Increasing the resilience of elite rice cultivars to sheath rot (*Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw]) in Rwanda through breeding for resistance

by

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Thesis abstract

Sarocladium oryzae [(Sawada) W. Gams & D. Hawksw] is currently regarded as one of most severe emerging disease of rice in Rwanda affecting mostly newly released long grain rice varieties by considerably reducing their yielding potential and deterioration in their grain quality characteristics. The disease hence poses a potential threat to the rice sector in Rwanda as it affects most of the dwarf varieties that have been bred for farmers and market preferred traits mainly yield and quality. The overall objective of this study was to increase the resilience of popular rice cultivars to sheath rot (ShR) of rice through breeding for disease resistance as a sustainable disease management strategy. Specifically the objectives were (i) to characterise rice germplasm for resistance to ShR and other agronomic traits; (ii) to assess genetic diversity among ShR resistant and susceptible parents prior to hybridization programmes; (iii) to determine the gene action and nature of inheritance of resistance to sheath rot disease in rice and (iv) to introgress rice sheath rot disease resistance genes into popular rice cultivars.

As part of achieving these objectives, genetic variability of local germplasm was assessed for important agronomic traits. Sixty-four varieties were evaluated in three different locations in an 8 x 8 lattice design with two replications in each environment. The REML procedure revealed significant differences between genotypes with minor environmental effects across the sites as confirmed by low differences between phenotypic and genotypic coefficients of variation. Genotypic variance was higher than environmental variance for most of the traits except for number of tillers and flag leaf length. Days to 50% flowering, plant height, days to maturity, flag leaf area, sheath length, total panicle per plot, single panicle weight and grain length had the highest heritability (80.27% - 96.42%) and genetic advance estimates, whereas heritability was moderate (59.47%) for grain yield. Thus parental selection based on ad hoc traits was suggested as an effective strategy.

Principal component analysis extracted seven components contributing to more than 72% of the total variation with the most important traits being plant height, number of branches per panicle, number of grains per panicle, single panicle weight, grain yield, number of tillers and grain length. Principal component biplots showed groups of genotypes suitable for specific breeding programmes, for instance clusters of genotypes combining high yielding potential with plant

stature, tillering ability as well as grain length. This study has thus provided useful information towards different hybridization programmes.

Sources of resistant genes to sheath rot of rice were also identified. Results showed that 10 late maturing, intermediate to tall, well exerted and short grain cultivars had different levels of resistance with a percent disease index (PDI) from 0.8 - 16.0%. Out of these, one immune cultivar (Yunyine) and five resistant cultivars (Nyiragikara, Nerica 1, Moroberekan, Cyicaro, and Yunertian) were considered suitable for various ad hoc breeding programmes. Four moderately resistant cultivars met the cost effective rice farming requirements. The remaining, early maturing, dwarf and semi-dwarf, enclosed panicles and mostly long grain cultivars were found to exhibit different levels of susceptibility with PDI ranging between 27.1 - 83.2%.

In addition, single nucleotide polymorphism (SNP) markers were used in assessment of genetic diversity with the aim of identifying potential candidates for various hybridization programmes. Ten resistant and fifteen susceptible varieties were analysed using 94 SNP markers. The total number of alleles amplified per locus ranged from 1 to 4 with a mean of 2.01 with a total of 189 alleles amplified from 25 genotypes. The number of observations per marker locus or the number of non-missing genotypes observed in the sample ranged from 11 to 25 with an average of 23. Mean major allele frequency was 76.2%, whereas the mean polymorphic information content was 0.263, and gene diversity estimated at 0.325 on average. These results showed that the markers were highly informative and revealed good estimates of genetic diversity among studied varieties. Genetic distance, ranging from 0 and 0.63 coupled with the UPGMA dendrogram, clearly distinguished sheath rot resistant and susceptible genotypes. The study indicated the possibility of improving levels of resistance to sheath rot with minimum risk of genetic depression or reduced variability among progenies through hybridization of locally adapted germplasm.

From this study 12 genetically distant varieties were selected for genetic studies to assess the mode and nature of inheritance of resistance to sheath rot of rice. Eight susceptible female parental materials (Buryohe, FAC 56, Fashingabo, Gakire, Intsinzi, Mpembuke, Ndamirabahinzi, Rumbuka) were crossed with four resistant parents (Cyicaro, Nyiragikara, Yunertian, Yunkeng) in an 8 x 4 North Carolina design II (NCDII) to produce F1 progenies. Results revealed that both additive and non-additive gene action were important in the inheritance of horizontal resistance to ShR of rice. Based on high GCA/SCA ratio, coupled with high heritability estimates, additive effects were more predominant than non-additive. The most promising resistant genotypes were

Cyicaro, Yunertian and Yunkeng as indicated by the SCA effects. Significance of both GCA and SCA effects suggested that rice improvement programmes should be directed towards selection of superior parents or good combiners emphasizing on GCA. Crosses exhibiting high SCA effects would produce desirable transgressive segregants in later generations if efforts could be made to modify the conventional breeding methodologies to capitalize on both additive and non-additive genetic effects. The predominance of additive genetic effects together with the relevance of dominant genes and high narrow and broad sense heritability estimates suggested that the rice improvement programme can be improved through phenotypic recurrent selection for horizontal resistance traits in a controlled environment.

Therefore recurrent selection was used to introgress ShR resistant genes into farmers' popular cultivars such as Rumbuka, Buryohe and Gakire. These varieties were involved in a series of backcrosses as recurrent parents with Yunertian and Yunkeng as donors of resistant genes. Subsequently, 8 BC2F1 introgression lines were developed and were tested in three different environments for adaptability and stability of traits related to ShR resistance and yielding potential. Phenotypic selection of BC2F1 genotypes led to the recovery of between 78 - 85% of the recurrent parents' genome with a remarkable increased resistance and grain yield (121 - 125%). From the additive main effects and multiplicative interaction AMMI model both main effects from genotype and environment had a significant effect on the performance of the different introgression lines for six traits; disease severity, grain yield, plant height, tillering ability, grain length and number of grains per panicle. The genotype by environment interaction also had a significant effect on disease severity, grain yield, tillering ability and number of grains per panicle. The AMMI analysis indicated that G6 (Intsinzi X Yunertian) had wide adaptation for the traits across the three test environments. Other genotypes showing relatively good general adaptation included G2 for disease severity and tillering ability, G8 for disease severity, and G4 for grain yield and number of grains per panicle, as they had IPCA1 values less than 0.5.

Stability analysis results showed that genotypes G5 and G6 (Intsinzi X Yunertian) were stable for disease severity whereas G6 (Intsinzi X Yunertian), G5 and G4 had good stability for yield and its related traits. Although these genotypes combined wide adaption and stability across the test environments, their yielding potential was not the best. This indicated the need to investigate yield performance in specific locations in further breeding programmes. This study

provides a breakthrough in breeding for resistance to sheath rot resistance as demonstrated by the progress made in introgressing resistant genes into susceptible cultivars through a few backcrossing generations with maximum recovery of the recurrent parents' genome.

Declaration

I, Simon Martin Mvuyekure, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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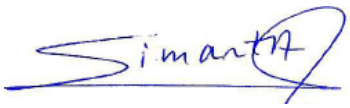

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As the candidate's supervisors, we agree to the submission of this thesis:

Dr. Julia Sibiya (Supervisor)

Prof. John Derera (Co-Supervisor)

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Dedication

To God Almighty this work is dedicated

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1. Thesis Introduction

1.1 Background

Rice is a major staple food for billions of human beings across the world. It is estimated that half of the world population depends on rice (Acquaah, 2012). From a nutritional point of view, rice is a very important cereal crop as it accounts for 35 - 75% of calories consumed worldwide (Khush and Jena, 2009). Its cultivation is the principal activity and source of income for more than 100 million households in developing countries in Asia, Africa and Latin America (Nguyen, 2012).

World rice production increased from 358.5 million tons in 1993 - 1994 to 408.8 million tons in 2002-2003 (Acquaah, 2012) and then to 482.7 million tons in 2011 - 2012 with forecasts estimating an increase of 7 annually (FAO, 2012a). The area under rice cultivation is also increasing gradually. It increased from 145.2 million to 155.1 million hectares in 1990-2000 (Acquaah, 2012) and reached 165 million hectares in 2012 (FAO, 2012b). It is estimated that 90% of rice is produced and consumed in Asia whereas Africa contributes only about 9%. As the world rice consumption continues to increase, especially in Africa, various estimates predict that the world will need to produce more rice in the near future, and accordingly, high yielding and stable varieties will need to be developed.

Rice production is becoming a significant component of the agricultural sector in Rwanda. This is due to the Crop Intensification Programme (CIP) set in place in 2007 by the government of Rwanda with the aim of increasing agricultural productivity of high potential food crops and ensuring food security and self-sufficiency. The National Agriculture Policy (NAP) in general and CIP in particular, set rice high on the list of priority agricultural commodities as it was recognized that, not only do most of Rwandan marshlands offer good ecosystems for rice growing but also rice offers a potential market in the country and in the East African Community region. As a result, the area under rice cultivation in Rwanda rose from around 5,000 ha to around 13,000 ha from 2000 and 2010, accounting for a growth in production from less than 20,000 tons to around 70,000 tons in 2010 involving about 62 000 farmers (Kathiresan, 2010). The demand and consumption of rice have risen substantially. However, despite the steep production increase, the country still imports about 40% of the rice it consumes (Promar, 2012). The National Rice

Development Strategy (NRDS) put in place in 2011 has set milestones trying to achieve the self-sufficiency in rice production and consumption by the year 2018 and to increase the competitiveness of Rwandan rice in local and regional markets. To attain these aims, the strategy targets an increase of productivity from 5.8 to 7.0 tons per hectare and expansion of the area under cultivation up to 28,500 ha by 2018 (Minagri, 2011). This strategy will integrate interventions on almost all aspects of the rice production value chain, including plant breeding.

1.2 Major constraints to rice production in Rwanda

1.2.1 Abiotic stress

Rice is cultivated in varied ecosystems in Rwanda on a gradient landscape from east to west. This gradient is based on increasing elevation and hence a decrease in temperature. High elevation combined with a drop in temperature overnight leads to damage from cold weather and hence a serious concern for non-tolerant varieties. Cold damage is the main yield-limiting factor of the low temperatures during the rice panicle formation stage causing average annual yield reductions of up to 40% (Jacobs and Pearson, 1994). As a matter of fact, in Rwanda, a new rice scheme called Rugeramigozi created in 2009 in Muhanga District failed to meet its expectations due to cold weather until some tolerant varieties were identified. Elsewhere, basmati rice is one of the most consumer preferred variety because of its long grain, aroma and low gelatinization, and consequently it is also the most traded even though its price is high compared to other varieties. However, because of cold problems that characterise most of the rice growing marshlands in Rwanda, basmati rice production is exclusively confined to Bugarama rice scheme, which is the hottest place in the country (Promar, 2012). Consequently, local production of long grain, less gelatinization and aromatic rice fails to meet local demand and therefore reliance on imports.

In addition, water and soil fertility have also been proven to hamper the productivity of rice in most of the rice schemes in Rwanda. According to Kathiresan (2010) these problems are related to poor management especially maintenance of irrigation systems and the lack of suitable fertilizer recommendations and the high fertilizer cost. Subsequently, the low input intensive mono-cropping pattern in the marshlands is constantly depleting the soil and water reserves.

1.2.2 Biotic stresses

Rwanda is characterized by a good hydrologic regime together with its subtropical climate allowing rice production throughout the year in exclusively irrigated schemes. Despite a number of strategies put in place to attain self-sufficiency, rice is generally grown in marshlands in an intensive mono-cropping system which favours a gradual build-up of various pests and diseases. The most important diseases are blast, *Pyricularia grisea*, rice sheath rot, rice yellow mottle virus and stalk-eyed borer (*Diopsis thoracica*). Because of the high cost of agrochemicals in Rwanda, chemical control of pests and diseases are not commonly applied. Hence all widely grown cultivars that are susceptible to major pests and diseases fail to meet their high yielding potential.

1.3 Problem statement

The sheath rot of rice caused by *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], is regularly reported to be among the major rice diseases in Rwanda and all popular cultivars are susceptible. It is also the least studied and there is a lack of clear and detailed information concerning geographic distribution, yield losses, genetic diversity of the pathogen, environmental interaction and management strategies.

However, a number of reports are available on the biology and pathogenicity of *S. oryzae*, epidemiology, and genetic diversity of the pathogen (Ayyadurai *et al.*, 2005). Tentative chemical, biological and cultural control methods have been proposed (Sakthivel and Gnanamanickam, 1987; Mukerji and Manoharachary, 2010; Prasanna Kumar *et al.*, 2013). Nevertheless, fungicide treatments have been unsuccessful or are too expensive as well as harmful to the environment (Ayyadurai *et al.*, 2005). Biological control of *S. oryzae* has been limited due to inconsistency of antagonists in field conditions (Sakthivel and Gnanamanickam, 1987). Genetic improvement of rice has always been a priority for breeders over the years. As far as rice sheath rot is concerned, little has been documented concerning improving resistance of commercial varieties in Rwanda.

Even though rice sheath rot resistant varieties have been developed in Asia (Sreenivasaprasad and Johnson, 2001; Mosharraf *et al.*, 2003), in most of the cases, imported germplasm fails to meet farmers agronomic preferences and market demand, as far as rice farming in Rwanda is concerned (Promar, 2012). Therefore, there is a need to initiate a rice breeding programme for resistance to rice sheath rot, among other constraints, that involves farmers in a participatory approach. Despite this need, there is limited or no information on the potential sources of resistance to sheath rot in available rice germplasm and studies on inheritance and combining ability for rice sheath rot are needed to inform the breeding programme.

As the current study is spatio-temporal and financially limited, emphasis was placed on breeding for resistance to sheath rot disease. If successful, this would constitute a sustainable disease management measure for rice production in Rwanda.

1.4 Research objectives

The overall goal of this study is to contribute to food security in Rwanda by increasing elite rice cultivars' productivity through varietal resistance to sheath rot disease.

The attainment of this goal will involve the following specific objectives:

1. To characterise rice germplasm for resistance to sheath rot disease and other agronomic traits.
2. To assess genetic diversity among sheath rot resistant and susceptible parents prior to hybridization programmes.
3. To determine the gene action and nature of inheritance of resistance to sheath rot disease in rice.
4. To introgress rice sheath rot disease resistance genes into popular rice cultivars.

1.5 Research hypotheses

i) Rice germplasm in Rwanda is sufficiently diverse to avoid genetic depression after hybridization programmes

ii) Sources of sheath rot disease resistant genes are available in locally adapted germplasm

iii) The inheritance of genes for resistance to sheath rot and associated traits is controlled by both additive and non-additive gene action.

iv) Popular rice cultivars improved for sheath rot disease resistance are high yielding and adapted to various agro-ecological conditions of Rwanda.

1.6 Structure of the thesis

The thesis is structured according to the objectives, where, each objective is presented in a chapter aimed at publishing in a refereed journal and, therefore, some contents may be found overlapping across the chapters. Crop Science journal style was used for referencing with minor adjustments. The thesis outline is as follows**:**

Thesis abstract

Introduction to thesis

Chapter 1: A review of the literature relevant to the research study.

Chapter 2: Evaluation of genetic variability of rice germplasm based on agro-morphological traits.

Chapter 3: Identification of sources of resistance to sheath rot of rice among Rwandan rice germplasm

Chapter 4: SNPs based assessment of genetic diversity of rice for selection of parents for sheath rot resistance breeding

Chapter 5: Genetic analysis for sheath rot disease resistance in rice

Chapter 6: Introgression of sheath rot resistant genes into popular rice cultivars and evaluation of introgression lines in multi-environment trials.

References

Acquaah, G., 2012. Principles of Plant Genetics and Breeding. Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK.

Ayyadurai, N., Kirubakaran, S.I., Srisha, S., and Sakthivel, N., 2005. Biological and molecular variability of *Sarocladium oryzae*, the sheath rot pathogen of rice *(Oryza sativa* L.). *Current Microbiology* 50: 319-323.

FAO, 2012a. Rice market monitor. Trade and Markets Division. Food and Agriculture Organization of the United Nations, Rome Italy.

FAO, 2012b. Food outlook: Global market analysis trade and market division, Food and Agriculture Organization of the United Nations, Rome Italy.

Jacobs, B.C., and Pearson, C.J., 1994. Cold damage and development of rice: a conceptual model. *Australian Journal of Experimental Agriculture* 34: 917-919.

Kathiresan, A., 2010. Enabling self sufficiency and competitiveness of Rwanda rice: Issues and policy options. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Khush, G.S., and Jena, K.K., 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: Wang, G.L. and Valent, B., (eds), Advances in Genetics, Genomics and Control of Rice Blast Disease. Springer Netherlands. p. 1-10.

Minagri, 2011. National rice development strategy: period 2011-2018. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Mosharraf, H., M.D. Shah, R. Munirur, K.M.Khalequzzaman, M.D. Nazrul Islam, and Ashrafuzzaman, M., 2003. Performance of breeding lines/cultivars of rice against bacterial leaf blight and sheath rot under field condition. *Journal of Biological Sciences* 3: 478-483.

Mukerji, K.G., and Manoharachary, C., 2010 Taxonomy and ecology of Indian fungi. J.K. International Publishing House Pvt Ltd, New Delhi, India. .

Nguyen, N.V., 2012: Global Climate change and rice food security. International Rice Commission, FAO, Rome.

Prasanna Kumar, M.K., D.K. Sidde Gowda, M. Rishikant, N. Kiran Kumar, K.T. Pandurange Gowda, and K. Vishwanath, 2013. Impact of fungicides on rice production in India. In

Mizuho (eds): Fungicides: Showcases of Integrated Plant Disease Management from around the World. In: Mizuho, N., (eds.), InTech, Rijeka, Croatia. p. 340-363.

Promar, 2012. Agriculture, forestry and fisheries of Rwanda: Fact-finding survey for the support of aid to developing countries (Fiscal year 2011 Research Project). Promar Consulting, Tokyo, Japan.

Sakthivel, N., and Gnanamanickam, S., 1987. Evaluation of *pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice (*Oryza sativa* L.). *Applied and Environmental Microbiology.* Vol. 53,: 2056-2059.

Sreenivasaprasad, S., and Johnson, R., 2001. Major fungal diseases of rice: recent advances. Kluwer Academic Publishers.

2 Chapter 1

Literature review

2.1 Introduction

In recent years, rice cultivation has become an integral part of the national food security strategies in Rwanda and increases in area under production and total production have occurred. Further increases are projected to ensure the country is, not only, food secure, but also self-sufficient in rice supply. However, rice productivity in Rwanda is hampered by biotic and abiotic constraints, among others, rice sheath rot (ShR). This is a fungal disease caused by *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], and yield losses up to 85% have been recorded. The disease is one of the most important emerging rice biotic stresses in Rwanda but it is the least studied, and no sustainable control measures have been established yet in the country. Fungicide treatment and biological control strategies have been initiated in Asia but till now little information is available concerning breeding for resistance. This review discusses various issues in terms of challenges and opportunities related to sheath rot management in Rwanda. It emphasizes mostly on breeding for resistance, as the development and use of resistant cultivars is the most sustainable sheath rot management strategy. It has several advantages such as being relatively inexpensive and cost effective, easy to deploy and having no adverse environmental consequences. It is also convenient for farmers to use, requiring no additional production costs and skills.

2.2 Sheath rot of rice as potential threat to rice farming systems in Rwanda

Rice is a major staple food for billions of human beings across the world. It is estimated that half of the world population depends on rice (Acquaah, 2012). From a nutritional point of view, rice is a very important cereal crop as it accounts for 35-75% of calories consumed worldwide (Khush and Jena, 2009). Rice production is becoming a significant component of the agricultural sector in Rwanda. Remarkably, the area under rice cultivation in Rwanda tripled in the last 10 years

accounting for a growth in production from less than 20,000 tons to around 70,000 tons with about 62 000 farmers involved (Kathiresan, 2010). Despite the steep increase in production, the country still imports about 40% of the rice it consumes (Promar, 2012). The current country's objectives are to attain self-sufficiency in rice by the year 2018. Even though a number of strategies have been put in place to attain self-sufficiency, rice is generally grown in marshlands, in an intensive mono-cropping system which favours a gradual build-up of various pests and diseases. The most devastating biotic stresses of rice in Rwanda include blast (*Pyricularia grisea)*, rice yellow mottle virus and stalk-eyed borer (*Diopsisi thoracica*).

Sheath rot of rice has been regularly reported to be a serious emerging rice problem, as it causes substantial yield losses in most of farmers' varieties and has been observed in almost all rice growing regions of Rwanda. Because of the fact that it was formerly classified among minor diseases, it is the least studied disease and no clear and detailed information is available in the country concerning geographic distribution, yield losses, genetic diversity of the pathogen, and environmental interaction and management strategies. On the other hand, according to Ayyadurai *et al.* (2005), fungicide treatments have been unsuccessful or are too expensive as well as harmful to the environment, whereas biological control of Sheath rot has been limited due to inconsistency of antagonists under the field conditions (Sakthivel and Gnanamanickam, 1987).

Even though rice sheath rot resistant varieties have been developed in Asia (Sreenivasaprasad and Johnson, 2001; Mosharraf *et al.*, 2003), in most of the cases, imported germplasm fails to meet farmers agronomic preferences and market demand, as far as rice farming systems in Rwanda are concerned (Promar, 2012).

2.3 Distribution of sheath rot and impact

Rice sheath rot has for long been considered as a minor disease, but in recent years, it has become one of the serious emerging rice diseases in almost all rice growing regions of the World (Mew and Gonzalez, 2002; Madhav *et al.*, 2013). The disease was first identified in 1922 and later became of great matter of concern in Japan, all of South East Asia, Africa, Latin America and the USA. The disease is also prevalent in rice growing areas of Western Australia (Ngala and Adeniji, 1986; Sakthivel 2001; Lanoiselet *et al.*, 2012). Currently sheath rot has

gained the status of a major disease in most rice growing regions (Lalan Sharma *et al.*, 2013). Similarly, the disease is now prevalent in all rice schemes of Rwanda with severe impact on the productivity of most farmer preferred varieties such as Buryohe, Intsinzi, Gakire, and Rumbuka. The disease may also be one of the reasons why dwarf and semi-dwarf long grain introductions (varieties) fail to adapt in most of Rwandan rice ecosystems.

Yield losses associated with sheath rot disease range between 26 and 50% in general and higher yield losses up to 85% were recorded in Taiwan (Sakthivel, 2001; Pearce *et al.*, 2001). These losses are a result of poor panicle formation and exsertion, tiller stunting, spikelet sterility (80 - 100%) reduced grain filling, husk and caryopses diseases, and losses in milling (Ngala and Adeniji, 1986). In addition to yield losses, the quality is also affected. Severe infections lead to chaffy, discoloured grains and affect viability and nutritional value of the grains followed by a decrease in the protein and starch contents of the infected seeds (Reddy *et al.*, 2000). Variability in yield losses depends upon prevailing conditions under which rice is grown that favour the development of the pathogen and the level of susceptibility of the grown cultivar. In most cases, higher yield losses occur mostly in lowland environments, particularly in rainy seasons in both rainfed and upland ecosystems (Mew and Gonzalez, 2002; Lanoiselet *et al.*, 2012).

2.4 Taxonomy of the pathogen *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw].

Sarocladium is a genus of hyphomycetes that causes the sheath rot of rice. Initially *Sarocladium* was described as *Acrocylindrium oryzae* from rice in Taiwan (Sawada, 1972 quoted by Ou (1985). However, the genus *Sarocladium*, was then documented in 1975 to accommodate the two species *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], and *Sarocladium attenuatum* (W. Gams & D. Hawksw), both of which were reported to be the causal agents of the sheath rot disease. The two species were differentiated primarily based on the more regularly verticilate conidiophores and long narrow acerose conidia produced by *S. attenuatum* (Pearce *et al.*, 2001). However, fifty strains of *Sarocladium* (11 dried specimens and 39 living cultures) were examined for variation in conidial size, utilization of several carbon sources, hydrolysis of several substrates, esterase isozymes after gel electrophoresis, and secondary metabolites after thin-layer chromatography (Bridge *et al.*, 1989). The results showed that there was insufficient justification to maintain the separation of the genus into the two species *S.*

oryzae and *S. attenuatum;* therefore *S. attenuatum* is now considered a synonym of *S. oryzae (*Bridge *et al.,* 1989*).*

2.5 Symptoms of sheath rot disease of rice

Sheath rot mainly attacks the uppermost leaf sheaths enclosing the young panicles. The lesions start as oblong or somewhat irregular spots 0.5 - 1.5 cm long, with brown margins and grey centres, or they may be greyish-brown throughout (Ou, 1985). They enlarge and often coalesce and may cover most of the leaf sheath. The young panicles remain within the sheath, do not develop further and rot, or only partial panicle emergence may be observed. Young lesions show whitish, powdery growth both outside and inside the affected sheaths. Old lesions have less or no powdery growth of the fungus and appear as dry, brownish lesions with the enclosed panicle rotted. Spikelets extended outside the rotted sheath normally develop, but are usually highly discoloured (brown) and may be partially filled and infected by the pathogen which causes empty grains in under severe infections (Pearce *et al.*, 2001). These symptoms have been seen on rice in different rice farming marshlands of Rwanda, in Rurambi and Cyili in Southern and Eastern Provinces (Figure 2.1).

Figure 2.1 *Sarocladium oryzae* [(Sawada*)* W. Gams & D. Hawksw], symptoms on rice in Rurambi (left) and Cyili (right) in Rwanda

Extracellular cellulases (as carboxymethyl cellulase), invertases, phenolases, nucleases and proteases have been reported to be produced by the fungus. The relative production of some of these enzymes has been linked to differential levels of pathogenicity on rice. The production of extracellular nucleases has also been linked with specific pathogen strains (Pearce *et al.*, 2001).

However, symptoms apparently caused by *S. oryzae* are similar to those caused by some strains of *Pseudomonas spp* mostly *Pseudomonas syringae* and this prevents reliable diagnosis based on symptoms (Zigler and Alvarez, 1990; Dariush *et al.*, 2012; Saberi *et al.*, 2013). Nevertheless, a pathogen characterization carried out in Rwanda in 2014 studied 96 leaf samples collected from 16 rice marshlands on six rice cultivars. Morphological and molecular assays were performed on potato dextrose agar (PDA) (Figure 2.2) cultures and analysed using Random Amplified Polymorphic DNA (RAPD) and internal transcribed agar sequence (ITS) methods respectively. The results confirmed the presence of various races of *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] instead of strains of *Pseudomonas spp*.

Figure 2.2 *Sarocladium oryzae* [(Sawada) [W. Gams & D. Hawksw] cultured on PDA by Mvuyekure *et.al* (unpublished) in Rwanda (left) and by Marín *et al.* (2013) in Cuba (right)

2.6 Epidemiology and disease cycle of sheath rot

The fungus severely infects the dwarf cultivars under water stressed conditions in both rain fed and upland ecosystems (Ou, 1985). The pathogen also attacks semi dwarf as well as traditional tall cultivars (Sakthivel and Gnanamanickam, 1986). It survives as mycelium in infected seeds,

plant residues and soil on the left over stubble and straw. In addition, the fungus infects a number of other cereal as well as bamboo and these can serve as alternate hosts (Pearce *et al.*, 2001). It survives for at least 4 months in seed and 7 months in sheath at room temperature and as long as 10 months in leaf sheaths in the field. Conidia are easily carried over long distances by wind (Amin, 1976).

Sarocladium oryzae [(Sawada) W. Gams & D. Hawksw] enters the host through stomata or injuries and ramifies intercellularly in the vascular bundles and mesophyll tissues. The disease has been associated with injuries caused and various insects (Ou, 1985; Lakshmanan, 1992; Lewin and Vidhyasekaran, 1987). *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] is also associated with other fungi, and such interactions with other stem-attacking pathogens of rice are believed to have contributed to *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] in becoming a dominant rice disease. Hot and humid weather favours the disease development. Temperatures of 20 to 30^0C and relative humidity in the range of 65 to 85% favour the development of sheath rot Lanoiselet *et al.* (2012).

These climatic conditions are similar to those in most of rice growing marshlands in Rwanda especially in Southern and Eastern Zones of the country which record annual average temperatures between 20 and 25^oC (Mutabazi, 2011). Thus conditions obviously favour the proliferation of the disease especially on the dwarf and high yielding varieties which are more susceptible to the disease.

2.7 Genetic variability of *Sarocladium oryzae* [(Sawada) [W. Gams & D. Hawksw]

The development of resistant cultivars needs a successful breeding programme and effective deployment of durable plant resistance. This is attained through an understanding of pathogen diversity and of the way in which virulence evolves in the pathogen population (Ayyadurai *et al.*, 2005). Significant variability occurs in the rate of coloration and conidial shape and size of isolates of *S.oryzae* on PDA cultures. Variations have also been reported in virulence of *Sarocladium oryzae* [(Sawada) [W. Gams & D. Hawksw] isolates on rice, and these differences have been associated with differing levels of extracellular enzymes. Intraspecific variation, that gave some indications to the genetic variability of isolates was reported by Bridge *et al.* (1989),

after studying electrophoretic properties of intracellular esterases. They observed isolates that produced multiple bands and detected seven different isoenzyme patterns in 13 isolates. Different strains isolated from different plants can be differentiated at the regional field and panicle scale. The evidence of existence of genetic variability was also confirmed by Ayyadurai *et al.* (2005), after a series of Random Amplified Polymorphic DNA (RAPD) markers based studies. Variability in pathogenicity, phytotoxic metabolite production, and DNA polymorphisms were detected among *S. oryzae* isolates. Variations were also reported by Pearce *et al.* (2001) in virulence of different isolates on rice and these differences were associated with differing levels of extracellular enzymes and the alternate plant hosts range. The molecular variability of *S. oryzae* isolates will be an important consideration in breeding programmes to develop durable resistance for sheath rot disease.

2.8 Sheath rot control strategies

2.8.1 Chemical control

A number of chemical fungicides against the sheath rot of rice have been intensively used across the world and remarkable control has been achieved. According to Prasanna Kumar *et al.* (2013), seed treatment with benlate (benomyl) and panoctine (guazatine) improves germination of sheath rot infected seeds. For field control of the disease, Mukerji and Manoharachary (2010), reported that the most effective fungicide for the control of sheath rot was derosal (carbendazim) followed by aureofungin and difolatan (captafol). These findings were also confirmed by Narayanaprasad *et al.* (2011) where the application of carbendazim (0.1%), mancozeb (0.2%) and difolatan (0.2%) produced better results for sheath rot management, when major growth and yield parameters were considered, resulting in less disease incidence and severity.

In an another separate study, Lalan Sharma *et al.* (2013) using systemic and non-systemic chemicals against *S. oryzae*, observed maximum inhibition of radial growth (76.53%) at 10.0 ppm of tebuconazole fungicides. In non-systemic fungicides, maximum inhibition of radial growth (78.86%) was recorded at 2000 ppm. Foliar sprays of tebuconazole were found superior to other treatments resulting in disease severity (59.01 - 64.33%) reduction, followed by carbendazim (48.70 - 55.28%) The treatments resulted in increased grain yield per plant (45.06

- 65.84%), grain yield per plot (45.57 - 65.85%), 1000-grain weight (10.80 - 52.58 gr) and reduction in chaffiness (48.07 - 53.80%). Among the non-systemic fungicides chorothalonil was found to be the best in managing sheath rot, giving reduction in disease severity (35.68 - 38.85%), and also increased grain yield per plant (24.78 - 44.74%), grain yield per plot (24.52 - 44.57%), 1000-grains weight (4.25 - 35.47%) and reduction in chaffiness (15.74 - 45.96%) as compared to the check.

However, sheath rot of rice is a new emerging rice disease in Rwanda and consequently the disease is almost unknown to farmers and extension workers and no chemical product have been tested efficient so far.

2.8.2 Biological control

Biological control of rice sheath rot as an alternative to chemical control is believed to be an effective strategy for disease management for resource poor farmers across the world, more particularly in developing countries (Pearce *et al.,* 2001). To this end, a study by Sakthivel and Gnanamanickam (1987) resulted in the identification of an antagonistic bacterial strain, *Pseudomonas fluorescens,* capable of suppressing the development of rice sheath rot. This was also confirmed by Saravanakumar *et al.* (2008) who, after a series of studies, concluded that fluorescent *pseudomonas* mixtures mediate disease resistance in rice plants against sheath rot disease. In contrast, Ayyadurai *et al.* (2005) reported that this kind of management method has limitations due to inconsistency under field conditions. This method remains unknown in Rwanda and consequently is not applied.

2.8.3 Varietal resistance and cultural practices

Only a few rice cultivars are resistant to sheath rot. Generally, the tall varieties have been observed to be moderately resistant to sheath rot in Rwanda and no information is available in the region regarding resistant varieties. However, photoperiod-sensitive tall varieties have been confirmed to be more resistant than photoperiod-insensitive and dwarf varieties (http://www.plantwise.org/KnowledgeBank/Datasheet.aspx?dsid=48393). A report by Sakthivel (2001) indicated that many of the high yielding international commercial cultivars were highly

susceptible to sheath rot of rice. Therefore, considerable effort should be made to screen for resistant cultivars. Few cultivars such as Tetep, ARC7117, Ramtulasi, Intran, and Zenith have been proven to be good sources of resistance in India (Amin, 1976). However, successful breeding and effective development of durable resistance requires an understanding of the pathogen diversity and the way in which virulence evolves in the pathogen population in order to breed against as many races as possible. Practices such as field sanitation, crop residue management, control of weeds and pathogen free seeds may enhance the effectiveness of the sheath rot control, but none of these control method is applied in Rwanda in regards to sheath rot of rice.

2.9 Improving rice existing germplasm for resistance to sheath rot of rice

The value of resistance in controlling plant diseases was recognized in the early 1900s and more recent realizations of the dangers of polluting the environment through chemical control of plant diseases gave additional impetus and importance to the breeding of resistant varieties (Agrios, 2005). However, the long-term success of breeding for disease resistance is influenced by many factors including; the nature of the pathogen and diversity of virulence in the population; availability, diversity and type of genetic resistance; screening methodology and selection environment for tracking resistance; selection of environments and methodologies for rapidly generating multiple stress resistant inbred lines and their use in hybrid or varietal development (Cairns *et al.*, 2012). Because sheath rot of rice was previously considered a minor disease, there is limited information in literature, regarding breeding programmes for resistance to the disease globally including Rwanda.

Nevertheless, other diseases, such as blast, bacterial blight, and yellow mottle virus have been studied on a large scale. Varietal resistance to the majority of ‘major’ diseases in rice has been documented (Mackill *et al.*, 1996; Khush *et al.*, 2003; Guimaraes, 2009; Wang and Valent, 2009). Most of the resistance mechanisms for these diseases are oligogenic, which implies that the resistance changes with changes in pathogens, resulting in its breakdown. Qualitative resistance typically confers a high level of resistance, is usually race-specific, and is based on single dominant or recessive genes. In contrast, quantitative resistance in plants is typically partial and race-nonspecific in phenotype, oligogenic or polygenic in inheritance and is conditioned by additive or partially dominant genes. Although it is easier to work with qualitative

resistance in crop genetic studies and in breeding, quantitative resistance is often the more useful in an agronomic context, due to its generally higher durability and broader specificity (Wisser *et al.*, 2006).

2.9.1 Phenotyping rice germplasm in breeding for resistance to sheath rot

The recent development of breeding technologies other than conventional methods has led to remarkable breakthroughs in molecular plant breeding for improvement of both qualitative and quantitative traits. However, the success and accuracy of conventional and biotechnology based breeding programmes must be coupled with accurate and standard uniform phenotyping techniques in various environments (Madhav *et al.*, 2013).

2.9.2 Screening rice germplasm for resistance to sheath rot

2.9.2.1 Isolation and inoculation of *S. oryzae* for germplasm screening for resistance

As far as artificial inoculation of *S.oryzae* is concerned, various techniques have been tested and validated. Several workers reported that insertion of grains of rice or sorghum or any other host plant colonized with the fungus between the flag leaf sheath and culm was more dependable and consistently produced severe infections (Madhav *et al.*, 2013). Estrada and Crill (1980) successfully induced typical sheath rot symptoms with *S. oryzae* spore suspension prepared in 25% beef extract peptone solution. Kang and Raltan (1983) induced sheath rot symptoms by injecting a spore suspension or inserting mycelial pieces from PDA culture. Reddy and Subbaya (1989) reported successful induction of the disease by inserting single leaf bit inoculum in between the culm and leaf sheath.

Narayanaprasad *et al*. (2011) tested four sheath rot artificial inoculation methods which are listed below:

i) **Seed inoculation method:** the infected seeds were kept in between the flag leaf sheath and in emerged sheath

ii) **Sheath inoculum method**: in this method infected sheaths were cut into small pieces then kept in between the flag leaf sheath and in emerged sheath.

iii) **Agar method**: involved growing the fungus on potato dextrose agar at room temperature and taking small bits of the mycelium using sterilized inoculum needle and inserting the fungus in a small hole in each tiller.

iv) **Spore suspension method**: the fungus was grown for ten days at room temperature on potato dextrose agar and then scraped from the surface and mixed in sterilized distilled water to obtain a spore suspension. One drop of spore suspension was placed inside the flag leaf sheath enclosing the unmerged panicle using a sterilized plastic dropping bottle

2.9.2.2 Phenotyping of partial physiological resistance and sources of resistant genotypes

Sarocladium oryzae [*(*Sawada) W. Gams & D. Hawksw] pathogen has a wide range of host plants and major genes that confer complete resistance in rice but have not been identified yet (Srinivasachary *et al.*, 2002). In addition, some other *oryzae* species have been reported as source of resistance. Resistance to sheath rot of rice may be associated with complex traits as is the case with most of the rice diseases as reported by Srinivasachary *et al.* (2002) that a very strong relationship exists between disease severity and panicle exsertion. Resistance to sheath rot may also be hypothesized to result from too many groups of mechanisms: physiological resistance and disease escape.

In general, dwarf cultivars of rice have been shown to be more susceptible, whilst taller ones have been found to be moderately resistant to sheath rot (Ngala and Adeniji, 1986). Based on the synthesis of momilactone (antifungal compounds produced by rice roots), Purkayastha *et al.* (1983), showed that there was a significant difference between tall resistant cultivars and semi-dwarf susceptible cultivars. These authors also confirmed the association of momilactone synthesis with differential resistance of tall and semi-dwarf rice cultivars to sheath rot. Asian germplasm was generally more susceptible than African (Ngala and Adeniji, 1986). For example, Moroberekan is a tall west African upland genotype, with sturdy stems and large fertile panicles with bold grains, and it exhibits resistance to sheath rot and blast and tolerance to other biotic and abiotic stresses like drought, disease and pests (Girish *et al.*, 2006). Some advanced dwarf lines of IR1544 (IR24 X Tetep) were also found to be resistant (Amin, 1976).

Therefore, these resistant genotypes can be used as sources of resistance to sheath rot in various breeding programmes.

2.9.3 Methods for screening rice germplasm for resistance to sheath rot of rice

In a rice breeding programme for resistance to sheath rot, morphological or molecular quantification and classification of resistance groups of parents and progenies are key components to be considered prior to choosing individuals to be involved in the crossing process. A number of sheath rot resistance evaluation methods have been discussed but the commonly used ones have been documented by Madhav *et al.* (2013). Satyanarayana and Reddy (1990) suggested a 1-9 scale for sheath rot disease based on the coverage of the lesions on the leaf sheath and damage on the panicles (Table 2.1).

Table 2.1 Qualitative and quantitative sheath rot scoring system

score	Description
1	Small brown lesions on boot leaf sheath and panicle emergence normal
3	Lesions enlarge, coalesce and cover about 5% of the leaf sheath and panicle emergence normal
5	Lesions cover 6 - 15% of the leaf sheath area and 75% of the panicle is emerged
7	Lesions covers 16 - 50% of leaf sheath area and 50% of the panicle emerged
9	Lesions cover > 50% leaf sheath area and about 25% of the panicle is emerged

The disease index (DI) is then calculated using the following formula:
DI= (1 x A) + (3 x B) + (5 x C) + (7x D) + (9 x E), where A, B, C, D, and E are percentage of tillers in grade 1, 3, 5, 7 and 9. The varietal reaction is then categorized based on DI as HR (0-99), R (DI=100-199), MR (DI= 200-299), MS (D=300-499), S (DI=500-699) and HS (DI=700-899).

Another sheath rot scoring method was used by Huang *et al*. (2012) and Lalan Sharma *et al.* (2013). The method consists of quantification of lesion length based on metric measurements

(mm or cm) and the proportion of lesion length (in centimetres) over the total length of the leaf sheath is transformed into percentage (

Table 2.2). This method seems more practical than the previous one.

Table 2.2: Rice sheath rot scoring system based on lesion length

Scale grade	Lesion length (cm)	Description
0	<1	No lesion or spot on flag leaf
1	1.1-3.0	Spots visible on tillers upon very careful examination (<1% flag leaf sheath area covered
3	3.1-5	Spots visible on tillers upon careful examination (1 - 5% flag leaf sheath area covered)
5	5.1-12	Spots easily visible on tillers (6 - 25% flag leaf area covered)
7	12.1-20.0	Spots present on almost whole the tillers parts (26 - 50% flag leaf area covered) damage conspicuous.
9	>20	Spots very common on whole the tillers parts (51 - 100 flag leaf sheath area covered) death of plant common, damage directly reduce induce severe yield loss

Percent disease index (PDI) can be calculated on the basis of rating scale, and varietal reactions observed, as illustrated on Table 2.3.

Table 2.3: Varietal reaction based on percent disease index

Percent disease index (PDI)	Varietal reaction (VR)
0%	Immune (I)
1 - 10%	Resistant (R)
11 - 25%	Moderately resistant (MR)
25 - 50%	Moderately susceptible (MS)
50 - 75%	Susceptible (S)
76 - 100%	Highly susceptible (HS)

2.9.4 Gene action and inheritance mechanisms in breeding for sheath rot resistance

For a breeder to take an appropriate decision regarding the direction of his/her breeding programme, genetics namely gene action and inheritance of resistance must be well understood. While, studies of genetics associated with resistance to major rice diseases have been widely documented for blast (Mackill and Bonman, 1992; Filippi and Prabhu, 1996), sheath blight (Wisser *et al.*, 2005; Wisser *et al.*, 2012; Sattari, 2014), and yellow mottle virus (Mogga *et al.*, 2010; Munganyinka *et al.*, 2015), gene action and inheritance mechanisms associated with resistance to sheath rot of rice have not been documented. This might be due to the previous status of sheath rot as a minor disease. Information on this disease has been limited and confined only to the physiology of the pathogen, pathogen genetics and control through fungicides (Srinivasachary *et al.*, 2002). However, Mackill *et al.* (1996) indicated that most of the genes associated with disease resistance in rice are under the control of additive and non-additive gene action, whereas the only information available is that resistance to sheath rot is associated with recessive genes (Chauhan and Bhatt, 1986.).

Therefore, genetic analysis of the disease is important in determining gene action controlling resistance, its inheritance and heritability of the trait prior to the start of a breeding programme per se. This can be done by utilising an appropriate mating design that can allow for estimation of different genetic components and variances that enable estimations of additive and non-additive gene action and heritability (both broad and narrow sense). The mating design can also result in determination of general and specific combining abilities (GCA and SCA), whereby predominance of GCA indicates additive gene action and SCA indicates non-additive. The nature of inheritance will inform whether resistance to sheath rot is monogenic or polygenic.

2.9.5 Introgression of resistant genes through backcross breeding

Backcross breeding is an effective method to transfer one or a few genes controlling a specific trait from one line into a second, usually, elite breeding line. The parent with the desired trait is called the donor parent, and may not perform as well as an elite variety (recurrent parent (RP) in most areas other than the trait of interest. If the trait of interest is controlled by a dominant gene,

this process will involve four cycles of backcrossing, whereas if the gene is recessive, the process requires more generations of selfing in between through progeny testing (Vogel, 2009).

The rate at which the donor parent genome is reduced and the recurrent parent genome recovered in the genetic makeup of the new plant can be calculated using the number of backcross generations utilized. This rate can be dramatically increased with the recent advances in marker technology which allow breeders to specifically target the gene of interest and control the genetic background (Vogel, 2009).

Backcrossing involves making an initial cross between the donor and recurrent parents. The resultant F1 progenies have 50% of their genetic material from each parent. F1 individuals are crossed to the recurrent parent to develop backcross one (BC1) progenies. Individuals from the BC1 are once again crossed to the recurrent parent. Each generation of backcrossing reduces the proportion of the donor parent present in the population by half. This cycle of crossing backcross progeny to the recurrent parent continues until a new line identical to the recurrent parent, but with the desired trait from the donor parent is created (Robbins, 2012).The number of BC generations will depend on how closely the breeder wants the isogenic line to resemble the RP or how well the BC genotype is performing. The proportion of RP recovery in each BC family increases with the BC generation. The following are proportions of the genes that are theoretically recovered from the recurrent parent according to number of backcrossing; F1=50%; BC1=75%; BC2=87.5%, BC3=93.8% and BC4=96.9% (Brown *et al.*, 2014).

The backcrossing process can often be accelerated using marker-assisted backcrossing by utilising markers for both foreground selection (for target gene) and background selection to recover the recurrent genome. One of the rapid and precise improvement of existing farmers and market preferred varieties is the incorporation of resistance genes into preferred cultivars through a series of methodologies mostly marker assisted backcross breeding (Singh *et al.*, 2012). When genes or QTLs of interest are known and linked to some of these markers, they can be used in marker assisted selection or MAS. Marker-assisted backcross breeding (MABB), which involves two steps: (1) MAS for the gene of interest, known as foreground selection and (2) MAS for recovery of the recurrent parent genome, known as background selection (Hospital et al., 1992), is the most effective way of transferring specific gene(s) to agronomically superior variety or parental lines. In rice, the feasibility of MABB to pyramid bacterial blight resistance

genes and incorporation of blast resistance genes into susceptible varieties has been well demonstrated (Singh *et al.*, 2012; Huang *et al.*, 2012). The MABC is currently becoming a common practice to introgress genes from donor parents into recurrent parents as it reduces considerably the time and number of required backcrosses. Hospital (2003) states a recovery of 98.6% of recurrent parent at BC3 using MABC.

As far as sheath rot of rice is concerned, QTLs associated with resistance have been identified, and mapped by Srinivasachary *et al.* (2002) and validated by Wisser *et al.* (2012). However, these QTLs have been identified based on RFLP markers which are no longer suitable for molecular biology based studies. According to Inoue and Cai (2004), despite their advantage of being highly reproducible, transferable, and co-dominant, RFLP markers require a large amount of genomic DNA. They are also time consuming and relatively highly expensive compared to other type of markers. Currently, it is advisable, where other type of markers are not available, to convert RFLP markers into more high throughput, cost and time effective sequence specific markers such as STS, SSR or SNPs (Inoue and Cai, 2004).

2.10 Summary

From the literature review, rice is becoming one of major component of food security strategies in Rwanda. The country aims at ensuring self-sufficiency in rice demand and supply as well as increased competitiveness of local rice in the international market. However, rice supplies currently are lagging far behind its demand and reasons contributing to the low yielding potential of available germplasm include a variety of biotic and abiotic constraints.

Despite the fact that rice sheath rot has been for long regarded as a minor disease, it recently emerged as one of most devastating disease especially for high yielding dwarf varieties resulting from the green revolution. The disease affects not only grain yield but also grain quality and is nowadays considered as a potential threat to rice sector in Rwanda as no commercial variety has been proven resistant and grown at a large scale so far. This led to farmers to keep growing tall and short grain varieties whereas the consumer market prefers long grain varieties mostly imported from Asia.

Substantial grain yield loss and quality deterioration have been reported all over the world in rice producing countries. The disease therefore needs a special consideration as far as rice pest and disease management research is considered in Rwanda. Even though a number of chemicals have been tested and found efficient, none of them have been recommended to Rwandan farmers. This is why a sustainable disease management strategy needs to be developed. The development and use of resistant cultivars is the best disease management method as it is relatively less expensive and cost effective. It is also easy to deploy, has no adverse environmental consequences and it is convenient for farmers to use requiring no additional costs and skills. To this end, a number of resistant varieties have been identified in Asia but none of them are adapted to Rwandan agro ecological conditions. This affirms the need to screen locally adapted germplasm and identify locally adapted sources of resistance. Various sheath rot screening methods have been described in this review as well as sheath rot inoculation methods.

However, because the disease has for long been regarded as minor, little has been done to determine the nature of inheritance of resistance. None is available on whether the resistance is completely or partially dominant as well as whether it is under monogenic or polygenic control. Suggestions of polygenic nature of inheritance for most of fungal disease needs also to be investigated as far as sheath rot of rice is concerned.

Backcross breeding has been proven to be an effective method to transfer one or a few resistance genes from one line to another, usually elite cultivar to be improved. The number of backcross cycles needed will depend on whether the gene is recessive or dominant with the latter requiring at least 4 cycles for maximum recovery of the recurrent parent's genome. Nevertheless these cycles can be accelerated using marker assisted foreground selection for sheath rot resistance and background selection for the other trait of interest. In this regard QTLs associated with resistance to sheath rot of rice have been mapped using restriction fragment length polymorphism RFLP markers and there is a need for higher throughput, less costly and time consuming markers; which include simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs).

Although most of the reports in this literature review provided potentially useful information to genetically manage sheath rot of rice, few resistant varieties have been identified in Rwanda. This consequently confirms the need for genetic studies associated with breeding for sheath rot resistance and subsequently improvement of locally adapted germplasm to meet farmers' and consumers' yield and quality requirements.

References

Acquaah, G., 2012. Principles of Plant Genetics and Breeding. Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK.

Agrios, G.N., 2005. Plant pathology, 5th ed. Elsevier Academic Press, London.

Amin, K.S., 1976. Sources of resistance to *Acrocylindrium* sheath-rot of rice. *Plant Disease Reporter* 60: 72-73.

Ayyadurai, N., Kirubakaran, S., Srisha, S., and Sakthivel, N., 2005. Biological and molecular variability of *Sarocladium oryzae*, the sheath rot pathogen of rice (*Oryza sativa* L.). *Current Microbiology* 50: 319-323.

Bridge, P.D., Hawksw, D.L., Kavishe, D.F., and Farnell, P.A., 1989. A revision of the species concept in *Sarocladium*, the causal agent of sheath-rot in rice and bamboo blight, based on biochemical and morphometric analyses. *Plant Pathology* 38: 239-245.

Brown, J., P. Caligari, and Campos, H., 2014. Plant breeding, 2nd ed. Wiley-Blackwell.

Cairns, J., Sonder, K., Zaidi, P., Verhulst, N., Mahuku, G., Babu, R., Nair, S., Das, B., Govaerts, B., and Vinayan, M., 2012. 1 Maize production in a changing climate: impacts, adaptation, and mitigatiosn strategies. *Advances in Agronomy* 114: 1-65.

Chauhan, V.S., and Bhatt, J.C., 1986. Inheritance of resistance to sheath rot in rice. *SABRAO* 18:2.

Dariush, S., Ebadi, A.A., Khoshkdaman, M., Rabiei, B., and Elahinia, A., 2012. Characterising the genetic diversity of *Pseudomonas syringae* pv. *syringae* isolated from rice and wheat in Iran. *Plant Protection Science* 48: 162–169.

Filippi, M.C., and Prabhu, A.S., 1996. Inheritance of blast resistance in rice to two *Pyricularia grisea* races, IB-1 and IB-9. *Brazilian Journal of Genetics* 19: 599-604.

Girish, T.N., Gireesha, T.M., Vaishali, M.G., Hanamareddy, B.G., and Hittalmani, S., 2006. Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza sativa* L.) from an indica × japonica cross for growth and yield traits under aerobic conditions. *Euphytica* 152: 149-161.

Guimaraes, P.E., 2009. Rice breeding, In Carena, M. J., (eds), Handbook of Plant Breeding, Cereals. Springer, Berlin.

Hospital, F., 2003 Marker-assisted breeding. In: Newbury, H.J. (eds), Plant molecular breeding. Blackwell Publishing, Oxford UK.

Huang, B., Xu, J.Y., Hou, M.S., Ali, J., and Mou, T.M., 2012. Introgression of bacterial blight resistance genes Xa7, Xa21, Xa22 and Xa23 into hybrid rice restorer lines by molecular marker-assisted selection. *Euphytica* 187: 449–459.

Inoue, M., and Cai, H., 2004. Sequence analysis and conversion of genomic RFLP markers to STS and SSR markers in Italian ryegrass *(Lolium multiflorum* Lam.). *Breeding Science.* 54: 245-251.

Kathiresan, A., 2010. Enabling self sufficiency and competitiveness of Rwanda rice: Issues and policy options. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Khush, G.S., Brar, D.S., and Hardy, B., 2003. Advances in rice genetics. Supplement to rice genetics IV. In: Proceedings of the Fourth International Rice Genetics Symposium, 22-27 October 2000. International Rice Research Institute Los Baños, Philippines

Khush, G.S., and Jena, K.K., 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: Wang, G.L. and Valent, B., (eds), Advances in Genetics, Genomics and Control of Rice Blast Disease. Springer Netherlands. p. 1-10.

Lakshmanan, P. 1992. Brevenia rehi (L), vector for the sheath rot (ShR) pathogen. *International Rice Research Notes* 17, 23.

Lalan Sharma, D.T., Nagrale, S.K., Singh, K.K., Sharma, and Sinha, A.P., 2013. A study on fungicides potential and incidence of sheath rot of rice caused by *Sarocladium oryzae* (Sawada). *Journal of Applied and Natural Science* 5 (1): 24-29

Lanoiselet, V., You, M.P., Li, Y.P., Wang, C.P., Shivas, R.G., and Barbetti, M.J., 2012. First report of *Sarocladium oryzae* causing sheath rot on rice (*Oryza sativa*) in Western Australia. *Plant Disease* 96: 1382-1382.

Lewin, H.D. and and Vidhyasekaran, P. 1987. Controlling sheath rot (ShR) in rice. *International Rice Research News letter* 12, 17.

Mackill, D.J., and Bonman, J.M., 1992. Inheritance of blast resistance in near-isogenic lines of rice. *Phytopathology* 82: 746-749.

Mackill, D.J., Coffman, W.R., and Garrity, D.P., 1996. Rainfed lowland rice improvement. International Rice Research Intitute, Manila, Philippines.

Madhav, M.S., Laha, G.S., Padmakumari, A.P., Somasekhar, N., Mangrauthia, S.K., and Viraktamath, B.C., 2013. Phenotyping rice for molecular plant breeding. In: Panguluri, S.K. and Kumar, A.A. (eds), Phenotyping for plant breeding. Springer New York. p. 1-40.

Marín, M., Wong, I., García, G., Morán, R., Basulto, R., Pimentel, E., and Mena, J., 2013. Actividad antagónica in vitro de Tsukamurella paurometabola C-924 frente a fitopatógenos. *Revista de Protección Vegetal* 28: 132-137.

Mew, T.W., and Gonzalez, P., 2002. A handbook of rice seedborne fungi. International Rice Research Institute, and Enfield, N.H. (USA). Science Publishers, Inc., Los Bafios, Philippines.

Mogga, M.L., Lamo, J., Asea, G., and Edema, R., 2010. Inheritance of resistance to rice yellow mottle virus disease in selected rice cultivars in Uganda. *RUFORUM Working Document 5:* 469-474.

Mosharraf, H., Shah, M.D., Munirur, R., Khalequzzaman, K.M., Nazrul Islam, M.D., and Ashrafuzzaman, M., 2003. Performance of breeding lines/cultivars of rice against bacterial leaf blight and sheath rot under field condition. *Journal of Biological Sciences* 3: 478-483.

Mukerji, K.G., and Manoharachary, C., 2010 Taxonomy And Ecology Of Indian Fungi. J.K. International Publishing House Pvt Ltd, New Delhi, India. .

Munganyinka, E., Edema, R., Lamo, L., Gibson, P., and Rukundo, P., 2015. Combining ability for resistance to rice yellow mottle virus disease in interspecific and intraspecific rice Genotypes. *African Journal of Crop Science* 3: 102-107.

Mutabazi, A., 2011: Rwanda country situational analysis. Camco report to assist the East African Community (EAC) in the development of a regional climate change master plan. http://rema.gov.rw/rema_doc/Climate%20change/Situational%20Analysis%20-%20RWANDA%20Final.pdf.

Narayanaprasad, Jagadeesh, B.R., Shivakumar, G.B., Prasad, P.S., Sudarshan, G.K., and Sunil Kumar, N., 2011. Studies on the nature and properties of sheath rot (*Sarocladium*

oryzae Sawada) causing seed borne pathogen on rice (*Oryza sativa* l.). *International Journal of Science and Nature* 2: 317-320.

Ngala, G.N., and Adeniji, M.O., 1986. Sheath rot disease in tropical Africa. In: Juo, A.S.R. and Lowe, J.A., (eds), The wetlands and rice in subsaharan Africa. IITA, Ibadan, Nigeria.

Ou, S.H., 1985. Rice diseases, 2nd ed. Oxford University Press.

Pearce, D.A., Bridge, P.D., and Hawksw, D.L., 2001. Species concept in sarocladium, the causal agent of sheath rot in rice and bamboo blight. In: Sreenivasaprasad S. and Johnson R., (eds), Major Fungal Diseases of Rice: Recent Advances. Springer Netherlands. p. 285-292.

Prasanna Kumar, M.K., Sidde Gowda, D.K., Rishikant, M., Kiran Kumar, N., Pandurange Gowda, K.T and Vishwanath, K., 2013. Impact of fungicides on rice production in India. In Mizuho (eds), Fungicides: Showcases of Integrated Plant Disease Management from around the World. InTech, Rijeka, Croatia. p. 340-363.

Promar, 2012. Agriculture, forestry and fisheries of Rwanda: Fact-finding survey for the support of aid to developing countries (Fiscal year 2011 Research Project). Promar Consulting, Tokyo, Japan.

Purkayastha, R., Ghosal, A., and Biswas, S., 1983. Production of momilactone associated with resistance of rice cultivars to sheath rot disease. Short communication. *Current Science 14:88.*

Reddy, M.M., Reddy, C.S., and Singh, B.G., 2000. Effect of sheath rot disease on qualitative characters of rice grain. *Journal of Mycology and Plant Pathology* 30: 68-72.

Saberi E., Safaie, N., and Rahimian, H., 2013. Characterization of bacterial strains associated with sheath rot complex and grain discoloration of rice in North of Iran (Mazandaran province). *Journal of Bacteriology Research* 5: 51-61.

Sakthivel, N., 2001. Sheath rot disease of rice: Current status and control strategies. In: Sreenivasaprasad S, Johnson R (eds). Major Fungal Diseases of Rice Recent Advances. Kluwer Academic Publishers Boston.

Sakthivel, N., and Gnanamanickam, S., 1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice (*Oryza sativa* L.). *Applied and Environmental Microbiology.* 53: 2056-2059.

Saravanakumar, D., Lavanya N., Muthumeena K., Raguchander T., and R., S., 2008. Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. *BioControl Journal*. 54: 273-286.

Sattari, A., B. Fakheri., M. Noroozi and K. Moazami gudarzi, 2014. Review: Breeding for resistance to sheath blight in rice. *International Journal of Farming and Allied Sciences* 3: 970-979.

Satyanarayana, K., Singh, S.K., and Reddy, A.P.K., 1990. Studies on morphology, growth habit, hypha anastomosis and virulence pattern of five isolates of sheath blight pathogen of rice. *Indian Phytopathology* 43: 368-371.

Singh, V.K., Singh, A., Singh, S.P., Ellur, R.K., Choudhary, V., Sarkel, S., Singh, D., Krishnan, S.G., Nagarajan, M., Vinod, K.K., Singh, U.D., Rathore, R., Prashanthi, S.K., Agrawal, P.K., Bhatt, J.C., Mohapatra, T., Prabhu, K.V., and Singh, A.K., 2012. Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crops Research* 128: 8-16.

Sreenivasaprasad, S., and Johnson, R., 2001. Major fungal diseases of rice: Recent advances. Kluwer Academic Publishers.

Srinivasachary, H., Hittalmani, S., Girish Kumar, K., Shashidhar, H. E., and Vaishali, M. G., 2002. Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exsertion in rice (*Oryza sativa* L.). *Current Science,* 82: 133-135.

Vogel, K.E., 2009. Backcross breeding. In: Scott, M.P., (eds.), Transgenic Maize: Methods and Protocols. Humana Press, Totowa, NJ. p. 161-169.

Wang, G.L., and Valent, B., 2009. Advances in genetics, genomics and control of rice blast disease. Springer Science+Business Media B.V. 2009.

Wisser, J. R., Qi, S., Hulbert, H. S., Kresovich, S., and. Nelson, J. R., 2012. Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. *Genetics* 169: 2277–2293.

Wisser, R.J., Balint-Kurti, P.J., and Nelson, R.J., 2006. The genetic architecture of disease resistance in maize: A synthesis of published studies. *Phytopathology* 96: 120-129.

Zigler, R.S., and Alvarez, E., 1990. Characteristics of *Pseudomonas* spp. causing grain discoloration and sheath rot of rice and associated Pseudomonas epiphytes. *Plant Disease* 74: 917-722.

3 Chapter 2

Evaluation of genetic variability of rice germplasm based on agro-morphological traits

Abstract

Characterization of germplasm in crop breeding contributes towards an understanding of genetic diversity, which is essential for genetic broadening of breeding populations. Due to the scarcity of information regarding diversity among rice germplasm in Rwanda, this study was carried out to determine agro-morphological variability among 64 varieties and relationships between parental materials suitable for various rice breeding programmes in Rwanda. Trials were laid in an 8 x 8 lattice design, replicated twice in each of three locations, in Rurambi, Cyili and Cyabayaga of Southern, South Eastern and North Eastern regions of Rwanda, between February and June, 2014. The analysis of variance revealed significant differences among genotypes with environmental effects being of relatively minor importance across the sites. Genotypic variance was higher than environmental variance for most of the traits except number of tillers and flag leaf length. Apart from these traits, the rest exhibited high levels of heritability. Days to 50% flowering, plant height, days to maturity, flag leaf area, sheath length, total panicle per plot, single panicle weight and grain length had the highest heritability (80.27% - 96.42%) and genetic advance estimates. Low environmental influence on phenotypic expression of genotypes was confirmed by least differences between phenotypic and genotypic coefficient of variation for a number of traits. Thus parental selection based on ad hoc traits was suggested as being effective. Principal component analysis extracted seven components contributing to more than 72% of total variation. Three components which were most reliable in explaining the variability were highly correlated with a number of traits such as plant height, number of branches per panicle, number of grains per panicle, single panicle weight, grain yield and number of tillers. Principal component based biplots revealed groups of genotypes suitable for specific breeding programmes. These included clusters of genotypes combining grain yield potential with different plant stature, tillering ability as well as grain length. This study has provided useful information on evaluation of genetic diversity of rice germplasm and its possible application to rice improvement programmes in Rwanda.

3.1 Introduction

Rice, as one of the major food crops, ranks only second to wheat among the most cultivated cereals in the world. It is estimated that half of the world population depends on rice (Acquaah, 2012). From a nutritional point of view, rice accounts for 35 - 75% of calories consumed worldwide (Khush and Jena, 2009). Rice production is becoming an important component of the agricultural sector in Rwanda. The area under rice cultivation in Rwanda has tripled in the last 10 years resulting in a substantial production increase, from less than 20,000 tons to around 70,000 tons, involving about 62,000 small scale farmers (Kathiresan, 2010). Despite this steep production increase, Rwanda still imports about 40% of the rice it consumes (Promar, 2012).

Even though, a number of strategies have been put in place to attain self-sufficiency by the year 2018 (Minagri, 2013), rice is generally grown in marshlands, in an intensive mono-cropping system which favours a gradual build-up of various biotic and abiotic stresses. These include diseases such as blast (*Magnaporthe oryzae.*), bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial sheath brown rot (*Pseudomonas fuscovaginae*), yellow mottle virus, insect pests like diopsis (*Diopsis thoracica*) as well as cold temperatures that characterize an important number of rice growing ecosystems. Besides low rice yield related constraints, grain quality is also a matter of concern given that the domestic rice market prefers relatively expensive long grain and aromatic rice, most of which is imported. This type of rice is from varieties mainly of Asian origin, especially the Green Revolution semi dwarf varieties that were bred for intensive production and high yield. However, despite their popularity, these varieties are poorly adapted to many of the African environments (WARDA, 2001). This is probably the reason why most of the rice grown in Rwanda is still the japonica type varieties which are tall and intermediate statured with short grains (ISAR *et al.*, 2003).

With evolving rice farming systems and changing farmers and consumer preferences, rice breeding programmes in Rwanda have received significant attention with respect to improvement of yield with best grain quality. In spite of thousands of rice varieties available in the Rwandan rice germplasm collections, little is known about the genetic variability, and consequently few elite rice varieties have been released that suit different agro-climatic conditions (Ndikumana and Gasore, 2010).

Agro-morphological variability characterization of local germplasm is very important to better understand existing diversity, attain targeted genetic broadening of breeding populations, and potentially link this knowledge to genotypic information (Pucher *et al.*, 2015). Studies of genetic diversity provide information on the genetic divergence and serves as a platform for specific breeding programmes. It also provides potential exploitable combinations to create segregating progenies with maximum genetic potential for further selections. Genetic diversity exposes the genetic variability in diverse populations and provides justification for introgression of desired genes to enhance performance.

In this study, 64 rice varieties were evaluated in three different agro-ecological conditions based on their agro-morphological traits with the objective to (i) assess genetic variability of the varieties across different agro-ecological conditions; (ii) assign different varieties into relationship groups; and (iii) assess the potential use of this rice germplasm in different breeding programmes.

3.2 Materials and methods

3.2.1 Experimental sites and design

Field trials were conducted in three different sites (locations) representing diverse agro-ecological conditions of rice growing marshlands in Rwanda, all of them being irrigated ecosystems. The locations included Cyili in Gisagara District of Southern Province ($2^0$27'16.25'', 29^{o}50'19.99''and 1398 m asl); Rurambi in Bugesera District of the Eastern Province (2^{o}04'16.55''), 30^{o}13'07.50'' and 1336 m asl) and Cyabayaga of Nyagatare District of Eastern Province (1^{o}24'31.15'', 30^o'16'43.52'', and 1358 m asl). Trials were conducted from January to June, 2014 and were laid out in a balanced 8 x 8 lattice design with 2 replications on each site. The Figure 3.1 illustrates the process of trial establishment.

Figure 3.1 Photographic illustration of the germinations beds (left) and experimental plots for different varieties evaluated in the course of the study

3.2.2 Planting materials

Planting materials consisted of 64 rice varieties (Table 3.1) kindly provided by the Rice Research programme of Rwanda Agriculture Board (RAB). All these varieties had been introduced in the country to meet not only farmers' preferences but also market demand. Hence a number of them have already been released and others are still in the multipurpose evaluation process prior to release.

Sun-dried seeds from each genotype were pre-germinated in plastic bags, and raised in a nursery to increase the germination rate. Seedlings were transplanted 21 days later as per recommendations. The experimental plots were 2 m wide and 2 m long with 10 rows in each. One seedling per hill was transplanted, maintaining 0.20 m × 0.20 m inter and intra-row spacing, respectively. The crop was raised under aerobic conditions by providing irrigation once in every 3 days-interval, and all the other cultural practices and crop protection measures were followed as recommended thus ensuring uniform and healthy crop growth.

3.2.3 Data collection

Agro-morphological traits were observed on randomly selected plants in each single plot at 50% flowering (where applicable) and at the maturity stages of the crop. These traits included; number of days to 50% flowering (DFF), plant height (cm) at 50% flowering (PHT50), number of tillers at 50% flowering (NT50), flag leaf length (cm) at 50% flowering (FLL50), days to maturity

(DM), plant height (cm) (PH), number of tillers (NT), number of productive tillers (NPT), flag leaf area (cm^2) (FLA), sheath length (cm) (ShL); panicle length (cm) (PL); number of branches per panicle (BrP); number of grains per panicle (GP); single panicle weight (g) (SPW); total number of panicles per plot (TPP); weight of 1000 grains (g) (W1000G); grain length (mm) (GL); and grain yield (g/plot) per plot (GY) converted in tons/ha.

3.2.4 Data analysis

Statistical analysis of data on each quantitative character was carried out using mean values of five randomly selected plants from each genotype in each replication**.** Genetic variability across sites for evaluated varieties was obtained through the analysis of variance performed on data of different traits. The following model was fitted in accordance with the description of Atlin (2006 a):

$$Y_{ijklm} = \mu + E_i + R(E)_{j(i)} + B(RE)_{m(ji)} + G_k + GE_{ik} + e_{ijklm}$$

where Y_{ijklm} = response of k^{th} genotype grown in i^{th} environment, j^{th} replication and m^{th} block; μ= general mean; E_i= effect of i^{th} environment; $R(E)_{j(i)}$ =effect of j^{th} replication within i^{th} environment; $B(RE)_{m(ji)}$=effect of m^{th} block within j^{th} replication and i^{th} environment; G_k= effect of k^{th} genotype; GE_{ik} = effect of interaction between k^{th} genotype and i^{th} environment; e_{ijklm}= term or plot residual.

The analysis of variance was performed using REML procedure of Genstat 17 edition (Payne *et al.,* 2013), where significant differences were declared at 5 and 1% significance levels. From the obtained mean square values, respective variance components and broad sense heritability (or actually repeatability for fixed genotypes) estimates were estimated according to formulae of, Annicchiarico (2002) and Atlin (2006 b). Genotypic and phenotypic coefficients of variation as well as genetic advance were estimated according to a method of Singh and Chaudhary (1985).

1) Genetic variance (Vg) = (MSg - MSg.e) / re
2) Genetic x environmental interaction variance (Vge) = (MSge - MSe) / r
3) Environmental variance (Ve) = MSe
4) Phenotypic variance (Vp)= $\frac{Ve}{re} + \frac{Vge}{e}$+Vg,

Where e= number of sites (location), r=number of replications

Table 3.1: List, types and origin of 64 varieties used in the study.

Code	Name	Type	Origin	Code	Name	Type	Origin	Code	Name	Type	Origin	Code	Name	Type	Origin
G1	Yunyine	Japonica	RAB	**G17**	Mpembuke	Indica	RAB	**G33**	Cyicaro	Indica	RAB	**G49**	AER 16	Indica	IRRI
G2	Nyiragikara	Japonica	RAB	**G18**	IRN 1-10	Indica	IRRI	**G34**	IUR 33	Indica	IRRI	**G50**	NERICA 10	**	ARC
G3	Buryohe	Indica	RAB	**G19**	IRN 5	Indica	IRRI	**G35**	IRC 9	Indica	IRRI	**G51**	NERICA 4	**	ARC
G4	Ndamirabana	Indica	RAB	**G20**	IUR 54	Indica	IRRI	**G36**	FAC 56	Indica	IRRI	**G52**	IR 56	Indica	IRRI
G5	IRN 41	Indica	IRRI	**G21**	Fashingabo	Japonica	RAB	**G37**	IRC 1-4	Indica	IRRI	**G53**	IRN 60	Indica	IRRI
G6	IUR 94	Indica	IRRI	**G22**	LL 29	Indica	IRRI	**G38**	Zongeng	Indica	RAB	**G54**	IIR 1-43	Indica	IRRI
G7	Ndamirabhinzi	indica	RAB	**G23**	IUR 69	Indica	IRRI	**G39**	IRF 13	Indica	IRRI	**G55**	IIR 1-18	Indica	IRRI
G8	IRC 22	Indica	IRRI	**G24**	IUR 98	Indica	IRRI	**G40**	IIR 47	Indica	IRRI	**G56**	IUR 60	Indica	IRRI
G9	Rumbuka	Indica	RAB	**G25**	IRF 18	Indica	IRRI	**G41**	IUR 53	Indica	IRRI	**G57**	IRN 44	Indica	IRRI
G10	Nerica 1	**	RAB	**G26**	IRN 63	Indica	IRRI	**G42**	LL 72	Indica	IRRI	**G58**	IRN 15	Indica	IRRI
G11	Moroberekan	**	ARC	**G27**	Imbaturabukungu	Indica	RAB	**G43**	IUR 48	Indica	IRRI	**G59**	AER 6	Indica	IRRI
G12	Intsindagira	Indica	RAB	**G28**	IRN 41	Indica	IRRI	**G44**	Gakire	Indica	RAB	**G60**	Yunertian	Japonica	RAB
G13	AER 17	Indica	IRRI	**G29**	IIR 1-27	Indica	IRRI	**G45**	IUR 30	Indica	IRRI	**G61**	IRF 10	Indica	IRRI
G14	IRN 40	Indica	IRRI	**G30**	Ndengera	Indica	RAB	**G46**	IRN 80	Indica	IRRI	**G62**	Jyambere	Indica	RAB
G15	IUR 31	Indica	IRRI	**G31**	IUR 56	Indica	IRRI	**G47**	Kigega	Japonica	RAB	**G63**	Tetep	japonica	ARC
G16	Intsinzi	Indica	RAB	**G32**	IUR 84	Indica	IRRI	**G48**	Yunkeng	Japonica	RAB	**G64**	Kimaranzara	Indica	RAB

** Nerica is a cross between *O. glaberrima* and *O. sativa*, Moroberekan is an *O. glaberrima* rice whereas the rest is *O. sativa* rice either *indica* or *japonica* types

RAB: Asian cultivars already released by the Rwanda Agriculture Board, rice research programme

IRRI: Rice varieties newly introduced from the International Rice Research Centre

ARC: Varieties introduced from the Africa Rice Centre

5) Broad sense heritability (H^2)= $\frac{Vg}{\frac{Ve}{re}+\frac{Vge}{e}+Vg}$

6) Genotypic coefficient of variation (GCV (%) = $\frac{\sqrt{Vg}}{\bar{X}}$ x 100

Where $\bar{X}$ is the grand mean

7) Phenotypic coefficient of variation (PCV (%))= $\frac{\sqrt{Vp}}{\bar{X}}$ x 100

8) Genetic advance (GA) =K$\sqrt{Vp}H^2$

Where, K= 1.40 for 20% selection index

The role played by each trait in overall variability was examined by the principal component analysis.

Principal component analysis (PCA) was performed on the mean values recorded on 18 phenotypic traits from 3 trial sites. The PCA clearly indicates the genetic variation of the varieties and measures the important characters with a greater impact to the total variables and each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. From this PCA, a biplot graph was drawn to classify varieties into similarity groups. Both PC and biplot analysis were computed with SPSS 16th edition (SPSS, 2006).

3.3 Results

3.3.1 Determination of genetic variability parameters

Results from the analysis of variance (Table 3.3) revealed a wide range of variability among 64 rice varieties for 18 studied traits, as indicated by the significant differences ($P<0.05$ and $P<0.01$) for the genotype component of variation. Significant differences were also apparent for the effect of sites (locations) on varietal performance for most of the traits considered except for a few including flag leaf length at 50% flowering and days to maturity.

Table 3.2 Mean square (ms) estimates from analysis of variance (ANOVA) of 18 agro morphological traits across the three sites

Source of variation	d.f.	DFF	PH50	NoT50	FLL50	DM	PH	NoT	NoPT	FLA
E	2	1825 **	2531.65**	53.41*	6.02	10.34	1185.24**	27.37**	75.36**	161.17
R(E)	3	8.98	129.78	2.47	38.73	9.68	70.35	0.6	5.17**	51.57
B(RE)	42	9.4	45.46	6.95	38.09	35.31	19.79	0.81	0.94	11.55
G	63	332.3**	1026.8**	12.21*	58.47*	470.18**	1270**	7.51**	10.99**	297.88
GE	126	9.03	70.19*	6.49	45.12	48.45	49.95*	2.55**	4.25**	17.63
Residual	147	11.91	51.31	8.7	42.2	35.94	35.51	0.73	1.32	16.51
Total	383	72.83	230.9	8.54	45.17	111.08	247.87	2.59	4.25	63.65
C.V.		3.09	8.97	28.2	27.29	4.1	6.82	6.95	11.41	18.97
Source of variation	**d.f.**	**ShL**	**PL**	**BrP**	**GP**	**TPP**	**SPW**	**W1000G**	**GL**	**GY**
E	2	129.63**	66.13**	136.65**	2259.5**	3126.3**	0.27**	33.99*	0.38**	1013772**
R(E)	3	9.37	3.57	0.46	2127.6	4687.7**	0.08	24.68	1.98	91461
B(RE)	42	8.26	10.1	0.84	304.1**	855.6	0.06	4.21	1.05	5278
G	63	57.16**	22.34**	3.23**	875.6**	7568.6**	0.36**	13.42**	6.61**	15525**
GE	126	11.68	7.25	2.23**	326.9*	1163.1**	0.07**	4.65	0.56	6887
Residual	147	9.63	7.28	0.67	236.1	681.5	0.04	5.81	1.08	5418
Total	383	18.6	10.33	2.33	404	2036.1	0.11	6.8	1.82	13488
C.V.		11.25	13.86	7.95	11.28	9.95	8.75	10.26	11.94	23.46

Means square estimates followed by ** and * indicate significant effects on 5 and 1% significance levels respectively.

where Y_{ijklm} = response of k^{th} genotype grown in i^{th} environment, j^{th} replication and m^{th} block; μ= general mean; Ei= effect of i^{th} environment; $R(E)_{j(i)}$ =effect of j^{th} replication within i^{th} environment; $B(RE)_{m(ji)}$=effect of m^{th} block within j^{th} replication and i^{th} environment; Gk= effect of k^{th} genotype; GE_{ik} = effect of interaction between k^{th} genotype and i^{th} environment.

DFF= number of days to 50% flowering; PHT50 = plant height at 50% flowering; NT50=number of tillers at 50% flowering; FLL50 = flag leaf length at 50% flowering DM=days to maturity; PH = plant height,NT = number of tillers; NPT= number of productive tillers; FLA = flag leaf area; ShL = sheath length, PL = panicle length; BrP = number of branches per panicle; GP = number of grains per panicle; SPW = single panicle weight; TPP = total number of panicles per plot; W1000G = weight of 1000 grains; GL = grain length; and GY=grain yield per plot.

se= standard error for means; C.V.=coefficient of variation; MSg= means square genotype; MSs = means square site; MS gs= mean square genotype x site; MSe= means square error; Vg= genotypic variance; Vge= genotype x site variance;

Table 3.3: Mean, Variance components, heritability and genetic advance estimates from 18 agro morphological traits across the three sites

Trait	Mean	s.e	variance components				H(%)	Coefficients of variation		GA
			Vg	Vgxe	Ve	Vp		GCV (%)	PCV (%)	
DFF	111.58	3.45	53.4	0	11.91	54.9	96.42	6.55	6.64	10
PH50	79.82	7.16	162.58	9.44	51.31	174.28	93.29	15.97	16.54	17.2
NoT50	10.46	2.95	0.58	0	8.7	1.67	28.73	7.31	12.34	0.52
FLL50	23.8	6.5	2.71	1.46	42.2	10.23	26.5	6.92	13.44	1.19
DM	146.14	6	72.37	6.26	35.94	80.45	89.96	5.82	6.14	11.3
PH	87.37	5.96	205.75	7.22	35.51	214.07	96.11	16.42	16.75	19.7
NoT	12.3	0.85	1.13	0.91	0.73	1.55	72.67	8.64	10.13	1.27
NoPT	10.09	1.15	1.61	1.46	1.32	2.32	69.47	12.58	15.1	1.48
FLA	21.42	4.06	46.9	0.56	16.51	49.83	94.1	31.97	32.95	9.3
ShL	27.59	3.1	7.92	1.03	9.63	9.87	80.27	10.2	11.39	3.53
PL	19.47	2.7	2.51	0	7.28	3.72	67.42	8.14	9.91	1.82
BrP	10.3	0.82	0.43	0.78	0.67	0.8	53.45	6.35	8.68	0.67
GP	136.24	15.36	106.58	45.4	236.1	161.07	66.17	7.58	9.32	11.8
TPP	262.22	26.11	1147.85	240.8	681.5	1341.7	85.55	12.92	13.97	43.9
SPW	2.37	0.21	0.05	0.01	0.04	0.06	81.59	9.65	10.68	0.29
W1000G	23.49	2.41	1.27	-0.58	5.81	2.04	62.06	4.79	6.08	1.24
GL	8.72	1.04	0.92	-0.26	1.08	1.01	83.62	11.01	11.55	1.18
GY	3.137	73.61	1684.5	734.5	5418	2832.33	59.47	13.08	16.96	44.3

DFF= number of days to 50% flowering; PHT50 = plant height at 50% flowering; NT50=number of tillers at 50% flowering; FLL50 = flag leaf length at 50% flowering DM=days to maturity; PH = plant height, NT = number of tillers; NPT= number of productive tillers; FLA = flag leaf area; ShL = sheath length, PL = panicle length; BrP = number of branches per panicle; GP = number of grains per panicle; SPW = single panicle weight; TPP = total number of panicles per plot; W1000G = weight of 1000 grains; GL = grain length; and GY=grain yield per plot.

se= standard error for means; Vp= phenotypic variance; H = broad sense heritability; GCV=genotypic coefficient of variation; PCV= phenotypic coefficient of variation; GA= genetic advance.

There were significant interactions between genotypes and sites for a few traits: plant height at 50% flowering and maturity, number of tillers, productive tillers, branches per panicle, grains per panicle, panicles per plot, and single panicle weight.

Results from the estimation of variance components, heritability, coefficient of variation and genetic advance are presented on Table 3.3.

Regarding variance components, phenotypic variance was higher than genotypic and environmental variance. Genotypic variance on the other hand, was greater than environmental variance for most of the traits except for number of tillers at 50% flowering, flag leaf length, sheath length, panicle length number of branches per panicle and grain yield. Similarly, phenotypic coefficient of variation was higher than genotypic variation for all the traits, with some traits exhibiting very high scores. These are flag leaf area (32.5%), grain yield (16.96%), plant height at 50% flowering (16.55%), plant height (16.75%) and number of tillers (15.10%). In contrast, lowest phenotypic coefficient of variation was observed on days to 50% flowering, days to maturity, weight of 1000 grains and number of branches per panicle.

Heritability (in broad sense) ranged from 26.50% (flag leaf length) to 96.42% (days to 50% flowering). Most of the traits exhibited a high level of heritability except number of tillers and flag leaf length which recorded lower estimates, 28.75% and 26.50% respectively. Number of branches per panicle and grain yield had moderate heritability estimates (53.45% and 59.47% respectively) while most heritable traits were days to 50% flowering (96.42%), plant height at 50% flowering (93.29%), plant height (96.11%), and flag leaf area (94.10%). These traits as expected, also had the highest estimates of genetic advance.

3.3.2 Individual trait contribution to total germplasm variability

Results from ANOVA suggested suitability of evaluated traits to reveal morphological differences among different genotypes. However, a further step was needed to demonstrate the contribution of each trait in the germplasm variability.

Principal components analysis is a powerful approach in evaluation of germplasm collections that allows a better understanding of the structure of the entire collection. It makes it possible to identify the most suitable variables among the studied varieties (Zimisuhara *et al.*, 2015). According to Sanni

et al. (2012), the PCA is a measure of how important the impact a certain trait has in explaining the total variability and each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. It is therefore a reliable guiding tool in the process of parental selection in various breeding programmes. For the present study, PCA were performed to detect which traits are most responsible in explaining genetic variability among the 64 genotypes.

Plotting the eigenvalues against the corresponding PCs produces a scree plot that illustrates the rate of change in the magnitude of the eigenvalues for the PC. The rate of decline tends to be fast initially then levels off. The 'elbow', or the point at which the curve bends, is considered to indicate the maximum number of PC to extract. According to this statement, the scree plot (Figure 3.2) revealed five principal components. However, the extracted sums of squared loadings and component correlation matrix revealed actually seven principal components which were correlated to 18 traits (Table 3.4). From this table seven principal components accounted for 72.17% of the total variation. Individual contribution for each of the seven components was: 20.57%; 13.30%; 8.86%; 8.45%; 8.27%; 6.92% and 5.78%, in respective order (Figure 3.2 and Table 3.4).

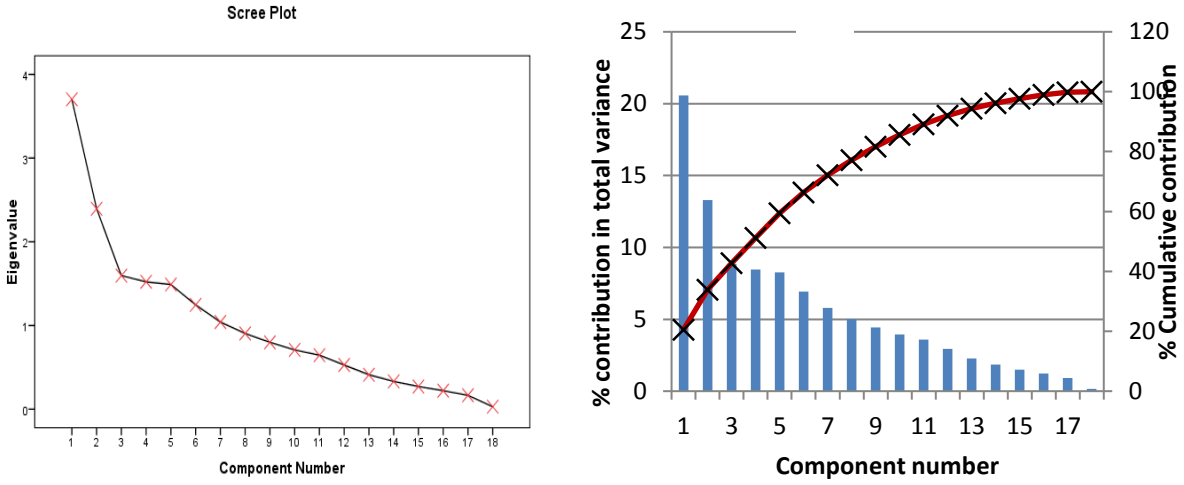

Figure 3.2 Scree plot with eigenvalues of 18 extracted principal components (A) and contribution of each component to overall variability

Analysis of the factor loadings of the characters in the retained PCs showed that phenotypic traits that contributed to yield showed high positive loadings in PC1, PC2 and PC3 (Table 3.4).

Table 3.4: Contribution of each extracted principal component in total variability and their coefficients of correlation with evaluated traits

	Component						
	1	2	3	4	5	6	7
Contribution (%)	20.568	13.302	8.864	8.454	8.272	6.922	5.783
Cumulative contribution (%)	20.568	33.87	42.734	51.188	59.46	66.382	72.165
DFF	0.019	-0.037	-0.007	-0.058	-0.071	-0.126	0.843
PH50	0.887	0.138	-0.194	0.129	0.053	0.041	-0.138
NoT50	-0.283	0.18	0.082	0.154	0.154	0.086	0.652
FLL50	0.102	-0.014	0.09	0.888	0.037	-0.141	0.087
DM	-0.009	0.27	0.105	-0.174	0.755	-0.301	-0.072
PH	0.902	0.064	-0.168	0.16	0.097	-0.029	-0.009
NoT	-0.427	0.072	0.642	-0.053	0.25	-0.161	0.194
NoPT	-0.347	0.036	0.823	0.088	0.162	-0.062	0.033
FLA	0.338	0.105	-0.208	0.006	0.002	0.633	-0.262
ShL	0.552	0.211	-0.012	-0.419	0.061	0.069	-0.091
PL	0.088	0.374	0.01	0.735	-0.088	0.131	-0.076
BrP	-0.188	0.714	-0.25	0.191	0.085	0.016	0.02
GP	0.281	0.634	0.091	0.082	-0.176	0.074	0.159
TPP	0.086	-0.091	0.832	0.059	-0.157	-0.008	-0.039
SPW	0.417	0.452	0.221	0.068	-0.348	-0.064	-0.173
W1000G	-0.237	0.288	-0.007	-0.142	-0.756	-0.297	-0.106
GL	-0.126	-0.049	0.003	-0.064	-0.017	0.843	0.054
GY	0.2	0.807	0.03	-0.039	0.104	-0.068	0.003

DFF= number of days to 50% flowering; PHT50 = plant height at 50% flowering; NT50=number of tillers at 50% flowering; FLL50 = flag leaf length at 50% flowering DM=days to maturity; PH = plant height, NT = number of tillers; NPT= number of productive tillers; FLA = flag leaf area; ShL = sheath length, PL = panicle length; BrP = number of branches per panicle; GP = number of grains per panicle; SPW = single panicle weight; TPP = total number of panicles per plot; W1000G = weight of 1000 grains; GL = grain length; and GY=grain yield per plot

These PCs represent exalted discriminatory and polymorphic traits that are strongly correlated with specific principal components. PC1 was strongly associated with plant height attributes (0.887 and 0.902). PC2 was associated with harvested plant parts related characters such as number of branches per panicle (0.714), number of grains per panicle (0.634) and grain yield (0.807) and single panicle weight (0.452). Tillering ability characters were found in association with PC3: that is, number of tillers (0.642), number of productive tillers (0.823) as well as total number of panicles per plot (0.832).

Elsewhere, PC4 was associated with panicle length (0.735) and flag leaf length (0.888), PC5 linked to days to maturity (0.755) and negatively related to weight of 1000 grains, PC6 with flag leaf area (0.633) and grain length and finally PC7 associated with days to 50% flowering.

3.3.3 Classification of varieties into relationship groups

As mentioned in section 3. 3. 2, the scree plots based on the morphological traits revealed the exact proportion of each component and its contribution to the total variation without any further information. Unlike the one-dimensional visualization of scree plot (Figure 3.2) and subsequent loadings associated with each trait in each component (Table 3.4), a biplot is a two-dimensional approach for grouping varieties taking into consideration the traits that are correlated with specific PCs. Consequently an attempt was made to visualize the clustering pattern using some selected principal component.

The first two principal components were correlated mostly with growth (vegetative) and yield (reproductive) characters that explained the 33.87% of the total variation. Plotting PC1 against PC2 resulted in grouping different varieties as shown in Figure 3.3. As the most compelling result, the biplot showed the distribution of varieties based on PC1, and PC2 (Figure 3.3 A) focused on 4 groups of genotypes. Genotypes appearing in quadrant 1 had highest scores of panicle and yield related characteristics such as panicle length, grains per panicle, single panicle weight and most importantly grain yield. Quadrant 2 had genotypes characterized by highest records of vegetative growth especially tall varieties with long panicle sheaths. Quadrant 1 gathers a group of varieties that are dwarf or semi-dwarf and high yielding potential, whereas quadrant 2 harbours a group of tall and high yielding varieties. With reference to relationship between rice tillering ability and yield, PC2 was correlated with yield characters whereas PC3 was correlated with tillering ability characters, as mentioned earlier. Accordingly, another attempt was to plot PC2 to PC3 (Figure 3.3 B). From this

biplot chart, all observations are centred on 4 scenarios. Quadrant 1 grouped high yielding and low tillering ability varieties while quadrant 2 grouped varieties combining high yielding and high tillering ability. On the other hand, quadrant 3 assembled low yielding and low tillering ability varieties and

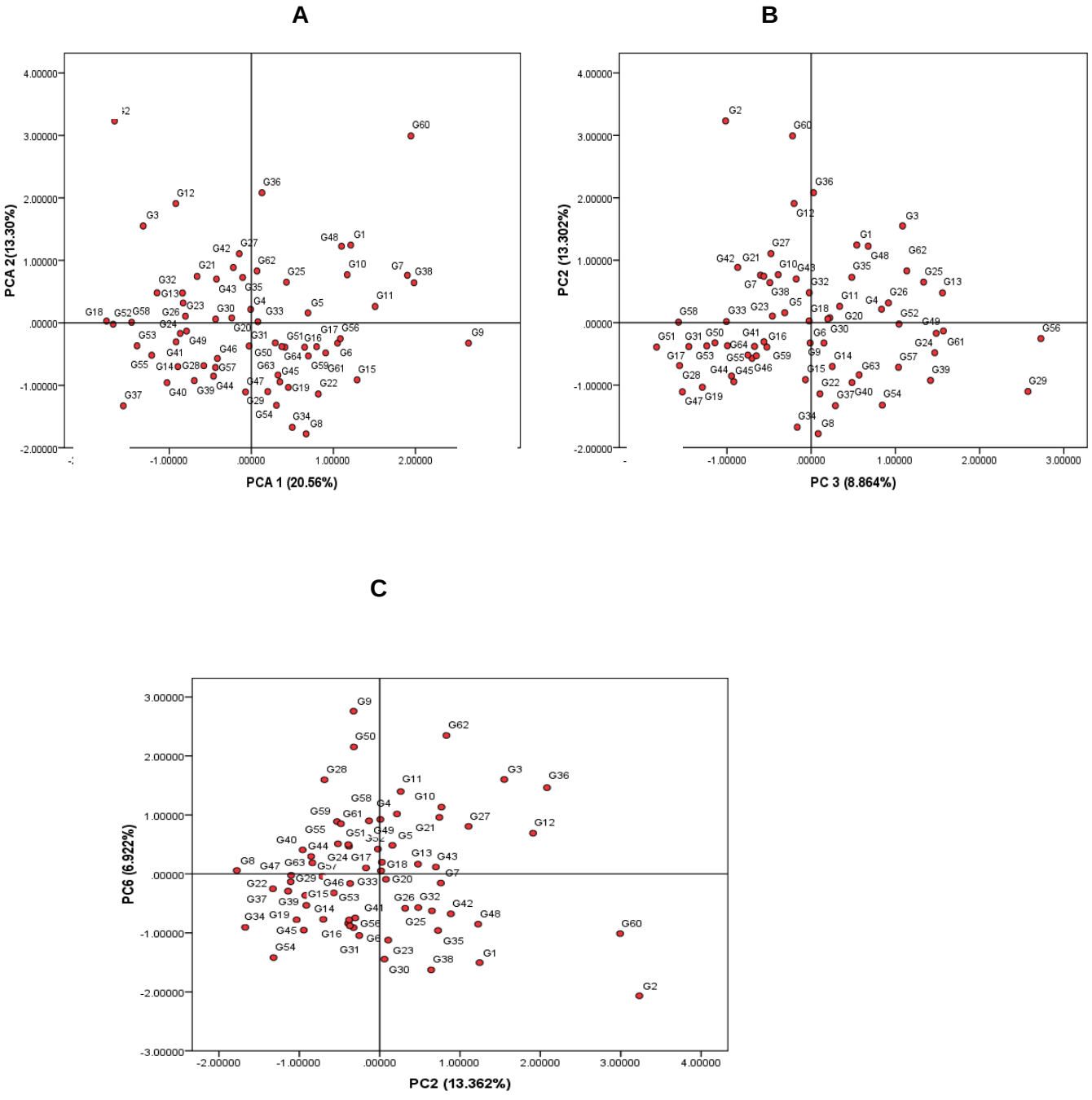

Figure 3.3 Biplot illustration of accession relationship based on specific PC1 versus PC2 (A), PCA 2 versus and PC3 (B) and PCA 2 versus PCA 6 (C). Quadrants are numbered from 1 to 4 in a serpentine order in each biplot

Quadrant 3 enclosed genotypes which are characterized by dwarf and semi dwarf plant stature as well as low yielding potential, while quadrant 4 contained tall and low yielding varieties.

As far as grain yield and quality are concerned PC2 was correlated to yield characteristics and PC6 was correlated with grain quality especially length. Plotting PC2 against PC6 on Figure 3.3 (C) resulted in quadrant 1 exhibiting long grain and low yielding genotypes whereas quadrant 2 contained high yielding and long grain genotypes. Quadrant 4 was made up of high yielding and short grain genotypes and finally quadrant 3 had short grain and low yielding genotypes.

3.4 Discussion

3.4.1 Genetic variability of different varieties across the environments

Significant differences observed among the genotypes indicate the existence of considerable genetic variability among the evaluated germplasm with regard to the 18 quantitative traits considered in this study. This was expected given that these varieties were developed from a diverse gene pool in different programmes with different breeding objectives; a suggestion also made by Oladosu *et al.* (2014). Despite the existence of considerable varietal diversity, it is important to consider the impact of site specific performance of studied varieties to facilitate the formulation of more effective breeding strategies.

The results from the present study are in general agreement with a number of findings obtained by other researchers. For instance, Pandey *et al.* (2011) reported highly significant differences among 40 rice varieties involving 12 quantitative characters with estimates of phenotypic variance being higher than genotypic and environmental variance. Similarly, Tuhina-Khatun *et al.* (2015) found a wide range and significant variation among 43 upland rice cultivars for the 22 quantitative agro-morphological characters they used.

The current results provide useful information that can be readily applied by plant breeders in the rice improvement programmes in Rwanda. This is because, as it has been demonstrated, effective evaluation of varietal diversity provides an excellent opportunity for utilization of any rice germplasm for genetic improvement. It offers, therefore, a considerable scope of choice of parents prior to hybridization (Pandey *et al.*, 2011). According to Acquaah (2012), a large phenotypic variance would

provide the breeder with a wide range of variability from which to select. However, variability in terms of genetic divergence for agronomic traits is not sufficient on its own, but also requires reliable estimates of heritability to plan an efficient hybridization programme (Akinwale *et al.*, 2011).

As yield is a quantitative trait involving a large number of genes that can be greatly influenced by environmental factors, the estimation of heritable and non-heritable component of genetic parameters is a key factor in determining the existence of high variability among studied varieties. In this regard, broad sense heritability (or repeatability for fixed genotypes) estimates which measures the portion of observed variability attributable to genetic differences provided some interesting information. Low heritability estimates were observed only for a few traits such as number of tillers and flag leaf length whereas the rest of traits had both high heritability as well as high genetic advance. This is understandable given the strong direct relationship between both parameters and therefore, an indication of the presence of considerable variation and additive gene effects (Gangashetty *et al.*, 2013). Consequently, improvement of these characters could be quite effective through mainly phenotypic selection.

Low heritability coupled with low genetic advance has also been reported for a number of traits in other studies. In their experiment, Seyoum *et al.* (2012) obtained heritability levels of 25.82 and 49.0% for number of tillers and grain yield, respectively. These results are very similar to those found in this study in addition to other traits. Sellammal *et al.* (2014) conducted a study on genetic variability for drought tolerance under varied moisture stress regimes. Results on heritability and genetic advance corroborate more or less what was obtained in the present study at least for some traits including plant height (94.3%), number of tillers (37.4%), and grain yield (54.4%).

In a population, observed variation is a result of both genetic and environmental factors, whereas genetic variability is the only heritable component of variation. Nevertheless, heritability alone does not provide definite information on the expected gain in the next generation as this has to be considered in conjunction with the genetic advance (Ahsan *et al.*, 2015). Even when the heritability of the trait of interest is very high, genetic advance would be small without a large amount of phenotypic variation. That is why heritability in conjunction with genetic advance would give a more reliable index of selection value Johnson *et al.* (1955) cited by Reddy *et al.* (2013).

Though phenotypic coefficient of variation was greater than genotypic coefficient of variation for all the traits, most of them exhibited small differences between the PCV and GCV except for the few

mentioned traits earlier in this section. Large differences between PCV and GCV estimates for the same traits have also been discussed by Akinwale *et al.* (2011), Dutta and Borua (2013) and Oladosu *et al.* (2014). The difference between PCV and GCV estimates indicates the relative influence of the environment on a specific trait which in turn determines the extent of its heritability (Gangashetty *et al*, 2013). According to Osman *et al.* (2012), small differences between PCV and GCV indicate less influence of the environment on a considered character. Thus, parental selection based on traits having less difference between PCV and GCV would be effective for future hybridization and selection programmes.

3.4.2 Individual trait contribution to overall variability of varieties

The varieties were further classified into groups based on their relatedness using the principal component analysis for future hybridization programmes. Results from the scree plot in Figure 3.2 exhibited five PCs whereas Table 3.4 showed seven PCs. According to various reports cited by Sanni *et al.* (2012) and Sinha and Mishra (2013), the first three components are the most reliable in explaining variation patterns among different genotypes. In addition, the characters associated with these PCs are the most important in differentiating various genotypes in question. However, as rice breeding targets not only grain yield but also quality, PC6 which was highly correlated with quality was also taken into consideration in then discussion.

The cumulative variance of 72.17% by the first seven axes with eigen value of > 1.0 (Table 3.4) indicates that the identified traits (especially the ones in the first three PCs) within the axes exhibited great influence on the phenotype of the varieties, and could effectively be used for selection among them. The principal component analysis method has been widely used on rice for various objectives, among others, germplasm characterization and diversity studies. For instance, Rabara *et al.* (2014), also observed 18 independent PCs, seven of which being responsible for 74.95% of the total variation among 307 rice landraces using 57 qualitative and quantitative characters in Philippines. Gana *et al.* (2013) observed five components that were strongly correlated with 12 traits among 39 genotypes and explaining 68.9% of total variation. Vishnu *et al.* (2014) also identified five PCs contributing to 85.12% of total variation, whereas, Chakravorty *et al.* (2013) identified six PCs explaining 75.83% of the total variation. The above findings suggest a common trend such that, in some circumstances, traits within a component contributed only one-sidedly, either reproductively (PC2) or vegetatively (PC1 and PC3). Same remark has also been formulated by Chakravorty *et al.* (2013).

Remarkably, to some extent, these findings corroborate with what has been observed in the present study. In fact, a number of traits were commonly found positively correlated with the first three components in general even the fourth one to some extent. These include plant height, number of tillers, number of panicle per plot, panicle ramification, weight of 1000 grains, panicle length and grain yield. According to correlation analysis performed on data set of this study (data not shown), all these traits were positively correlated with grain yield. Therefore, the prominent characters coming together in different principal components and contributing towards explaining the variability have the tendency to remain together. This may be taken into consideration during utilization of these traits in breeding programmes.

As rice farming systems in Rwanda aim mainly at grain yield and quality, the biplot in Figure 3.3 (A) exhibits only two clusters of varieties of interest. Quadrant 1 had a group of varieties that are dwarf or semi-dwarf and high yielding potential, whereas quadrant 2 harbours a group of tall and high yielding varieties. This can be explained by the fact that grain yield has been found to be most directly determined by three yield components: number of panicles per unit land area, the average number of grain produced per panicle and the average weight of the individual grains (Moldenhauer *et al.*, 2013). However, vegetative traits (plant stature and tillering ability) exercise either a positive or negative impact on grain yield. With reference to varietal characteristics in quadrant 1 and 2, short and high yielding varieties have been reported all over the world. Mishra *et al.* (2003) and Pandey (2006) described a list of high yielding and short statured Indian varieties. In Rwanda, newly released varieties, Ndikumana and Gasore (2010) characterized a number of short statured varieties that can potentially yield more than 6 tons per hectare, including some with very good quality characteristics. These include dwarf and semi dwarf varieties, including Buryohe, Ndamirabana,and Ndengera. They also mentioned high yielding tall and intermediate varieties such as Rumbuka, and set cultivars commonly known as Kigori variants.

In reference to Figure 3.3 (B), normally, tillering ability should be in direct relationship with grain yield. However, situations like the ones in quadrant 1 and 2, where high tillering varieties resulted in low yield is not a special case for this study but happens quite often. The reason is that number of tillers per hill for some varieties had negative impact on other traits by creating a bushy stand. It is evident that there should be an optimum number of tillers per hill for a given crop spacing and for a specific variety. This should permit adequate resource use efficiency, especially light, soil nutrient uptake and translocation to various parts of the plant as well as good pest and disease management

at the most sensitive physiological and growth stages (Kang and Kim, 2012). These authors also argue that components of a new type concept for greater resource-use efficiency and yield potential may include among other factors, enhanced foliar growth with reduced tillering. This scenario has also been reported by Dingkuhn *et al.* (1993) who explained that growth and limiting properties of the canopy at flowering indicated that tillers and leaf area production was excessive while foliar nitrogen concentration was suboptimal during reproductive and ripening phase. According to De Datta (1981), in some growing areas of rice, especially in temperate climate, high tillering capacity is not essential, but low tillering capacity is compensated by high number of seedlings per plot. Farmers in Australia and China manage to get 8-9 t/ha yield with one or two tillers per plant. These results suggest that it is necessary to obtain high panicle or grain number or both per unit area and these can be achieved partly through breeding and improved management practices.

Apart from yield, grain quality is the most important factor considered by plant breeders. Yield is the most noticeable characteristic to farmers but when the product reaches the market, quality becomes the key determinant of its marketability. If consumers do not accept the taste, texture, aroma, or appearance of a newly developed variety, its usefulness is greatly diminished. There are several traits associated with physical quality of grain, which include length and width of the rice grain. These are important attributes that determine the class of the rice. There are three main classes of rice based on grain length: short, medium and long. Consumers in Rwanda prefer mostly aromatic and long grain rice which is imported and subsequently highly priced. Therefore, as far as grain length is concerned, a further attempt was made to plot PC6 (positively and highly correlated with grain length) with PC2 (correlated with grain yield).

In view of grain yield and quality, logically quadrant 1 and 2 of the Figure 3.3 (C) are more interesting from the breeding point of view because of a number of reasons. Grain yield and quality have largely been reported to be negatively correlated as far as grain quality parameters, except length and width, are concerned (http://irri.org/about-us/our-organization/grain-quality-and-nutrition-center) but this depends on the meaning attached to high yields for different rice producers. High-grain quality rice has different meaning in different countries, depending on consumer preferences or preferences on the international market. For instance, in Thailand like in the Rwandan rice market, good quality rice is defined as that with a long, slender, translucent grain with a length of more than 7 mm (De Datta, 1981). According to the same author, in developed countries and in other rice exporting countries, physical appearance of the grain is often more important than grain yield. In the

developing countries, grain quality takes greater importance as the countries become more prosperous and self-sufficient in rice.

For rice breeding purposes, and the traits considered in this study, the varieties appearing in quadrant 2 of Figure 3.3 (A) are suitable for breeding objectives focusing in improving yield and tall or intermediate stature. These include varieties such as G60 (Yunertian), G36 (FAC56), G48 (Yunkeng), and G1 (Yunyine), among others. Quality for this group is questionable as generally tall varieties have short grains and subsequent quality characteristics. Varieties appearing in quadrant 1 are potentially interesting for breeding programmes aimed at grain yield and improved quality with short stature. Generally, dwarf genotypes are Asian imported for their quality characteristics and dwarf stature and long grain are widespread in these rice growing regions. Yield is associated with their greater resource use efficiency in terms of less foliar growth and enhanced assimilate export from leaves to stems along with sustained high nitrogen concentration (Kang and Kim, 2012). Varieties in this group include G2 (Nyiragikara), G12 (Intsindagira), G3 (Buryohe), G32 (IUR 48), and G42 (LL72).

Quadrant 2 and 4 of Figure 3.3 (B) had varieties that can be considered by breeding programmes focusing on tillering ability and improved yield. These include high yielding and tillering ability varieties G36 (FAC56), G3 (Buryohe), G1 (Yunyine), G62 (Jyambere), and G25 (IRF 18) on one hand, and high yielding and low tillering ability varieties G2 (Nyiragikara), G60 (Yunertian), G12 (Intsindagira), G27 (Imabturabukungu, and G42 (LL 72) on the other hand. For breeding programmes aimed at physical quality of the grain (length for instance), varieties in quadrants 2 of Figure 3.3 (C) i.e high yielding potential and long grain, are more suitable for parental selection prior to hybridization. These include, respectively in this order, G62 (Nyiragikara), G3 (Buryohe), G36 (FAC 56), G27 (Imbaturabukungu) and G12 (Intsindagirabigega).

3.5 Conclusion

The development of rice varieties that have potential to ensure food security should focus on exploitation of genetic diversity especially associated with quantitative traits. This study highlighted existence of high level of genetic variability among 64 rice varieties available in Rwanda's rice germplasm collection. Much of the variability was found to be due to the genetic component of variation with little influence from environmental factors within the sites. This led to high levels of

heritability and genetic advance for most of the traits, except for number of tillers and flag leaf length. Hence most of traits in this study can be considered to be of considerable importance in future breeding programmes.

Principal components analysis extracted 8 most polymorphic traits mostly responsible for genetic variability. These traits are plant height at both growth stages, number of branches per panicle, number of grains per panicle, single panicle weight, grain yield, number of tillers and total panicle per plot. These traits should therefore be given special prominence during parental selection in hybridization process. Based on these traits, biplot analysis proposed clusters of varieties to be considered in varietal improvement strategies involving grain yield on one hand and vegetative characteristics (plant stature, tillering ability) and physical quality of grains (length) on the other hand. Hence these results are of significant importance in identifying potential parental materials for improving various morphological traits considered in this study.

References

Acquaah, G., 2012. Principles of plant genetics and breeding. Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK.

Ahsan, M. Z., Majidano, M. S., Bhutto, H., Soomro, A. W., Panhwar, F. H., Channa, A. R., and Sial, K. B., 2015. Genetic variability, coefficient of variance, heritability and genetic advance of some *Gossypium hirsutum L*. varieties. *Journal of Agricultural Science* 7: 147-151.

Akinwale, M. G., Gregorio, G., Nwilene, F., Akinyele, B. O., Ogunbayo, S. A., and Odiyi, A.C., 2011. Heritability and correlation coefficient analysis for yield and its components in rice (*Oryza sativa L*.). *African Journal of Plant Science* 5: 207-212.

Annicchiarico, P., 2002. Genotype x environment interactions: challenges and opportunities for plant breeding and cultivar recommendations. Food and Agriculture Organization of The United Nations, Rome, Italy.

Atlin, G., 2006 a. Multi-environment trials: design and analysis. International Rice Research Institute, IRRI HQ, Los Baños, Philippines.

Atlin, G., 2006 b: Heritability in multi-location trials and response to selection. In: IRRI, (eds), Planning rice breeding programs for impact. International Rice Research Institute, IRRI HQ, Los Baños, Philippines. p 15-163.

Chakravorty, A., Ghosh, P. D., and Sahu, P. K., 2013. Multivariate analysis of phenotypic diversity of landraces of rice of West Bengal. *American Journal of Experimental Agriculture* 3: 110-123.

De Datta, S. K., 1981. Principles and practices of rice production. John Wiley and Sons, Singapore.

Dingkuhn, M., Penning De Vries, F. W. T., and Miezan, K. M., 1993. Improvement of rice plant type concepts: Systems research enables interaction of physiology and breeding. In: Penning de Vries, F., Teng, P. and Metselaar, K., (eds), Systems Approaches for Agricultural Development. Springer, Netherlands. p. 19-35.

Dutta, P., and Borua, P., 2013. Morphological traits as selection indices in rice: a statistical view. *Universal Journal of Agricultural Research* 1: 85-96.

Gana, A. S., Shaba, S. Z., and Tsado, E. K., 2013. Principal component analysis of morphological traits in thirty-nine varieties of rice (*Oryza sativa L*.) grown in a rainfed lowland ecology of Nigeria. *Journal of Plant Breeding and Crop Science* 4: 120-126.

Gangashetty, P. I., Salimath, P., and Hanamaratti, N., 2013. Genetic variability studies in genetically diverse non-basmati local aromatic genotypes of rice (Oryza sativa (L.). *Rice Genomics and Genetics* 4: 4-8.

Hallauer , A. R., Carena, M. J., and Miranda, J. B., 2010. Quantitative genetics in maize breeding. Springer. New York.

ISAR, ATDT, and CIAT, 2003: Marketing survey of the rice sub-sector in Rwanda. *I*nstitut des Sciences Agronomiques du Rwanda (ISAR) and Iternational Institute of Tropical Agriculture (IITA-FOODNET), Butare Rwanda.

Kang, K. K., and Kim, Y. G., 2012. Temperate rice in Korea. In: Jena, K.K., (eds.), Advances In temperate rice research. Interantional Rice Research Institute, Los Baños (Philippines).

Kathiresan, A., 2010. Enabling self sufficiency and competitiveness of Rwanda rice: Issues and policy options. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Kathiresan, A., 2013. Rwanda's rice commodity chain-facing globalization. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Khush, G. S., and Jena, K. K., 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa L*.). In: Wang, G. L. and Valent, B., (eds), Advances in Genetics, Genomics and Control of Rice Blast Disease. Springer Netherlands. p. 1-10.

Minagri, 2013: National rice development strategy: period 2011-2018. Ministry Of Agriculture And Animal Resources, Kigali Rwanda.

Mishra, B., Singh, R. K., and Senadhira, D., 2003. Advances in breeding salt tolerant rice varieties. In: Khush, G. S., Brar, D. S., and Hardy, B., (eds), Advances In Rice Genetics. International Rice Research Institute, Los Banos Philippines. p. 5-7.

Moldenhauer, K., Wilson, E. C., Counce, P., and Hardke, T. J., 2013. Rice growth and development In: Hardke, T. J., (eds.), Arkansas Rice Production Handbook. University of Arkansas: Division of Agriculture Cooperative Extension Service, Little Rock, Arkansas 72204. p. 212.

Ndikumana, I., and Gasore, E. R., 2010. Description of new rice varieties introduced in Rwanda, Institut des Sciences Agronomiques du Rwanda, Kigali Rwanda.

Oladosu, Y., Rafii, M. Y., Norhani, A., Mohammad, A. M., Rahim, H. A., Ghazali, H., Mohammad, A. L., and Isiaka, K., 2014. Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits. *The Scientific World Journal* 2014: 1-12.

Osman, K. A., Mustafa, A. M., Ali, F., Yonglain, Z., and Fazhan, Q., 2012. Genetic variability for yield and related attributes of upland rice genotypes in semi arid zone (Sudan). *African Journal of Agricultural Research* 7: 4613-4619.

Pandey, B., 2006. A Textbook of Botany: Angiosperms - Taxonomy, Anatomy, Embryology and Economic Botany, Fifth ed. S. Chand and Co. Ltd., New Delhi.

Pandey, P., Anurag, P.J., Tiwari, D.K., Yadav, S.K., and Kumar, B., 2011. Genetic variability, diversity and association of quantitative traits with grain yield in rice (*Oryza sativa L*.). *Journal of Bio-Science* 17: 77-82.

Payne, R., Welham, S., and Harding, S., 2014. A guide to reml in genstat. VSN International, Hertfordshire, Uk.

Promar, 2012. Agriculture, forestry and fisheries of Rwanda: Fact-finding survey for the support of aid to developing countries (Fiscal year 2011 Research Project). Promar Consulting, Tokyo, Japan.

Pucher, A., Sy, O., Angarawai, I. I., Gondah, J., Zangre, R., Ouedraogo, M., Sanogo, M. D., Boureima, S., Hash, C. T., and Haussmann, B. I. G., 2015. Agro-morphological characterization of west and central african pearl millet varieties. *Crop Science* 55 : 737-748.

Rabara, R. C., Ferrer, M. C., Diaz, C. L., Newingham, M. C. V., and Romero, G. O., 2014. Phenotypic diversity of farmers' traditional rice varieties in the Philippines. *Agronomy* 4: 217-241.

Reddy, M., Reddy, B.N., Arsul, B., and Maheshwari, J., 2013. Genetic variability, heritability and genetic advance of growth and yield components of linseed (*Linum usitatissimum L*.). *International Journal of Current Microbiology and Applied Sciences* 2: 231-237.

Sanni, K. A., Fawole, I., Ogunbayo, A., Tia, D., Somado, E. A., Futakuchi, K., Sié, M., Nwilene, F. E., and Guei, R. G., 2012. Multivariate analysis of diversity of landrace rice germplasm. *Crop Science* 52: 494-504.

Sellammal, R., Robin, S., and Raveendran, M., 2014. Association and heritability studies for drought resistance under varied moisture stress regimes in backcross inbred population of rice. *Rice Science* 21: 150-161.

Seyoum, M., Alamerew, S., and Bantte, K., 2012. Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice (*Oryza sativa L*.). *Journal of Plant Sciences* 7: 13-22.

Singh, R. K., and Chaudhary, B. D., 1985. Biometrical methods in quantitative genetics analysis. Kalyani Publishers.

Sinha, A. K., and Mishra, P. K., 2013. Agro-morphological characterization and morphology based genetic diversity analysis of landraces of rice variety (*Oryza sativa* L.) of Bankura Ddistrict of West Bengal. *International Journal of Current Research* 5 (10): 2764-2769.

Tuhina-Khatun, M., Hanafi, M. M., Rafii Yusop, M., Wong, M. Y., Salleh, F. M., and Ferdous, J., 2015. Genetic variation, heritability, and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. *BioMedical Research International* 2015: 1-7.

Vishnu, V. N., Robin, S., Sudhakar, D., Raveendran, M., Rajeswari, S., and Manonmani, S., 2014. Evaluation of rice genetic diversity and variability in a population panel by principal component analysis. *Indian Journal of Science and Technology,* 7: 1555–1562.

WARDA, 2001: Nerica rice for life. West Africa Rice Development Association (WARDA/ADRAO), Bouaké 01, Côte d'Ivoire.

Zimisuhara, B., Valdiani, A., Shaharuddin, N. A., Qamaruzzaman, F., and Maziah, M., 2015. Structure and principal components analyses reveal an intervarietal fusion in Malaysian mistletoe fig (*Ficus deltoidea* Jack) populations. *International Journal of Molecular Sciences* 16: 14369-14394.

4 Chapter 3

Identification of sources of resistance to sheath rot disease of rice among Rwandan rice germplasm

Abstract

Sheath rot of rice, *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], is currently regarded as one of most seriously emerging rice diseases, not only in Rwanda but also worldwide. It was formerly regarded as a minor disease and consequently little information is available for its management in the country. Varietal resistance is the most cost-effective and eco-friendly management strategy for the small scale farmers. An investigation was conducted to determine the economic threats caused by this disease and identify resistant and genetically distant parental materials that could be employed in varietal development programme. Sixty four varieties were evaluated in field trials at three different sites, using morphometric markers. Results indicated 10 late maturing, intermediate to tall, well exerted and short grain cultivars which showed different levels of resistance with a percent disease index (PDI) from 0.8 - 16.0%. Out of these, one immune cultivar (Yunyine) and five resistant cultivars (Nyiragikara, Nerica 1, Moroberekan, Cyicaro, and Yunertian) were found to be suitable for various ad hoc breeding programmes. Four moderately resistant cultivars were found to meet cost effective rice farming requirements. The remaining, early maturing, dwarf and semi-dwarf, enclosed panicles and mostly long grain cultivars were found to exhibit different levels of susceptibility with PDI ranging between 27.1 - 83.2%. Based on Pearson's correlation coefficients, a number of agro-morphological traits were significantly and negatively correlated with sheath rot. These were plant height, number of branches per panicle, number of grains per panicle, weight of 1000 grains, panicle length and grain yield. Sheath rot was positively and significantly correlated with flag leaf sheath length. Based on these results, ShR can be regarded as potential threat to rice sector as far as farming systems are concerned especially since the rice consumer market in Rwanda prefers mostly long grain varieties.

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4.1 Introduction

The sheath rot (ShR) of rice caused mainly by a seed borne fungal pathogen, *Sarocladium oryzae* [(Sawada*)* W. Gams & D. Hawksw], is one of most serious emerging and devastating rice diseases in wetland rice growing regions (Lanoiselet *et al.*, 2012). The pathogen attacks flag leaf sheaths and grains and yield losses result mainly from poor panicle formation and exertion, spikelet sterility (80-100%), reduced grain filling, and losses in milling (Ngala and Adeniji, 1986). Quality is also affected as severe attacks lead to chaffy, discoloured grains and affect viability and nutritional value of the grains followed by a decrease in the protein and starch contents of infected seeds (Reddy *et al.*, 2000). Losses range between 26 and 50% in general but higher yield losses up to 85% were recorded in Taiwan (Sakthivel, 2001; Pearce *et al.*, 2001). Variability in yield losses depends upon prevailing favourable conditions under which rice is grown and the level of susceptibility of the grown cultivar (Ayyadurai *et al.*, 2005). The disease is among many diseases which were earlier considered as minor, and have assumed the proportion of major ones. This is due to introductions of high yielding varieties as a result of the green revolution on one hand, and the change in cultivation practices which are heavily dependent on chemical fertilizers and the apparent changes in climate, on the other hand (Madhav *et al.*, 2013). Because of this, sheath rot of rice has been inadequately studied; yet it is a serious threat to increased rice production in tropical Africa (Ngala and Adeniji, 1986).

Sheath rot management in rice fields relies on integration of chemical control with cultural practices. According to Ayyadurai *et al.* (2005) fungicide treatments are most of the time unsuccessful under farmers conditions or are very expensive as well as harmful to the environment. In the same context, biological control has been of limited effect due to inconsistency of antagonists under field conditions (Gnanamanickam, 2009). Therefore, the most sustainable solution is the development and deployment of resistant varieties. A number of resistant varieties have been developed in different countries (Lakshmanan and Velusamy, 1991; Pearce *et al.*, 2001), but none of them has been developed in Rwanda. There is therefore, a need to develop local varieties resistant to rice sheath rot by introgressing resistance genes from locally adapted parents. This is the most effective strategy in terms of sustainability. However, the genetic diversity of Rwandan rice germplasm; its variation in resistance to sheath rot has not been documented yet. This study aimed at (i) highlighting the evidence of ShR as a potential threat to Rwanda's rice sector (ii) identifying adequate sources of

resistance to be incorporated in varietal improvement programmes, and (iii) determining the correlation between resistant germplasm with yield and yield components.

4.2 Materials and methods

3.2.1 Experimental sites and design

Field trials were conducted in three different sites representing diverse agro-ecological conditions of rice growing marshlands in Rwanda. These sites are Cyili located in Gisagara District of Southern Province ($2^{o}27'16.25''$, $29^{o}50'19.99''$and 1398 m asl); Rurambi in Bugesera District of the Eastern Province ($2^{o}04'16.55''$, $30^{o}13'07.50''$ and 1336 m asl) and Cyabayaga of Nyagatare District of Eastern Province ($1^{o}24'31.15''$, $30^{o'}16'43.52''$, and 1358 m asl). These trials were conducted from January to June, 2014 and trials were laid out in a balanced 8 x 8 lattice design with 2 replications in each site.

4.2.1 Planting materials and establishment of trials

Planting materials consisted of 64 rice varieties from the rice research programme of Rwanda Agriculture Board (RAB). The description of these varieties is detailed in Table 4.1. Sun-dried seeds from each genotype were pre-germinated in plastic bags, and raised in a nursery to increase the germination rate. Seedlings were transplanted 21 days after sowing as per rice farming routine recommendations. The experiment was conducted in a 2 m wide and 2 m long plot. One seedling per hill was transplanted, maintaining 20 cm × 20 cm for inter- and intra-row spacing respectively. The crop was raised under irrigated conditions by providing irrigation once at 3 days interval and the rest of the cultural practices followed recommendations for rice production ensuring uniform and healthy crop growth.

Prior to establishment of the trials, the experimental sites were considered as disease hot spots and hence plant materials were to be exposed to sufficient natural inoculum. However, according to Madhav *et al.* (2013), results may fluctuate due to inconsistent and uneven degree of natural infection.

Table 4.1: List, type and origins of 64 varieties used in the study

Code	Name	Type	Origin	Code	Name	Type	Origin
G1	Yunyine	Japonica	RAB	**G33**	Cyicaro	Indica	RAB
G2	N'gikara	Japonica	RAB	**G34**	IUR 33	Indica	IRRI
G3	Buryohe	Indica	RAB	**G35**	IRC 9	Indica	IRRI
G4	N'rabana	Indica	RAB	**G36**	FAC 56	Indica	IRRI
G5	IRN 41	Indica	IRRI	**G37**	IRC 1-4	Indica	IRRI
G6	IUR 94	Indica	IRRI	**G38**	Zongeng	Indica	RAB
G7	N'bahinzi	indica	RAB	**G39**	IRF 13	Indica	IRRI
G8	IRC 22	Indica	IRRI	**G40**	IIR 1- 47	Indica	IRRI
G9	Rumbuka	Indica	RAB	**G41**	IUR 53	Indica	IRRI
G10	Nerica 1	**	RAB	**G42**	LL 72	Indica	IRRI
G11	Moroberekan	**	ARC	**G43**	IUR 48	Indica	IRRI
G12	I'bigega	Indica	RAB	**G44**	Gakire	Indica	RAB
G13	AER 17	Indica	IRRI	**G45**	IUR 30	Indica	IRRI
G14	IRN 40	Indica	IRRI	**G46**	IRN 80	Indica	IRRI
G15	IUR 31	Indica	IRRI	**G47**	Kigega	Japonica	RAB
G16	Intsinzi	Indica	RAB	**G48**	Yunkeng	Japonica	RAB
G17	Mpembuke	Indica	RAB	**G49**	AER 16	Indica	IRRI
G18	IRN 1-10	Indica	IRRI	**G50**	NERICA 10	**	ARC
G19	IRN 5	Indica	IRRI	**G51**	NERICA 4	**	ARC
G20	IUR 54	Indica	IRRI	**G52**	IR 56	Indica	IRRI
G21	Fashingabo	Japonica	RAB	**G53**	IRN 60	Indica	IRRI
G22	LL 29	Indica	IRRI	**G54**	IIR 1-43	Indica	IRRI
G23	IUR 69	Indica	IRRI	**G55**	IIR 1-18	Indica	IRRI
G24	IUR 98	Indica	IRRI	**G56**	IUR 60	Indica	IRRI
G25	IRF 18	Indica	IRRI	**G57**	IRN 44	Indica	IRRI
G26	IRN 63	Indica	IRRI	**G58**	IRN 15	Indica	IRRI
G27	I'bukungu	Indica	RAB	**G59**	AER 6	Indica	IRRI
G28	IRN 41	Indica	IRRI	**G60**	Yunertian	Japonica	RAB
G29	IIR 1-27	Indica	IRRI	**G61**	IRF 10	Indica	IRRI
G30	Ndengera	Indica	RAB	**G62**	Jyambere	Indica	RAB
G31	IUR 56	Indica	IRRI	**G63**	Tetep	japonica	ARC
G32	IUR 84	Indica	IRRI	**G64**	Kimaranzara	Indica	RAB

** Nerica is a cross between *O. glaberrima* and *O. sativa*, Moroberekan is an *O. glaberrima* rice whereas the rest is *O. sativa* rice either *indica* or *japonica* types. RAB: Asian cultivars already released by the Rwanda agriculture Board, rice research programme, IRRI: Rice varieties newly introduced from the International Rice Research Centre, ARC: Varieties introduced from the Africa Rice Centre.

To obtain uniform infections of the disease, artificial inoculation of the plants is required. In this regard, natural inoculum was supplemented by artificial inoculation according to a simple and efficient method known as sheath inoculum technique as described by Narayanaprasad *et al.* (2011).This consisted of cutting infected sheath into small pieces then inserting them in between the flag leaf sheath and in emerged sheath.

Because rice sheath rot is sometimes confused with rice sheath brown rot (*Pseudomonas fuscovaginae),* the disease on every selected and tagged hill was confirmed by isolation of the pathogen on PDA (Figure 4.1).

Figure 4.1: *Sarocladium oryzae* [(Sawada) [W. Gams & D. Hawksw] cultured on PDA for this study (left) and in another study in Cuba (right) by Marín *et al.* (2013).

4.2.2 Data collection

Disease symptom development was monitored on a fortnightly basis after appearance of first spots around 90 days after planting. For disease severity, observations were recorded at mature flag leaf sheath on randomly selected and tagged 5 plant tillers in each plot by using 0 – 9 rating scale given by standard disease estimation system of rice by IRRI (1996) as described in Table 4.2. Minor adjustments were carried out and these concerns metric based measurements (in centimetres) of spot length over the total length of the flag leaf sheath instead of visual assessment of lesion coverage on the leaf.

Table 4.2: Disease severity rating scale based on proportion of lesion length over flag leaf sheath length

Score	Disease severity description
0	No lesion or spot on flag leaf
1	Spots visible on tillers upon very careful examination (<1% flag leaf sheath area covered
3	Spots easily visible on tillers upon careful examination (1 - 5% flag leaf area covered)
5	Spots easily visible on tillers (6 - 25% flag leaf area covered)
7	Spots present on almost whole tillers parts (26 - 50% flag leaf area covered) damage conspicuous.
9	Spots very common on whole tillers parts (51 - 100% flag leaf sheath area covered) death of plant common, damage directly induce severe yield loss

Morphological data were also recorded in a bid to detect eventual evidence of relationship between ShR of rice with some growth and yield characters. These included plant height (cm), panicle exsertion measured as the distance from the flag leaf ligule to the panicle node, in centimetres (cm), number of grains per panicle, weight of 1000 grains (g), grain yield (g/plot converted to t/ha).

4.2.3 Data analysis

The numerical values were further used for the calculation of PDI (Percent disease index) using the following formula by (Lalan Sharma *et al.*, 2013).

$$\text{PDI} = \frac{Sum\ of\ individual\ rating}{No\ of\ leaves\ examined} \times \frac{100}{Maximum\ disease\ rating}$$

Based on PDI, varietal reaction was categorized as follows: I (immune = 0%), R (resistant = 1 - 10%), MR (moderately resistant= 11 - 25%), MS (moderately susceptible = 26 - 50%), S (susceptible= 51 - 75%) and HS (highly susceptible= 76 - 100%).

Data for the last severity scores were submitted for analysis to assess the mean performance of each accession for some selected traits. A combined analysis of variance across sites was performed to estimate the effect of genotypes, site and their interaction following a mixed model where the factor genotype was fixed and site random (Paris *et al.* 2011). The ANOVA was computed according to REML procedure using Genstat 17 edition (Payne *et al.*, 2014) to fit the following model as described by IRRI (2006):

$$Y_{ijklm} = \mu + E_i + R(E)_{j(i)} + B(RE)_{m(ji)} + G_k + GE_{ik} + e_{ijklm}$$

where Y_{ijklm} = response of k^{th} genotype grown in i^{th} environment, j^{th} replication and m^{th} block; μ= general mean; Ei= effect of i^{th} environment; $R(E)_{j(i)}$ =effect of j^{th} replication within i^{th} environment; $B(RE)_{m(ji)}$=effect of m^{th} block within j^{th} replication and i^{th} environment; Gk= effect of k^{th} genotype; effect of interaction between k^{th} genotype and i^{th} environment; e_{ijklm}= term or plot residual.

Mean squares were estimated and significant differences were declared using 1 and 5% significant levels and genotypic mean performance for various agro morphological traits were compared using least significant difference. However, some selected traits were described according to rice descriptors as established by Bioversity-International in conjunction with IRRI and WARDA (Bioversity-International *et al.*, 2007). The relationship between studied rice varieties using disease severity scores and agronomic traits was determined with correlation analysis using SPSS 16 edition.

4.3 Results

Results from the combined analysis of variance of final ShR severity ratings as well as other important agronomic traits are presented in Table 4.3. Mean squares estimates revealed the effect of interaction between genotype and site were only significant ($P>0.05$) for plant height, grains per panicle and number of branches per panicle, whereas there was no effect of site on panicle exsertion. Mean square estimates revealed significant effect ($P<0.05$) of genotype and site (location) on ShR, and other agronomic traits for the different varieties.

Table 4.3: Mean square estimates for ShR severity ratings and other agronomic characters for 64 varieties over 3 sites

Source of variation	df	ShR(PDI)	PE	ShL	PH	GP	BrP	WTG	GY
E	2	180.02**	1.27	222.194**	1185.24**	2259.50**	136.65**	33.99*	1013772.00**
R (E)	3	23.20	0.187	10.58	70.35	2127.60	0.46	24.68	91461.00
B(RE)	42	0.98	0.911	3.746	19.79	304.10*	0.84	4.21	5278.00
G	63	1095.58**	45.1**	148.57**	1270.00**	875.60**	3.23**	13.42**	15525.00**
GE	126	17.31	1.045	9.03	49.95*	326.90*	2.23**	4.65	6887.00
Residual	1683	20.01	1.107	9.598	35.51	236.10	0.67	5.81	5418.00
Total	1919		8.299	???	247.87	404.00	2.33	6.80	13488.00
CV (%)		30.40	49.27	10.70	6.82	11.28	7.95	10.26	23.46
se.		4.47	1.05	3.10	5.96	15.36	0.82	2.41	73.61

df= degree of freedom; ShR (PDI)= Percentage disease index for sheat rot; PE= panicle exsertion; ShL= flag leaf sheath length; PH= plant height; GP= grains per panicle; BrP= number of branches per panicle, WTG= weight of 1000 grains; GY= grain yield

where Yijklm = response of kth genotype grown in ith environment, jth replication and mth block; μ= general mean; Ei= effect of ith environment; R(E)j(i) =effect of jth replication within ith environment; B(RE)m(ji)=effect of mth block within jth replication and ith environment; Gk= effect of kth genotype; effect of interaction between kth genotype and ith environment. CV= coefficient of variation and se= standard error.

4.3.1 Reaction of different varieties to sheath rot of rice

The sheath rot symptom ratings using percentage disease index (PDI) (Figure 4.2) revealed that amongst 64 varieties assessed, only 10 genotypes showed different resistance levels (immunity, resistance and moderate resistance). Eleven genotypes were moderately susceptible whereas the rest of genotypes were either susceptible or highly susceptible. Percent disease index ranged from 0.79% to 83.22% for genotype G2 (Nyiragikara) and genotype G45 (IUR 30) respectively (Table 4.4). Moroberekan the resistant check was rated 1.8% while Buryohe, the susceptible check was rated 76.2%. However, only one accession, Nyiragikara, recorded fewer PDI (more resistant) than the resistant check, whereas ,6 genotypes G58 (IRN 15), G9 (Rumbuka), G55 (IIR 1-18), G19 (IRN 5), G31 (IUR 56), and G45 (IUR 30) were more susceptible than the susceptible check with PDI values of 76.6%, 77.3%, 79.8%, 80.7% and 83.2%, respectively. The remaining 36 varieties were less susceptible than the check regardless of statistical significances (Table 4.4). The newly imported good quality rice varieties were remarkably susceptible to sheath rot.

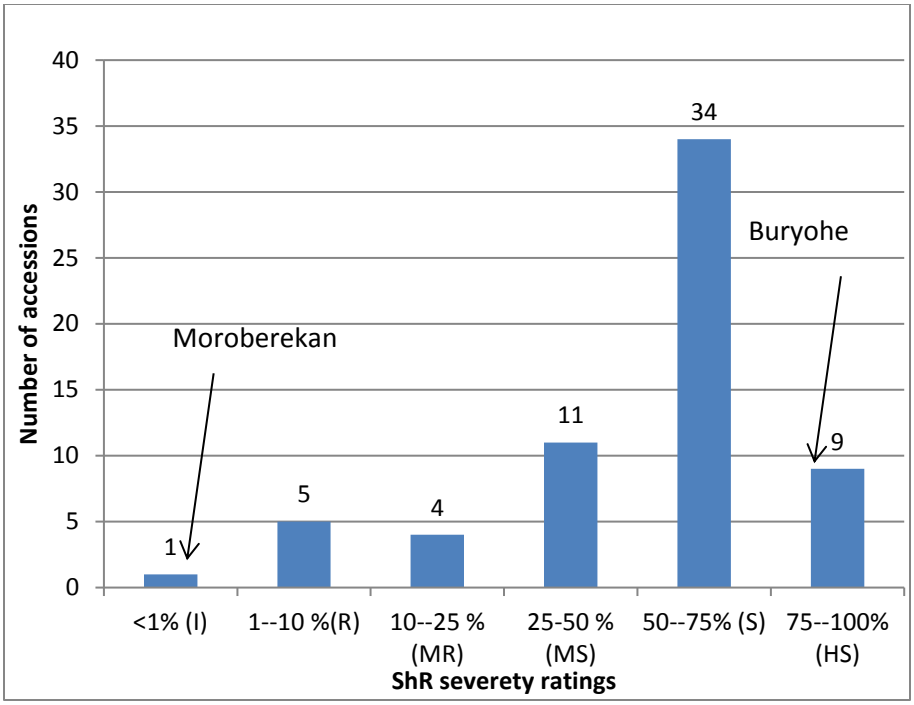

Figure 4.2 Distribution of sheath rot severity ratings among 64 varieties and two checks, resistant (Moroberekan) and susceptible (Buryohe).

Table 4.4: Response of different accession in terms of percent disease index and varietal reaction to sheath rot of rice

Genotype	Name	MIR	VR	Genotype	Name	MIR	VR	Genotype	Name	MIR	VR
G2	Nyiragikara	0.8	I	**G15**	IUR 31	51.6	S	G32	IUR 84	67.6	S
G11 (RC)	Moroberekan	1.8	R	**G62**	Jyambere	51.9	S	G52	IR 56	67.7	S
G1	Yunyine	2.2	R	**G21**	Fanshingabo	52.2	S	G43	IUR 48	68.4	S
G60	Yunertian	2.3	R	**G29**	IIR 1-27	54.4	S	G46	IRN 80	71.0	S
G51	Nerica 4	6.0	R	**G35**	IRC 9	55.6	S	G24	IUR 98	71.4	S
G33	Cyicaro	6.2	R	**G14**	IRN 40	51.2	S	G49	AER 16	73.1	S
G10	Nerica 1	11.2	MR	**G36**	FAC 56	56.1	S	G59	AER 6	73.1	S
G50	Nerica 10	13.3	MR	**G13**	AER 17	56.6	R	G57	IRN 44	73.2	S
G38	Zongeng	16.7	MR	**G44**	Gakire	57.4	S	G22	LL29	73.4	S
G63	Tetep	16.0	MR	**G23**	IUR 69	57.9	S	G42	IUR 48	66.0	S
G48	Yunkeng	27.1	MS	**G56**	IUR 60	72.5	S	G34	IUR 33	74.2	S
G61	IRF 10	32.0	MS	**G16**	Intsinzi	57.9	S	G53	IRN 60	66.2	S
G17	Mpembuke	36.3	MS	**G54**	IIR 1-48	61.0	S	G47	Kigega	75.7	HS
G12	Intsindagira	38.0	MS	**G30**	Ndengera	61.5	S	G28	IRN 41	75.8	HS
G7	N'bahinzi	39.6	MS	**G41**	IUR 53	61.9	S	*G3 (SC)*	Buryohe	76.2	HS
G27	I'bukungu	40.5	MS	**G5**	IRN 41	62.0	S	G58	IRN 15	76.6	HS
G39	IRF 13	45.3	MS	**G40**	IIR 1-47	62.5	S	G9	Rumbuka	77.3	HS
G26	IRN 63	46.2	MS	**G4**	N'bana	63.2	S	G55	IIR 1-18	79.5	HS
G8	IRC 22	47.8	MS	**G37**	IRC 1-4	63.3	S	G19	IRN 5	79.8	HS
G64	Kimaranzara	48.3	MS	**G20**	IUR 54	65.4	S	G31	IUR 56	80.7	HS
5	IRN 41	50.8	S	**G18**	IRN 1-10	65.7	S	G45	IUR 30	83.2	HS
G6	IUR 94	51.5	S								

MIR= mean individual rating in terms of percent disease index (PDI) across environments, VR= varietal reaction to the disease I= immune; R=resistant; MR= moderately resistant; MS= moderately susceptible; S= susceptible, HS=highly susceptible, RC= resistant check and SC= susceptible check

4.3.2 Relationship between ShR symptoms ratings with yield and yield characters

Sheath rot of rice occurs in late stages of rice growth and affects mostly reproductive parts of the plants namely panicle and panicle related traits. Consequently, an attempt was made to link observed ShR ratings with yield and yield components such as plant stature, panicle traits and overall grain yield. Pearson's correlation matrix (

Table 4.5) showed negative and significant correlation coefficients between ShR with plant height, panicle exsertion, number of branches per panicle, number of grains per panicle, and grain yield. On the contrary, ShR was positively and significantly correlated with flag leaf sheath length. Even though ShR negatively affected weight of 1000 grains and panicle length, the effect was not statistically significant. The highest correlation coefficient was observed between ShR and ShL, while the least correlation coefficient was obtained between ShR and panicle length.

Table 4.5: Pearson's correlation coefficient matrix between yield and yield components traits

	PDI	PH	PE	ShL	BrP	GP	WTG	GY
PDI	1							
PH	-0.369**	1						
PE	-0.662**	0.515**	1					
ShL	0.728**	0.362**	-0.222	1				
BrP	-0.306*	-0.002	0.425**	-0.273*	1			
GP	-0.397**	0.219	0.337**	-0.23	0.292*	1		
WTG	-0.056	-0.223	0.144	-0.195	0.095	0.121	1	
GY	-0.524**	0.267*	0.519**	-0.309*	0.435**	0.415**	0.154	1
PL	-0.045	0.175	0.137	0.091	0.297*	0.215	0.058	0.294*

PH= plant height (cm), PE= panicle exsertion (cm), ShL= flag leaf sheath length (cm), BrP=number of branches per panicle (no), WTG= weight of 1000 grains (g), GY= grain yield (t/ha), CH= chaffy grains, GD= grain discoloration and PDI= percent disease index (%).

4.3.3 Agro morphological descriptions of resistant selected varieties

The mean performance of different varieties across the three sites is given in Table 4.6. The following description was based on the data generated in sheath rot resistance experiments, exclusively. Some recommended cultural practices, for instance, fungicides application, were purposely omitted; therefore the data should be used with caution in germplasm selection.

In general, all ShR resistant varieties were described as late maturing with plant stature between intermediate (90 - 110 cm) to intermediate-to-long (110 - 130 cm). Tillering ability and panicle number of all varieties were described as intermediate. Panicle exsertion for resistant genotypes was described as moderately to well exserted. Panicle length was described as short to medium (15 - 25 cm), grain length was classified as short and intermediate. All these genotypes, except Nericas, had grain length less than 6.3 mm and consequently described as short grain varieties with a thousand grain weight ranging between 20.99 and 23.98 gr.

All susceptible genotypes were generally characterized by dwarf and semi dwarf plant stature except a few such as Rumbuka and Mpembuke which were intermediate. These varieties showed enclosed and partially exserted panicles, except Mpembuke and Ndamirabana which were exserted. Grain length was classified as intermediate to long with a thousand grain weight ranging from 20.34 to 25.79 g. Panicle length was between short and intermediate.

Table 4.6: Mean values of agro morphological traits of selected resistant and most susceptible varieties across the three sites

Code	Genotypes	VR	DM	PH	NoT	FLA	PE	ShL	PL	BrP	GP	TPP	SPW	W1000G	GL	GY (t/ha)
G1	Yunyine	R	144.83	102.67	12.82	15.07	9.97	31.57	18.75	11.22	154.2	271.87	2.92	23.96	5.07	3.378
G2	Gikara	I	153.17	109.3	13.37	9.38	9	23.66	21.6	12.65	140.4	240.07	2.25	26.16	5.48	5.35
G10	Nerica 1	R	139.17	98.48	10.12	42.51	4.22	31.64	19.89	10.72	154.4	288.97	2.43	24.08	8.2	3.435
G11	Moroberekan	R	145.17	108.7	12.42	33.65	7.01	30.37	17.52	9.63	156.77	245.63	2.44	20.99	5.17	3.528
G33	Cyicaro	R	139.33	98.81	11.82	26.6	7.45	27.38	20.22	10.23	143.47	250.23	2.02	24.13	4.08	3.324
G38	Yunertian	R	151.83	115.97	10.57	16.92	8.58	30.68	20.28	11.17	151.53	279.23	2.55	23.98	6.33	3.338
G50	Nerica 10	MR	136.33	92.77	11.68	40.29	9.4	31.47	20.77	10	135.07	211.93	2.06	22.2	9.68	3.049
G16	Posiyani	MS	142.62	105.94	9.78	46.12	8.21	31.82	17.4	9.15	139.13	229.87	2.07	24.88	4.66	2.895
G60	Zongeng	MR	174	125.75	12.2	24.63	9.06	32.78	22.05	11.72	147.53	242.23	2.77	22.53	4.63	4.844
G63	Tetep	MR	133.67	96.25	12.53	20.05	6.21	31.87	19.31	9.77	117	281.53	2.78	24.1	7	2.642
G3	Buryohe	HS	145.67	77.65	13.77	23.46	4.22	28.86	20.82	10.93	153.2	290.03	2.55	25.06	10.7	3.531
G4	Ndamirabana	MS	145.5	77.78	14.12	29.5	0.78	29.88	18.01	9.12	131.3	267.97	2.2	22.6	8.85	3.979
G7	Bahinzi	HS	144.33	111.39	10.07	24.94	10.22	30.34	21.65	11.27	144.23	273.67	2.75	24.1	8.75	3.355
G9	Rumbuka	HS	141	121.05	9.92	44.67	2	28.91	22.97	10.15	140.07	303.9	2.53	20.34	10.25	3.342
G12	Intsindagira	MS	148	83.21	11.9	30.59	0.55	30.96	21.3	11.08	156.77	229.03	2.76	24.98	9.02	3.555
G16	Intsinzi	S	140.83	92.05	11.47	23.79	0.93	28.21	17.8	10.35	139.47	248.27	2.53	23.61	7.38	2.818
G17	Mpembuke	MS	135.17	109.21	11.75	30.12	7.53	28.63	18.21	9.87	138.03	193.3	2.32	25.79	9.57	3.065
G21	Fashingabo	S	163.67	71.11	11.43	22.69	4.08	29.89	20.26	10.4	133.87	227.47	2.2	22.07	10.23	3.618
G27	Bukungu	MS	148	81.8	12.05	26.11	0.45	29.79	19.26	11.08	147.27	233.47	2.45	22.81	9.37	3.482
G30	Ndengera	S	151.17	82.65	13.62	17.44	0.7	28.46	17.5	10.68	128.03	278.63	2.1	24.51	7	3.175
G36	Fac 56	S	143.83	86.75	12.12	26.24	1.65	30.86	20.5	10.95	163.1	232.63	2.79	24.76	10.18	3.59
G44	Gakire	S	141	79.99	11.27	15.62	0.57	29.8	16.22	9.7	124.53	220	2.21	24.74	10.05	2.846
G62	Jyambere	S	141.5	84.55	13.12	29.14	1.07	31.11	19.02	11.07	143.73	301.67	2.18	21.93	10.55	3.591
G64	Kimaranzara	MS	135.67	97.35	9.88	22.49	5.7	31.25	19.11	10.37	125.37	288.8	2.41	25.69	7.77	3.382
G48	Yunkeng	MR	142.31	105.77	10.99	16	8.16	29.11	18.7	12.64	158.8	265.17	2.92	25.66	4.19	3.309

DM= days to maturity; PH= plant height; NoT=number of tillers; FLA= flag leaf area; PE= panicle exsertion; ShL= sheath length; PL= panicle length; BrP= number of branches per panicle; GP= number of grains per panicle; TPP= total number of panicles per plant; SPW = single panicle weight; W1000G= weight of 1000 grains; GL = grain length; GY= grain yield.

I= immune; R=resistant; MR= moderately resistant; MS= moderately susceptible; S= susceptible, HS=highly susceptible. The user of these figures must be cautious as no chemical control measure was applied for all the varieties except insecticide against *Diopsis thoracica*

4.4 Discussion

4.4.1 Sheath rot as a potential threat to rice sector and varietal resistance

In this study, only 10 out of 64 varieties evaluated showed some level of resistance to ShR, with six of these showing high levels of resistance and all being characterized by intermediate to tall plant stature, well exerted panicles and short grains. This is in agreement with reports by Ngala and Adeniji (1986) who identified dwarf cultivars of rice to be more susceptible whilst taller ones were resistant to sheath rot. According to various reports, the fungus severely infects the high yielding dwarf cultivars under water stressed conditions in both rainfed and upland ecosystems (Ou, 1985; Gnanamanickam, 2009). The pathogen attacks both semi dwarf as well as traditional tall cultivars (Sakthivel and Gnanamanickam, 1987).

The relative few numbers of resistant genotypes among studied germplasm agrees with (Sakthivel, 2001) who stated that many of the high yielding international commercial cultivars are highly susceptible to sheath rot of rice. As sheath rot attacks panicle parts, an attempt was made to correlate panicle related traits with disease severity. Panicle related traits and grain yield were significantly and negatively correlated with disease severity, whereas severity was positively correlated with flag leaf sheath length and no relationship was identified between severity and panicle length. This strong relationship can be explained by the fact that panicles from affected plants do not, or partially emerge and are compressed inside the sheath and this affects all panicle parts (Sakthivel, 2001). These results agree with reports by Ngala and Adeniji (1986) who concluded that the attack by the fungus of the flag leaf sheaths and grains also affected yield through poor panicle formation and exsertion, spikelet sterility, reduced number of spikelets per panicle, reduced grain filling, and losses in milling (Pearce *et al.*, 2001). In a similar study, Lakshmanan and Velusamy (1991), concluded that ShR was associated with reduced 1000 grains weight, healthy grain percentage, increased chaffy and discoloured grains, and overall grain yield.

In their efforts to detect ShR resistant varieties in India, Shivakumar *et al.* (2011) screened 3000 entries and only seven accession showed high levels of resistance recording a score of zero. Mosharraf *et al.* (2003) obtained 3 resistant genotypes out of 38 from screening trials in Pakistan. A good number of ShR resistant materials have been reported in breeding lines derived from *Oryza oficinalis* by Lakshmanan and Velusamy (1991).

As far as rice value chain is concerned, studies conducted by Kathiresan, (2013) show that 54% of the consumers in Rwanda prefer long grains and only 14% of the consumers prefer short and bold grains. About 16% of the consumers prefer aromatic basmati grains and 20% of the consumers accept all types of grains and hence do not have any specific preferences. Consequently, there is a significant market space for long grain types. Kathiresan, (2013) reports that only 30% of the local rice farmers cultivate long grain varieties and about 70% of the farmers grow short- and bold varieties. This suggests that the local farmers are producing more of what is less preferred by the market, and grow less of what is preferred (long grain) in the market and therefore, much of this rice is imported. However, efforts have been made to introduce long grain cultivars by the Rwanda Agriculture Board and 20 cultivars, all of them semi-dwarf and indica varieties were recently released to meet this demand (Ndikumana and Gasore, 2010). All the varieties were bred for resistance / tolerance to blast, yellow mottle virus, cold temperatures but apparently ShR was given less emphasis because of its status as a minor disease.

Despite, their improved grain length (over 7mmm) coupled with a good yield potential of semi-dwarf indica varieties, none of them is grown on a large scale. In reference to this study, dwarf and semi-dwarf indica varieties (including newly released ones) were seriously affected by sheath rot disease and their yields were far below their potential yields as per reports by Ndikumana and Gasore (2010). Yield losses associated with sheath rot in these genotypes have been widely reported ranging between 26 and 50% in general; however, higher yield losses of up to 85% were recorded in Taiwan (Sakthivel, 2001; Pearce *et al.*, 2001). Furthermore, they are reports where ShR is a common problem on densely planted, nitrogen-responsive, high yielding semi-dwarf as well as tall rice cultivars in all rice growing areas in Asia (Sakthivel, 2001). Likewise, Mew and Gonzalez (2002) and Lanoiselet *et al.* (2012), suggested that higher yield losses occur most commonly in lowland environments, and particularly in rainy seasons in both rainfed and upland ecosystems. This is to be taken seriously because these farming systems are more or less identical to those under which rice is grown in Rwanda, that is, an intensive growing system in irrigated marshlands, much of them characterized by low temperatures.

Farmers repeatedly reported that one of the reasons they fail to adopt newly released varieties, is because of high levels of “ibihuhwe” (chaffy grains) and consequently they prefer to remain with their traditional tall and short grain varieties, locally known as ‘kigoli’ (Yunkeng, Yunyine, Posiyani, and Zongeng). Based on results of this study, sheath rot of rice is potentially constraining the development and adoption of long grain varieties, regardless of other factors.

Chemical and biological control of ShR has been largely been discussed and a list of efficient fungicides was released by Lalan Sharma *et al.* (2013). However, the development of resistant cultivars is a potential, more sustainable management measure as it is inexpensive to deploy, has no adverse environmental hazards, and is more convenient for farmers to use. Because a number of susceptible varieties subjected to this study are already released and adapted to various agro ecological conditions, their genetic improvement through a series of backcrosses will yield quick results. These varieties should be considered as recurrent parents while varieties such as Moroberekan, Yunyine, Yunertian, should serve as resistance donor parents.

4.5 Conclusion

The aim of this study was to raise awareness of ShR as one of the most important emerging rice diseases that could seriously threaten rice productivity in Rwanda as well. Because of the fact that the disease was formerly regarded as minor, little has so far been done in Rwandan rice breeding programmes towards the control of this disease. Genetic improvement of locally adapted cultivars through breeding for resistance to this disease would be the most sustainable and cost effective strategy to tackle the threat caused by the disease. To this end, identifying sources of resistance among local germplasm was the first major step and results from this study revealed 10 cultivars with different levels of resistance to ShR. Six cultivars were found to be resistant with one of them (Yunyine) more resistant than the resistant check. All these six cultivars were considered the best sources of resistant genes for ShR as far as varietal improvement is concerned especially since they are already adapted to most of Rwanda's rice growing ecosystems. Four cultivars were found moderately resistant and hence appeared to meet the farmers' requirements as far as high yields are concerned regardless of market preferences.

However, regarding small scale farmers and market preferences (high yielding and long grain varieties) ShR can be considered as a serious potential threat to rice sector development as far as farming systems are concerned. A set of varieties were released to meet a number of requirements including high yields and grain quality (length). Though all of them are dwarf and semi-dwarf varieties with potential high yield, they are mostly susceptible to ShR and hence their yielding potential is seriously affected. This study also revealed useful information to be taken into consideration in the development of high yielding and ShR resistant cultivars, not only in Rwanda but also elsewhere in the World.

References

Ayyadurai, N., Kirubakaran, S. I., Srisha, S., and Sakthivel, N., 2005. Biological and molecular variability of *Sarocladium oryzae*, the sheath rot pathogen of rice (*Oryza sativa* L.). *Current Microbiology* 50: 319-323.

Bioversity-International, IRRI, and WARDA, 2007. Descriptors for wild and cultivated Rice (*Oryza* spp.). Bioversity International, Rome, Italy.

Gnanamanickam, S., 2009. Biological control of sheath-rot and other fungal diseases. Biological Control of Rice Diseases. Springer Netherlands.

IRRI, 1996. Standard evaluation system for Rice. International Rice Research Institute, Manila, Philippines.

IRRI, 2006: Breeding theory: multi-environment trials, design and analysis. Rice breeding course. International Rice Research Institute, Manilla, Phillipines. .

Kathiresan, A., 2013. Rwanda's rice commodity chain-facing globalization. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Lakshmanan, P., and Velusamy, R., 1991. Resistance to sheath rot (ShR) of breeding lines derived from *Oryza officinalis*. *International Rice Research Newsletter* 16: 8-9.

Lalan Sharma, D.T., Nagrale, S. K., Singh, K. K., Sharma, and Sinha, A. P., 2013. A study on fungicides potential and incidence of sheath rot of rice caused by *Sarocladium oryzae* (Sawada). *Journal of Applied and Natural Science* 5: 24-29.

Lanoiselet, V., You, M. P., Li, Y. P., Wang, C. P., Shivas, R. G., and Barbetti, M. J., 2012. First report of *Sarocladium oryzae* causing sheath rot on rice (*Oryza sativa L.*) in Western Australia. *Plant Disease* 96: 1382-1382.

Madhav, M.S., Laha, G.S., Padmakumari, A.P., Somasekhar, N., Mangrauthia, S.K., and Viraktamath, B.C., 2013. Phenotyping rice for molecular plant breeding. In: Panguluri, S.K. and Kumar, A.A., (eds), Phenotyping for Plant Breeding. Springer New York. p. 1-40.

Marín, M., Wong, I., García, G., Morán, R., Basulto, R., Pimentel, E., and Mena, J., 2013. Actividad antagónica in vitro de Tsukamurella paurometabola C-924 frente a fitopatógenos. *Revista de Protección Vegetal* 28: 132-137.

Mew, T.W., and Gonzalez, P., 2002. A Handbook of rice seedborne fungi. International Rice Research Institute, and Enfield, N.H. (USA). Science Publishers, Inc., Los Bafios, Philippines.

Mosharraf, H., Shah, M.D., Munirur, R., Khalequzzaman, K.M., Nazrul Islam, M. D., and Ashrafuzzaman, M., 2003. Performance of breeding lines / cultivars of rice against bacterial leaf blight and sheath rot under field condition. *Journal of Biological Sciences* 3: 478-483.

Narayanaprasad, Jagadeesh, B.R., Shivakumar, G.B., Prasad, P.S., Sudarshan, G.K., and Sunil Kumar, N., 2011. Studies on the nature and properties of sheath rot (*Sarocladium oryzae Sawada*) causing seed borne pathogen on rice (*Oryza sativa* L.). *International Journal of Science and Nature* 2: 317-320.

Ndikumana, I., and Gasore, E.R., 2010. Description Of New Rice Varieties Introduced in Rwanda, Institut des Sciences Agronomiques du Rwanda, Kigali Rwanda.

Ngala, G.N., and Adeniji, M.O., 1986. Sheath rot disease in tropical Africa. In: Juo, A.S.R. and Lowe, J.A., (eds), The Wetlands And Rice In Subsaharan Africa. IITA, Ibadan, Nigeria.

Ou, S.H., 1985. Rice diseases, 2nd ed. Oxford University Press.

Paris, T.R., Manzanilla, D., Tatlonghari, G., Libios, R., Cueno, A., and Villanova, D., 2011. Guide to participatory varietal selection for submergence tolerance in rice. International Rice Research Institute, Los Baños (Philippines).

Payne, R., Welham, S., and Harding, S., 2014. A guide to reml in genstat. VSN International, Hertfordshire, Uk.

Pearce, D.A., Bridge, P.D., and Hawksw, D.L., 2001. Species Concept in Sarocladium, the Causal Agent of Sheath Rot in Rice and Bamboo Blight. In: Sreenivasaprasad, S. and Johnson, R. (eds). Major fungal diseases of rice: Recent advances. Springer Netherlands, Dordrecht. p. 285-292.

Reddy, M.M., Reddy, C.S., and Singh, B.G., 2000. Effect of sheath rot disease on qualitative characters of rice grain. *Journal of Mycology and Plant Pathology* 30: 68-72.

Sakthivel, N., 2001. Sheath rot disease of rice: Current status and control strategies. In: Sreenivasaprasad S, Johnson R (eds). Major Fungal Diseases Of Rice Recent Advances Kluwer Academic Publishers Boston.

Sakthivel, N., and Gnanamanickam, S., 1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice (*Oryza sativa* L.). *Applied and Environmental Microbiology* 53: 2056-2059.

Shivakumar, G., Prasad, P., and Sudarshan, G., 2011. Screening of rice genotypes for resistance against sheath rot of rice (*Sarocladium oryzae Sawada*). *Trends in Biosciences* 4: 114-115.

5 Chapter 4

SNPs based assessment of genetic diversity of rice for selection of parents for sheath rot resistance breeding

Abstract

Sheath rot of rice is currently regarded as one of most important emerging rice diseases not only in Rwanda, but also in many other rice growing countries around the World. Since varietal resistance has been considered as most sustainable management strategy for small scale farmers, the aim of this study was to identify genetically distant parental materials for sheath rot resistance breeding in Rwanda. Ten resistant and fifteen susceptible varieties were analysed using 94 single polymorphic nucleotides (SNPs) markers. The total number of alleles amplified per locus ranged from 1 to 4 with a mean of 2.01 with 189 alleles, in total, amplified from 25 genotypes. The number of observations per marker locus or the number non-missing genotypes observed in the sample ranged from 11 to 25 with an average of 23. Mean major allele frequency was 76.2%, whereas the mean polymorphic information content was 0.263, and gene diversity estimated at 0.325 on average. Therefore, markers were highly informative and revealed good estimates of genetic diversity among studied varieties. Genetic distance, ranging from 0 and 0.63 coupled with the UPGMA dendrogram, clearly distinguished sheath rot resistant and susceptible genotypes. This study revealed the possibility of improving levels of resistance to sheath rot with minimum risk of genetic depression or reduced variability among progenies through hybridization of locally adapted germplasm.

5.1 Introduction

The sheath rot of rice (ShR) caused by a seed borne fungal pathogen, *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], is one of most important emerging and devastating rice diseases in rice growing regions (Lanoiselet *et al.*, 2012; Hittalmani *et al.*, 2016). Losses range between 26 and 50% in general but higher yield losses up to 85% were recorded (Sakthivel, 2001).

The disease is nowadays among many diseases which were formerly considered as minor, but have recently acquired the status of major ones (Ngala and Adeniji, 1986). This is, probably, due to change in cultivation practices as a result of green revolution on one hand, and apparent climate change effects, on the other hand (Madhav *et al.*, 2013). Crop intensification practices such as increased plant density, a high rate of nitrogen fertilizers and the use of semi-dwarf and photoperiod-insensitive cultivars, favour the susceptibility of rice to some diseases in general and ShR in particular (Bigirimana *et al.*, 2015).

ShR management in fields relies on integration of chemicals with cultural practices. However, according to Ayyadurai *et al.* (2005) fungicide treatments are most of the time unsuccessful under farmers conditions or are very expensive as well as harmful to environment. In the same context, biological control has been of limited effect due to inconsistency of antagonists in field conditions (Gnanamanickam, 2009). Therefore, the most sustainable solution is the development and deployment of resistant varieties, as this is easy for farmers to use with no additional cost, and environmentally friendlier. A number of resistant varieties have been developed in different countries (Lakshmanan and Velusamy, 1991; Pearce *et al.*, 2001), but most of the times such varieties fail to adapt to harsh environmental conditions of ecosystems they are introduced in (WARDA, 2008). There is therefore, a need to develop resistant genotypes using locally adapted parents. In this regard, breeding investigations for sheath rot resistance requires the identification of sufficiently genetically distant parental materials for hybridization. This aims at avoiding genetic depression and reduced genetic variability in subsequent progenies. Based on this, assessment of genetic diversity, relationships, and structure within a given set of germplasm is useful in plant breeding for different reasons including: (i) assisting in the selection of parental combinations for developing progenies with maximum genetic variability for genetic mapping or further selection; (ii) determining the level of genetic variability when defining core subsets selected for specific traits (iii) estimating possible loss of genetic diversity during conservation or selection programmes (Reif *et al.*, 2005).

Morphological characterization of rice germplasm has been regarded as a central component of plant breeding programmes to carry out selection, study genetics of traits, associate markers with traits and understand traits diversity (Nascimento *et al.*, 2011). Despite their usefulness, morphometric markers lead to more reliable indications once coupled with molecular markers (Kilian and Graner, 2012).

Molecular markers are particularly useful for the evaluation of genetic diversity in various crop species with a narrow genetic base (Soleimani *et al.,* 2002). More recently, single nucleotide polymorphism (SNP) markers acquired significant consideration because they are bi-allelic in nature and occur at a much higher frequency in the genome than any other markers (Ren *et al.*, 2013). This study aims at (i) to provide substantial information to maintain and use rice locally available genetic resources in breeding, (ii) to identify genetically distant parental materials to be involved in various varietal improvement programmes with regards to resistance to ShR.

5.2 Materials and methods

5.2.1 Plant materials, DNA extraction, and SNP genotyping

Plant materials subjected to this study comprise of 25 agronomically useful rice varieties that were selected as potential parental materials in varietal improvement programmes towards resistance to sheath rot of rice (Mvuyekure et al., 2014 unpublished). Selection of genotypes was based on agro-morphological attributes, reaction to sheath rot of rice as well as farmers and consumer preferences. The list and key agronomical characteristics of assessed varieties are given in Table 5.1.

Table 5.1: Key agronomic features of sheath rot resistant and susceptible varieties used in the study

Code	Genotypes	Varietal Reaction to ShR	Panicle exsertion	Plant stature	Tillering ability	Weight of 1000 grains	Grain length
2	Intsindagira	Moderately susceptible	Just exserted	Short	Intermediate	24.98	Long
4	Imbaturabukungu	Moderately susceptible	Partly exserted	Short	Intermediate	22.81	Long
6	Yunertian	Resistant	Well exserted	Intermediate to long	low	23.98	Medium
7	Zongeng	Resistant	Moderately well exserted	Intermediate to long	Intermediate	22.53	Short
12	Fac 56	Susceptible	Partly exserted	Short	Intermediate	24.76	Long
15	Jyambere	Susceptible	Enclosed	Short	Intermediate	21.93	Long
16	Posiyani	Moderately susceptible	Moderately well exserted	Intermediate to long	low	24.88	Short
18	Ndamirabana	Highly susceptible	Partly exserted	Short	Intermediate	22.6	Long
19	Fashingabo	Susceptible	Partly exserted	Short	low	22.07	Long
24	Yunyine	Resistant	Well exserted	Intermediate to long	Intermediate	23.96	Short
25	Nerica 1	Resistant	Moderately well exserted	Short to Intermediate	Low	24.08	Medium
27	Gakire	Susceptible	Enclosed	Short	low	24.74	Long
31	Ndengera	Susceptible	Partly exserted	Short	Intermediate	24.51	Medium
32	Buryohe	Highly susceptile	Partly exserted	Short	Intermediate	25.06	Long
33	Intsinzi	Susceptible	Enclosed	Short to Intermediate	low	23.61	Medium
34	Kimaranzara	Moderately susceptible	Just exserted	Short to Intermediate	low	25.69	Medium
40	Yunkeng	Resistant	Well exserted	Intermediate to long	low	25.66	Short
43	Nyiragikara	Highly resistant	Well exserted	Intermediate to long	Intermediate	26.16	Short
44	Nerica 10	Moderately Resistant	Moderately well exserted	Short to Intermediate	low	22.2	Medium
48	Cyicaro	Resistant	Moderately well exserted	Short to Intermediate	Intermediate	24.13	Short
50	Ndamirabahinzi	Highly susceptible	Partly exserted	Intermediate to long	low	24.1	Medium
51	Mpembuke	Moderately susceptible	Partly exserted	Intermediate	low	25.79	Long
53	Rumbuka	Highly susceptible	Just exserted	Intermediate to long	low	20.34	Long
59	Moroberekan	Resistant	Well exserted	Intermediate to long	Intermediate	20.99	Short
60	Tetep	Resistant	Just exserted	Short to Intermediate	Intermediate	24.1	Medium

Leaf samples for DNA extraction were collected on 30 days old seedlings using the LGC genomics plant sample collection kit (http://www.lgcgroup.com/plant-kit/#.Vsb0KFR97IU) and shipped to LGC genomics, UK. DNA extraction and all SNPs genotyping processes were performed by LGC genomics, according to their validated protocol and working conditions. Genetic diversity among 10 sheath rot resistant and 15 susceptible cultivars was assessed using 94 SNPs that were obtained from the Integrated Plant Breeding Platform: https://www.integratedbreeding.net/544/communities/genomics-crop-info/crop-information/gcp-kaspar-snp-markers/crop-snp-markers/rice?map=1 .

Selection of SNPs to use was guided by a number of conditions. SNPs were evenly distributed along the 12 linkage groups corresponding to all 12 rice chromosomes. While each linkage group contains between 100 and 120 markers, 7 - 9 markers were randomly and evenly chosen from each linkage group for this study. The list of these SNPs is given in Appendix 5.1.

5.2.2 Data analysis

Genotyping data were analysed using Power Marker V 3.25 for estimation of SNPs summary statistics including, allele number, major allele frequency, heterozygosity, number of observed genotypes per marker locus, gene diversity, polymorphic information content (PIC), and Nei frequency based distance (Nei *et al.*, 1983). Based on this distance a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed using Mega 5.2 software, for cluster analysis in a bid to evaluate relationship groups among studied varieties.

5.3 Results

5.3.1 Polymorphic levels of SNP markers

The summary SNP statistics are presented in Appendix 5.1. Results indicated that 90.4% of marker alleles (85 markers out of 94) were polymorphic whereas only 9 markers out of 94 SNPs, were monomorphic. The number of observations for a marker locus or the number of non-missing genotypes observed in the sample ranged from 11 to 25 with an average of 23. A genotype is generally regarded as missing if one of its two alleles is missing. The number of alleles amplified per locus varied between 1 and 4. A total of 189 alleles were amplified with an average of 2.011 alleles per locus in 25 varieties.

The major allele frequency was also calculated for all 94 markers and ranged from 50% to 100% with an average of 76.2%. More than 60% of the polymorphic loci presented a major allele frequency higher than 70% and 11 loci showing more than 90%. The mean PIC value for markers was 0.263 with a range between 0 (monomorphism) and 0.555. According to marker informative level established by (Botstein *et al.*, 1980), 48 markers (51.06%) and 21 others (22.3%) were highly and reasonably informative, respectively whereas only 12 (15.8%) were less informative.
Heterozygosity, being a measure of allelic diversity at a locus ranged from 0.018 to 0.160 and its expected estimations or gene diversity, ranged from 0 to 61.2% with the mean gene diversity being 32.5%. Therefore the high allelic richness coupled with estimates of gene diversity indicates a high level of genetic diversity among studied genotypes useful for further genetic studies.

5.3.2 Genetic distance among varieties

The average genetic distances between and within Indica / Japonica rice groups is presented in Table 5.2. The lowest distance (0) was recorded between Ndamirabana (G18) and Gakire (G27), both varieties belonging to indica group. This was followed by the distance between Rumbuka (G 53) and Yunkeng (G40), which belong to different groups Indica and Japonica respectively. Highest distance (0.63) was recorded between Yunyine (G24) and Tetep (G60), a Japonica and Indica groups respectively. Yunyine recorded high genetic distance (>0.5) from most of genotypes (19 genotypes) except Zongeng (G7), Yunertian (G6), Yunkeng (G40), Fac 56 (G12), Rumbuka (G53), and Moroberekan (G59). In general, genetic distance, clearly distinguishes Japonica and Indica subspecies at a large scale. It also revealed low distance within subspecies and high distance between subspecies.

5.3.3 Cluster analysis and relationship groups

The Nei frequency based distance was used to assess similarity between studied varieties and construct a UPGMA dendrogarm for evaluation of relationship groups between varieties.
The phylogenetic tree of Figure 5.1 summarizes evolutionary relationships among genotypes and categorizes them into distinct genetic groups.

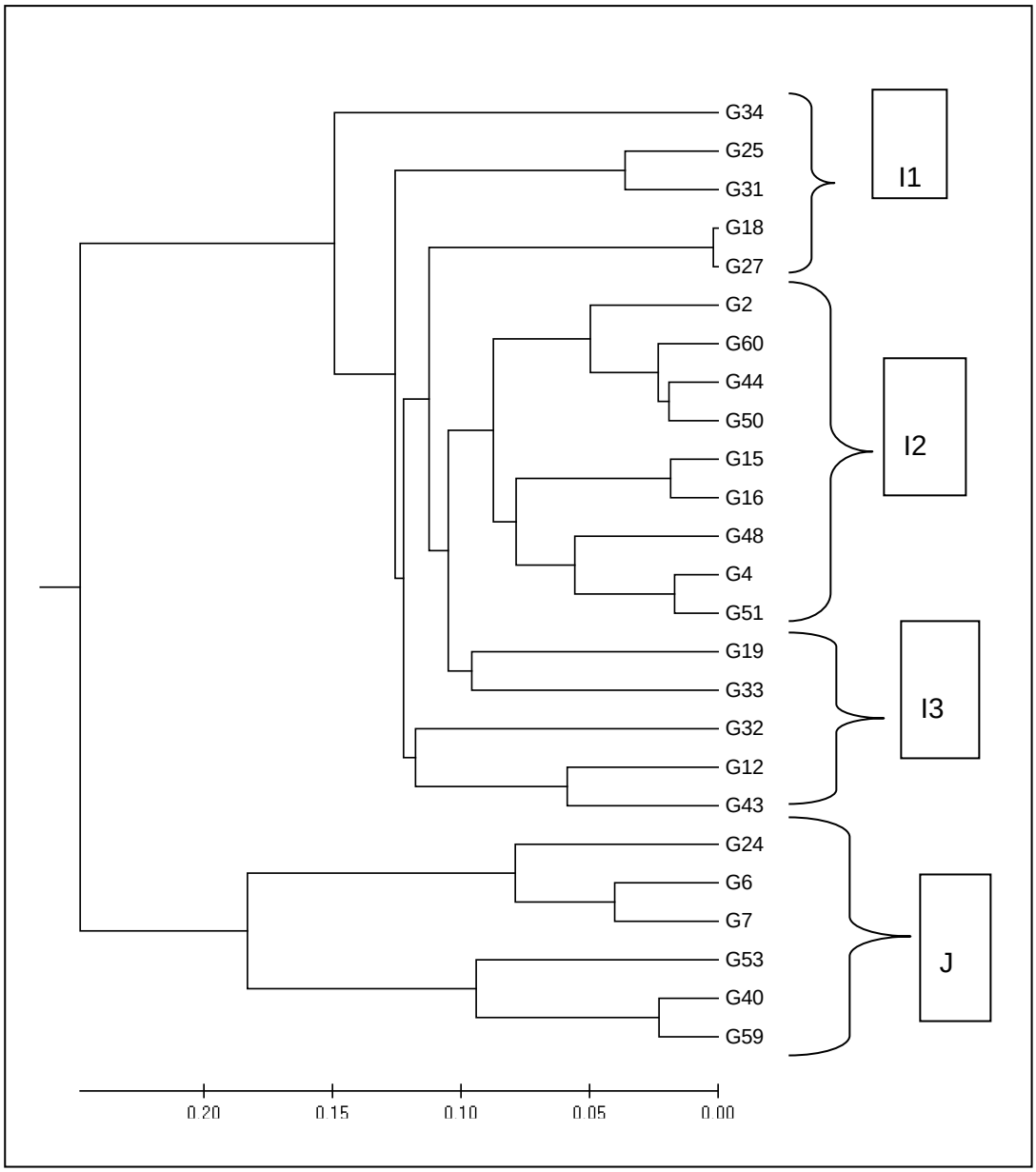

Figure 5.1: UPGMA dendrogram of sheath rot resistant and susceptible genotypes based on Nei genetic distance.

Names of different varieties are given in this order: G34=Kimaranzara, G25= Nerica 1, G31=Ndengera, G18= Ndamirabana, G27=Gakire, G2=Intsindagirabigega, G60=tetep, G44=Nerica 10, G50=Ndamirabahinzi, G15=Jyambere, G16=Posiyani, G48=Cyicaro, G4=Imbaturabukungu, G51=Mpembuke, G19=Fashingabo, G33=Intsinzi, G32=Buryohe, G12= FAC56, G43=Nyiragikara; G24=Yunyine, G6= Yunertian, G7=Zongeng, G53= Rumbuka, G40= Yunkeng, G59 = Moroberekan

In this dendrogram, nodes represent different genotypes whereas branches are graphical estimates of the genetic distance between the genotypes. Therefore, this is an indication of genetic relationships between genotypes. In the dendrogram, varieties were distinguishably separated into two major different groups, representing indica (referred as I in dendrogram) on one hand, and japonica (referred as J in dendrogram) on the other hand. However, varieties such as Nerica 1 and Nerica 10 were found closely related to indica type varieties, whereas they are actually crosses between *Oryza glaberrima* and *Oryza sativa*. These varieties have also been found resistant to sheath rot of rice, in contrast to the rest of group. Similarly, Moroberekan was similar to japonica type varieties whereas it is actually an *Oryza glaberrima.* Japonica genotypes are easily distinguishable by their taller plant stature, well exserted panicles and short grain and more particularly, resistance to sheath rot comparatively to indica type varieties.
Susceptible genotypes of the indica group, in the same cluster were less genetically dissimilar while those in different clusters were more genetically dissimilar. This was the case for instance between Rumbuka and Kimaranzara which are both indica with high level of dissimilarity.

Based on previously known morphological characteristics of studied genotypes and Nei similarity matrix (Table 5.2), the dendrogram in Figure 5.1 revealed also three subgroups in indica group; I1, I2 and I3.

Table 5.2 Nei frequency based distance among studied germplasm using 94 rice SNPs

	G12	G15	G16	G18	G19	G2	G24	G25	G27	G31	G32	G33	G34	G4	G40	G43	G44	G48	G50	G51	G53	G59	G6	G60
G12																								
G15	0.28																							
G16	0.25	0.04																						
G18	0.34	0.22	0.25																					
G19	0.25	0.2	0.21	0.32																				
G2	0.23	0.17	0.23	0.26	0.24																			
G24	0.26	0.61	0.61	0.57	0.6	0.54																		
G25	0.32	0.28	0.29	0.28	0.2	0.24	0.61																	
G27	0.29	0.2	0.24	0	0.29	0.24	0.56	0.29																
G31	0.31	0.27	0.28	0.25	0.25	0.19	0.6	0.07	0.26															
G32	0.26	0.27	0.26	0.29	0.25	0.28	0.57	0.29	0.27	0.28														
G33	0.35	0.19	0.21	0.28	0.19	0.19	0.58	0.3	0.26	0.31	0.24													
G34	0.36	0.28	0.33	0.33	0.28	0.31	0.59	0.3	0.32	0.29	0.31	0.33												
G4	0.26	0.14	0.18	0.17	0.22	0.16	0.61	0.25	0.17	0.22	0.26	0.21	0.25											
G40	0.27	0.46	0.45	0.48	0.41	0.45	0.36	0.5	0.47	0.46	0.39	0.44	0.45	0.44										
G43	0.12	0.23	0.24	0.29	0.19	0.2	0.55	0.23	0.26	0.22	0.22	0.25	0.31	0.26	0.45									
G44	0.23	0.19	0.22	0.23	0.17	0.12	0.56	0.21	0.22	0.23	0.19	0.22	0.32	0.23	0.44	0.15								
G48	0.24	0.16	0.21	0.2	0.23	0.2	0.52	0.3	0.19	0.24	0.28	0.26	0.27	0.13	0.41	0.24	0.22							
G50	0.2	0.11	0.13	0.18	0.15	0.06	0.58	0.16	0.17	0.16	0.17	0.18	0.23	0.08	0.4	0.15	0.04	0.13						
G51	0.25	0.1	0.15	0.21	0.22	0.15	0.6	0.27	0.19	0.22	0.26	0.21	0.26	0.03	0.42	0.26	0.21	0.1	0.05					
G53	0.34	0.49	0.47	0.49	0.42	0.45	0.42	0.47	0.47	0.42	0.41	0.4	0.53	0.46	0.18	0.43	0.43	0.45	0.39	0.43				
G59	0.32	0.48	0.48	0.51	0.44	0.47	0.39	0.52	0.5	0.48	0.44	0.47	0.5	0.46	0.05	0.5	0.47	0.43	0.43	0.45	0.2			
G6	0.15	0.6	0.57	0.57	0.53	0.52	0.13	0.56	0.56	0.55	0.51	0.59	0.59	0.56	0.3	0.51	0.51	0.54	0.51	0.55	0.38	0.31		
G60	0.25	0.18	0.22	0.26	0.22	0.12	0.63	0.22	0.22	0.21	0.22	0.24	0.31	0.24	0.48	0.16	0.05	0.2	0.05	0.2	0.43	0.51	0.57	
G7	0.23	0.58	0.59	0.56	0.58	0.51	0.19	0.57	0.55	0.56	0.53	0.62	0.61	0.56	0.37	0.54	0.5	0.57	0.5	0.56	0.39	0.39	0.08	0.55

Regarding their genetic similarity coefficients, clusters I1 included varieties such as Kimaranzara, Nerica 1, Ndengera, Ndamirabana, and Gakire. Cluster I2 gathers varieties such as Intsindagirabigega, Tetep, Ndamirabahinzi, Jyambere, Posiyani, Cyicaro, Imbaturabukungu, and Mpembuke. Cluster I3 is made of varieties such as Fashingabo, Intsinzi, Buryohe, FAC 56 and Nyiragikara. The rest of varieties; Yunyine, Yunertian, Zongeng, Rumbuka, Yunkeng belong to Japonica type varieties. In fact, cluster I1 groups cultivars generally characterized by dwarf plant stature, high tillering ability, poor panicle exsertion and long grain. Cluster J groups intermediate to long plant stature, well exserted panicles, low tillering ability and short grain cultivars; characteristics of Japonica rice types. Clusters I2 and I3 group genotypes between both extremes. As far as resistance to ShR is concerned, most susceptible cultivars appeared in cluster I1, I2 and I3 except Nericas and Nyiragikara. Most resistant genotypes appear in cluster J except Rumbuka.

5.4 Discussion

Accurate identification of genetic relationship and divergence of genetic resources is most useful for efficient choice of parental materials in breeding and genetic conservation strategies (Guimarães, 2009). This will assist in minimizing the use of closely related parents in breeding programmes with a high risk of leading to genetic depression and reduced genetic variation (Weddell, 2002).

The present investigation was conducted in a bid to establish genetic variability and relationship among 25 selected rice varieties 10 resistant and 15 susceptible to ShR. This was an attempt to identify potential parental materials suitable for various hybridization processes, with particular regard to resistance to the sheath rot of rice. To this end, SNPs were used because of their low cost per data point, high genomic abundance, locus-specificity, codominance, high throughput analysis, and lower genotyping error rates (Rafalski, 2002). As heterozygosity is a measure of genetic variation within a population, its high average at a locus could be expected to correlate with high levels of genetic variation at loci with critical importance for adaptive response to environmental changes (Kotzé and Muller, 1994) quoted by (Ojango *et al.*, 2011). Moreover, high values of heterozygosity and PIC statistics are a sign of marker informativeness, which is a desirable property in linkage association test (Manikanda, 2013). In these regards, all the 94 SNP markers provided adequate informative polymorphism to evaluate genetic diversity of studied cultivars.

The results of this study are in close agreement with findings by Chen *et al.* (2011) who performed SNPs genotyping on over 300 inbred lines and obtained average mean PIC value of 0.277 and 0.35 gene diversity against 0.25 and 0.32 for PIC and gene diversity respectively for this study. However, mean allele number and PIC values were relatively low compared to other genetic diversity studies where SNPs were used. For instance Das *et al.* (2013) obtained 182 alleles with an average of 5.13 alleles per locus. One of reasons for low allelic variation may be due the use of already released and locally adapted cultivars with a high selection pressure, instead of using landraces, wild relatives or segregating populations (Ram *et al.*, 2007; Thomson *et al.*, 2007).
In their work on comparison of effectiveness of SSR and SNPs, Singh et al. (2013) obtained for the SNP markers PIC values ranging from 0.03 to 0.37 with an average PIC value 0.23. These values were slightly below the values of this study. Due to bi-allelic nature of SNPs, their PIC values can range from 0 to 0.5, and consequently, results from this study demonstrated that this set of SNPs are sufficiently informative and can be used as a tool for large scale genotyping in rice molecular breeding research involving japonica x japonica, indica x japonica and indica x indica crosses. Based on cluster analysis of this study, SNPs markers were useful and provided two distinct major genetic groups and three other subgroups enabling breeders to design targeted crosses for development of ShR resistant genotypes, while conserving genetic diversity.

The results revealed high level of polymorphism among sheath rot resistant and susceptible parents and therefore confirming a suggestion that a good portion of the genetic diversity and specific adaptation of the investigated rice ShR resistant and susceptible genotypes had been achieved. This polymorphism is due to the fact that the studied germplasm consisted of morphologically distant subspecies of *Oryza sativa*; japonica subspecies in one hand and indica subspecies in other hand. Japonica and indica rice are easy to distinguish by obvious distinct morphological and physiological characters (Oka and Morishima, 1982; Hung-Ying *et al.*, 2012; Lin *et al.*, 2012). Clear SNPs based distinction between indica and japonica subspecies of *Oryza sativa* have also been largely described by Feltus *et al.* (2004) and Chen et al. (2011). Their SNP results revealed some common and contrasting patterns of the haplotype diversity along different rice chromosomes in the indica and Japonica varieties, which suggest different evolutionary forces possibly acting in specific regions of the rice genome during domestication and evolution of rice. Elsewhere, subgroups within indica group based on SSR and SNP markers was also reported by Singh, et al. (2013). Within indica and Japonica types, variability is probably a result of pedigree evolved in different gene pool in the same subspecies, as suggested by Lu *et al.* (2009). The indica varieties from IRRI (Philippines) were closely related because of selection under similar environments for specific breeding aims (Lin *et al*., 2012). However, it should be noted that the

SNPs employed were selected as polymorphic in a large number of rice SNPs, and therefore their use may not be ideal to detect private polymorphisms or rare alleles potentially involved in directional selection of landraces as suggested by Hamilton *et al.* (2012).

As far as ShR of rice is concerned, since indica types are generally susceptible and Japonica varieties having different levels of resistance (Mvuyekure *et.al*., 2014, unpublished), genetic diversity among both groups as confirmed by SNP markers is a breakthrough in varietal improvement towards resistance to the ShR of rice. Varieties at both ends of the dendrogram can be considered as potential parental materials for this purpose in whatever breeding strategy is used. In these regards, improving resistance levels of indica varieties subjected to this study, except Rumbuka, should involve cultivars such as Yunyine, Yunertian and Moroberekan. Rumbuka should be improved through hybridization with Nyiragikara. Involvement of Nyiragikira in improvement programmes targeting indica, varieties except Rumbuka, would not be a good deal because, due to low genetic distance, there are risks of genetic depression or reduced diversity in resulting progenies.

5.5 Conclusion

This study concluded that 94 SNPs markers were highly informative and sufficiently polymorphic to distinguish relationship groups from 25 rice cultivars that are considered potential parental lines in breeding for sheath rot resistance programmes. The studied varieties revealed the existence of high genetic variability than can be exploited for crop improvement with minimized risks of genetic depression and reduced diversity among progenies. The information generated will contribute significantly to further breeding studies mainly, in determination of gene action and nature of inheritance governing resistance to sheath rot. It will also be helpful to design an adequate breeding strategy to introgress sheath rot resistance genes into popular cultivars. To this end, three cultivars, Yunyine, Yunertian and Moroberekan were found good candidate source of resistant genes for improving most of indica varieties except Rumbuka. The improvement of Rumbuka, as a variety of great yielding potential, should lead to better results once hybridized with Nyiragikara. Finally these are valuable findings as breeding for disease resistance has always been considered as one of the most sustainable disease management strategies, is the deployment of resistant varieties as it is easy for farmers, requires no additional cost, and is also environmental friendlier.

References

Ayyadurai, N., Kirubakaran, S.I., Srisha, S., and Sakthivel, N., 2005. Biological and molecular variability of *Sarocladium oryzae*, the sheath rot pathogen of rice (*Oryza sativa L*.). *Current Microbiology* 50: 319-323.

Bigirimana, V.d.P., Hua, G.K.H., Nyamangyoku, O.I., and Höfte, M., 2015. Rice sheath rot: An emerging ubiquitous destructive disease complex. *Frontiers in Plant Science* 6: 1066-1082.

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314-331.

Chen, H., He, H., Zou, Y., Chen, W., Yu, R., Liu, X., Yang, Y., Gao, Y.M., Xu, J.L., Fan, L.M., Li, Y., Li, Z.K., and Deng, X.W., 2011. Development and application of a set of breeder-friendly SNP markers for genetic analyses and molecular breeding of rice (*Oryza sativa L*.). *Theoretical and Applied Genetics* 123: 869-879.

Das, B., Sengupta, S., Parida, S., Roy, B., Ghosh, M., Prasad, M., and Ghose, T., 2013. Genetic diversity and population structure of rice landraces from Eastern and North Eastern States of India. *BMC Genetics* 14: 71-85.

Feltus, F.A., Wan, J., Schulze, S.R., Estill, J.C., Jiang, N., and Paterson, A.H., 2004. A SNP resource for rice genetics and breeding based on subspecies indica and japonica genome alignments. *Genome Research* 14: 1812-1819.

Gnanamanickam, S., 2009. Biological control of sheath-rot and other fungal diseases. In: Gnanamanickam, S., (eds.), Biological Control of Rice Diseases. Springer Netherlands. p. 91-93.

Guimarães, P.E., 2009. Rice breeding. In: Carena, J.M., (eds.), Cereals. Springer US, New York, NY. p. 99-126.

Hamilton, J.P., Sung-Chur, S., Stoffel, K., Van Deynze, A., Buell, C.R., and Francis, D.M., 2012. Single nucleotide polymorphism discovery in cultivated tomato via sequencing by synthesis. *The Plant Genome* 5: 17-29.

Hittalmani, S., Mahesh, H.B., Mahadevaiah, C., and Prasannakumar, M.K., 2016. De novo genome assembly and annotation of rice sheath rot fungus *Sarocladium oryzae* reveals genes involved in helvolic acid and cerulenin biosynthesis pathways. *BMC Genomics* 17: 271-283.

Hung-Ying, L., Yong-Pei, W., Ai-Ling, H., Sheng-Wei, H., Fu-Jin, W., YI, H., and Lin, Y.-R., 2012. Genetic diversity of rice germplasm used in Taiwan breeding programs. *Botanical. Studies* 53: 363-376.

Kilian, B., and Graner, A., 2012. NGS technologies for analyzing germplasm diversity in genebanks. *Briefings in Functional Genomics* 11: 38-50.

Lakshmanan, P., and Velusamy, R., 1991. Resistance to sheath rot (ShR) of breeding lines derived from *Oryza officinalis*. *International Rice Research Newsletter* 16: 8-9.

Lanoiselet, V., You, M.P., Li, Y.P., Wang, C.P., Shivas, R.G., and Barbetti, M.J., 2012. First report of *Sarocladium oryzae* causing sheath rot on rice (*Oryza sativa*) in western Australia. *Plant Disease* 96: 1382-1382.

Lin, H.Y., Wu, Y.P., Hour, A.L., Ho, S.W., Wei, F.J., Hsing, C., and Lin, Y.R., 2012. Genetic diversity of rice germplasm used in Taiwan breeding programs. *Botanical Studies* 53: 363-376.

Lu, B.-R., Cai, X., and Xin, J., 2009. Efficient indica and japonica rice identification based on the InDel molecular method: Its implication in rice breeding and evolutionary research. *Progress in Natural Science* 19: 1241-1252.

Madhav, M.S., Laha, G.S., Padmakumari, A.P., Somasekhar, N., Mangrauthia, S.K., and Viraktamath, B.C., 2013. Phenotyping rice for molecular plant breeding. In: Panguluri, S.K. and Kumar, A.A., (eds), Phenotyping for plant breeding. Springer New York. p. 1-40.

Manikanda, B., N., 2013. Genotyping of mapping population. In: Boopathi, N.M., (eds.), Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits. Springer India, New Delhi. p. XV, 293.

Mvuyekure, SM, Sibiya J, Derera J, Nzungize J, Nkima G. 2014: Genetic variability of rice germplasm for resistance to sheath rot of rice and important agronomic traits in Rwanda. Rwanda Agriculture Board (RAB) and African Center for Crop Improvement (ACCI), Kigali. pp 21.

Nascimento, W.F.d., Silva, E.F.d., and Veasey, E.A., 2011. Agro-morphological characterization of upland rice varieties. *Scientia Agricola* 68: 652-660.

Nei, M., Tajima, F., and Tateno, Y., 1983. Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19: 153-170.

Ngala, G.N., and Adeniji, M.O., 1986. Sheath rot disease in tropical Africa. In: Juo, A.S.R. and Lowe, J.A., (eds), The Wetlands and Rice in Subsaharan Africa. IITA, Ibadan, Nigeria.

Ojango, J., Mpofu, N., Marshall, K., and Andersson-Eklund, L., 2011. Quantitative methods to improve the understanding and utilisation of animal genetic resources. In: Ojango, J.M., Malmfors, B. and Okeyo, A.M., (eds), Animal Genetics Training Resource, version 3, 2011. International Livestock Research Institute. Nairobi, Kenya.

Oka, H.-I., and Morishima, H., 1982. Phylogenetic differentiation of cultivated rice, XXIII. Potentiality of wild progenitors to evolve the indica and japonica types of rice cultivars. *Euphytica* 31: 41-50.

Pearce, D.A., Bridge, P.D., and Hawksw, D.L., 2001. Species concept in sarocladium, the causal agent of sheath rot in rice and bamboo blight. In: Sreenivasaprasad, S. and Johnson, R., eds), Major Fungal Diseases of Rice. Springer Netherlands. p. 285-292.

Rafalski, A., 2002. Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology* 5: 94-100.

Ram, S.G., Thiruvengadam, V., and Vinod, K.K., 2007. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *Journal of Applied Genetics* 48: 337-345.

Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L., van Ginkel, M., Hoisington, D., Bohn, M., and Melchinger, A.E., 2005. Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics* 110: 859-864.

Ren, J., Sun, D., Chen, L., You, F.M., Wang, J., Peng, Y., Nevo, E., Sun, D., Luo, M.-C., and Peng, J., 2013. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. *International Journal of Molecular Sciences* 14: 7061-7088.

Sakthivel, N., 2001. Sheath rot disease of rice: Current status and control strategies. In: Sreenivasaprasad S, Johnson R (eds). Major Fungal Diseases of Rice Recent Advances. Kluwer Academic Publishers Boston.

Singh, N., Choudhury, D.R., Singh, A.K., Kumar, S., Srinivasan, K., Tyagi, R.K., Singh, N.K., and Singh, R., 2013. Ccomparison of ssr and snp markers in estimation of genetic diversity and population structure of indian rice varieties. *PLoS ONE* 8: e84136.

Soleimani, D.V., Baum, R.B., and Johnson, A.D., AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum L. subsp. durum* (Desf.) Husn.]. *Theoretical and Applied Genetics* 104: 350-357.

Thomson, M., Septiningsih, E., Suwardjo, F., Santoso, T., Silitonga, T., and McCouch, S., 2007. Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics* 114: 559-568.

WARDA, 2008. NERICA : The new rice for africa – a compendium Africa Rice Center (WARDA). Cotonou Benin.

Weddell, B.J., 2002. Conserving Living Natural Resources: In the Context of a Changing World. Cambridge University Press, Cambridge, UK.

Appendix 5.1 List of used markers and summary statistics

Marker	Major allele frequency	Genotype number	Sample size	Number of observations	Allele number	Gene diversity	Heterozygosity	PIC
K_id1002308	0.6	2	25	25	2	0.48	0	0.365
K_id1006954	0.667	2	25	24	2	0.444	0	0.346
K_id1008787	0.848	3	25	23	2	0.258	0.043	0.225
K_id1011568	0.56	3	25	25	2	0.493	0.08	0.371
K_id1014143	0.545	4	25	11	4	0.612	0	0.555
K_id1024233	0.545	3	25	22	2	0.496	0.091	0.373
K_id1025888	0.82	4	25	25	3	0.306	0.04	0.278
K_id1026656	0.96	2	25	25	2	0.077	0	0.074
K_id2000096	0.571	3	25	21	3	0.526	0	0.429
K_id2001992	0.841	3	25	22	2	0.268	0.045	0.232
K_id2004058	0.82	3	25	25	2	0.295	0.04	0.252
K_id2006621	0.72	2	25	25	2	0.403	0	0.322
K_id2007797	0.818	3	25	11	3	0.314	0	0.292
K_id2008480	0.88	2	25	25	2	0.211	0	0.189
K_id2010564	0.6	3	25	25	2	0.48	0.08	0.365
K_id2010969	0.696	3	25	23	3	0.446	0	0.378
K_id2013007	0.833	3	25	24	2	0.278	0.083	0.239
K_id3000111	0.7	3	25	25	2	0.42	0.04	0.332
K_id3002805	0.522	2	25	23	2	0.499	0	0.375
K_id3006808	0.708	2	25	24	2	0.413	0	0.328

Marker	Major allele frequency	Genotype number	Sample size	Number of observations	Allele number	Gene diversity	Heterozygosity	PIC
K_id3007703	0.74	4	25	25	3	0.402	0.04	0.347
K_id3008390	0.714	3	25	21	3	0.431	0	0.37
K_id3010318	0.826	2	25	23	2	0.287	0	0.246
K_id3010628	0.913	2	25	23	2	0.159	0	0.146
K_id3013806	0.563	3	25	24	2	0.492	0.042	0.371
K_id3017084	0.905	3	25	21	3	0.177	0	0.169
K_id4001365	0.6	2	25	15	2	0.48	0	0.365
K_id4002780	0.543	3	25	23	2	0.496	0.043	0.373
K_id4004294	0.636	2	25	22	2	0.463	0	0.356
K_id4005120	0.636	2	25	22	2	0.463	0	0.356
K_id4005867	0.7	2	25	20	2	0.42	0	0.332
K_id4007444	0.587	3	25	23	2	0.485	0.043	0.367
K_id4010621	0.5	3	25	24	3	0.538	0	0.432
K_id4012434	0.714	2	25	21	2	0.408	0	0.325
K_id5000128	0.913	2	25	23	2	0.159	0	0.146
K_id5001534	0.92	2	25	25	2	0.147	0	0.136
K_id5003785	0.696	2	25	23	2	0.423	0	0.334
K_id5006332	0.565	2	25	23	2	0.491	0	0.371
K_id5007714	0.587	3	25	23	2	0.485	0.043	0.367
K_id5008723	1	1	25	18	1	0	0	0

Marker	Major allele frequency	Genotype number	Sample size	Number of observations	Allele number	Gene diversity	Heterozygosity	PIC
K_id5011704	0.708	2	25	24	2	0.413	0	0.328
K_id5013100	0.696	2	25	23	2	0.423	0	0.334
K_id6000134	0.88	2	25	25	2	0.211	0	0.189
K_id6004862	1	1	25	22	1	0	0	0
K_id6007386	1	1	25	23	1	0	0	0
K_id6010534	0.909	2	25	22	2	0.165	0	0.152
K_id6012080	0.74	3	25	25	2	0.385	0.04	0.311
K_id6012658	0.86	3	25	25	2	0.241	0.04	0.212
K_id6016125	0.708	2	25	24	2	0.413	0	0.328
K_id6002535	0.88	2	25	25	2	0.211	0	0.189
K_id7000063	0.75	2	25	24	2	0.375	0	0.305
K_id7001596	0.913	2	25	23	2	0.159	0	0.146
K_id7002534	0.913	2	25	23	2	0.159	0	0.146
K_id7003748	0.6	2	25	25	2	0.48	0	0.365
K_id7004442	0.96	2	25	25	2	0.077	0	0.074
K_id7005111	0.842	2	25	19	2	0.266	0	0.231
K_id7005689	0.86	3	25	25	2	0.241	0.04	0.212
K_id8000131	0.625	2	25	24	2	0.469	0	0.359
K_id8001667	0.76	2	25	25	2	0.365	0	0.298
K_id8003220	0.761	3	25	23	2	0.364	0.043	0.298

Marker	Major allele frequency	Genotype number	Sample size	Number of observations	Allele number	Gene diversity	Heterozygosity	PIC
K_id8004986	0.52	3	25	25	2	0.499	0.16	0.375
K_id8006032	0.75	2	25	24	2	0.375	0	0.305
K_id8006950	0.826	2	25	23	2	0.287	0	0.246
K_id8007951	0.81	2	25	21	2	0.308	0	0.261
K_id9000045	1	1	25	19	1	0	0	0
K_id9001558	0.739	2	25	23	2	0.386	0	0.311
K_id9002532	0.604	3	25	24	2	0.478	0.042	0.364
K_id9003471	1	1	25	25	1	0	0	0
K_id9004347	0.739	2	25	23	2	0.386	0	0.311
K_id9005089	0.87	2	25	23	2	0.227	0	0.201
K_id9006757	1	1	25	19	1	0	0	0
K_id9007001	0.64	2	25	25	2	0.461	0	0.355
K_id9007259	0.7	3	25	25	2	0.42	0.04	0.332
K_id10000028	0.565	2	25	23	2	0.491	0	0.371
K_id10001624	0.58	3	25	25	2	0.487	0.04	0.369
K_id10002912	0.66	3	25	25	2	0.449	0.04	0.348
K_id10004275	1	1	25	25	1	0	0	0
K_id11000399	0.804	3	25	23	2	0.315	0.043	0.265
K_id11001993	1	1	25	25	1	0	0	0
K_id11003845	0.935	2	25	23	2	0.122	0.13	0.114

Marker	Major allele frequency	Genotype number	Sample size	Number of observations	Allele number	Gene diversity	Heterozygosity	PIC
K_id11005657	0.813	2	25	16	2	0.305	0	0.258
K_id11006897	0.96	2	25	25	2	0.077	0	0.074
K_id11007625	0.848	3	25	23	2	0.258	0.13	0.225
K_id11008403	1	1	25	25	1	0	0	0
K_id11008862	0.64	2	25	25	2	0.461	0	0.355
K_id11010309	0.771	3	25	24	2	0.353	0.125	0.291
K_id12000266	0.75	2	25	24	2	0.375	0	0.305
K_id12001996	0.773	2	25	22	2	0.351	0	0.29
K_id12004271	0.583	2	25	24	2	0.486	0	0.368
K_id12005822	0.917	2	25	24	2	0.153	0	0.141
K_id12006560	0.708	2	25	24	2	0.413	0	0.328
K_id12008285	0.65	2	25	20	2	0.455	0	0.351
K_id12008894	0.875	2	25	24	2	0.219	0	0.195
K_id12006515	0.739	2	25	23	2	0.386	0	0.311
Mean	0.762	2.2872	25	23	2.0106	0.325	0.018	0.263

6 Chapter 5

Genetic analysis for sheath rot disease resistance in rice

Abstract

Sheath rot of rice is one of the less studied rice diseases and thus little information is available on its management especially through breeding for disease resistance. Understanding genetic mechanisms controlling inheritance of disease resistance traits is an important component of breeding investigations targeting development of resistant genotypes. Using a North Carolina design II, 32 F1 hybrids were generated by crossing 8 susceptible to 4 resistant parents and evaluated under field conditions for sheath rot resistance. Significance of both general and specific combining ability (GCA and SCA) effects indicated involvement and magnitude of additive and non-additive gene action in controlling the inheritance of traits associated with horizontal resistance to sheath rot of rice. Based on high GCA/SCA ratio, coupled with high heritability estimates, additive effects were more predominant in the expression of lesion size, area under disease progress curve and panicle exsertion. In addition, results indicated that dominant genes were more important than recessive genes. As far as sources of resistance are concerned, the most promising genotypes were Cyicaro, Yunertian and Yunkeng. Results from GCA and SCA analysis suggested that crop improvement programmes should be directed towards selection of superior parents or good combiners emphasizing on GCA. Crosses exhibiting high SCA effects would produce desirable transgressive segregants in later generations if efforts could be made to modify the conventional breeding methodologies to capitalize on both additive and non-additive genetic effects. The predominance of additive genetic effects together with the relevance of dominant genes suggested possibilities of improving the resistance by introgression of resistance genes through recurrent selection coupled with phenotypic selection. However, high estimates of heritability observed for all three traits, indicated these traits should be selected in controlled environment.

6.1 Introduction

Rice is among the most widely consumed staple food in the world. It is the most important grain for human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide (Raja, 2013). However, rice production frequently faces constraints due to both biotic and abiotic stresses and among these is the sheath rot caused by *Sarocladium oryzae* [(Sawada) [W. Gams & D. Hawksw]. The disease has become endemic in almost all the rice growing regions around the world in both rain-fed and irrigated ecosystems and is now considered as one of most important emerging and destructive disease of rice (Madhav *et al.*, 2013; Raja, 2013; Hittalmani *et al.*, 2016).

The disease affects both local and modern rice varieties with high incidences being reported in modern cultivars (Miah *et al.*, 1985). Dwarf and high yielding Asian varieties are more susceptible, whereas tall varieties with well exserted panicles are resistant. Sheath rot damages the uppermost flag leaf sheath covering the young panicles (Amin, 1976; Miah et al., 1985). Under severe conditions, panicles fail to fully emerge and remain enclosed in the flag leaf sheaths (Estrada *et al.*, 1979; Naeimi *et al.*, 2003). This leads to poor panicle formation, followed by increased number of chaffy, discoloured, and shrivelled grains thus reducing the weight and number of healthy grains. Yield losses range between 20 and 30% in general but severe losses up to 70 - 85% have been recorded in several parts of the world (Sakthivel, 2001; Pearce *et al.*, 2001).

The management of sheath rot disease involves all measures aimed at reducing its impact on rice productivity and hence ensuring food security. Similar to other pests and diseases of rice, the deployment of varietal resistance has always been considered the most economically and environmentally friendly approach. In breeding for resistance to endemic diseases, horizontal resistance is often preferred to vertical resistance (Vanderplank, 1984). Horizontal resistance is advantageous in that it operates against all pathotypes and so there is no differential interaction between pathotypes and cultivars. Additionally, horizontal resistance is in most cases not liable to breakdown because there is no strong selection pressure in favour of some pathotypes against others (Keane and Brown, 1997). Improving the levels of horizontal resistance for

sheath rot in rice is the most durable strategy to genetically overcome massive yield losses attributed to the disease. While Vanderplank (1984) and Mulbah *et al.* (2015) attested that components of horizontal resistance include traits such as lesion size, and speed at which lesion spreads over the affected leaf area or area under the disease progress curve (Vinod *et al.*, 1990).

Vinod et *al*. (1990) and Srinivasachary *et al.* (2002) reported a strong relationship between sheath rot of rice and panicle exsertion. However, because the disease has for a long time been considered as minor, little information is available on its genetic variation, mode of gene action and nature of inheritance. One of the ways of understanding the gene action is through analysis of the combining ability of the breeding lines and their hybrids. According to Falconer *et al.* (1996) and Sprague and Tatum (1942), determination of combining ability is important not only for gene action but for parental selection in hybridization programmes and identification of promising recombinants for a given breeding programme. This information can serve as a tool for determining the most appropriate breeding procedure for trait-specific improvement programme. It is therefore important for a plant breeder to determine at the start of a breeding programme whether the resistance is controlled by a few or many genes, and whether resistance is additive, dominant or recessive to susceptibility (Russell, 1978). Therefore, this study was conducted to estimate the combining ability effects for resistance to sheath rot among selected rice lines and determine the gene action controlling sheath rot resistance.

6.2 Materials and methods

6.2.1 Plant materials

Plant materials used in this study (Table 6.1) consisted of eight and four sheath rot susceptible and resistant varieties, respectively, all kindly availed by the Rice Research Programme of Rwanda Agriculture Board (RAB). Hybridization was performed following an 8 x 4 North Carolina Design II mating design, in the RAB screen house, Rubona station (Figure 6.1), during the season of September to December, 2014. Thirty two F1 progenies were obtained from crosses between eight female sheath rot (ShR) susceptible parents and four male ShR resistant parents.

Figure 6.1 Crossing block established in one of RAB tunnels in Rubona

Table 6.1: List of parental lines used and their reaction to sheath rot of rice

	Code	Name	VR to ShR	Origin	Height	PE
Females	P1	Buryohe	HS	RAB	Semi-dwarf	Partially exserted
	P2	Fac 56	S	RAB	Semi-dwarf	Partially exserted
	P3	Fashingabo	S	RAB	Semi-dwarf	Partially exserted
	P4	Gakire	S	RAB	Semi-dwarf	Enclosed
	P5	intsinzi	HS	RAB	Semi-dwarf	Enclosed
	P6	Mpembuke	S	RAB	Intermediate	Partially exserted
	P7	Ndamirabahinzi	S	RAB	Intermediate	Partially exserted
	P8	Rumbuka	HS	RAB	Intermediate	Partially exserted
Males	P9	Cyicaro	MR	RAB	Intermediate	Exserted
	P10	Nyiragikara	HR	RAB	Intermediate	Well exserted
	P11	Yunertian	HR	RAB	Long	Well exserted
	P12	Yunkeng	R	RAB	Long	Well exserted

VA=varietal reaction; ShR= sheath rot; MR= moderately resistant; HR = highly resistant; R= resistant; S = susceptible; HS = highly susceptible; PE= Panicle exsertion.

All the 32 crosses and the 12 parental lines were subjected to field evaluation in Rurambi; one of the District irrigated rice schemes in the Eastern Province of Rwanda. The location had been cultivated with rice for a long time as an intensive monoculture without rotation and ShR of rice was previously confirmed to be endemic in the area and hence a good disease hotspot.

The NCDII crosses did not generate enough F1 seeds; hence the F1 plants were multiplied through clonal propagation by tiller transplanting method. The method consisted of uprooting a rice hill and separating tillers from each other and then re-transplanting the individually separated tillers. The method was initially tested in a separate study where it resulted in a multiplication rate of at least four times the number of tillers which were identical to the original plant for most of the quantitative and qualitative traits.

6.2.2 Experimental layout and design

The experiment was laid out in an 11 x 4 alpha lattice design and replicated two times, between January and June 2015. The experimental plots consisted of 1 m wide and 1 m long plots with 20 cm between and within rows, and five rows in each plot. The small size of the plot was in accordance with Portmann and Ketata (1997) who states that it may be desirable to keep the number of replicates and plot size small when seed supplies are limited, so that each genotype is grown at as many locations as possible.

Sun-dried seeds from each genotype were pre-germinated in plastic bags, and raised in a nursery to increase the germination rate. Seedlings were transplanted 21 days later as per recommendations. Despite the experimental site being a disease hot spot and hence plant materials exposed to sufficient natural inoculum, Madhav *et al*. (2013) suggested that results may fluctuate due to inconsistent and uneven distribution of natural infection. Therefore to obtain uniform disease infection, artificial inoculation of the plants was done according to a simple and efficient method known as sheath inoculum technique, described by Narayanaprasad *et al.* (2011). The technique consists of cutting infected sheath into small pieces then inserting them in between the flag leaf sheath and the emerged sheath. In each experimental plot, 5 plants out of 25 were randomly selected and tagged for artificial inoculation and various measurements. The crop was raised under aerobic conditions by providing continuous irrigation, while the rest of the cultural practices and crop protection measures were applied as recommended, thus ensuring uniform and healthy crop growth.

6.2.3 Data collection and analysis

Data were collected on a fortnightly basis on ShR horizontal resistance related traits, namely lesion size and panicle exsertion, starting a few days after booting stage. Lesion size was evaluated as a percentage ratio between the lesion length (cm) and the total length of the sheath of the flag leaf. From the lesion size, the area under disease progress curve (AUDPC) was determined using the following formula as described by Simko and Piepho (2012).

$$\text{AUDPC} = \left[Y_1 \frac{t_2 - t_1}{2}\right] + \left[\sum_{1=2}^{n=1} \left(Y_i \frac{t_i + 1}{2}\right)\right] + \left[Y_n \frac{t_n - t_n - 1}{2}\right]$$

Where Y_1 and Y_n are assessments at the first and last observations, respectively, and t_1, t_2, t_{n-1}, and t_n are the times of the first, second, penultimate, and last observations.

Panicle exsertion was evaluated by metric measurement (in cm) of the length of uppermost inter-node above the flag leaf sheath or panicle rachis. Mean performance of each cross and parental line was determined through the analysis of variance using REML procedure of Genestat 17th edition (Payne *et al.*, 2014). Genetic parameters were determined from the expectations of mean squares from the analysis of variance of the North Carolina Design II performed on F1 progenies as described by Acquaah (2012).

In the analysis of combining ability, the variation in the crosses were partitioned into general combining ability (GCA) and specific combining ability (SCA) according to the methods described by Simmonds (1979) quoted by Mzengeza (2010). The genetic model used is given below:

$$Y_{ijk} = \mu + m_i + f_j + (mf)_{ij} + w_{ijk} + r_k + e_{ijk}$$

Where: μ = the population mean; m_i = the effect of the i^{th} male (GCA_{male}); f_j = the effect of the j^{th} female (GCA_{female}), mf_{ij} = the interaction effect obtained in the cross between lines i and j (SCA_{fm}); w_{ijk} = the effect of the k^{th} progeny from the cross between lines i and j; r_k = the replication effect, and e_{ijk} = the experimental error.

Consequently, general and specific combining abilities (GCA and SCA respectively) for the parents and crosses were determined according to the following formulae as described by Acquaah (2012).

The GCA for each of the male parents was calculated using the following formulas:

$GCA_m = X_m – \mu$,

$GCA_f = X_f – \mu$.

The SCAs of the crosses were computed from the formula:

$SCA_X = X_X - E(X_X) = X_X – [GCA_m + GCA_f + \mu]$

Where: GCA_m = general combining ability of male parent; X_m = mean of the male parent; μ = overall mean of all crosses; GCA_f = general combining ability of the female parent, X_f = mean of the female; SCA_X = specific combining ability of the two parents in the cross; X_X = observed mean value of the cross; $E(X_X)$ = expected values of the cross basing on the GCAs of the two parents

Using mean squares for GCA (MSg), SCA (MSs) and Error (MSe) extracted from the ANOVA table, variance components; additive (σ^2A), dominance (σ^2D:), and Environmental (σ^2E) variances; were estimated as follows according to Acquaah (2012).

$\sigma^2m = [MSf - MSfm]/rm = 1/4\ VA$

$r\sigma^2f = [MSm - MSfm]/rf = 1/4\ VA$

$r\sigma^2\ mf = [MSfm - MSe]/r = 1/4\ VD$

$\sigma^2e = MSe = 1/2\ VA + 3/4\ VD + E$

Heritability in broad sense (H^2) and narrow sense (h^2) were estimated as follows:

$H^2 = (\sigma^2A + \sigma^2NA)/(\sigma^2A + \sigma^2D + \sigma^2E)$

$h^2 = (\sigma^2A)/(\sigma^2A + \sigma^2D + \sigma^2E)$

Other parameters estimated include maternal effects, GCA/SCA ratio and level of dominance.

6.3 Results

6.3.1 Mean performance of crosses and parents

Results from the analysis of variance using REML procedure revealed highly significant differences (P<0.001) amongst all genotypes evaluated, that is, twelve parental lines and their derived 32 crosses (F1 progenies) for lesion size, area under disease progress curve and panicle exertion. The mean value for each of the three traits varied significantly with specific genotypes (Table 6.2).

Table 6.2: Mean values for parental lines and crosses for lesion size, area under disease progress curve and panicle exsertion

	Code	Genotype	Lesion size (cm)	Panicle exsertion (cm)
Female parents	P1	Buryohe	18.64	4.73
	P2	Fac 56	17.92	2.22
	P3	Fashingabo	4.93	5.00
	P4	Gakire	14.96	5.21
	P5	Intsinzi	15.96	4.59
	P6	Mpembuke	10.27	8.16
	P7	Ndamirabahinzi	11.36	7.18
	P8	Rumbuka	18.97	4.82
Male parents	P9	Cyicaro	1.67	7.83
	P10	Nyiragikara	0.51	9.23
	P11	Yunertian	0.47	9.32
	P12	Yunkeng	1.47	7.93
Crosses	P1XP9	Buryohe x Cyicaro	13.21	5.22
	P2XP9	Fac 56 x Cyicaro	12.27	5.48
	P3XP9	Fashingabo x Cyicaro	11.00	6.43
	P4XP9	Gakire x Cyicaro	9.92	5.84
	P5XP9	Intsinzi x Cyicaro	9.44	4.54
	P6XP9	Mpembuke x Cyicaro	13.60	4.52
	P7XP9	Ndamirabahinzi x Cyicaro	8.47	5.14
	P8XP9	Rumbuka x Cyicaro	9.57	4.47

Code	Genotype	Lesion size (cm)	Panicle exsertion (cm)
P1XP10	Buryohe x Nyiragikara	8.60	5.55
P2XP10	Fac 56 x Nyiragikara	9.29	6.54
P3XP10	Fashingabo x Nyiragikara	8.26	6.02
P4XP10	Gakire x Nyiragikara	8.06	5.80
P5XP10	Intsinzi x Nyiragikara	10.66	5.73
P6XP10	Mpembuke x Nyiragikara	9.39	5.95
P7XP10	Ndamirabahinzi x Nyiragikara	9.15	5.83
P8XP10	Rumbuka x Nyiragikara	8.63	5.87
P1XP11	Buryohe x Yunertian	8.44	5.17
P2XP11	Fac 56 x Yunertian	9.89	5.79
P3XP11	Fashingabo x Yunertian	8.73	6.17
P4XP11	Gakire x Yunertian	7.31	5.14
P5XP11	Intsinzi x Yunertian	9.53	7.23
P6XP11	Mpembuke x Yunertian	12.28	7.07
P7XP11	Ndamirabahinzi x Yunertian	9.30	8.08
P8XP11	Rumbuka x Yunertian	9.09	7.20
P1XP12	Buryohe x Yunkeng	8.09	6.16
P2XP12	Fac 56 x Yunkeng	11.57	7.09
P3XP12	Fashingabo x Yunkeng	7.22	6.79
P4XP12	Gakire x Yunkeng	9.40	6.41
P5XP12	Intsinzi x Yunkeng	12.33	4.78
P6XP12	Mpembuke x Yunkeng	14.02	5.70
P7XP12	Ndamirabahinzi x Yunkeng	11.96	6.47
P8XP12	Rumbuka x Yunkeng	12.44	5.50
	s.e.d	1.10	0.33
	LSD(0.05)	2.17	0.64
	CV (%)	24.75	12.03

The mean lesion size for parental lines and derived crosses ranged from 0.47 cm to 18.97 cm with the female parents recording highest scores than male parents. Mean lesion size for crosses ranged between 7.22 cm and 14.02 cm. Crosses involving P9 (Cyicaro) as a source of resistance recorded highest values for lesion size in average with an average of 10.99 cm per cross, followed by crosses involving Yunkeng (10.88 cm) in average. Crosses involving

Yunertian recorded an average lesion size of 9.32 cm whereas; the least average (9.01 cm) lesion size was recorded on crosses involving Nyiragikara. Mean panicle exsertion values for all the genotypes varied between 2.22 cm and 9.32 cm with, evidently, male parental lines (sources of resistant genes) recording highest values compared to female parents and crosses. Most well exserted among crosses were the ones involving Yunertian with an average panicle exsertion of 6.38 cm, followed by Yunkeng (6.11 cm), Nyiragikara (5.91 cm) whereas the least values were obtained with crosses involving Cyicaro (5.21 cm).

6.3.2 Analysis of combining ability effects

The results of analysis of North Carolina Design II are presented in Table 6.3. Mean squares estimates for male and female parents as well as their interactions led to the estimation of both general and specific combining abilities (GCA and SCA). Variances due to GCAs for both male and female parents were highly significant ($P<0.01$) for lesion size (LS), area under disease progress curve (AUDPC) and panicle exsertion (PE). Variances due to SCA were significant ($P<0.05$) for only AUDPC and PE. On the other hand, GCAm/SCA ratio and GCAf/ SCA ratios were greater than one for all the traits but the highest ratio was for PE. Maternal effects were not significant for all the traits.

Table 6.3: Analysis of variance for the 8 x 4 North Carolina Design II for lesion size (LS), area under disease progress curve (AUDPC) and panicle exsertion (PE)

Source of variation	DF	LS	AUDPC	PE
GCAm	3	94.772**	16458.884**	9.122**
GCAf	7	58.329**	14865.770**	25.362**
SCA mf	21	9.669ns	2266.817*	0.966*
Error	288	6.558	1286.791	0.597
s.e		2.576	35.87	0.773
CV (%)		25.4	35.3	13.2
GCAm/SCA		9.802	7.261	9.443
GCAf/SCA		6.0326	6.5580	26.2547
Maternal effects		1.625 ns	1.107 ns	0.360 ns

** and * represent significant effects of GCA and SCA at 1 and 5% respectively

In addition to GCA and SCA estimates, individual GCA and SCA effects for both parental lines and crosses revealed considerable variations among different genotypes (Table 6.4, Figure 6.2 and Figure 6.3).

Table 6.4: Estimates of effects of GCA of parental lines for area under disease progress curve (AUDPC), lesion size (LS) and panicle exsertion (PE)

Genotype	Code	AUDPC	LS	PE
Buryohe	P1	19.756 **	1.58**	-0.178
Fac 56	P2	6.056	0.27	-1.253**
Fashingabo	P3	-18.394 **	-1.49**	0.049
Gakire	P4	-10.044	-0.62*	-0.078
Intsinzi	P5	-19.194 **	-1.47**	-0.361**
Mpembuke	P6	0.031	-0.01	1.467**
Ndamirabahinzi	P7	-12.894 *	-0.94**	0.669**
Rumbuka	P8	34.681**	2.67**	-0.313*
Cyicaro	P9	-0.031	0.08	-0.345**
Nyiragikara	P10	20.294**	1.47**	0.094
Yunertian	P11	-10.194**	-0.71	0.428**
Yunkeng	P12	-10.069**	-0.84*	-0.177*

GCA estimates followed by * and ** are statistically significant at 5 and 1% levels of significance, respectively.

From the Table 6.4, Rumbuka had the highest positive GCA for AUDPC (34.68) and LS (2.67) essentially for susceptibility, whereas the highest GCA effect for panicle exsertion was recorded on Mpembuke (1.47). In contrast, Intsinzi had the highest negative GCA for both AUDPC (-19.194) and LS (-1.470) and Fac 56 for PE (-1.253). Figure 6.2 and Figure 6.3 illustrate SCA of crosses. Highest positive effects were recorded on Buryohe x Cyicaro x for AUDPC (27.23), Fc 56 x Nyiragikara (1.89) for LS and Fashingabo x Nyiragikara for PE (0.45). The highest

negative effects were observed for Gakire x Nyiragikara for AUDPC (-25.294) and LS (-1.64), and Rumbuka x Cyicaro for PE (-0.45).

For disease resistance studies, negative values of SCA were more genetically useful due to the rating scale used as they reflect less disease severity. Hence, out of 32 crosses, 18 had negative SCA effects for AUDPC and LS.

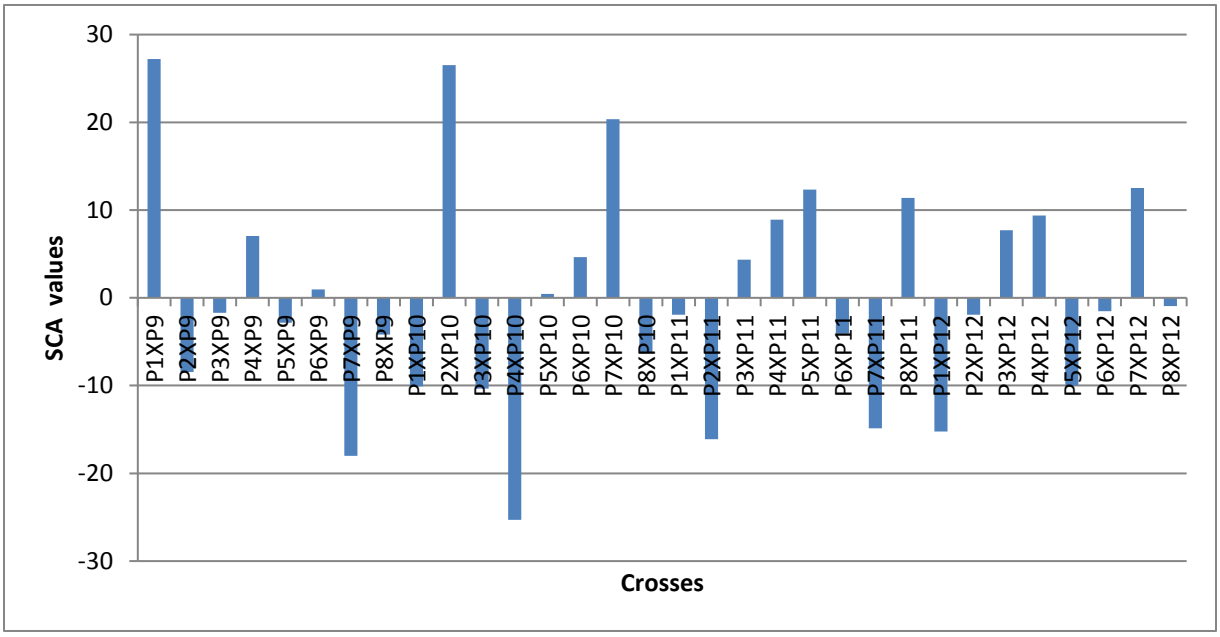

Figure 6.2 Specific combining ability for area under disease progress curve

For AUDPC these 18 included the following crosses: P2XP9, P3XP9, P5XP9, P7XP9, P8XP9, P1XP10, P3XP10, P4XP10, P8XP10, P1XP11, P2XP11, P6XP11, P7XP11, P2XP1, 2P1XP12, P5XP12, P6XP12, P8XP12 for AUDPC (Figure 6.2). For LS the crosses were P2XP9, P5XP9, P6XP9, P7XP9, P8XP9, P1XP10, P3XP10, P4XP10, P5XP10, P8XP10, P1XP11, P2XP11, P6XP11, P7XP11, P1XP12, P5XP12, P6XP12, P8XP12 for LS (Figure 6.3). On the other hand, 16 crosses out 32 had the desirable positive SCA effects for PE.

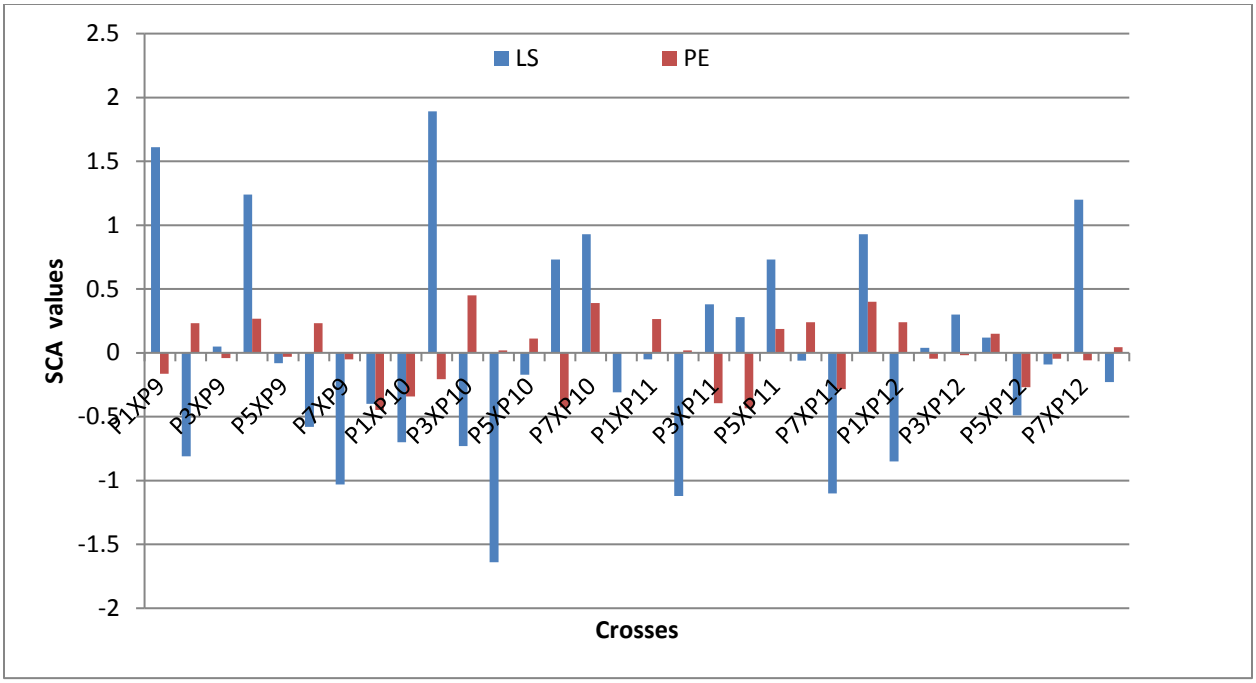

Figure 6.3: Specific combining ability estimates for Lesion size and Panicle exsertion

These 16 crosses included: P1XP9, P3XP9, P5XP9, P7XP9, P8XP9, P1XP10, P2XP10, P6XP10, P3XP11, P4XP11, P7XP11, P2XP12, 3XP12, 5XP12, P6XP12, P7XP12. A number of crosses with high SCA effects involved parents with high x low or low x high GCA values, low x low GCA or low x average GCA effects.

6.3.3 Variance components and genetic parameters

The analysis of genetic effects on the mechanisms of inheritance to ShR of rice was estimated based on variance components as shown in Table 6.5

Table 6.5: Variance components and related genetic parameters for lesion size (LS), area under disease progress curve (AUDPC) and panicle exsertion (PE)

Estimated parameter	LS	AUDPC	PE
Variation between males or GCAm variance (σ^2m)	5.319	887.004	0.510
Variation between females or GCAf variance (σ^2f)	6.083	1574.869	3.050
Variation due to interaction between males and females SCA variance (σ^2mf)	1.556	490.013	0.185
Variation within full sibs	6.558	1286.791	0.507
Additive variance of males (σ^2Am)	21.276	3548.017	2.039
Additive variance of females (σ^2Af)	24.330	6299.477	12.198
Non-additive variance (dominance or epistasis) (σ^2D)	6.222	1960.052	0.738
Environmental variance (σ^2e)	15.304	3244.047	1.573
Broad sense heritability based on females (H^2m)	0.666	0.718	0.892
Narrow sense heritability based on females (h^2f)	0.637	0.637	0.889
Level of dominance based on males (dm)*	0.332	0.035	1.192
Level of dominance based on females (df)	0.290	0.020	0.199

* Relevant only when maternal effects are significant

Results from the analysis of genetic effects revealed that a large proportion of variation was due to parental lines rather than crosses based on low level of SCA variance and estimates of variation within full sibs. On the other hand, the additive component of genetic variation was greater than non-additive and environmental component of variation for all the studied traits. Variations within full sibs were greater than GCA and SCA only for lesion size. Following the same trend, additive variance either based on male and female parents was greater than non-additive variance.

Heritability in broad sense was higher than heritability in narrow sense. Broad sense heritability ranged between 66.6% and 89.2% for all the studied traits, whereas narrow sense heritability varied from 63.7 to 88.9%. Panicle exsertion recorded the highest heritability estimates whereas lesion size showed lowest heritability. The analysis of level of dominance was between 0 and 1 for all the studied traits except PE. This is an indication of incomplete dominance or partial

dominance effects of genes for the expression of LS, AUDPC, and PE. Highest estimates were obtained on lesion size followed by panicle exertion.

6.4 Discussion

6.4.1 Performance of parental lines and crosses for resistance to ShR of rice

The analysis of variance revealed significant differences at both 5 and 1% for the three evaluated traits associated with horizontal resistance to sheath rot of rice, namely, lesion size, area under disease progress curve and panicle exsertion. These results demonstrated the existence of considerable variability among parental materials and progenies that can be exploited for cultivar improvement programme for resistance to sheath rot of rice.

This variability among genotypes might be due to genetic makeup of each of parental line, based on their origins from different gene pools. This was also alluded to by Ngala and Adeniji (1986) who indicated that African tall rice cultivars are more resistant to sheath rot than Asian dwarf varieties. In this study, resistant rice cultivars originated from intermediate or tall japonica types whereas susceptible parents were from Asian indica types. A single nucleotide polymorphism (SNP) based study in chapter 5 of this thesis showed a large genetic diversity among resistant and susceptible parental materials, involved in this study.

6.4.2 Combining ability effects

Combining ability effect is one of the most important parameters commonly used by plant breeders to evaluate the genetic potential of materials. This is useful, especially, for efficient selection of parents for hybridization, effective and efficient selection within a segregating population, and prediction of response to selection, among others (Acquaah, 2012). In most instances, the analysis of combining ability provides reliable information on the potential of parents to produce superior progenies following hybridization, and the magnitude of additive and non-additive gene action (Shattuck *et al.*, 1993).

Sprague and Tatum (1942), defined GCA as the average performance of a line in hybrid combination, and SCA as cases in which certain combinations are relatively better or worse

than would be expected on the basis of the GCA of their parents. Generally, good combiner parents result in higher frequency of heterotic hybrids than poor combiners (Virmani, 2012). In this study, both GCA and SCA revealed significant differences for the traits evaluated except for the SCA for lesion size. This significance of GCA suggests that crop improvement programmes for resistance to ShR of rice should be directed towards selection of superior parents, that is, good combiners. The significance of SCA effects for AUDPC and PE suggests that gains can be achieved through hybridization emphasizing on non-additive gene effects. According to Bokmeyer *et al.* (2009), negative GCA and SCA effects are desirable for disease resistance, based on a scale where the highest value corresponds to more disease attack.

However, male and female parents revealed considerable variability in estimates of GCA. Those with highest and positive scores were considered bad combiners, as positive effects for disease resistance related traits indicate increased levels of disease susceptibility. This is why genotypes such as Nyiragikara, Buryohe and Rumbuka were regarded as bad combiners as far as AUDPC and LS are concerned. Conversely, genotypes such as Ndamirabahinzi, Intsinzi, Fashingabo, Yunkeng and Yunertian were identified as good combiners for LS and AUDPC as they recorded the highest negative GCA values. Consequently, they will be considered in hybridization programmes aiming at the improvement of resistance to sheath rot, as male and female parents. The superiority of these good x combiner parents was also observed in F1 progenies due to high negative SCA effects of crosses involving the above mentioned good combiners. However, in some progenies, a number of high negative SCA effects for F1 progenies were obtained by crossing a good combiner, either as male or female parents, to a bad combiner. This is an indication that crossing a good combiner to another good combiner does not necessarily lead to desired progenies.

Some of the crosses showing high SCA effects involved parents with high x low or low x high GCA, low x low GCA or low x average GCA. The high SCA effects of such crosses might be attributed to additive x additive type of gene action and the high disease resistance potential of these crosses can be fixed in subsequent generations (Chakraborty *et al.*, 2009). According to the same source, the crosses that originated from high general combiner parents reflecting high negative SCA effects are expected to produce useful transgressive segregants, which can be identified following simple conventional breeding techniques like pedigree method of selection.

Conversely, high SCA effects of the crosses that resulted from high x low combining parents are attributed to additive x dominance type of gene action (Sharma *et al.*, 2014). The high level of resistance from such crosses would be unfixable in subsequent generations but these crosses would produce desirable transgressive segregants in later generations by modifying the conventional breeding methodologies to capitalize on both additive and non-additive genetic effects (Chakraborty *et al*., 2009). Various investigations reported by Virmani (2012) showed evidence of high x high general combiners resulting in crosses showing low SCA effects and concluding that crosses between good general combiners did not always result in good F1 crosses.

In general parents possessing high general combining ability also possess good performance *per se* in crosses, although exceptions to this rule are not uncommon. However, the magnitude and direction of combining ability effects are useful concepts to take into account for parental selection in crop improvement hybridization programmes (Singh *et al.*, 2012). In this study, crosses exhibiting high negative specific combining ability effects for AUDPC and LS were derived from parents with various types of general combining ability effects (good combiner x good combiner, good combiner x bad combiner, bad combiner x bad combiner etc).

High SCA effects of the crosses that resulted from high x low combining parents may be due to additive x dominance type of gene action. The high performance from such crosses would be unfixable in subsequent generations and therefore cannot be exploited by standard selection procedure (Chakraborty *et al*., 2009). However, these crosses would produce desirable transgressive segregants in later generations if efforts could be made to modify the conventional breeding methodologies to capitalize on both additive and non-additive genetic effects.

Consequently, a breeding method involving the fixable gene effects and at the same time, maintaining considerable heterozygosity for exploiting the dominance effects, may prove most efficient for the performance of the targeted trait. In this regard, recurrent selection can be considered to be the most efficient selection procedure. However, in self-pollinated crops like rice, recurrent selection in true sense is difficult to practise due to large numbers of hand emasculation and pollination. Under such a situation, biparental mating in early segregating generations might be practised to ensure higher utilization of both additive and non-additive

gene actions. The high SCA effects of the cross combinations involving low x low combiners could be due to dominance and dominance x dominance type of gene action. Such specific crosses can be exploited for heterosis breeding.

6.4.3 Gene action

The occurrence of crosses with high negative specific combining ability effects involving bad combiners x bad combiners indicates that although the parents in such crosses lacked additive gene effects compared to high general combining ability parents, heterozygotes were highly responsive to the environment due to non-additive effects such as dominance and epistasis (Kamaluddin *et al.*, 2007). Intermating between crosses followed by selection may be a useful strategy for obtaining desirable segregants in crosses from good combiner x bad combiner and bad combiner x bad combiner. The negative SCA effects for LS, AUDPC and PE observed indicates that dominance gene action was the main aspect in the non-additive effects in the inheritance of resistance to ShR and hence, it was suggested that AUDPC, PE and LS were quantitatively inherited. The results of this study corroborate with those of a number of other reports on combining ability in rice available in literature. Apart from a few exceptions, most studies showed significant GCA and SCA effects for most of fungal rice diseases indicating, therefore, that both additive and non-additive gene action were important in the inheritance of disease resistance related traits (Pereira *et al.*, 2012).

However, according to Melchinger *et al.* (1987), the ratio of SCA over GCA is important for predicting hybrid performance from GCA effects. According to Brown *et al.* (2014), when SCA is relatively small in comparison with GCA, it should be possible to predict the performance of a particular cross combination based only on the values obtained for GCA of parents. For inbred lines, the closer the SCA/GCA ratio is equal to one, the greater predictability based on GCA will be possible. Consequently, because of the fact that GCA estimates were much greater than SCA estimates in this study, good combiner parents in this study will be useful for prediction of introgression of sheath rot resistant genes into further progenies in crop improvement programmes. For good combiners, as far as sources of resistance are concerned, potential male parental lines with significant negative GCA included Cyicaro, Nyiragikara and Yunkeng. On the other side, best combiners programme involving susceptible parents, included

Fashingabo, Gakire, Intsinzi, and Ndamirabahinzi as they all had significant and high negative GCA effects.

The importance of estimating GCA and SCA effects is not only about identification of parental lines to be involved in selection programmes, but also understanding gene effects governing the expression of specific traits. From the genetic point of view GCA effects measure additive gene action while SCA effects indicate non-additive effects (Bradshaw, 2016). In the present study, the mean squares associated with GCA were highly significant for all the traits, whereas SCA was not significant only for LS. This is an indication of the presence of both additive and non-additive gene effect in the mechanisms of expression of these traits associated with resistance to sheath rot of rice. Non-significant SCA for lesion size suggests that non- additive effect of genes were less important, and consequently, it would be useful to consider genotypes with inherently smaller lesions in further crossing programmes aimed at developing progenies with resistance to ShR. However, reports by Reif *et al.* (2007) suggest that in the absence of epistasis, GCA seems predominant over SCA and the relevance of dominance effects tends to decrease. Similarly, a relatively large SCA/GCA ratio implies the presence of dominance and epistatic gene effects. In this regards, the ratios of GCA/SCA in this study were all greater that one, suggesting additive effects were most predominant than non-additive ones. The involvement of mostly additive gene effects in the mechanism of resistance to sheath rot was not unique for this study only, as, this was also reported by Chauhan and Bhatt (1986.) and Srinivasachary et al. (2002).

Although, very little information on mode of inheritance of resistance to sheath rot of rice is available in literature, predominance of additive effects is common to most of the rice diseases. This was reported for blast (Roumen, 1994; Mulbah et al., 2015), rice yellow mottle virus (Munganyinka *et al.*, 2015), bacterial blight (Jeung *et al.*, 2006) and rice sheath brown rot (Sthapit, 1995). With the predominance of additive effects, recurrent selection should be useful in improving sheath rot resistance related traits as according to (Hallauer, 2007), once additive gene effects are important, breeding methods that emphasize on GCA should be used for improving targeted traits. Also breeding methods based on phenotypic selection would be effective. Since additive genes are largely fixable, unlike non-additive genes (Dabholkar, 2006), the best combiners identified in this study are potential candidates for use in breeding

programmes aimed at improvement of levels of resistance to rice sheath rot, as also suggested by Mulbah *et al*. (2015). Therefore selecting the best progenies as parents for the next generation would likely lead to future gains.

On the other hand, predominance of additive gene effects was also revealed by narrow sense heritability levels obtained in this study. Since heritability is a measure of the heritable portion of variability, higher heritability values of quantitative traits are useful as they provide the basis of selection for phenotypic performance (Girish *et al.*, 2006). A high narrow sense heritability is an indication that the expression of targeted trait is mainly due to the additive gene effects (Brown *et al.*, 2014). Heritability estimates ranging from 63.7 to 89.2% for both broad and narrow sense were observed for LS, AUDPC and PE. These high levels of heritability might also reflect the environmental conditions in which the trial was established. The trial site is a continuously irrigated scheme with less variability of environmental conditions, especially rainfall and temperatures.

However, even if little information is available in literature concerning inheritance of resistance of sheath rot, various reports identified a strong connection between sheath of rice and panicle exsertion (Vinod *et al.*, 1990; Lalan Sharma *et al.*, 2013; Hittalmani *et al.*, 2016). In the absence of information on heritability for sheath rot based traits, one can use information on panicle exsertion. High heritability estimates for panicle exertion corroborates results of a number of authors, including Sellammal *et al.* (2014) and are in contrast with those of Girish et al. (2006), Cruz *et al.* (2008) and Neelima *et al.* (2015) who reported moderate estimates of heritability.

High estimates of heritability observed for all three traits, indicated these traits could be selected in controlled environments in a recurrent selection programme. Based on heritability estimates of sheath rot resistance components, resistance to this disease can be achieved through mass selection or any other methods based on progeny testing. This is in accordance with (Lopes and Boiteux, 2012), who suggested that the selection of resistance traits that display high heritability and simple genetic control, mainly of the additive type, might be achieved through the individual performance or, rather, the performance *per se* of inbred lines or populations. Notably, if there is a significant maternal effect, then there will be a difference in the selection of the female parent for a particular crossing**.** For this study, nevertheless, maternal effects were not significant.

Concepts of the proportion of dominant and recessive genes occurring in a group of parents, as well as the degree and direction of dominance have been clearly elaborated by Viana *et al.* (2001). The level of dominance estimated in this study were all between 0-1 except for PE when estimated based on male parents. This indicates partial dominance of genes involved in resistance to sheath rot according to Chahal and Gosal (2002). The evidence of predominance of additive genetic effect on inheritance of resistance to ShR paves the way for a possibility of improving the resistance by introgression of resistance genes through recurrent selection or series of backcrossing.

6.5 Conclusion

Because sheath rot of rice has been, for long, regarded as a minor disease, little is known about the mode of action associated with mechanism of inheritance of resistance for this disease. It was therefore, important to conduct genetic studies in a bid to identify possible breeding strategies aimed at developing resistance to one of the most important emerging rice diseases, not only in Rwanda but also in regions where the disease is endemic.

The North Carolina Design II was chosen as the mating design for this study, and the analysis revealed significant general and specific combining ability estimates for lesion size, area under disease progress curve and panicle exertion. Based on these results, it was evident that both additive and non-additive gene effects played a role in the mechanism governing inheritance of resistance to sheath rot of rice. However, high heritability estimates indicated that additive gene effects were predominant over non-additive gene effect with the analysis of level of dominance revealed indicating partial dominance of the genes involved. The existence of additive gene effects coupled with non-additive gene effects indicated that one of the ways for crop improvement should be the introgression of resistance genes into new varieties through recurrent selection strategies focusing mainly on best GCA of parental materials. The varieties Cyicaro, Yunertian and Yunkeng, that were found as best combiners should be considered potential sources of resistant genes in breeding programmes aimed at improving the level of resistance to rice sheath rot, followed by selecting the best progenies as parents for the next generation to obtain substantial future breeding gains. These results represent an important development towards breeding for resistance to sheath rot of rice as they will help breeders to devise strategies for resistance to this disease in their rice breeding programmes.

References

Acquaah, G., 2012. Principles of Plant Genetics and Breeding. Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK.

Amin, K.S., 1976. Sources of resistance to *Acrocylindrium* sheath-rot of rice. *Plant Disease Reporter* 60: 72-73.

Bokmeyer, J.M., Bonos, S.A., and Meyer, W.A., 2009. Inheritance characteristics of brown patch resistance in tall fescue. *Crop Science* 49: 2302-2308.

Bradshaw, J.E., 2016. Plant Breeding: Past, Present and Future. Springer International Publishing.

Brown, J., P. Caligari, and Campos, H., 2014. Plant breeding, 2nd ed. Wiley-Blackwell.

Chahal, G.S., and Gosal, S.S., 2002. Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science International, Pangbourne, UK.

Chakraborty, R., Chakraborty, S., Dutta, B., and Paul, S., 2009. Combining ability analysis for yield and yield components in bold grained rice (*Oryza sativa L.*) of Assam. *Acta Agronómica* 58: 9-13.

Chauhan, V.S., and Bhatt, J.C., 1986. Inheritance of resistance to sheath rot in rice. *SABRAO* 18 (2).

Cruz, R.P.d., Milach, S.C.K., and Federizzi, L.C., 2008. Inheritance of pinacle exsertion in rice. *Scientia Agricola* 65: 502-507.

Dabholkar, A.R., 2006. General plant breeding. Concept Publishing Company.

Estrada, B.A., Sanchez, L.M., and Crill, P., 1979. Evaluation of screening methods for sheath rot [caused by *Acrocylindrium oryzae]* resistance of rice. *Plant Disease Reporter* 63: 908-911.

Falconer, D.S., Mackay, T.F., and Frankham, R., 1996. Introduction to quantitative genetics. *Trends in Genetics* 12: 280.

Girish, T.N., Gireesha, T.M., Vaishali, M.G., Hanamareddy, B.G., and Hittalmani, S., 2006. Response of a new IR50/Moroberekan recombinant inbred population of rice (*Oryza*

sativa L.) from an indica × japonica cross for growth and yield traits under aerobic conditions. *Euphytica* 152: 149-161.

Hallauer, A.R., 2007. History, contribution, and future of quantitative genetics in plant breeding: lessons from maize. *Crop Science.* 47: 4-19.

Hittalmani, S., Mahesh, H.B., Mahadevaiah, C., and Prasannakumar, M.K., 2016. De novo genome assembly and annotation of rice sheath rot fungus *Sarocladium* oryzae reveals genes involved in Helvolic acid and Cerulenin biosynthesis pathways. *BMC Genomics* 17: 271-283.

Jeung, J., Heu, S., Shin, M., Vera Cruz, C., and Jena, K., 2006. Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology* 96: 867-875.

Kamaluddin, Singh, R.M., Prasad, L.C., Abdin, M.Z., and Joshi, A.K., 2007. Combining ability analysis for grain filling duration and yield traits in spring wheat (*Triticum aestivum L. em. Thell.*). *Genetics and Molecular Biology* 30: 411-416.

Keane, P., and Brown, J., 1997. Disease management: resistant cultivars. In: Brown, J.F. and Ogle, H.J., (eds), Plant pathogens and plant dseases. Rockvale Publications, Armidale NSW 2350 Australia.

Lalan Sharma, D.T., Nagrale, S.K., Singh, K.K., Sharma, and Sinha, A.P., 2013. A study on fungicides potential and incidence of sheath rot of rice caused by *Sarocladium oryzae* (Sawada). *Journal of Applied and Natural Science* 5 (1): 24-29

Lopes, C.A., and Boiteux, L.S., 2012. Breeding for resistance to bacterial diseases. In: Fritsche-Neto, R. and Borém, A., (eds), Plant Breeding for Botic Stress Resistance. Springer Berlin Heidelberg, Berlin, Heidelberg. p. 37-55.

Madhav, M.S., Laha, G.S., Padmakumari, A.P., Somasekhar, N., Mangrauthia, S.K., and Viraktamath, B.C., 2013. Phenotyping rice for molecular plant breeding. In: Panguluri, S.K. and Kumar, A.A., (eds), Phenotyping for Plant Breeding. Springer New York. p. 1-40.

Melchinger, A.E., Geiger, H.H., Seitz, G., and Schmidt, G.A., 1987. Optimum prediction of three-way crosses from single crosses in forage maize (*Zea mays* L.). *Theoretical and Applied Genetics* 74: 339-345.

Miah, S.A., Shahjahan, A.K.M., Hossain, M.A., and Sharma, N.R., 1985. A survey of rice diseases in Bangladesh. *Tropical Pest Management* 31: 208-213.

Mulbah, Q.S., Shimelis, H.A., and Laing, M.D., 2015. Combining ability and gene action of three components of horizontal resistance against rice blast. *Euphytica* 206: 805-814.

Munganyinka, E., Edema, R., Lamo, L., Gibson, P., and Rukundo, P., 2015. Combining ability for resistance to rice yellow mottle virus disease in interspecific and intraspecific rice Genotypes. *African Journal of Crop Science* 3 (1): 102-107.

Mzengeza, T. 2010. Genetic studies of grain and morphological traits in earlygeneration crosses of Malawi Rice (*Oryza sativa L*.). PhD thesis. University of KwaZulu Natal, Pitermaritzburg South Africa.

Naeimi, S., Okhovvat, S.M., Hedjaroude, G.A., and Khosravi, V., 2003. Sheath rot of rice in Iran. *Communications in Agricultural and Applied Biological Sciences*. 68: 681-694.

Narayanaprasad, Jagadeesh, B.R., Shivakumar, G.B., Prasad, P.S., Sudarshan, G.K., and Sunil Kumar, N., 2011. Studies on the nature and properties of sheath rot (*Sarocladium oryzae Sawada*) causing seed borne pathogen on rice (*Oryza sativa L*.). *Internation Journal of Science and Nature* 2 (2): 317-320.

Neelima, P., Rani, K.J., Raju, C.D., and Keshavulu, K., 2015. Evaluation of rice genotypes for cold tolerance. *Agricultural Science Research Journal* 5(9): 124-133.

Ngala, G.N., and Adeniji, M.O., 1986. Sheath rot disease in tropical Africa. In: Juo, A.S.R. and Lowe, J.A., (eds), The wetlands and rice in subsaharan Africa. IITA, Ibadan, Nigeria.

Payne, R., Welham, S., and Harding, S., 2014. A Guide to REML in Genstat. VSN International, Hertfordshire, UK.

Pearce, D.A., Bridge, P.D., and Hawksw, D.L., 2001. Species concept in *sarocladium*, the causal agent of sheath rot in rice and bamboo blight. Major fungal diseases of rice. Springer Netherlands. p. 285-292.

Pereira, D.S.A., Gomes, C.B., Castro, C.M., and Silva, G.O.d., 2012. Breeding for Fungus Resistance. In: Fritsche-Neto, R. and Borém, A., (eds), Plant Breeding for Biotic Stress Resistance. Springer Berlin Heidelberg, Berlin, Heidelberg. p. 13-35.

Portmann, P., and Ketata, H., 1997. Field plot technique. In: Kempton, R.A., Fox, P.N. and Cerezo, M., (eds), Statistical methods for plant variety evaluation. Springer Netherlands, Dordrecht. p. 9-18.

Raja, I.Y., 2013. Management of Rice Sheath Rot Caused by *Sarocladium oryzae*. LAP LAMBERT Academic Publishing.

Reif, J.C., Gumpert, F.M., Fischer, S., and Melchinger, A.E., 2007. Impact of Interpopulation Divergence on Additive and Dominance Variance in Hybrid Populations. *Genetics* 176: 1931-1934.

Roumen, E.C., 1994. The inheritance of host plant resistance and its effect on the relative infection efficiency of Magnaporthe grisea in rice cultivars. *Theoretical and Applied Genetics* 89: 498-503.

Russell, G.E., 1978. General principles and methods of breeding for resistance. Plant Breeding for Pest and Disease Resistance. Butterworth-Heinemann. p. 15-44.

Sakthivel, N., 2001. Sheath rot disease of rice: Current status and control strategies. In: Sreenivasaprasad S, Johnson R (eds). Major fungal diseases of rice recent advances Kluwer Academic Publishers Boston.

Sellammal, R., Robin, S., and Raveendran, M., 2014. Association and heritability studies for drought resistance under varied moisture stress regimes in backcross inbred population of rice. *Rice Science* 21: 150-161.

Sharma, C., Singh, N., Mall, A., Kumar, K., and Singh, O., 2014. Combining ability for yield and yield attributes in rice (*Oryza sativa* L.) genotypes using CMS system. *SAARC Journal of Agriculture* 11: 23-33.

Shattuck, V.I., Christie, B., and Corso, C., 1993. Principles for Griffing's combining ability analysis. *Genetica* 90: 73-77.

Simko, I., and Piepho, H.P., 2012. The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology* 102: 381-389.

Singh, A., Kumar, A., Ahmad, E., and Jaiswal, J., 2012. Combining ability and gene action studies for seed yield, its components and quality traits in bread wheat (*Triticum aestivum* L. em Thell.). *Electronic Journal of Plant Breeding* 3: 964-972.

Sprague, G.F., and Tatum, L.A., 1942. General vs. specific combining ability in single crosses of corn. *Agronomy Journal* 34: 923-932.

Srinivasachary, H., E. Shailaja Hittalmani, M. Girish Kumar, G. Shashidhar, and Vaishali., 2002. Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exsertion in rice (*Oryza sativa* L.). *Current Science,* 82: 133-135.

Sthapit, B.R., 1995. Inheritance and selection of field resistance to sheath brown rot disease in rice. *Plant Disease* 79: 1140.

Vanderplank, J.E., 1984. Slow rusting of cereal crops. Disease Resistance in Plants. Academic Press. p. 154-161.

Viana, J.M.S., Cruz, C.D., and Cardoso, A.A., 2001. Theory and analysis of partial diallel crosses. Parents and F2 generations. *Acta Scientiarum* 23: 627-634.

Vinod, K.K., P. Vivekanandan, and M. Subramanian, 1990. Effect of cytoplasmic male sterility (CMS) on panicle exsertion and sheath rot (ShR) incidence in F2 rice hybrids. . *International Rice Research Newsletter* 15: 5.

Virmani, S.S., 2012. Heterosis and hybrid rice breeding. Springer Science & Business Media.

7 Chapter 6

Introgression of sheath rot resistant genes into popular rice cultivars and evaluation of introgression lines in multi-environment trials

Abstract

Crop improvement for disease resistance of elite varieties in disease endemic regions is among the important objectives of rice breeding programme especially through backcross breeding. In this study, BC2F1 lines were generated from four popular cultivars and two donors of sheath rot resistant genes. Phenotypic selection of BC2F1 genotypes in three multi-environmental trials using six agronomic traits led to recovery of between 78 - 85% of the recurrent parents' genome with increased resistance and grain yield (121 - 125%). From AMMI analysis, both main effect from genotype and environment had a significant effect on the performance of different introgression lines for six traits; disease severity, grain yield, plant height, tillering ability, grain length and number of grains per panicle. The genotype by environment interaction also had significant effect on disease severity, grain yield, tillering ability and number of grains per panicle. The introgression line G6 (BC2F1 involving Intsinzi x Yunertian) had a wide adaptation for the traits across the three test environments. Other genotypes showing relatively good general adaptation included the introgression line G2 (BC2F1 involving Rumbuka x Yunertian) for disease severity and tillering ability, G8 (BC2F1 involving Gakire x Yunyine) for disease severity, and G4 (BC2F1 involving Buryohe x Yunertian) for grain yield and number of grains per panicle, as they had IPCA1 values less than 0.5. Stability analysis using AMMI stability values, static and dynamic stability showed genotypes G5 (BC2F1 involving Intsinzi x Yunyine) and G6 (BC2F1 involving Intsinzi x Yunertian) as being stable for disease severity across the environments whereas G6 (BC2F1 involving Intsinzi x Yunertian), G5 (BC2F1 involving Intsinzi x Yunyine) and G4 (BC2F1 involving Buryohe x Yunertian) showed good stability for yield and its related traits. However, even though these genotypes combined wide adaption and stability across the test environments, their yielding potential was low. suggesting the need of few other backcrossing generations The results of this study indicate an important innovation in breeding for resistance to sheath rot resistance and, according to them progress can be made in introgressing resistant genes into susceptible cultivars through a few backcrossing generations with maximum recovery of the recurrent parents' genome.

7.1 Introduction

In recent years, Rice has been gradually increasing in importance as a cereal crop for both food security and source of income especially for small scale farmers in Rwanda. Consequently, recent agricultural surveys in Rwanda have ranked rice third after maize and sorghum for area under cultivation and second after maize for yield and gross production (NISR, 2015). Despite a steep increase in local rice production and consumption, the country still imports more than 50% of its needs (Ruganzu *et al.*, 2015). This is because rice production in Rwanda is dominated by short grain varieties commonly known as Kigoli, whereas, the consumer market has shown a preference of long grain and aromatic rice mostly imported from Tanzania, Pakistan and Thailand (Kathiresan, 2013). Therefore, various strategies have been put in place to introduce long grain and aromatic rice in Rwanda's rice farming systems and a few varieties with adaptability to various agro ecological conditions of Rwanda have been released. High yielding, long grain and aromatic varieties such Basmati are only grown in Bugarama marshlands whereas for some other few varieties, the yield potential has been greatly reduced by various biotic and abiotic stresses. These include dwarf varieties that are very susceptible to cold and mostly sheath rot of rice or *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw], an emerging threat for rice cultivation globally (Bigirimana, *et al.,* 2015; Hittalmani *et al.*, 2016). The disease has been regarded as minor for a long time, hence has been least studied.

Even though chemicals have been proven effective in other parts of the World (Thapak *et al.*, 2003), varietal resistance is the most sustainable disease control method in terms of ease for farmers to use and less harm to the environment There is, therefore, a need to develop resistant genotypes that meet farmer and consumer preferred traits and are stable and adaptable to the various agro-environments of Rwanda. To this end, varietal improvement programmes should include locally adapted cultivars as parental materials for both sources of resistance and sources of preferred characteristics. One of the best ways to achieve this is through introgression of resistant genes into susceptible cultivars by means of backcrosses and using foreground and background selection for targeted traits and recovery of recurrent parent genome.

A crop improvement programme has two major components; the breeding phase and the evaluation for performance phase. This performance is a function of genotype, environment, and their interaction (GEI). Genotype x environment interaction is the differential response of a genotype to changing environmental conditions (Gangashetty *et al.*, 2016) and occurs when different cultivars or genotypes respond differently to diverse environments (Yan and Kang, 2003). Understanding the structure and nature of GEI is important in plant breeding programmes because a significant GEI can seriously impair efforts in selecting superior genotypes from new crop introductions and cultivar development programmes (Shafii and Price, 1998). Information on the structure and nature of GEI is particularly useful to breeders because it can help determine if they need to develop cultivars for all environments of interest (broad adaptation) or if they should develop specific cultivars for specific target environments (Bridges, 1989).

The objectives of this study were to (i) develop a rice breeding programme targeting improving levels of resistance to sheath rot of rice simultaneously with farmer and consumer preferred traits; and (ii) to evaluate the adaptation and stability of generated introgression lines across different rice growing environments.

7.2 Materials and methods

7.2.1 Plant materials and development of BC2F1 introgression lines

Cultivars Yunyine and Yunertian which were identified as best combiners for sheath rot disease resistance in combining ability studies in chapter 5 were selected as donor parents for resistant genes for improvement of sheath rot (ShR) resistance in some of the popular rice cultivars; Rumbuka, Buryohe, Gakire and Intsinzi. These popular cultivars were chosen based on their farmer and consumer market preferred characteristics especially grain length, aroma, colour, palatability and threshability. The list of parental lines is given in Table 7.1.

Table 7.1: List of parental lines to be improved (recurrent parents) and characteristics of their selected agronomic traits

Name	Disease resistance	LS (%)	PH (cm)	TA	GP	GY (gr)	GL (cm)
Rumbuka	Susceptible	72.6	81.7	12	127	353.2	11.9
Buryohe	Susceptible	81.3	96.9	9	136	361.1	10.2
Gakire	Susceptible	68.8	85.2	14	149	323.2	10.3
Intsinzi	Susceptible	75.1	83.7	12	139	324.7	10.0

LS: lesion size; GY: grain yield per plot; PH: plant height; TA: tillering ability; GL: grain length; GP: number of grains per panicle.

Each one of the four recurrent parents was crossed to each one of sheath rot resistant parent to produce F1 progenies as illustrated in Table 7.2 The F1 progenies of the recurrent parents in Table 6.1 that exhibited negative SCA for ShR's lesion size and positive SCA for panicle exsertion were involved in crossing programme to introgress ShR resistant genes into these cultivars. The F1 progenies were evaluated in the field for heterozygosity confirmation. True crosses from four selected parental materials were selected and crossed back to each of the recurrent parents to generate BC1F1.

The BC1F1s were then evaluated in the field and segregant progenies with levels of resistance to ShR in foreground and recovery of recurrent parent's phenotype in background were selected. Selected individuals were advanced to BC2F1 by crossing them back to their recurrent parents. The whole process from F1 to BC2F1 was conducted in three rice cropping seasons between July 2014 and December 2015. From eight crosses, a total of 1101 seeds were generated corresponding to eight introgression lines as illustrated in Table 7.2. The scheme of introgression of resistant genes into susceptible varieties is illustrated in Figure 7.1.

Table 7.2: List of planting materials and number of introgression lines generated for each cross

Code	Code for introgression line (IL)	Susceptible and recurrent Parent	Resistant parent	Number of F1 seeds produced	BC1F1 seeds produced	BC2F1 seeds produced
G1	IL 1	Rumbuka	Yunyine	30	36	126
G2	IL 2	Rumbuka	Yunertian	32	42	122
G3	IL 3	Buryohe	Yunyine	20	45	120
G4	IL 4	Buryohe	Yunertian	35	36	127
G5	IL 5	Intsinzi	Yunyine	40	58	140
G6	IL 6	Intsinzi	Yunertian	23	66	129
G7	IL 7	Gakire	Yunyine	38	71	204
G8	IL 8	Gakire	Yunertian	42	57	133

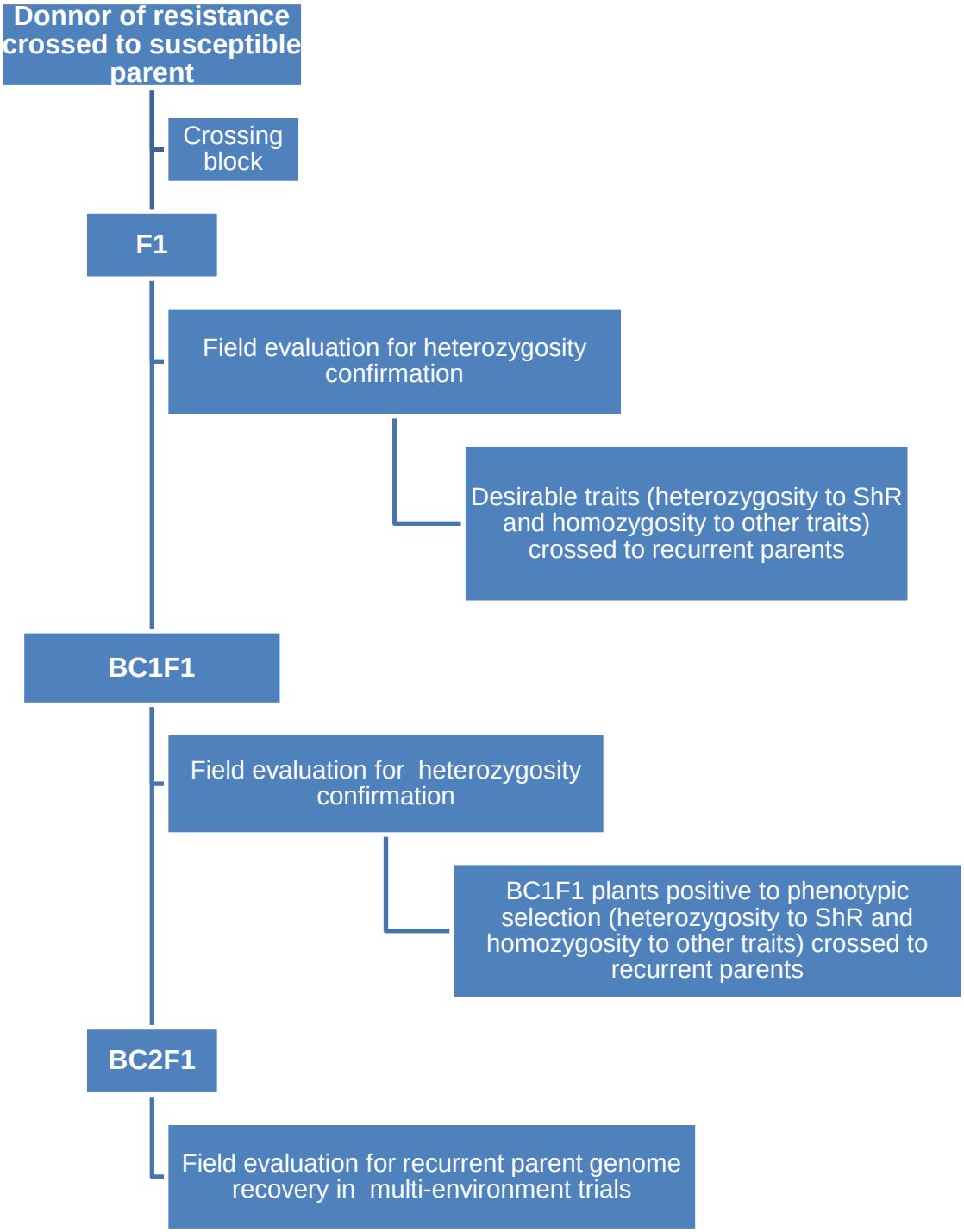

Figure 7.1 Graphical illustration of the scheme of introgression of sheath rot resistant genes into commercial varieties

7.2.2 Field evaluation of introgression lines for resistance to sheath rot and other important agronomic traits

Eight advanced BC2F1 progenies or introgression lines were evaluated in three different environmental conditions. Experimental sites were located in South Eastern, North Eastern and South Western zones of the country. Characteristics of each site are summarized in the Table 7.3. The sites were selected based on their diverse agro-ecological conditions and they have been previously identified as sheath rot hot spots.

Table 7.3 Location and climatic parameters of test environments

District	Rice Marshland	Elevation (masl)	latitude	Longitude	Daily mean temperature (min-max) ^{O}C*	Mean Monthly rainfalls (mm)*
Nyagatare	Kirimburi	1343.5	01^{o}17'58.29"	30 ^{o}18'47.40"	15.1-28.6	270.1
Bugesera	Rurambi	1340.51	02 ^{o}02'23.53"	30 ^{o}10'58.92"	15.9-25.8	106.6
Rusizi	Bugarama	957.072	02 ^{o}42'01.77"	29 ^{o}01'06.19"	15.5-31.3	96.29

* Data provided by Rwanda meteorological agency based on nearby stations

All the eight genotypes (introgression lines) were evaluated in a randomized complete block design (RCBD) with two replications in each experimental site between January and June 2016.

Since the rice crossing resulted in a few seeds, planting materials were increased through tiller transplanting method. The experimental plot consisted of 1 m wide and 1 m long with 20 cm left between and within rows, hence 5 rows in a plot. This is in accordance with Portmann and Ketata (1997) who suggested it may be desirable to keep the number of replicates and plot size small when seed supplies are limited, so that each genotype is grown at as many locations as possible. A 50 cm border was left between two successive plots to avoid inter-plot interferences (Portmann and Ketata, 1997). Fields were left to natural inoculum for disease evaluations as the sites had been tested for several times and considered excellent disease hot spots. Other

cultural practices such as weeding, fertilizer, irrigation, pest management, bird scaring and post-harvest, were applied as recommended in rice farming systems of Rwanda.

7.2.3 Data collection and analysis

Data collection consisted of reaction to sheath rot of rice, one hand, and growth and yield characteristics on the other hand. Disease reaction was monitored on a fortnightly basis after the initial appearance of symptoms by measuring the lesion size which reflected disease severity. This was then expressed as a percentage (%) of lesion length over the total length of the flag leaf sheaths. Growth and yield parameters assessed included tillering ability (number of tillers), plant height (cm), grain yield (g/plot), grain length (mm), and numbers of grains per panicle. Different measurements were taken on ten selected and tagged hills in the middle of the plot to avoid interplot interference.

Least square means for disease severity, growth and yield parameter disease scores were analyzed using additive main effects and multiplicative interactions (AMMI) procedure in Genstat 17th edition (Payne *et al.*, 2014).

The combined AMMI analysis was performed across locations using the model suggested by Kang and Gauch (1996) as follows:

$$\mathrm{Y}ij = \mu + \mathrm{g}i + \mathrm{e}j + \sum_{n=1}^{N} \lambda_n \alpha_{in} \gamma_{jn} + \rho_{ge} + \varepsilon_{ij}$$

Where: $\mathrm{Y}ij$: yield of genotypes; μ: grand mean; gi: genotypic main effect; ej: environmental main effect; N: number of PCA axes considered; λ_n: singular value of the nth PCA axis; α_{in}: scores for the ith genotype on the nth axis; and γ_{jn}: scores for the jth; ρ_{ge}: residual for IPCAs not fitted; ε_{ij}: error term.

The AMMI model was also used to rank genotypes across environments and to rate differential genotypic adaptation to various environmental conditions. The stability of different introgression lines across the three test environment was also assessed using statistic and dynamic stability analysis as well as AMMI stability values (ASV).

As per description by Becker and Leon (1988), the static and dynamic yield stability concepts reflect the differential response of genotypes to variable environments. The static stability concept indicate that the performance of a given trait of a given genotypes remains constant in different environments, whereas the dynamic stability indicate that the response of genotype in a given environment is parallel to the average response of all genotypes in the trial (Becker and Leon, 1988).

In this regard, superiority index (SUP) measures the distance in grain yield of a given genotype to the genotype with the maximum performance in each environment (Lin and Binns, 1988). A small SUP value indicates a better fit of a genotype to the dynamic stability concept. According to Lin *et al.* (1986) and Becker and Leon (1988), static stability coefficient (SSC) is an estimation of the consistency of genotype performance for grain yield across the test environments. A low value (closer to zero) of this coefficient indicates a better fit of a genotype to the static stability concept. Both SUP and SSC were estimated using GenStat 17th edition (Payne *et al*., 2014).

On the other hand, The AMMI stability value (ASV) proposed by Purchase *et al*. (2000) was used to quantify and rank genotypes according to the yield stability. This value is calculated from the IPCA1 and IPCA2 scores of each genotype in the AMMI model and the two main principal component axes (PC1 and PC2) (Zali *et al.*, 2012).

$$\mathbf{ASV} = \sqrt{\left[\frac{\text{SSIPCA1}}{\text{SSIPCA2}}(\text{IPCA1 score})\right]^2 + (\text{IPCA2 score})^2}$$

This parameter also follows the static stability concept and ranks genotypes with low values as more stable (Purchase *et al*., 2000).

7.3 Results

7.3.1 Effects of genotype and environment on disease severity, growth and yield traits of eight introgression lines

The combined analysis of variance was performed on six agronomically important traits that are closely correlated to sheath rot of rice and across three different environments. Results from

AMMI analysis Table 7.4 indicated significant differences (P<0.05) among main effects (genotypes and environments) for all the traits except plant height whose environmental effect was not significant. The effect of interaction between genotype and environment was also significant for four traits out of six, with the non-significant effect observed for plant height and grain length. The interaction effect on grain yield, tillering ability and number of grains per panicle was only significant at 5% significant level, whereas, lesion size was significant at 1% significant level. Blocks displayed significant differences for only two traits namely plant height and number of grains per panicle.

The interaction principal component axis one (IPCA1) was significant (P<0.01) for disease severity, grain yield, tillering ability and number of grains per panicle, while, effects were not significant for plant height and grain length.

Table 7.4: Combined AMMI analysis for selected growth and yield traits of 8 introgression lines across environments

Source of variation	d.f.	LS (%)	GY/Plot	PH	TA	GL	GP
Treatments	23	124.02**	20746**	500.3**	78.87**	14.3	3027**
Genotypes (G)	7	284.04**	47458**	1450.6**	202.99**	24.11*	5171**
Environments (E)	2	220.09**	35788**	219	114.76**	51.58**	12823
Block (B)	3	15.08	477	270.2*	6.95	2.71	9950**
Interactions (GxE)	14	30.28**	5242*	65.4	11.68*	4.07	556*
IPCA 1	8	42.42**	7994**	105.8	18.91**	3.97	832**
IPCA 2	6	14.09	1572	11.5	2.05	4.21	189
Error (e)	453	9.3	2928	93	6.64	10.84	299
% Variation explained by G		37.663	37.673	51.269	44.814	19.023	15.511
% Variation explained by E		29.184	28.409	7.74	25.336	40.698	38.465
% Variation explained by GxE		4.015	4.161	2.311	2.579	3.211	1.668
% Variation explained by e		1.233	2.324	3.287	1.466	8.553	0.897

* and ** indicate significant differences at 0.05 and 0.01 respectively. df: degree of freedom; LS: lesion size; GY: grain yield per plot; PH: plant height; TA: tillering ability; GL: grain length; GP: number of grains per panicle; IPCA: Interaction Principal Component axe.

The interaction principal component axis two (IPCA2) did not show any significant effects for all the traits, therefore only AMMI 1 model was applied for traits with significant IPCA1. Variations due to error were the least among all sources of variation, reflecting minimum effects of extrinsic factors involved in the trait expression. Variations due to genotype accounted for higher values for four traits including lesion size, grain yield, plant height and tillering ability, while variations due to diverse environmental conditions were higher for only grain length and number of grains per panicle. Variations due to GxE interaction were less than those due to main effects for all the studied traits. Main effects as well as their interactions accounted for more than 70% of variations of lesion size, grain yield and tillering ability. Variations of plant height, grain height, and number of grains per panicle were 61.32%, 62.93%, and 55.64%, respectively. Variations due to residual effects were less than the main effects (genotype and interaction) for all the traits. However, as far as GxE interaction is concerned, variation due to experimental error was higher for plant height and grain length.

7.3.2 Mean performance and ranking of introgression lines for ShR resistance, growth and yield traits across the environments

The IPCA1 was significant for four traits, that is, lesion size, grain yield, tillering ability and number of grains per panicle, whereas IPCA2 was not significant for all the traits. Consequently, classification of genotypes and environments will only consider AMMI 1 model. The mean values of agronomic traits and ShR resistance of eight introgression lines across the three test environments are illustrated on Table 7.5. Lesion size ranged between 6.8 and 17.1% with the best genotype in terms of resistance to ShR being G7 grown in Nyagatare. G4 grown in Bugarama recorded the highest level of ShR susceptibility. The highest grain yield was recorded on G3 at Rurambi, and the least yielding genotype was G6 at Nyagatare. The best tillering genotype was G2 at Rurambi and least number of tillers was observed for G3 at Nyagatare. The highest number of grains per panicle was obtained for G5 at Bugarama and the lowest values were observed for G2 at Rurambi.

The ranking of the eight introgression lines based on ShR resistance, growth and yield trait is presented on Table 7.6. Based on selected traits, genotypes performed differently across environments for specific traits as a result of significant effect of genotype by environment

interaction. For instance genotype G4 dominated other genotypes for susceptibility; G3 dominated for high yield, whereas G5 dominated for tillering ability and number of grains per panicle. G7 performed well for disease resistance as it ranked among the last two genotypes for disease severity. However, G7 did not perform well for grain yield and number of grains per panicle. Genotype G6 was the last in rank in terms of grain yield and G3 performed poorly for tillering ability.

All the genotypes exhibited improved resistance to sheath rot from highly susceptible status to resistant. On the other hand, all the genotypes had increased grain yield compared to their parental materials or recurrent parent by between 121 and 125%. Other traits which showed increased performance included plant height (112 - 142%) and number of grains per panicle (111-125%). Tillering ability and grain length of the eight introgression lines was reduced by 86-91% and 78-90%, respectively compared to their respective recurrent parent.

Table 7.5: Mean performance of eight introgression lines based on selected traits across environments

Genotype	LS (%)			GY (gr)			TA			GP		
	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi
G1	9.84	9.69	9.71	442.9	355.4	413.9	7.3	10.9	11.75	161.65	169.05	153.45
G2	13.89	10.57	12.31	454.5	435.9	434.8	11.2	12.35	13.05	156.75	166.95	139
G3	9.02	12.19	11.68	465.9	411.3	467.9	7.5	7.8	8.05	173.9	170.65	158.2
G4	16.05	12.92	17.01	422.7	410.4	423.2	7.65	8.95	9	160.8	168.3	148.2
G5	12.32	10.25	13.2	425.4	409.6	400.2	12.85	12.85	13	187.6	185.1	172.7
G6	13.08	11.27	14.46	367.8	347.3	353.4	11.45	12.75	12.9	167.05	173.45	153.95
G7	9.4	6.8	10.1	410	396.8	403.8	10.4	11.55	10.65	152	152	152.2
G8	14.62	12.06	15.68	417.7	401.1	397.9	9.5	11.4	12	168.7	180.95	150.15
LSD		1.895			33.63			1.602			10.742	
SED		0.964			17.11			0.815			5.466	
CV (%)		25.4			13.2			24.1			10.6	

LSD: least significant differences; s.e.d: standard error of differences, C.V.: coefficient of variations

LS= lesion size; GY= grain yield; TA=tillering ability; GP= number of grains per panicle

Table 7.6: Ranking of introgression lines based on selected traits across environments*

	LS			GY			TA			GP		
Rank	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi	Bugarama	Nyagatare	Rurambi
1	**G4**	**G4**	**G4**	**G3**	**G2**	**G3**	**G5**	**G5**	**G2**	**G5**	**G5**	**G5**
2	**G3**	**G8**	**G8**	**G2**	**G3**	**G2**	**G6**	**G6**	**G5**	**G8**	**G3**	**G3**
3	**G8**	**G2**	**G6**	**G1**	**G4**	**G4**	**G2**	**G2**	**G6**	**G6**	**G8**	**G6**
4	**G6**	**G6**	**G5**	**G5**	**G5**	**G1**	**G7**	**G7**	**G8**	**G3**	**G6**	**G1**
5	G2	G5	G2	G4	G8	G7	G8	G8	G1	G1	G1	G7
6	G5	G1	G3	G8	G7	G5	G4	G1	G7	G4	G4	G8
7	G1	G7	G7	G7	G1	G8	G3	G4	G4	G2	G2	G4
8	G7	G3	G1	G6	G6	G6	G1	G3	G3	G7	G7	G2

***** Genotypes highlighted in bold are the first four AMMI selection in each test environment

LS= lesion size; GY= grain yield; TA=tillering ability; GP= number of grains per panicle

7.3.3 Analysis of adaptation potential of introgression lines across test environments

The analysis of variance using AMMI model resulted in significant effect of genotype by environment interaction. Partitioning this effect into principal component axes 1 and 2 (IPCA1 and IPCA2) resulted in significant effects only for IPCA1 for four traits and consequently played a major role in explaining variation due to interaction more than IPCA2.

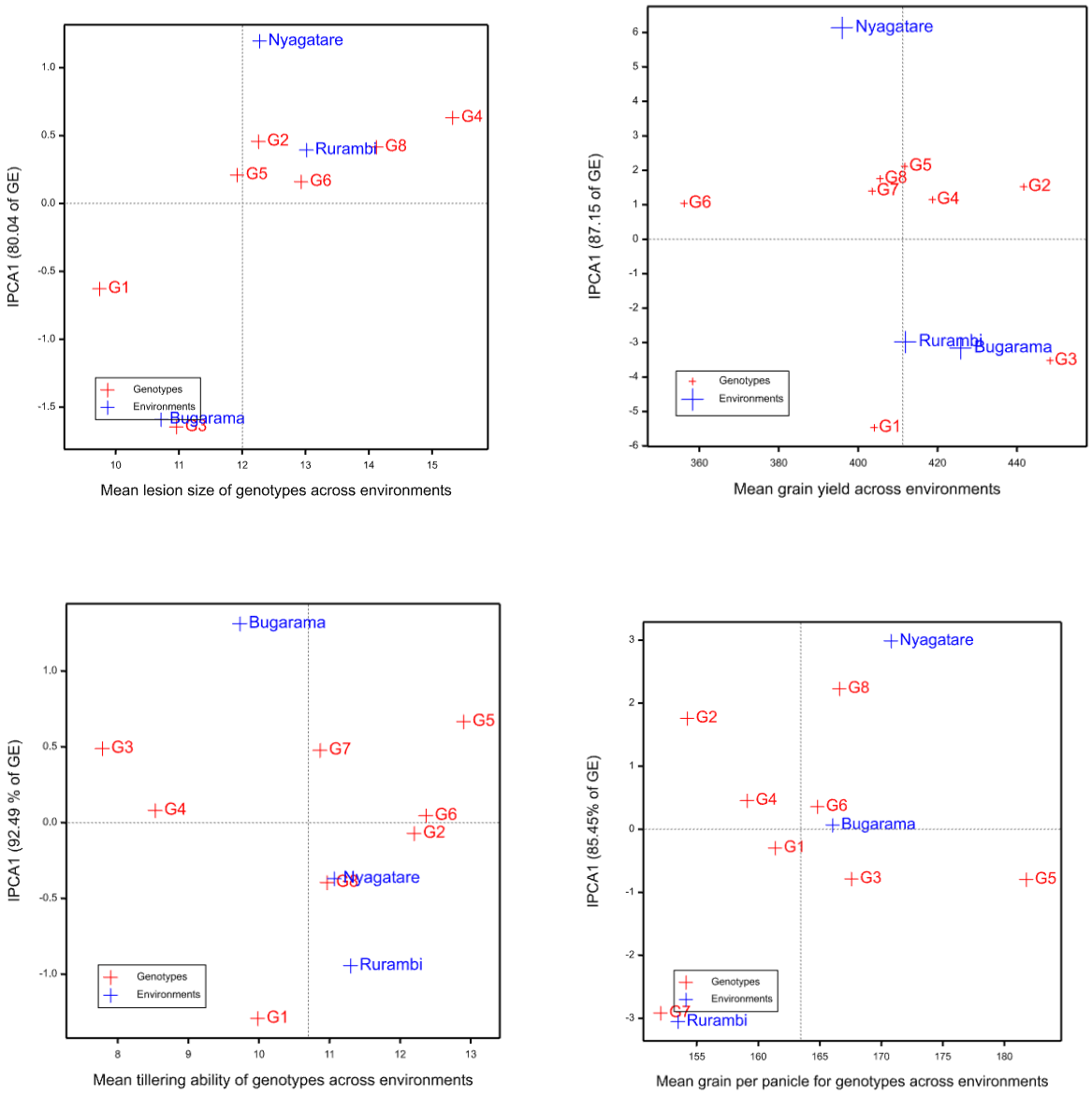

Figure.7.2 AMMI biplots for adaptation analysis of introgression lines across environments

The AMMI1 model was therefore used to classify genotypes across test environments for disease severity, grain yield, tillering ability and number of grains per plot (GP) by plotting the IPCA1 against the means obtained from genotypes and environments (Figure.7.2).

For mean lesion size, a cluster of genotypes was identified with mean lesion size higher than the grand mean and IPCA1 values close to 0 - 0.5. These included the cluster of G2, G6, and G8. Another genotype, G4 had large values of IPCA1. Another cluster comprised of genotypes that recorded negative values of IPCA1 and mean lesion size less than the average. These were G7, G1 and G3. Genotype G5 recorded less mean lesion size than the grand mean, but positive IPCA1 values close to zero. Environmental means were distributed in two totally opposite quadrants, with Nyagatare and Rurambi in the same quadrant.

For grain yield, four genotypes were distributed slightly close to the grand mean axis indicating average grain yield in two quadrants appearing in the positive values of IPCA1 but greater than 0.5. Only two genotypes (G4 and G2) recorded positive values of IPCA1 coupled with mean yield above the average. Two genotypes recorded negative values of IPCA and two test environments fell in the same quadrant. Rurambi recorded the mean yield equal to average yield, whereas, Nyagatare was below the average and Bugarama above. In this regard each genotype, except G6, exhibited specific adaptation for particular environments for grain yield. For tillering ability genotypes were scattered in all the 4 quadrants and test environments distinctively occurring in two opposite quadrants. This was the same with number of grains per panicle. The AMMI biplot for tillering ability showed positive IPCA1 values close to zero and mean number of tillers greater than the general mean for G6 and G7.

7.3.4 Stability analysis of introgression lines across the environments

Results from the stability analysis using static and dynamic indices as well as AMMI stability values are shown in Table 7.7.

Table 7.7 Estimated trait stability parameters of 8 introgression lines evaluated across three test environments

	LS			GY			TA			GP		
Genotype	ASV	SUP	SSC	ASV	SUP	SSC	ASV	SUP	SSC	ASV	SUP	SSC
G1	2.228 (6)	17.041 (7)	0.007 (1)	37.130 (8)	1653 (7)	1989.2 (8)	16.254 (8)	6.049 (6)	5.581 (8)	2.267 (2)	216.9 (5)	60.9 (2)
G2	1.170 (3)	5.368 (4)	2.749 (4)	10.417 (4)	204 (2)	122.5 (5)	1.229 (3)	0.495 (3)	0.873 (6)	10.342 (6)	402.8 (7)	200.1 (7)
G3	6.668 (8)	13.074 (6)	2.907 (5)	23.762 (7)	100 (1)	1030.4 (7)	6.190 (6)	13.188 (8)	0.076 (2)	4.247 (4)	101.1 (2)	68.7 (4)
G4	2.681(7)	0 (1)	4.582 (8)	7.509 (2)	753 (3)	52.4 (2)	0.890 (2)	9.775 (7)	0.586 (4)	2.823 (3)	266.8 (6)	103.2 (6)
G5	1.016 (1)	5.928 (5)	2.292 (2)	14.474 (6)	1151 (4)	162.2 (6)	8.413 (7)	0 (1)	0.007 (1)	4.370 (5)	0 (1)	63.7 (3)
G6	1.094 (2)	3.013 (3)	2.56 (3)	7.066 (1)	5093 (8)	110.9 (3)	0.491 (1)	0.332 (2)	0.636 (5)	2.186 (1)	151.6 (4)	98.8 (5)
G7	1.695 (4)	21.582 (8)	3.03 (6)	9.374 (3)	1459 (6)	43.6 (1)	5.893 (5)	2.242 (4)	0.366 (3)	17.197 (8)	463.9 (8)	0 (1)
G8	1.863 (5)	0.756 (2)	3.453 (7)	11.995 (5)	1406 (5)	112.8 (4)	4.994 (4)	2.405 (5)	1.703 (7)	13.114 (7)	147.2 (3)	240.5 (8)

Numbers in brackets give the position of each genotype, ranked according to the stability coefficient in the same column.
LS= lesion size; GY= grain yield; TA=tillering ability; GP= number of grains per panicle; ASV= AMMI stability value; SUP=Superiority index and; SSC= static stability coefficient.

As shown in Table 7.7, genotypes ranked differently with respect to trait stability across test environments. For lesion size, the AMMI stability value (ASV) ranged from 1.016 to 6.668 and superiority index (SUP) values from 0 to 21.582 whereas the static stability coefficient (SSC) values varied from 0.007 to 4.582. ASV for grain yield ranged from 7.066 and 37.130; SUP between 100 and 5093 and SSC between 43.6 and 1989.2. The stability of tillering ability across environment ranged from 0.491 to 16.254 for ASV; 0 to 13.188 for SUP and 0.007 to 5.581 for SSC. The stability of introgression lines for number of grains per panicle across the environments ranged from 2.186 to 17.197 for ASV; 0 to 463.9 for SUP and 0 to 240 for SSC.

G5 (introgression line between Intsinzi and Yunyine) was more stable than any other genotype across test environments followed by G6 (introgression line between Intsinzi and Yunertian), G2 (introgression line between Rumbuka and Yunertian) and G7 (introgression line between Gakire and Yunyine) in respective order, as far as lesion size is concerned. However, because breeding for disease resistance always targets least lesion size, G3 (introgression line involving Buryohe x Yunyine), followed by G4 (introgression involving Buryohe x Yunertian) and G1 (introgression line involving Rumbuka x Yunyine) should be selected for stability based on ASV. G7 (introgression line involving Gakire x Yunyine) showed good dynamic stability whereas G1 (introgression line involving Gakire x Yunyine) was the best genotype for static stability. Stability analysis of introgression lines across environment for yielding potential revealed G6 (Intsinzi x Yunertian) as the most stable genotype followed by G4 (Buryohe x Yunertian) and G7 (Gakire x Yunyine) based on ASV. G3 (Buryohe x Yunyine) and G2 (Rumubuka x Yunertian) had the best stable yield across environments considering dynamic stability while static stability revealed G7 (Gakire x Yunyine) and G4 (Buryohe x Yunertian). The most stable introgression lines for tillering ability were G6 (Intsinzi x Yunertian) and G4 (Buryohe x Yunertian) based on ASV, G5 (Intsinzi x Yunyine) and G6 (Intsinzi x Yunertian) for SUP and G5 (Intsinzi x Yunyine) and G3 (Buryohe x Yunyine) for SSC. Finally stability of introgression lines for number of grains per panicle revealed G6 (Intsinzi x Yunyine) and G6 (Intsinzi x Yunyine) for ASV, G5 (Intsinzi x Yunyine) and G3 (Buryohe Yuneyine) for SUP and G7 (Gakire x Yunyine) and G1 for SSC.

In general, G6 (Intsinzi x Yunertian) showed more stability for any other genotype based on ASV as it ranked first for grain yield, tillering ability and number of grains per panicle and ranked second for lesion size. Other genotypes revealed various ranking across the environments with respect to the stability analysis method considered.

Averaging the ranks of all traits for ASV, G6 (Intsinzi x Yunertian) was the most stable genotype followed by G4 (Buryohe x Yunertian) and G2 (Rumbuka x Yunertian) whereas G7 (Gakire x Yunyine), G8 (Gakire x Yunertian) and G1 (Rumbuka x Yunyine) were the least stable genotypes in the respective order. Averaging the ranking of genotypes for all the traits indicated G6 (Intsinzi x Yunertian) and G5 (Intsinzi x Yunyine) as the best genotypes in terms of dynamic stability with an overall rank of 2 and 3, respectively, whereas G6 (Intsinzi x Yunertian), G5 (Intsinzi x Yunyine and G7 (Gakire x Yunyine) where the best for static stability with overall rank of 3 for the three traits. Based on the average ranking of the three stability analysis method, G6 was the best genotype in terms of stability across the environments for lesion size, tillering ability and number of grains per panicle whereas it was the second best for grain yield just after G4, while G4 and G5 also ranked well for all the traits.

7.4 Discussion

7.4.1 Effect of genotype, environment and their interaction on the performance of introgression lines

The mean performance of different introgression lines for resistance to sheath rot of rice and other agronomic traits across different environments was evaluated through the AMMI analysis. In this study, the AMMI analysis revealed significant differences due to main effects (genotype and environment) of eight introgression lines for all the traits at 1% and 5% significance levels. The performance of introgression lines for four traits was modified by the environments in which they were exposed to as indicated by the significant effect of GxE interaction. Blocks displayed significant differences for only two traits namely plant height and number of grains per panicle. This indicates possible differences in soil characteristics or uneven distribution of irrigation water with blocks.

As suggested by Hongyu *et al.* (2014) the AMMI model is a very useful tool to evaluate differential response [genotype by environment interaction (GEI)] of genotypes across environments in multi-environment trials. Multi-environment trial data summarizes data into genotype means in rows and environments in columns. In the case where a genotype ranks differently in different environments, the GEI occurs in various forms, and the most significant one being a cross over interaction (Rodrigues *et al.*, 2014). Results of this study reflect obvious existence of considerable variation in the mean performance of all the tested genotypes over the test environments and on the environmental means over tested genotypes.

The fact that the main effects (genotype) accounted for a greater proportion of the total variation compared to other sources of variation for all the traits indicated large genetic diversity among the generated BC2F1 introgression lines. The highly significant influence of the environment indicated high differential performance of introgression lines across the different environments. Test environments presented a wide set of environmental factors, among others, temperature and rainfall variations as well as soil characteristics. Other biotic stresses such as pests and diseases not subjected to this study could also have played a considerable role in environmental variability that occurred in the test environments. Both variations due to genotype and environment as main effects were higher in values than variations due to GxE interaction. This reflects a minimum influence of genotype by environment interaction on the expression of the above mentioned traits, compared to the influence of main effects.

The high proportions of variation due to error compared to variation due to GxE interaction for two traits, plant height and grain length suggested that variability was due to unknown extrinsic factors in GxE interactions. The large proportion of genotypic effects have also been reported by various authors in rice breeding and multi-environmental trials. For instance for grain yield, Bose *et al.* (2014) reported that genotype effect explained 82.37% of the total variation against 37.67% reported in this study. In breeding for blast resistance, Mukherjee *et al.* (2013) observed 84.04% variation due to genotype which was much higher compared to 37.66% for ShR reported in this study. However, the large proportion of environmental effects compared to genotypic effects is common in rice breeding programmes as reported by many researchers (Tariku *et al.*, 2013; Maji *et al.*, 2015; Liang *et al.*, 2015).

Nevertheless, understanding the relationship between crop performance and environment has long been a key issue for plant breeders and geneticists. Crop performance or phenotype is a function of genotype, variety or cultivar, environment and their interaction (GEI). GEI is said to occur when different cultivars or genotypes respond differently to diverse environments (Yan and Kang (2003). It is important only when it is significant and causes significant changes in genotype ranks in different environments, that is, different genotypes are superior in different environments. For GEI to be detected via statistical procedures, there must be at least two distinct genotypes or cultivars evaluated in at least two different environments (Yan and Kang, 2003).

The genotype x environment interaction revealed significant effects on the performance of introgression lines for four traits, namely, disease severity, grain yield, tillering ability and number of grains per panicle. This significance confirmed the necessity of conducting multi-environmental trials in a bid to identify stability and type of adaptation of introgression lines of this study. The AMMI analysis partitioned the effect of GxE interaction into two principal components IPCA1 and IPCA2. IPCA1 was significant for disease severity, grain yield, tillering ability and number of grains per panicle. This explains why classification of genotype performance across the test environments was carried out based on AMMI1 model.

7.4.2 AMMI ranking of introgression lines across the environment

Within environments, AMMI1 frequently ranked genotypes differently except for G4 and G5 which consistently ranked first in all environments for disease severity and number of grains per panicle, respectively. In most of the cases, AMMI1 selected the same winner in two out of the three environments but selected different winners in the remaining environment. Based on reports by Aina *et al.* (2007) and Sibiya (2009), the differences in ranking of genotypes across environment could have been due to the residual or random variation (noise). In unadjusted means, the level of random variation could have been responsible for the elevation of some of the introgression lines to higher positions due to differences observed with this AMMI ranking. Consequently, the existence of site specific adaptation of some genotype for a given trait should be considered and more accurate classification tools should be used in order to increase chances of making accurate selection of genotypes based on precise environmental variability.

The mean performance of introgression lines across test environments revealed reduced disease severity, increased grain yield, number of grains per panicle and plant height of between 121 - 125%, 111 - 125% and 112 - 142%, respectively. On the other hand, grain length and tillering ability exhibited reduced values but recovered the recurrent parent genome by between 78 - 90% and 86 - 91%, respectively. The recurrent parent recovery rate is reasonable as the proportion of recurrent parent recovery in each backcross family increases with the backcross generation. The following are proportions of the genes that are theoretically recovered from the recurrent parent according to number of backcrossing; F1=50%; BC1=75%; BC2 = 87.5%, BC3 = 93.8% and BC4 = 96.9% (Brown *et al.*, 2014). These figures can be explained by the crossing schemes involved for genetically diverse genotypes.

Donors of resistance were characterized by tall plant stature, low tillering ability and short grain whereas susceptible ones were characterised by dwarf plant stature, high tillering ability and long grains. These results are in close corroboration with those obtained by many other researchers who reported faster recovery of the recurrent parent genome with marker assisted selection (92.2% in average) compared to conventional backcrossing breeding (87.5% on average) when foreground and background selection are combined in BC2 progenies (Hospital, 2003; Boopathi, 2013; Hasan et al., 2015). Therefore in this study, for some of the traits the percentage recovery was more than the expected for BC2 using conventional backcrossing. This can be explained by the predominance of additive gene action, high heritability of the traits coupled with selecting for phenotypic attributes that may have fostered deviation of the selection in favour of the donor parent, accelerating recovery of the recurrent genome.

7.4.3 Evaluation of adaptation potential of introgression lines across test environments

When AMMI1 provides a good description of the data, the main effects and IPCA1 scores for genotypes and environments are plotted on the same diagram, facilitating inference about the specific interactions of individual genotypes and environments by using the sign and magnitude of IPCA1 values (Fox *et al.*, 1997). These authors explain that any genotype with IPCA1 values close to zero shows general or wide adaptation to the tested environments. Its response pattern across the environment parallels the mean of all the genotypes in the trial. A large genotypic

IPCA1 score reflects more narrow or specific adaptation to environments with IPCA1 scores of the same sign.

In this study a number of genotypes exhibited IPCA scores close to zero and mean performance greater than the general mean and hence good general or wide adaptation to all the three environments. Genotypes observed in this category included G6 (Intsinzi x Yunertian), G2 (Rumbuka x Yunertian) and G8 (Gakire x Yunertian) for disease severity. Another cluster comprised of genotypes such as G7 (Gakire x Yunyine), G1 (Rumbuka x Yunyine) and G3 (Buryohe x Yunyine) recorded negative values of IPCA1 and mean lesion size less than the average. These genotypes were considered as more resistant to sheath rot but with narrow or specific adaption to environments. Genotype G5 (Intsinzi x Yunertian) recorded less mean lesion size than the grand mean, but positive IPCA1 values close to zero. This is also a genotype with good general adaptation for sheath resistance. For yield and its related traits a number of genotypes showed a good general adaptation to environments. These include G4 (Buryohe x Yunertian) and G6 (Intsinzi X Yunertian) for grain yield, G6 (Intsinzi X Yunertian) and G2 (Rumbuka x Yunertian) for tillering ability and G6 (Intsinzi X Yunertian) for number of grains per panicle. The performance of other genotypes for all the four traits was considerably influenced by test environments as they were distantly scattered all around the quadrants of the biplot. These genotypes were categorized as genotypes exhibiting higher means than general means coupled with large values of IPCA for all the traits. This situation reflects site specific or narrow adaptation of the genotypes to test environments. The results of this study provide good and considerable information for further breeding efforts for sheath rot resistance. This is because in breeding for wide adaptation, the aim is to obtain a variety which performs well in nearly all environments whereas in breeding for specific adaptation, the aim is to obtain a variety which performs well in a definite subset of environments within a target region (Annicchiarico, 2002). Consequently, with further few backcrosses, genotypes will be recommended in specific or a wide range of environments.

7.4.4 Stability analysis of introgression lines across the test environments

Breeding for wide adaptation and for high yield stability have sometimes been considered one and the same, in so far as the latter two terms indicate a consistently good yield response

across environments. However, besides wide and specific adaptation concept of genotypes, yield stability and its related traits are also a matter of concern. In these regards, stability of our introgression lines was assessed through types of analysis i.e AMMI stability value, dynamic stability and static stability.

Being analogous to the biological concept of homeostasis; a stable genotype tends to maintain a constant yield across environments; the static stability is more useful than dynamic stability which rather implies for a stable genotype a yield response in each environment that is always parallel to the mean response of the tested genotypes, i.e. zero GE interaction (Annicchiarico, 2002). The measure of dynamic stability depends on the specific set of tested genotypes, unlike the measure of static stability (Lin *et al*., 1986).

On the other hand, the AMMI stability value (ASV) proposed by Purchase *et al*. (2000) was used to quantify and rank genotypes according to the yield stability. Though there are other statistical methods widely used to measure stability, the ASV statistic is the most suitable for AMMI analysis (Farshadfar, 2008). The present study revealed large stability values whatever estimation method was used. This is an indication of large differences among generated introgression lines. These differences may have been due to large genetic diversity observed (chapter 4) within the parental materials that were used to generate these introgression lines.

Based on the three stability analysis G7 (Gakire x Yunyine), G4 (Buryohe x Yunertian) and G6 (Intsinzi X Yunertian) can be recommended having high yielding potential for a variety of environments. Yield stability was high for G6 (Intsinzi X Yunertian), G4 (Buryohe Yunertian) and G7 (Gakire x Yunyine). The results of ASV and SSC were almost the same with minor differences on the order of ranks. The dynamic stability comes with another quite different ranking with G3 (Buryohe x Yunyine), G2 (Rumbuka x Yunertian) and G4 (Buryohe x Yunertian) as best genotypes. This is obvious as the measure of dynamic stability depends on the specific set of tested genotypes, unlike the measure of static stability (Lin *et al*., 1986) whereas ASV parameter also follows the static stability concept. The results of yield stability more or less corroborate with those of lesion size where ASV ranked the first three genotypes as G5 (Instinzi x Yunyine), G6 (Intsinzi X Yunertian) and G2 while SSC ranked them as G1 (Rumbuka x

Yunyine), G5 (Intsizni x Yunyine) and G6 (Intsinzi x Yunertian). SUP ranked them differently. This is an indication of an already close relationship between sheath rot of rice and yield potential.

However, from a farmer's point of view, yield consistency in space also deserves consideration in the presence of sizeable genotype – location (GxL) interaction, since a selected or recommended genotype should be stable-yielding both across years and across locations in its area of adaptation or recommendation (Piepho, 1998). This is particularly so when there is a prospect of wide adaptation or recommendation, because in the context of a specific adaptation or recommendation the GL effects are minimized by the division of the target region into sub regions.

In these regards, a number of genotypes deserve much attention in further backcrossing generation for maximum recovery of the recurrent parents genomes. These include genotypes G6 (Intsinzi X Yunertian), G4 (Buryohe x Yunertian), and G2 (Rumbuka x Yunertian) which showed not only a good wide adaption potential but also yield stability across environments. However, genotypes with good wide adaptation and good yield stability across environment are not necessarily the high yielding ones. G2 (Rumbuka x Yunertian) and G3 (Buryohe x Yunyine) were the high yielding genotypes across the environments but revealed specific adaptation and low yield stability across environments. This is normal because according to (Annicchiarico, 2002) high yield stability usually refers to a genotype's ability to perform consistently, whether at high or low yield levels, across a wide range of environments. These results are in close corroboration with a number of other rice breeders. Bose et al. 2014 obtained genotypes with high yielding potential, wide adaptation and good yield stability on one hand and low yielding and good stable genotypes on the other hand. Nearly same results were obtained by Krishnamurthy *et al.* (2016).

As farmers always seek high yield, further selection programme should focus on high yielding genotypes disregarding stability across environments and specific adaptation in targeted environments would be exploited.

7.5 Conclusion

This study allowed the generation of BC2F1 introgression lines with resistance to sheath rot of rice selected in foreground and other important agronomic traits in background, with respect the improvement of some of commercial cultivars. The evaluation of generated introgression lines in multi-environmental trials led to identification of cultivars with improved sheath rot resistance, improved grain yields and between 75-85% recovery of parental genome for grain length, plant height and tillering ability few more backcross generations will be required for maximum recovery of recurrent parent's genome.

Multi-environmental trials of generated introgression lines in three test environments showed a significant influence of test environments for some selected traits; disease severity, grain yield, grain length and number of grains per panicle. The AMMI analysis revealed wide adaptation for disease resistance in terms of severity, and number of grains per panicle whereas the adaptation potential for grain yield per plot and tillering ability was characterized as narrow or site specific.

Using three stability analysis methods revealed genotypes with high yield stability [G6 (Intsinzi X Yunertian) and G4 (Buryohe x Yuertian)] showed less yielding potential and these led to suggestions that in further selection programs, focus should be on yield performance in targeted environment will be focussed on.

The results of this study represent a major step in breeding rice for sheath rot resistance as they indicated that more selections for a specific trait in specific environments and few more backcrossing generations would lead to maximum recovery of the recurrent parents' genome.

The newly generated Sheath rot resistant genotypes are expected to be easily adopted by farmers as they were developed from commercial varieties. The results will also contribute to rice production sustainability in Rwanda in regards to the impact of sheath rot of rice on yield and quality of rice.

References

Aina, O., Dixon, A., and Akinrinde, E., 2007. Additive main effects and multiplicative interaction (AMMI) analysis for yield of cassava in Nigeria. *Journal of Biological Sciences* 7: 796-800.

Annicchiarico, P., 2002. Genotype x environment interactions: Challenges and opportunities for plant breeding and cultivar recommendations. Food and Agriculture Organization of the United Nations, Rome, Italy.

Becker, H., and Leon, J., 1988. Stability analysis in plant breeding. *Plant Breeding* 101: 1-23.

Bigirimana, V.d.P., Hua, G.K.H., Nyamangyoku, O.I., and Höfte, M., 2015. Rice Sheath Rot: An Emerging Ubiquitous Destructive Disease Complex. *Frontiers in Plant Science* 6: 1066-1082.

Boopathi, N.M., 2013. Marker-Assisted Selection. Springer India, India.

Bose, L.K., Jambhulkar, N.N., Pande, K., and Singh, O.N., 2014. Use of AMMI and other stability statistics in the simultaneous selection of rice genotypes for yield and stability under direct-seeded conditions. *Chilean Journal of Agricultural Research* 74: 3-9.

Bridges, W.C., Jr., 1989. Analysis of a plant breeding experiment with heterogeneous variances using mixed model equations,. In: S-189, S.R.P., (eds.), Applications of mixed models in agriculture and related disciplines. Louisiana Agricultural Experiment Station, Baton Rouge, LA. p. 145–151.

Brown, J., Caligari, P., and Campos, H., 2014. Plant breeding, 2nd ed. Wiley-Blackwell.

Farshadfar, E., 2008. Incorporation of AMMI stability value and grain yield in a single non-parametric index (GSI) in bread wheat. *Pakistan Journal of Biological Sciences* 11: 1791-1796.

Fox, P.N., Crossa, J., and Romagosa, I., 1997. Multi-environment testing and genotype x environment interaction. In: Kempton, R.A. and P.N. Fox, (eds), Statistical Methods for Plant Variety Evaluation. Chapman &. Hall, London SE1 8HN, UK.

Gangashetty, P.I., Motagi, B.N., Pavan, R., and Roodagi, M.B., 2016. Breeding crop plants for improved human nutrition through biofortification: Progress and prospects. In: Al-Khayri,

M.J., Jain, M.S. and Johnson, V.D., (eds), Advances in Plant breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits. Springer International Publishing, Cham. p. 35-76.

Hasan, M.M., Rafii, M.Y., Ismail, M.R., Mahmood, M., Rahim, H.A., Alam, M.A., Ashkani, S., Malek, M.A., and Latif, M.A., 2015. Marker-assisted backcrossing: a useful method for rice improvement. *Biotechnology, Biotechnological Equipment* 29: 237-254.

Hittalmani, S., Mahesh, H.B., Mahadevaiah, C., and Prasannakumar, M.K., 2016. De novo genome assembly and annotation of rice sheath rot fungus *Sarocladium* oryzae reveals genes involved in Helvolic acid and Cerulenin biosynthesis pathways. *BMC Genomics* 17: 271-283.

Hongyu, K., García-Peña, M., Araújo Lúcio Borges, D., and Santos Dias Carlos Tadeu, D., 2014. Statistical analysis of yield trials by AMMI analysis of genotype × environment interaction. *Biometrical Letters* 51: 89-102.

Hospital, F., 2003 Marker-assisted breeding. In: Newbury, H.J. (ed) Plant molecular breeding. Blackwell Publishing, Oxford UK.

Kang, M.S., and Gauch, H.G., 1996. Genotype-by-environment interaction. CRC press.

Kathiresan, A., 2013. Rwanda's rice commodity chain-facing globalization. Ministry of Agriculture and Animal Resources, Kigali Rwanda.

Krishnamurthy, S., Sharma, S., Sharma, D., Sharma, P., Singh, Y., Mishra, V., Burman, D., Maji, B., Bandyopadhyay, B., and Mandal, S., 2016. Analysis of stability and G× E interaction of rice genotypes across saline and alkaline environments in India. *Cereal Research Communications* 44: 349-360.

Liang, S., Ren, G., Liu, J., Zhao, X., Zhou, M., McNeil, D., and Ye, G., 2015. Genotype-by-environment interaction is important for grain yield in irrigated lowland rice. *Field Crops Research* 180: 90-99.

Lin, C.S., Binns, M.R., and Lefkovitch, L.P., 1986. Stability analysis: Where do we stand? *Crop Science* 26: 894-900.

Maji, A., Bashir, M., Odoba, A., Gbanguba, A., and Audu, S., 2015. Genotype× environment interaction and stability estimate for grain yield of upland rice genotypes in Nigeria. *Journal of Rice Research* 3: 136-141.

Mukherjee, A., Mohapatra, N., Bose, L., Jambhulkar, N., and Nayak, P., 2013. Additive main effects and multiplicative interaction (AMMI) analysis of GxE interactions in rice-blast pathosystem to identify stable resistant genotypes. *African Journal of Agricultural Research* 8: 5492-5507.

NISR, 2015: Seasonal Agricultural Survey. National Institute of Statistics of Rwanda, Kigali.

Payne, R., Welham, S., and Harding, S., 2014. A Guide to REML in Genstat. VSN International, Hertfordshire, Uk.

Piepho, H.P., 1998. Methods for comparing the yield stability of cropping systems. *Journal of Agronomy and Crop Science* 180: 193-213.

Portmann, P., and Ketata, H., 1997. Field plot technique. In: Kempton, R.A., Fox, P.N. and Cerezo, M., (eds), Statistical methods for plant variety evaluation. Springer Netherlands, Dordrecht. p. 9-18.

Purchase, J., Hatting, H., and Van Deventer, C., 2000. Genotype× environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. *South African Journal of Plant and Soil* 17: 101-107.

Rodrigues, P.C., Malosetti, M., Gauch, H.G., and van Eeuwijk, F.A., 2014. A weighted AMMI algorithm to study genotype-by-environment interaction and QTL-by-environment interaction. *Crop Science* 54: 1555-1570.

Ruganzu, V., Shirimpumu, A., and Mutegi, J.K., 2015. Salinity reducing food security and financial returns from rice production in Rwanda. *Journal of Agriculture and Sustainability* 7: 114-127.

Shafii, B., and Price, W.J., 1998. Analysis of genotype-by-environment interaction using the additive main effects and multiplicative interaction model and stability estimates. *Journal of Agricultural, Biological, and Environmental Statistics* 3: 335-345.

Sibiya, J. 2009. Breeding investigations for resistance to *Phaeosphaeria Leaf Spot* (PLS) and other important foliar diseases and a study of yield stability in African maize germplasm. PhD Thesis. University of KwaZulu Natal, Pietermartzburg.

Tariku, S., T. Lakew, M. Bitew, and M. Asfaw, 2013. Genotype by environment interaction and grain yield stability analysis of rice (*Oryza sativa* L.) genotypes evaluated in North Western Ethiopia. *Net Journal of Agricultural Science* 1: 10-16.

Thapak, S., Thrimurty, V., and Dantre, R., 2003. Sheath rot management in rice with fungicides and biopesticides. *International Rice Research Notes* 28: 41.

Yan, W., and Kang, M.S., 2003. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC press.

Zali, H., Farshadfar, E., Sabaghpour, S.H., and Karimizadeh, R., 2012. Evaluation of genotype× environment interaction in chickpea using measures of stability from AMMI model. *Annals of Biological Research* 3: 3126-3136.

8 Chapter 7

General overview of the thesis

8.1 Introduction

This research was undertaken in the framework of investigating sustainable management of sheath rot of rice through breeding for disease resistance. The very first step towards initiating effective breeding efforts was to investigate and understand the genetic variability and source of resistance genes among locally adapted germplasm; and therefore identify inheritance pattern associated with resistance to sheath rot of rice. Additionally, introgressing resistant genes into farmers popular cultivars is essential in a such a way that it will ease farmers' adoption of new genotypes as well as adaptability of the developed genotypes on a wide range of environments.

The research was carried out to meet thesis objectives and test set hypothesis. It highlights key findings and their implications and paves the way for future breeding research as far as resistance to sheath rot of rice is concerned.

The following hypotheses were tested:

i) Rice germplasm in Rwanda is sufficiently diverse to avoid genetic depression after hybridization programmes
ii) Sources of sheath rot disease resistant genes are available in locally adapted germplasm
iii) The inheritance of genes for resistance to sheath rot traits is controlled by both additive and non-additive gene action.

8.2 Popular rice cultivars improved for sheath rot disease resistance are high yielding and adapted to various agro-ecological conditions of Rwanda.

Summary of key findings

8.2.1 Evaluation of genetic variability of rice germplasm based on agro-morphological traits

- Sixty-four rice germplasm adapted to Rwandan agro ecological conditions were evaluated for genetic diversity using morphological characters.
- REML procedures revealed significant differences among different varieties for all the studied traits with less influence of environmental factors.
- Genotypic variance was higher than environmental variance for most of the traits except number of tillers and flag leaf length
- Apart from number of tillers per hill and flag leaf length the rest of the traits exhibited high levels of heritability and genetic advance.
- Low environmental influence on phenotypic expression of genotypes was confirmed by low differences between phenotypic and genotypic coefficient of variation for most of the traits
- Principal component analysis extracted seven components contributing to more than 72% of total variation.
- Principal component based biplots revealed groups of genotypes suitable for specific breeding programmes.
- Breeding programmes involving tillering ability and improved yield are suggested for high yielding and tillering ability varieties [G36 (FAC 56), G3 (Buryohe), G1 (Yunyine), G6 (IUR 94) G62 (Jyambere), G25 (IRF 18)] on one hand, and high yielding and low tillering ability [(G2 (Nyiragikara), G60 (Yunertian), G12 (Intsindagirabigega), G27 (Imbaturabukungu), G42 (LL72)] on the other hand.
- Breeding investigations aimed at physical quality of the grain (length for instance) should focus on high yielding potential and long grain, i.e. [G62 (Jyambere), G3 (Buryohe), G36 (FAC 56), G27 (Imbaturabukungu) and G12.(Intsindagirabigega)].

8.2.2 Identification of sources of resistance to sheath rot disease of rice among Rwandan rice germplasm

- Sixty-four varieties were evaluated in field trials at three different sites, using morphometric markers.
- Out of 64 varieties; ten late maturing, intermediate to tall, well exerted and short grain genotypes showed different levels of resistance with a percent disease index (PDI) from 0.8 - 16.0%.
- Out of these, one immune cultivar (Yunyine) and five resistant cultivars (Nyiragikara, Nerica 1, Moroberekan, Cyicaro, and Yunertian) were found to be suitable for various ad hoc breeding programmes.
- Four moderately resistant cultivars were found to meet cost effective rice farming requirements.
- The remaining, early maturing, dwarf and semi-dwarf, enclosed panicles and mostly long grain cultivars were found to exhibit different levels of susceptibility with PDI ranging between 27.1 - 83.2%.
- Based on Pearson's correlation coefficients, a number of agro-morphological traits were significantly and negatively correlated with sheath rot (ShR). These were plant height, number of branches per panicle, number of grains per panicle, weight of 1000 grains, panicle length and grain yield. Sheath rot was positively and significantly correlated with flag leaf sheath length.

8.2.3 SNPs based assessment of genetic diversity of rice for selection of parents for sheath rot resistance breeding

Ten sheath rot resistant and fifteen susceptible varieties were analysed using 94 single polymorphic nucleotides (SNPs) markers.

- The total number of alleles amplified per locus ranged from 1 to 4 with a mean of 2.01 with 189 alleles, in total, amplified from 25 genotypes.

- The number of observations per marker locus or the number non-missing genotypes observed in the sample ranged from 11 to 25 with an average of 23.
- Mean major allele frequency was 76.2%, whereas the mean polymorphic information content was 0.263, and gene diversity estimated at 0.325 on average. Therefore, markers were highly informative and revealed good estimates of genetic diversity among studied varieties.
- Genetic distance, ranging from 0 and 0.63 coupled with the UPGMA dendrogram, clearly distinguished sheath rot resistant and susceptible genotypes.

8.2.4 Genetic analysis for sheath rot disease resistance in rice

Using a North Carolina design II, 32 F1 hybrids were generated by crossing 8 susceptible to 4 resistant parents and evaluated under field conditions for sheath rot resistance.

- Significance of both general and specific combining ability (GCA and SCA) effects indicated involvement and magnitude of both additive and non-additive gene action in controlling the inheritance of traits associated with horizontal resistance to sheath rot of rice.
- Based on high GCA/SCA ratio, coupled with high heritability estimates; additive effects were more predominant in the expression of lesion size, area under disease progress curve and panicle exsertion. In addition, results indicated that dominant genes were more important than recessive genes.
- As far as sources of resistance are concerned, the most promising genotypes were Cyicaro, Yunertian and Yunkeng.

8.2.5 Introgression of sheath rot resistant genes into popular rice cultivars and evaluation of introgression lines in multi-environment trials

In this study, BC2F1 lines were generated from four popular cultivars and two donors of sheath rot (ShR) resistant genes.

- Phenotypic selection of BC2F1 genotypes in three multi-environmental trials using six agronomic traits led to recovery of between 78 - 85% of the recurrent parents' genome with a remarkable increased resistance and grain yield (121 - 125%).
- The AMMI model indicated the nature and magnitude of adaptation and stability of developed introgression lines.
- Both main effect from genotype and environment had a significant effect on the performance of different introgression lines for six traits; disease severity, grain yield, plant height, tillering ability, grain length and number of grains per panicle.
- The genotype by environment interaction also had significant effects on disease severity, grain yield, tillering ability and number of grains per panicle.
- The AMMI analysis indicated that G6 (Intsinzi X Yunertian) had wide adaptation for most of the traits across the three test environments.
- Other genotypes showing relatively good general adaptation included G2 for disease severity and tillering ability, G8 for disease severity, and G4 for grain yield and number of grains per panicle, as they had IPCA1 values less than 0.5.
- AMMI stability value (ASV), Static stability (SSC) and dynamic stability analysis revealed that genotypes referred as G5 and G6 (Intsinzi X Yunertian) were stable for disease severity across the environment whereas G6 (Intsinzi X Yunertian), G5 and G4 showed good stability for yield and its related traits.

8.3 Implications of findings on breeding for sheath rot resistance and the way forward

Results from genetic variability studies led to the identification of potential parental materials for improving various morphological traits considered in this study. The genetic variability of local rice germplasm for traits under study coupled with little environmental influence signifies that parental selection based on individual traits would be quite effective.

Results on identification of sources of resistance genes indicated that out of 64 evaluated varieties only 10 were very important from a breeding point of view. This testifies to the potential threat of sheath rot disease on the rice sector as far as farming systems are concerned

especially since the rice consumer market in Rwanda prefers mostly long grain varieties. These 10 resistant varieties were found to be suitable for various ad hoc breeding programmes.

The assessment of SNPs based genetic diversity among sheath rot resistant and susceptible varieties indicated that large diversity among local germplam. This suggested the possibility of improving levels of resistance to sheath rot with minimum risk of genetic depression or reduced variability among progenies through hybridization of locally adapted germplasm.

Crossing sheath rot resistant parents to susceptible ones revealed the predominance of additive gene action over non-additive which suggests that crop improvement programmes should be directed towards selection of superior parents or good combiners emphasizing on GCA. The predominance of additive genetic effects together with the relevance of dominant genes suggested possibilities of improving the resistance by introgression of resistance genes through recurrent selection coupled with phenotypic selection. However, high estimates of heritability observed for all three traits, indicated these traits should be selected in controlled environment.

Generation and testing of BC2F1 introgression lines in multi-locational trials led to a breakthrough in breeding for resistance to sheath rot resistance as they demonstrate that progress can be made in introgressing resistant genes into susceptible cultivars through a few backcrossing generations with maximum recovery of the recurrent parents' genome.

In these regards it is suggested to advance these introgression lines to few further BC generations using, where possible, marker assistance selection with resistance to sheath rot of rice in foreground and other important agronomic traits in background. This would lead to quick recovery of the initial farmers preferred cultivars with resistance to sheath rot incorporated.