GENETIC LOCI FOR RESISTANCE TO BLAST (*Pyricularia oryzae*) POPULATIONS IN IRRIGATED RICE IN KENYA

BY

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DECLARATION

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DEDICATION

This thesis is dedicated to my late father Danstern Ouma Bideru and daughter Samantha Cynthia Akinyi.

ABSTRACT

Rice (*Oryza sativa*) is the third most important food crop after maize and wheat in Kenya. Blast (*Pyricularia oryzae*) is one of the most destructive and widespread diseases of rice in Kenya but minimal research attention have been given to this disease. Differential lines and recombinant inbred lines (RILs) were grown under irrigation in Mwea, West Kano and Gamba to characterize virulence spectrum of blast populations; identify resistance genes and quantitative trait loci (QTL) that confer adaptability to the local agricultural conditions. RILs were genotyped using single nucleotide polymorphisms (SNP) markers. Virulence spectrum of blast populations in irrigated ecosystems in Kenya varied significantly within and across the locations. West Kano had 68.63% of rice differential lines susceptible to blast population, followed by 49.02% in Mwea and 40.02% in Gamba. Some rice differential lines carrying the same genes from different donors showed different reaction to blast population, suggesting that background effects of the donor varieties had a role in the susceptibility as well as resistance of the lines. Environment had a significant effect on the expression of genes. Further, some rice differential lines in different genetic background showed varied response to blast population at each location and across the locations. Lines with *Pik-s*, *Pik-p, Pik-h*, *Piz-5*, *Piz*, *Pit*, *Pish Pi1*, *Pi5 (t)*, *Pi12 (t)*, *Pik-m*, *Pita-2*, *Pib* and *Pik* were resistant to blast population. IRBLzt-IR56 [CO] carrying *Piz-t* gene showed resistance to blast across the locations. Reaction of RILs to blast differed significantly within and across the locations. Linkage map was constructed with 2416 polymorphic SNP markers and QTL declared at logarithm of odds (LOD) score of 3.0. Genetic map had a total distance of 1526.8 cM with an average distance of 0.63 cM between adjacent markers, and covered 95.48% (364.73 cM) of the rice genome. Eighteen QTL on on chromosomes 1, 2, 3, 4, 5, 6, 8, 9 and 11 with variable effects co-segregated with blast resistance. Most QTL mapped to the genomic regions where blast resistance genes have been observed, suggesting that QTL in this study may be identical or allelic to the specific resistance genes described previously. Eight QTL namely; *qrbr-1-4*, *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3*, *qrbr-4-4*, *qrbr-5*, *qrbr-8-2* and *qrbr-9-2* mapped to QTL for agronomic traits and bacterial blight and sheath blight. Such corresponding locations suggest that some of the genes underlying QTL are commonly involved in the defense response against pathogens and may be linked to other traits of agricultural value. Most promising R genes and QTL observed in this study would be introgressed into preferred yet susceptible rice varieties in Kenya. Characterization of R gene(s) in BW196 and QTL that confer resistance to multiple diseases and agronomic traits is required for inclusion in breeding programs.

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ABBREVIATIONS AND ACRONYMS

AFLP- Amplified Fragment Length Polymorphism
BECA- Bio-science East and Central Africa
CAPS- Cleaved Amplified Polymorphic Sequences
cM- centiMorgan
COMESA- Common markets for Eastern and Southern Africa
CTAB- Cetyl trimethylammonium bromide
EAC- East African Community
ESA- East and Southern Africa
EDTA- Ethylenediaminetetraacetic acid
FAO- Food Agricultural Organization
GAIN- Global Agricultural Information Network
GOK- Government of Kenya
IEA- Institute of Economic Affairs
ILRI- International Livestock Research Institute
IRGSP- International Rice Genome Sequencing Project
IRRI-ESA- International Rice Research Institute- East and Southern Africa
IRRI- International Rice research Institute
JIRCARS- Japan International Research Center for Agricultural Sciences
JKUAT- Jomo Kenyatta University of Agriculture and Technology
KALRO- Kenya Agricultural Livestock Research Organization
KEPHIS- Kenya Plant Health Inspectorate Services
LTH- Lijiangxituanheigu
MAS- Marker-assisted Selection
MIAD- Mwea Irrigation Agricultural Development
MSU7- Michigan State University version 7
MLs- Monogenic lines
MOA- Ministry of Agriculture
NBS-LRR- Nucleotide binding site-Leucine Rich Repeats
NIB- National Irrigation Board
NILs-Near isogenic lines
PAMPs- Pathogen associated molecular patterns
PhilRice- Philippine Rice
QTL- Quantitative Trait Loci
RILs- Recombinant inbred lines
RFLP- Restriction Fragment Length Polymorphism
SFP- Single Feature Polymorphism
SNP- Single Nucleotide Polymorphism
SSR- Simple Sequence Repeats
TARDA- Tana and Athi River Development Authority
TBE- Tris borate EDTA
TE- Tris EDTA
TDIP- Tana Delta Irrigation Project
USDA- United States Department of Agriculture

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Rice (*Oryza sativa* L.) cultivation was introduced in Kenya in 1907 from Asia (MOA, 2008). The crop is the third most important staple food in Kenya after maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Short *et al*., 2012). It is mainly grown by small-scale farmers as a commercial and food crop. Rice provides 20% of the world's dietary energy supply compared to maize and wheat that supply 5% and 19%, respectively (FAO, 2011). The national rice consumption estimated at 540, 000 tonnes has been rising steadily at an average rate of 12% compared to wheat 4% and maize 1% per year (Onyango, 2014; GAIN Report, 2015). The upward trend has been attributed to population growth and altered feeding habits.

The local rice production stands 126, 400 tonnes and this can barely cope with increased demand culminating in a high import dependency ratio of over 84% (GAIN Report, 2015). In 2014, Kenya imported 420, 000 tonnes of milled rice to offset the deficit (GAIN Report, 2015). The East African Community (EAC) and Common Market for Eastern and Southern Africa (COMESA) allowed Kenya to reduce the *ad valorem* tariff on rice from 75% to 35% (GAIN Report, 2015). This concession is renewed on yearly basis and applies only to countries in the EAC and COMESA. This placed a strain on Kenya's national budget that allocated USD 383 million for agriculture in the year 2014/2015 fiscal year (IEA Report, 2014). Import of rice into Kenya was estimated at over USD 168 million in the year 2014 (GAIN Report, 2015).

In Kenya, the increased demand for rice indicates that importation of rice is no longer a viable sustainable strategy. To cab this increased rice demand, the Government of Kenya (GOK) through 2008-2018 national rice development strategies, embarked on a plan to exploit its largely unexplored rice production potential to boost domestic production to 178,580 tonnes by 2018 (Irea, 2010). Therefore, rice production is expected to benefit from the increased investment by GOK and partners in new irrigation projects and rehabilitation of the existing schemes. However, to achieve this goal, the country would require rice varieties with high yield potential and yield stability. Yield reduction is caused by both biotic and abiotic stresses (Leung, 2008; Khush & Jena, 2009). Among the biotic stresses, rice blast is one of the most destructive disease causing significant yield losses in farmers' field worldwide (Fukuoka *et al*., 2015).

Rice blast is caused by the fungus *Pyricularia oryzae* Cavara [anamorph] *Magnaporthe oryzae* B.Couch [teleomorh] (Couch & Kohn, 2002). The disease is common in rice-growing environments worldwide (Africa Rice Center, 2011). Blast disease presents a serious threat to food security as rice is the staple food for more than 60% of the world population (Skamnioti & Gurr, 2009; Kumar *et al*., 2010). Emergence of new races of the blast pathogen have resulted in frequent breakdown of resistance causing up to 100% of crop losses in China, Japan, India and Philippines (Ghazanfar *et al*., 2009; Khush & Jena, 2009; FAO, 2010). Rice blast epidemics lead to absolute yield losses in various countries in West Africa (Yanagihara *et al*., 2010; Séré *et al*., 2011). The disease continues to cause significant yield losses in high yielding but susceptible rice varieties in Kenya. For

example, about 5, 600 hectares under Basmati 370 in Mwea irrigation schemes that produces the bulk of Kenya's rice were infected with blast in 2008 (Kihoro *et al*., 2013).

Virulence spectrum of blast populations has been observed in Latin America, Asia and West Africa (Fukuta *et al*., 2010; Koide *et al*., 2011; Séré *et al*., 2011). However, such studies have remained scarce in East and Central African countries including Kenya (Yanagihara *et al*., 2010; Kihoro *et al*., 2013). Although some studies have been carried out on Kenyan blast populations under natural conditions using local varieties (Kouko, 1997), virulence spectrum of blast populations in Kenya is not well documented. This is because the resistance sources in the local varieties were not known making it difficult to characterize virulence spectrum of blast populations in the farmer's fields. Further, most studies on Kenyan rice blast as reported by Kouko (1997) were carried out in early 1980's. It is likely that the blast pathogens have evolved during the last 35 years as the old cultivars have been replaced with new ones. This is because the host genotype grown in a given area has been reported to influence the composition of the blast pathogens (Chen *et al*., 1996; Park *et al*., 2003; Akator *et al*., 2014). Furthermore, major rice growing areas in Kenya like Mwea and the coastal region were not adequately covered in earlier studies. Therefore, the data collected then may not reflect the current blast populations in Kenya.

In the field, virulence spectrum of blast populations can be identified by using rice differential lines that carries a single resistance gene (Odjo *et al*., 2011; Idowu *et al*., 2013; Akator *et al*., 2014). This is premised on assumption that each *Pyricularia* gene

(*Pi*) confers resistance to a race of *P. oryzae* that carries the corresponding avirulence (*AVR*) gene (Yanagihara *et al*., 2010; Séré *et al*., 2011). The International Rice Research Institute (IRRI), Africa Rice Center and Japan International Research Center for Agricultural Sciences (JIRCAS) have proposed use of rice differential systems to characterize virulence spectrum of blast populations and accelerate use of *Pi* genes in rice breeding programs (Leung *et al*., 2004; Yanagihara *et al*., 2010).

Genetic studies of resistance to rice blast began with the establishment of differential system in Japan in the early 1960s (Ou, 1985). After the first molecular linkage map was published in 1988 (McCouch *et al*., 1988), efforts to map resistance genes increased, especially once a draft rice genome was sequenced in 2002 (Goff *et al*., 2002; Yu *et al*., 2002). To date, over 100 genes for blast resistance have been identified in rice germplasm, of which 60 are genetically mapped (Wang *et al*., 2010; Jia *et al*., 2012; Sharma *et al*., 2012). A number of *Pi* genes that confer resistance to rice blast under natural conditions have been identified in West Africa (Odjo *et al*., 2011; Séré *et al*., 2011; Akator *et al*., 2014). There are no rice varieties carrying genes that confer resistance to rice blast in Kenya (MOA, 2008; Kihoro *et al.*, 2013). Consequently, Kenya Agricultural Livestock Research Organization (KALRO) planned to screen rice varieties with a view of breeding blast resistant varieties by 2018 (MOA, 2008; Irea, 2010). This research aimed at identifying genes for resistance against blast populations in irrigated ecosystems in Kenya.

Although breeding of resistant cultivars using single blast resistance (*Pi*) genes is a major strategy for managing the disease, these genes are vulnerable to counter-evolution of pathogens (Kou & Wang, 2012; Fukuoka *et al*., 2015). This is because the genome of the blast fungus is rich in repetitive sequences and retro-transposons (Dean *et al*., 2005; Thon *et al*., 2006), which allow the fungus to rapidly evolve to form an endless array of new pathotypes from a single asexual spore that becomes highly adapted to infect the rice cultivars. New sources of resistance are therefore needed to continue a cycle referred to as an evolutionary 'arms race' between the host and blast pathogens (Jones & Dangl, 2006; Fukuoka *et al*., 2014). Rice cultivars carrying quantitative trait loci (QTL) that have small individual effects than *Pi* genes have maintained resistance for a long time, presumably because of decreased selection pressure against the pathogen (Kou & Wang, 2010; Fukuoka *et al*., 2014; Fukuoka *et al*., 2015). Attempts to map and identify QTL conferring resistance to rice blast in Kenya have not been made. Therefore, identification of QTL in resistant rice cultivars is crucial to our understanding of the genetic control of QTL-mediated blast resistance (Huang *et al*., 2011; Jia & Liu, 2011a; Liu *et al*., 2011a).

In most experiments on rice blast resistance, populations were artificially inoculated with only a single pathogen race that allowed measurement of the interaction between a resistance gene and a single pathotype under controlled conditions (Chen *et al*., 2003; Shi *et al*., 2010; Immanuel *et al*., 2011). Under natural conditions, however, numerous and complex pathotypes of *P.oryzae* are simultaneously involved in the induction of the plant resistance response. The varieties evaluated for resistance to a single pathotype are exposed to a number of pathogenic variations once released into the natural environment

(Zeigler & Correa, 2000). These varieties stand little chances of surviving under the onslaught of such variation because most breeding programs are designed in a way that a blast resistant variety is not exposed to pathogen variants as it would encounter under natural conditions. Therefore, genes or QTL identified under natural conditions confer broad-spectrum and durable resistance that has practical application in rice breeding programs (Tabien *et al*., 2002; Li *et al*., 2008a).

Molecular markers such as simple sequence repeat (SSR) have been used for construction of linkage map, genetic diversity analysis, marker-assisted selection (MAS) and marker-trait association (Miah *et al*., 2013; Thakur *et al*., 2014). Despite the fact that SSRs have been useful in genetic studies, they have a limited number of motifs in the genome and this limits their ability to saturate a chromosomal region and identify gene-based markers (Thomson, 2014). Similarly, the high information content generated by SSRs from multiple alleles per locus presents difficulties in merging SSR data from different platforms and documenting/presenting allele sizes in the databases (Thomson, 2014). Resolution of QTL map generated using SSR markers is frequently low because of limited molecular markers for a particular population and QTL are often mapped to intervals covering large DNA fragments (Hu *et al*., 2008). Pyramiding such chromosomal regions through crosses between different genetic backgrounds may bring undesirable traits into an improved cultivar due to linkage drag. Therefore, there is a need to use genotyping platforms that would map precisely the location of the resistance QTL to accelerate their usage in molecular breeding.

The introduction of DNA sequence technologies has made it possible to obtain single nucleotide polymorphism (SNP) markers for population genotyping (Mardis, 2008; Huang *et al*., 2009; Varshney *et al*., 2009). This method produces a high density map that allows precise genetic mapping (Yu *et al*., 2011; Chen *et al*., 2014). The known physical positions of the sequencing-based SNP markers can be used to resolve the false double crossovers between adjacent markers that would be incorrectly incorporated in genetic maps by restriction fragment length polymorphisms (RFLPs) or SSRs causing inconsistency in the analysis (Chen *et al*., 2014; Thomson, 2014). SNPs have emerged as the genetic marker of choice for mapping quantitative traits because of their co-dominant inheritance, multi-allelic nature, chromosome-specific location and genome wide distribution (Thakur *et al*., 2014). Further, they are highly amenable to automation and have the ability to reveal hidden polymorphisms (Thomson, 2014). Some SNPs that are tightly linked to blast resistance genes have been observed in rice (Hayashi *et al*., 2004; Thakur *et al*., 2014). Functional markers linked to disease resistance present an opportunity for breeders to incorporate a targeted approach in the breeding plan to select and combine beneficial alleles that control traits of interest. This is likely to bridge the gap between the discovery of useful genes or QTL and their incorporation in breeding programs and accelerate the release of marker-assisted selection products that have a significant impact on commercially grown rice (Thomson, 2014).

1.2 Statement of the problem

About 80% of the rice grown in Kenya is under irrigation schemes established by the Government while the remaining 20% is produced under rain-fed conditions (MOA, 2008). Due to climate change and cultural methods such as monoculture, excessive

nitrogen application and inadequate spacing often practiced by local farmers provide a favourable environment for blast to thrive in irrigated ecosystems (Piotti *et al*., 2005; Jia *et al*., 2012). Rice blast is a common problem in Kenya (Figure 1.1) and yet minimal research attention has been given to this disease. The disease is a major constraint to sustainability and expansion of rice cultivation in areas where rice production has not kept up with increasing demand from growing population. Rice blast accounts for crop yield reductions of up to 40% in Kenya (Irea, 2010; Kihoro *et al*., 2013). The quantity of yield reduction in rice by blast annually is sufficient to feed over 16 million people in the country (Kihoro *et al*., 2013).

Figure 1.1 ITA 310, a local rice cultivar infected by *Pyricularia oryzae* in a farmer's field at Gamba (Source: Author, 2013)

There are no locally bred blast resistant varieties, and farmers rely on introduced varieties that lose their resistance within a few years of intensive agricultural cultivation because of genetic instability and pathogenic variability of *P. oryzae* as well as likely occurrence of blast races to which these cultivars do not bear resistance (Chen *et al.,* 2001; Akator *et al.,* 2014). In addition, most of the major rice blast resistance (R) genes so far identified confer resistance to only a small set of blast isolates in a limited geographical region (Ballini *et al*., 2008; Liu *et al*., 2010). The effectiveness and durability of host resistance is determined by the dynamics of pathogen variation (Wu *et al*., 2005a; Séré *et al*., 2011). Therefore, it is necessary to determine the diversity of blast pathogen populations in the farmers' field and thereby develop effective control strategies to prevent devastation of the crop.

A number of methods have been used by farmers to control rice blast in Kenya. Use of chemicals such as *Topsin*, *Goldazim*, *Rodazim* and *Bavastin* has been effective in minimizing the disease in Kenya (Kouko, 1997; Kihoro *et al*., 2013). However, chemical control is no longer a sustainable practice for the resource-poor rice farmers in Kenya. Moreover, the chemicals could have negative impacts on the environment as well as human health. Other methods of blast control include field abandonment, burning of diseased straws and split application of fertilizer. Many rice farmers abandoned attempts at controlling blast using the aforementioned methods because they are ineffective, labour-intensive and too expensive given the rate of infection of rice plants. Host plant resistance coupled with good agronomic practices remains the most promising option for

managing the blast problem. Therefore, developing resistant varieties against highly variable blast pathogens continues to be a priority for rice breeding programs in Kenya.

1.3 Justification of the study

Rice is one of the major cereal crops that constitute the staple diet all over the world. About half the population of the world consumes rice on daily basis (Khush & Jena, 2009). Population growth and altered feeding habits has been rapid in Africa, creating an increased demand for rice (Africa Rice Center, 2011). Rice production would therefore be required to increase by more than 30% to meet the staple food requirement by 2030 (Sharma *et al*., 2012). However, yields are very low currently making Kenya a net importer of rice on yearly basis (GAIN Report, 2015).

Rice blast is one of the most devastating diseases in Kenya and many other rice-growing areas, causing significant yield reduction in farmers' fields. Given the substantial losses caused by the pathogen, meeting the demand would require concerted efforts to improve blast resistance in rice. Further, the demand for rice would have to be met in the face of significant global climate change (Luck *et al*., 2011; Pautasso *et al*., 2012). The expected climate variability could increase the number of epidemics in a given locality, as well as the yearly fluctuations of their prevalence. The Great Lakes region is among the most vulnerable regions to climate change in Africa (www.africarice.org/warda/newsbrief.asp, 2012). Hence, a further increase in yield losses from rice blast can be expected. Incorporating rice blast resistance genes and QTL into improved varieties remains the most promising strategy to reduce the impact of the devastation of this disease. In fact, blast resistant genotypes have a better capacity for compensation in grain mass than do

blast susceptible genotypes, leading to improved grain yield and quality of the seeds (Bonman, 1992). Therefore, there is need to search for novel genetic resources that confer adaptability to the local agricultural conditions.

Characterization of blast populations in irrigated ecosystems in Kenya using known resistance genes in the differential rice lines would facilitate identification of the most effective resistance genes for incorporation into local rice varieties either individually or in gene pyramid form. There is good evidence that gene pyramiding confers increased resistance to rice blast disease (Fukuoka *et al*., 2015). The increased level of resistance to blast pathogen races has been found to be proportional to a number of genes accumulated in rice lines (Liu *et al*., 2004; Jiang *et al*., 2012a). Furthermore, mapping QTL conferring resistance would complement the major resistance genes identified in this study and permit use of directly linked DNA markers to enhance marker assisted introgression of blast resistance QTL into high yielding but blast sensitive rice varieties in Kenya. Therefore, MAS offers a powerful strategy to select genotypes with resistance genes with increased precision compared with conventional disease resistance breeding. This would reduce the yield lost to the pathogen, increase rice production in blast 'hot spot' areas and subsequently enhancing food security in Kenya. The outcome of this research would also enable rural communities in blast 'hot spot' areas to develop capacity and resilience to climate change by adopting a cheaper alternative for disease control.

1.4 Objectives of the study

The general objective of the study was to contrinute the understanding host genes conferring resistance to blast populations in irrigated ecosystems in Kenya. The specific objectives of this research were to:-

1. Characterize virulence spectrum of blast populations in irrigated ecosystems in Kenya using rice differential lines;
2. Identify resistance genes (*Pi* genes) conferring resistance to blast population in irrigated ecosystems in Kenya using rice differential lines;
3. Identify and map QTL associated with resistance to blast population in irrigated ecosystems in Kenya using SNP markers.

1.5 Research hypotheses

1. Virulence spectrum of blast population in irrigated ecosystems in Kenya varies within and across experimental locations.
2. There are resistance (*Pi*) genes that confer resistance to blast population at each site and across sites in irrigated ecosystems in Kenya.
3. There are QTL associated with resistance that could complement the *Pi* genes in improving resistance of blast sensitive rice cultivars in irrigated ecosystems in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of rice

2.1.1 Rice taxonomy

Rice (*Oryza sativa* L.) belongs to the genus *Oryza* and the tribe *Oryzeae* of the family poaceae. The genus *Oryza* has 25 species comprising 23 wild and 2 cultivated (*O. sativa* L and *O. glaberrima* Steud) with a pan-tropical and sub-tropical distribution (Brar & Khush, 2003; Appendix 1). *O. sativa* is widely grown in different parts of the world while *O. glaberrima* is restricted only in West African countries. African *O. glaberrima* has two ecotypes: the floating and the non-floating (Agnoun *et al*., 2012). These ecotypes have some negative features with respect to the Asian *O. sativa*: the seed scatters easily, the grain is brittle and difficult to mill and the yields are low, producing between 75 and 100 grains per plant (Linares, 2002). But the *O. glaberrima* types have luxurious wide leaves that shade out weeds and are more resistant to diseases and pests than the Asian *O. sativa* (Linares, 2002; Agnoun *et al*., 2012).

The *O. sativa* has two sub species; *indica* and *japonica* that originated from two independent domestication events involving different populations of wild rice *O.rufipogon* with gene flow occurring from *japonica* to *indica* between 8, 200 and 13,500 years ago (Molina *et al*., 2011; Huang *et al*., 2012; Wei e*t al*., 2012). They show significant diversity in single nucleotide polymorphisms, inter-genic sequences, and individual gene duplications, indicating the occurrence of dynamic genome evolution in

O. sativa (Wei *et al*., 2012). *O. glaberrima* also evolved independently from different population of *O. barthii* (Nayar, 2010; Li *et al*., 2011).

2.1.2 Geographic origin of rice

It is evident that *O. sativa* was first domesticated in the Pearl River basin near the Tropic of Cancer in Southern China (Huang *et al*., 2012; Wei *et al*., 2012). *O. glaberrima* is thought to have been domesticated from the wild ancestor *O. barthii* by people living in the floodplains at the bend of the Niger and Sahelian Rivers (Li *et al*., 2011).

2.1.3 Rice gene pool and species complexes

The basic chromosome number of the genus *Oryza* is 12 (Brar & Khush, 2003). *O. sativa*, *O. glaberrima* and 14 other wild species are diploids (2n = 24) while some wild species are tetraploids (2n = 4× = 48) (Brar & Khush, 2003). The species belonging to *Oryza* are classified into 10 distinct genome types: six diploid genomes (AA, BB, CC, EE, FF and GG) and four tetraploids (BBCC, CCDD, HHKK and HHJJ) (Brar & Khush, 2003, Wing *et al*., 2005). This grouping is based on chromosomal affinity during meiosis, fertility in interspecific hybrids and degree of sexual compatibility. The genus *Oryza* is classified into four complexes: Sativa, Officinalis, Ridley and Meyeriana (Brar & Khush, 2003). The sativa complex comprises the cultivated species; *O. sativa* (2n =24), *O. glaberrima* (2n = 24) and five wild species namely; *O. longistaminata*, *O.barthii*, *O. rufipogon*, *O. nivara* and *O. spontanea* that have the AA genome type (Kovach & McCouch, 2008). The species with the same genome type can be intercrossed, whereas those with different genome types are incompatible because of reproductive barriers. Therefore, the wild rice species; *O. rufipogon* and *O. barthii* that share similarity with

cultivated species can be used in hybridization-based breeding to develop new cultivars with favourable traits.

2.1.4 Ecology of rice

Rice ecosystems are characterized by elevation, rainfall pattern, depth of flooding and drainage, and the adaptation of the crop to agro-ecological conditions (Kouko, 1997). Due to its versatility in nature, rice is grown in diverse ecosystems including irrigated, rain-fed upland and lowland, mangrove and deep water ecosystems (Africa Rice Center, 2011). Cultivation of paddy rice requires a warm and wet climate with sufficient water. Traditionally, young seedlings are submerged under water to reduce pests and growth of weeds. Owing to climate change and cultivation requirements, diseases are inevitably widespread in rice paddies, and this often places major constraints on production (Africa Rice Center, 2011).

2.2 The blast fungus

2.2.1 Reproduction

The blast fungus is hemibiotrophic in nature and occurs in asexual and sexual forms. The asexual stage (anamorph) is *Pyricularia oryzae* while the sexual stage (teleomorph) is called *Magnaporthe oryzae* (TeBeest *et al*., 2007). *P. oryzae* produces piriform shaped conidia with 1-2 transversal septa, slightly hyaline and are linked to conidiophore by its large bottom (Scheuermann *et al*., 2012). Conidiophores are septated, simple, rarely branched, showing sympodial growth and slightly browned. Teleomorph stage produces hyaline, fusiform, three septate ascospores in unitunicate asci. The teleomorphic stage is heterothallic with a bipolar mating system controlled by two different alleles at a single

locus with additional genes controlling the sexual cycle (Talbot, 2003; TeBeest *et al*., 2007). *P. oryzae* are predominant in nature but presence of recombining populations forming *M. oryzae* has been observed in northern India (Zeigler, 1998; Kumar *et al*., 1999) and Yunnan Province in China (Saleh *et al*., 2014). The importance of the teleomorphic stage to disease epidemiology is not well understood. However, the presence of the sexual stage may contribute to greater pathogen virulence since sexual reproduction creates new isolates via recombination.

2.2.2 Genome plasticity of *P. oryzae*

Most plant pathogens evolve rapidly and have an unusual capacity to shift their virulence and overcome host resistance resulting in disease epidemics (Ziegler *et al*., 1995; Séré *et al.,* 2007). This depends on a number of factors including weather conditions, disease pressure and genome stability of the pathogen. The genome of *P. oryzae* contains transposons inserted into copies of themselves or other element (Thorn *et al*., 2004). These repetitive sequences enable the fungus to frequently change pathogenicity or escape from host recognition by inactivation or deletion of pathogen-associated molecular patterns (PAMPs) encoding genes whose products trigger the plant adaptive immune system (Kang *et al*., 2001; Farman *et al*., 2002). Some of *P. oryzae* genes are located in telomeric and sub-telomeric regions of the chromosome as in the case of *Avr-Pita* (Orbanch *et al*., 2000), *Avr-Piz* (Luo *et al*., 2002), *Avr-Pi15* (Ma *et al*., 2006), *Avr-Pit* and *Avr-Pia* (Chen *et al*., 2007). These telomeric and sub-telomeric regions contain about 24% of transposable elements (Rehmeyer *et al*., 2006), indicating that they have a role in niche adaptation by promoting deletion/disruption of genes whose non-uniform distribution within the pathogen population can be adaptive. Deletions in these telomeric

and sub-teolomeric regions result in the loss of avirulence genes leading to gain in virulence as in the case of *AVR-Pita* (Orbach *et al*., 2000).

2.2.3 Diversity of blast populations

P. oryzae exhibits a high degree of genetic variability, and novel pathogenic variants capable of infecting previously resistant host plants arise at a high frequency during rice cultivation (Bonman, 1992). Diversity of blast populations has been observed in a number of studies worldwide (Chen *et al*., 2001; Fuentes *et al*., 2003; Mithrasena *et al*., 2012). Diversity of *P. oryzae* populations has been inferred from field disease severities on sets of diverse rice cultivars carrying single resistance genes (Koide *et al*., 2011). A high diversity of rice blast populations that showed a different reaction pattern on near-isogenic rice lines was observed in West Africa (Odjo *et al*., 2011; Séré *et al*., 2011). Research studies to characterize virulence spectrum of blast population in rice ecosystems in Kenya are very scarce (Kouko, 1997; MOA, 2008). Recent studies in Kenya have focused on the control of rice blast using fungicides and biocontrol measures as well as surveys on the impact of rice blast on the livelihood of local farmers (Maina *et al*., 2012; Kihoro *et al*., 2013).

2.3 Rice blast

2.3.1 Distribution of rice blast

Rice blast disease caused by the fungus *P. oryzae* is the most devastating disease distributed worldwide where rice is grown (Africa Rice Center, 2011). The disease occurs with variable intensities depending on the environmental conditions and cropping system.

In Kenya, the disease is common in irrigated ecosystems as well as upland rain-fed ecosystems (Kouko, 1997).

2.3.2 Rice blast symptoms

Blast symptoms on leaves vary according to the environmental conditions, the age of the plant and resistance level of the host cultivars (Puri *et al*., 2006; Anwar *et al*., 2011; Scheuermann *et al*., 2012). Susceptible cultivars develop a greyish center with a brown margin while lesions in resistant cultivars remain small in size (1-2mm) and are dark brown in colour (Figure 2.1). Under favourable conditions, lesions on the leaves of susceptible lines expand rapidly and tend to coalesce, leading to complete necrosis of infected leaves (Nutsugah *et al*., 2008). Foliar lesions reduce significantly the leaf area available for photosynthesis causing the whole tiller to fall off (Séré *et al*., 2011). In collar rot, lesions are located at the junction of the leaf blade and leaf sheath and may lead to death of the entire leaf.

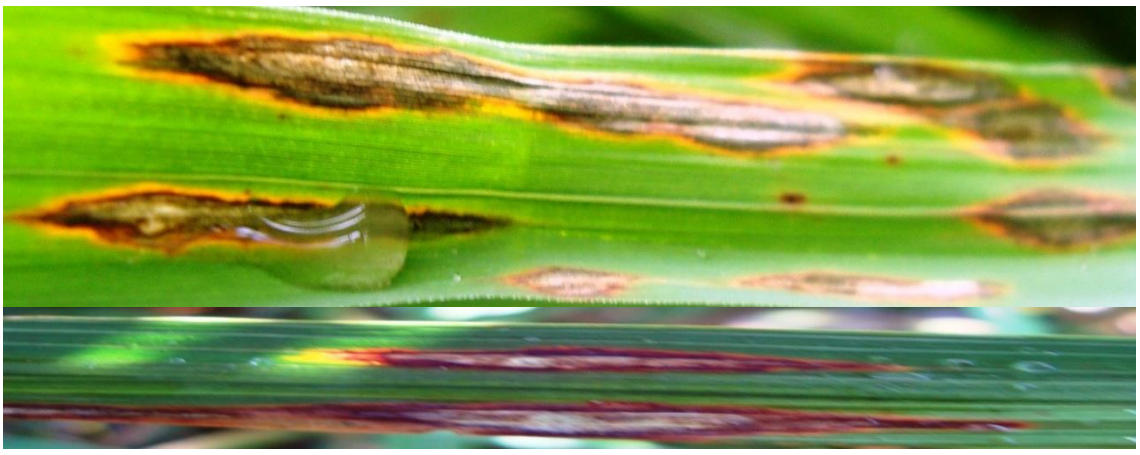

Figure 2.1 Typical lesions on breeding rice line caused by *P. oryzae* at Gamba (Source: Author, 2014)

Infection to the neck node is characterized by triangular purplish lesions and lesions have the ability to elongate to both sides of the neck node. Neck blast leads to early maturity of the panicles, causing indirect yield losses through grain shedding (Ou, 1985; TeBeest *et al*., 2007). Although neck and leaf blast are caused by the same pathogen, different genes determine the reaction to the two phases of the disease (Ou 1985; Puri *et al*., 2009). Node blast appears black-brown in colour and occurs in a banded pattern. This kind of infection breaks the culm resulting in the death of the entire rice plant. Panicle blast symptoms include the panicle appearing brown or black. Infected panicles are partly or completely unfilled.

2.3.3 Environmental conditions favourable for disease development

The occurrence of blast disease is highly unpredictable. The temperature ranging from 22°C to 27°C, nights without wind and clouded skies favour extended moisture on rice plants and high humidity of about 80-90% promote blast disease development (Castejón-Muñoz, 2008; Taguchi *et al*., 2014; Derren, 2010). Excessive nitrogen application promotes the disease. In addition, intermittent aerated soil root zone environments that are common in rice production systems due to scarcity of water also favour blast infection and development causing significant yield loss in rice production systems (Xiong & Yang, 2003; Derren, 2010; PhilRice-IRRI, 2010). In general, the severity of the blast disease is dependent on the ability of the pathogen to infect a compatible host and produce spores.

2.3.4 Epidemiology

In both tropical and temperate regions, the fungus overwinters in straw piles or kernel (TeBeest *et al*., 2007). The infected straw and seeds on the soil surfaces are able to produce between 20, 000 and 60, 000 spores of *P .oryzae* (TeBeest *et al*., 2007; Séré *et al*., 2011). Pathogen spores also survive through infection of grasses and volunteer plants (Kato, 2001; Nutsugah *et al*., 2008). Most conidia are released at night in the presence of dew and are dispersed by air, wind or rain to the neighbouring healthy plants.

Infection by the rice blast fungus begins with the attachment of conidium to the host leaf anchoring itself to the leaf cuticle with spore-tip mucilage (Appendix 2). Germination proceeds with the extension of a germ tube, which undergoes swelling at its tip and then differentiates into an infection structure called the appressorium (Talbot, 2003; Liu *et al*., 2011b). Differentiated appressorium becomes melanized except for a well-defined pore between the appressorium and the rice leaf (Talbot, 2003). The formation of this infection structure on the host surface marks the onset of the disease. A penetration peg is then driven through the host surface and the infection hypha invades and grows through the rice leaf (Liu *et al*., 2011b). At this stage, the symptoms become evident and small oval lesions begin to appear, accompanied by local chlorosis. Eventually, the growing lesions become necrotic and host cells die resulting in characteristic ‘blast’ disease symptoms (Kato, 2001; Kim *et al*., 2009).

2.4 Control of rice blast

2.4.1 Cultural control

Cultural strategy involves use of high quality and disease-free seeds for planting. This method is commonly used to reduce the blast damage because infected seeds left on the soil surface provide inoculum from which epidemics develop. Crop rotation as a cultural method has been found to reduce blast development in the fields by providing a mechanism that separates viable spores in crop residue from newly emerging seedlings (Peters *et al*., 2003; TeBeest *et al*., 2007). Split application of nitrogen fertilizers based on the actual requirements of the crop reduces the incidence and severity of rice blast (Talukder *et al*., 2005; Ballini *et al*., 2013). Other cultural methods including maintaining anaerobic conditions in paddy field and sowing early after the onset of the rains also reduce the severity of the blast (TeBeest *et al*., 2007; PhilRice-IRRI, 2010). Vijaya (2002) observed a highest blast incidence at 10 × 10 cm than at 20 × 15 cm plant spacing, indicating that reduced spacing increase plant density and consequently favours disease development.

2.4.2 Chemical control

Chemicals such as *benomyl, ediphenphos, tricyclazole, Coratop, kitazine* and *mancozeb* have been effective against leaf and neck blasts (Ganesh *et al*., 2012; Kihoro *et al*., 2013). Although use of chemicals has been effective in reducing the damage caused by the blast pathogen, they have toxic effects on non-target organisms and cause undesirable changes in the environment when used persistently. Moreover, most chemicals are too expensive for the resource poor farmers in developing countries and their use generates additional

costs in rice production. In Japan, sales of anti-blast fungicides have been estimated at €160 million annually (Yamaguchi, 2004).

2.4.3 Biocontrol

A number of microbial agents including *Enterobacter agglomerans* Ch2D, *Xanthomonas luminescens* Ch3D, *Enterobacter agglomerans* Ch4B, *E. agglomerans* Gg14D, *Serratia liquefaciens* Gh13D, *Bacillus firmus* E65, *Serratia marcescens* E31, *Pseudomonas aeruginosa* C32b, *Bacillus cereus* II.14, *Pseudomonas fluorescens* AUPF25, *Streptomyces sindeneusis*, actinomyetes and phyloplane fungi have been used to control rice blast disease (Maina *et al*., 2012; Suryadi *et al*., 2013; Suprapta *et al*., 2014). Although microbial agents have been useful in managing rice blast disease, this method is vulnerable to environmental variation in the field. Moreover, inconsistent temperature and moisture in the field could render the biocontrol agent populations to reach densities that are too small to have significant effects and may not recover as rapidly as the blast pathogen when conducive-conditions occur.

2.4.4 Genetic control

Host plant resistance has been proposed as an alternative and the most effective way of managing rice blast disease because it offers an environmentally safe option for controlling the blast pathogen. Rice blast disease resistance falls into two main categories: qualitative and quantitative resistance (Kou & Wang, 2012).

2.5 Sources of blast resistance genes

Rice has been subjected to selection both by nature and man, reducing diversity in the cultivated species (Leung *et al*., 2003). Domesticated rice genotypes which were

subjected to mass cultivation occupy major areas under rice crop compared to the land races, traditional farmer cultivars and wild species. Increased uniformity of the domesticated rice cultivars and lines narrowed down the genetic base that favoured plant pathogens for better survival (Mahender *et al*., 2012).

A number of rice cultivars and lines have served as source of blast resistance genes worldwide. The cultivar Tetep was found to contain two blast resistant genes; *Pitp (t)* and *Pi-kh (Pi54)* (Sharma *et al*., 2002; Barman *et al*., 2004; Sharma *et al*., 2005). In addition, *Pi38* and *Pi-42(t)* were identified in cultivars *Tadukan* and *DHR9* (Gowda *et al*., 2006; Kumar *et al*., 2010). Other rice cultivars including *Oryzica Llanos5*, *NP-125, Raminad Str.3*, *WC 299* and *WC 277* have been used as sources of resistance genes in Brazil (Scheuermann *et al*., 2012). Moroberekan is a source of blast resistance traits and drought tolerant factors and has been used extensively in the development of mapping populations (Wang *et al*., 1994; Chen *et al*., 1999; Jeon *et al*., 2003; Wu *et al*., 2004). Genetic variation in resistance to blast disease in the NERICA varieties has been observed in West Africa countries (Blaise & Moussa, 2004; Odjo *et al*., 2011). Since NERICA are being promoted in Kenya, there is a need to undertake preliminary screening to determine their resistance to blast pathogen populations. Blast resistance genes have been obtained in the wild rice including *Pi-40(t)* from *O. australiensis* and *Pirf2-1(t)* from *O. rufipogon* (Jeung *et al*., 2007; Dwinita *et al*., 2008).

Even though during the course of rice improvement many genes and their alleles from available rice landraces, cultivars, elite lines and related wild species have been explored,

still there is potential to tap the rice germplasm for the improvement of important traits in the crop. Of 100 blast resistance genes identified, 45% are from *japonica* cultivars, 51% from *indica* cultivars and the remaining 4% from wild species of rice (Sharma *et al*., 2012). Since only a few such genes have been identified from wild species of rice, there still remain unexplored genes among these species which can be a rich source of more useful resistance genes. In addition, functional genes from maize (*Zea mays*), sorghum (*Sorghum bicolor* Moench) and brachypodium (*Brachypodium distachyon*) were tested in transgenic rice and confirmed to confer resistance against rice blast (Yang *et al.*, 2013). Therefore, high genome collinearity that has been observed in different species of the poaceae family presents opportunity for mapping novel genes against blast by synteny (Chen *et al*., 2003).

2.6 Breeding for durable blast resistance

Using conventional and molecular breeding, many blast-resistant varieties have been developed. For example, blast resistance genes were successfully introgressed into *Luhui 17*, *G46B*, *Zhenshan 97B*, *Jin 23B*, *CO39*, *IR50*, *Pusa1602* and *Pusa1603* lines using marker assisted selection (MAS) (Jiang *et al*., 2012a; Scheuermann *et al*., 2012; Miah *et al*., 2013). Four QTL derived from upland rice cultivars that control partial resistance to blast have been introgressed into lowland rice cultivars using MAS (Yamamoto *et al*., 2009). These studies provide evidence that several blast resistance genes could be combined using MAS in a single genetic background to develop rice cultivars with broad-spectrum resistance to blast (Jena & Mackill, 2008). However, the successful application of MAS requires extremely tight linkages between markers and scorable

traits. Therefore, to accelerate the effectiveness of MAS, the map locations of target QTL need to be determined and flanking markers developed (Yamamoto *et al*., 2009).

Breeding for improved varieties with disease resistance attributes requires previous knowledge on the pathogen population in each region and / or site where future cultivars will be grown (Scheuermann *et al*., 2012). However, information regarding diversity of blast pathogen populations in Kenya are limited (Kouko, 1997; MOA, 2008). The documented studies were conducted more than three decades ago and do not reflect diversity of blast pathogen population. Consequently, information concerning the genes or QTL that could be deployed in breeding programs to improve blast resistance in the local rice varieties is not well docuemented in Kenya.

2.7 Disease resistance genes in rice

2.7.1 Qualitative resistance

Qualitative resistance is conferred by one or few disease resistance (R) genes whose protein products interact either directly or indirectly with corresponding pathogen effectors (avirulence gene products) (Hammond-Kosack & Parker, 2003; Poland *et al*., 2009; Sharma *et al*., 2012). This type of resistance is pathogen race-specific and interaction results in a reduced ability of the pathogen to grow or multiply. The mechanism of resistance is premised on a gene for gene theory, meaning that for every disease resistance gene in the host plant, there is avirulence gene on the pathogen (Flor, 1971). Some genes such as *Pi35 (t), Pi40(t)* and *Pi34* confer broad spectrum resistance to blast (Nguyen *et al*., 2006; Jeung *et al*., 2007; Zenbayashi-Sawata *et al*., 2007).

Rice blast disease resistance is also controlled by recessive R genes. The recessive gene, *pi21*, encodes for a protein with a heavy metal transfer/detoxify domain in the N-terminal and a proline-rich domain in the C-terminal (Fukuoka *et al*., 2009). This gene has lost between 18 and 42 base pair (bp) segments in the proline-rich region that might have led to the slow-blast development in the resistant cultivar. Gene, *pi55 (t)* is another recessive gene that confer resistance to *M. oryzae* (He *et al*., 2012). The *pi21* gene confers quantitative resistance while *pi55 (t)* conveys complete resistance against *M. oryzae*.

So far, over 100 major blast resistance (R) genes have been identified in rice (Sharma *et al*., 2012). Although R-genes like sequences are distributed throughout the rice genome, nearly half of these genes are localized on chromosome 6, 11 and 12 (Yang *et al*., 2009). Major R genes encode five different classes of proteins with additional subclasses defined on the basis of domain structures as well as their membrane topology (Luo, *et al*., 2012; Jacob *et al*., 2013). The nucleotide binding site and leucine rich repeats (NBS-LRR) form the largest class of R proteins that have a coiled coil (CC) or a TOLL/interleukin 1 receptor (TIR) domain at the N-terminus. NBS-LRR genes are clustered in the genomes and sequences of many clusters are highly homologous to one another (Michelmore & Meyers, 1998). NBS domains are involved in ATP binding and/ or hydrolysis and the LRR domain that contain highly conserved segments as well as variable segments participate in protein-protein or protein-ligand interactions (Matsushima & Miyashita, 2012).

Resistance genes have been cloned and used in crop breeding programs to increase resistance to specific pathogens (Jiang *et al*., 2012b). Although breeding and deployment of resistance cultivars using R genes have been effective in managing rice blast, these genes are rapidly overcome due to the rapid evolution of the pathogens and adaptation to cultivated varieties (Ballini *et al*., 2008; Kou & Wang, 2012). Therefore, pyramiding different R genes or use of quantitative trait loci (QTL) probably represents the best available means to achieve durable control (Jiang *et al*., 2012a; Sharma *et al*., 2012; Fukuoka *et al*., 2015).

2.7.2 Quantitative resistance

Quantitative resistance is governed by multiple genes or QTL, with each gene or QTL providing a partial increase in resistance (Poland *et al*., 2009; Kou & Wang, 2012; Fukuoka *et al*., 2012). This type of resistance tends to follow a normal distribution, does not conform to Mendelian inheritance and is greatly influenced by the environment (Hu *et al*., 2008; Lestari *et al*., 2011). It is a measurable trait that depends on the cumulative action of many genes and their interaction with the environment, which varies among individuals over a given range to produce a continuous distribution of phenotypes. The accumulation of such small effects may provide a long life span in crop production systems than the resistance conferred by a single *Pi* gene because each gene involved has a small effect to the disease resistance (Kou & Wang, 2010; Fukuoka *et al*., 2012; Fukuoka *et al*., 2014). There are two possible explanations for the extended durability of QTL. The first reason is that a pathogen would require the combination of a larger number of mutations in its genome to overcome quantitative resistance than to overcome qualitative resistance. The second possibility is that selection pressures exerted on the

pathogen by quantitative resistance would be low and distributed among several genes, reducing the risk of emergence of virulent variants from the pathogen population (Poland *et al*., 2009). In addition, quantitative resistance is race non-specific as resistance QTL for rice disease caused by different blast isolates or different species of plant pathogens have been mapped to the same or overlapping loci (Hu *et al*., 2008; Carrillo *et al*., 2009).

2.8 Quantitative trait loci

2.8.1 Definition of quantitative trait loci

Quantitative trait loci (QTL) is a statistically significant locus that may include one gene or a cluster of genes having quantitative effects on a phenotype or trait of interest (e.g., yield, drought tolerance and disease resistance) with physical boundaries defined by linked molecular markers (Kottearachchi, 2013). Molecular markers are used in mapping QTL because such loci depend on the chromosome recombination between linked markers in biparental populations (Yang, 1996). Once the linkage map is constructed, QTL can be identified by dissecting the association between markers and the variation of phenotype through statistical analyses (Wang *et al.,* 2014a). The statistical analysis yields two types of QTL: main-effect QTL (M-QTL) and epistatic QTL (E-QTL) based largely on the presence or absence of epistasis.

2.8.2 Principle of QTL mapping

Molecular markers give unambiguous, single site genetic differences that can easily be scored and mapped in most segregating populations. It is possible to identify and map between 10 and 50 segregating markers per chromosome in a population of most species (Semagn *et al*., 2010). Most markers are in non-coding regions and do not affect any trait

directly, but some are linked to a QTL that have an effect on the trait of interest (Prasanna, 2011). QTL analysis depends on the fact that where such linkage occurs, the marker locus and the QTL do not segregate independently and so differences in those marker, genotypes are associated with distinctive trait phenotypes (Kearsey, 1998; Semagn *et al*., 2010). Identification of genomic regions that carry QTL allows breeders to use marker-aided selection to precisely move beneficial QTL into elite lines for crop improvement in breeding programs.

2.8.3 Precision and QTL detection power

The size of a mapping population cannot be underscored in quality QTL mapping. A mapping population size of 200-300 individuals is sufficient to generate phenotypic data and determine trait- association by genotyping with linked markers spaced about 10 to 15 centimorgans (cM) apart (Bernardo, 2008; Semagn *et al*., 2010; Prasanna, 2011). In contrast, small population size often results in the detection of a few QTL with large phenotypic effects and the magnitude of QTL effects can also be biased (Utz *et al*., 2000; Schon *et al*., 2004). The probability of detecting a QTL at a given level of statistical significance is determined by the number of progeny in the population, heritability of the trait, genetic dissimilarity among progeny, effect of various QTL and the environment (Semagn *et al*., 2010). The accuracy and precision of phenotyping raise the heritability value that increases the statistical power of QTL detection. Cross-population and environment comparison of phenotyping is therefore needed in order to determine how the marker-trait association identified under one environment can be used for selection under another (Semagn *et al*., 2010).

2.8.4 Mapping populations

QTL mapping has been used as an approach for studying complex and polygenic forms of disease resistance. Mapping refers to placing the markers in order, indicating the relative distance between them, and assigning them to their linkage groups on the basis of recombination values from all pairwise combination between the markers (Prasanna, 2011; Wang *et al*., 2014a). Hence, QTL mapping begins with the collection of phenotypic and genotypic data from a mapping population, followed by statistical analysis to establish a significant association between markers and traits (Yang, 1996).

Different types of mapping populations such as F_2, backcross (BC), recombinant inbred lines (RILs) and doubled haploid (DH) lines derived from a cross between two inbred lines have been used in QTL analyses in rice (Chen *et al*., 2003; Wu *et al*., 2004; Li *et al*., 2007a; Carrillo *et al*., 2009; Ashikani *et al*., 2011). F_2 is useful for detecting QTL with additive effects, and also serves to estimate the degree of dominance for detected QTL. In contrast, when dominance is observed, backcrosses give biased estimates of the effects because additive and dominant effects are completely confounded in this design (Semagn *et al*., 2010). However, both F_2 and BC populations have a number of limitations. One limitation is the occurrence of relatively fews meiosis in these populations such that markers that are far from the QTL remain strongly associated with it (Semagn *et al*., 2010). Such long-distance associations are known to interfere with precise localization of the QTL. The second limitation is that they are temporary populations, highly heterozygous and cannot be evaluated several times in different environmental conditions. Hence, their performance is not repeatable. Also, epistatic interactions can

hardly be resolved in both F_2 and BC populations (Semagn *et al*., 2010; Wang *et al*., 2014a). Although DH lines are quicker to generate, have improved purity and genetic uniformity compared to RILs, F_2 and BC populations making it easy to carry out genetic studies, their use in mapping quantitative traits has been limited in rice by segregation distortion (Xu *et al*., 1997).

Recombinant inbred lines are advanced homozygous lines that have undergone several rounds of meiosis and have through multiple generations of mating increased in the potential number of recombination events, which helps improve map resolution (Semagn *et al*., 2010). The resulting lines are essentially homozygous, have little within-line genetic variation and only the genetic difference between lines is considered (Prasanna, 2011). The phenotypic value of the complex quantitative traits obtained from RILs can be repeatedly measured through a replicated experimental design. The same genotype can be tested in different environments, allowing the study of the QTL by environment interaction (Prasanna, 2011, Wang *et al*., 2014a). RILs have the potential to resolve epistatic interactions that cannot be determined by other mapping populations such as F_2 and backcross populations (Li *et al*., 2007a; Semagn *et al*., 2010). Further, random experimental errors can be controlled by using RILs, improving the QTL mapping in the process (Semagn *et al*., 2010; Wang *et al*., 2014a). Therefore, using the selected parental combination in the development of RILs and subsequent QTL analysis offers opportunities for a direct breeding program.

2.8.5 QTL mapping for rice blast resistance

Several studies have identified QTL conferring resistance to rice blast (Ballini et al., 2008; Carrillo *et al*., 2009; Shi et al., 2010). These results provide evidence that QTL confer broad-spectrum resistance to rice blast. Blast resistance genes and QTL have been mapped in the same position (Wang *et al*., 1994; Huang *et al*., 2011; Sharma *et al*., 2012). The relationship between R genes and QTL is not well understood. Regardless of the fact that many QTL for blast resistance have been observed, they have not been adequately used in rice improvement because of the complexity of expression of genes governing these QTL and limited knowledge of resistance mechanisms underlying these genes (Ballini *et al*., 2008; Fukuoka *et al*., 2012; Fukuoka *et al*., 2014).

Most QTL mapping studies have used rice plants inoculated with few blast isolates under controlled environments. The number and effects of QTL detected under natural infection of rice blast and artificial inoculations differ (Li *et al*., 2007a; Li *et al*., 2008a). Twenty nine QTL were observed using natural infection conditions compared to twelve under artificial conditions (Chen *et al.,* 2003). The variation was attributed to the fact that there are unknown and complex pathotypes of *P. oryzae* with their genetic instability in the natural conditions that are simultaneously involved in the induction activities of plant resistance QTL expression than that of artificial inoculation (Bilgic *et al*., 2006). However, the effects of QTL detected by natural infection were much smaller than under artificial inoculation (Chen *et al*., 2003) and this demonstrate lack of single elicitors of pathotypes in the natural environment. The expression of QTL for blast resistance are unstable and influenced by the developmental status of the plants, epistatic effects and the

environmental interactions (Wu *et al*., 2005a). QTL identified based on natural infection (i.e., with a mixture of races) are more useful than those obtained by artificial inoculation with few isolates because they are able to reveal interaction between genotype and environmental conditions (Li *et al*., 2008a). This underlines the importance of conducting experiments under natural conditions to understand the impact of environmental conditions on the expression of resistance gene (s).

2.8.6 Molecular markers for QTL mapping

Molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and array-based single feature polymorphisms (SFP) have been used to map genes or loci that confer resistance to rice blast disease (Wang *et al*., 1994; Shan *et al.*, 1999; Miah *et al*., 2013). Although these markers have been useful in mapping genes in a number of populations, many regions were sparsely represented and low density on whole genome was reported making it difficult to obtain precise information about the numbers and location of the QTL (Yu *et al*., 2011; Wang *et al*., 2011; Chen *et al*., 2014). The following section reviews single nucleotide polymorphism (SNP) markers and their application in rice breeding programs to improve blast disease resistance.

Single nucleotide polymorphism is defined as a single base change in the DNA sequences at which different nucleotides occur in different individuals of a population (McCouch *et al*., 2010; Tung *et al*., 2010). SNPs are defined by two nucleotides, say A and G, at a specific location in the genome. Since DNA is double-stranded, the complementarity of

DNA would indicate that all the four nucleotides are present at the base-pair location since A and G individuals also carry T and C, respectively on the complementary strand. However, in defining a SNP only one of the complementary strands is normally used. SNPs are found in coding, noncoding and inter-genic regions. SNPs in coding region may or may not alter the protein structure made by the coding region owing to degeneracy nature of the genetic code. Different combinations of SNPs that occur along a stretch of DNA are termed haplotypes. Hence, a haplotype is a set of closely linked genetic markers present on chromosome that tends to be inherited together (Thomson, 2014).

SNP genotyping offers opportunity for gene mapping, map-based cloning, and MAS in crops (Thomson, 2014). Many polymorphic SNPs are present in the genomic regions containing *Piz* and *Piz-t* that averaged one SNP every 248 base pair Hayashi *et al.*, 2004). Rice SNP50 array successfully tagged a fragment containing *Pigm(t)* that conditioned resistance to blast in elite parental line R608 of Gumei4 rice cultivar (Chen *et al*., 2014). Thakur et al. (2014) using GoldenGate assay identified 96 SNPs in a set of six major rice blast resistance genes: *Pita, Piz (t)*, *Pi54*, *Pi9*, *Pi5(t)* and *Pib*. Thermo genic lines that are used to generate hybrid rice in China were verified and found to contain *Pi2* gene in their genetic background using SNP markers (Jiang *et al*., 2015).

SNPs have been used to locate QTL for seed shattering traits and yield and its components in rice (Konishi *et al*., 2006; Yu *et al*., 2011; Wang *et al*., 2014b). In addition, fine mapping approach was used to clone the rice bacterial blight (*Xanthomonas oryzae*) resistance gene Xa_5, by isolating the recombination breakpoints to a pair of SNPs

followed by sequencing of the corresponding 5 kilo base (kb) region (Iyer & McCouch, 2004). The availability of SNPs in the databases identified in diverse rice germplasm presents an opportunity to identify sets of genome-wide polymorphisms for use in high throughput QTL and association mapping (McNally *et al*., 2009; McCouch *et al*., 2010; Chen *et al*., 2014).

Many different SNP genotyping platforms are available that address a variety of needs for different marker densities and cost per sample (Thomson, 2014). Early SNP genotyping platforms relied on gel-based methods such as cleaved amplified polymorphic sequence (CAPS) markers (Thiel *et al*., 2004; Komori & Nitta, 2005) or allele-specific amplification methods (Hayashi *et al*., 2004). The expansion of the field has led to the highly multiplexed fixed arrays providing over 1 million SNP loci to high-sample-throughput platforms that allow hundreds of samples to run per day (Thomson, 2014). Three major SNP array platforms built on separate assays principles have been used in rice. Affymetrix 44k gene-chip detects SNPs based on differential hybridization efficiency between DNA probes and template sequences and has been used in genome-wide association studies (McCouch *et al*., 2010; Tung *et al*., 2010; Zhao *et al*., 2011). Illumina GoldenGate SNP chip detects SNPs based on DNA extension and differential ligation and various SNP chips of this type have been developed and used in different genetic analysis and breeding projects (Thomson *et al*., 2012; Thakur *et al*., 2014).

The Illumina Infinium SNP (6k) array technology is based on differential single nucleotide extension that can be combined with high-density beadchips to create

platforms capable of genotyping millions of SNPs per slide (Steemers & Gunderson, 2007; Yu *et al*., 2014). The 6k Infinium technology consists of 5636 markers including 5556 SNPs for genetic diversity and 80 SNPs for specific gene functions in rice (Yu *et al*., 2014). These SNPs were developed by sequencing more than 500 rice land races. Development of the SNP array was based on genetic background selection and genotyping of target genes. Illumina Beadarray technology and Infinium SNP assay platforms were chosen for the SNP array fabrication because of its demonstrated high specificity, reproducibility and accuracy in SNP call (Oliphant *et al*., 2002; Steemers & Gunderson, 2007). Two sorts of DNA variations were considered in designing the array including SNPs with adequate coverage and representation of the genome diversity as judged on the basis of re-sequence diverse germplasm collections and allelic variations of characterized functional genes controlling important breeding traits (Huang *et al*., 2010; Jiang *et al*., 2012b). This technology has been used successfully in human disease diagnosis (HumanOmni, www.illumin.com), genetic and breeding studies in maize, wheat and barley (*Hordeum vulgare*) (Ganal *et al*., 2011; Cook *et al*., 2012; Miedaner & Korzun, 2012). This platform has advantages of high specificity, reproducibility and call rate and therefore provides options for breeding applications.

2.9 Map construction

A genetic map describes orders and positions of identifiable landmarks which might be genes. Two types of map namely, genetic and physical are commonly used in cytogenetics and molecular techniques. These maps provide comparable information on marker or gene order along the chromosomes. They are used for fine mapping and physical characterization of QTL. Estimating recombination frequency between two

positions generates a genetic map (Meng *et al*., 2015). In contrast, having the complete sequence makes it possible to determine directly the order and spacing of the genes, which is a type of physical map (Weeks & Lange, 1987). Molecular marker permits construction of high-density genetic maps for any species amenable to genetics and utilize them for detecting, mapping, and estimating the effects of QTL (Huang *et al*., 2010).

The analysis involves construction of linkage maps and identification of markers that are associated to QTL while minimizing the occurrence of false positives (Young, 1996). False positives lead to type I errors that declare an association between a marker and QTL when in fact one is not available. For each DNA marker, the individuals are split into classes according to marker genotype (Semagn *et al.,* 2010; Prasanna, 2011). Means of the phenotypic data is calculated and compared among the classes. A significant difference between the means suggests that there is a relationship between the DNA marker and the trait of interest (Wang *et al.,* 2014a). In other words, the DNA marker is associated with a QTL. Since the traits of interest are genetically complex, environmental factors and genetic background potentially have an influence on the results (Sharma *et al.,* 2012). This constitutes one of the most powerful applications of QTL mapping (i.e. analyzing gene x gene and gene x environment interactions).

2.10 Statistical methods for QTL analysis

Statistical methods frequently used to map QTL include single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM) and inclusive composite interval mapping (ICIM).

2.10.1 Single marker analysis

The single marker analysis (SMA) method divides the mapping population into classes based on the genotype at each marker locus, and a QTL is declared based on significant difference in the mean phenotypic score for each of the groups (Prasanna, 2011). *F*-tests provide evidence whether differences between marker locus genotype classes are significant or not. This method was used only for the first time in the identification of QTL for rice blast resistance in Moroberekan (Wang *et al*., 1994). Although computationally simple, this approach is least informative due to the likelihood of QTL detection significantly reduces as the distance between the marker and QTL increases, SMA cannot determine whether the markers are associated with one or more QTL and the effects of QTL are likely to be underestimated because they are confounded with recombination frequencies (Lander & Botstein, 1989).

2.10.2 Simple interval mapping

Simple interval mapping (SIM) uses the flanking molecular markers to associate them to QTL (Lander and Botstein, 1989). SIM is more precise in locating QTL compared to SMA, however, major problem with SIM is that linked and unlinked QTLs affect the result of the analysis and may give false QTL identification. This method was used in mapping QTL for rice blast resistance recessive gene *pi21* in Japanese upland rice cultivar Owarihatamochi (Fukuoka & Okuno, 2001).

2.10.3 Composite interval mapping

Composite interval mapping (CIM) developed by Jansen and Stam (1994) builds on SIM that places certain markers into the model as cofactors. Composite interval mapping uses

the subset of markers as linked as well as unlinked QTL (Sharma *et al*., 2012). SMA, SIM and CIM have been applied to identify QTL associated to rice blast resistance (Ballini *et al*., 2008). Composite interval mapping (CIM) represents one of the statistical methods that have been extensively used in mapping QTL. However, the algorithm used in CIM cannot ensure complete background control (Li *et al*., 2007b; Wang, 2009; Wang *et al*., 2014a).

2.10.4 Inclusive composite interval mapping

Inclusive composite interval mapping (ICIM) developed by Wang et al. (2014a) has proved to be more efficient than CIM for background control via a two-step mapping strategy (Wang *et al*., 2009: Zhang *et al*., 2012). In the first step of ICIM, stepwise regression identifies the most significant regression variables while in the second step; interval mapping is performed using phenotypes adjusted by the markers identified in the first step. ICIM therefore, retains all advantages of CIM over the simple interval mapping, and avoids the possible increase of sampling variance and the complicated background marker selection process in CIM (Li *et al*., 2010; Li *et al*., 2012). ICIM has multi-environment trial (MET) functionality tool that can be used to estimate QTL by environment interaction (Wang *et al*., 2014a; Meng *et al*., 2015). The ICIM method has been used in mapping QTL associated with yield and its component traits in rice (Wang *et al*., 2014b) and for leaf rust resistance in wheat (Zhou *et al*., 2014). Since ICIM has the ability to identify QTL with increased precision than CIM, it was used to map QTL in the present study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental locations

Field experiment was conducted in Mwea [0°39′ N, 37º17′E, 1195 m above sea level], West Kano [0°20′63″ S, 34°81′43″ E, 1138 m above sea level] and Gamba [2°16' S, 40°18′52″ E, 17 m above sea level] during the long rain seasons of 2013 and 2014 (Appendix 3). These sites were chosen because they form major rice growing areas under irrigation in Kenya and diversity of blast populations and genes conferring resistance to blast races has not been adequately studied. The micro-climate at the three sites also favours rice blast disease development year after year.

3.1.1 Mwea

The Mwea Irrigation Agricultural Development (MIAD) scheme, which is elevated at 1195 m above sea level, is located in the lower slopes of Mount Kenya, in Kirinyaga county of Kenya. It is bounded by 0º32′N and 0º46′N and 37º13′E and 37º30′E. An annual average precipitation for Mwea is about 950 mm, with the long rains occurring between March and May, while the short rains period is between October and December (Kihoro *et al*., 2013). The area covers three agro-climatic zones, with maximum moisture availability ratios ranging from 0.65 for zone III toward the highland slopes, to 0.50 for the vast area covered by zone IV, and to 0.4 for the semi-arid zone V (Sombroek *et al*., 1982). Moisture availability zones are based on the ratio of the measured average annual rainfall to the calculated average annual evaporation. The area is generally hot, with average temperatures ranging between 23°C and 25°C, having about 10°C difference

between the minimum temperatures in June/July and the maximum temperatures in October/March (Kihoro *et al*., 2013; Appendix 4).

Vertisols are predominant in rice-growing areas of Mwea (Sombroek *et al*., 1982). The soil is characterized by imperfectly drained clays that are very deep, dark gray to black, firm and prone to cracking when deficient in moisture. Rice is mainly grown in Mwea from August to December, when temperatures are optimum for grain filling and with less risk of disease incidence (Mukiama & Mwangi, 1989). However, this period coincides with reduced water level in River Tana that supplies water for irrigation exerting pressure on water availability for irrigation and to address this limitation, staggered planting is implemented in the region (Ijumba *et al*., 1990).

3.1.2 West Kano

West Kano irrigation scheme in Kisumu County is surrounded by Lake Victoria to the West, Nyando and Nyabongo escarpments and footsteps of Tinderet highlands. It occupies the major part of Kano plains (34°48' and 35°02' and 00°04' and 00°20' South) (D'Costa, 1973). This area lies to the eastern side of the shores of Winam Gulf of Lake Victoria. It occupies 841 hectares at an altitude of 1137 m above sea level. It receives a mean annual precipitation of 1100 mm, long rains occurring in March and May, while short rainy period occurs between October and December. This area has a potential evapo-transpiration loss of 2200 mm per annum with a mean diurnal temperature of 23ºC, and a relative humidity of 68-70% (Appendix 5). West Kano irrigation scheme has vertisol type soils belonging to group 1 of the USDA soil irrigation suitability

classification (D'Costa, 1973). They are fine textured, dark, blocky soils low in organic matter that shrink and crack as result of reduction in moisture content (Afullo, 2009).

3.1.3 Gamba

The Tana Delta irrigation scheme (40°10' E and latitude 2°16' S) that covers an area of approximately 400 hectares and elavted at between 10 and 20 m above sea level is located in the Tana River Delta, Tana River County, 110 km north of Malindi. The area stretches along the left bank of the Tana River from Sailoni Village, at the northern end to Gamba village at the southern with approximate area of 4000 hectares (TDIP Report, 2013). The delta area falls under ecological zone IV, within which the natural vegetation comprises Acacia-rich dry forms of woodland and bush land vegetation. The terrace land contains low forest, thicket or bushed grassland. There are two types of soil namely: fluvisols and vertisols that occur in the Tana Delta. The area experience high relative humidity (Appendix 6).

3.2 Plant materials

3.2.1 Rice differential lines

Thirty one monogenic lines developed by transferring various blast resistance genes into the *japonica* rice cultivar Lijiangxintuanheigu (LTH) and 20 near-isogenic lines (NILs) carrying resistance genes in the background of the *indica* cultivar CO39 (Appendix 7) were provided by IRRI, The Philippines. These lines were designated as IRBL, followed by the resistance gene and the abbreviation of the resistance donor. Rice differential lines, recurrent parents (LTH and CO39) and three local rice varieties Basmati 217, ITA 310

and BW196 were used in this experiment. The local varieties were chosen because they are commonly grown by the farmers in the irrigated ecosystems in Kenya.

3.2.2 Development of recombinant inbred lines

Recombinant inbred lines (RILs) were used to map QTL conditioning resistance to rice blast populations in irrigated ecosystems in Kenya. The RILs were developed by single seed descent in a greenhouse at the International Rice Research Institute (IRRI) in the Philippines. The breeding line, IR64-21, an *indica* elite rice line which is widely grown in the Philippines was used as a female parent while Moroberekan, a tropical *japonica* upland landrace from Guinea, West Africa was used as the source of resistance. The IR64-21 and Moroberekan were chosen because they are highly diverse; contain good agronomic traits and SNP data for the two varieties is also available (Appendix 8). IR64-21 was a progeny line derived from a single IR64 plant (Wu *et al*., 2005b). About 128 F_1 seedlings generated from the cross were validated to be hybrid using SSR markers (Kenneth McNally personal communication, IRRI). The F_1 were selfed to obtain F_2 seeds. Single plant selection was made from F_2 through $F_{6:7}$ and the seeds were bulked forming F_7-bulk lines. The 320 $F_{6:7}$ RILs, parents and two local varieties (IR2793-80-1 and Yala-1) were screened for leaf blast resistance under natural infection conditions in irrigated ecosystems in Kenya.

3.3 Field evaluation of rice differential lines and RILs

3.3.1 Nursery bed preparation

Rice seeds were dipped in a tub containing sodium chloride solution and contaminated seeds were discarded. Healthy seeds were soaked for 24 hours to accelerate germination,

drained and incubated on a wet gunny bag for 48 hours in the greenhouse to allow the seeds to sprout. The nursery bed for the test entries, susceptible and resistant checks was prepared following the procedure described by Wang et al. (1994). The pre-germinated seeds of each test line and local checks were sown in 50 cm long double rows maintaining 10 cm spacing. The seedbed was irrigated 2-3 days after sowing and water level was maintained at 2 cm above the soil surface for 21 days as seedling grew before transplanting. Urea was applied at the rate of 50 gm^{-2}, 10 days after sowing to ensure good growth of the seedlings. Before obtaining seedlings for transplanting the nursery was flooded a day earlier to reduce damage to roots. Seedlings were carefully uprooted, excess mud removed by washing in water, labelled seedlings and transported to the main experimental field for transplanting.

3.3.2 Land preparation

The field was flooded with water for one week to losen the soil and water drained. A rotorvator was connected to the tractor and used to plough the field. Then, the field was flooded for one week to kill the weeds. Field was levelled to allow uniform movement of water. The field was puddled to render the soil impervious. Water as drained out and field of the field layout marked to demarcate the experimental area.

3.3.3 Experimental design

The test entries were randomized independently in all three sites using R/DiGGeR software package (Coombes, 2008). Field planting for fifty six test entries consisting of 51 differential lines, 2 recurrent parents (LTH and CO39) and 3 local varieties (ITA 310, BW 196 and BAS 217) followed a randomized incomplete block design in order to allow

correction for local fertility difference (Li et al., 2008). The experiment was replicated three times per site within a year. Each replication had seven incomplete blocks and eight test entries were grown in each of the blocks. In each block, 16 plants per line were transplanted at 21 days after sowing in single row plots measuring 3 m long and spaced at 20 cm between plants within a row and 20 cm between the rows. The susceptible variety, Basmati 370 was planted on either side of each row and around the test entries to act as natural source of inoculum in order to adequately induce blast disease infection. Field management followed normal agricultural practices of weeding, fertilizer and irrigation with the exception of fungicide and bactericide application (Ghaley *et al.,* 2012). Phosphorus in form of diammonium phosphate (DAP) at 130 kg ha-1[equivalent to 60 kg P ha^{-1}] and potassium in form of Muriate of potash (MOP) at 100 kg ha^{-1} [equivalent to 60 kg K ha^{-1}] was applied one week before transplanting as basal fertilizer. Nitrogen fertilizer was applied two times at seedling stage and mid-tillering stage (200 kg ha^{-1} of 46% urea applied two times, equal to 92 kg N ha^{-1} per application). Field management followed normal agricultural practices of weeding and irrigation with the exception of fungicide and bactericide application (Li *et al*., 2008a).

Mapping population consisting 320 $F_{6.7}$ RILs, IR64-21, Moroberekan and two local varieties (IR2793-80-1 and Yala-1) were transplanted in 12 randomized incomplete blocks, replicated three times in each site within a year. Twenty seven lines were grown in each of the 12 incomplete blocks. Rice seedlings were also transplanted in single rows as indicated in the paragraph above under rice differential section. In each block, 12 plants per line were grown in a single row plots and spaced as indicated above. Test

entries were separated by a single row of a resistant check, IR2793-80-1 to minimize the interferences between adjacent rows as described by Wang et al. (1994). The test entries were surrounded by susceptible variety (Basmati 370) and field management was carried out as stated above.

3.3.4 Disease evaluation

The infection type was scored in the field at 60 days after transplanting (DAT) using 0-5 scale rating system (Bonman *et al*., 1986). The predominant lesion type on the leaves was scored as follows: 0- no evidence of infection; 1- presence of pin-point size brown specks; 2- slightly larger brown specks of about 0.5 mm in diameter but no necrotic spots; 3- small, round, or elliptical lesion about 1 to 2 mm or slightly more in diameter with grey centers and brown margins; 4- typical spindle-shaped blast lesion on the leaf, 3 mm or more in length, with large necrotic grey center and water soaked or reddish brown margin; 5- many large blast lesion as in 4 or larger and the upper portion of one or two leaves may be killed by collapsed lesion. The ratings of 0 to 2 was regarded as an incompatible (resistant) reaction, 3 as moderate resistant while rating of 4 and 5 indicated a compatible (susceptible) reaction (Appendix 9). All the 16 plants per differential line in each replication were evaluated for rice blast infection while ten middle plants per RIL in each replication were scored.

3.4 Genotyping of recombinant inbred lines

3.4.1 DNA extraction

Three week old rice leaves of the $F_{6.7}$ RILs and parents were sampled and stored at -80ºC in the Bio-Science Eastern and Central Africa (BECA) laboratory at ILRI. Leaf samples

were lyophilized for 48 hours. About 100 mg of lyophilized leaves was placed in 2 ml sterile Eppendorf tube with two tungstein beads one at the bottom and another at the top and the tubes capped tightly. The leaf samples were grinded to a fine powder in a GenoGrinder® 2010 at 1500 rpm for 2 min. Genomic DNA was extracted from the lyophilized leaves using modified Dellaporta et al. (1983) method. The modification included addition of CTAB step in the protocol.

Approximately, 750 µl of pre-warmed extraction buffer (100 mM *Tris*-HCl, 50mM EDTA, 500 mM NaCl, pH 8.0) was added to each sample and mixed thoroughly by inverting the tubes placed on Styrofoam (Appendix 10). Then, 50 µl of sodium dodecyl sulphate (SDS) was added to the tubes, capped and mixed thoroughly by gently inverting the tubes to disrupt cell membranes. All tubes were incubated in a water bath at 65ºC for 30 minutes with occasional inversion of the tubes at a 10 minute interval to denature nucleases. Then, 250 µl of 3 M sodium acetate (NaAc) (pH 5.2) was added and mixed thoroughly by inverting the tubes and incubated on ice for 30 minutes to precipitate SDS-bound proteins. The contents in the tubes were centrifuged at 14,000 rpm for 15 minutes at room temperature in a microfuge to pellet debris. The supernatant was decanted into a new sterile Eppendorf tube and nucleic acids precipitated by adding equal volume of chilled isopropanol and incubated at -20ºC for 30 minutes. Thereafter, the contents were spinned at 14, 000 rpm for 10 minutes at room temperature in Eppendorf centrifuge to obtain a nucleic acid pellet.

The pellet was washed with 70% ethanol, dried and dissolved in 250 µl of 1× TE (10 mM Tris, 1 mM EDTA). Then, 250 µl of pre-warmed 2 x CTAB buffer (200 mM Tris, 50 mM EDTA, 2 M NaCl, 2% CTAB) was added to each DNA pellet and mixed gently by inverting the tubes to selectively precipitate nucleic acids. Thereafter, the contents in the tubes were incubated at 65ºC for 15 minutes. Then, 500 µl of chloroform: isoamylalcohol (24:1) was added and mixed gently by inverting the tube to remove proteins and organic compounds including lipids, phenolics and tannins. The sample tubes were centrifuged at 14,000 rpm for 10 minutes at room temperature (≈ 20°C) to separate the debris. Supernatant was carefully transferred to sterile tubes without touching the chloroform interface. The chloroform: isoamylalcohol step was repeated once more. About 400 µl of supernatant was transferred to 1.5 ml sterile Eppendorf tube and nucleic acid precipitated by adding equal volume of chilled isopropanol and incubated at -20ºC for 30 minutes.

The sample tubes were centrifuged at 14,000 rpm for 15 minutes to pellet genomic DNA. The supernatant was decanted and DNA pellet washed twice with 500 µl of 70% ethanol and the tubes blotted to drain excess ethanol by carefully inverting the open tube onto a clean sterile tissue. The nucleic acid was air-dried at room temperature (≈ 20°C) on the bench. DNA pellet from each sample was dissolved in 50 µl of 1 × TE (10 mM Tris, 1 mM EDTA). To each dissolved DNA sample, 2 µl of RNAse A (10 mg/ml) was added and incubated at 37ºC for 30 minutes to enhance the activity of RNAse A in the digestion of RNA.

3.4.2 DNA Quality check and quantification

DNA quality and quantity was assessed using agarose gel electrophoresis method as described by Sambrook and Russell (2001). Quantification was done on standard agarose gel (0.8%) and electrophoresed in 0.5× Tris borate EDTA (TBE) buffer. Four microliter of stock DNA was diluted by adding 46 µl of TE to normalize the concentration of DNA to 50 ng/ µl. From the diluted DNA, 5µl was mixed with 5 µl of 2 × loading dye (15% Ficoll® 400, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4% Orange G, 10 mM *Tris*-HCl (pH 7.5), 50 mM EDTA). Then, 1 µl of the sample was loaded into separate wells of the submerged agarose gels. The samples were run alongside 50 ng/µl and 100 ng/µl uncut methylated Lambda (λ) DNA at 70 volts for 30 minutes. The gel was removed and photographed under UV light using a UV gel documentation system. DNA concentration was estimated by comparing band sizes and staining intensities of the test DNA samples with those of the standard lambda DNA (Appendix 11). The concentration and purity of the rice genomic DNA sample was also measured using a NanoDrop system, 500c (Appendix 12). The concentration of the DNA was adjusted to 50 ng/µl by diluting stock DNA with TE and the sample stored at -20°C.

3.5 Infinium SNP chip

Infinium SNP chip containing 4,606 SNP markers evenly distributed on 12 rice chromosomes were used to genotype 320 RILs and the two parents. SNP genotyping was carried out in the Genotyping Service Laboratory (GSL) at IRRI, The Philippines. DNA amplification, fragmentation, precipitation, resuspension, hybridization, washing, staining and scanning of the beadchip were performed according to the Infinium assay standard protocol (Infinium® HD Assay Ultra, Manual, http://www.illumina.com; Appendix 13).

3.5.1 Sample preparation

Four microliter of DNA normalized to 50 ng/µl was dispensed into a 96 sample well plate using 8-channel pipette. The plates were labeled with a barcode sticker supplied with the kit and span down in a centrifuge for 30 seconds. The plates were covered with a lid to keep out dust.

3.5.2 Amplification

The Illumina hybridization oven was preheated and allowed temperature to equilibrate to 37°C. The MSA3 barcode label was applied to a new MIDI plate (Infinium). The pack of tubes labeled MA1, MA2, and MSM (patented reagents) from Illumina were removed from the -20°C freezer, set on the bench at room temperature to thaw. These tubes were pulse centrifuged to 280 ×g. Using 8-channel pipette, 20 µl of MA1 was dispensed into each well of the MSA3 plate wells. Then, 4 µl of DNA sample from 96-well plate was transferred to the corresponding wells in the MSA3 plate. The original DNA sample identification for each well in the MSA3 plate was recorded on the laboratory tracking form. Then, 4 µl of 0.1 M NaOH was added into each well of the MSA3 plate containing MA1 and DNA sample. The MSA3 plate was sealed with the 96-well cap mat and vortexed at 1,600 rpm for 1 minute on a microplate shaker. The MSA3 plate was centrifuged at 280 ×g for 1 minute. The MSA3 plate was incubated at room temperature for 10 minutes. Then, 34 µl of MA2 was dispensed into each well of the MSA3 plate containing the DNA samples. This was followed by dispensing 38 µl of MSM into each well of the MSA3 plate. The MSA3 plate was resealed with the cap mat. The sealed MSA3 plate was vortexed at 1600 rpm for 1 minute and pulse centrifuged at 280 × g for 1

minute. The MSA3 plate was incubated in the Illumina hybridization oven for 24 hours at 37°C.

3.5.3 Fragmentation

The FMS (patented reagent) tube was removed from the freezer and thawed at room temperarure (≈ 20°C) and gently inverted to homogenize the contents. The FMS tube was pulse-centrifuged at 280 ×g. MSA3 plate was removed from the Illumina hybridization oven and centrifuged at 50 ×g for 1 minute. Then, 25 µl of FMS was dispensed into each well of the MSA3 plate containing sample, covered with 96-well cap mat. The MSA3 plate was vortexed at 1600 rpm for 1 minute and centrifuged at 50 ×g for 1 minute at 22°C. The sealed MSA3 plate was incubated on a preheated heat block set at 37°C for 1 hour.

3.5.4 Precipitation

The PM1 (patented reagent) tube was thawed to room temperature and pulse-centrifuged at 50 ×g for 1 minute. The 96-well cap mat was removed from the MSA3 plate and 50 µl of PM1 dispensed into each well of the plate. The MSA3 plate was sealed with the cap mat and vortexed at 1600 rpm for 1 minute. Then MSA3 plate was incubated on a preheated block set at 37°C for 5 minutes. Thereafter, the MSA3 plate was centrifuged to 50 ×g at 22°C for 1 minute. To the MSA3 plate containing the sample, 155 µl of 100% isopropanol was dispensed into each well, covered tightly with a new dry cap mat. The MSA3 plate was inverted at least 10 times to mix contents thoroughly. The contents in the MSA3 plate was incubated at 4°C for 30 minutes, centrifuged to 3, 000 ×g at 4°C for 20 minutes. The cap mat was removed from the MSA3 plate and discarded. The

supernatant was discarded by inverting the MSA3 plate and smacking it down onto an absorbent pad. The MSA3 plate was tapped firmly several times until all wells were devoid of liquid. The uncovered and inverted MSA3 plate was left on the tube rack for 1 hour at room temperature to air dry the pellet.

3.5.5 Resuspension

The RA1 (patented reagent) tube that was stored at -20°C was thawed in a water bath set at room temperature (≈ 20°C). After thawing, 23 µl of RA1 was dispensed into each well of the MSA3 plate containing DNA pellet. The remaining RA1 was maintained at 4°C for later use during staining step. The MSA3 plate was sealed using a foil by firmly holding the heat-sealer sealing block down for 5 seconds. The sealed MSA3 plate was placed in the Illumina hybridization oven preheated to 48°C and incubated for 1 hour. The MSA3 plate was vortexed at 1800 rpm for 1 minute and pulse-centrifuged at 280 ×g.

3.5.6 Hybridization

The hybridization (Hyb) chamber gaskets (Illumina) were placed into Hyb chambers (Appendix 14). Then, 400 µl of PB2 (patented reagent, Illumina) was dispensed into each of the humidifying buffer reservoirs in each Hyb chamber (Illumina). The lid of each Hyb chamber was secured and kept on the bench at room temperature until ready to load beadchips. The re-suspended MSA3 plate was incubated on preheated heat block set at 95°C for 20 minutes to denature the DNA samples. As the MSA3 plate was being denatured, a box of beadchips was removed from 4°C and set on the bench. After 20 minutes incubation, MSA3 plate was removed from the heat block and placed on the benchtop at room temperature for 30 minutes. After cooling for 30 minutes, the MSA3

plate was pulse centrifuged at 280 ×g. Just before loading the DNA samples, all the beadchips from their packages were removed making sure the beadstripe area and sample inlets are not touched.

The beadchip was placed in a Hyb chamber insert while orienting the barcode end so that it matches the barcode symbol on the insert. Using a multi-channel precision pipette, 12 µl of each DNA sample was dispensed onto the appropriate beadchip section, according to the chart on the laboratory tracking form. The samples were loaded by directly placing pipette tip to the array surface and the pipette held straight above the array surface making sure that all arrays contain the sample. DNA samples A1-F1 from the MSA3 plate were loaded into the left side beadchip inlet ports A1-F1 (every other inlet port on the left side of the beadchip). DNA samples in G1 and H1 from the MSA3 plate were loaded into the left side beadchip inlet ports G1 and H1 (Appendix 15). DNA samples A2-D2 from the MSA3 plate were loaded into the left side beadchip inlet ports A2-D2. DNA samples E2-H2 from the MSA3 plate were loaded into the right side beadchip inlet ports E2-H2. DNA sample A3 and B3 from the MSA3 plate was loaded into the right side beadchip inlet ports A3 and B3. DNA samples C3-H3 from the MSA3 plate were loaded into the right side beadchip inlet ports C3-H3 (every other inlet port on the right side of the beadchip). The remaining DNA samples were loaded following the colour-coded sections shown in the graphics supplied by Illumina. The beadchip barcode for each group of DNA samples was recorded on the laboratory tracking form. The sections of the beadchips were visually inspected ensuring that the DNA samples entirely covered each beadstripe.

The Hyb chamber inserts (Illumina) containing beadchips were loaded into the Hyb chamber. The barcode end was positioned over the ridges indicated on the Hyb chamber. The back side of the lid was placed onto the Hyb chamber and the front end slowly brought down to avoid dislodging the hybridization chamber inserts. The clamps on both sides of the Hyb chamber were closed. The Hyb chamber was placed in a preheated Illumina Hyb oven set to 48°C ensuring the clamps of the Hyb chamber face the left and right sides of the oven. The complete Hyb chamber containing the beadchips was incubated at 48°C for 16 hours and the MSA3 plate discarded.

3.5.7 Washing of beadchip

The Hyb chambers were removed from the Illumina hybridization oven and incubated at room temperature for 25 minutes prior to opening. Two wash dishes each were filled with 200 ml of PB1 (patented reagent, Illumina) and labelled as "PB1". Then, beadchip alignment fixture was filled with 150 ml of PB1 and clear plastic spacers were separated from the white glass backs. The glass back plates were cleaned as described in the Infinium Assay Laboratory Set up and Procedures Guide. The wire handle was attached to the rack and submerged in the first wash dish containing 200 ml PB1. Hyb chamber inserts were removed from the Hyb chambers and the beadchips removed from the Hyb chamber inserts one at a time (Appendix 16). IntelliHyb seal was removed from each beadchip using powder-free gloved hands. This was done by holding the beadchip in one hand with the thumb and forefinger on the long edges of the beadchip while avoiding contact with the sample inserts. The barcodes were ensured that are facing up, close to the handler and the top of the beadchip angled slightly. The seal was removed in a single,

slow, consistent motion by pulling it off in a diagonal direction. Beadchips were slide into the wash rack one at a time, making sure that the beadchips are completely submerged in the PB1. After placing all the beadchips in the wash rack, it was moved up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation to remove bubbles. The wash rack was then moved to the second wash dish containing PB1 making sure that the beadchips are completely submerged. Wash rack was moved up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation to remove air bubbles.

The flow-through chamber was assembled by filling the multi sample beadchip alignment with 150 ml of PB1 (Appendix 17). For each beadchip, a black frame was placed into the multiple-sample beadchip alignment fixture (Illumina). Each beadchip was placed into a black frame, aligning its barcode with the ridges stamped onto the alignment fixture and immersed fully in PB1. A clear spacer was placed onto the top of each beadchip using the alignment fixture grooves to guide the spacers into proper position. Alignment bar was put onto the alignment fixture. Using a laboratory air gun, any accumulated dust from the glass plates were removed just before placing them onto the beadchips. A clean glass back plate was placed on top of the clear spacer covering each beadchip. The plate reservoir was placed at the barcode end of the beadchip facing inward to create a reservoir against the beadchip surface.

Metal clamps were attached onto each flow-through chamber by gently pushing the glass back plate up against the alignment bar with one finger. The first metal clamp was placed

around the flow-through chamber so that the clamp was about 5 mm from the top edge while the second metal clamp was placed around the flow-through chamber at the barcode end, 5 mm from the reagent reservoir. Using pair of scissors, the spacers were trimmed at the non-barcode end of the assembly. Then, Hyb chamber reservoir was washed with distilled water and scrubbed with a cleaning brush ensuring that no PB1 remained. The assembled flow-through chambers were placed on the laboratory bench in a horizontal position while performing the preparation steps for beadchip staining.

3.5.8 Staining preparation

The water circulator was filled to the appropriate level. It was switched on and set to 44ºC to equilibrate. Bubbles trapped in the chamber rack were removed. The flow-through chambers were verified to ensure it reached 44°C in multiple positions with an Illumina temperature probe. The reagent tubes containing XC1, XC2, TEM, STM and ATM (patented reagents) were removed from the freezer, allowed to thaw at room temperature, centrifuged to 3000 ×g for 3 minutes and arranged in order in which they would be used. The XC4 re-suspended by adding 330 ml of 100% ethanol. The XC4 bottle was vortexed to ensure complete resuspension and incubated at room temperature (≈ 20°C) overnight.

As the chamber rack reached 44ºC, each flow-through chamber assembly was placed into the chamber rack. In each reservoir of the flow-through chamber, 150 µl of RA1 was added and incubated for 30 minutes. This was followed by adding 450 µl XC1 to each reservoir of the flow-through chamber and incubated for 10 minutes. Then, 450 µl of XC2 was added to the flow-through assemblies and incubated for 10 minutes. This was

followed by addition of 200 µl TEM to the flow-through assemblies and incubated for 15 minutes. Then, 450 µl of 95% formamide/1mM EDTA was added to the flow-through assemblies and incubated for 1 minute. The 95% formamide/ 1 mM EDTA was prepared by mixing 95% formamide, 0.2% EDTA (0.5 M), 4.8% H_2O by volume and frozen in separate 15 ml increments. Then 450 µl of XC3 was dispensed into each of the flow-through assemblies and incubated for 1 minute. The beadchip was stained by adding 250 µl STM and incubated for 10 minutes. Then, 250 µl of ATM was added to the flow-through reservoir and incubated for 10 minutes. The flow-through chambers were removed from the chamber rack and placed horizontally on a laboratory bench at room temperature.

About 310 ml of PB1 per 8 beadchips was poured into a wash dish and covered. The staining rack was placed inside the wash dish. For each beadchip, metal clamps were removed from the flow-through chamber followed by the glass back plate, spacer and the beadchip. Beadchips were placed into the staining rack that was placed in the wash dish with barcodes facing away from the handler ensuring that all the chips were submerged. Staining rack was moved up and down for 10 minutes, breaking the surface of the reagent to remove bubbles and soaked for 5 minutes. Then, 310 ml of re-suspended XC4 was poured into a wash dish ensuring that it does stay unused for more than 10 minutes.

The beadchip staining rack was moved into the XC4 dish ensuring that the barcodes are facing away from the handler. Staining rack was moved slowly up and down 10 times, breaking the surface of the reagent to remove air bubbles and soaked for 5 minutes.

Staining rack was lifted out of the solution and placed on a tube rack with the staining rack and beadchips horizontal while barcodes facing up. The beadchips were removed from the staining rack with locking tweezers while working from top to bottom. Beadchips were dried in the vacuum desiccator for 50-55 minutes at 508 mmHg. The underside of each beadchip was cleaned with a ProStat EtOH wipe. The beadchips were stored overnight in the Illumina beadchip slide storage box inside a vacuum desiccator at room temperature (≈ 20°C).

3.5.9 Scanning the beadchip

After the beadchips had dried, the vacuum was turned off by slowly returning the pressure to atmospheric. The iScan Reader software (Illumina) was activated and the beadchips moved into scanning tray. Beadchip's decoded files were downloaded by activating the Decode File Client software and inputting the desired beadchip barcodes along with their corresponding box identification. Once the chips were properly seated in the tray and decode files recognized by the software, scanning was initiated (Appendix 18). The iScan Reader uses a laser to excite the fluorescence of the single-base extension product on the beads of the beadchip sections. Light emissions from the fluorescence are then recorded in high-resolution image of the beadchip sections.

3.6 Data analyses

3.6.1 Rice differential lines

Rice blast disease lesion type scores were used to calculate disease severity index (DSI) as described by Grau et al. (1982). Disease incidence was calculated as described by Taguchi et al. (2014) using the following formula:

$$DSI(\%) = (PDR \times Number\ of\ plants\ with\ the\ scale) \times 100) / HDR \times Total\ number\ of\ plants$$

$$Disease\ incidence = \left(\frac{Number\ of\ diseased\ plants}{Total\ number\ of\ inspected\ plants} \right) \times 100$$

Where PDR is the predominant disease rating scale for a given differential line, HDR is the highest disease rating scale. In the present study, HDR = 5.

Percent disease severity data was transformed as described by Maharijaya et al. (2015) in order to stabilize variances as follows: $y = \arcsin e[\ sqrt\ (x)]$

Where y = transformed variable, *sqrt* = square root, x = percent disease severity

The transformed disease severity data was subjected to analysis of variance (ANOVA) using generalized linear model (GLM) procedures of statistical analysis software (SAS) version 9.1.3 with blocks nested within replications (SAS, 2004). The rice lines (genotypes) were considered as fixed while season, location, genotype × season, genotype × location and genotype × season × location as random effects. Means of rice lines and varieties were separated using least significance difference at $P \leq 0.05$. The overall statistical model used in this analysis was as follows:

$$Y_{ijklm} = \mu + R_i + B_{j(i)} + G_k + S_l + L_m + GS_{kl} + GL_{km} + GSL_{klm} + \varepsilon_{ijklmn}$$

Where Y_{ijklm} is the observation on the $ijklm^{th}$ plot corresponding to genotype k in block j of replicate i season l in the location m, μ = general mean, R_i is the effect due to replication in i^{th} season and m^{th} location, $B_{j(i)}$ is the effect due to j^{th} block in i^{th} replicate in l^{th} season and m^{th} location, G_k is the effect due to k^{th} genotype in the j^{th} block, in i^{th} replicate, l^{th} season and m^{th} location, S_l is the effect due to l^{th} season in m^{th} location, L_m is the effect due to m^{th} location, GS_{kl} is the effect due to k^{th} genotype by l^{th} season interaction, GL_{km} is iffect due to k^{th} genotype by m^{th} location, GSL_{klm} is the effect due to

k^{th} genotype by l^{th} season by m^{th} location and ε_{ijklmn} represents the residual for the $ijklm^{th}$ plot.

Disease severity mean of the three replications per site was used to calculate dissimilarity coefficient as described by Sneath and Sokal (1973). The resulting distance coefficient was used for cluster analysis by applying unweighted pair group method for arithmetic average analysis (UPGMA) using SAHN-UPGMA clustering method (NTSYS-pc version 2.11X) as described by Rohlf (2000).

3.6.2 Recombinant inbred lines

Disease severity index data for RILs were calculated as stated in section 3.8.1 above. Analysis of variance for RILs was performed as described above. The genotypes were considered as fixed effects while environment, G × E and replications were considered as random effects. As there was a significant genotype × environment interaction effect, each season per location was considered as a specific environment for QTL analysis. Therefore, QTL analysis was first analyzed separately for each season per experimental site. The genotype means calculated by averaging the three replications per environment was used for QTL analysis. Thereafter, the means per experimental site were also computed and used to map QTL. The data from the six environments were analyzed using the following linear mixed effect model:

$Y_{ijk} = \mu + R_i + G_j + E_k + GE_{jk} + \varepsilon_{ijkl}$ Where *Yijk* is the observation on the ijk^{th} plot corresponding to genotype *j* in replicate *i* in the environment *k*, μ is the general mean, R_i is the effect due to i^{th} replicate in the k^{th} environment, G_j is the effect due to j^{th} genotype in the i^{th} replicate, k^{th} environment, E^k is the effect due to k^{th} environment, GE_{jk} is the

effect due to j^{th} genotype by k^{th} environment and ε_{ijkl} represents the residual error for the ijk^{th} plot.

Broad sense heritability was calculated according to the formula described by Zhou et al. (1960) to determine if the variation observed is due to genetic component as follows:

$H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2 / e + \sigma_\varepsilon^2 / re)$ $\sigma_g^2 = (MS_f - MS_{fe}) / re$ $\sigma_{ge}^2 = (MSfe + MSe) / r$

$\sigma_e^2 = MS_e$ Where H^2 is broad sense heritability, σ^2_g is the genotypic variance, σ^2_{ge} is the genotype × environment interaction variance, σ^2_ε is the error variance, MS_f is the genotype mean square, MS_{fe} is the mean square for genotype × environment interaction, MS_e is the mean square for error, r is the number of replications and e is the number of environments.

3.6.3 Genetic map construction

A total of 2416 polymorphic SNP markers between the two parents were tested for goodness-of-fit to establish the deviation of observed frequencies from the expected 1:1 Mendelian segregation ratio for RIL population. The SNP markers that showed high segregation distortion were excluded from the analysis. The remaining markers were subjected to the binning (BIN) tool in the QTL IciMapping to identify and remove redundant markers (i.e., markers that are completely correlated in a population and therefore, cannot provide additional information, if more than one of them is considered) in a data set (Wang et al., 2014a). The SNP markers with high missing rate of greater than 30% were removed. Further filtering was done by removing SNP markers with χ^2 score >10 as described by Tabien et al. (2000). In addition, markers that mapped to the

same spot in the rice genome were removed to avoid overpopulating the map with SNPs that are not informative.

A logarithm of odds (LOD) criteria of 3.0 was used to construct genetic map (Wang *et al*., 2014a). Ordering of marker was done by input command since physical positions of the markers on the 12 chromosomes were known. After ordering, markers were rippled using sum of adjacent recombination frequencies (SARF) that gave a short linkage map. Conversion of recombination frequencies between linked loci into centimorgan (cM) was obtained using Kosambi's mapping function (Kosambi, 1944). The final map was drawn by QTL IciMapping software version 4 (Wang *et al*., 2014a). Correspondence of linkage groups and order of the markers on chromosomes was inferred from the physical map of Nipponbare reference genome Michigan State University version 7 (MSU7) (Kawahara *et al*., 2013; http://rice.plantbiology.msu.edu/).

3.6.4 QTL analysis

QTL for leaf blast resistance were detected using disease severity means of the three replications in each environment. QTL analysis was conducted using the inclusive composite interval mapping (ICIM) software QTL IciMapping version 4 (Wang *et al*., 2014a). A logarithm of odds (LOD) threshold of 2.5 was initially used to declare major QTL in this study. Thereafter, 1,000 permutations at a probability of 0.05 were used to declare definitive QTL. A chromosomal walk speed of 1.0 cM and default window size of 8 cM was used for all QTL estimations. QTL effects were estimated as the proportion of phenotypic variance explained (PVE) by the QTL.

3.6.5 Naming of QTL

QTL were named following Tabien et al. (2002) with the following modifications. A '*qrbr'* prefix indicates a QTL for rice blast resistance. This is followed after a hyphen by the chromosome onto which the QTL was mapped. When multiple QTL were mapped in the same chromosome, an additional hyphen and number was added to clearly distinguish between them. For example, a locus name *qrbr-1-1* means a QTL for blast resistance located towards the top of chromosome 1.

CHAPTER FOUR

RESULTS

4.1 Virulence spectrum of blast populations at three locations

The response of rice differential lines carrying known blast resistance genes and local varieties to blast population differed significantly ($P \leq 0.05$) within and among the experimental locations (Table 4.1). Effects due to season and location were significant for the reaction of rice differential lines and local varieties, meaning that blast population differed within and across the experimental locations. Varieties contributed the highest source of variation at 43.3% followed by location × varieties (23.84%), season × location × varieties (7.82%), location (5.93%), season × varieties (5.78%) and season (2.57%).

Table 4.1 Analysis of variance of disease severity of rice differential lines and local varieties evaluated at three locations in Kenya

Source of variation	Df	SS	MS	Sig.
Replication	2	0.03	0.01	0.44
Blocks within replication	6	0.04	0.01	0.85
Season	1	2.19	2.19**	0.00
Location	2	5.06	2.53**	0.00
Genotypes	55	36.10	0.66**	0.00
Season × Genotypes	55	4.93	0.09**	0.00
Location × Genotypes	110	20.34	0.19**	0.00
Season × Location × Genotypes	112	6.67	0.06**	0.00

**Significant at $P \leq 0.05$, SS = sum of squares, df = degree of freedom, MS = mean square

The grouping of differential rice lines and local varieties based on disease severity index varied within and among the experimental locations. Mwea formed 3 distinct groups at a similarity coefficient of 0.17 (Figure 4.1). The first group consisted of 39 susceptible differential rice lines, recurrent parents and local varieties with a mean disease severity

index ranging from 73.3 to 100% (Appendix 19). The second group had 10 resistant differential rice lines including IRBLz-FU, IRBLta2-IR64[CO], IRBLzt-IR56[CO], IRBLb-IT13[CO], IRBLsh-S[CO], IRBLsh-KU[CO], IRBLz5-CA[CO], IRBLkp-K60[CO], IRBLta2-PI[CO] and IRBLta2-RE[CO] with a mean disease severity index ranging from 0 to 15%. The third group comprised of 6 resistant differential lines and one local variety with a mean disease severity index ranging 27 to 40%. These were IRBLt-K59, IRBLsh-B [CO], BW196, IRBL12-M, IRBLk-KA [CO], IRBLkm-TS [CO] and IRBLk-KU [CO].

Disease severity index classified differential rice lines and local varieties into four groups that were demarcated at a cut-off similarity coefficient of 0.17 at West Kano (Figure 4.2). Group I had 45 susceptible differential rice lines, recurrent parents and local varieties with mean disease severity index ranging from 73.3 to 87% (Appendix 19). Six moderately resistant lines namely; IRBLkm-TS [CO], IRBLta2-PI [CO], IRBLta2-RE [CO], IRBLta2-IR64 [CO] and IRBL7-M [CO] were clustered in group II with a mean disease severity index of 60%. Group III consisted of IRBLta-CT2, IRBL11-ZH and BAS 217 that were highly susceptible with a mean disease severity index ranging from 93 to 100%. IRBLkp-K60, IRBLzt-IR56 [CO] and a local variety (BW196) were clustered in group IV with a mean disease severity index that ranged from 26.67 to 33.33%.

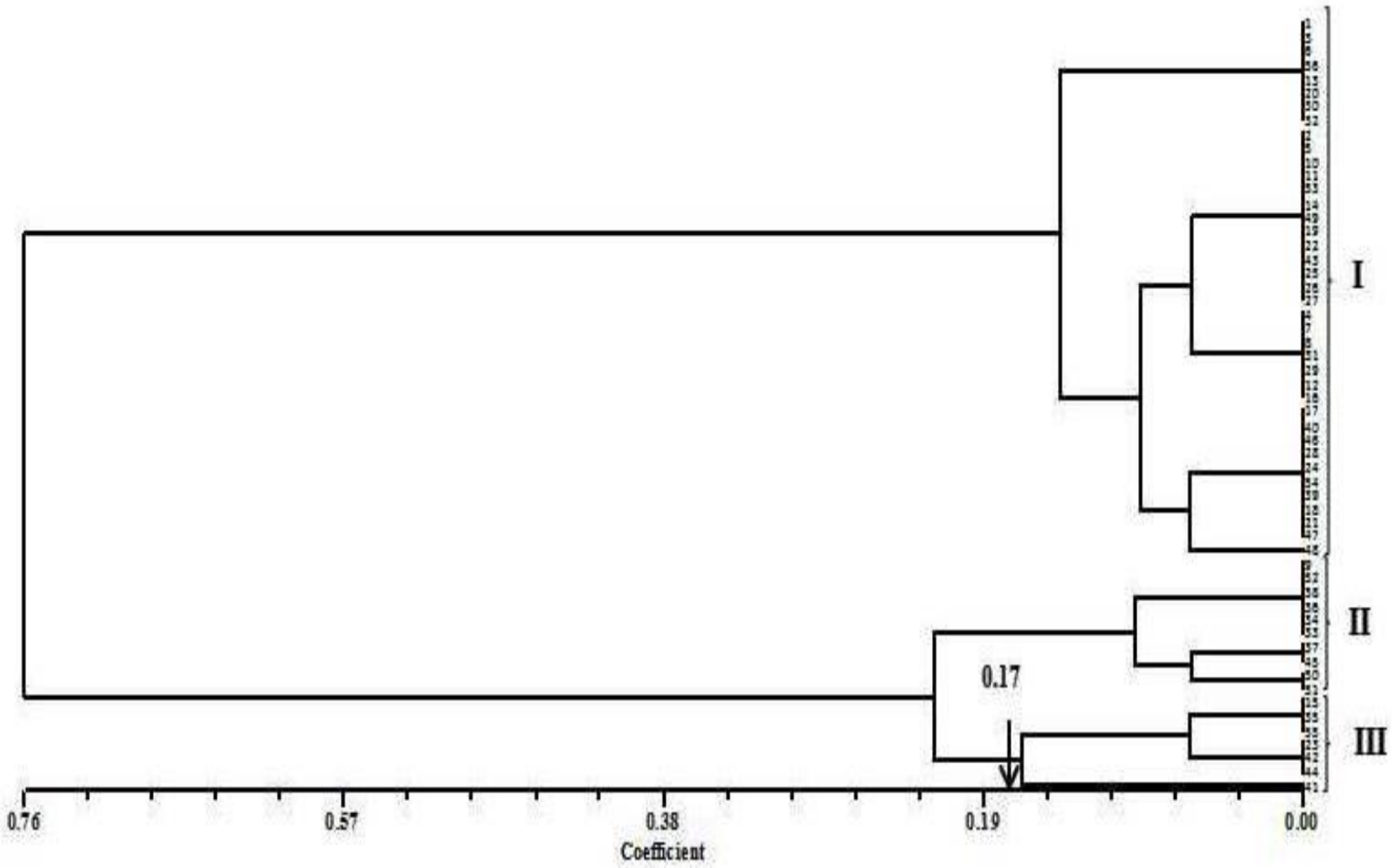

Figure 4.1 Relationship among ric differential lines and local varieties at Mwea. Arrow indicates the clustering point. Serial numbers correspond to the rice lines and local varieties listed in the appendix 7

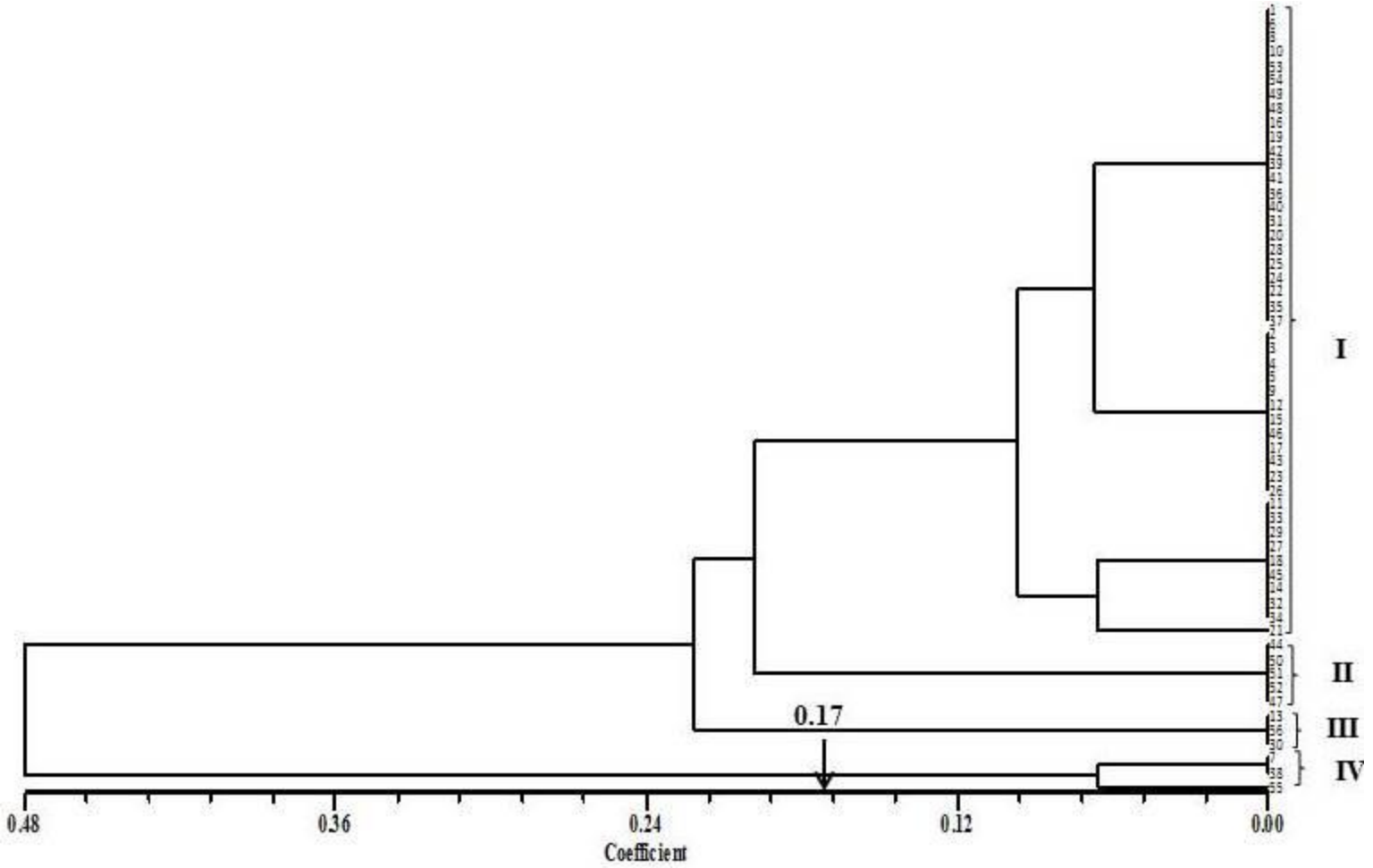

Figure 4.2 Relationship among rice differential lines and local varieties at West Kano. Arrow indicates the clustering point. Serial number corresponds to the rice lines and local varieties listed in the appendix 7

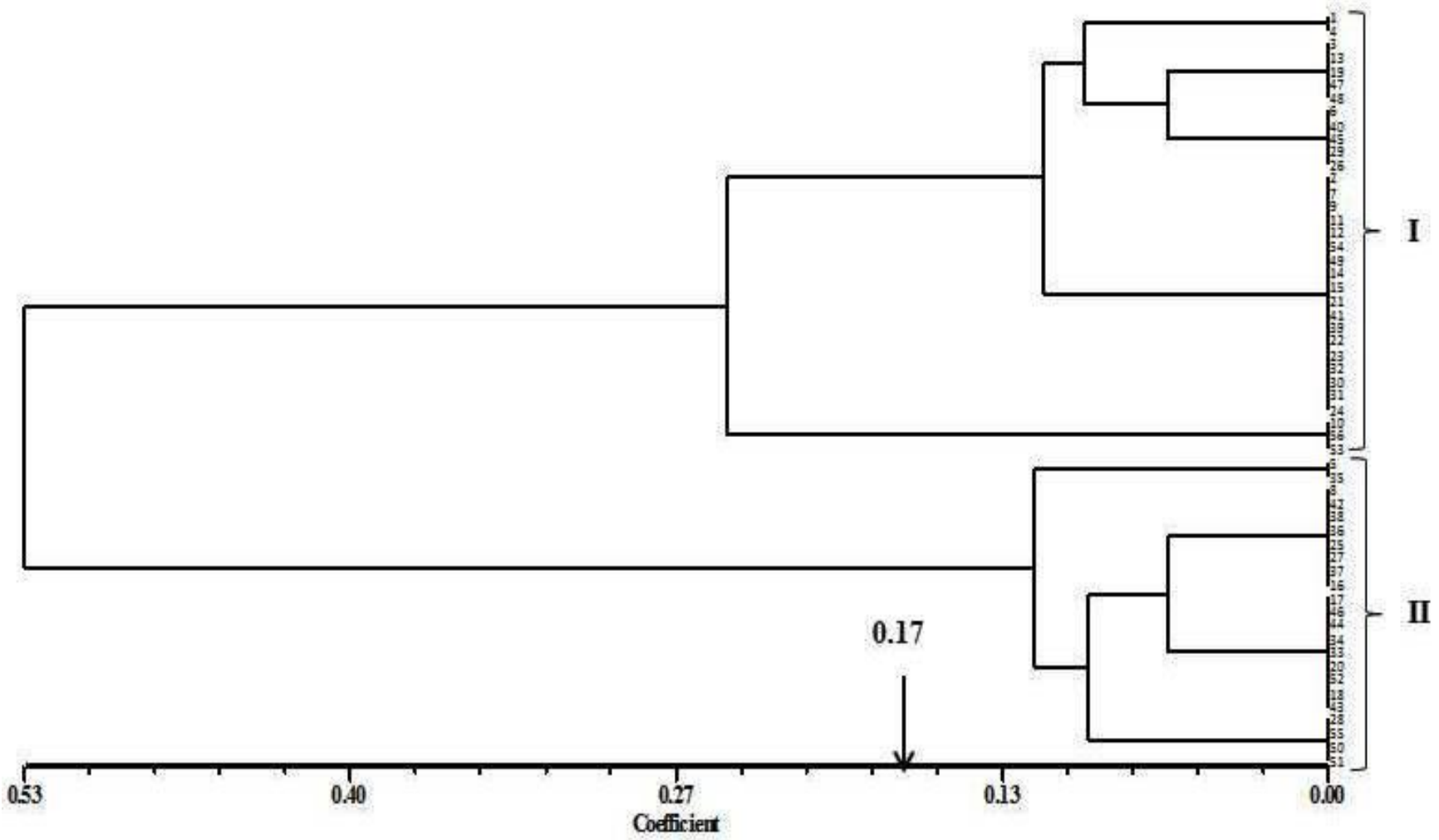

Figure 4.3 Relationship among rice differential lines and local varieties at Gamba. Arrow indicates the clustering point. Serial mumbers corresponds to the rice lines and local varieties listed in the appendix 7.

Rice differential lines and local rice varieties evaluated at Gamba were distributed into three groups at a coefficient of 0.17 (Figure 4.3). The first group comprised 30 susceptible differential rice lines, a recurrent parent (LTH) and a local variety (ITA 310) with mean disease severity index ranging from 80 to 100% (Appendix 19). IRBLz5-CA, CO39 and BAS217 were clustered in the second group. They were moderatetly resistant against blast population with a mean disease severity index of 60%. Twenty two resistant lines namely; IRBLks-S, IRBLsh-B [CO], IRBLkh-K3, IRBLk-KA[CO], IRBLzt-IR56[CO], IRBLb-IT13[CO], IRBLkm-TS, IRBLta2-PI, IRBLz5-CA[CO], IRBLsh-S, IRBLsh-B, IRBLkp-K60[CO], IRBLkm-TS[CO], IRBLsh-S[CO], IRBLsh-KU[CO], IRBL5-M, IRBLta2-IR64[CO], IRBL1-CL, IRBLkh-K3[CO], IRBLta2-RE, IRBLta2-PI[CO], IRBLta2-RE[CO] and a local variety, BW196 were clustered in group three. The mean disease severity index for group three ranged from 20 to 40%.

Field evaluation at Mwea, West Kano and Gamba showed that 29.41%, 50.98% and 25.49% of rice lines carrying different resistance genes were attacked by blast population, respectively in the year 2013 (Figure 4.4, 4.5 & 4.6; Appendix 20). In 2014, West Kano had 86.27% of differential rice lines showing susceptibility to blast population, followed by 68.63% in Mwea and 54.90% in Gamba. The virulence spectrum of blast population based on the number of susceptible lines carrying resistance genes differed across the experimental locations. West Kano therefore, had the highest diversity of blast population followed by Mwea and Gamba.

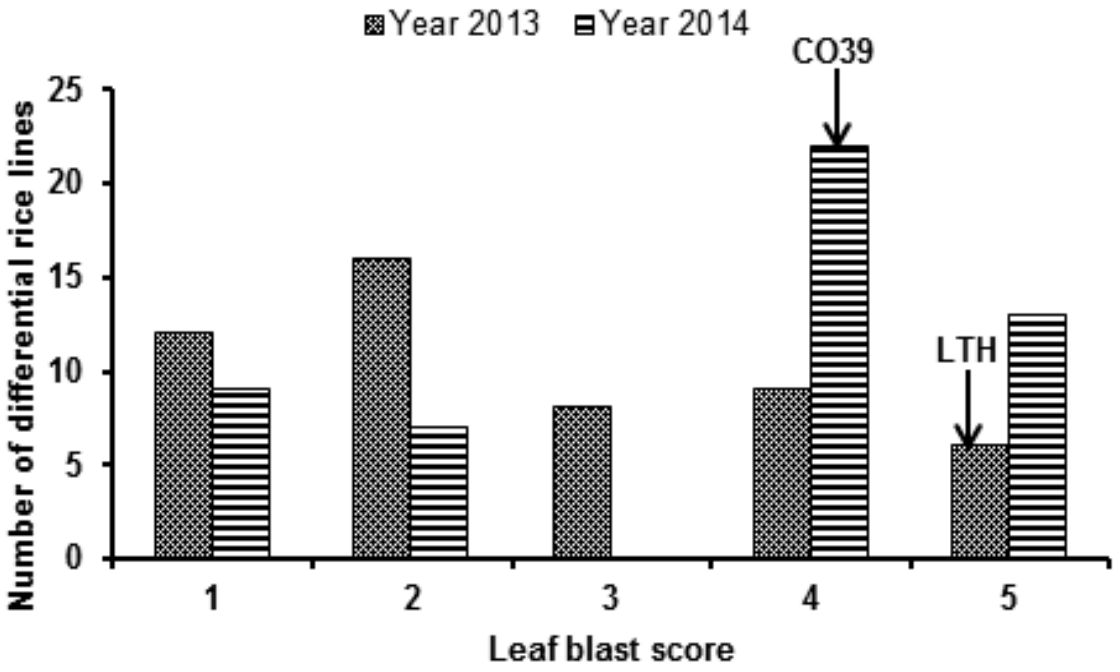

Figure 4.4 Leaf blast score of rice differential lines evaluated at Mwea in the year 2013 and 2014

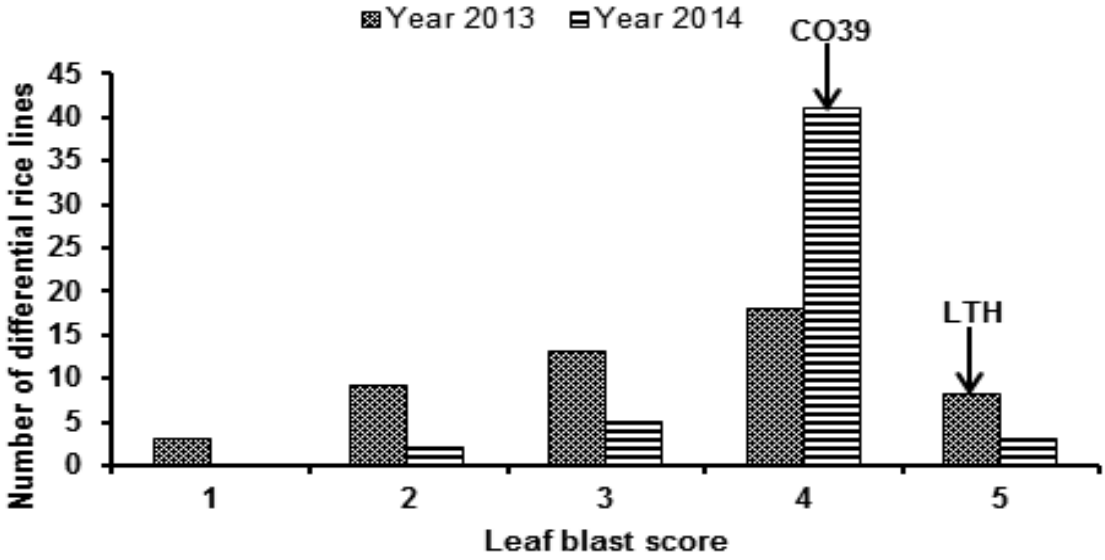

Figure 4.5 Leaf blast score of rice differential lines evaluated at West Kano in the year 2013 and 2014

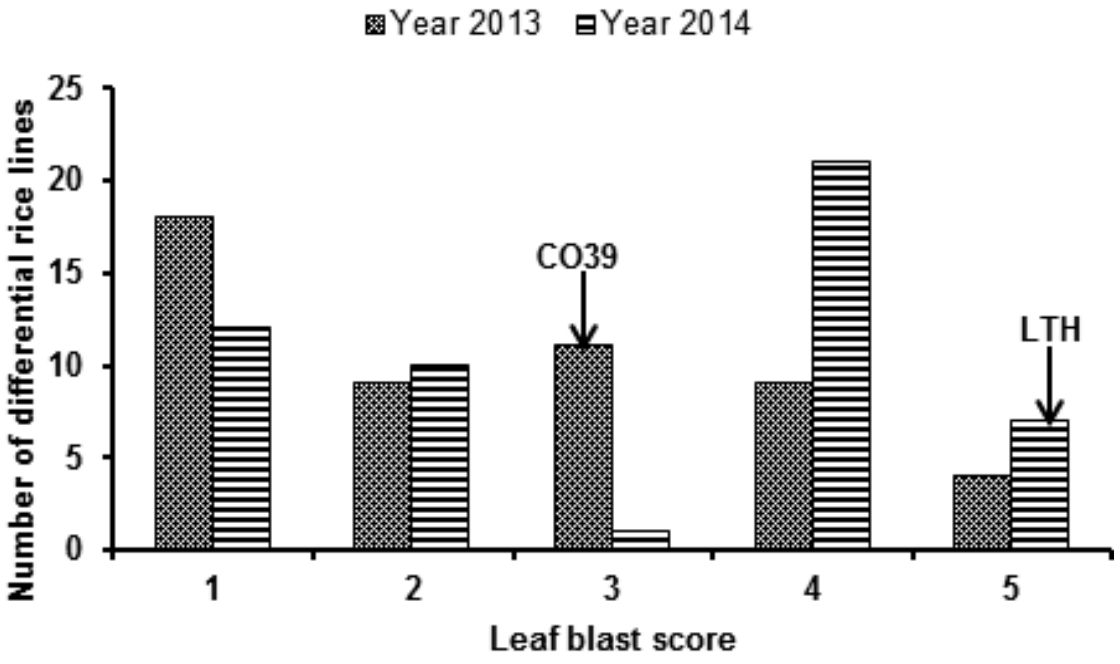

Figure 4.6 Leaf blast score of rice differential lines evaluated at Gamba in the year 2013 and 2014

Blast disease incidence varied significantly across the three experimental locations (Table 4.2). In the year 2013, mean incidence level of 34.76%, 99.58% and 89.82% was observed in Mwea, West Kano and Gamba, respectively. The blast incidence also varied significantly across the experimental locations with Gamba showing the highest mean of 89.64%, followed by West Kano with 84.63%; Mwea had the lowest at 40.41% in the year 2014. The overall incidence at Mwea, West Kano and Gamba was 37.58%, 92.11% and 89.73%, respectively.

Table 4.2 Mean comparison of rice blast incidence at three locations in Kenya

Locations	Mean blast incidence (%)		
	Year 2013	Year 2014	Overall
Mwea	34.76c	50.41c	37.58c
West Kano	99.58a	84.63b	92.11a
Gamba	89.82b	89.64a	89.73b

Significant at $P \leq 0.05$

4.2 Genes conferring resistance to blast populations

Sixteen rice differential lines carrying 11 known resistance genes including *Pik-p*, *Piz*, *Pit*, *Pish*, *Pi12 (t)*, *Pik-m*, *Pib*, *Piz-5*, *Piz-t*, *Pik* and *Pita-2* were resistant to blast population at Mwea (Table 4.3). Thirteen lines IRBLkp-K60[CO], IRBLsh-B[CO], IRBLkm-TS[CO], IRBLsh-KU[CO], IRBLsh-S[CO], IRBLb-IT13[CO], IRBLz5-CA[CO], IRBLzt-IR56[CO], IRBLk-KA[CO], IRBLk-KU[CO], IRBLta2-PI[CO], IRBLta2-RE[CO] and IRBLta2-IR64[CO] exhibited resistance genes in the CO39 genetic background. The remaining three lines namely; IRBLz-FU, IRBLt-K59 and IRBL12-M had resistance genes in the LTH genetic background. Two differential rice lines IRBLkp-K60 and IRBLzt-IR56 [CO] carrying *Pik-p* and *Piz-t* genes displayed resistance to the blast population at West Kano. Rice lines, IRBLkp-K60 [CO], IRBLta2-PI [CO], IRBLta2-RE [CO] and IRBLta2-IR64 [CO] carrying *Pik-p* and *Pita-2* exhibited moderate resistance at West Kano. Rice differential lines with resistance genes *Pik-s*, *Pik-h*, *Pish*, *Pi1*, *Pi5 (t)*, *Pik-m*, *Pita-2*, *Pib*, *Piz-5*, *Piz-t* and *Pik* were resistant to the blast population at Gamba (Table 4.3).

Rice line IRBLzt-IR56 [CO] carrying *Piz-t* showed resistance to the blast populations across the three experimental locations (Table 4.3). Also, IRBLta2-PI [CO], IRBLta2-RE

[CO] and IRBLta2-IR64 [CO] harbouring *Pita-2* showed either moderate or resistant reaction against blast populations across the three experimental locations. In addition, BW196, which is not popularly grown by local farmers in Kenya due to poor grain quality, was resistant to the blast populations across the experimental locations. The resistance gene (s) in this variety is unknown.

Table 4.3 Rice differential lines carrying genes effective against blast populations over the two years at three locations in Kenya

Rice differential lines	R gene	Locations		
		Mwea	West Kano	Gamba
IRBLks-S	*Pik-s*	S	S	R
IRBLkp-K60	*Pik-p*	S	R	S
IRBLkp-K60[CO]	*Pik-p*	R	MR	S
IRBLkh-K3	*Pik-h*	S	S	R
IRBLkh-K3[CO]	*Pik-h*	S	S	R
IRBLz-FU	*Piz*	R	S	S
IRBLt-K59	*Pit*	R	S	S
IRBLsh-B	*Pish*	S	S	R
IRBLsh-S	*Pish*	S	S	R
IRBLsh-B[CO]	*Pish*	R	S	R
IRBLsh-KU[CO]	*Pish*	R	S	R
IRBLsh-S[CO]	*Pish*	R	S	R
IRBL1-CL	*Pi1*	S	S	R
IRBL1-CL[CO]	*Pi1*	S	S	R
IRBL5-M	*Pi5(t)*	S	S	R
IRBL12-M	*Pi12(t)*	R	S	S
IRBLkm-TS[CO]	*Pik-m*	R	S	R
IRBLkm-TS	*Pik-m*	S	S	R
IRBLta2-PI	*Pita-2*	S	S	R
IRBLta2-RE	*Pita-2*	S	S	R
IRBLta2-PI[CO]	*Pita-2*	R	MR	R
IRBLta2-RE[CO]	*Pita-2*	R	MR	R
IRBLta2-IR64[CO]	*Pita-2*	R	MR	R
IRBLb-IT13[CO]	*Pib*	R	S	R
IRBLZ5-CA[CO]	*Piz-5*	R	S	R
IRBLzt-IR56[CO]	*Piz-t*	R	R	R
IRBLk-KA[CO]	*Pik*	R	S	R
IRBLk-KU[CO]	*Pik*	R	S	S
BW196	*Unknown*	R	R	R

R = Resistant, MR = moderately resistant, S = susceptible,

Rice differential lines carrying similar genes from different donor parents showed different reaction to the blast population (Table 4.4; Appendix 20). For example, rice lines IRBLb-B and IRBLzt-T were susceptible at Mwea, unlike IRBLIT13 [CO] and IRBLzt-IR56 [CO] containing the same resistance genes *Pib* and *Piz-t*. Although rice lines IRBLzt-T and IRBLzt-IR56 [CO] carry the same resistance gene (*Piz-t*), the former was susceptible to blast population at West Kano. Further, IRBLks-F5 and IRBLks-CO [CO] carrying the same gene as IRBLks-S (*Pik-s*) were susceptible to the blast population at Gamba. Furthermore, IRBLa-A and IRBLa-C were susceptible at Gamba, unlike CO39 carrying the same resistance gene *Pia*.

Table 4.4 Reaction of rice lines carrying similar genes against blast populations across two years at three locations in Kenya

Rice differential lines	R gene	Locations		
		Mwea	West Kano	Gamba
IRBLb-B	Pib	S	S	S
IRBLIT13[CO]	Pib	R	S	R
IRBLzt-T	Piz-t	S	S	S
IRBLzt-IR56[CO]	Piz-t	R	R	R
IRBLks-F5	Pik-s	S	S	S
IRBLks-CO[CO]	Pik-s	S	S	S
IRBLks-S	Pik-s	S	S	R
IRBLa-A	Pia	S	S	S
IRBLa-C	Pia	S	S	S
CO39	Pia	S	S	MR

R = Resistant, MR = moderately resistant and S = susceptible

The recurrent parents were susceptible to blast populations at Mwea and West Kano (Appendix 20). However, CO39 showed moderate resistance to the blast population at Gamba while LTH was highly susceptible (Figure 4.7). Further, rice lines IRBLa-A and IRBLa-C were susceptible at Gamba, unlike CO39 carrying the same resistance gene *Pia*.

Figure 4.7 Reaction of rice differential lines and the recurrent parents to blast at Gamba (Source: Author, 2014)

Gene placed in CO39 and LTH genetic backgrounds showed a different infection types against blast populations (Figure 4.8). It was clear that IRBLk-KA, IRBLkp-K60, IRBLz5-CA, IRBLsh-S, IRBLsh-B, IRBLta2-PI and IRBLta2-RE containing *Pik*, *Pik-p*, *Piz-5*, *Pish* and *Pita2* genes in the LTH genetic background were susceptible to blast population at Mwea. These same lines in the CO39 genetic background were resistant to the blast population at Mwea. Rice line IRBLk-KA from LTH genetic background carrying the same resistance gene *Pik* as IRBLk-KA [CO] was susceptible to blast population at Gamba. In addition, IRBL5-M carrying resistance gene *Pi5(t)* in the LTH background was resistant to blast population at Gamba, unlike IRBL5-M[CO] in the

CO39 background. Rice lines carrying similar genes in different genetic backgrounds showed a comparable reaction pattern to blast population at West Kano.

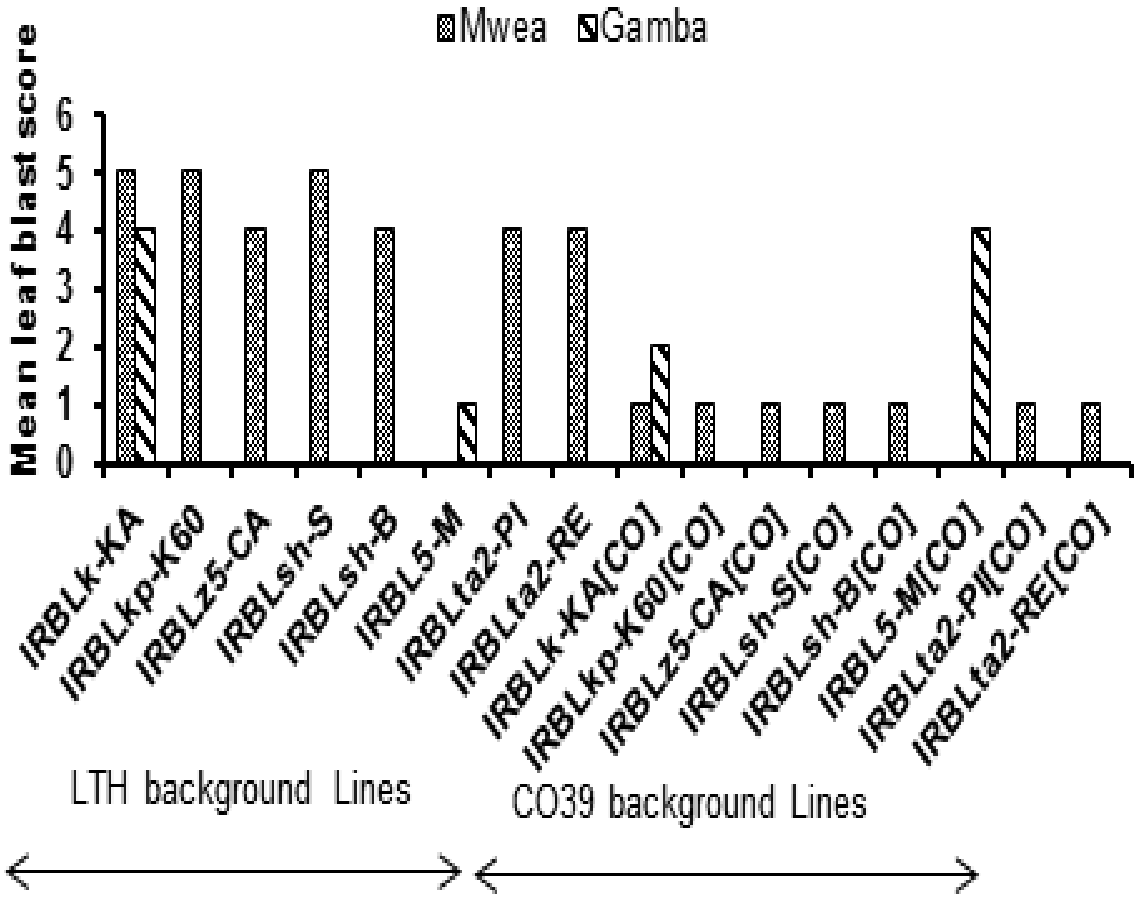

Figure 4.8 Reaction patterns of resistance genes in CO39 and LTH genetic backgrounds

4.3 Mapping of QTL conferring resistance to blast populations

4.3.1 Phenotypic variation of the recombinant inbred lines

Two RILs were lines were not genotyped due to poor DNA quality. Further, 25 RILs were excluded because they showed variation in disease reaction among plants within a line, indicating possibility of heterozygosity at one or more resistance loci. The remaining

33 were excluded from the analysis due to high percentage of missing marker data. Therefore, 260 RILs that showed less than 30% missing rate across the entire genotypic data set were used to map QTL in the present study.

The frequency distribution of the lesion type scores obtained in the six environments was examined to determine if they approached normality. *Chi* square analyses for Mwea-2013 and 2014 showed that no normal distributions were followed (Table 4.5; Figure 4.9). The segregation ratio of resistant (rating ≤3) versus susceptible (rating ≥3) RI lines varied considerably at Mwea-2013 and Mwea-2014 and most RI lines were susceptible. The number of plants per line attacked by blast population at Mwea was low and therefore, the highest disease rating score was used to represent the reaction type. However, this was not the case in West Kano and Gamba because blast disease was evenly distributed in the experimental plots. Normal distribution was not followed in West Kano-2013 while a near normal distribution was observed at West Kano-2014(Table 4.5; Figure 4.10). The *chi* square analyses for the data collected at Gamba-2013 and Gamba-2014 also showed that the data did not follow a normal distribution (Table 4.5 Figure 4.11). The distribution of lesion type among RI lines at Gamba was skewed toward Moroberekan.

Table 4.5 *Chi* square test for normality of disease severity scores at three sites in Kenya

Site	Year	Chi Square (χ^2)	Df	P-Value
Mwea	2013	78.01254529	3	8.1899E-17
	2014	14.56583843	3	0.0022279
West Kano	2013	4.173150285	3	0.24336384
	2014	14.17355184	3	0.00267819
Gamba	2013	17.0308587	3	0.00069649
	2014	109.2574607	3	1.5854E-23

χ^2-test for normality of the data ($P \leq 0.05$)

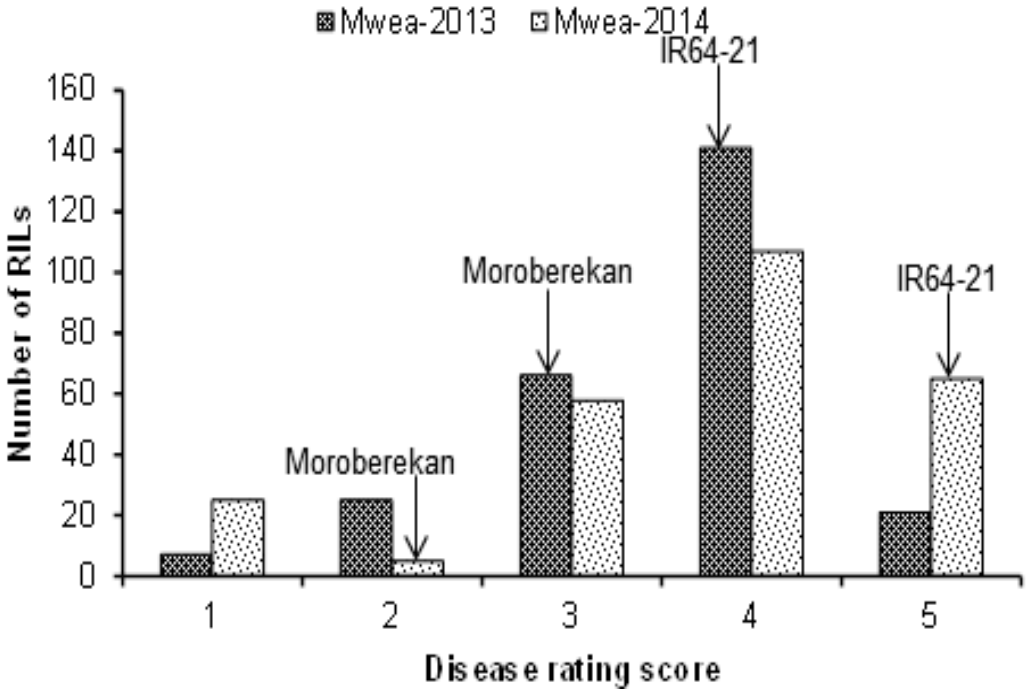

Figure 4.9 Frequency distribution of disease rating for for recombinant inbred population at Mwea

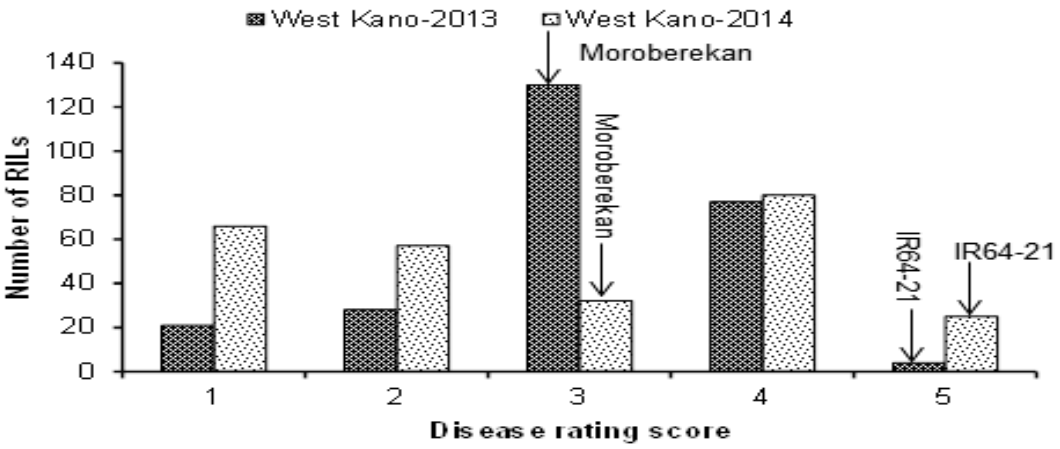

Figure 4.10 Frequency distribution disease rating for recombinant inbred population at West Kano

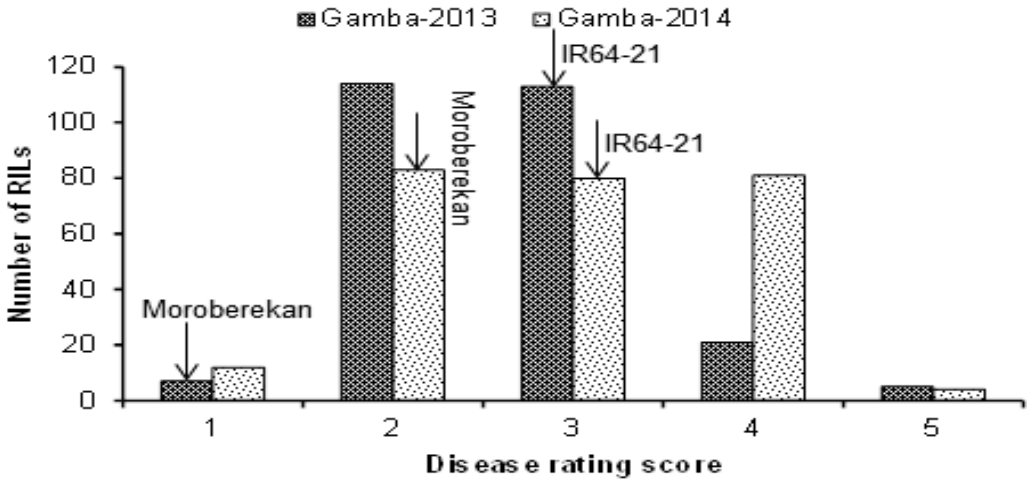

Figure 4.11 Frequency distribution disease rating for recombinant inbred population at Gamba

The RI population exhibited transgressive segregation in both directions for disease rating measured in the year 2013 and 2014 at all the experimental locations (Figure 4.9-4.11). Some of the RILs were highly resistant than the Moroberekan. IR64-21 was susceptible to blast populations at both Mwea and West Kano while Moroberekan was resistant. However, both parents were resistant to blast population at Gamba.

The results of the combined analysis of variance showed that effects due to the environments, genotype (lines) as well as genotype × environment interaction significantly (P≤0.05) differed for rice blast infection (Table 4.6). Genotype × environment contributed the highest source of variation at 50.69% followed by genotype (25.38%) and environments (6.28%). A significant interaction observed reflected different blast populations in the experimental locations. This environmental effect highlights the importance of conducting the experiments under natural infestation conditions. The broad sense heritability was 0.60 across the six environments, indicating that genotype of the RILs contributed largely to the source of variation. As there was a significant genotype × environment interaction effect, QTL analyses was done separately for each of the six test environment used.

Table 4.6 Analysis of variance of disease severity scores for recombinant inbred population

Source of variation	df	MS	Sig.
Replication	2	0.50	0.17
Genotypes	259	4.79**	0.00
Environments	5	153.53**	0.00
Genotypes × Environments	1295	1.91**	0.00
Error	3118	0.28	

**Significant at P ≤ 0.05, df = degree of freedom, MS = mean square

4.3.2 Detection of polymorphism

Single nucleotide polymorphism survey between IR64-21 and Moroberekan was carried out using 4606 markers. Among 4606 SNP markers tested, 2422 were polymorphic between the two parents. Of the 2422 polymorphic markers identified, 6 markers located on chromosome 4 (1), 5 (1), 8 (2) and 10 (2) gave different parental genotypes across the replications. Hence, they were excluded from further analysis leaving 2416 markers that

showed consistent polymorphism between the two parents. The overall level of polymorphism detected by the SNP markers was 52.45%. These markers were evenly distributed across the 12 chromosomes covering 95.48% (364.73Mb) of the rice genome published by the International Rice Genome Sequencing Project (IRGSP, 2005), ranging from 91.73% on chromosome 11 to 98.7% on chromosome 12 (Table 4.7). There was an average of 1.59 SNPs per centimorgan (cM) across the whole genome. The linkage map that was generated by polymorphic SNP markers had a total distance of 1526.8 cM with an average distance of 0.63 cM between adjacent markers.

Table 4.7 Information on polymorphic SNP markers between IR64-21 and Moroberekan

Chr[a]	TM[b]	SP[c]	EP[d]	GC[e]	SWC[f]	CP[g]	GD[h]	SNP[i]/cM
1	274	194844	42492399	42.3	45.06	93.87	181.8	1.51
2	260	134511	35691841	35.56	36.82	96.58	157.9	1.65
3	301	470708	35737093	35.27	37.26	94.66	166.4	1.81
4	204	222177	34935052	34.71	35.86	96.79	129.6	1.57
5	157	277001	29584298	29.31	30.04	97.57	122.3	1.28
6	161	244274	30809492	30.57	32.12	95.17	124.4	1.29
7	182	184193	28989771	28.81	30.36	94.89	118.6	1.53
8	168	250632	28058830	27.81	28.53	97.48	121.1	1.39
9	189	348460	22680525	22.33	23.84	93.67	93.5	2.02
10	136	649600	23033344	22.38	23.66	94.59	83.8	1.62
11	198	681662	28958989	28.28	30.83	91.73	117.9	1.68
12	186	119957	275206623	27.4	27.76	98.7	109.5	1.70
Total	2416			364.73	382.14	95.48	1526.8	

[a]Chromosome,[b]Total polymorphic markers per chromosome, [c]Starting position in base pair, [d]Ending position in base pairs, [e]Genome coverage in mega base (Mb) pair, [f]Size of whole chromosome in Mb as described by International Rice Genomic Sequencing Plan (IRGSP, 2005), [g]Coverage percentage (%), [h]Genetic distance in centimorgan and [h]Genetic distance and [i]SNP per centimorgan

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4.3.3 Segregation of polymorphic markers in $F_{6.7}$ RI lines

A total of 2416 markers showing polymorphism between the two parents were chosen for mapping and QTL analysis for the F_7 RI lines. On average, allele frequencies were 57.7%

and 42.3% for IR64-21(recipient parent) and Moroberekan (donor parent), respectively. This slightly deviated from the expected 50%: 50% for RI population. Some of the markers segregated into a 1: 1 ratio for the maternal and paternal parent. Of 2416 markers, 537 loci were skewed toward *indica* (IR64-21) alleles and 29 toward *japonica* (Moroberekan) alleles (Table 4.8). In total, 23.43% of the markers showed a distorted segregation ratio. The allelic frequencies for IR64-21 ranged from 13.6 to 100%, and the 12 chromosomes showed varying level of segregation distortion (Table 4.8). Chromosome 3, 7 and 12 had the most skewed (>70%) segregation favouring *indica* alleles. Segregation was most distorted for locus 12852964 located on chromosome 12 where all the alleles were skewed toward *indica* alleles. Chromosome 2, 4, 5, 6, 7, 8, and 9 had between 2 and 10 markers skewed (>70%) toward Moroberekan alleles.

Table 4.8 Distorted markers on rice chromosome in the IR64-21 and Moroberekan cross

Chr	No. of markers	Markers skewed to IR64-21	Markers skewed to Moroberekan	Distorted markers
1	274	41	0	41
2	260	23	2	25
3	301	100	0	100
4	204	17	10	27
5	157	9	6	15
6	161	25	2	27
7	182	81	3	84
8	168	27	4	31
9	189	50	2	52
10	136	52	0	52
11	198	34	0	34
12	186	78	0	78
Total	2416	537	29	566
Allele frequency		57.7	42.3	
Range (%)		13.6-100	0-86.4	

*χ^2-test to expected allelic frequency of 1:1 ($P \leq 0.01$).

4.3.4 QTL for blast resistance

Eighteen putative QTL were identified and mapped onto rice chromosomes 1, 2, 3, 4, 5, 6, 8, 9 and 11 (Table 4.9, Figure 4.12). No QTL was detected on chromosome 7, 10 and 12. The identified loci were associated with the $\log_{10}$-likelihood ratio (LOD) score threshold of 2.5 or above. Of the 18 QTL, 12 putative resistance loci were contributed by Moroberekan while IR64-21 provided six loci. Among the 18 QTL detected, 4 QTL (*qrbr-5*, *qrbr-6*, *qrbr-9-2*, and *qrbr-11*) conferred resistance to blast at Mwea-2013 and Mwea-2014. These QTL were detected with LOD scores of 2.67, 3.65, 3.37 and 4.47, respectively. The QTL explained 5.02%, 7.00%, 7.77%, and 10.92%, respectively of the observed phenotypic variance of resistance against blast (Table 4.10). Together, *qrbr-5*, *qrbr-6*, *qrbr-9-2*, and *qrbr-11* explained 30.71% variation for resistance to blast at Mwea. On combining the data of the two environments, *qrbr-9-1* and *qrbr-11* were identified at Mwea (Table 4.10).The identified QTL exhibited LOD scores of 3.35 and 4.17 and explained 8.15% and 10.19% of the phenotypic variation.

Four putative namely; *qrbr-1-1*, *qrbr-1-2*, *qrbr-1-4* and *qrbr-3* QTL located on chromosome 1 and 3 conferred resistances to blast population at West Kano-2013 and West Kano-2014 (Table 4.9; Figure 4.12). These QTL were detected with LOD scores of 3.45, 3.55, 6.91 and 4.76, respectively. They explained 8.54%, 9.09%, 16.83% and 12.05%, respectively of the observed phenotypic variance for blast severity (Table 4.9). A QTL *qrbr-1-4* on chromosome 1 that was flanked by markers id1019016 and 1086147 contributed to high phenotypic variation for rice blast severity. The QTL *qrbr-3* was mapped between markers 3495053 and 3550679 on chromosome 3. The QTL identified

at West Kano contributed to 46.51% of the total variation of lesion type observed under natural infection conditions. Combined data analysis at West Kano showed the existence of one QTL located on chromosome 3 with LOD score of 4.91 that explained 13.04% of the phenotypic variation (Table 4.10).

Table 4.9 Putative QTL associated with field blast resistance in the recombinant inbred population as determined by LOD>2.5

[a]QTL	[b]Chr	Pos (Mb)	Nearest marker	Location and Year	[c]LOD	[d]ADD	[e]P	[f]PVE
qrbr-1-1	1	27.77	926187	West Kano-2013	3.45	-0.23	P1	8.54
qrbr-1-2	1	30.50	1011161	West Kano-2013	3.55	-0.23	P1	9.09
qrbr-1-3	1	30.71	1047154	Gamba-2013	3.25	0.15	P1	7.07
qrbr-1-4	1	31.52	id1019016	West Kano-2013	6.91	0.32	P1	16.83
qrbr-2-1	2	24.77	2181296	Gamba-2013	4.22	-0.18	P2	10.03
qrbr-2-2	2	29.69	2326123	Gamba-2013	4.62	-0.16	P2	7.79
qrbr-3	3	35.74	3550679	West Kano-2014	4.76	-0.43	P2	12.05
qrbr-4-1	4	27.84	4601965	Gamba-2014	3.32	-0.19	P2	7.23
qrbr-4-2	4	30.6	4678550	Gamba-2014	4.67	-0.23	P2	11.01
qrbr-4.3	4	32.30	4733006	Gamba-2013	7.81	-0.21	P2	13.44
qrbr-4-4	4	34.10	4776434	Gamba-2013	7.69	-0.21	P2	13.48
qrbr-5	5	3.45	4909480	Mwea-2014	2.67	0.25	P2	5.02
qrbr-6	6	15.59	6385433	Mwea-2014	3.65	-0.29	P2	7.00
qrbr-8-1	8	0.86	id8000315	Gamba-2014	2.56	0.17	P1	6.36
qrbr-8-2	8	18.10	8725484	Gamba-2014	2.81	0.15	P1	5.09
qrbr-8-3	8	27.45	9035803	Gamba-2013	2.66	0.17	P1	6.36
qrbr-9-2	9	21.02	9844166	Mwea-2013	3.37	-0.24	P2	7.77
qrbr-9-2	9	21.02	9844166	Mwea-2014	3.23	-0.31	P2	8.06
qrbr-11	11	19.34	11566032	Mwea-2013	4.47	-0.28	P2	10.92

[a]QTL were detected with a minimum Logarithm of odds (LOD) threshold of 2.5, [b]Chromosome number, [c]LOD score supporting the existence of a QTL, [d]ADD= additive effects of resistance allele, [e]P = parent contributing resistance allele; P1 = IR64-21, P2 = Moroberekan and [f]PVE = Percent phenotypic variance explained by the QTL

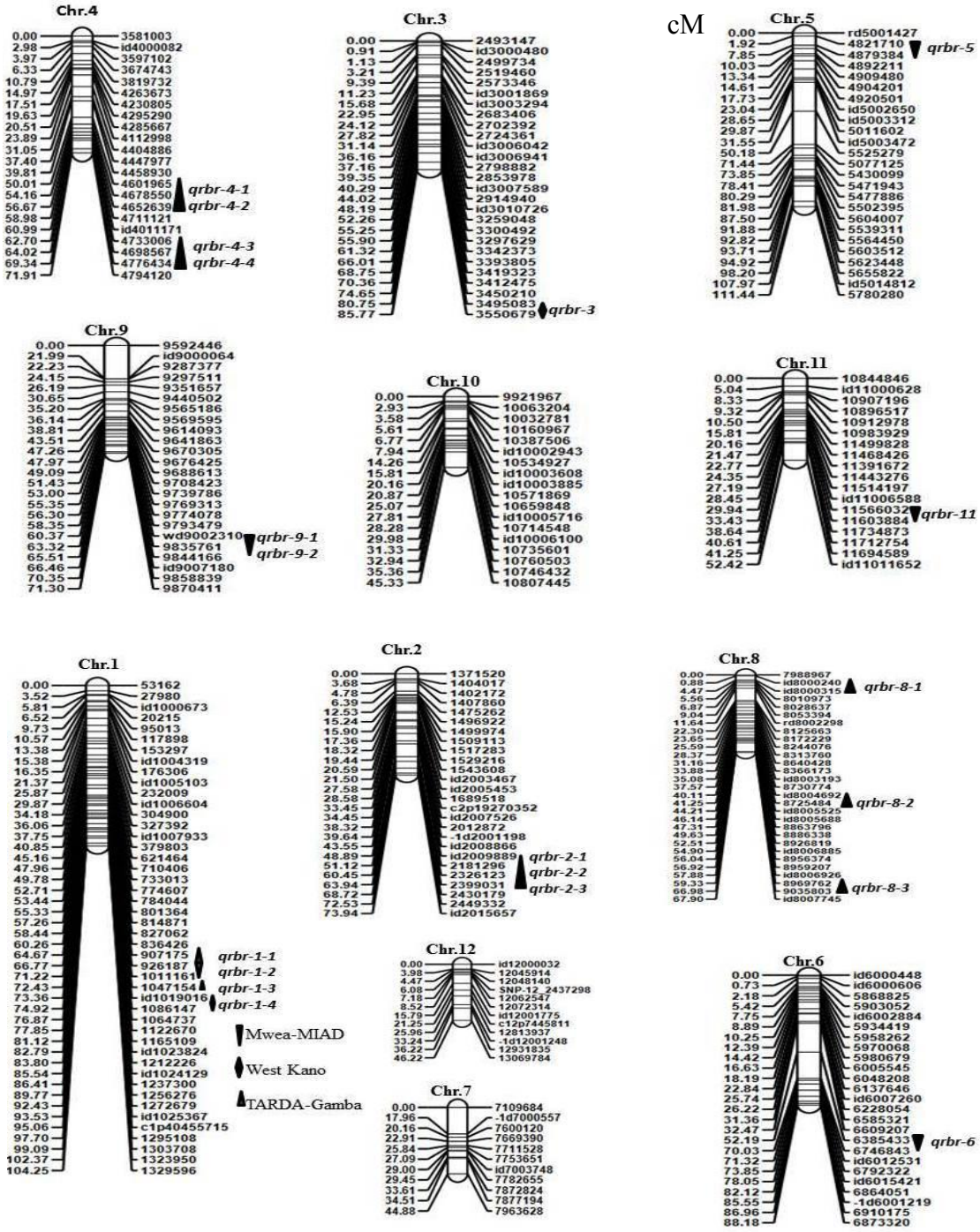

Figure 4.12 Genomic locations of quantitative trait loci affecting blast resistance in the recombinant inbred population at the three sites. Map units are expressed in centiMorgan (cM)

Ten QTL namely; *qrbr-1-3*, *qrbr-2-2*, *qrbr-2-3*, *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3*, *qrbr-4-4*, *qrbr-8-1*, *qrbr-8-2* and *qrbr-8-3* were detected on chromosome 1, 2, 4 and 8 at Gamba (Table 4.9; Figure 4.12). These QTL were identified with LOD scores of 3.25, 4.22, 4.62, 3.32, 4.67, 7.81, 7.69, 2.56, 2.81 and 2.66, respectively. They explained 7.07%, 10.03%, 7.79%, 7.23%, 11.01%, 13.44%, 13.48%, 6.36%, 5.09% and 4.51%, respectively of the observed phenotypic variation for resistance against blast population. Three QTL, *qrbr-2-1*, *qrbr-4-3*, and *qrbr-4-5* located on chromosome 2 and 4 with LOD scores of 3.44, 8.89, and 8.24 were identified from combined data for Gamba. These QTL explained 6.73%, 15.99%, and 18.14% of the observed phenotypic variation, respectively.

Table 4.10 Summary of quantitative trait loci associated with field blast resistance in the recombinant inbred population derived between IR64-21 and Moroberekan

[a]QTL	[b]Chr	Pos (Mb)	Nearest marker	Location and Year	[c]LOD	[d]ADD	[e]PVE
qrbr-1-1	1	27.77	926187	West Kano-2013	3.45	-0.23	8.54
qrbr-1-3	1	30.50	1011161	West Kano-2013	3.55	-0.23	9.09
qrbr-1-4	1	30.71	id1019016	West Kano-2013	6.91	0.32	16.83
qrbr-2-1	2	23.98	id2009889	Gamba	3.44	-0.14	6.73
qrbr-2-2	2	24.77	2181296	Gamba-2013	4.22	-0.18	10.03
qrbr-2-3	2	29.69	2326123	Gamba-2013	4.62	-0.16	7.79
qrbr-3	3	35.74	3550679	West Kano-2014	4.76	-0.43	12.04
qrbr-3	3	35.74	3550679	West Kano	4.91	-0.304	13.04
qrbr-4-1	4	27.84	4601965	Gamba-2014	3.32	-0.19	7.23
qrbr-4-2	4	30.60	4678550	Gamba-2014	4.67	-0.23	11.02
qrbr-4-3	4	32.30	4733006	Gamba-2013	7.81	-0.21	13.44
qrbr-4-3	4	32.30	4733006	Gamba	8.89	-0.21	15.99
qrbr-4-4	4	34.10	4698567	Gamba-2013	7.69	-0.21	13.47
qrbr-4-4	4	34.10	4698567	Gamba	8.24	-0.22	18.14
qrbr-6	6	15.59	6385433	Mwea-2014	3.65	-0.29	7.00
qrbr-9-1	9	20.68	9835761	Mwea	3.35	-0.26	8.15
qrbr-9-2	9	21.02	9844166	Mwea-2013	3.37	-0.24	7.77
qrbr-11	11	19.34	11566032	Mwea-2013	4.47	-0.28	10.92
qrbr-11	11	19.34	11566032	Mwea	4.17	-0.29	10.19

[a]QTL were detected with a minimum Logarithm of odds (LOD) threshold of 2.5, [b]Chromosome number, [c]LOD score supporting the existence of a QTL, [d]ADD= additive effects of resistance allele and [e]PVE = Percent phenotypic variance explained by the QTL.

When the 18 putative QTL identified with LOD>2.5 were subject to 1000 permutations (P≤0.05), 13 QTL across the six environments were retained with LOD scores above the threshold value of 3.3 (Table 4.10). These QTL that accounted for 7.00 to 13.47% were located on chromosome 1, 2, 3, 4, 6, 9 and 11, respectively at LOD scores of 3.32 to8.89.

A number of blast resistance QTL co-localized on the same chromosome region. Four QTL associated with rice blast resistance were located on chromosome 1 between SNP marker 907175 and 10886147 (Table 4.9 and 4.10). Two QTL *qrbr-2-1* and *qrbr-2-2* linked to SNP markers 2181296 and 2399031 on chromosome 2. Four QTL on chromosome 4 mapped to the same chromosome region. In addition, two blast resistance loci on chromosome 8 and 9 co-localized on the same chromosomal region. QTL with both minor and relatively major effects (PVE > 10%) were identified in this study. Some putative QTL sharing common SNP markers were located closely in the same chromosome region.

Most of the QTL identified were influenced by environment. The QTL *qrbr-11* was specific to Mwea-2013. Similarly, *qrbr-5* and *qrbr-6* were specific to Mwea-2014. However, *qrbr-9-2* located at the same position on chromosome 9 and flanked by the same SNP markers wd9009310 and 98411166 was observed in Mwea-2013 and Mwea-2014. At West Kano, environment specific QTL were observed on chromosome 1 and 3. Three QTL *qrbr-1-3*, *qrbr-2-2* and *qrbr-2-3* located on chromosome 1 and 2 were observed in Gamba-2013. QTL *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3*, *qrbr-4-4*, *qrbr-8-2* and *qrbr-8-3* were detected at Gamba across the two years (Table 4.9 and 4.10).

CHAPTER FIVE

DISCUSSION

5.1 Virulence spectrum of blast populations across the locations

Knowledge about the virulence spectrum of blast population using rice differential lines may provide a useful guide for the design of strategies to increase durable resistance genes in rice. There was a significant variation in the response of the rice genotypes against blast population within and among locations, indicating that phenotypic expressions of varietal reaction to blast infection under diverse environmental conditions vary considerably. Further, rice blast incidence varied significantly, suggesting the differences in disease pressure at the three locations. Furthermore, there was a significant interaction between seasons, locations and rice genotypes suggesting that blast population differed within and among the locations. Differences in clustering of rice genotypes in the dendrogram were also observed demonstrating a varied response to blast population at each location exists. Environmental conditions in different locations influence the expression of blast resistance genes and consequently the variation in blast population (Castejón-Muñoz, 2008; Mousanejad *et al.*, 2009; Ghaley *et al.*, 2012; Akator *et al.*, 2014). In this study, high level of environmental variation in terms of relative humidity, strength of wind, temperature and rainfall was observed and these could influence the amount of fungal sporulation thereby affecting the virulence of blast population. The population of *P. oryzae* in the mid altitude site exhibited wide spectrum of virulence than at high altitude site (Thinlay *et al*., 2000a, 2000b; Ghaley *et al*., 2012). Consequently in this study, the diferences in altitude could also explain the varied response of rice differential lines and local varieties to blast population within and among the locations.

Many rice differential lines carrying known resistance genes were susceptible to rice blast in the year 2014 than 2013 at Gamba. The rice differential lines in the year 2014 were subjected to intermittent water stress compared to the same crop in the year 2013 due to breakdown in irrigation facility at Gamba. Therefore, the high number of rice differential lines susceptible to blast population in the year 2014 could be attributed to water stress effects. Plants accumulate abscisic acid (ABA) under water deficit conditions and ABA accumulation correlates with susceptibility of rice plants to *P. oryzae* (Mayek-Perez *et al*., 2002; Xiong & Yang, 2003; Koga *et al*., 2004). Further, ABA has been detected in the hyphae and conidia of *P. oryzae* implying that blast fungus-derived ABA could play a role in triggering ABA signaling in host infection sites (Jiang *et al*., 2010). As the pathogen requires ABA to infect the host, the secretion of ABA by host plant under water stress conditions may have accelerated penetration of the host at Gamba in 2014 unlike 2013.

West Kano exhibited 68.63% of rice differential lines showing susceptibility to blast population followed by 49.02% in Mwea and 40.2 % at Gamba across the two years. This shows that blast population at West Kano had a wide spectrum of virulence at Mwea and Gamba. The high virulence spectrum observed at West Kano and Mwea may be attributed to the different rice varieties cultivated over the years. This is because host genotypes grown in a given location are known to influence the composition of the blast population (Chen *et al.,* 1996; Park *et al.,* 2003; Akator *et al.,* 2014). The findings in this study are in agreement with those observed in West African countries and Bhutan using rice differential lines (Odjo *et al*., 2011; Ghaley *et al.,* 2012; Idowu *et al*., 2013).

The low number of rice differential lines susceptible to blast population at Gamba may be due to the few rice varieties grown by farmers as well as a short history of rice cultivation. Rice farming in Gamba commenced in 1993 with four cultivars namely; IR-50, Mutant-Z, Tana 1 and UPR-103-80-1-2 unlike in West Kano and Mwea where rice cultivation using different varieties has been in existence for many years (TDIP report, 2013). In 1997, rice farming in Gamba was suspended due to heavy rains until the year 2009 when the scheme was revived. Since then, ITA310 and BAS 370 have been interchangeably planted by the local farmers. Roumen et al. (1997) and Fuentes et al. (2003) investigated the diversity of blast populations and observed a high genetic similarity in areas that grew a reduced number of rice cultivars as well as experiencing recent introduction of the crop. Owing to the continued cultivation of few cultivars, the blast population at Gamba might not have been subjected to the extreme bottlenecks imposed by the introduction and widespread cultivation of exotic rice materials containing broad-spectrum resistance genes (Mekwatanakarn *et al*., 2000). Therefore, the population at Gamba may offer a glimpse into the composition of *P. oryzae* population before the advent of modern plant breeding that disseminated multiple major and locally novel resistance genes.

5.2 Resistance genes effective against blast populations

The rice line IRBLzt-IR56 [CO] carrying Piz-t had an extremely high resistance spectrum at each site and across the sites. In addition, IRBLta2-PI [CO], IRBLta2-RE [CO] and IRBLta2-IR64 [CO] carrying *Pita-2* were also top resistant lines across the experimental sites. However, blast populations were able to cause a compatible reaction in a number of rice differential lines including those carrying *Pii*, *Pia* and *Pita* genes at different sites.

Similarly, this phenomenon has been observed in Central and Southern China, West Africa and Bhutan (Chen *et al*., 2001; Séré *et al*., 2007; Ghaley *et al*., 2012). This may be related to the use of resistance gene (s) in rice cultivars grown in these experimental sites. The ease with which blast populations caused compatible interaction with rice lines carrying genes *Pii, Pia,* and *Pita* may be an indication that these genes have been extensively used in the past and hence lost their ability to confer resistance due to variation of the blast pathogens. The upland rice variety 'Sensho' carrying *Pi34* and widely cultivated in Japan was found to be susceptible to a blast isolate that originated from an upland rice variety (Zenbayashi-Sawata *et al*., 2005). Further, *Ingngoppor-tinawon,* a landrace carrying resistance loci *qBFR4* that is widely grown in the Philippines was found to be susceptible to blast isolates (Mizobuchi *et al*., 2014).

The resistance reaction exhibited by rice lines carrying resistance genes *Piz-t, Pik-m, Pita-2*, *Pish*, *Piz,* and *Pit* may suggest that these genes may not be present in cultivars used in rice production in the irrigated ecosystems in Kenya. Further, the environmental variation at the locations could have influenced the virulence of the blast populations, leading to the resistance reaction. Furthermore, the rice differential lines with genes that conferred resistance to blast population at each and across sites may have a wide spectrum of resistance or are more adaptive than other genes. Several resistance genes such as *Pi9*, *Pi2, Piz-5*, *Piz-t*, *Piz*, *Pigm* and *Pita-2* confer broad-spectrum resistance against blast races when introgressed in sensitive cultivars (Ghaley *et al*., 2012; Hua *et al*., 2012; Lei *et al*., 2013). The knowledge on R genes in rice varieties grown in Kenya would be essential in differentiating one possibility form another. This is because a local

variety, BW196 carrying unknown gene (s) was resistant to blast population across the experimental locations.

The effectiveness *Piz-t* gene in controlling *P. oryzae* suggests that it could be a useful gene in breeding programs in Kenya. However, in this study, there was differential response of rice lines to blast populations suggesting that diversifying the resistance genes in various rice breeding programs may be a useful strategy. Accumulating different resistance genes into a single genetic background provides durable resistance to blast as the resistance of cultivars that carry several resistance genes is longer lasting than in those with single genes (Chen *et al*., 1996; Chen *et al*., 2001; Fukuoka *et al*., 2015). Consequently, introgression of resistance genes such as *Piz-t*, *Pish*, *Pik-m* and *Pita-2* into a susceptible rice cultivar would provide significantly stable and durable resistance against blast populations in irrigated ecosystems in Kenya. However, introgression of several resistance genes into the genetic background of susceptible cultivars should be carried out cautiously because most rice differential lines in this study were susceptible to blast population and therefore, are virulent to multiple resistance genes. Gene pyramiding may exert selection on the pathogen that could lead to the emergence of virulent variants capable of overcoming multiple resistance genes (Chen *et al*., 2001; Poland *et al*., 2009). Therefore, use of either varieties carrying single resistance genes or those carrying several genes may be a good strategies in controlling rice blast as evidenced in China and Japan (Wang *et al*., 1998; Ishizaki *et al*., 2005; Jiang *et al*., 2015).

In this study, rice differential lines IRBLb-B, IRBLzt-T, IRBLks-F5, IRBLks-CO [CO] and IRBLz5-CA (R) were susceptible at Mwea, West Kano and Gamba, unlike IRBLb-IT13 [CO], IRBLzt-IR56 [CO], IRBLks-S, and IRBLz5-CA containing the same resistance genes *Pib*, *Piz-t*, *Pik-s* and *Piz-5*. CO39 also showed moderate resistance to blast population at Gamba unlike IRBLa-C and IRBLa-A carrying the same *Pia* gene. Variation in resistance may be attributed to the background effect of donor varieties. Some donor varieties carry several resistance genes that are involved in conferring resistance to *P. oryzae* (Mekwatanakarn *et al.*, 2000; Chauhan *et al*., 2002; Jia, 2009; Yu *et al.*, 2014). CO39 carries both *Pia* and *Pi-CO39(t)* and transgenic rice lines expressing either *Pia* or *Pi-CO39(t)* were susceptible to blast isolates (Cesari *et al*., 2013), suggesting that these two genes are linked and an association between the proteins coded by these two genes is required to recognize the avirulence factors released by the pathogen. Therefore, interaction between *Pia* and *Pi-CO39(t)* may partly explain the moderate resistance of CO39 that carries the same *Pia* gene as rice line IRBLa-C and IRBLa-A. Similar results were observed in Asia and West Africa where rice differential lines carrying similar genes from different donor varieties differed in their reaction to *P. oryzae* populations (Chen *et al.*, 2001; Odjo *et al.*, 2011; Akator et *al.*, 2014).

Alternatively, the unexpected results could be explained by either a suppressor gene or a mutation in the rice differential lines showing susceptible reaction. The rice cultivar IR36 was found to be susceptible to certain blast isolates unlike IR64 containing the same resistance gene *Pi33* (Ballini *et al*., 2007), suggesting that the loss of function could be attributed to a suppressor gene or mutation in IR36. Suppression of *Pm8*, *Pm17*, *Lr3*,

Rcr3/Cf2 and *Rme/Mi* genes for resistance to powdery mildew (*Erysiphe graminis* f.sp *tritici*), leaf rust (*Puccinia triticina*) and stem rust (*Puccinia graminis*) was observed in wheat (*Triticum eastivum* L.) (Zeller & Hsam, 1996; Kerber & Aug, 1999; Knott, 2000; Martin *et al*., 2003).

Rice differential lines IRBLk-KA, IRBLkp-K60, IRBLz5-CA, IRBLsh-S, IRBLsh-B, IRBLta2-PI and IRBLta2-RE carrying *Pik*, *Pik-p*, *Piz-5*, *Pish* and *Pita-2* genes in the LTH genetic background were susceptible to the blast population at Mwea. However, the same lines in the CO39 genetic background were resistant to blast population at Mwea. Similarly, rice lines IRBLk-KA and IRBL5-M showed variable pattern of gene expression in the two genetic backgrounds at Gamba. The two sets of rice differential lines have varied length of introgression segement that carry additional genes that may interact with known R genes resulting in lack of recognizition of the avirulence factors generated by the blast isolates (Tsunematsu *et al*., 2000; Fukuta et *al*., 2009; Kobayashi *et al*., 2009). Further, the varying levels of differentially expressed genes were observed in isogenic lines carrying the same R gene in different genetic backgrounds after infection with blast isolates (Onaga *et al.,* 2013), suggesting that background effects of LTH and CO39 could play a role in the reaction pattern observed in this study. Rice differential lines carrying the same genes in LTH and CO39 genetic backgrounds respond differently to *P. oryzae* population (Onaga *et al.,* 2013; Divya *et al.,* 2014).

In this study, some rice differential lines carrying the same genes in different genetic backgrounds showed resistant reaction to blast population at Gamba. The high

temperatures of up to 36°C was observed at Gamba may have contributed to the up-regulation of R genes in different genetic backgrounds resulting in comparable reaction pattern. High temperature of up to 35°C delayed progression of blast in isogenic lines carrying the same R gene in different genetic backgrounds (Onaga *et al*., 2013). Further, the expression of rice blast resistance gene, *Pib* was induced at high temperature (Wang *et al*., 2001b). A resistance gene, *Xa7* was effective against bacterial blight (*Xanthomonas oryzae pv. oryzae*) at high temperature (Webb *et al*., 2010). In wheat, *Yr36* was up-regulated at high temperatures leading to enhanced resistance against stripe rust (Fu *et al*., 2009).

5.3 Mapping of QTL conferring resistance to blast populations

5.3.1 Phenotypic variation of recombinant inbred lines

Transgressive segregation was observed as many RILs were more susceptible or resistant than the parents suggesting successfull introgression of gene (s) conferring resistance to rice blast. The difference in the frequency distributions of blast rating score was observed within and among the experimental locations. Further, analysis of variance showed significant differences in disease severity scores among RILs, environments and genotype × environment interaction. The variation observed indicates that the environment influenced resistance to blast populations. This might reflect the segregation of different blast resistance genes in the RI population that function at different stages in the infection process. The three locations in this study fall in different ecological zones (Jaetzold *et al*., 2007) and consequently experience variation in rainfall, temperature and relative humidity that may influence sporulation of blast races. In this study, environment specific QTL were observed indicating that there is existence of different blast races due

to variation in abiotic factors at the experimental locations. Therefore, breeding for specific environment is essential for blast resistance trait and each environment should be targeted separately. Environment specific QTL have been observed in other QTL mapping analyses (Wang *et al.*, 1994; Talukder *et al.*, 2005; Li *et al.*, 2008a).

5.3.2 Segregation distortion

In this study, 23.43% of the total polymorphic markers (2416) showed segregation distortion in RILs. Since the genetic backgrounds of IR64-21 (*indica*) and Moroberekan (*japonica*) are different and differ in genetic distance a certain proportion of segregation distortion was expected in this sub-specific cross (Taylor and Ingvarsson, 2003; Fishman *et al*., 2008; Alheit *et al*., 2011). Segregation distortion ranging from 5- 43.7% has been observed in different mapping populations (Xu *et al*., 1997; Matsushita *et al*., 2003; Liu *et al*., 2008; Reflinur *et al*., 2014). Wang et al. (1994) detected extremely distorted segregation ratios of up to 80% in RILs derived from a cross between CO39 and Moroberekan. The percentage of distorted markers observed in this study was low, consequently they are suitable for QTL mapping. Significant segregation distortion may affect linkage analysis in the estimation of linkage distance (Wu *et al*., 2010; Reflinur *et al*., 2014), and as a result, distorted markers observed in this study were excluded from the data set for linkage mapping.

Most distorted markers in this study were observed on chromosomes 3, 7 and 12. Segregation distortion regions 3, 4, 6, 7, 11, and 12 were found near gametophytic gene loci (*ga*) and sterility loci (*s*) (Xu et al., 1997; Zhao *et al*., 2006; Wu *et al*., 2010). Gametophyte and sterility genes observed on chromosome 3, 7 and 12 may be

responsible for segregation this study in the IR64-21/Moroberekan RI population. Segregation distortion detected in different crops is due to physiological, genetic factors such as gametic or zygotic selection, chromosomal rearrangement, genetic incompatibility, pollen competition, preferential fertilization, and environmental factors and these factors are commonly observed during recombinant inbred line development (Xu *et al*., 1997; Matsushita *et al*. 2003; Liu *et al*., 2008; Wang *et al*., 2009).

5.3.3 Comparative analysis of QTL for rice blast resistance

In this study, QTL mapping of the IR64-21 × Moroberekan population revealed eighteen QTL that conferred resistance to blast populations in irrigated ecosystems in Kenya. Three QTL namely; *qrbr-1-1*, *qrbr-1-2* and *qrbr-1-3* were located at 27.77 Mb, 30.5 Mb and 30.71 Mb on chromosome 1 mapped close to blast resistance genes *Pitp(t)* [25-28.67 Mb] and *Pit* [22.70-30.40 Mb] (Barman *et al.,* 2004; Hayashi *et al.,* 2010). The QTL *qrbr-1-4* at 31.52 Mb on chromosome 1 occurred in close proximity to resistance genes *Pi37(t)* [33.11-33.49 Mb] , *Pish* (33.38-35.28 Mb) and *Pi35(t)* [33.11-33.16 Mb] (Chen *et al.,* 2005; Takashashi *et al.,* 2010; Fukuoka *et al.,* 2014). Wang et al. (1994) detected a large DNA fragment conferring resistance to blast between 14.62 Mb and 34.94Mb on chromosome 1. A QTL located in the same position as *Pi35(t)* on chromosome 1 was observed in *O. rufipogon* (Hirabayashi *et al.,* 2010). This common correspondence in locations suggests that the QTL in this study may be identical to the specific resistance genes and QTL already described in other mapping populations. This provides evidence that at least the QTL observed in this study represent actual QTL from Moroberekan and IR64-21 lines.

This study clearly showed that QTL *qrbr-2-1*, *qrbr-2-2* and *qrbr-2-3* detected between 23.98 Mb and 32.56 Mb on chromosome 2 mapped in close proximity to *Pid(t)1* [20.14-22.6 Mb], *Pig(t)* [34.35-35.14 Mb] and *Pi-tq5* [34.61-35.66 Mb] (Chen *et al*., 2004; Zhou *et al*., 2004). These QTL also occurred in the same vicinity as *qBLASTads-2* [34.61-35.66 Mb] and *qLN2* [25.87-31.5 Mb] (Tabien *et al.,* 2002; Wu *et al*., 2005a). The phenotypic variance explained by each of QTL *qrbr-2-1*(6.73%), *qrbr-2-2* (10.03%) and *qrbr-2-3* (7.79%) in this study was lower than *qBLASTads-2* (21.2%) but had comparable effects as *qLN2* (8.4%). These differences in phenotypic effects could be attributed to the allelic variation at these and *qBLASTads-2* may be different from those observed in this study. A genomic region observed at 24.13 Mb corresponded well with *qrbr-2*-1 (Wang *et al*., 2014c). The comparable phenotypic effects observed on chromosome 2 in this study uggest that the genes controlling blast resistance may be the same.

QTL *qrbr-3* located between 33.71 Mb and 35.74Mb on chromosome 3 mapped in the same genomic interval as QTL for blast resistance, *qBFR-3a* [24.87-36.16 Mb], and *qBFR-3b* [33.80-36.16 Mb] (Sabouri *et al*., 2011). The QTL effects of the three QTL were comparable indicating that the genes underlying these QTL could be similar. Three chromosome regions conditioning rice blast resistance have been observed near SSR markers at 24.59 Mb, 28.6 Mb and 28.09 Mb (Chen *et al.,* 2003; Wu *et al*., 2004; Hirabayashi *et al*., 2010). Differences in the position of QTL in diverse mapping populations might be due to chromosomal reorganization brought about by inversions and translocation (Luo *et al.,* 2012).

The QTL *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3* and *qrbr-4-4* on chromosome 4 were located at 27.84 Mb, 30.60 Mb, 32.30 Mb and 34.10 Mb, respectively. They corresponded well with *qBR4-2* [30-34 Mb], *qBFR4* (31.5 Mb) and *Pi63* (31.2-31.5 Mb), respectively (Fukuoka *et al.*, 2012; Mizobuchi *et al.*, 2014; Xu *et al.*, 2014). A chromosomal region derived from wild relative of rice, *O. rufipogon* was detected in a similar position (Hirabayashi *et al.*, 2010). The individual QTL on chromosome 4 exhibited lower phenotypic effect than *qBR4-2* (29.40%) and *qBFR4* (73.50%). It is likely that there is allelic variation in QTL observed on chromosome 4 in this study and those previously described and this variation may be responsible for the differences in phenotypic effects. The predicted protein products of *qBR4-2a, qBR4-2b* and *Pi63* resembled previously observed disease resistance proteins containing a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (McHale *et al.*, 2006). Genetic variation in one or more NBS-LRR genes account for the differences in the phenotypic effects observed in rice (Bryan *et al.*, 2000; Zhou *et al.*, 2006; Ashikawa *et al.*, 2008; Wu *et al.*, 2013; Fukuoka *et al.*, 2014). Beneficial QTL alleles with the ability to alter disease resistance in a quantitative manner have been generated by duplication and substitution events in the NBS-LRR genes (Meyers *et al.*, 2003; Rao *et al*., 2005; Fukuoka *et al*., 2014).

A QTL *qrbr-5* at 3.45 Mb on chromosome 5 mapped near blast resistance gene *Pi26(t)* [2.07-2.76 Mb] and genomic region, *qRBr5.1* at 2.21Mb (Sallaud *et al*., 2003; Ashkani *et al*., 2013). Sabouri et al. (2011) detected *qBFR-5* on chromosome 5 with LOD threshold less than 3.0. The QTL *qrbr-5* and *qRBr5.1* explained 5.02% and 2.00%

phenotypic effects, respectively, and were observed with LOD threshold less than 3.0, suggesting existence of minor QTL at this position.

The QTL *qrbr-6* located between 15.59 Mb and 24.22 Mb on chromosome 6 in this study mapped in the same genomic interval as blast resistance genes *Pi13(t)* [12.46-16.30 Mb], *Pi25* [18.08-19.26 Mb], *Pid2* [17.16 Mb] and *Pi40(t)* [16.27-17.53 Mb], respectively (Pan *et al*., 1996; Zhuang *et al*., 2001; Chen *et al*., 2006; Jeung *et al*., 2007). The similarity in locations suggest that blast resistance genes previously observed may be the ones underlying QTL on chromosome 6 in this study. A large genomic region was mapped between 9.54 Mb and 24.45 Mb on chromosome 6 in RI lines derived from the CO39 and Moroberekan (Wang *et al*., 1994). Fukuta et al. (2004) identified one QTL *BR-1* at 19.96 Mb that mapped in the same interval as *qrbr-6*. In this study, *qrbr-6* was located in the same position with *qRBr-6.1*, *qRBr-6.2* and *qRBr-6.3* (Ashkani *et al*., 2013). The phenotypic effect contributed by *qrbr-6* (7%) in this study was higher than *qRBr-6.2* (4%) and *qRBr-6*.3(4%) but lower than *qRBr-6.1* at 16%. This variation in percent variance explained may suggest that alleles conditioning resistance to blast populations in this study may be different from those previously observed in other mapping populations.

The QTL *qrbr-8*-1 localized at 0.86 Mb was found in the vicinity of *rbr8* and *qBLAST8.1* that mapped to RFLP and SSR markers at 4.1 Mb and 4.0 Mb, respectively (Chen *et al.*, 2003; Jia & Liu, 2011). Apparently, these three QTL flank a major blast resistance gene, *Pi36* that mapped at 2.0 Mb (Liu *et al.*, 2007). The *qBLAST8.2* detected at 6.8 Mb and

coincides with the gene for sheath blight resistance also mapped close to *qrbr-8-1*(Jia & Liu, 2011). A blast resistance gene, *Pi33* identified between 5.92 Mb and 6.12 Mb lie in the vicinity of *qrbr-8.1* (Sallaud et al. 2003). A QTL *qrbr-8-2* at 18.10 Mb and blast resistance gene, *Pi-29(t)* at 16.24 Mb was detected almost in the same genomic position (Sallaud *et al.,* 2003). QTL *qrbr-8-2* and *qrbr-8-3* (27.45 Mb) mapped in close proximity to *BR8-1* (20.66-22.89Mb) (Fukuta *et al.,* 2004). QTL *BR8-1*, *qrbr-8-1*, *qrbr-8-2* and *qrbr-8-3* were observed with LOD threshold less than 3.0.

The QTL *qrbr-9-1* and *qrbr-9-2* (20.19-21-20.02 Mb) on chromosome 9 mapped in the same genomic interval as *rbr9a* (14.65-20.17 Mb), *rbr9c* (19.95-20.48 Mb) and *qtl9-5-1* (16.87-22.21 Mb), *qBLAST9.*1 (17.7 Mb), *qBLAST9.2* (17.9 Mb), respectively (Chen *et al.*, 2003; Shi *et al.,* 2010; Jia & Liu, 2011). The QTL effect of *qrbr-9* at 7.77% was comparable to *qtl9-5-1* (7.49%) and *qBLAST9.2* (7.62%). The remaining *rbr9a*, *rbr9c* (5.3%) and *qBLAST9.1* (4.64%) had a lower phenotypic effect compared to *qrbr-9*. These variations in the phenotypic effects suggest variation at these loci as well as differential expression of genes underlying the QTL on chromosome 9.

The QTL *qrbr-11* l at 19.34 Mb on chromosome 11 corresponded with blast resistance genes, *Pi7(t)* [18.18- 20.34 Mb], *Pi44* [20.55-26.00 Mb], *Pilm2*[13.64-28.38 Mb], *Pi34* [19.42-19.49 Mb], *Pi38* [19.14-21.98 Mb] and *Pi47*[22.01 Mb], respectively (Wang *et al*., 1994; Chen *et al*., 1999; Tabien *et al*., 2000; Zenbayashi-Sawata *et al*., 2002; Gowda *et al*., 2006; Huang *et al*., 2011). There QTL namely; *qLB11.1* (11.8-18.41Mb), *qtl11-2-2* and *qtl11-3-1* (18.62-23.77 Mb) mapped in close proximity as *qrbr-11* observed in this

study (Cho *et al.*, 2008; Shi *et al.*, 2010). Blast resistance genes *Pi-7(t)* and *Pi44* have been observed in Moroberekan and therefore, they are potential candidates for the genes conferring resistance in the IR64-21 × Moroberekan mapping population. This suggests that genes underlying *qrbr-11* on chromosome 11 may be similar to the blast resistance genes described previously in other rice cultivars and mapping populations. Blast resistance genes *Pi47* and *Pi48* genes in Xiangzi 3150 were observed in the same position as the QTL conditioning resistance to blast isolate 193-1-1 and to the *P. oryzae* in the field (Huang *et al*., 2011). Location of QTL and R genes in the genomic regions has also been detected in potato (*Solanun tuberosum*) and maize (*Zea mays*) (Gebhardt & Valkonen, 2001; Xiao *et al*., 2007).

5.3.4 Relationship of identified QTL with other diseases and agronomic traits

This study clearly showed that two QTL, *qrbr-1-4* and *qrbr-3* corresponded with *qBBR1* (32.47 Mb) and *qBBR3* (36.25 Mb) for bacterial blight (*Xanthomonas oryzae*) resistance (Han *et al*., 2014). Four QTL, *qrbr-5*, *qrbr-8-2*, *qrbr-9-1* and *qrbr-9-2* coincided with *qShB5*(3.25 Mb), *QSbr8a* (17.44 Mb), *qShB9-2* and *qshb9.2* (21.19- 22.72 Mb) conferring resistance to sheath blight (*Rhizoctonia solani*) in chromosomal locations (Li *et al.*, 1995; Liu *et al*., 2009; Han *et al.*, 2014; Yadav *et al*., 2015). Similarly, *qrbr-9-2* observed in this study coincided with one of the seven QTL for rice yellow mottle virus (Albar *et al*., 1998). Correspondence in location for QTL detected for resistance to various diseases suggests that some of the genes underlying QTL are commonly involved in the defense response against pathogens. *Benzothiadiazole* has been observed to induce systemic acquired resistance responses against rice blast, sheath blight and bacterial blight (Ge *et al*., 1999; Song *et al*., 2001; Zhang *et al*., 2004), suggesting that these

diseases may share common resistance pathways in rice. This provides an opportunity to study the relationships between QTL conferring resistance to other diseases or blast in rice.

In this study, four QTL *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3* and *qrbr-4-4* also coincided with a cluster of QTL associated with gall midge resistance gene, *Gm7* (Sardesai *et al*., 2002), root traits detected between 29.86 Mb and 30.77 Mb (Courtois *et al*., 2003), cold tolerance at booting stage (Saito *et al*., 2004) and chlorophyll content at 30.45 Mb (Takai *et al*., 2010) in rice. These results suggest that the QTL in this study may be functionally associated with important agronomic trraits in rice. Therefore, this observation highlights the importance of this chromosome region as a target for selection in breeding.

5.3.5 Blast resistance loci in the recipient parent

Six blast resistance QTL was observed in IR64-21. This is not unexpected since IR64 the progenitor of IR64-21 possess at least six blast resistance genes from over 38 different resistant cultivars (Sallaud *et al*., 2003; Ballini *et al*., 2007; Kobayashi *et al*., 2009). The progeny of these crosses were tested in blast nurseries and such screening experiments may have led to the selection of progenies that accumulated different resistance genes. Since IR64-21 was derived from a single IR64 plant, this progeny may have accumulated different resistance genes from IR64 and these genes might be the ones conditioning resistance to blast population in Kenya. This is because the QTL, *qrbr-8-2* located on chromosome 8 in this study mapped closely to the blast resistance gene *Pi29(t)* identified in IR64 (Sallaud *et al*., 2003). QTL conferring resistance to blast races have been

observed in susceptible parents in multiple rice mapping populations (Wu *et al*., 2004; Hu *et al*., 2008; Shi *et al*., 2010).

5.3.6 Implications for breeding

Since blast resistance in rice cultivars is short-lived and genetic analyses have focused on resistances that confer durable resistance (Wisser *et al*., 2005; Hu *et al*., 2008). Rice cultivars with durable resistance against blast races have maintained resistance over time, and multiple QTL underlie high levels of resistance (Wang *et al.,* 1994; Miyamoto *et al*., 2001; Saka, 2006). Pyramiding resistance gene (s) or QTL can achieve the same level or even a higher level of resistance than that controlled by a single R gene (Fukuoka *et al*., 2014; Fukuoka *et al*., 2015). Resistance genes and QTL observed in this study provide an ideal genetic stock for improving resistance of rice varieties through marker assisted breeding against blast pathogen populations in irrigated ecosystems in Kenya.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Blast population at West Kano had wide spectrum of virulence than at Mwea and Gamba, suggesting that high diversity of blast population exists at the three locations in irrigated ecosystems in Kenya.
2. Rice differential lines carrying *Pik-s*, *Pik-h*, *Piz-5*, *Piz*, *Pit*, *Pish Pi1*, *Pi5 (t)*, *Pi12 (t)*, *Pik-m*, *Pita-2*, *Pib* and *Pik* were resistant to blast population at different locations. *Piz-t* and *Pita*-2 were the most effective R genes across the locations. A very resistant local variety, BW196 carries unknown R gene(s).
3. Eighteen QTL with variable effects co-segregated with blast resistance. Several genes underlie QTL: *Pitp(t)*, *Pi7(t)*, *Pi25*, *Pi29(t)*, *Pi34*, *Pi35(t)*, *Pi36*, *Pi37 (t)*, *Pi38*, *Pi40(t)*, *Pi44*, *Pi47* & *Pi63*. Seven QTL *qrbr-1-4*, *qrbr-4-1*, *qrbr-4-2*, *qrbr-4-3*, *qrbr-3*, *qrbr-9-1*, and *qrbr-9-2* mapped to QTL for agronomic traits & bacterial blight and sheath blight.

6.2 Recommendations

1. In this study, a mixed population of blast was used. There is need to isolate and characterize blast races, and challenge them on rice differential lines under controlled conditions.
2. Most promising R genes and QTL should introgressed into preferred yet susceptible rice varieties in Kenya. Identification of resistance gene(s) in BW196 for inclusion in breeding programs.
3. Characterize QTL that confer resistance to multiple diseases & agronomic traits.

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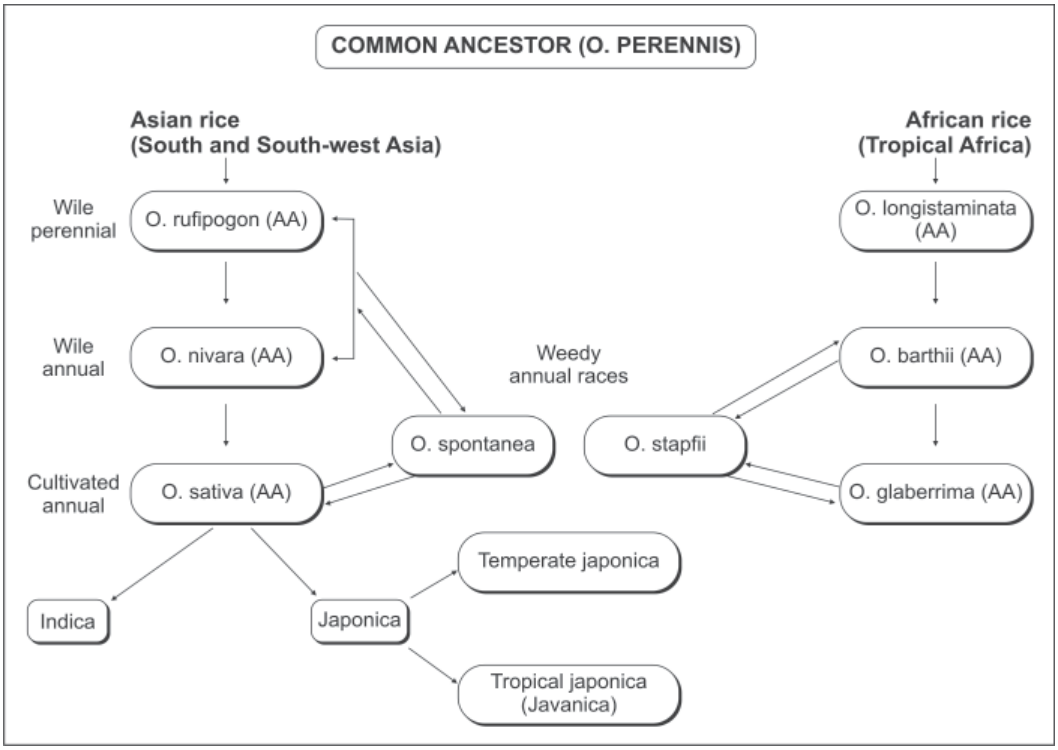
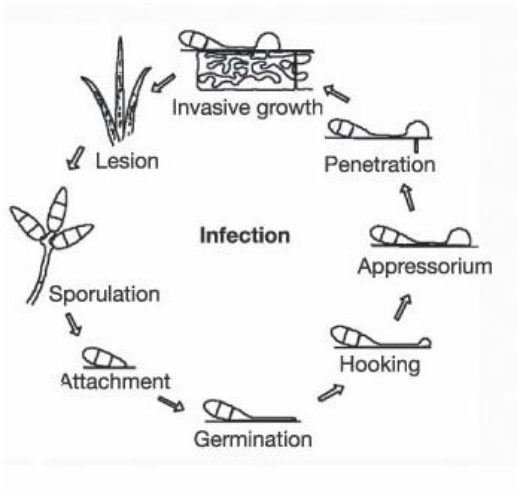
APPENDICES

Appendix i Schematic representation of the evolutionary pathways of Asian and African rice

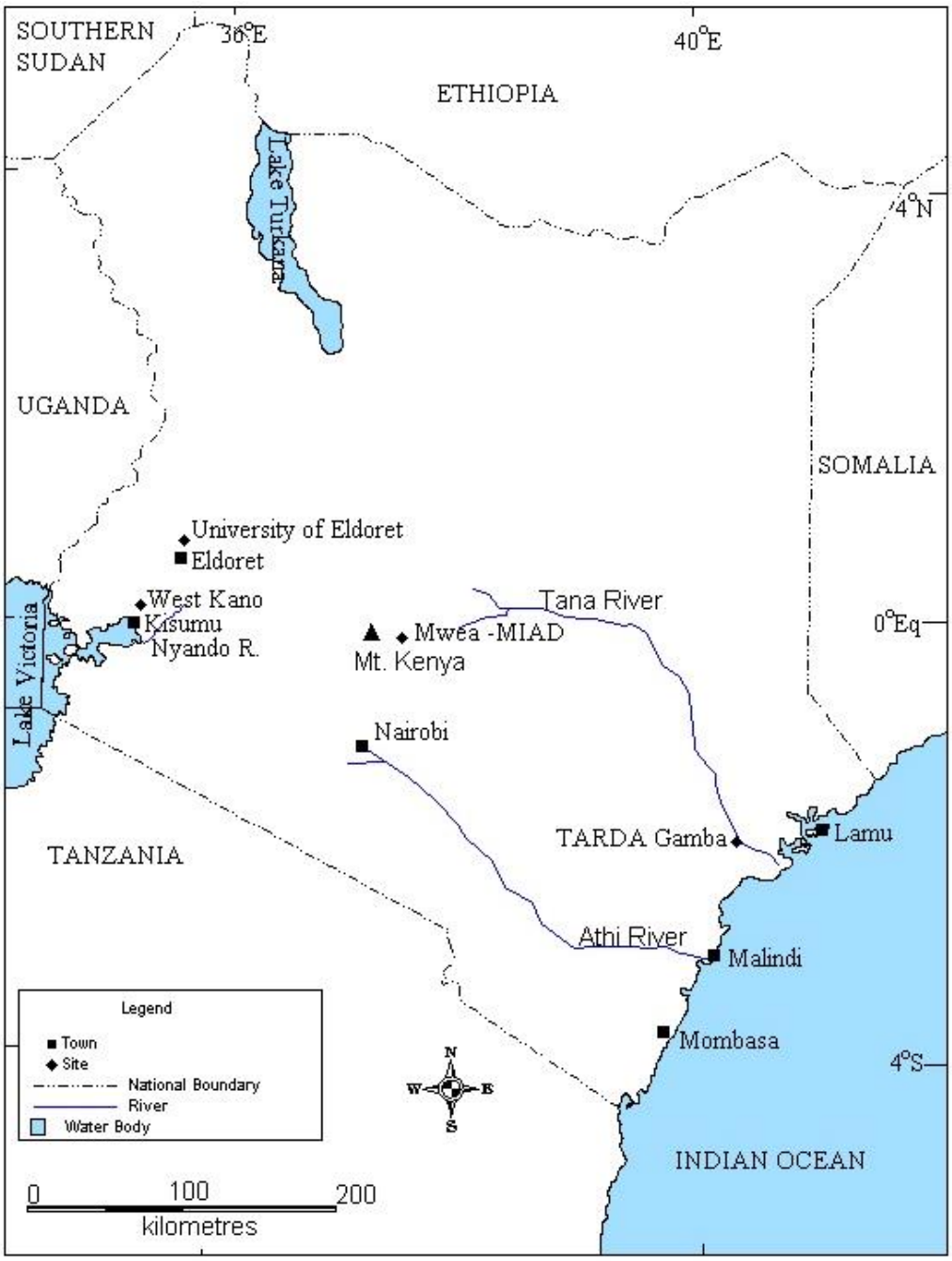


Apppendix ii Infection cycle of *P. oryzae*



Source: Dean et al. (2005)

Appendix iii Map of Kenya showing sites where field experiments were conducted



Map drawn by Luka Kanda, Department of Geography, Moi University.

Appendix iv Weather parameters at Mwea for the year 2013 and 2014

	Year 2013					Year 2014				
Month	Rain(mm)[a]	Temp(°C)[b]	Temp(°C)[c]	RH(%)[d]	Evp[e]	Rain(mm)[a]	Temp(°C)[b]	Temp(°C)[c]	RH (%)[d]	Evp[e]
Jan	2.02	29.28	18.45	77.13	5.20	0	30.15	16.77	71.48	6.16
Feb	0	31.38	19.14	73.18	6.64	5.54	30.52	19.64	75.04	5.69
Mar	**8.91**	**31.46**	**21.13**	**76.19**	**6.08**	**5.75**	**30.13**	**18.09**	**77.32**	**5.38**
Apr	13.72	28.9	21.51	77.7	5.28	4.17	28.70	16.40	82.53	4.52
May	1.81	27.79	20.40	78.61	4.35	3.25	28.18	17.82	80.01	4.54
Jun	0.5	25.07	17.84	80.73	3.33	1.43	26.76	16.36	78.71	3.75

a= Rainfall in millimeter, b = Maximum temperature, c = Minimum temperature, d = Relative humidity, e = evaporation rate f= Wind speed.

Appendix v Weather parameters at West Kano for the year 2013 and 2014

	Year 2013					Year 2014				
Month	Rain(mm)[a]	Temp(°C)[b]	Temp(°C)[c]	RH(%)[d]	WS[f]	Rain(mm)[a]	Temp(°C)[b]	Temp(°C)[c]	RH (%)[d]	WS[f]
Jan	4.52	28.80	17.30	58.00	4.51					
Feb	2.79	30.20	16.70	51.00	4.62					
Mar	9.31	29.80	18.00	62.00	5.36					
Apr	10.39	27.6	18.2	69.8	4.27		28.67	18.20	54.33	5.27
May	8.98	28.00	17.70	62.00	3.47	4.42	28.84	17.68	57.10	4.02
June	1.37	28.00	17.20	57.70	3.61	2.13	27.87	17.67	62.00	4.04
July	2.45	28.80	16.50	53.00	4.69	3.75	28.19	17.48	63.10	4.56

Appendix vi Weather parameter at Gamba for the year 2013 and 2014

Month	Year 2013					Year 2014				
	Temp (°C)[a]	Temp (°C)[b]	RH (%)[c]	RH(%)[d]	Rain (mm)[e]	Temp (°C)[a]	Temp (°C)[b]	RH (%)[c]	RH(%)[d]	Rain (mm)[e]
Jan	35.8	21.15	96.78	43.11	0.28					
Feb	35.96	21.21	95.68	41.81	0.19	35.42	22.11	86.77	43.49	0
Mar	36.06	23.14	96.45	46.89	2.83	36.05	23.01	90.79	45	2.79
Apr	34.45	23.72	95.51	51.94	0.65	35.19	22.91	94.59	48.93	1.45
May	30.61	22.74	95.62	61.29	4.88	**31.02**	**22.2**	**95.88**	**61.09**	**5.35**
Jun	**29.65**	**20.45**	**96.08**	**58.75**	**1.37**	30.18	20.35	96.77	58.25	2.97
Jul	29.62	19.33	96.3	53.5	0.18	30.57	19.56	96.49	52.68	0.9
Aug	29.55	19.73	96.98	54.29	3.05	30.83	18.91	97.1	51.93	1.81

Bold = Transplanting period, a = Maximum temperature, b= minimum temperature, c = maximum relative humidity, d = minimum relative humidity, e = rainfall in millimeter

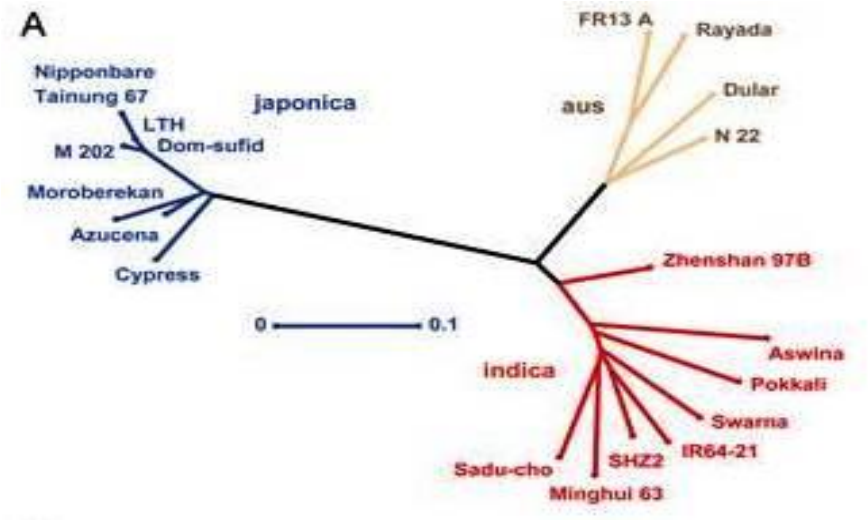
Appendix vii Rice genotypes evaluated for blast resistance in this study

ID	Designation	R gene	Remarks
1.	IRBLa-A	Pia	LTH monogenic line
2.	IRBLa-C	Pia	LTH monogenic line
3.	IRBLi-F5	Pii	LTH monogenic line
4.	IRBLks-F5	Pik-s	LTH monogenic line
5.	IRBLks-S	Pik-s	LTH monogenic line
6.	IRBLk-KA	Pik	LTH monogenic line
7.	IRBLkp-60	Pik-p	LTH monogenic line
8.	IRBLkh-K3	Pik-h	LTH monogenic line
9.	IRBLz-FU	Piz	LTH monogenic line
10.	IRBLz5-CA	Piz-5	LTH monogenic line
11.	IRBLzt-T	Piz-t	LTH monogenic line
12.	IRBLta-K1	Pita	LTH monogenic line
13.	IRBLta-CT2	Pita	LTH monogenic line
14.	IRBLb-B	Pib	LTH monogenic line
15.	IRBLt-K59	Pit	LTH monogenic line
16.	IRBLsh-S	Pish	LTH monogenic line
17.	IRBLsh-B	Pish	LTH monogenic line
18.	IRBL1-CL	Pi1	LTH monogenic line
19.	IRBL3-CP4	Pi3	LTH monogenic line
20.	IRBL5-M	Pi5(t)	LTH monogenic line
21.	IRBL7-M	Pi7(t)	LTH monogenic line
22.	IRBL9-W	Pi9	LTH monogenic line
23.	IRBL12-M	Pi12(t)	LTH monogenic line
24.	IRBL19-A	Pi19	LTH monogenic line
25.	IRBLkm-TS	Pik-m	LTH monogenic line
26.	IRBL20-IR24	Pi20	LTH monogenic line
27.	IRBLta2-PI	Pita2	LTH monogenic line
28.	IRBLta-RE	Pita2	LTH monogenic line
29.	IRBLta-CP1	Pita	LTH monogenic line
30.	IRBL11-ZH	Pi11(t)	LTH monogenic line
31.	IRBLz5-CA(R)	Piz5	LTH monogenic line
32.	LTH	None	LIjiangxintuanheigu, japonica cultivar with no blast R gene
33.	IRBLsh-KU[CO]	Pish	CO39 near-isogenic line
34.	IRBLsh-S[CO]	Pish	CO39 near-isogenic line
35.	IRBLsh-B[CO]	Pish	CO39 near-isogenic line
36.	IRBLb-IT13[CO]	Pib	CO39 near-isogenic line
37.	IRBLz5-CA[CO]	Piz5	CO39 near-isogenic line
38.	IRBLzt-IR56[CO]	Piz-t	CO39 near-isogenic line
39.	IRBL5-M[CO]	Pi5(t)	CO39 near-isogenic line
40.	IRBLks-CO[CO]	Pik-s	CO39 near-isogenic line
41.	IRBLk-KU[CO]	Pik	CO39 near-isogenic line
42.	IRBLk-KA[CO]	Pik	CO39 near-isogenic line
43.	IRBLkh-K3[CO]	Pik-h	CO39 near-isogenic line
44.	IRBLkm-TS[CO]	Pik-m	CO39 near-isogenic line
45.	IRBLkp-K60[CO]	Pik-p	CO39 near-isogenic line
46.	IRBL1-CL[CO]	Pi1	CO39 near-isogenic line
47.	IRBL7-M[CO]	Pi7(t)	CO39 near-isogenic line
48.	IRBLta-YA[CO]	Pita	CO39 near-isogenic line
49.	IRBLta-ME[CO]	Pita	CO39 near-isogenic line
50.	IRBLta2-PI[CO]	Pita2	CO39 near-isogenic line
51.	IRBLta2-RE[CO]	Pita2	CO39 near-isogenic line
52.	IRBLta2-IR64[CO]	Pita2	CO39 near-isogenic line

Appendix vii Rice genotypes evaluated for blast resistance in this study (Continued)

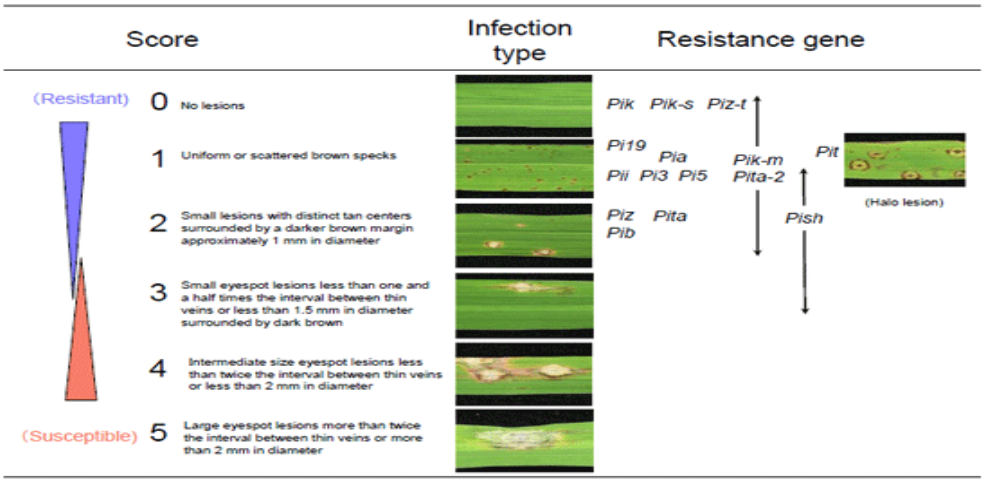
ID	Designation	R gene	Remarks
53.	CO39	Pia	*Indica* cultivar
54.	ITA310	Unknown	Local variety
55.	BW196	Unknown	Local variety
56.	BAS217	Unknown	Local variety

Appendix viii Dendrogram showing the relationship between IR64-21 and Moroberekan based on SNP information



Adapted from McNally et al. (2009).

Appendix ix Criterion used for leaf blast evaluation in the present study



Adapted from Bonman et al. (1986)

Appendix 1 Reagents prepared for DNA extraction at BECA/ILRI

Extraction buffer (1 liter)

100 mM Tris-HCl (100 µl of 1 M Tris-HCl, pH 8.0)

50 mM EDTA (10 ml of 0.5 M EDTA, pH 8.0)

500 mM NaCl (10 ml of 5 M NaCl)

2% CTAB (hexacetyl-ammoniumbromide) beffer

200 mM Tris-HCl (5 ml of 1 M Tris-HCl, pH 8.0)

50 mM EDTA (10 ml of 0.5 M EDTA, pH 8.0)

2 M NaCl (2.5 ml of 5 M NaCl)

Chloroform: isoamyl alcohol (24:1) (100 ml)

Chloroform (48 ml) and Isoamyl alcohol (2 ml)

Low salt TE buffer (10 mM) (1 liter)

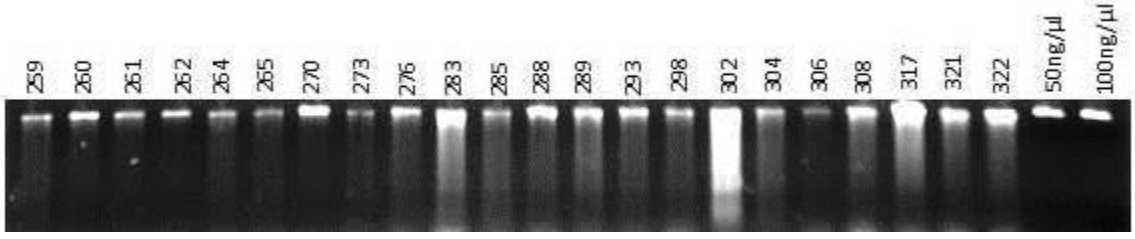
10 mM Tris-HCl (10 ml of 1 M Tris-HCl, pH 8.0)

1 mM EDTA (500 ml of 0.5 M EDTA)

Ethanol 70% (stored at -20°C)

Ethanol (absolute): 70 ml adjust to 100 ml with double distilled water.

Appendix xi Extracted genomic DNA from a sample of the recombinant inbred population derived from IR64-21 and Moroberekan cross



Appendix 2 Concentration and quality of genomic DNA measured using Nanodrop spectrophotometer

Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280
RIL_1	2234.9	1.95	RIL_29	4927.3	1.93	RIL_57	1771.6	1.89	RIL_85	4344.9	1.92
RIL_2	3482.9	1.98	RIL_30	4623.2	1.93	RIL_58	2446.3	1.89	RIL_86	5796.2	1.89
RIL_3	3459.7	1.96	RIL_31	3537.5	1.95	RIL_59	3753.4	1.94	RIL_87	5253.9	1.91
RIL_4	2172	1.94	RIL_32	5072.6	1.92	RIL_60	1583.3	1.88	RIL_88	4919.1	1.89
RIL_5	2622.4	1.96	RIL_33	2463.4	1.92	RIL_61	2624.1	1.88	RIL_89	3775.4	1.88
RIL_6	2744.8	1.96	RIL_34	4033.3	1.94	RIL_62	4588.7	1.93	RIL_90	3002.6	1.9
RIL_7	3352.3	1.94	RIL_35	285.2	1.64	RIL_63	2629.6	1.9	RIL_91	5106.1	1.89
RIL_8	3018	1.88	RIL_36	2722.4	1.92	RIL_64	3772.3	1.91	RIL_92	2945.6	1.85
RIL_9	4038.5	1.93	RIL_37	4423.6	1.95	RIL_65	4601	2.12	RIL_93	3538.2	1.89
RIL_10	4746.4	1.93	RIL_38	5197.1	1.91	RIL_66	2487.4	1.88	RIL_94	2935	1.86
RIL_11	3561.9	1.95	RIL_39	4651	1.93	RIL_67	4202.9	1.91	RIL_95	4956.1	1.9
RIL_12	4232.9	1.93	RIL_40	4337.2	1.95	RIL_68	4620	1.91	RIL_96	3231.5	1.91
RIL_13	3860.8	1.94	RIL_41	3713.3	1.93	RIL_69	3514.1	1.91	RIL_97	3278.6	1.94
RIL_14	3967.7	1.93	RIL_42	3309.9	1.93	RIL_70	2915.8	1.86	RIL_99	3836.6	1.89
RIL_15	2541.5	1.93	RIL_43	3427.8	1.92	RIL_71	2844	1.88	RIL_100	2984.8	1.85
RIL_16	3520.8	1.97	RIL_44	5983.4	1.89	RIL_72	3750.5	1.89	RIL_101	4823.5	1.89
RIL_17	3028.9	1.9	RIL_45	3172.8	1.93	RIL_73	4212.6	1.91	RIL_102	2673.7	1.85
RIL_18	4582	1.93	RIL_46	4420	1.93	RIL_74	4402.2	1.89	RIL_103	4097.8	1.86
RIL_19	4179	1.96	RIL_47	5202.7	1.94	RIL_75	3617.6	1.89	RIL_104	4261	1.88
RIL_20	4118.2	1.95	RIL_48	4034.5	1.96	RIL_76	4913	1.92	RIL_105	4856.3	1.89
RIL_21	4068.6	1.91	RIL_49	2458.4	1.89	RIL_77	2674.4	1.86	RIL_106	3865	1.88
RIL_22	4439.3	1.95	RIL_50	2748.1	1.91	RIL_78	2653.1	1.88	RIL_107	5705.6	1.89
RIL_23	4352.6	1.91	RIL_51	3001.7	1.85	RIL_79	6484.6	1.85	RIL_108	6141.9	1.87
RIL_24	3611.2	1.93	RIL_52	4685	1.96	RIL_80	5860.2	1.88	RIL_109	2996.1	1.85
RIL_25	2724.7	1.93	RIL_53	4163.8	1.95	RIL_81	5057.6	1.92	RIL_110	5319.9	1.91
RIL_26	4614.1	1.92	RIL_54	2396.5	1.9	RIL_82	5226.1	1.91	RIL_111	3530.5	1.91
RIL_27	4162.5	1.95	RIL_55	4794	1.94	RIL_83	11482.8	1.92	RIL_112	2556.8	1.85
RIL_28	3589.9	1.95	RIL_56	8421.3	1.93	RIL_84	32.3	0.92	RIL_113	6380.4	1.85

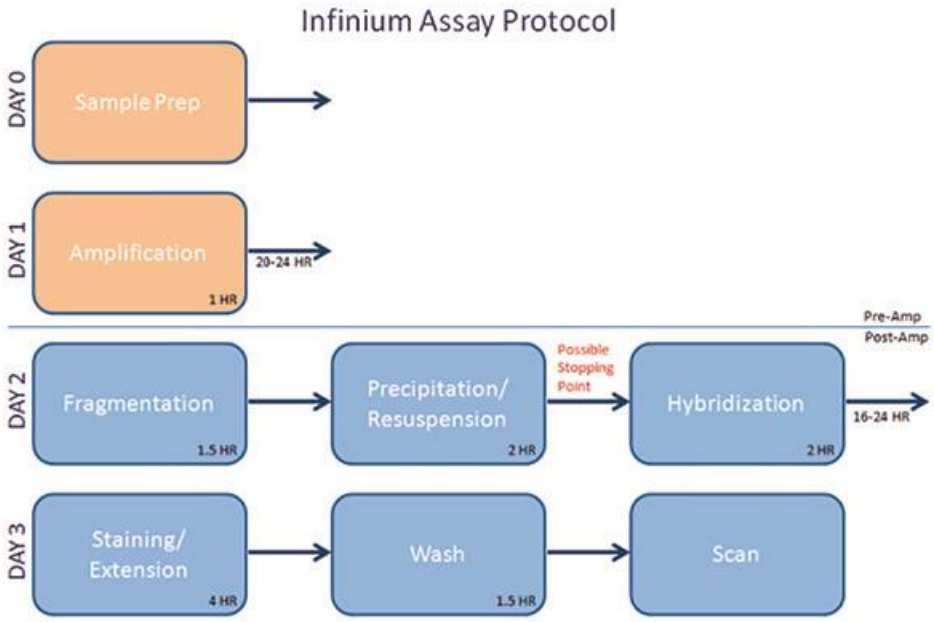
Appendix xii Concentration and quality of genomic DNA measured using Nanodrop spectrophotometer (Continued)

Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280
RIL_114	4133.2	1.94	RIL_142	4485.4	1.93	RIL_170	2857.7	1.94	RIL_198	1836.4	1.95
RIL_115	3742.5	1.89	RIL_143	4950.5	1.95	RIL_171	3496	1.96	RIL_199	4014	1.96
RIL_116	5135	1.91	RIL_144	4544.6	1.92	RIL_172	2608.6	1.95	RIL_200	5239.6	1.93
RIL_117	4509.6	1.9	RIL_145	3887.6	1.93	RIL_173	560.3	1.86	RIL_201	4795.1	1.93
RIL_118	3567.1	1.92	RIL_146	4961.3	1.93	RIL_174	3109.1	1.89	RIL_202	1990.6	1.96
RIL_119	3890.5	1.89	RIL_147	21.1	0.73	RIL_175	101	1.39	RIL_203	4389	1.96
RIL_120	2902.4	1.87	RIL_148	6222.7	1.86	RIL_176	4043.4	1.95	RIL_204	3988.9	1.93
RIL_121	2147.9	1.87	RIL_149	4023.3	1.96	RIL_177	4238.4	1.97	RIL_205	4407.5	1.95
RIL_122	5927.7	1.88	RIL_150	5094.4	1.93	RIL_178	2044.1	1.93	RIL_206	4285.6	1.95
RIL_123	2644.1	1.89	RIL_151	2836.1	1.9	RIL_179	2435.3	1.93	RIL_207	1633.9	1.94
RIL_124	3233.6	1.91	RIL_152	5545.4	1.9	RIL_180	4036.3	1.96	RIL_208	3138.8	1.94
RIL_125	3406	1.9	RIL_153	5352.9	1.91	RIL_181	4040.8	1.96	RIL_209	315.2	1.85
RIL_126	2206.6	1.87	RIL_154	6108.6	1.9	RIL_182	2454.3	1.95	RIL_210	3849.3	1.97
RIL_127	5361.8	1.89	RIL_155	5407.4	1.92	RIL_183	3565.9	1.97	RIL_211	3285.5	1.95
RIL_128	2936.2	1.87	RIL_156	4907.4	1.93	RIL_184	4157.7	1.97	RIL_212	2318.9	1.95
RIL_129	2945.6	1.86	RIL_157	3053.7	1.93	RIL_185	1049.5	1.91	RIL_213	1338.3	1.93
RIL_130	3088.6	1.86	RIL_158	3746.4	1.97	RIL_186	2220.9	1.94	RIL_214	3036.7	1.91
RIL_131	6222.6	1.89	RIL_159	3191.6	1.96	RIL_187	3664.8	1.97	RIL_215	2500.4	1.95
RIL_132	5104.7	1.92	RIL_160	2449.9	1.93	RIL_188	4912.9	1.96	RIL_216	4139.4	1.95
RIL_133	5362	1.92	RIL_161	4220.6	1.96	RIL_189	2931.8	1.93	RIL_217	737.3	1.86
RIL_134	6234.3	1.87	RIL_162	2918.8	1.94	RIL_190	54.3	1.14	RIL_218	4446.9	1.94
RIL_135	4639.2	1.92	RIL_163	2136.8	1.95	RIL_191	3552.1	1.97	RIL_219	3068.2	1.91
RIL_136	5951	1.88	RIL_164	5180.1	1.95	RIL_192	3083.8	1.96	RIL_220	1176.7	1.95
RIL_137	5313.3	1.91	RIL_165	3432.5	1.96	RIL_193	4582.9	1.96	RIL_221	1545.4	1.94
RIL_138	6378.8	1.87	RIL_166	2665.3	1.93	RIL_194	3831.4	1.95	RIL_222	2117	1.93
RIL_139	5264.8	1.93	RIL_167	136.7	1.53	RIL_195	2508.4	1.94	RIL_223	996.2	1.89
RIL_140	3776.8	1.9	RIL_168	3805.2	1.98	RIL_196	3973.1	1.96	RIL_224	3478.1	1.95
RIL_141	2254.6	1.92	RIL_169	3027.6	1.9	RIL_197	2935.1	1.91	RIL_225	1240.5	1.93

Appendix xii Concentration and quality of genomic DNA measured using Nanodrop spectrophotometer (Continued)

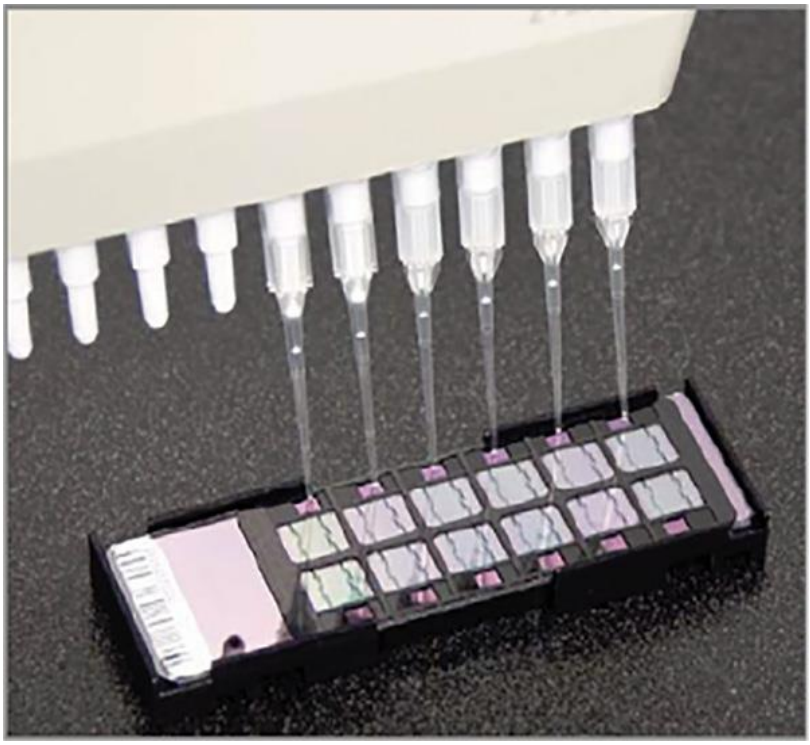
Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280	Line	ngµl-1	260/280
RIL_226	3627.1	1.96	RIL_254	3405.5	1.93	RIL_282	251.8	1.72	RIL_310	2375.4	1.94
RIL_227	2229.1	1.94	RIL_255	2276	1.97	RIL_283	5478.9	1.91	RIL_311	3334.2	1.95
RIL_228	2652.2	1.94	RIL_256	3223.3	1.95	RIL_284	3050	1.92	RIL_312	4421.3	1.95
RIL_229	2939.8	1.92	RIL_257	2512.5	1.93	RIL_285	2398.5	1.97	RIL_313	4593.2	1.93
RIL_230	2423.3	1.94	RIL_258	1729.7	1.95	RIL_286	274.1	1.68	RIL_314	3642	1.97
RIL_231	4695.2	1.93	RIL_259	2525.6	1.94	RIL_287	1009.1	1.91	RIL_315	4063.2	1.96
RIL_232	3089.5	1.96	RIL_260	1901.7	1.93	RIL_288	3622.6	1.96	RIL_316	2986.4	1.92
RIL_233	3898	1.96	RIL_261	533.2	1.85	RIL_289	2437.1	1.92	RIL_317	2407.8	1.91
RIL_234	3008.9	1.92	RIL_262	1827	1.95	RIL_290	4468.7	1.93	RIL_318	5725.3	1.91
RIL_235	2214.3	1.95	RIL_263	2533.2	1.95	RIL_291	488.5	1.88	RIL_319	4251.1	1.96
RIL_236	2632.9	1.94	RIL_264	3627.3	1.96	RIL_292	1794.2	1.94	RIL_320	2335.8	1.92
RIL_237	3256.3	1.93	RIL_265	2779.4	1.93	RIL_293	2772.9	1.92	P1_321	4287	1.95
RIL_238	2793.1	1.93	RIL_266	193.5	1.6	RIL_294	779.3	1.91	P2_322	3858.1	1.97
RIL_239	4733	1.95	RIL_267	1840.8	1.93	RIL_295	1802.8	1.94			
RIL_240	4683.2	1.96	RIL_268	2438.8	1.93	RIL_296	3264.6	1.95			
RIL_241	5287.3	1.92	RIL_269	1242.1	1.92	RIL_297	3130.5	1.95			
RIL_242	2535.9	1.93	RIL_270	1270.9	1.91	RIL_298	2511.5	1.94			
RIL_243	2089.8	1.94	RIL_271	63.5	1.23	RIL_299	4564	1.95			
RIL_244	2948.6	1.9	RIL_272	407.9	1.8	RIL_300	106.3	1.43			
RIL_245	312.7	1.73	RIL_273	3448.5	1.95	RIL_301	774.6	1.86			
RIL_246	2066.5	1.94	RIL_274	3932.9	1.96	RIL_302	1319.3	1.9			
RIL_247	3403.9	1.95	RIL_275	2681.6	1.9	RIL_303	1035.5	1.89			
RIL_248	2906.5	1.92	RIL_276	2672.8	1.92	RIL_304	626.5	1.87			
RIL_249	3525.8	1.98	RIL_277	3117.8	1.93	RIL_305	2025.1	1.94			
RIL_250	3785.1	1.95	RIL_278	1205.9	1.91	RIL_306	2377.1	1.93			
RIL_251	2182.9	1.95	RIL_279	1635.8	1.94	RIL_307	4681.8	1.95			
RIL_252	5229.2	1.92	RIL_280	2032.3	1.94	RIL_308	4162.4	1.95			
RIL_253	2958.8	1.93	RIL_281	2389.9	1.93	RIL_309	4642.8	1.95			

Appendix xiii Summarized Infinium 6 k assay protocol

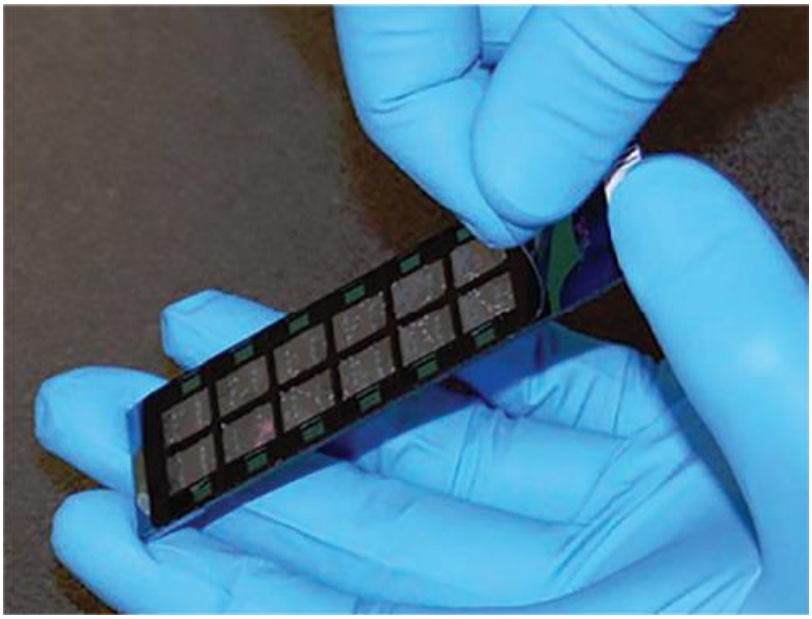


Appendix 3 Complete hybridization chamber base, mat and the lid

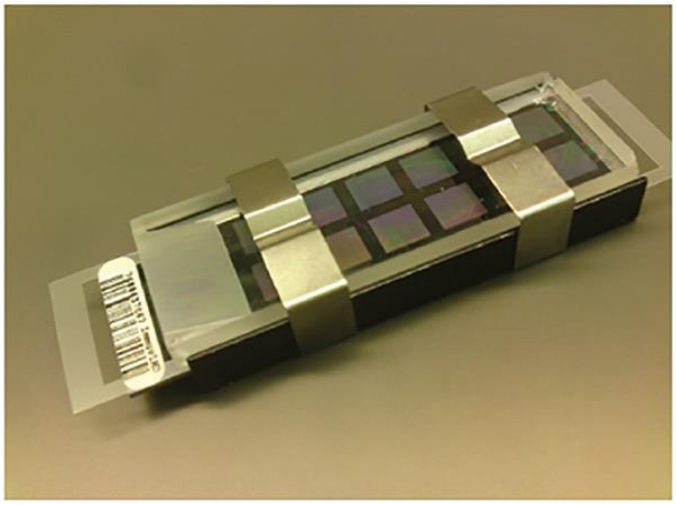
Appendix xv Loading beadchip by dispensing the sample on the inlet ports



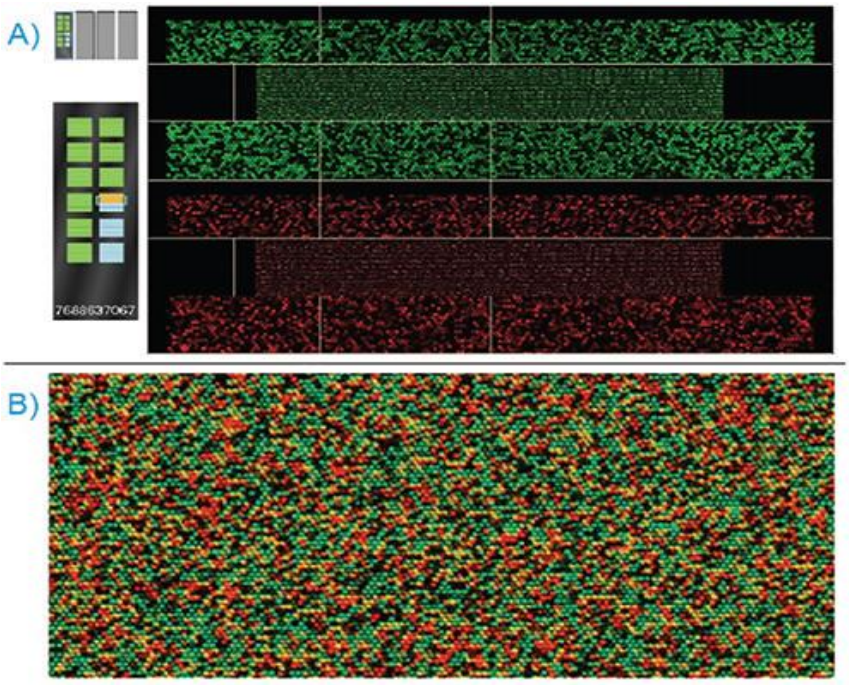
Appendix xvi Removal of beadchip cover by grasping the seal at the corner



Appendix xvii Complete flow through assemby



Appendix xviii Scanning of the beadchip



A) The beadchip is scanned with both a red and green laser; the scanning software displays both simultaneously. Sections passing intensity quality control (QC) highlights green on the beadchip display to the left. Sections failing intensity QC highlight red on the beadchip display. **B)** Once scanning is complete, the software overlay the red and green displays. A zoomed-in image is shown. The color and intensity of each individual bead indicates the allele present

Appendix xix Mean values of disease severity index at three locations in Kenya

S. No	Line/Variety	R genes	Locations		
			Mwea	West Kano	Gamba
1	IRBLa-A	*Pia*	1.00a	0.80bc	1.00a
2	IRBLa-C	*Pia*	0.87ab	0.73cd	0.80b
3	IRLi-F5	*Pii*	1.00a	0.73cd	0.93ab
4	IRBLks-F5	*Pik-s*	0.93ab	0.73cd	1.00a
5	IRBLks-S	*Pik-s*	0.87ab	0.73cd	0.40d
6	IRBLk-KA	*Pik*	1.00a	0.80bc	0.87ab
7	IRBLkp-K60	*Pik-p*	0.93ab	0.33e	0.80b
8	IRBLkh-K3	*Pik-h*	0.93ab	0.80bc	0.33de
9	IRBLz-FU	*Piz*	0.00e	0.73cd	0.80b
10	IRBLz5-CA	*Piz-5*	0.87ab	0.80bc	0.60c
11	IRBLzt-T	*Piz-t*	0.87ab	0.87abc	0.80b
12	IRBLta-K1	*Pita*	0.93ab	0.73cd	0.80b
13	IRBLta-CT2	*Pita*	1.00a	1.00a	0.93ab
14	IRBLb-B	*Pib*	0.87ab	0.87abc	0.80b
15	IRBLt-K59	*Pit*	0.20cde	0.73cd	0.80b
16	IRBLsh-S	*Pish*	0.93ab	0.80bc	0.33de
17	IRBLsh-B	*Pish*	0.80ab	0.73cd	0.27de
18	IRBL1-CL	*Pi1*	0.80ab	0.87abc	0.27de
19	IRBL3-CP4	*Pi3*	0.87ab	0.80bc	0.93ab
20	IRBL5-M	*Pi5(t)*	1.00a	0.80bc	0.27de
21	IRBL7-M	*Pi7(t)*	0.80ab	0.93ab	0.80b
22	IRBL9-W	*Pi9*	0.87ab	0.80bc	0.80b
23	IRBL12-M	*Pi12(t)*	0.27cd	0.73cd	0.80b
24	IRBL19-A	*Pi19*	0.80ab	0.80bc	0.80b
25	IRBLkm-TS	*Pik-m*	0.87ab	0.80bc	0.33de
26	IRBL20-IR24	*Pi20*	0.87ab	0.73cd	0.87ab
27	IRBLta2-PI	*Pita2*	0.87ab	0.87abc	0.33de
28	IRBLta2-RE	*Pita2*	0.80ab	0.80bc	0.20e
29	IRBLta-CP1	*Pita*	0.93ab	0.87abc	0.87ab
30	IRBL11-ZH	*Pi11(t)*	1.00a	1.00a	0.80b
31	IRBLz5-CA (R)	*Piz5*	0.93ab	0.80bc	0.80b
32	LTH	*No gene*	1.00a	0.87abc	0.80b
33	IRBLsh-KU[CO]	*Pish*	0.00e	0.87abc	0.27de
34	IRBLsh-S[CO]	*Pish*	0.00e	0.87abc	0.27de
35	IRBLsh-B[CO]	*Pish*	0.20cde	0.80bc	0.40d
36	IRBLb-IT13[CO]	*Pib*	0.00e	0.80bc	0.33de
37	IRBLz5-CA[CO]	*Piz-5*	0.13de	0.80bc	0.33de
38	IRBLzt-IR56[CO]	*Piz-t*	0.00e	0.33e	0.33de
39	IRBL5-M[CO]	*Pi5(t)*	0.80ab	0.80bc	0.80b
40	IRBLks-CO[CO]	*Piks*	0.80ab	0.80bc	0.87ab
41	IRBLk-KU[CO]	*Pik*	0.40c	0.80bc	0.80b
42	IRBLk-KA[CO]	*Pik*	0.27cd	0.80bc	0.33de
43	IRBLkh-K3[CO]	*Pik-h*	0.87ab	0.73cd	0.27de
44	IRBLkm-TS[CO]	*Pik-m*	0.27cd	0.60d	0.27de

Values followed by different lowercase letters in a column are significantly different at the 0.05 level. Disease severity index (DSI) ≤ 0.4 resistant reaction, DSI = 0.6 susceptible reaction, DSI > 0.6 susceptible reaction.

Appendix 4 Mean values of disease severity index at three locations in Kenya (Continued)

S. No	Line/Variety	R genes	Locations		
			Mwea	West Kano	Gamba
45	IRBLkp-K60[CO]	*Pik-p*	0.13de	0.60d	0.87ab
46	IRBL1-CL[CO]	*Pi1*	0.80ab	0.73cd	0.27de
47	IRBL7-M[CO]	*Pi7(t)*	0.80ab	0.87abc	0.93ab
48	IRBLta-YA[CO]	*Pita*	0.73b	0.80bc	0.93ab
49	IRBLta-ME[CO]	*Pita*	0.87ab	0.80bc	0.80b
50	IRBLta2-PI[CO]	*Pita-2*	0.07de	0.60d	0.20e
51	IRBLta2-RE[CO]	*Pita-2*	0.07de	0.60d	0.20e
52	IRBLta2-IR64[CO]	*Pita-2*	0.00e	0.60d	0.27de
53	CO39	*Pia*	0.87ab	0.80bc	0.60c
54	ITA310	*Unknown*	0.80ab	0.80bc	0.80b
55	BW196	*Unknown*	0.20cde	0.33e	0.20e
56	BAS217	*Unknown*	1.00a	0.93ab	0.60c

Values followed by different lowercase letters in a column are significantly different at the 0.05 level. Disease severity index (DSI) ≤ 0.4 resistant reaction, DSI = 0.6 susceptible reaction, DSI > 0.6 susceptible reaction.

Appendix xx Reaction of rice differential lines and local varieties to blast population at three sites in Kenya

S. No	Line/Variety	R gene	Year 2013			Year 2014		
			MW	WK	G	MW	WK	G
1	IRBLa-A	*Pia*	S	S	S	S	S	S
2	IRBLa-C	*Pia*	S	S	S	S	S	S
3	IRLi-F5	*Pii*	MR	S	S	S	S	S
4	IRBLks-F5	*Pik-s*	S	S	S	S	S	S
5	IRBLks-S	*Pik-s*	R	S	R	S	S	R
6	IRBLk-KA	*Pik*	S	S	R	S	S	S
7	IRBLkp-K60	*Pik-p*	MR	R	R	S	R	S
8	IRBLkh-K3	*Pik-h*	R	R	R	S	S	R
9	IRBLz-FU	*Piz*	MR	S	S	R	S	S
10	IRBLz5-CA	*Piz-5*	R	S	R	S	S	MR
11	IRBLzt-T	*Piz-t*	R	S	MR	S	S	S
12	IRBLta-K1	*Pita*	S	S	MR	S	S	S
13	IRBLta-CT2	*Pita*	S	S	S	S	S	S
14	IRBLb-B	*Pib*	R	MR	S	S	S	S
15	IRBLt-K59	*Pit*	R	S	MR	R	S	S
16	IRBLsh-S	*Pish*	MR	MR	R	S	S	R
17	IRBLsh-B	*Pish*	S	MR	R	S	S	R
18	IRBL1-CL	*Pi1*	MR	S	R	S	S	R
19	IRBL3-CP4	*Pi3*	S	MR	S	S	S	S
20	IRBL5-M	*Pi5(t)*	S	MR	R	S	S	R
21	IRBL7-M	*Pi7(t)*	S	S	MR	S	S	S
22	IRBL9-W	*Pi9*	R	MR	R	S	S	S

Where MW = Mwea, WK = West Kano, G = Gamba, R = Resistant, MR = moderately resistant and S = susceptible

Appendix xx Reaction of rice differential lines and local varieties to blast population at three sites in Kenya (continued)

S.No	Line/Variety	R gene	Year 2013			Year 2014		
			MW	WK	G	MW	WK	G
23	IRBL12-M	*Pi12(t)*	R	S	MR	R	S	S
24	IRBL19-A	*Pi19*	R	S	S	S	S	S
25	IRBLkm-TS	*Pik-m*	S	S	R	S	S	R
26	IRBL20-IR24	*Pi20*	MR	S	MR	S	S	S
27	IRBLta2-PI	*Pita2*	R	S	R	S	S	R
28	IRBLta2-RE	*Pita2*	S	S	R	S	S	R
29	IRBLta-CP1	*Pita*	S	S	S	S	S	S
30	IRBL11-ZH	*Pi11(t)*	R	S	MR	S	S	S
31	IRBLz5-CA (R)	*Piz5*	R	MR	MR	S	S	S
32	LTH	No gene	S	S	S	S	S	S
32	LTH	No gene	S	S	S	S	S	S
33	IRBLsh-KU[CO]	*Pish*	R	R	R	R	S	R
34	IRBLsh-S[CO]	*Pish*	R	R	R	R	S	R
35	IRBLsh-B[CO]	*Pish*	R	MR	R	R	S	R
36	IRBLb-IT13[CO]	*Pib*	R	R	R	R	S	R
37	IRBLz5-CA[CO]	*Piz-5*	R	MR	R	R	S	R
38	IRBLzt-IR56[CO]	*Piz-t*	MR	R	R	R	R	R
39	IRBL5-M[CO]	*Pi5(t)*	S	S	S	S	S	S
40	IRBLks-CO[CO]	*Piks*	S	S	S	R	S	S
41	IRBLk-KU[CO]	*Pik*	R	R	R	R	S	S
42	IRBLk-KA[CO]	*Pik*	R	R	R	R	S	R
43	IRBLkh-K3[CO]	*Pik-h*	MR	MR	R	S	S	R
44	IRBLkm-TS[CO]	*Pik-m*	R	S	R	R	S	R
45	IRBLkp-K60[CO]	*Pik-p*	R	R	MR	R	MR	S
46	IRBL1-CL[CO]	*Pi1*	R	MR	R	S	S	R
47	IRBL7-M[CO]	*Pi7(t)*	R	MR	MR	S	MR	S
48	IRBLta-YA[CO]	*Pita*	R	MR	MR	S	S	S
49	IRBLta-ME[CO]	*Pita*	R	S	S	S	S	S
50	IRBLta2-PI[CO]	*Pita-2*	R	R	R	R	MR	R
51	IRBLta2-RE[CO]	*Pita-2*	R	R	R	R	MR	R
52	IRBLta2-IR64[CO]	*Pita-2*	R	R	R	R	MR	R
53	CO39	Pia	S	S	MR	S	S	MR
54	ITA310	Unknown	S	S	S	S	S	S
55	BW196	Unknown	R	R	R	R	R	R
56	BAS217	Unknown	S	S	MR	S	S	MR

Where MW = Mwea, WK = West Kano, G = Gamba, R = Resistant, MR = moderately resistant and S = susceptible