	RM24469	9	NA	NA	NA	17054142	17054167
76	RM23772	9	CTTGCACAAGAGGCAACACTCC	GTTTGGTAGGTCGCATTGTTTGG	146	3749595	3749621
77	RM24015	9	TTTCGTGGATGGAGGGAGTACG	TGGCGACTTATGAGCGTTTGTAGG	184	9509462	9509497
78	RM24433	9	ACATCTCTCTCGCTCCCTTTCC	GGCTTTAAAGGGTGGATTGAACC	436	16440886	16440933
79	RM24715	9	GAGCAGCAAGAGCAGCAGAGG	CATGCTCGACTTCAGAAGCTTGG	174	20888227	20888258
80	RM24513	9	CCGTGCAACTTAAATCCAAACAGG	GGAATCCTATATGAGCCAGTGATGG	172	17666088	17666147
81	RM25930	10	AACTGAGAAACATGGGACAGAAGC	CTCAACCACACCCTCATTCACC	180	22565078	22565129
82	RM25816	10	TCGGAGTAATTAAGCAGCAGACG	ACAATTCTCCCATCTCCATCACC	182	21122935	21122972
83	RM26166	11	ACACCGTGAATGCTCTGCTTCG	ATGCACCCTTCGTCACAGAAGATCC	175	3838197	3838223
84	RM27280	11	GGGTGGGTCTGGTCACTATGTCAGG	TCCTAACTCCACCCACTTTGATTTGC	367	26668627	26668653
85	RM27281	11	GGGTGGGTCTGGTCACTATGTCAGG	TCCTAACTCCACCCACTTTGATTTGC	367	26668656	26668730
86	RM27387	11	TAGGTCCATCCAAATCTCGATCC	TGGCAGAGGAGATTAGAGTAATACGG	295	28326750	28326839
87	RM27289	11	NA	NA	NA	26796502	26796522
88	RM26139	11	GTCGAGGACGAGTTCCACACG	CCTTCTCGTACCCGAGGTTCTGC	328	3247205	3247228

89	RM26474	11	CCCATATCTTTCGGCACAAGC	TTCCCAGATGCCTAGCAATTTCC	272	10600408	10600431
90	RM28258	12	GGCTCACCTCGTTCTCGATCC	CATAAATAAATAGGGCGCCACACC	196	19156149	19156178
91	RM28009	12	GGGCGTTCGGTTTACTTGGTTACTCG	GGCGGCATAGGAGTGTTTAGAGTGC	212	13258404	13258435
92	RM28163	12	ATGGTAGAGACACAAGTCCATGC	GACAAATTGGTGTAGGTGAAGG	218	17578154	17578185
93	RM28705	12	TGTGAATGCCCGTATGGATGG	TCTGAAACCATATCGTCGCATACC	100	25972751	25972776
94	RM28712	12	TTGCTACTACCACAACAGGGTTCC	GCAGCCACAGCTTTGAATAGAGC	154	26032594	26032629
95	RM28791	12	GGAGAAAGAGAGGTGATCCTTTCC	CATGTCTTGGTGAGTGATGTTGC	291	27024527	27024556
96	RM27565	12	AAGGCGAACTGTCCTAGTGAAGC	CAGGATGTTCTTGCCAAGTTGC	198	3185544	3185599
97	RM27440	12	TCACCAGGGTCGTTAAAGTACTGC	GCAATACCACATCTGATCCACACC	518	747006	747041
98	RM27554	12	TCTTGTTCGACATTCCCATTCC	TCACAGCACATTCTCAGCATTCC	264	3023729	3023749

Summary

Genetic relationships are essential information in crop improvement programs. A microsatellite is a specific sequence of DNA bases or nucleotides which contains repeats. In the present study rice microsatellite (RM) markers were used to study the parental polymorphism between the selected six rice verities. Cultivar selected to study parental polymorphism were Tetep, Chugoku 45, CN_4-4-2, 29S and 32R. 1338 RM markers located in rice chromosomes based on the reported distribution were used to detect genetic relation. Out of 1338 SSR markers tested 91 produced polymorphic bands between the two parents and 85 primers amplified clear and scorable bands. Most of the polymorphic SSR marker in between 32R and Nipponbare were derived from Tetep in 32R and only few markers were derived from Chugoku 45. By the cluster analysis, six rice line were categorized into two big cluster *Indica* dominant group and *Japonica* dominant group. The two *Japonica* dominant group and *Indica* dominant group had 41% similarity. Cluster analysis of genetic information showed that 32R had 45% similarity with Tetep and 29S had 97% similarity with Chugoku 45. Furthermore, 29S, CN_4-4-2, Chugoku 45 and Nipponbare were belonged to the same cluster of *Japonica* variety and *Japonica* dominant group had 91% similarity.

CHAPTER 4

Identification of QTLs involved in resistance to sheath blight disease in rice line 32R derived from Tetep

Introduction

Rice sheath blight disease caused by the soil borne fungal pathogen *Rhizoctonia solani* Kuhn is one of the most serious rice diseases worldwide, severely impairing both grain yield and quality (Lee and Rush, 1983). Disease response is not consistent. The non-genetic environmental factors affecting disease severity are numerous and strong (Pinson *et al*., 2005). A crop with a high plant density and closed canopy associated with high nitrogen management favors disease build-up from panicle initiation onwards. The development of sheath blight disease is sensitive to surrounding environment including humidity, temperature, light intensity, nitrogen fertilizer rate, silica level in soil, lodging and water depth as well as plant morphology such as plant height, growth stage, and plant architecture (Lee *et al*., 1983; Bollich *et al*., 1985; Li *et al*., 1995; Savary *et al*., 1995; Eizenga *et al*., 2002 and Tang *et al*., 2007). Crop losses generally vary from 0 to 50% depending on the severity of the disease and the stage at which the crop is infected and environmental conditions (Marchetti *et al*., 1991). Nowadays, the damage caused by the sheath blight disease has also increased because of the introduction of high yielding compact semi-dwarf cultivars and the application of high levels of nitrogen fertilizers in rice fields (Taheri and Tarighi, 2011). In addition, the epidemic area of sheath blight disease is increasing because of global warming (Iizumi and Yokozawa, 2008).

The research progress of sheath blight disease in rice is lagging behind, as compared to rice blast or bacterial blight. Some genes conferring resistance to blast or bacterial blight have been

cloned, but no gene for resistance to sheath blight disease in rice has been cloned yet (Jia *et al*., 2012). In addition, breeding of rice varieties with blast resistance or bacterial blight resistance has achieved great success, but the breeding of sheath blight disease resistant varieties has been hindered due to the lack of highly resistant rice germplasm (Xiang *et al*., 2011).

Wasano *et al*. (1985) developed a short culm elite line 32R from the progeny of Tetep. The rice line 32R was continuously screened for sheath blight disease resistance over 15 years, along with another sheath blight susceptible rice line 29S, which is genetically related to 32R. Wasano and Hirota (1986) reported that the 32R showed more resistance than the recurrent parent Tetep while 29S showed more susceptibility than the Nipponbare. Lignin, a physical defense barrier against the sheath blight fungus, deposition is high in 32R as compared to susceptible line (Danson *et al*., 2000). Proteomic analysis of defense responses in 32R showed the expression level of enzymes of glycolytic pathways and pentose phosphate pathway increase while susceptible line 29S remained unchanged (Miyagi *et al*., 2006). Similarly, the study of the changes in metabolic pathways shows that the expression pattern of the pentose phosphate and glycolytic pathways were higher in the resistance line than that of the susceptible line (Mutuku and Nose, 2010, 2012a and 2012b). In agronomy side, the tendency towards lodging and spikelets falling in Tetep had been improved in 32R, other agronomic characters especially fertility and yield, remains extremely poor (Gaihre and Nose, 2013). Furthermore, it has grown capacity in different temperature regime (Kiet and Nose, 2011). Thus, the genetic influence and adaption of strong agronomic traits of *Japonica* cultivar in Tetep might have strong role to develop sheath blight resistance capacity in the rice line 32R. Without identification of causative polymorphisms, the association of differential gene expression or metabolite concentration with the phenotypic variation does not always disclose the responsible genetic factors, due to genetic linkage of additional genes with the genomic region. To

address this fact, study of QTL analysis was determined on the important sheath blight resistance rice line 32R, based on findings and information of earlier study, after examined the polymorphism between 32R and Nipponbare. During the material development of this study, the yielding capacity of F_1 progeny also examined and attributed 12.5 metric ton yield per hectare with ideotype plant structure (Gaihre and Nose, 2013). This work is also the part of developing rice cultivar resistance to the sheath blight disease having high yielding capacity by the marker assisted selection method for the sustainable rice development.

Quantitative characters have been a major area of study in genetics for over many years because they are a common feature of natural variation (Kearsey and Farquhar, 1997). The rapid progress in the development of molecular marker technique and available complete genomic information about rice plant has led to the intensive use of quantitative trait loci (QTL) mapping of quantitative resistance against pathogens in rice plant. Identification and mapping of QTLs is a valuable in the interpretation of the molecular and biochemical mechanisms involved in host-pathogen interaction and for the improvement crop quality by the process of marker-associated selection (Channamallikarjuna *et al*., 2010). Several quantitative trait loci (QTL) for sheath blight resistance have been identified using mapping populations derived from *Indica* or *Japonica* rice. QTLs associated with the resistance of sheath blight disease have been reported on all 12 chromosomes (Srinivasachary *et al*., 2010 and Zeng *et al*., 2011). More than 70 QTLs for sheath blight resistance have been reported in different study (Fu *et al*., 2011). To our knowledge, there is no report of development of sheath blight disease resistance cultivar by utilizing these QTLs. The failure of marker-assisted breeding for resistance of sheath blight disease in rice might be due to the insufficient study of parent cultivar and affection of QTL by physiological, morphological and environmental factors. QTL analysis of sheath blight resistance in under field conditions is

affected by environmental factors. Sato et al. (2004) identified two sheath blight resistance QTLs on chromosomes 3 and 12 on rice line WSS2 by using a BC_1F_1 population derived from a cross of Hinohikar/WSS2//Hinohikari. Channamalikarjuna et al. (2010) identified sheath blight QTLs on chromosomes 1, 3, 7, 8, 9 and 11 on the rice line Tetep in four years by using the recombinant inbreed line. However, the identification and utilization of QTLs of sheath blight disease resistance is still important to develop sheath blight disease resistance rice line. For this purpose the rice line 32R and Nipponbare are selected as the parent cultivar.

The objective of the present study was to identify QTLs related to sheath blight resistance in a field environment in the rice line 32R for future breeding program along with the defense response of morphological character in field environments. And also clarify the parental segregation on rice line 32R.

Materials and Methods

Plant materials

The experiment was conducted at the agronomy field of Saga University, Saga, Japan (33^o 16" N and 130^o 18" E) during April 2007 to October 2010 in a heavy clay soil. Rice lines used for this experiment are Nipponbare (*Japonica* variety), susceptible to sheath blight disease, as a male parent and 2F18-7-32 (32R), resistance to sheath blight disease, as a female parent. The later cultivar was developed by crossing Tetep as the female parent and CN_4-4-2 as the male parent. Crossing Chugoku 45 with Nipponbare resulted in the development of CN_4-4-2. F_1 rice line was developed by artificial emasculation and pollination at the flowering stage of 32R with Nipponbare. F_2 rice was developed by self-fertilization method. The rice line 29S also developed from the parents of 32R by the similar breeding method along with 32R and the rice line 29S was

used in this study to clarify the genetic mechanism of 32R. 29S was susceptible to sheath blight disease than that of the recurrent parent (Miyagi *et al*., 2006).

Plant cultivation

Seeds of parent cultivars and F_2 were treated with a systemic insecticide and fungicide. 0.1% of Sumichion, an insecticide (Yashima Chemicals Industry Co., Ltd) and 0.5% Tekurido C, a fungicide (Kumiai Chemicals Industry Co., Ltd) for 24 h and then washed by tap water and incubated at 28^{o}C for 48 h for germination. Pre- germinated seeds were sown in seedling trays. A common procedure was followed in rising of seedling in bed. Seedlings of 30 days old plant were transplanted in the well-puddled experimental plots as single plant per hill with a spacing of 30 x 25 cm. Nitrogen, phosphorus and potash were applied at 50, 33 and 33 kg/ha respectively just before transplanting. Phenotypic characters related to sheath blight resistance, such as culm length, first leaf angle, second leaf angle, third leaf angle and sheath blight resistance evaluated for each individual. Data were analyzed for statistical significance following least significant difference value at 5% and 1%. (SPSS 16.0 SPSS Inc. 2007).

Evaluation of sheath blight resistance

The *R. solani* isolate C-154, number 305229 maintained in the Agricultural Resource Gene Bank, Tsukuba, Japan was used for the inoculation. Culture and inoculations of the mycelium were based on the syringe inoculation method (Wasano *et al.* 1983). The potato sucrose agar (PSA) was made containing 300 g potato, 20 g sucrose and 15 g agar. *R. solani* fungus was grown at 28^{o}C for about 4 days. It was chopped into small pieces that it would fit in a syringe and then it was homogenized by syringe. Then, 0.2 ml of prepared inoculums was injected to the third leaf sheath from the flag leaf using plastic syringe during the heading date, selecting five tiller from each hill. Disease symptoms were scored based on the ratio of lesion area to leaf sheath area one month after

inoculation, according to method of Wasano *et al*. (1983). Lesion area and whole leaf sheath area were measured by using LIA 32 scanner software (Yamamoto, 2004).

Environmental conditions

The experimental field of this study, Saga, Japan lies in the subtropical humid region. The weather during fungus inoculation period in 2010 was conducive for sheath blight infection with the temperature ranging from 23.7 to 36.7°C and average relative humidity was 69% (Tables 4.1 and 4.2). The average rainfall and humidity in 2010 was higher than 2007, 2008 and 2009. The average temperature of August and September in 2010 was greater than 2008 and 2009. (Source: Japan Meteorological Agency; www.jma.go.jp)

Linkage map construction

Total genomic DNA of 32R, Nipponbare and F_2 population was extracted from the leaves of rice according to the modified CTAB method described by Murray et al. (1995). Temperature cycles for the polymerization chain reaction (PCR) parameters were maintained according to Chen *et al*. (1997). The detail method of PCR is mentioned in Chapter 3. PCR products were detected on 4% agarose gel. A linkage map was constructed using MAPMAKER/EXP V3.0 (Lander et al. 1987 and Lincoln *et al.* 1992). The distance was expressed in centiMorgans (cM) using the Kosambi mapping function. From the polymorphism study in Chapter 3, 91 SSR marker were found producing polymorphic bands between the two parents and 85 primers amplified clear and scorable bands and were used for genotyping of F_2 population. The use of 100 marker in 100 plants can cover whole rice genome with 20 cM distance. A marker density of 10-20 cM is by far sufficient for precise QTL detection (Stange *et al*., 2013).

QTL analysis

The QTL analysis using composite interval mapping (CIM), for the detection of main-effect QTL was conducted using QTL CARTOGRAPHER V2.5 (Wang *et al*., 2006). Standard model involving forward and backward regression method was employed with a probability in and out of 0.01. The permutation test was performed to estimate the appropriate threshold value of the logarithm of the odds ratio (LOD) score for determining the presence of the QTL. Permutation test for each trait was done 1000 times at 0.05 significance level. When the LOD score exceeded over the permutation threshold value of each trait, the position with the highest LOD on each interval was estimated as the definitive QTL.

Results

Phenotypic characters

The disease symptom is shown in Figure 4.1. The resistant rice line 32R and susceptible rice line Nipponbare had significantly different sheath blight disease score (Fig. 4.2A). The average disease score of 32R was 11.7 ± 4.90 and the average disease score of Nipponbare was 41.7 ± 23.5. The disease score of the F_2 population ranged from 0.00 to 53.0. The culm length of 32R was 76.9 ± 4.31 cm and Nipponbare was 79.1 ± 9.49 cm (Fig. 4.2B). Phenotypic frequency distribution of culm length was varied continuously with transgressive segregants in F_2 population. The important trait for sheath blight disease development, leaf angle is higher in Nipponbare than that of 32R (Fig. 4.2C, 4.2D and 4.2E). The first leaf angle, second leaf angle and third leaf angle of 32R was 9.83 ± 2.71º, 16.8 ± 3.82º and 18.1 ± 3.19º, respectively. Similarly, the first leaf angle, second leaf angle and third leaf angle of Nipponbare was 10.7 ± 1.89º, 21.1 ± 2.24º and 23.3 ± 2.93º, respectively.

Table 4.1. Monthly weather condition of Saga city in 2010

Month	Temperature ((℃)				Average of daily total insolation (MJ/m^2)
	Maximum	Average of daily maximum	Minimum	Average of daily minimum	
May	31.2	24.8	7.8	14.3	19.2
June	32.5	28.3	15.1	20.2	15.3
July	35.8	31.4	22.6	24.3	16.3
August	36.7	34.7	23.7	26.2	20.0
September	36.4	30.3	16.6	21.8	15.4
Octomber	29.1	24.1	9.1	15.7	11.3

Data were collected from Japan Metrological Agency (www.data.jma.go.jp).

Table 4.2. Annual weather report of Saga city

Year	Average temperature (℃)				Relative Humidity (%)				Rainfall (mm)			
	2007	2008	2009	2010	2007	2008	2009	2010	2007	2008	2009	2010
May	20.0	19.9	19.8	19.1	62	61	57	61	132.0	196.5	68.0	213.0
June	24.0	22.5	23.5	23.6	70	73	69	70	123.0	622.5	305.0	315.5
July	26.4	28.9	26.5	27.4	74	68	75	72	593.5	44.5	537.5	424.0
August	28.9	27.5	28.0	29.6	68	71	68	69	213.5	295.0	113.0	93.5
September	26.9	25.2	24.5	25.7	68	71	65	70	95.5	115.5	45.0	128.5
October	20.5	19.7	18.9	19.5	63	66	63	66	103.5	25.0	132.5	60.0

Data were collected from Japan Metrological Agency (www.data.jma.go.jp).

32R Nipponbare F_2

Figure 4.1. Sheath blight disease symptom in rice line 32R, Nipponbare and one of the selected F_2. The oval gray spot with the black brown margins shows the sheath blight disease infection.

The correlation coefficients among the five traits assessed in the F_2 population are shown in Table 4.3. Correlations among the different traits were evaluated at 0.05% and 0.01% levels. A significant negative correlation was found between the sheath blight disease score and culm length. No significant correlation was found between sheath blight disease score and leaf angles in F_2 population. The negative significant correlation was observed in between culm length and third leaf angle in the present study. First leaf angle and second leaf angle were not correlated with culm length. First leaf angle, second leaf angle and third leaf angle were significantly correlated among each other. The strongest correlations among the leaf angles indicate that the inclination pattern of all leaf is similar.

QTL analysis

The polymorphic marker utilized in this study are almost derived from Tetep. There were only a few markers derived from Chugoku45 on rice line 32R polymorphic with Nipponbare in chromosome 3 and 7 (Fig. 4.3). The seven prominent QTLs were detected for the sheath blight disease resistance on chromosomes 1, 3, 4, 5, 7, 8 and 9 (Fig. 4.5 and Table 4.4). These QTLs were designated *qSBR* and these QTLs were derived from 32R allele. *qSBR1*, *qSBR3*, *qSBR4*, *qSBR5*, *qSBR7*, *qSBR8* and *qSBR9* with LOD scores 2.95, 2.94, 3.79, 3.77, 2.80, 4.29 and 4.60, respectively were detected and phenotypic variance explained by these QTLS were 7.50, 8.43, 7.84, 6.28, 8.70, 7.60 and 6.84, respectively. The additive effects of QTLs were ranged from 0.14 to 0.88. The QTLs for culm length (qCL) were located on chromosomes 3, 4 and 5 with LOD value 2.52, 3.26 and 3.45, and phenotypic variance of 7.53, 13.6 and 15.4%, respectively. QTLs for flag leaf angle were located on chromosomes 1, 3, 4 and 8 with LOD values 10.2, 18.8, 9.16 and 9.74, and phenotypic variance 6.13, 5.42, 6.34 and 6.40, respectively. QTLs for second leaf angle from the flag leaf were located on chromosome 8 with LOD value 6.77. Similarly, QTLs for

third leaf angle from the flag leaf were located on chromosomes 1 and 3 with LOD values 3.49 and 5.69 explaining 8.37 and 7.10 phenotypic variance, respectively.

Discussion

Identifying useful disease resistance genes are the most important for the sustainable crop development. The present study was carried out to identify chromosomal regions for stable field resistance to sheath blight disease in hybrid cultivars using an F_2 population from the cross between 32R and Nipponbare. This study enabled to identify seven genomic regions affecting sheath blight disease resistance in chromosome 1, 3, 4, 5, 7, 8 and 9. These all QTLs of sheath blight disease were derived from resistance cultivar 32R. The QTLs of culm length were derived from the Nipponbare and the QTLs of leaf angles were derived from the 32R. These QTLs can play a vital role for the management of sheath blight disease and ideotype plant structure. The rice line 32R could be a potential donor for the resistance to sheath blight disease races in the field.

32R is a rice line developed by conventional breeding. The study of different aspects of sheath blight disease including disease symptoms in field, metabolic pathways and proteomics analysis after *R. solani* induction was continuing more than 15 years. The study showed that 32R has strong resistance capacity against sheath blight disease (Wasano and Hirota, 1986; Danson 2000; Miyagi et al., 2006; Mutuku and Nose, 2010; Mutuku and Nose, 2012a and Gaihre and Nose, 2013). Nevertheless, the genetic relation of 32R with parent cultivar and 29S was not known clearly. The study of genetic relation among 32R, Tetep, Chugoku 45, Nipponbare, CN_4-4-2 and 29S in Chapter 3 elucidated that most of the polymorphic markers in between 32R and Nipponbare were derived from Tetep in the rice line 32R. Only RM3372 and RM4352 in chromosome 3 and RM481 and RM505 in chromosome 7 were polymorphic in between 32R and Nipponbare, derived from Chugoku 45 and near these markers, there is no QTL position of sheath blight disease in this

study. Because of the above reason, we conclude that the resistance mechanism of 32R is induced character of Tetep.

Among the selected 96 F_2 plants, the scores for disease severity in the sheath blight test ranged from 0.0 to 53.0 (Fig. 4.2A). Some F_2 progeny had a higher resistance compared to 32R and some F_2 progeny were more susceptible than Nipponbare. This suggests multigenics inheritance of QTLs for resistance to races in the field confirmed by QTL analysis. Sheath blight disease resistance has been considered as a polygenic, making it difficult to evaluate the effect of individual QTLs on resistance. Phenotyping of sheath blight disease could be confounded by variable humidity and temperature in the plant microenvironment under field conditions during the development of sheath blight disease.

Factors such as plant types, plant density, tiller number, lodging and water depths can all affect the field condition (Pinson *et al*., 2005). Therefore, the accuracy of QTL mapping can be confounded environmental factors under field conditions and selected parents (Lu *et al*., 1996).

Generally, partial resistance of sheath blight disease is influenced by morphological traits (Wasano *et al*., 1983; Li *et al*., 1995; Zou *et al*., 2000 and Sato *et al*., 2004). In this study, negative significant correlation was observed in between culm length and disease score of sheath blight disease (Table 4.3). Therefore, plant height is unfavorable for the mushrooming of sheath blight disease. Rice genotypes corresponding to taller plant can have a higher probability to have low disease intensity. QTLs of culm length were derived from the parent cultivar Nipponbare (Table 4.4). QTLs of culm length might be independent with QTLs for sheath blight resistance.

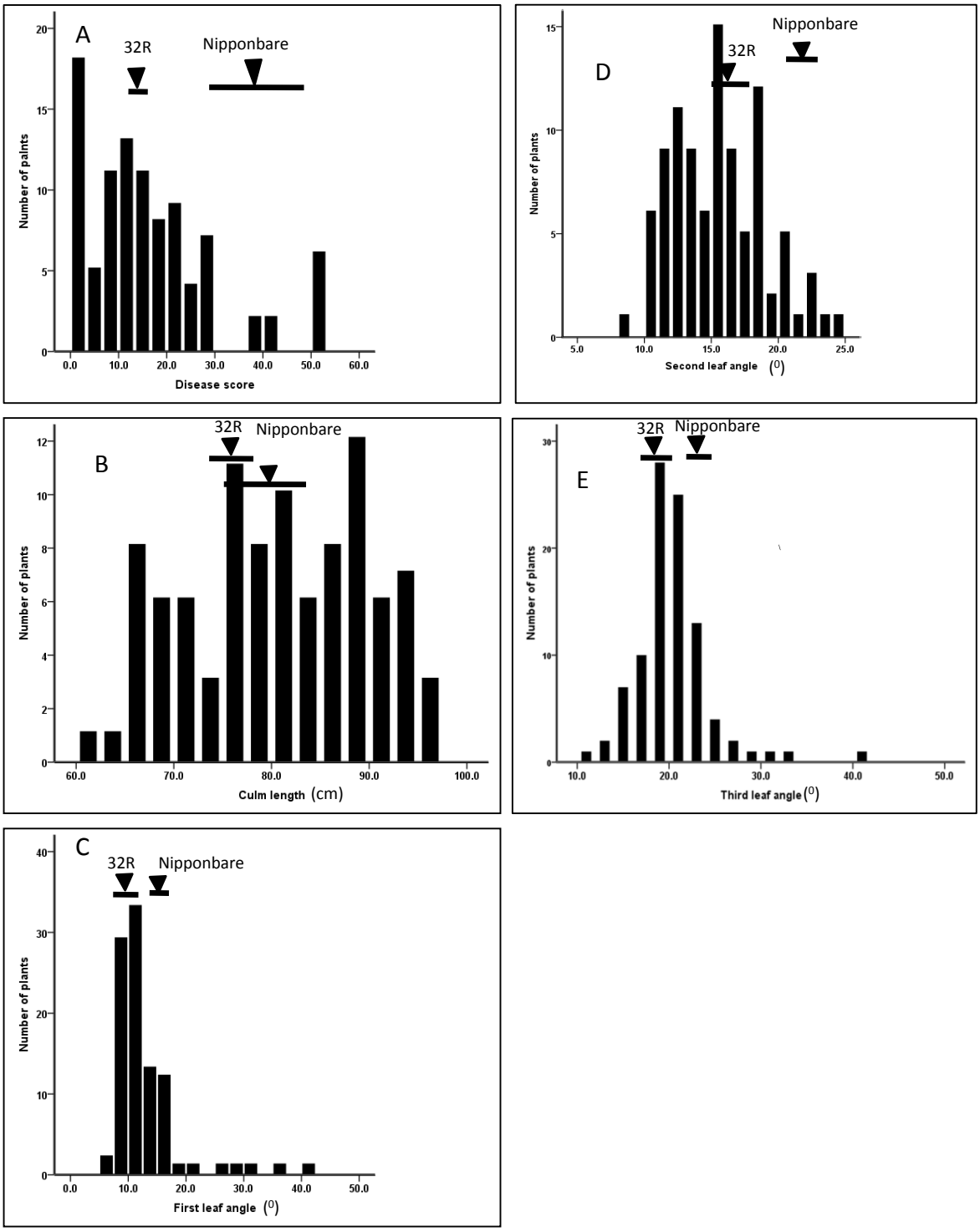

Fig. 4.2. Frequency distribution of different traits in the F_2 population derived from 32R and Nipponbare. Values for the parents are marked by arrows. Horizontal lines under the arrows indicate the standard deviations. The letters in figure are denotes A, disease score; B, culm length, first leaf angle; D, second leaf angle and E, third leaf angle.

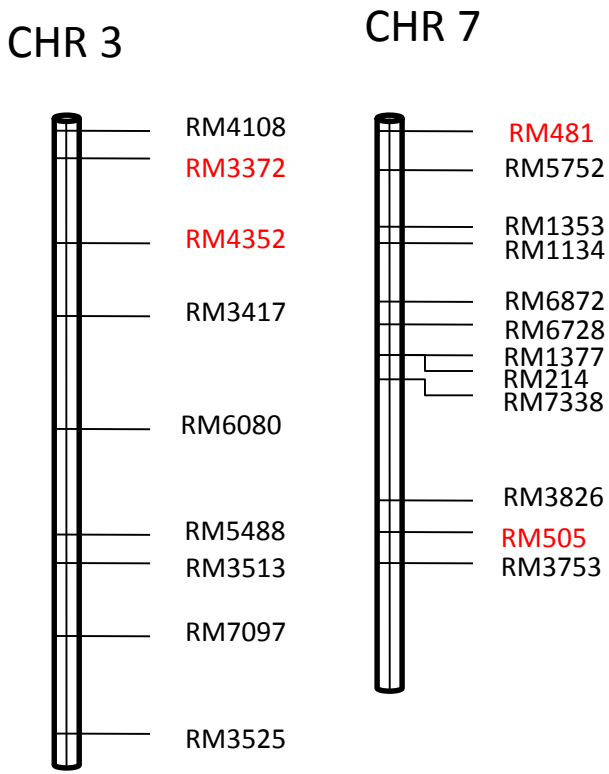

Fig. 4.3. Graphical genotype of 32R in chromosome 3 and 7. Markers are indicated to the right of each chromosome. Red markers indicate the marker derived from Chugoku 45.

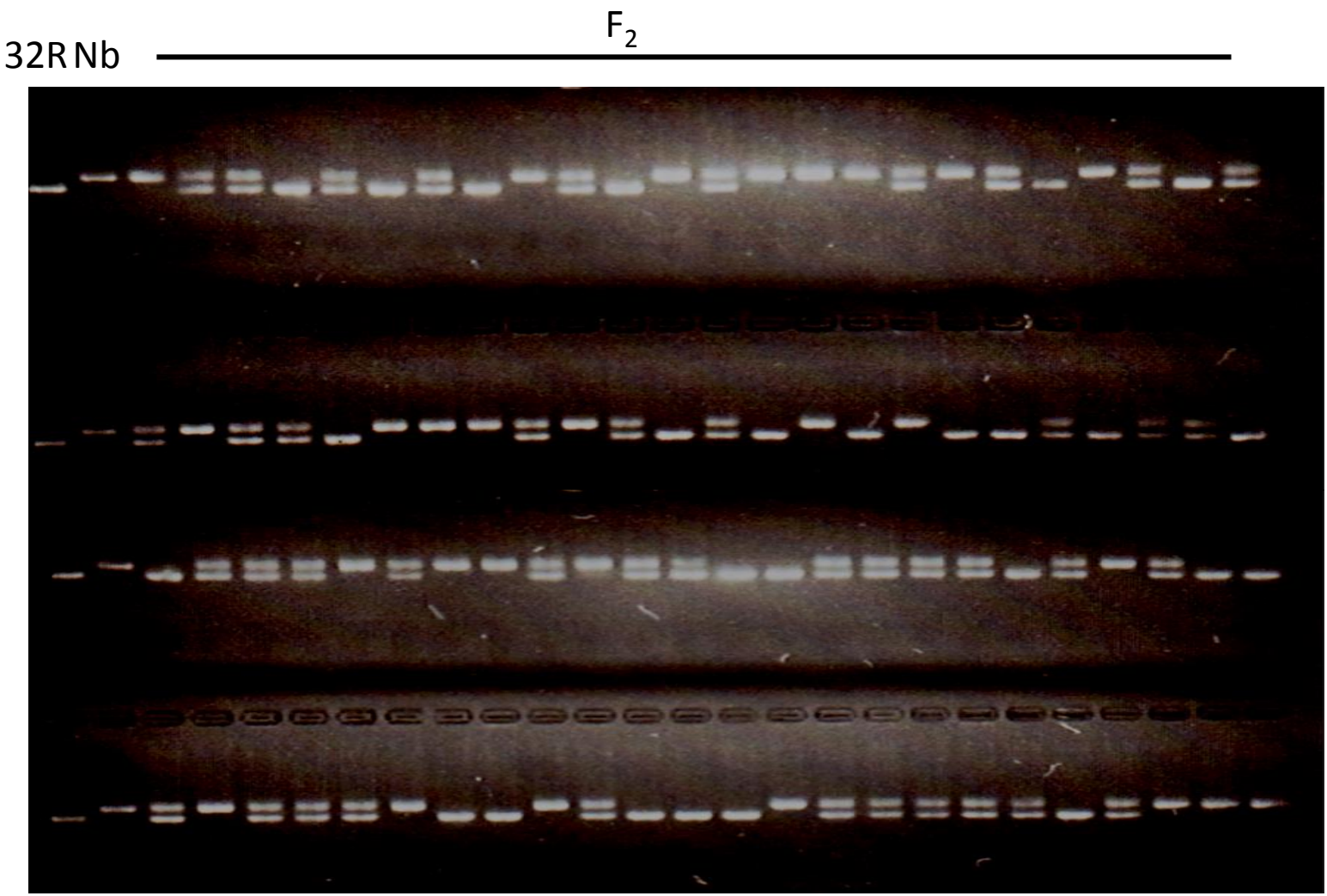

Fig. 4.4. PCR gel image of genotyping of F_2 population by one marker.

Table 4.3. Correlation coefficients among different traits in the F_2 population developed from 32R and Nipponbare

	Disease score	Culm length	First leaf angle	Second leaf angle
Culm length	-0.146*			
First leaf angle	-0.035	0.025		
Second leaf angle	0.037	-0.042	0.393**	
Third leaf angle	-0.002	-0.124*	0.238**	0.289**

All the traits were measured at the physiological maturity stage. The leaf angle made by upper leaf of rice with vertical stem is taken as first leaf angle. Similarly, the angle made by second leaf and third leaf counting from the top is taken as second leaf angle and third leaf angle, respectively. * and ** indicate significance at the 0.05 and 0.01 levels, respectively.

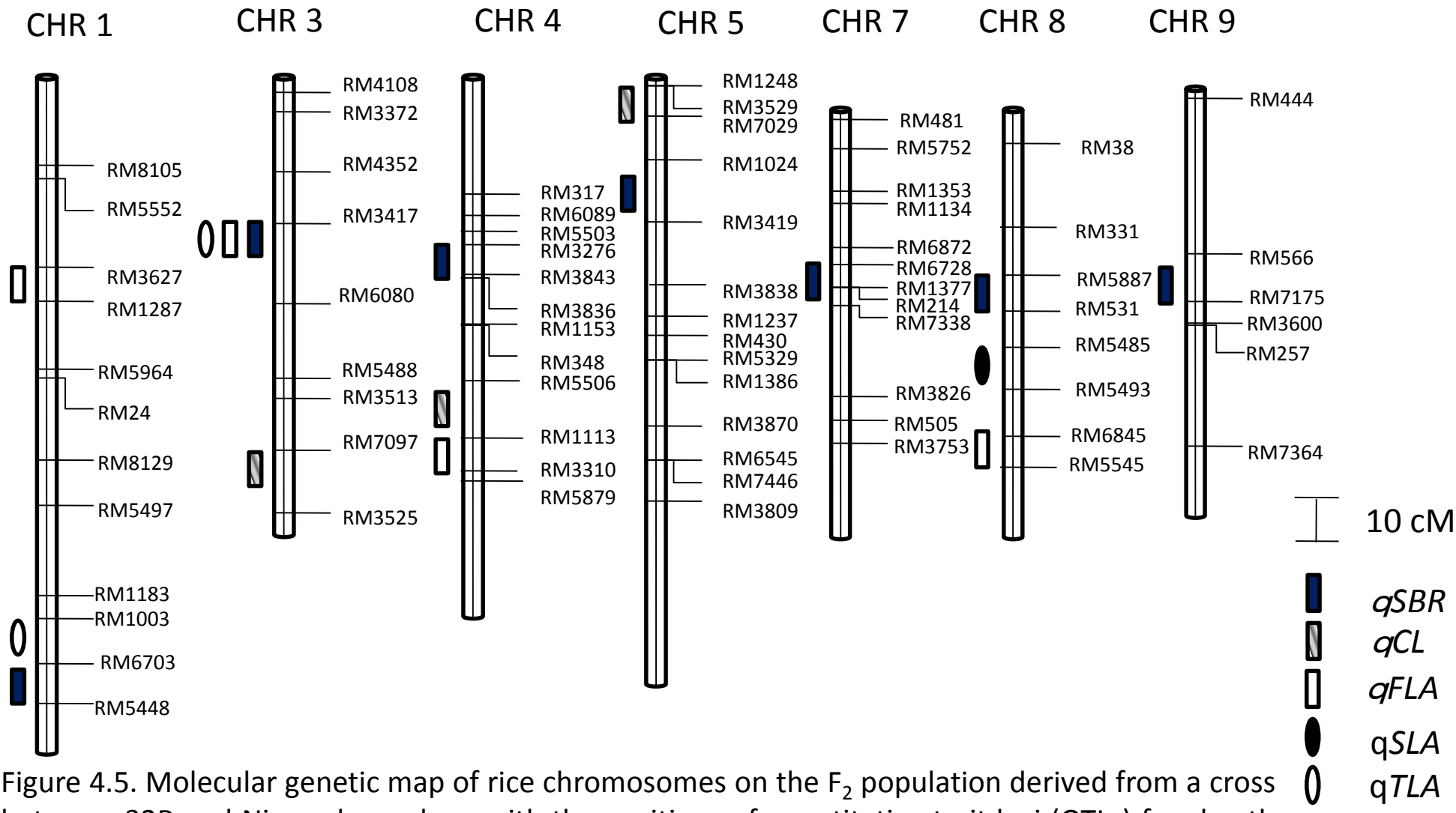

Figure 4.5. Molecular genetic map of rice chromosomes on the F_2 population derived from a cross between 32R and Nipponbare along with the positions of quantitative trait loci (QTLs) for sheath blight resistance and other traits. Where SBR, sheath blight resistance; CL, Culm length; FLA, First leaf angle; SLA, Second leaf angle and TLA; Third leaf angle.

Table 4.4. The quantitative trait loci (QTLs) identified for sheath blight resistance and other trait evaluated by composite interval mapping

Traits	QTL	CHR	Markers	Marker interval	LOD	R^2 (%)	Additive effect
Sheath blight disease	qSBR1	1	RM6703	RM6703-RM5448	2.95	7.50	0.17
	qSBR3	3	RM3417	RM3417-RM6080	2.94	7.43	0.14
	qSBR4	4	RM3276	RM3276-RM3843	3.79	8.42	0.59
	qSBR5	5	RM1024	RM1024-RM3419	3.77	7.79	0.88
	qSBR7	7	RM6728	RM6728-RM214	2.88	7.42	0.17
	qSBR8	8	RM5887	RM5887-RM531	4.29	8.71	0.33
	qSBR9	9	RM566	RM566-RM7175	6.73	9.19	0.37
Culm length	qCL3	3	RM7097	RM7097-RM3525	2.52	7.53	-0.98
	qCL4	4	RM5506	RM5506-RM1113	3.26	13.59	-3.87
	qCL5	5	RM3529	RM3529-RM7029	3.45	15.4	-5.94
Leaf angle	qFLA1	1	RM3627	RM3627-RM1287	10.23	8.50	9.84
	qFLA3	3	RM3417	RM3417-RM6080	18.78	9.12	10.69
	qFLA4	4	RM1113	RM1113-RM3310	9.16	8.50	9.63
	qFLA8	8	RM6845	RM6845-RM5545	9.74	8.60	9.80
	qSLA8	8	RM5485	RM5485-RM5493	3.46	6.77	5.28
	qTLA1	1	RM1003	RM1003-RM6703	3.49	8.37	4.77
	qTLA3	3	RM3417	RM3417-RM6080	5.69	7.10	6.20

SBR, sheath blight disease resistance; CL, culm length; FLA, First leaf angle; SLA, Second leaf angle and TLA, Third leaf angle.

The QTLs of culm length identified in this study might be used for the increase of culm length. QTLs of leaf angle were derived from the resistance cultivar 32R. The QTLs of leaf angle identified here can be utilized to decrease the leaf angle. However, there was not significant relation between leaf angles and disease score (Table 4.3). QTLs for first leaf angle and third leaf angle detected on chromosome 3 were on the same locus to QTL of sheath blight resistance. QTL of third leaf angle is close to *qSBR1*. Except in chromosome 1 and 3, other QTLs of leaf angles were not located close to the QTLs of sheath blight disease. Among seven QTLs of sheath blight disease, only three of them were close to the QTLs of culm length and leaf angles. This suggested that the relation between culm length and leaf angle with the sheath blight resistance was not likely to be due to pleiotropic but close linkage. Therefore, it is reasonable for sheath blight resistance breeding to select appropriate plant height and the erect leaf (Han *et al*., 2003). Earlier report has shown a correlation of sheath blight with plant height, heading date and leaf angle (Li *et al*., 1995). However, noticeable potential role of leaf angle for the resistance of sheath blight disease did not find in this study. The morphological traits might affect the infection and development of the *R. solani* by an influence microclimate on field such as air ventilation, relative humidity and light transmission rate (Han *et al*., 2003). Creating ideal plant morphology is also important for the high yielding cultivar development. The study of yielding capabilities during the material development of this study identified the 12.5 t/ha yielding capacity with sheath blight resistance in F_1 progeny because of the ideal physio-morphological trait development by the cross combination of 32R and Nipponbare (Gaihre and Nose 2013). Thus, the influence of the morphological traits is favorable for both sheath blight resistance and yield traits. Plant height and leaf angles are important traits associated with the morphology of ideal plant type. The QTLs related to plant height, identified in the chromosome 1, 4 and 5 might be important for the plant height management. Similarly, QTL

of leaf angle in the chromosome 8 around the SSR marker RM6845 might be used for leaf angle management.

The validation of QTLs of sheath blight disease is determined by using various genetic background, populations and environment. Among the several QTLs, some QTLs share the same locus on the chromosome suggesting the existence of common locus for differentiation among rice varieties. These QTLs are stable, specific and considered as valuable QTL (Sabouri *et al*., 2011). The *qSBR1* in the long arm of chromosome 1 is one of the important QTL identified in this study (Fig 4.5 and Table 4.4). Channamalikarjuna *et al*. (2010) reported QTLs of sheath blight disease resistance in the rice line Tetep close to *qSBR1*. Similarly, a major QTL of sheath blight disease was predicted near to this locus in the cross between Baiyequie and Maybelle (Xu *et al*., 2011). The locus of *qSBR7* identified in 32R was in the same region as a QTL of sheath blight disease mentioned in the field studies of rice lines Jasmine 85 (Pan *et al*., 1999; Costanzo *et al*., 2011 and Liu *et al*., 2013), TeQing (Pinson *et al*., 2005) and Tetep (Channamalikarjuna *et al*., 2010). Furthermore, Costanzo *et al*. (2011) reported that a gene (cytokinin-*O*-glucosyltransferases) conferring resistance to sheath blight disease contained in this locus. Similarly, the location of *qSBR9* identified in this study is at the same location of QTL identified in rice lines Jasmine 85, TeQing, Tetep, Pecos (Zou *et al*., 2000; Sharma *et al*., 2009; Channamalikarjuna *et al*., 2010 and Liu *et al*., 2013). Furthermore, The LOD value 4.60 and additive effect of 0.37 indicate that *qSBR9* is a major QTL for sheath blight resistance. In addition, *qSBR9* can be taken as major QTL because of the identification in similar chromosomal location of different mapping population at different global locations (Liu *et al*., 2013). The *qSBR4* is novel QTL identified only in rice line 32R in this study. Similarly, *qSBR8* is also identified in rice line 32R only but near to this QTL of sheath blight resistance has been reported in rice line Tetep (Channamalikarjuna *et al*., 2010). Furthermore, near

to this location QTL of second leaf angle has also been identified. Therefore, the *qSBR4* and *qSBR8* appeared in this study might be novel QTLs or influenced by environmental factors under field condition. The *qSBR3* identified in this study is more than 30 cM away from *qSBR3* identified in the rice line WSS2 (Sato *et al*., 2004). WSS2 is genetically similar to the rice line 32R. Furthermore, in this locus QTL of leaf angles were also found and also markers are not tightly linked. The *qSBR5* are might not be better to use for further breeding of sheath blight disease resistance because we could not get the tight linkage between polymorphic markers in this region.

The most important outcome of the present study is the *qSBR1*, *qSBR7* and *qSBR9* as well as their interactions of plant morphological character with sheath blight resistance in rice under natural field conditions. This study also clarifies the genetic mechanism of sheath blight resistance rice line 32R. The resistance capacity of sheath blight disease of 32R might be due to the molecular mechanisms involved in host- pathogen interaction. This finding and analytical method might help to unravel more important information for molecular mechanism of sheath blight disease resistance of rice and for creating disease-resistant rice varieties by the method marker assisted selection. *qSBR1*, *qSBR7* and *qSBR9* are closely linked to sheath blight resistance genes identified in present investigation may be useful for marker assisted selection in the improvement of sheath blight disease resistance rice breeding programs.

Summary

Rice sheath blight disease, caused by the soil borne pathogen *Rhizoctonia solani* Kuhn, annually causes severe losses in yield and quality in many rice production areas worldwide. Rice line 32R is a well-documented source of durable and broad spectrum resistance to sheath blight disease. Rice line 32R was developed from the *Indica* cultivar Tetep by crossing with the *Japonica*

cultivar CN_4-4-2 by using classical breeding techniques. CN_4-4-2 is the progeny of Chugoku 45 and Nipponbare. The study of polymorphism among the rice lines 32R, Tetep, Chugoku 45, Nipponbare, CN_4-4-2 and 29S (genetically similar to 32R) revealed that the resistance mechanism of rice line 32R is induced character of Tetep. Quantitative trait loci (QTL) analysis of the sheath blight disease resistance using simple sequence repeat (SSR) markers was conducted in F_2 population derived from the crossing of 32R with Nipponbare (susceptible to the sheath blight). Sheath blight resistance in F_2 population and its cross parents were studied using syringe inoculation method. Seven QTLs for the sheath blight resistance (*qSBR1*, *qSBR3*, *qSBR4*, *qSBR5*, *qSBR7*, *qSBR8* and *qSBR9*) were identified in chromosome 1, 3, 4, 5, 7, 8 and 9, respectively. Their resistance alleles derived from the resistance parent 32R. Additionally, the negative significant correlation between sheath blight disease and culm length suggest that plant height is unfavorable for sheath blight development. *qSBR1*, *qSBR7* and *qSBR9* shows characteristic of valuable QTLs that would enable to breed a resistance rice variety of sheath blight disease by the method of marker assisted selection (MAS).

CHAPTER 5

General Discussion

Rice has been extremely studied as favorite genetic experimental plant species because of its small genome size and staple food of the large proportion of people. In Asia, people are consuming 35-60% of the dietary calories from rice (Fageria, 2003). It is estimated that rice production should be increase about more than 1.2% per year to fulfill the global demand of rice (Normile, 2008). However, rice is subject to diseases that place major biological constraints on its production. Fungal and insect pests continually evolve and overcome host plant resistance. Therefore, great efforts is required to breed new rice varieties with higher yield potential and to improve rice management in order to enhance average farm yields of rice (Peng *et al*., 2008).

Breeding resistant varieties is an effective method of controlling harmful sheath blight disease. Despite optimism about continued yield improvement from conventional breeding, new technologies such as biotechnology will be needed to maximize the probability of success (Ortiz 1998 and Huang *et al*. 2002). One area of biotechnology, DNA marker technology, derived from research in molecular genetics and genomics, offers great promise for plant breeding. Present achievements in DNA marking technology allow finding new solutions for producing resistant rice genotypes, when assessment and selection of breeding material are carried out directly with respect to genotype based on molecular analysis. Plant breeding in combination with developments in agricultural technology such as agrochemicals has made remarkable progress in increasing crop yields for over a century. However, plant breeders must constantly respond to many changes. Specially, agricultural practices change, which creates the need for developing genotypes with specific agronomic characteristics, target environments, the organisms within them are constantly changing and disease infection because of the environmental change (Collard and Mackill 2008).

For the sustainable crop, the high yielding capacity with disease resistance is the basic requirements. The polygenic resistance cultivar with the proper management by human activity is thought to be important for sustainable agriculture. In the rice and other cereals, the product of sink capacity and grain filling efficiency is defined as yield potential (Kato and Takeda, 1996). To increase the yield potential, breeding efforts have expanded source and sink capacity, the maximum size of sink organs to be harvested, mainly by increasing the number of spikelets per panicle (Kato *et al*., 2007). In modern new rice cultivars, low percentage of grain filling is another problem. The low filled grain percentage was mainly attributed to poor sink strength of inferior spikelets. Pre-anthesis NSC reserves in the stem are closely associated with the sink strength during grain filling of rice, and nitrogen application at the spikelet differentiation stage would be a good practice to increase pre-anthesis NSC reserves, and consequently to enhance sink strength for rice varieties with large panicles, such as super rice varieties (Fu *et al*., 2014). To develop the modern cultivar plant selection by the deep study of phenotypic and genotypic characteristics to determine the cross combination is required. The rice 32R has combining ability with the earlier parent Nipponbare (Chapter 2). The cross combination of rice line 32R with Nipponbare, developed 12.5 t/ha yielding capacity in the F_1 progeny with ideotype plant structure. The number of filled grain per panicle, Rubisco content, NSC, panicle length, culm length, number of tiller, seed weight, leaf area and DMA in F_1 progeny have important role for the yielding capacity. Furthermore, the analysis of physio-morphological traits and disease symptom after inoculation in progeny developed by 32R and Nipponbare gives the clear image of plant structure required for high yielding potential and sheath blight resistance.

Both wild species and landraces of the *Oryza* genus possess under exploited alleles that may have a strong potential for the improvement of Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* Steud.). Wild rice has been used to successfully develop resistance against many rice diseases (Brar and Khush 1997; Jena and Mackill 2008 and Srinivasachary *et al*., 2011). Rice disease resistance is generally classified into two main categories: qualitative resistance (or complete resistance) and quantitative resistance (or partial resistance). Qualitative resistance is conferred by a single disease resistance gene that or its encoded protein can directly or indirectly interact with a corresponding pathogen effector. Thus, qualitative resistance is pathogen race-specific (Kou and Wang 2012). However, the presence of qualitative resistance of sheath blight disease in rice is thought to be difficult because of interaction of number of external factors.

Segregating populations derived from crosses between contrasting parents have been used to map QTL associated with sheath blight disease resistance and its associated traits. The frequently used mapping populations are recombinant inbred lines (RILs), doubled haploids (DH), F_2 progenies or their derivatives, and backcross (BC) derived populations. Sheath blight disease of rice caused by *R. solani* is a major biotic constraint of rice in most of the rice growing countries. Although the disease can be managed by fungicides in some percentage, breeding resistant varieties with durable resistance is a more ecologically sound and sustainable approach. Resistance to the sheath blight pathogen is quantitative in nature (Pinson *et al*., 2005). There are number of quantitative trait loci (QTLs) providing sheath blight resistance across different genetic backgrounds have been identified in different QTL mapping studies (Srinivasachary *et al*., 2011). The *qSBR11-1* is a major QTL that has been found to be effective against the sheath blight pathogen consistently over time and at different locations in rice line Tetep (Channamallikarjuna *et al*., 2010). However, in this study we could not find any polymorphic marker in this region in

between the rice line 32R and Nipponbare (Chapter 4). Furthermore, *qSBR11-1* was used in MAS breeding to make sheath blight resistance Basmati rice. The transfer of *qSBR11-1* developed Basmati rice moderately resistance from the susceptible rice. Improved Basmati rice line was susceptible than Tetep (Singh *et al*., 2012). The location of *qSBR9* identified in this study is at the similar location of QTL identified in rice lines Jasmine 85, TeQing, Tetep, Pecos and Jarjan (Zou *et al*., 2000; Sharma *et al.,* 2009; Channamalikarjuna *et al*., 2010; Liu *et al*., 2013 and Taguchi-Shiobara *et al*., 2013). The QTL *qSBR9* can be taken as major QTL because of the identification in similar chromosomal location of different mapping population at different global locations (Liu *et al*., 2013).

The association between increased plant height and decreased sheath blight incidence is not surprising, as sheath blight disease spreads from the water line up the leaf sheath and toward the panicle, and our rating was based on the percentage of vegetative height displaying sheath blight disease lesions. A comparatively smaller portion of tall plants would therefore be infected if the disease spreads at the same rate, irrespective of plant height. Furthermore, the shortened internodes of semi dwarf plants may create a more tightly closed canopy and a microclimate that is more conducive for disease development in comparison to tall plants. The QTLs of plant height identify here can be used for the plant height management in rice (Chapter 4, Table 4.4) The present study further supports the previous finding that agronomic traits, particularly plant height (Marchetti, 1983; Li *et al*., 1995a, b; Pinson *et al*., 2005 and Sharma *et al*., 2009), have a major impact on sheath blight resistance in rice. Identification of sheath blight resistance QTLs are more likely to be used in breeding programs when they are independent of mechanisms of disease escape, like delayed heading and increased plant height, which are considered undesirable agronomic traits.

The structure of plant morphology for the high yielding sheath blight resistance rice based on the concept involving combinations of many morphological and physiological traits into a multi-dimensional plant structure that maximizes the biomass and it's partitioning. Leaf angle is an important agronomic traits associated with the morphology of ideal plant type for the sheath blight resistance. Under the conditions of modern agriculture with increased application of N fertilizer, larger tiller and leaf angles may cause mutual shading, increased humidity favoring diseases and insects, and lodging. On the other hand, a highly compact plant type with completely vertical tillers and erect leaves tends to be inefficient at utilizing solar energy at early growth stages, and is vulnerable to those diseases which are favored by high humidity such as sheath blight disease. The plant structure that the most breeders favor tends to be intermediate between these extremes with erect leaves and relatively small tiller angle, allowing a high leaf area index without causing mutual shading (Li *et al*., 1998). In this study we found, the inclination pattern of all leaf angle is similar. The QTLs of leaf angle in the chromosome 8 around the SSR marker RM6845 might be used for leaf angle management.

The basic requirements of high yield in rice is to improve leaf photosynthetic efficiency and coordinate the source-sink relationship in rice plants (Takai *et al*., 2013 and Zang *et al*., 2014). The crossing of *Japonica* cultivar with *Indica* increase source capacity of rice. The yield potential also controlled by polygene like sheath blight disease. Grain yield, complex trait, is regulated by a number of elementary factors, but it is likely that the factors are not equally effective in determining the trait. The major trait that determined the grain yield are panicle length, number of grain per panicle and grain weight. These trait are directly depend on source capacity. The source capacity is depend on photosynthetic rate determined by leaf architecture. Thus QTL of grain yield is indirectly determined by QTL of panicle length, number grain per panicle, grain weight, leaf

angle, leaf thickness, Rubisco content in the leaf and chlorophyll content in the leaf. There are number QTLs were mentioned related to grain yield (www.gramene.org). The selected QTLs of yield related traits of Nipponbare mentioned different literature and q*SBR* identified in this study were presented in Figure 5.1. The *Japonica* allele narrow leaf 1 (NAL1) from Nipponbare effectively increased leaf width and length, leaf area and chlorophyll content in leaf (Wang *et al*., 2011 and *Zang et al*., 2014). The allele NAL1 of chromosome 4 may be a key gene for the development of plant type and yield-related traits in rice. Although we did not conducted the genetic study of chlorophyll content in leaf and leaf photosynthesis rate. The sequence comparison analysis was done. Ishimaru *et al*. (2005) reported that a QTL for yield potential on chromosome 5, rg5, which represented an allele from the *Indica* cultivar Kasalath studied in the background of *Japonica* Nipponbare, was thought to be of use in different genetic backgrounds. Similarly, Venu *et al*. (2014) also reported number of QTL of yield and yield related traits in different chromosome. The effective improvement of yield potential in the progeny of 32R and Nipponbare may the combination of *Indica* and *Japonica* traits to enhance the source capacity and sink size.

Comparing the location of QTLs makes it possible to determine the genetic relations among traits and the genetic limiting factors for a complex trait. It is very difficult to determine the direct relation of QTLs of sheath blight disease and yield potential. But yield potential can be converted into yield if the rice cultivar has disease resistance capacity. The detail relation of resistance capacity, QTL of sheath blight disease and yield potential is shown in systematic diagram (Figure 5.2). Diagram shows that that when sheath blight disease is not present, yield potential determines grain production. Moving to the right in the figure, sheath blight disease becomes more severe yield potential cannot be achieved and yield loss will be happen. So, escape of sheath blight or sheath blight tolerance becomes more important. The vertical axis represents the resistance and

susceptibilities of sheath blight disease. If the cultivar is resistance, the sheath blight disease escape through changing phenology. As the rice becomes susceptible (moving up on the axis), sheath blight resistance trait becomes important. 32R developed from Nipponbare, Chugoku 45 and Tetep is a highly valuable source of genes for resistance to multiple diseases, the contribution from this donor parent cannot be ruled out. The QTLs of sheath blight resistance, plant height, and leaf angles can be used for further study of sheath blight disease management. Super yielding rice with ideotype plant architecture and sheath blight resistant can be developed from the cross combination of 32R and Nipponbare by general selection.

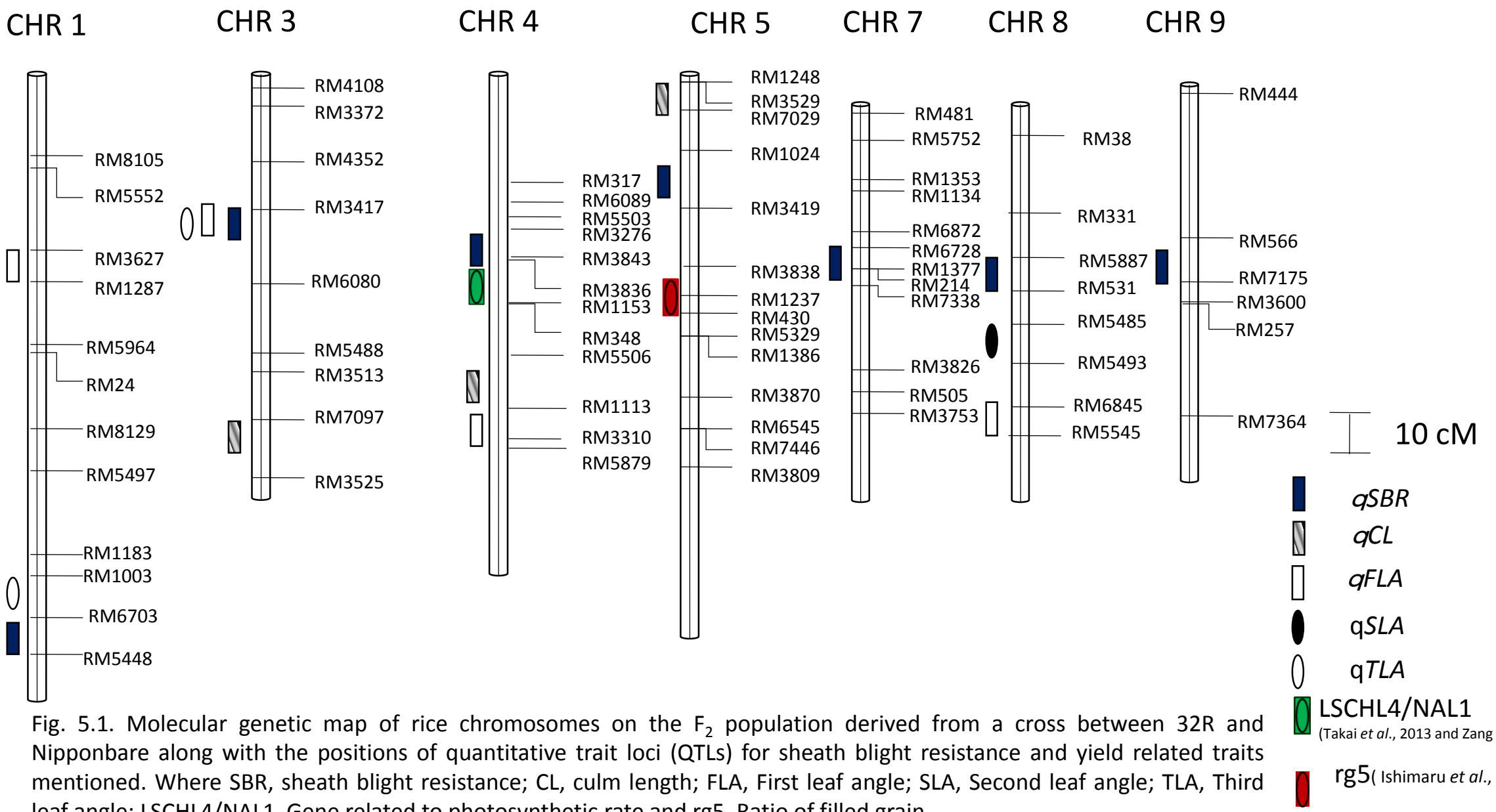

Fig. 5.1. Molecular genetic map of rice chromosomes on the F_2 population derived from a cross between 32R and Nipponbare along with the positions of quantitative trait loci (QTLs) for sheath blight resistance and yield related traits mentioned. Where SBR, sheath blight resistance; CL, culm length; FLA, First leaf angle; SLA, Second leaf angle; TLA, Third leaf angle; LSCHL4/NAL1, Gene related to photosynthetic rate and rg5, Ratio of filled grain.

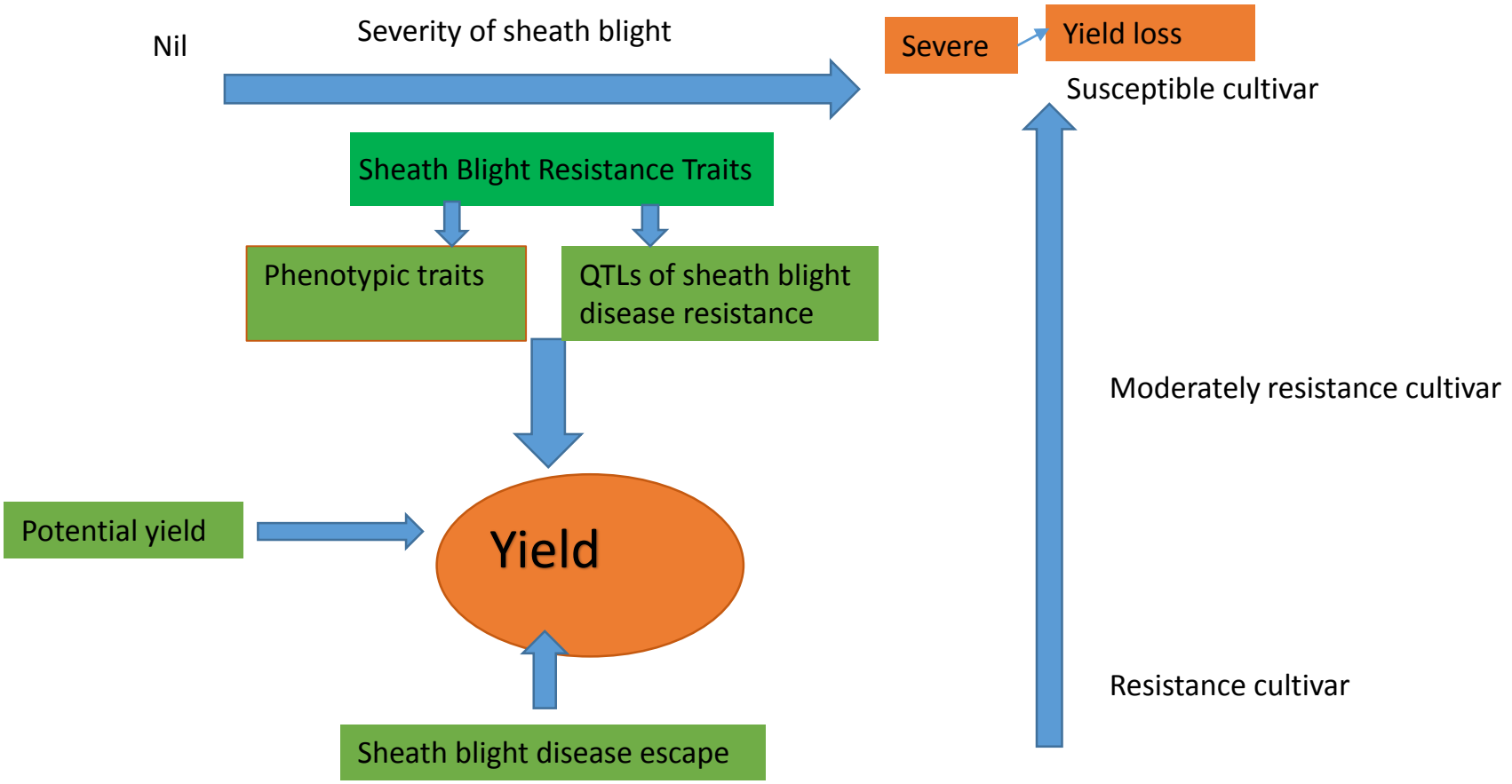

Fig.5.2. Schematic presentation of three components of yield in relation with sheath blight disease: potential yield, Sheath blight escape and Sheath blight resistance. These components play different roles, depending on predictability and severity of sheath blight disease in the target environment.(Partial concept was adapted from Fisher *et al*.,2012)

Future studies

Although QTL mapping has many potential practical outcomes, it is considered as a basic research process in molecular breeding. Data from single QTL mapping experiment may not accurate and will not be suitable for the marker-assisted breeding (Collard and Mackill 2008). Many factors may affect the accuracy of a QTL mapping study such as the level of replication used to generate phenotypic data, population size, environment, field condition etc. The accuracy of the QTL mapping study is critical to the success of MAS. This is particularly important when QTL mapping is undertaken for more complex traits, such as yield and fungal disease resistance that are controlled by many QTLs with small effects compared with simple traits (Kearsey and Farquhar 1998).

For plant breeding, the final 'product' is a new variety. Classical breeding method and molecular breeding method are the existing way to develop new variety. In Chapter 2, the study of relation between yield and sheath blight disease by the anatomical and physio-morphological pathways shows great possibility to develop sheath blight resistance cultivar with high yielding capabilities, for this both classical and molecular breeding method are suitable. To release new variety from the material developed in this study, needs to inspect agronomy traits as well as resistance capacity and appropriate selection until the desired uniformity achieved. Although it is time consuming, by the classical breeding also high yielding resistance cultivar can be released in low cost by general selection.

The final target of this study is to develop new high yielding cultivar having sheath blight disease resistance by the molecular breeding method, which is on the way. As we know, sheath blight disease resistance is a complex trait. Sheath blight severity was studied in the field.

Therefore, it may have influence of environmental factors. In Chapter 4, Seven QTLs for the sheath blight resistance (*qSBR1*, *qSBR3*, *qSBR4*, *qSBR5*, *qSBR7*, *qSBR8* and *qSBR9*) were identified. Among them, *qSBR1*, *qSBR7* and *qSBR9* are validated by matching the locus of the published QTLs of the Tetep as well as sheath blight disease resistance cultivar studied in field as well as in controlled environment. These validated QTLs *qSBR1*, *qSBR7* and *qSBR9* can be utilized for MAS breeding in future. The accuracy of the QTL mapping study is critical to the success of MAS. Therefore, considering the existence of QTL and environment interaction, it can be suggested that these QTLs should be again tested in closed environment different environment of different location. Consequently, tight linkage of SSR marker better to develop by increasing SSR marker in the target region.

Summary

Sheath blight disease, caused by *Rhizoctonia solani* Kuhn, is one of the most important diseases of rice. Despite extensive searches of the rice germ plasm, major genes, which give a complete resistance to the fungus, have not been identified. The use of resistant rice cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Sheath blight disease severity of the rice can be affected by environmental and chemical factors, and the epidemic area of sheath blight disease is also increasing because of climate change.

The objective of this study is to determine the genetic components of yielding capabilities and quantitative trait loci (QTL) of sheath blight disease resistance rice line 32R. Rice line 32R is a well-documented source of durable and broad-spectrum resistance to sheath blight disease. Rice line 32R was developed from the *Indica* cultivar Tetep by crossing with the *Japonica* cultivar CN_4-4-2 by using classical breeding techniques. CN_4-4-2 is the progeny of Chugoku 45 and Nipponbare

The rice line 32R, having the poor yielding capacity, crossed with Nipponbare produced 12.5 t/ha yield in the F_1 progeny with an ideotype plant structure. The high yielding capacity is due to the improved performance of culm length, panicle length, number of tiller, tillering angle, Rubisco content in leaf, nonstructural carbohydrate (NSC), dry matter accumulation (DMA), leaf area and number of filled grain per panicle. The mid parent and better parent heterosis was 42.3 and 29.8% for grain yield, respectively. Multiple regressions showed that number of filled grain per panicle, panicle length and leaf area contributed 83.0, 28.4 and 29.9% of its effort to the grain yield, respectively in F_1 progeny.

The genetic similarity and genetic diversity among selected six cultivar Tetep, 32R, 29S, CN_4-4-2, Chugoku 45 and Nipponbare were determined by selecting 1338 SSR markers. The polymorphic markers among all six cultivar were detected. Most of the polymorphic SSR marker

in between 32R and Nipponbare were derived from Tetep in 32R and only few markers were derived from Chugoku 45. By the cluster analysis, six rice line were categorized into two big cluster *Indica* dominant group and *Japonica* dominant group. The rice line 32R and Tetep were belonged to *Indica* dominant group and had 45% similarity. The rice line 29S had 97% similarity with Chugoku 45. Furthermore, 29S, CN_4-4-2, Chugoku 45 and Nipponbare were belonged to the same cluster of *Japonica* dominant group and had 91% similarity.

QTL analysis of the sheath blight disease resistance using simple sequence repeat (SSR) markers, was conducted in F_2 population derived from the crossing of 32R with Nipponbare (susceptible to the sheath blight). Sheath blight resistance in F_2 population and its cross parents were studied using syringe inoculation method. Seven QTLs for the sheath blight resistance (q*SBR1*, *qSBR3*, *qSBR4*, *qSBR5*, *qSBR7*, *qSBR8* and *qSBR9*) were identified in chromosome 1, 3, 4, 5, 7, 8 and 9, respectively. Their resistance alleles derived from the resistance parent 32R. Similarly, the QTLs of culm length and leaf angles also identified. The QTLs of plant height and Leaf angles can be utilize for the development of plant structure suitable for resistance of sheath blight. Additionally, the negative significant correlation between sheath blight disease and culm length suggest that plant height is unfavorable for sheath blight development. *qSBR1*, *qSBR7* and *qSBR9* shows characteristic of valuable QTLs that would enable to breed a resistance rice variety of sheath blight disease by the method of marker assisted selection (MAS).

This study led to conclusion that the cross combination of 32R and Nipponbare can develop high yielding rice with sheath blight resistance capacity. The yield potential of the expected rice line might depend on source capacity and sink size. The rice line 32R containing valuable QTLs of sheath blight resistance and high yielding potential with cross combination of Nipponbare, could be a potential donor for the resistance to sheath blight disease in the field in future.

Abstract in Japanese

Rhizoctonia solani Kuhn によって引き起こされる紋枯病は、イネで最も重要な病害のひとつである。現在までの広範なイネ遺伝資源の探索にもかかわらず、本菌に対する完全な抵抗性を示す主導遺伝子は同定されていない。紋枯病抵抗性イネ品種の開発は、農薬の使用を減らした持続型稲作実現の有力な手法となる。また、イネ紋枯病の罹病地域は地球温暖化に伴い拡大し、温帯域稲作の大きな課題となりつつある。

本研究の目的は、イネ系統 32R の紋枯病抵抗性に関与する QTL を明らかにし、同時に多収性の可能性と実現のための形質特性を明らかにすることである。本研究で用いた抵抗性系統 32R、感受性系統 29S は、インディカ品種 Tetep とジャポニカ品種 CN4-4-2 との交雑集団から選抜育成され、CN4-4-2 は中国 45 号と日本晴の後代である。

収量性が劣る 32R を日本晴と交配した F_1 集団は、12.5t/ha と高い収量を示し、その要因は、稈長、穂長、分げつ数、分げつ角度、葉中の Rubisco 含有量、非構造性炭水化物（NSC）、乾物蓄積（DMA）、葉面積および穂あたりの子実数等の改善によることが明らかになった。中間親及び優良親系統に対する子実収量のヘテロシス効果は、それぞれ 42.3、29.8%であった。F_1 後代における収量への形質の貢献度は、穂あたりの子実数、穂長および葉面積がそれぞれ、83.0、28.4、29.9%であった。

1338 個の SSR マーカーを用いて、32R 育成に関するイネ 5 品種、Tetep、29R、 CN_4-4-2、Chugoku45 と日本晴の間における遺伝的類似性と遺伝的多様性を検討した。

全 6 品種間で多型マーカーが検出され、32R と日本晴の間におけるほとんどの多型 SSR マーカーは、Tetep に由来し、わずかのマーカーが Chugoku45 に由来していた。クラスター解析によって、6 つのイネ系統は、インディカ優性グループとジャポニカ優性

グループの 2 つの大きなクラスターに分類された。32R と Tetep はインディカ優性グループに属し、45%の類似性があった。29S は Chugoku45 と 97%の類似性を示し、29S、CN_4-4-2、Chugoku45 及び日本晴は、ジャポニカ優性グループの同じクラスターに属し、91%の類似性を示した。

単純反復配列（SSR）マーカーを用いた紋枯病抵抗性の量的形質遺伝子座（QTL）解析を日本晴（イネ紋枯病感受性）と 32R の交配由来の F_2 集団において実施し、紋枯病抵抗性に関与する 7 つの QTL（*qSBR1*、*qSBR3*、*qSBR4*、*qSBR5*、q*SBR7*、*qSBR8* と *qSBR9*）を、染色体 1、3、4、5、7、8 および 9 で同定することができ、それらの抵抗性対立遺伝子は、抵抗性親 32R に由来するものであった。同時に、草丈と出葉角度に関与する QTL も同定され、これらの QTL は、紋枯病抵抗性改善に有効な草型の改良に活用できることが明らかになった。また、紋枯病と稈長との間に有意な負の相関が認められ、長稈性が紋枯病と収量改善に有利に働くことを示すことが明らかになった。 さらに、*qSBR1*、*qSBR7* および *qSBR9* は、マーカー利用選抜法（MAS）を活用した紋枯病抵抗性イネ品種の育種に有効な QTL と考えられる。

以上ように、本研究においては 32R と日本晴の交雑により紋枯病抵抗性を持つ多収性イネを改良し得る可能性が示唆され、同時に期待されるイネ系統の収量ポテンシャルは、ソース容量（子実数）とシンクサイズ（葉面積）の改善によって実現できることが示唆された。紋枯病抵抗性に関与する QTL を有し、日本晴の交雑により多収性の実現を可能にするイネ系統 32R は、今後地球温暖化に伴い紋枯病の拡大が懸念さる温帯域における紋枯病抵抗性と多収を実現する有望なドナーとなり得るものと考えられる。

References

Ao, H., Peng, S., Zou, Y., Tang, Q. and Visperas, R.M. 2010. Reduction of unproductive tillers did not increase the grain yield of irrigated rice. *Field Crops Research* 116: 108-115

Anees, M., Edel-Hermann, V. and Steinberg, C. 2010. Buildup of patches caused by *Rhizoctonia solani*. *Soil Biology and Biochemistry* 42: 1661-1672.

Brar, D.S. and Khush, G.S. 1997. Alien introgression in rice. *Plant Molecular Biology* 35:35-47.

Bray, J.R. and Curtis, J.T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27: 325-349.

Bertin, J., Hermardinquer, J., Keul, M. and Randles, W.G.L. 1971. Atlas of food crops. Paris.

Brooks, S.A. 2007. Sensitivity to a phytotoxin from *Rhizoctonia solani* correlates with sheath blight susceptibility in rice. *Phytopathology* 97: 1207-1212.

Bollich, C.N., Webb, B.D., Marchetti, M.A. and Scott, J.E. 1985. Registration of 'Lemont' rice. *Crop Science* 25: 883-885.

Carling, D.E., Baird, R.E., Gitaitis, R.D., Brainard, K.A. and Kuninaga S. 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* 92: 893-899.

Castillo-Muneraa, J.D., Cardenasa, M., Pinzona, A., Castanedab, A., Bernala, A.J. and Restrepoa, S. 2013. Developing a taxonomic identification system of *Phytophthora* species based on microsatellites. *Revista Iberoamericana de Micologia* 30: 88-95.

Chang, T.T. 1985. Crop history and genetic conservation: rice a case study. *Iowa State Journal of Research* 59: 425-455.

Channamallikarjuna, V., Sonah, H., Prasad, M., Rao, G.J.N., Chand, S., Upreti, H.C., Singh, N.K. and Sharma, T.R. 2010. Identification of major quantitative trait loci *qSBR11-1* for sheath blight resistance in rice. *Molecular Breeding* 25: 155-166.

Chen, L.Y., Xiao, Y.H., Tang, W.B. and Lei, D.Y. 2007. Practices and prospects of super hybrid rice breeding. *Rice Science* 14: 71-77.

Choudhary G., Ranjitkumar, N., Surapaneni, M., Deborah, D.A., Vipparla A, Anuradha,G., Siddiq, E.A. and Vemireddy, L.R. 2013. Molecular genetic diversity of major Indian rice cultivars over decadal periods. *PLoS ONE* 8: e66197. doi:10.1371/journal.pone.0066197

Collard, B.C.Y. and Mackill, D.J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society of London. Series B: *Biological Sciences* 363: 557-572.

Costanzo, S., Jackson, A.K. and Brooks, S.A. 2011. High-resolution mapping of Rsn1, a locus controlling sensitivity of rice to necrosis-inducing phytotoxin from *Rhizoctonia solani* AG1-IA. *Theoretical and Applied Genetics* 123: 33-41.

Counce, P.A. and Wells, B.R., 1990. Rice plant population density effect on early-season nitrogen requirement. *Journal of Production Agriculture* 3: 390-393.

Cu, R.M., Mew, T.W., Cassman, K.G. and Teng, P.S. 1996. Effect of sheath blight on yield in tropical, intensive rice production system. *Plant Disease* 80: 1103-1108.

Danson, J. 2000. Studies on carbohydrate and secondary metabolism of rice plants (*Orzya sativa*) in relation to resistance response to sheath blight fungus (*Rhizoctonia solani*) infection. Kagosima University Japan, PhD thesis.

Eizenga, G.C., Lee, F.N. and Rutger, J.N. 2002. Screening *Oryza* Species plants for rice sheath blight resistance. *Plant Disease* 86: 808-812.

Fernando, P.C. 2006. Agriculture, pesticides, food security and food safety. *Environmental Science and Pollution Research* 9: 685-692.

Fageria, N.K. 2003. Plant tissue test for determination of optimum concentration and uptake of nitrogen at different growth stages in low land rice. *Communications in Soil Science and Plant Analysis* 34: 259-270.

Fisher, K.S., Fukai, S., Kumar, A., Leung, H. and Jongdee, B. 2012. Field phenotyping strategies and breeding for adaptation of rice to drought. *Frontiers in Physiology* doi: 10.3389/fphys.2012.00282.

Fu, J., Huang, Z., Wang, Z., Yang, J. and Zang, J. 2011. Pre-anthesis non-structural carbohydrate reserve in the stem enhances the sink strength of inferior spikelets during grain filling of rice. *Field Crops Research* 123: 170-182.

Gaihre, Y.R., Yamagata, Y., Yoshimura, A. and Nose, A. 2015. QTL analysis of sheath blight disease resistance in the rice line 32R derived from Tetep. *Tropical Agriculture Development*. (In press).

Gaihre, Y.R. and Nose, A. 2013. High yielding capabilities and genetic variation in crossing of sheath blight disease resistant rice line. *Field Crops Research* 149: 133-140.

Gonzalez Garcıa, V., Portal Onco, M.A. and Susan, R. 2006. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research* 4: 55-79.

Groth, D.E. and Bond, J.A. 2007. Effects of cultivars and fungicides on rice sheath blight, yield and quality. *Plant Disease* 91: 1647-1650.

Groth, D.E. and Nowick, E.M. 1992. Selection for resistance to rice sheath blight through number of infection cushion and lesion type. *Plant Disease* 76: 721-723.

Han, Y.P., Xing, Y.Z., Gu, S.L., Chen, Z.X., Pan, X.B. and Chen, X.L. 2003. Effect of morphological traits on sheath blight resistance in rice. *Acta Botinica Sinica* 45: 825-831.

Huang, J.K., Pray, C. and Rozelle, S. 2002. Enhancing the crops to feed the poor. *Nature* 41: 678-684.

International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. *Nature* 436: 793-800.

Iizumi, T., Yokozawa, M., Hayasi, Y. and Kimura, F. 2008. Climate Change Impact on Rice Insurance Payouts in Japan. *Journal of Applied Meteorology and Climatology* 47: 2265-2277.

Ishimaru, K., Kashiwagi, T., Hirotsu, N. and Madoka, Y. 2005. Identification and physiological analyses of a locus for rice yield potential across the genetic background. *Journal of Experimental Botany* 56: 2745-2753.

Jena, K.K. and Mackill, D.J. 2008. Molecular markers and their use in marker-assisted selection in rice. *Crop Science* 48: 1266-1276.

Jia, L., Yan, W., Zhu, C., Agarma, H.A., Jackson, A, Yeater, K., Li, X., Huang, B., Hu, B., McClung, A. and Wu, D. 2012. Allelic analysis of sheath blight resistance with association mapping in rice. *PLoS ONE* 7, e32703. doi:10.1371/journal.pone.0032703.

Jia, Y., Correa-Victoria, F., McClung, A., Zhu, L., Liu, G., Wamishe, Y., Xie, J., Marchetti, M. A., Pinson, S.R.M., Rutger, J.N. and Correll, J.C. 2007. Rapid determination of rice cultivar response to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. *Plant Disease* 91: 485-489.

Jiang-shi, Z. and Chuan-gen, L. 2005. Practice and thoughts on developing hybrid rice for super high yield by exploiting inters specific heterosis. *Rice Science* 12: 1-6.

Kato, T., Shinmura, D. and Taniguchi, A. 2007. Activities of enzymes for sucrose-starch conversion in developing endosperm of rice and their association with grain filling in extra-heavy panicle types. *Plant Production Science* 10: 442-450.

Kato, T. and Takeda, K. 1996. Associations among characters related to yield sink capacity in space-planted rice. *Crop Science* 36: 1135-1139.

Kanbe, T., Sasaki, H., Aoki, N., Yamagishi, T. and Ohsugi, R. 2009. The QTL analysis of Rubisco in flag leaves and non-structural carbohydrates in leaf sheaths of rice using chromosome segment substitution lines and backcross progeny F_2 populations. *Plant Production Science* 12: 224-232.

Katsura, K., Maeda, S., Horie, T. and Shiraiwa, T. 2007. Analysis of yield attributes and crop physiological traits of Liangyoupeijiu, a hybrid rice recently bred in china. *Field Crops Research* 103: 170-177.

Kearsey, M.J. and Farquhar, A.G.L. 1998. QTL analysis in plants; where are we know? *Heredity* 80: 137-142.

Khush, G.S. 1977. Disease and insect resistance in rice. *Advances in Agronomy* 29: 268-341.

Khush, G.S. 1995. Breaking the yield frontier of rice. *Geo Journal* 35: 329-332.

Kiet, H.V. and Nose, A. 2011. Temperature effects on the plant growth of sheath blight disease resistance and susceptible rice line. *Japanese Journal of Crop Science* 232 (Extra Issue 2): 148-149.

Kou, Y and Wang, S. 2012. Toward an understanding of the molecular basis of quantitative disease resistance in rice. *Journal of Biotechnology* 159: 283-292.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Etoh, T. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. *Genomics* 1: 174-181.

Lee, F.N. and Rush, M.C. 1983. Rice sheath blight: a major rice disease. Plant Disease 67: 829-832.

Leung, H., Fan, J.X., Zou, Y., Chen, H., Revilla-Molina, I., Punga, I., Cruz, C.V. and Mew, T.W. 2003. Using genetic diversity to achieve sustainable rice disease management. *Plant Disease* 87: 1156-1169.

Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Fujimoto, H., Yuan, M., Luo, D., Han, B. and Li, J. 2003. Control of tillering in rice. *Nature* 422: 618-621.

Li, Z.K., Pinson, S.R.M., Marchetti, M.A., Stansel, J.W. and Park, W.D. 1995. Characterization of quantitative trait loci (QTL) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). *Theoretical and Applied Genetics* 91: 382-388.

Li, Z.K., Paterson A.H., Pinson, S.R.M. and Khush G.S. 1998. A major gene, Ta1 and QTLs affecting tiller and leaf angles in rice. *Rice Genetics Newsletter* 15: 154-156.

Lincoln, S.E., Daly, M.J. and Lander, E. 1992. Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report, 3rd edition. Whitehead Institute, Cambridge.

Liu, G., Jia, Y., McClung, A., Oard, J.H., Lee, F.N. and Correll, J.C. 2013. Conforming QTLs and finding additional loci responsible for resistance to rice sheath blight disease. *Plant Disease* 97: 113-117.

Lore, J.S., Hunjan, M.S., Singh, P., Willocquet, L., Sri, S. and Savary, S. 2013. Phenotyping of partial physiological resistance to rice sheath blight. *Journal of Phytopathology* 161: 224-229.

Lu, C., Shen, L., Tan, Z., Xu, Y., He, P., Chen, Y and Zhu, L. 1996. Comparative mapping of QTL for agronomic traits of rice across environments using a doubled haploid population. *Theoretical and Applied Genetics* 93: 1211-1217.

Marshall, D. and Rush, M. 1980. Infection cushion formation on rice sheaths by *Rhizoctonia solani*, *Phytopathology* 70: 947-950.

Marchetti, M.A. and Bollich, C.N. 1991. Quantification of the relationship between sheath blight severity and yield loss in rice. *Plant Disease* 75: 773-775.

Marchetti, M. 1983. Potential impact of sheath blight on rice yield and milling quality of short-statured rice lines in the Southern United States. *Plant Disease* 67: 162-165.

Martin, S.B., Lucas, L.T. and Campbell, C.L. 1984. Comparative sensitivity of *Rhizoctonia solani* and *Rhizoctonia*-like fungi to selected fungicides in vitro. *Phytopathology* 74: 778-781.

Miyagi, S., Agarie, S., Ohno, Y., Ishida, T. and Nose, A. 2006. Proteomics analysis of defence response in rice resistance line against infection by sheath blight fungus *Rhizoctonia solani*. Fourth International Rice Functional Genomics Symposium, France.

Miller, B.C., Hill, J.E. and Roberts, S.R. 1991. Plant population effects on growth and yield in water-seeded rice. *Agronomy Journal* 83: 291-297.

Morgante, M., Hanafey, M., Powell, W. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics* 30: 194-200.

Morita, S. and Nakano, H. 2011. Nonstructural carbohydrate content in the stem at full heading contributes to high performance of ripening in heat-tolerant rice cultivar Nikomaru. *Crop Science* 51: 818-828.

Murchie, E.H., Yang, J., Hubbart, S., Horton, P. and Peng, S. 2002. Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? *Journal of Experimental Botany* 53: 2217-2224.

Murry, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Research* 8: 4321-4325.

Mutuku, J.M. and Nose, A. 2010. *Rhizoctonia solani* infection in two rice lines increases mRNA expression of metabolic enzyme genes in glycolytic, oxidative pentose phosphate pathways and secondary metabolism. *Tropical Agriculture Development* 54: 119-131.

Mutuku, J.M. and Nose, A. 2012a. Changes in the contents of metabolites and enzyme activities in rice plants responding to *Rhizoctonia solani* Kuhn infection: activation of glycolysis and connection to phenylpropanoid pathway. *Plant Cell Physiology* 53: 1017-1032.

Mutuku, J.M. and Nose, A. 2012b. High activities and mRNA expression of pyrophosphate-fructose-6-phosphatephosphotransferase and 6-phosphofructokinase are induced as a response to *Rhizoctonia solani* infection in rice leaf sheaths. *Physiological and Molecular Plant Pathology* 77: 41-51.

Nagarajkumar, M., Bhaskaran, R., and Velazhahan, R. 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluoresces* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiological Research* 159: 73-81.

Nagata, K., Hiroyuki, S. and Tomio, T. 2002. Quantitative trait loci for nonstructural carbohydrate accumulation in leaf sheaths and culms of rice (*Oryza sativa* L.) and their effects on grain filling. *Breeding Science* 52: 275-283.

Normile, D. 2008. Reinventing rice to feed the world. *Science* 321: 330-333.

Ohsumi, A., Takai, T., Ida, M., Yamamoto, T., Arai-sanoh, Y., Yano, M., Ando, T. and Kondo, M. 2011. Evaluation of yield performance in rice near-isogenic lines with increased spikelet number. *Field Crops Research* 120: 68-75.

Ookawa, T., Yasuda, K., Kato, H., Sakai, M., Seito, M., Sunaga, K., Motobayasi, T., Tojo, S. and Hirasawa, T. 2010. Biomass production and lodging resistance in 'Leaf Star', a new long culm rice forage cultivar. *Plant Production Science* 13: 58-66.

Ortiz, R. 1998. Critical role of plant biotechnology for the genetic improvement of food crops: perspectives for the next millennium. *Electronic Journal of Biotechnology* (Doi: 10.2225/vol1-issue3-fulltext-7).

Pan, X.B., Rush, M.C., Sha, X.Y., Xie, Q.J., Linscombe, S.D., Stetina, S.R. and Oard, J.H. 1999. Major gene, nonallelic sheath blight resistance from the rice cultivars Jasmine 85 and Teqing. *Crop Science* 39: 338-346.

Peng, S., Khush, G.S., Virk, P., Tang, Q. and Zou, Y. 2008. Progress in ideotype breeding to increase rice yield potential. *Field Crops Research* 108: 32-38.

Pinson, S.R.M., Capdevielle, F.M. and Oard, J.H. 2005. Confirming QTL and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. *Crop Science* 45: 503-510.

Powell, W., Machray, G.C. and Proven, J. 1996. Polymorphism revealed by simple sequence repeat. *Trends in Plant Science* 1: 215-222.

Qian, Y., Chen, C., Zhang, Q., Li, Y., Chen, Z. and Li, M. 2010. Concentrations of cadmium, lead, mercury and arsenic in Chinese market milled rice and associated population health risk. *Food Control* 21: 1757-1763.

Sabouri, H., Sabouri, A., Jafarzadeh, M.R. and Mollashahi, M. 2011. Detection of QTLs controlling field blast resistance in rice (*Oryza sativa* L.). *Plant Omics Journal* 4: 1-5.

Sato, H., Ideta, O., Ando, I., Kunihiro, Y., Hirabayashi, H., Iwano, M., Miyasaka, A., Nemoto, H. and Imbe, T. 2004. Mapping QTL for sheath blight resistance in the rice line WSS2. *Breeding Science* 54: 265-271.

Sarkarung, S., 1991. A simplified crossing method for rice breeding: a manual. *Centro Internacional de Agricultura Tropical, Cali, Colombia* pp. 32.

San-oh, Y., Mano, Y., Ookawa, T. and Hirasawa, T. 2004. Comparison of dry matter production and associated characteristics between direct-sown and transplanted rice plants in a submerged paddy field and relationships to planting patterns. *Field Crops Research* 87: 43-58.

Savary, S., Castilla, N.P., Elazegui, F.A., McLaren, C.G., Ynalvez, M.A. and Teng, P.S. 1995. Direct and indirect effects of nitrogen supply and disease source structure on rice sheath blight spread. *Phytopathology* 85: 959-965.

Sharma, A., McClung, A.M., Pinson, S.R.M., Kepiro, J.S., Shank, A.R., Tabien, R.E. and Fjellstrom, R. 2009. Geneting mapping of sheath blight resistance QTLS within tropical *Japonica* rice Cultivars. *Crop Science* 49: 256-264.

Singh, A., Singh, V.K., Singh, S.P., Pandian, R.T.P., Ellur, R.K., Singh, D., Bhowmick, P.K., Gopala Krishnan, S., Nagarajan, M., Vinod, K.K., Singh, U.D., Prabhu, K.V., Sharma, T.R., Mohapatra, T. and Singh, A.K. 2012. Molecular breeding for the development of multiple disease resistance in Basmati rice. *AoB Plant* pls029; doi:10.1093/aobpla/pls029.

SPSS Inc., 2007. SPSS 16.0 Brief Guide SPSS Inc. 233 South Wacker Drive, Chicago.

Srinivasachary, Willocquet, L. and Savary, S. 2010. Resistance to sheath blight (*Rhizoctonia solani* Kuhn) [(teleomorph: Thanatephorus cucumeris (A.B. Frank) Donk.] disease: current status and perspectives. *Euphytica* 178: 1-22.

Stange, M., Friedrich Utz, H., Schrage, T.A., Melchinger, A.E. and Würschum, T. 2013. High-density genotyping: an overkill for QTL mapping? Lessons learned from a case study in maize and simulations. *Theoretical and Applied Genetics* 126: 2563-2574.

Sumner, D. R. 1996. Sclerotia formation by *Rhizoctonia* species and their survival. In Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst G. (Eds.), *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control. Pages 207-217. The Netherlands: Kluwer Academic Publishers.

Taheri, P. and Tarighi, S. 2011. Cytomolecular aspects of rice sheath blight caused by *Rhizoctonia solani*. *European Journal of Plant Pathology* 129: 511-528.

Taguchi-Shiobara, F., Ozaki, H., Sato, H., Maeda, H., Kojima, Y., Ebitani, T. and Yano, M. 2013. Mapping and validation of QTLs for rice sheath blight resistance. *Breeding Science* 63:301-308.

Takai, T., Adachi, S., Taguchi-Shiobara, F., Sanoh-Arai,Y., Iwasawa, N., Yoshinaga, S., Hirose, S., Taniguchi, Y., Yamanouchi, U., Wu, J., Matsumoto, T., Sugimoto, K., Kondo, K., Ikka, T., Ando, T., Kono, I., Ito, S., Shomura, A., Ookawa, T., Hirasawa, T., Yano, M., Kondo, M. and Yamamoto, T. 2013. A natural variant of NAL1, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Scientific Reports* 3: 2149.

Tang, Q., Peng, S., Buresh, R.J., Zou, Y., Castilla, N.P., Mew, T.W. and Zhong, X. 2007. Rice varietal difference in sheath blight development and its association with yield loss at different levels of N fertilization. *Field Crops Research* 102: 219-227.

Timsina, J. and Connor, D.J. 2001. Productivity and management of rice-wheat cropping systems: issues and challenges. *Field Crops Research* 69: 93-132.

Tiwari, R.K.S. and Chaure, N.K. 1997. Studies on factors influencing appearance and severity of sheath blight *Rhizoctonia solani* f.sp. Sasakii of rice. Advances in Plant Sciences 10: 223-226.

Tsukaguchi, T., Horie, T. and Ohnishi, M. 1996. Filling percentage of rice spikelets as affected by availability of nonstructural carbohydrates at the initial phase of grain filling. *Japanese Journal of Crop Science* 65: 445-452 *.

Torres-Escribano, S., Leal, M., Vélez, D. and Montoro, R. 2008. Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. *Environmental Science & Technology* 42: 3867-3872.

Vanniarajan C., Vinod, K. K. and Pereira, A. 2012. Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa* L.). *Journal of Genetics* 91: 9-19

Venu RC, Ma J, Jia Y, Liu G, Jia MH, et al. 2014. Identification of candidate genes associated with positive and negative heterosis in rice. *PLoS ONE* 9: e95178. doi:10.1371/journal.pone.0095178.

Vidhyasekaran, P., Ponmalar, T.R., Samiyappan, R., Velazhahan, R., Vimala, R., Ramanathan, A., Paranitharan, V. and Muthukrishnan, S. 1997. Host specific toxin production by *Rhizoctonia solani*, the rice sheath blight pathogen. P*hytopathology* 87: 1258-1263.

Virmani, S.S., Aquino, R.C. and Khush, G.S. 1982. Heterosis breeding in rice, *Oryza sativa* L. *Theoretical and Applied Genetics* 63: 373-380.

Wasano, K., Hirota, Y. and Kido, Y. 1985. Varietal differences in the resistance to sheath blight of rice, *Rizoctonia solani* Kuhn, and effectiveness of selection for the resistance from the cross, Tetep x CN_4-4-2. *Report of the Kyushu Branch of the Crop Science Society of Japan* 52: 16-22*.

Wasano, K., Oro, S. and Kido, Y. 1983. The syringe inoculation method for selecting rice plants resistant to sheath blight, *Rizoctonia solani* Kuhn. *Japanese Journal of Tropical Agriculture* 27: 131-139.

Wasano, K. and Hirota, Y. 1986. Varietal resistance of rice to sheath blight disease caused by *Rhizoctonia Solani* Kuhn; by the syringe inoculation method. *Bulletins of the Faculty of Agriculture of Saga University* 60: 49-59.

Wang, L., Wang, A., and Huang, X. 2011. Mapping 49 quantitative trait loci at high resolution through sequencing-based genotyping of rice recombinant inbred lines. *Theoretical and Applied Genetics* 122: 327-340.

Wang, S., Basten, C.J. and Zeng, Z.B. 2006. Windows QTL Cartographer2.5. Department of statistics, North Carolina State University, Raleigh, NC, USA. Available from: http://www.statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Webster, R.K. and Gunnell, P.S. 1992. Compendium of rice diseases. APS**,** St. Paul.

Wu, W., Huang, J., Cui, K., Nie, L., Wang, Q., Yang, F., Shah, F., Yao, F., and Peng, S. 2012. Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. *Field Crops Research* 128: 101-108.

Xu, Q., Yuan, X., Yu, H., Wang, Y., Tang, S. and Wei, X. 2011. Mapping quantitative trait loci for sheath blight resistance in rice using double haploid population. *Plant Breeding* 130: 404-406.

Yamamoto, K. 2004. Lia 32 ver. O.377e software. Nagoya University.

Yoshida, S. 1981. Fundamentals of rice crop science. IRRI, Los Banos, Philippines.

Yoshinaga, S., Takai, T., Arai-sanoh, Y. and Kondo, M. 2011. Yield potential and growth characteristics of the recent high-yielding rice varieties in Japan. Crop Science Symposium in East Asia, Fukuoka, Japan pp. 21-25.

Zhang, G.H., Li, S.Y., Wang, L., Ye, W.J., Zeng, D.L, Rao, Y.C., Peng, Y.L., Hu, J., Yang, Y.L. Xu, J. Ren D.Y., Gao, Z.Y., Zhu, L., Dong, G.J., Hu, X.M., Yan, M.X., Guo, L.B., Li, C.Y. and Qian, Q. 2014. Lschl4 from *Japonic*a cultivar, which is allelic to NAL1, increases yield of *Indica* super rice 93-11. *Molecular Plant* 7: 1350-1364.

Zhang, Y. J., Zhou, Y. R., Du, B. and Yang, J. C. 2008. Effects of nitrogen nutrition on grain yield of upland and paddy rice under different cultivation methods. *Acta Agronomica Sinica* 34:1005-1013.

Zeng, Y., Ji, Z., Ma, L., Li, X. and Yang, C. 2011. Advances in mapping loci conferring resistance to sheath blight and mining *Rhizoctonia solani* Resistance Resources. *Rice Science* 18: 56-66.

Zou, J.H., Pan, X.B., Chen, Z.X., Xu, J.Y., Lu, J.F., Zhai, W.X. and Zhu, L.H. 2000. Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). *Theoretical and Applied Genetics* 10: 569-573.

* In Japanese.

Acknowledgements

The work presented here and the dissertation have been possible with the sincerely help, guidance and support from number of people. It is a pleasure to convey my gratitude to all of them in my humble acknowledgement.

I would like to express my deepest gratitude to the Prof. Emirates Akihiro Nose for his guidance during my studies at Saga University. His profound insight and optimistic attitude have always provided a strong support for my research and preparation of thesis. I am also deeply indebted to him for allowing me to carry out the study here. I extremely grateful to my advisor Prof. Shao-Hui Zheng for his invaluable suggestion and consistent encouragement. I am also grateful to Prof. Toyoaki Anai, Prof. Yosinobu Kawamitsu, Prof. Jun-Ichi Sakagami and Prof. Akihiro Suzuki.

I appreciate the invaluable contributions of Mr. Kenzo Oshima, Mr. Takashi Arita and Dr. Yosuke Okimoto for his support and trouble-shooting during the study and in the course of my stay in Japan. I also appreciate invaluable support, suggestion and guidance for study from Dr. Yoshiyuki Yamagata and Prof. Atsusi Yoshimura of Kyushu University.

I would like to extend my profound gratitude to Mrs. Yosiko Yamamoto, principal of Codo International College, Tosu, Japan for support and timely advice for the study in Japan. I am also highly grateful to the staff and management of Hotel Grande Hagakure Saga, Japan for giving me to do part time work during my difficult time. I also wish to thank the Doctorate, Graduate and Undergraduate students of Tropical Crop Science Laboratory for their support and encouragement. Similarly, I am thankful to all Nepalese of Saga University for the helps, encouragement and cooperation in daily life.

I am deeply indebted to my wonderful family. I appreciate their support and encouragement, their love and respect in every single moment of my life. I would like to offer the deepest gratitude to my parents Shiva Lal Gaihre and Tika Devi Gaihre, my wife Bishnu Basyal, my daughter Yubisha Gaihre and my son Yatish Gaihre whose love, understanding, patience and encouragement have supported me to walk through every hurdles and obstacles.

Last, but not the least, I acknowledge my previous educational institutions, teachers that helped me make my way up to this mark and my friends both in Japan and Nepal.