UNIVERSITY OF CAPE COAST

MARKER-ASSISTED BREEDING FOR RESISTANCE TO BLAST AND RICE YELLOW MOTTLE DISEASES IN GHANA

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BY

KIRPAL AGYEMANG OFOSU

Thesis submitted to the Department of Crop Science of the College of Agriculture and Natural Science, University of Cape Coast, in partial fulfillment of the requirements for the award of Doctor of Philosophy degree in Crop Science (Plant Breeding)

JULY, 2024

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date:.....

Name: Kirpal Agyemang Ofosu

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

Rice is consumed by over half of the world's population and accounts for 19% of calorie intake. Biotic stresses cause substantial yield losses in rice. Rice blast and Rice yellow mottle disease (RYMD) are the two main biotic stressors in sub-Saharan Africa. In Ghana, the major rice varieties cultivated by farmers namely, CRI-Agra Rice, CRI-Amankwatia, Jasmine 85 and Togo Marshall are susceptible to blast and *Rice yellow mottle virus* (RYMV) diseases and can cause up to 100% yield losses. There is thus the need to tackle these two diseases in order to reduce these yield losses. The objective of this research was to introgress resistance genes for rice blast and RYMD into four popular aromatic rice varieties in Ghana. To achieve this objective, a donor parent, Gigante which had *RYMV1 (rymv1-2)* and blast (*Pi54)* resistance genes was crossed to the four popular aromatic rice varieties to produce BC_3F_2 populations. A total of 71 BC_3F_2 *rymv1-2* and *Pi54* introgressed lines were screened for resistance to RYMV and blast and evaluated in a Preliminary Yield Trial (PYT). All the lines were found to be highly resistant to RYMV and rice blast. The results of the PYT indicated that RYMV-B-03-84-36-10-57, RYMV-B-01-6-37-1-91 and RYMV-B-03-84-36-10-46 yielded 7.25 ton/ha, 7.23 ton/ha, and 7.12 ton/ha respectively, which were significantly higher ($P<0.05$) than the highest yielding recurrent parent, CRI-Agra Rice (7.09 ton/ha). Through marker-assisted backcrossing, lines that are resistant to rice blast and *Rice yellow mottle* disease were produced. These can be evaluated further and released as resistant versions of the four popular but susceptible aromatic varieties.

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DEDICATION

In memory of my mother, Mary Adwoa Fremah. May you continue to rest in the Lord.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AGRA	Alliance for a Green Revolution in Africa
ANOVA	Analysis of Variance
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
DArT	Diversity Array Technology
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GDP	Gross Domestic Product
GL	Grain Length
GW	Grain Width
IPM	Integrated Pest Management
IRRI	International Rice Research Institute
KASP	Kompetitive Allele-Specific PCR
MAB	Marker-Assisted Breeding
MABB	Marker-Assisted Backcross Breeding
MAS	Marker-Assisted Selection
MoFA	Ministry of Food and Agriculture
NVRRC	National Variety Release and Registration Committee
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PYT	Preliminary Yield Trial

RNA	Ribonucleic Acid
RYMV	Rice Yellow Mottle Virus
RYMVD	Rice Yellow Mottle Virus Disease
SES	Standard Evaluation System for Rice
SNP	Single Nucleotide Polymorphism
SSA	sub-Saharan Africa
SSR	Simple Sequence Repeat
USDA	United States Department of Agriculture
VPg	Viral Protein genome-linked

CHAPTER ONE

GENERAL INTRODUCTION

Background to the study

Rice is the second most important staple food crop in Ghana (Asante et al., 2019; Tawiah et al., 2021). The annual per capita consumption of rice in Ghana stood at 45 kg/person in 2021, and it has been increasing annually (MoFA, 2021). There has been a high demand for the grain in recent times due to changing lifestyles, urbanization and increase in population (MoFA, 2021). The country's annual consumption stood at about 1.3 million MT of milled rice in 2021 whilst local production stood at 600,000 MT of milled rice (GCB Bank, 2022).

Notwithstanding this high demand for the grain in the past few years, production has not been able to meet up with this demand. Even though the area under cultivation has increased for the past decade from 216,000 ha in 2013 to 325,000 ha in 2023, the average yield on farmers' fields has not increased that much. It has only moved from 2.6 tons/ ha in 2013 to an estimated 3.5 tons/ ha in 2023 (USDA, 2023).

A lot of factors account for the low production of rice in sub-Saharan Africa in general, and in Ghana in particular. These include production factors, processing factors and marketing factors. Among the production factors, biotic stresses play a key role in yield losses. Rice yellow mottle disease and rice blast are the most important biotic factors that reduce grain yield. They cause 10-100% yield losses (Abo et al., 1997; Agnoun et al., 2019). Thus, there is the need to tackle these two biotic factors in order to increase rice yield in Africa.

Rice yellow mottle disease is caused by the *Rice yellow mottle virus*. The disease is characterized by stunting of plants, yellowish streaking of leaves, leaf mottling, incomplete emergence of panicles, delayed flowering, and in extreme cases, death of plants (IRRI, 2013). Because it is a viral disease, its control is very difficult, thus, breeding resistant varieties seems to be the most effective way to mitigate the impact of this disease on rice production in Africa.

Rice yellow mottle virus disease was first discovered in 1966 in the Kisumu region, near the Victoria lake in Kenya (Bakker, 1970, 1971). From this spot, the disease has spread to almost all parts of the African continent (Onasanya et al., 2006). RYMV has been reported in 29 countries in Africa (CABI, 2021). However, the disease is more concentrated in West Africa (Oludare et al., 2016).

Six strains of the disease have been reported so far. These are S1, S2, S3, S4, S5 and S6 (Longue et al., 2018; Rakotomalala et al., 2019; Odongo et al., 2021). The S1, S2 and S3 strains of the virus are found in West and Central Africa (Pinel-Galzi et al., 2006), whereas the other strains are found in East Africa (Fargette et al., 2004; Kouassi et al., 2005). In Nigeria, the S1 strain of the virus is the most common (Fargette, et al., 2004), just as in Togo, Chad and Niger (Traore et al., 2005). In Ghana and Burkina Faso, the S2 strain of the virus predominates (Traore et al., 2015).

A survey was conducted recently by the CSIR-Crops Research Institute to find out whether a new strain of the virus apart from the S2 strain was present in Ghana. Different isolates of the disease were collected from 11 out of the 16 regions of the country where there is high production of rice. Results of the sequencing of the various isolates sampled from the 11 regions showed that the

S2 strain still predominated in the country with only a few places recording the S1 strain of the virus (Omiat et al., 2023).

Rice blast disease is seen as the second most important rice disease in Ghana even though it is the number one rice disease in the world. The disease can affect many parts of the rice plant such as the nodes, panicles, grains, leaves, leaf sheaths and the collar (IRRI, 2013). Agronomic management of rice blast is more feasible, compared to the management of RYMV since the latter is a viral disease. Nonetheless, these two major diseases cause substantial yield losses.

One way to increase rice production in Africa and, particularly, West Africa, is to breed for lines that are resistant to these biotic stressors. Breeding for disease resistance is the most effective way to increase the productivity of crops (Acquaah, 2012). In fact, research has shown that breeding for *R* genes into the background of crops can increase crop yield recovery substantially. Breeding for disease resistance is preferable since it is easy to deploy and has no adverse effect on the environment (Acquaah, 2012). It thus becomes the most effective way to manage diseases that affect crop plants.

In the case of rice blast disease, management practices such as reduction in the application of nitrogen fertilizers, good farm sanitation, wider spacing to reduce humidity in the farm and to allow proper air circulation, burning before planting, application of recommended fungicides, both as seed treatment or during the time of infection, are some of the ways to manage the disease. However, the management of RYMV is very difficult and less effective because, like any other viral disease, chemical treatment is mostly not practical. Though elimination of the main vector of the disease – beetles – and the removal

and burning or burying of infected plants have been adopted by farmers to manage the disease, these control measures have not been effective in RYMV control (Miah et al., 2017). Therefore, breeding for resistance still remains the most effective way to control most plant diseases (Acquaah, 2012).

In Ghana, popular aromatic rice varieties such as CRI-Agra Rice, Jasmine 85, CRI-Amankwatia and Togo Marshall, are susceptible or moderately susceptible to RYMV and blast diseases (Asante et al., 2020a; Tawiah et al., 2021). Rice consumers in Ghana prefer aromatic rice as opposed to American long grain rice (Asante, 2013; Asante et al., 2020b) and, thus, breeding for resistance to these two major diseases in the genetic background of these four popular aromatic rice varieties would go a long way towards increasing rice production in the country.

Problem statement

Rice yellow mottle disease and rice blast disease are the two main biotic stressors of rice in sub-Sahara Africa (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a; Tawiah et al., 2021). Rice yellow mottle disease alone can cause up to 100% yield losses in rice (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020). Rice production in SSA is low, and in Ghana the average yield on farmers' field is 3.5 ton/ha (USDA, 2023) compared to 8.0 ton/ha average potential yield on research fields.

Both RYMV and blast are difficult to manage since this would mean buying more chemicals to treat the disease in the case of rice blast, whilst with RYMV, spraying can only be done to control the insect vectors, though there are other means of transmitting the virus from infected rice plants to uninfected

ones (Sarra & Peters, 2003). In this situation, breeding for disease resistance becomes as the most effective way to tackle these two diseases. According to Acquaah (2012), resistance breeding is an effective way to control plant diseases since it is cost-effective and less harmful to the environment, compared to the use of pesticides.

To date, no rice variety has been bred in Ghana specifically for RYMV resistance (M. D. Asante, personal communication, September 12, 2022).

Objectives of the study

The main objective of this research was to breed resistance for blast and *Rice yellow mottle virus* into popular aromatic rice varieties in Ghana.

Specifically, the research sought to:

1. profile QTL for known genes of blast and *Rice yellow mottle virus* (RYMV) in the core germplasm at CSIR-CRI, Ghana.
2. introgress *RYMV1* (allele *rymv1-2*) and *Pi54* resistance genes into selected high-yielding aromatic rice varieties through marker-assisted backcrossing.
3. assess the reaction of introgressed rice lines to blast and *Rice yellow mottle virus* (RYMV).
4. assess the yield performance of RYMV and blast resistant lines generated from crosses between resistant donor and recurrent susceptible parents.

Significance of the study

RYMV and rice blast are the two main devastating biotic stressors in SSA, including Ghana, and substantial yield losses are attributed to these two main diseases. In the meantime, rice yields in farmers' fields in Ghana are way below the world average (FAOSTAT, 2022). Breeding resistant and high-yielding varieties is a major way to tackle this problem.

It must also be stated that, to date, no RYMV-resistant rice variety has been bred in Ghana. In Ghana, CRI-Agra Rice, Jasmine 85, Togo Marshall and CRI-Amankwatia are among the most popular aromatic rice varieties in the country. All these four popular varieties are either susceptible or moderately susceptible to RYMV disease. Thus, breeding for resistance to these two diseases would be a step in the right direction, helping improve the yield of these varieties on farmers' fields. This would go a long way to improve the yield of these varieties and improve the income of rice farmers and food security in Ghana.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter is structured into four parts. The first part covers relevant research on the origin and distribution of Rice yellow mottle virus (RYMV) disease, the structure and genome organization of the virus, characteristics of RYMV infection, vectors of RYMV, viral strains, the effect of RYMV on rice production in Africa, disease management, viral resistance and breeding for resistance.

The second part of this chapter delves into the causative agent of rice blast disease, symptoms of rice blast, economic impact of rice blast disease, various races of blast, scoring and detection of rice blast, control of rice blast and breeding for blast resistance.

The third part of this chapter deals with breeding for disease resistance, marker-assisted breeding (MAB), marker-assisted backcross breeding (MABB), the use of Kompetitive Allele-Specific PCR (KAS), Single Nucleotide Polymorphism (SNP) markers, and Diversity Array Technology (DArT) marker technology in MAB. The last part of this chapter deals with yield and yield component traits in rice in a preliminary yield trial (PYT).

Origin and distribution of the Rice Yellow Mottle Virus (RYMV)

The *Rice yellow mottle virus* (RYMV) disease, as the name suggests, is a viral disease of rice that is endemic in Africa (Ndjiondjop et al., 1999; Nwilene et al., 2013). The disease was first discovered in 1966 in the Kisumu region, near the Victoria lake in Kenya (Bakker, 1970, 1971). From this spot, the disease has spread to almost all parts of the African continent (Onasanya et al., 2006). The virus was discovered in West Africa in 1975 (Fauquet & Thouvenel, 1977), and it has been confined to the African continent (Kouassi et al., 2005). Köklü & Yilmaz (2004) reported a case of RYMV infection in sampled rice germplasm in Edirne, Turkey. However, this report has not been confirmed by any further research to date. It must also be stated that cases of symptoms of disease infections of rice samples resembling RYMV were recently reported in Russia and Ukraine. However serological results showed the absence of RYMV in the sampled rice plants (EPPO, 2021). These scanty unconfirmed reports still make the disease confined to the African continent to date.

Out of the 29 countries in Africa in which the disease has been reported (CABI, 2021), its concentration is seen more in West Africa (Oludare et al., 2016). Figure 1 shows the distribution of the disease on the African continent. In Ghana, RYMV was first described in 1984 (Salaudeen et al., 2010). Since then, the disease has been the major biotic constraint to rice production in the country.

Figure 2.1: Distribution of Rice yellow mottle disease in Africa. Countries in orange colour have recorded cases of RYMV (Source: https://www.cabi.org/isc)

Taxonomy, structure and genome organization of Rice yellow mottle virus

Rice yellow mottle virus (RYMV) belongs to the genus *Sobemovirus* (Oliver, 2019) of the family *Solemoviridae* (King et al., 2018). It is an icosahedral-shaped virus measuring 25-30 nm in diameter and contains about 77% protein (Bakker, 1974; Ling et al., 2013). The genome of RYMV is a simple one made up of a single-stranded positive-sense RNA that contains about 4,450 nucleotides (Ling et al., 2013), similar to the genome of the *Cocksfoot mottle virus* (Fargette et al., 2004). Rice yellow mottle virus, like other sobemoviruses, exhibits obligatory dependence on divalent cations (thus, Ca^{2+} and Mg^{2+}) for structural stability. The viral capsid (coat protein) contains 180 structural subunits made to a T=3 design which forms its icosahedral shape (Opalka et al., 2000).

The genome of RYMV is organized into five open reading frames (ORFs). Thus, ORF1, ORF2a, ORF2b, ORF3 and ORFx (Ling et al., 2013). ORF1 is located at the 5' end of the genome of the virus. It has 157 aa (17.8 kDa) and it encodes P1, a protein that is seen to play the role of RNA silencing and viral movement (Sarmiento et al., 2007; Siré et al., 2008; Chowdhury & Savithri, 2011). ORF2a is seen to encode a polyprotein which is serine protease-like and viral protein genome-linked (VPg). VPg determines the virulence of the virus strain as well as the virus' ability to break the resistance (Kouassi et al., 2005). ORF2b encodes an RNA-dependent RNA polymerase. This polymerase is produced as a result of transframe fusion with ORF2a through ribosomal frameshifting of around 140 to 170 codons found upstream of the 3' end of ORF2a (Ling et al., 2013). The fourth ORF, ORF3 is responsible for coat protein production which aids in virus spread within the plant. It is composed of 239 aa (26 kDa) (Suvi et al., 2019).The full function of the fifth ORF, ORFx is not fully known, though it has been proven that mutations in this sequence that limit the expression of ORFx also prevents the establishment of infection. Therefore, ORFx has been found to be involved in the establishment of RYMV infection (Ling et al., 2013; Suvi et al., 2019).

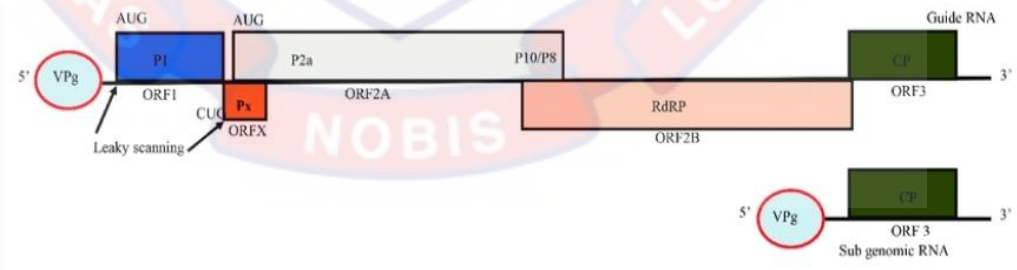

Figure 2.2: Genomic organization of *Rice yellow mottle virus* (RYMV) showing VPg, ORFs and ribosomal frameshifting between ORF2a and ORF2b. (Source: Ling et al., 2013)

Strains of RYMV

Serological differences between different isolates of RYMV have resulted in the discovery of six different strains of the virus in Africa, designated as S1, S2, S3, S4, S5 and S6 (Longue et al., 2018; Rakotomalala et al., 2019; Odongo et al., 2021;). The S1, S2 and S3 strains of the virus are prevalent in West and Central Africa (Pinel-Galzi et al., 2006). All the three strains (S1, S2 and S3) of the virus stated above have been discovered in Cote d'Ivoire and Mali (Fargette, et al., 2004; Traore et al., 2005). In Nigeria, the S1 strain of the virus is the most common (Fargette et al., 2004), just as in Togo, Chad and Niger (Traore et al., 2005). In Ghana and Burkina Faso, the S2 strain of the virus predominates (Traore et al., 2015).

Research conducted by Omiat et al. (2023) confirms the earlier work done by Traore et al. (2015) on the most predominant RYMV strain in Ghana. After about 10 years down the line, the S2 strain of the virus predominates in Ghana, with only a few places recording the S1 strain of the virus (Omiat et al. 2023).The S2 strain detected from this survey is similar to those found in Ivory Coast and Guinea, and higher than those from Mali or Benin in terms of number of sequence substitutions per site (Omiat et al., 2023).

The other three strains, S4, S5, and S6 are found in East Africa and Madagascar (Traore et al., 2005; Pinel-Galzi et al., 2006; Longue et al., 2018; Rakotomalala et al., 2019; Odongo et al., 2021;). The S4 strain is found in almost all parts of eastern Africa, including Uganda, Tanzania, Kenya, Ethiopia and Madagascar (Traore et al., 2005; Rakotomalala et al., 2019). Two additional strains, S5 and S6 are found in Tanzania (Fargette et al., 2004). The S4 and S6 strains are the two main strains of the virus in Malawi (Ndikumana et al., 2017).

Signs and symptoms of RYMV disease

The main symptoms of RYMV, which easily distinguishes the disease from other pathogenic or non-pathogenic infections are yellowing and leaf mottling (IRRI, 2013; Nwilene et al., 2013). The yellowing is caused by the presence of the virus in the leaf tissue and it is interspersed with mottling that differentiates the disease from yellowing caused by nitrogen deficiency. The leaf mottling caused by the virus starts as a linear chlorotic mottle on new leaves and latter changes into broken or continuous pale-green to yellowish streaks that can measure up to 10 cm long (Bakker, 1974). Aside from leaf mottling, the presence of the disease causes stunting in susceptible varieties.. The disease also causes delay in flowering, spikelet sterility, reduction in tillering, incomplete emergence of panicles and, in extreme cases, death of plants (Bakker, 1974; IRRI, 2013).

In field conditions, symptoms of RYMV usually occurs from the periphery. Symptoms occur from 1 to 2 weeks post-inoculation (Kouassi et al., 2005). The appearance of symptoms is dependent on the type of variety and the age of the plant (Bakker, 1970; Salaudeen et al., 2010). Figure 2.3 shows typical symptoms of the viral infection both in screenhouse and field conditions.

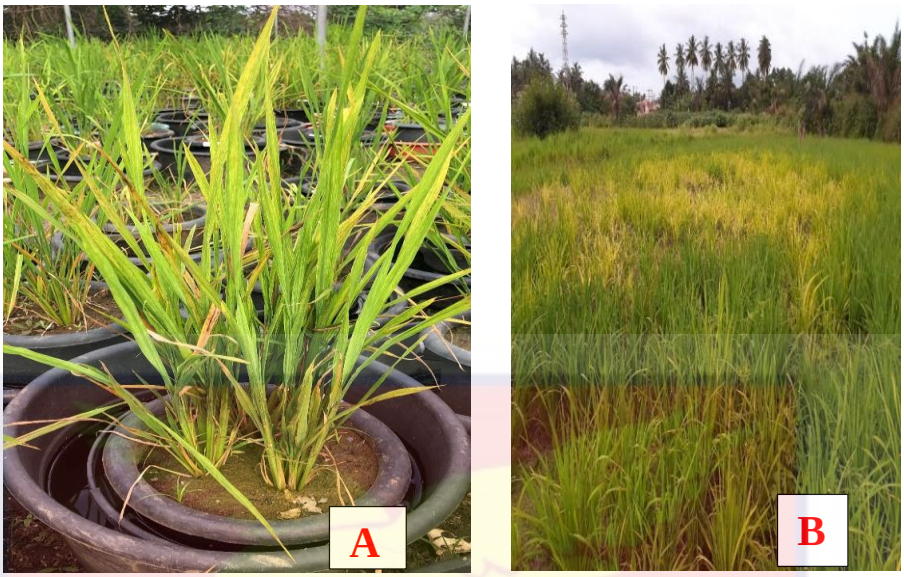

Figure 2.3: Sample plant in the screen house at CSIR-CRI showing symptoms of RYMV (A). Infected rice field at Asotwe, Ashanti Region (B)

Vectors of RYMV disease

Bakker (1970) discovered the beetle *Sesselia pusilla* of the family *Chrysomelidae* as the main vector for the transmission of Rice yellow mottle disease. Three additional species of beetles belonging to the same family were later discovered as major transmitters of RYMV. These are *Chaetocnema pulla*, *Trichispa sericea* and *Dicladispa (Chrysispa) viridicyanea* (Bakker, 1971).

Aside from beetles in the family *Chrysomelidae*, other insects such as grasshoppers (*Conocephalus spp.*), leafhoppers and flies have been known to transmit Rice yellow mottle virus (Kouassi et al., 2005; Koudamiloro et al., 2015).

RYMV can also be transmitted by mechanical inoculation (Kouassi et al., 2005; Salaudeen et al., 2010). Animals such as cows, rats and donkeys, have been identified to transmit RYMV (Sarra & Peters, 2003). Also, there is

evidence of wind-mediated transmission of the virus on fields (Sarra et al., 2004).

Another way through which RYMV can be transmitted is through contaminated soil water. This occurs when soil contaminated with roots, ratoons and debris of RYMV-infected plants left after harvesting come in contact with uninfected plants (Uke et al., 2014). However, there is no evidence of transmission of RYMV through seed (Allarangaye et al., 2016).

Scoring of RYMV disease

Two methods of assessment of RYMV are generally adopted by researchers. First is the scoring system developed by John and Thottappilly (1987). Here, scoring of disease severity ranges from 0 to 9, where 0 is "Highly Resistant"; 2 is "Resistant"; 4 is "Moderately resistant"; 7 is "Susceptible" and 9 is "Highly Susceptible". The second scoring system is based on the Standard Evaluation System for Rice (SES). Here, disease severity scoring ranges from 1 to 9, where 1 is "no symptoms observed"; 3 is "Leaves green but with sparse dots or streaks and less than 5% height reduction"; 5 is "Leaves green or pale green with mottling and 6% to 25% height reduction, flowering slightly delayed"; 7 is "Leaves pale yellow or yellow and 26-75% of height reduction, flowering delayed" and 9 is "Leaves turn yellow or orange, more than 75% of height reduction, no flowering or some plants dead" (IRRI, 2013).

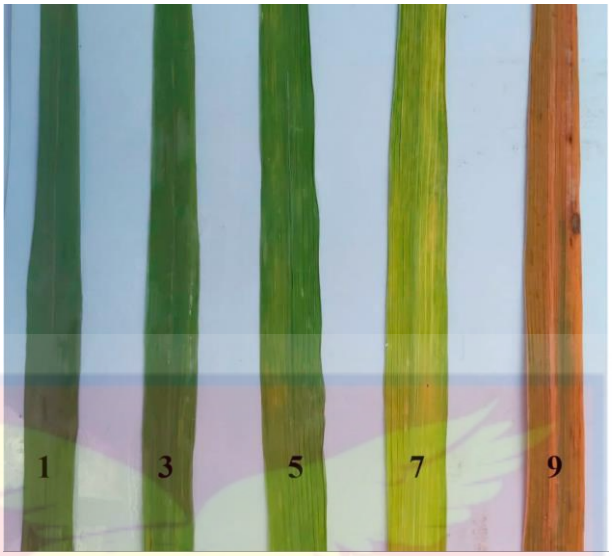

Figure 2.4: RYMV scoring system based on the Standard Evaluation System for Rice (SES). (Source: Asante et al., 2020)

Effect of RYMV on rice production in Africa

Depending on the stage of infection, the type of cultivar grown and other related factors, RYMV can cause up to 100% yield loss (Agnoun et al., 2019). The disease severely affects the growth of the rice plant and takes a toll on its flowering, maturity and yield. The disease causes stunted growth, delay in flowering and can even cause the death of plants in severe cases (IRRI, 2013).

Heinrichs et al. (1997) reported yield losses of 84%, 67% and 4% for Bouake 189, BG 90-2 and Moroberekan respectively. Research done by Onwughalu et al. (2010) revealed yield losses of 12.68% for Gigante, 78.06% for the rice variety Moroberekan and as high as 94.4% yield loss for Bouake 189 when these lines were inoculated with RYMV isolate under a screenhouse environment. Sérémé et al. (2016) assessed yield losses due to RYMV under field conditions in Burkina Faso. The researchers assessed the effect of RYMV on the yield of resistant, tolerant, susceptible and popular varieties. Mean yield loss of 33.23% was recorded among the varieties, with the susceptible variety

IR64 recording the highest grain yield loss (51.28%). This indicates how infection of RYMV, if not controlled, can lead to substantial yield losses.

N'Guessan et al. (2001) observed yield losses which ranged from 1% to 49% in the rice accession Ita 212. They also observed yield losses of 10% to 78% in the variety, Ngoyumaboi when they inoculated these lines with different isolates of RYMV. It must, however, be noted that, some partially resistant lines did not experience substantial yield losses.

Management of RYMV disease

Control of RYMV disease is a difficult one due to the fact that the disease is a viral disease, and viral diseases are generally difficult to control. Various strategies, such as chemical control, cultural control and biological control, have been adopted (Suvi et al., 2019).

Chemical control involves the use of recommended pesticides to control the main vector of the disease – beetles in the *Chrysomelidae* family – as well as other insect vectors, such as grasshoppers, leafhoppers and flies (Kouassi et al., 2005; Koudamiloro et al., 2015). However, the use of chemicals to control the insect vectors of RYMV has been found to be ineffective (Suvi, 2020). There is, therefore, the need to combine other control measures by means of integrated pest management (IPM).

Cultural control is one way of managing the disease. This involves creating a condition that would not be favourable for the virus to thrive. It mainly focuses on planting at an ideal period to escape the disease, careful rogueing to remove infected plants, removal of diseased residues and ratoons, practising crop rotation to break the disease cycle, disinfection of farms tools

and implements before moving them to new plots/fields, as well as field isolation (Suvi et al., 2019). Other known cultural methods used to manage the disease include growing different varieties or multi-lines on a single plot, changing nursery sites as well as ensuring proper phytosanitary measures to prevent the introduction of new strains of the disease from other countries or regions (Traore et al., 2015).

Rice blast disease epidemiology

The first description of rice blast disease was made by Soong Ying-shin in his book, "Utilization of Natural Resources" published in 1637. Here, he described the disease as a "fever" of rice seedlings because of the warmth absorbed into the grains in the course of drying in warm sunshine and thereafter being stored before cooling off (Ou, 1985). In Japan, the disease was referred to as Imochi-byoby Tsuchiya in 1704 and in Italy, it was reported in 1828 (Ou, 1985). In India, it was discovered and first reported in Tamil Nadu in 1913 (Padmanabhan, 1965). In Africa, the disease was reported in 1922 (Zewdu, 2021).

The Green Revolution of the 1960s, which brought the introduction of the first high-yielding, semi-dwarf rice cultivars with the excessive use of inputs, such as nitrogen fertilizers, increased the susceptibility of these improved rice varieties to blast and set the degree for the invasion of novel blast pathogen into contemporary-day rice (Kush, 2001).

Rice blast disease is caused by the fungus *Magnaporthe oryzae* B. C. Couch (Couch et al., 2005; Suwannual et al., 2017; Ning et al., 2020). The pathogen belongs to the Kingdom, *Fungi*; Phylum, *Ascomycota*; Class,

Sordariomycetes; Order, *Incertae sedis*; Family, *Magnaporthaceae*; Genus, *Magnaporthe* (Bussaban et al., 2005). Based on current phylogenetic, molecular and morphological data, isolates of the fungus from rice and carefully associated isolates from different grasses like *Eragrostris curvula*, *Elusine coracana*, *Lolium perenne*, and *Setaria spp*. are taxonomically defined as *Pyricularia oryzae*, while isolates from *Digitaria sanguinalis* (crab grass) are unique and classified as *Magnaporthe grisea* (Klaubauf et al., 2014).

Pyricularia oryzae, the teleomorphic form of *M. oryzae* is a pyrenomycete that produces fusiform, curved ascospores in perithecia in an unorganized manner. This sexual form can only be produced in the laboratory but not under field conditions (Zewdu, 2021). Under field conditions, the anamorphic form of the fungus is usually produced. The pathogen follows a chain of developmental and metabolic pathways from the time the spores land on the waxy leaf floor till the production of sporulating lesions (Wilson & Talbot, 2009). Theoretically, infested seed starts with the development of the disease through root colonization and then lesion formation followed by aerial dispersal of conidia (Ebbole, 2008).

During one developing season, one lesion can produce 2000-6000 conidia within a day for as many as 14 days. This is accompanied by a couple of cycles of contamination and reproduction, serving as a supply for secondary dispersal. However, the number of cycles and the quantity of spores which are produced on each lesion may be influenced by many factors such as temperature, rainfall, the depth of the water inside the paddy, the quantity of nitrogen used to fertilize the rice and the extent of genetic resistance within the cultivar that is infected (Couch et al., 2005).

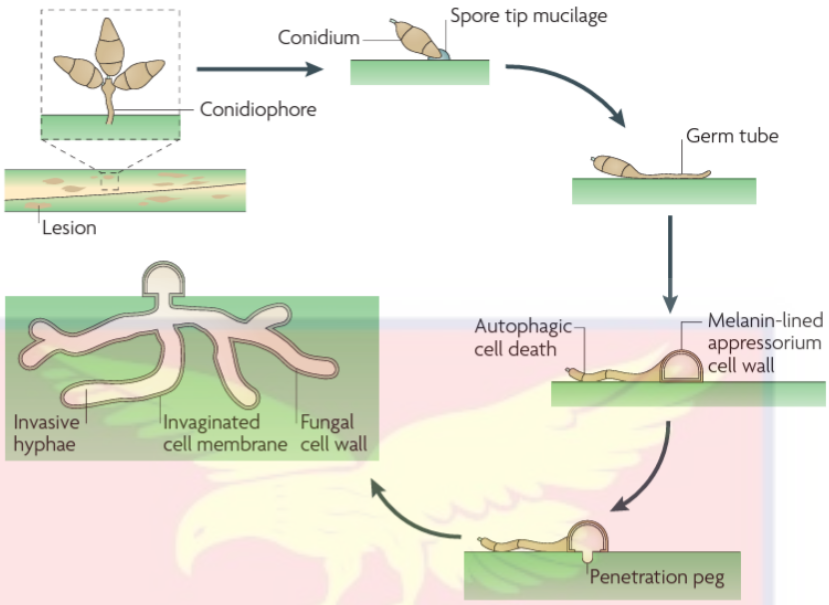

Figure 2.5: Life cycle of rice blast pathogen, *Magnaphorthe oryzae*.
(Source: Wilson & Talbot, 2009)

The disease infection starts when a spore of the fungus falls on parts of the plant such as the leaf, leaf sheath, culm, nodes or the panicle. The spore develops an adhesive that enables it stick to the cuticle of the plant. The conidium then develops a structure called appressorium which eventually penetrates the tissue of the plant to cause infection (Talbot, 2003). The presence of a chitinous cell wall and melanin-rich cell membrane aids the appressorium to cause damage to the host plant which appears in the form of lesions (Yarden et al., 2003). These lesions eventually produce spores that are transmitted usually by wind to other parts of the plant or other neighboring plants to begin a new cycle. The disease can overwinter, thereby making it persistent and the cycle difficult to break.

Detection and scoring of rice blast disease

Rice blast disease starts with the development of lesions, which come about as a result of invasive hyphae in the tissue of the plant. Lesions develop in almost all parts of the rice plant, most especially the leaves.

Rice blast can easily be misinterpreted as a rice brown spot caused by the fungus *Cochliobolus miyabeanus*. However, the difference between these two diseases is that, brown spot is oval or circular in shape and has a yellow halo around the lesions whilst rice blast has diamond-shaped lesions without a halo around the lesions (IRRI, 2013).

Scoring for blast is based on a scale of 0 to 9. The Standard Evaluation System for Rice (IRRI, 2013) describes scoring for leaf blast and another for panicle blast. For leaf blast, 0 = no lesion observed; 1 = small brown specks of pin-point size observed, or larger brown specks with no sporulation at the center; 2 = small roundish to slightly elongated necrotic spots which are about 1-2 mm in diameter with a distinct brown margin. These lesions are usually found on the basal leaves; 3 = lesions here are the same as in score 2 but a higher number of these lesions are found in the upper leaves; 4 = typical susceptible blast lesions are seen here which are 3 mm or greater but infect less than 4 % of the leaf area; 5 = typical blast lesions that infect 4 – 10 % of the total leaf area;

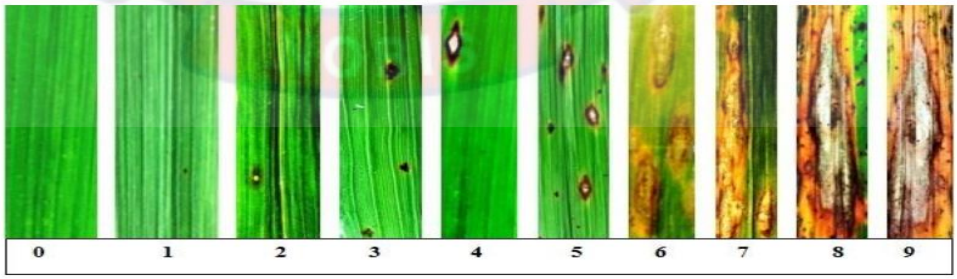

Figure 2.6: Scoring chart for rice blast disease

6 = typical blast lesions that infect 11 – 25% of total leaf area; 7 = typical blast lesions that infect 26 – 50% of total leaf area; 8 = typical blast lesions with infection ranging from 51 – 75% with many dead leaves; 9 = typical blast lesions greater than 75% (Figure 2. 6).

Effect of blast disease on rice production

Rice blast is one of the most important rice diseases in the world (Couch et al., 2005; Kumari et al., 2013; Yadav et al., 2019; Jamaloddin et al., 2020; Ramalingam et al., 2020;). As such, its effect on rice production cannot be overemphasized. The disease affects almost all parts of the rice plant, viz, roots, culm, leaves, nodes, panicles, leaf sheath, and seeds (IRRI, 2013). The disease has significant adverse effect of both grain yield and quality.

Talbot (2003) in his paper, "On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*" stated that on the average, rice blast disease causes yield losses of 10 – 30% yearly. The author cited the instance in Bhutan in 1995 where rice blast outbreak destroyed more than 700 hectares of rice fields, translating into a loss of 1,090 tons of rice. In Uganda, yield losses ranging from 40 – 75% have been reported and, in some cases, losses have reached up to 100% (Chuwa, 2015). Between 2001 and 2005, blast epidemics in China destroyed 5.7 million hectares of rice fields which translated into loss of millions of US dollars (Wilson & Talbot, 2009). In fact, yearly losses due to blast is estimated at US $ 55 million in Southern and South East Asia (Zewdu, 2021).

In sub-Sahara Africa, blast is one of the most economically important rice diseases. Survey in farmers' fields in Burkina Faso revealed yield losses ranging from 22 – 45% (Séré et al., 2013). In Ghana, rice blast and RYMV are the two most important rice diseases (Asante et al., 2020a; Tawiah et al., 2021). Séré et al. (2013) report of cases in Ghana where yield losses due to blast have reached 100% and, in Gambia and Sierra Leone, losses of up to 80% have been reported on experimental fields when susceptible varieties were planted.

Management of rice blast disease

Management and control of rice blast disease is a complex one due to its persistence on fields. However, various strategies have been adopted to manage the disease to reduce its impact on yield. These management practices are broadly classified into cultural, chemical, biological and host plant resistance (Pooja & Katoch, 2014; Asibi et al., 2019).

The cultural control method of managing rice blast disease involves manipulating practices, such as the rate of water supply, nutrient management, time of planting or transplanting of rice seedlings, spacing, weed control and burning of crop residues (Pooja & Katoch, 2014). The pathogen requires high relative humidity (RH, 92 – 96%), warm temperature (25 to 28º C) and extended periods of leaf dumpiness (Padmanabhan, 1965; Kankanala et al., 2007). This would mean that reducing the amount of water supplied to the rice plants may reduce dumpiness and the RH and may thus reduce the impact of blast on the plants. However, factors such as temperature and relative humidity are not under the control of the farmer, hence, manipulating these to the farmer's advantage may be difficult.

Cultural practices, such as proper farm sanitation, can be maintained, which include burning of crop residues to prevent the overwintering of the pathogen (Devkota, 2020). Also, research shows that too much application of nitrogenous fertilizers promotes the spread of rice blast disease (Long et al., 2000). Hence, reduction in or splitting of N application can help reduce the spread of the disease. Plant spacing is important because it influences the penetration of sunlight and, thus, determines how dumpy the field would be. Therefore, adopting the recommended spacing and, in some cases, increasing the recommended spacing can reduce the impact of blast on the rice plants. Using temporal escape is another cultural method that can be adopted. In this case, planting is done at a time where relative humidity is low and the temperature is not favorable for the growth of the fungus.

Chemical control methods involve the use of fungicides to control the fungus. Japan is noted to be the first country to extensively use copper-based fungicides to control rice blast (Ou, 1985). However, because of its adverse effect on the environment and on humans, copper-based fungicides were banned by the Japanese government in 1968 (Ou, 1985). Organophosphorus fungicides were later introduced but their use was discontinued due to the resistance of *P. oryzae* to these compounds. More recently, fungicides such as Mancozeb applied at 1000 ppm and 10000 ppm has proven to be effective against rice blast (Hajano et al., 2012). Magar et al. (2015) reported that a combination of Tricyclazole 22% and Hexaconazole 3% SC applied from booting stage on weekly bases was capable of controlling 87.03% of leaf blast and 79.62% of neck blast and this translated into the highest grain yield of 4.23 tons/ha as against other fungicide combinations.

Other chemicals used for the control of rice blast are Carpropamid, Fenoxanil, Tiadinil (Pooja & Katoch, 2014), Benomyl, Carbendszim 12% + Mancozeb 63%, Iprobenfos and Tebuconazole (Devkota, 2020). The different types of fungicides have different modes of action. Some act as melanin inhibitors whilst others act as ergosterol biosynthesis inhibitors (Zewdu, 2021). However, chemical control of rice blast is not an effective method, coupled with the fact that these chemicals have adverse effect on the environment.

One way of managing rice blast infection is the use of biological agents or compounds. Popular among biological agents used for the control of rice blast are *Pseudomonas fluorescens* and *Streptomyces sp*. These bacterial formulations have proven to be effective in the control of the spread of rice blast disease, with the latter being more widely used than the former (Asibi et al., 2019; Devkota, 2020). An experiment conducted by Vidhyasekaran et al. (1997) using the *P. fluorescens* strain Pf1 at 10 g/kg was able to inhibit the growth of *M. oryzae* from 21 days to 45 days after which the infection rose, but not as widespread as the control. Vaiyapuri et al. (2006) experimented *in vitro* and *in vovo* with SPM5C-1 and SPM5C-2, two aliphatic compounds which were derived from *Streptomyces sp*., PM5. Results from the experiment indicated considerable reduction of mycelial growth of *P. oryzae.* The use of biological agents, specifically antibiotics to control rice blast has been found to be ineffective due to resistant mutants of *P. oryzae* that develop in due cause (Pooja & Katoch, 2014).

The ineffectiveness of the above control methods has prompted scientists to look at the use of host-plant resistance in the control of rice blast disease. Resistance can be vertical, where there is the presence of one or two

major resistance genes against the disease, in which no symptoms of the pathogen is seen upon inoculation, or horizontal resistance where many minor genes come together to build resistance in the host plant in which the plant tolerates some levels of the presence of the disease but this does not adversely affect it physiological performance (Acquaah, 2012; Pooja & Katoch, 2014). Vertical resistance or complete resistance tend to breakdown when virulent isolates of the blast pathogen emerge. This was evident in South Korea in 1976 when the resistance in Tongil varieties was broken after this variety enjoyed a boom for only 5 years (Lee et al., 1976). In other places, complete resistance lasted between 1 and 3 years (Pooja & Katoch, 2014).

The problem with the breakdown of complete resistance due to the emergence of virulent isolates has caused scientists to shift attention to partial resistance which looks more durable. Korean pathologists define horizontal (partial) resistance as resistance that causes varieties to have blast score rating of 4 to 5 (Pooja & Katoch, 2014). The action of many minor genes coming together to create partial resistance makes it more tolerant to different races of blast pathogens. Partial resistance remains when complete resistance is broken (Zewdu, 2021).

Breeding for rice blast disease resistance

Breeding for host-plant resistance is seen as one of the most effective ways to mitigate the effect of diseases on crops. It is relatively less costly and not harmful to the environment (Acquaah, 2012).

Over one hundred blast resistance genes have been discovered and put into use in various breeding programmes (Sharma et al., 2012). Of these, *Pi54* has been identified to confer single gene broad spectrum resistance to rice blast disease (Rai et al., 2011; Das et al., 2012; Kumari et al., 2013; Thakur et al., 2015). *Pita* is seen as the most popular rice blast resistance gene. It confers broad spectrum, durable resistance to rice blast and it is in tight linkage with the send blast gene *Pita2* which is same as *Ptr* (Zhao et al., 2018; Meng et al., 2020). When *Pita* is inherited together with *Ptr*, the resistance becomes more durable (Meng et al., 2020). Other popular blast resistance genes such as *Pi46* (Xiao et al., 2016), *Piz-t* and *Pik* (Wang et al., 2016), *Pib*, *Pia*, *Pi1*, *Pikh*, *Pi2* and *Pi4* (Koizumi, 2007), *Pi21*, *Pi35*, *Pi63*, *Pid3-11*, *Pi-d2* and *Pi5* (Ning et al., 2020) and *Pik-m* (Suwannual et al., 2017) have been deployed by breeders to pyramid blast resistance into susceptible varieties.

Pyramiding two or more resistance genes into the background of susceptible varieties is seen as an effective way to improve resistance to rice blast disease (Divya et al., 2014; Zhi-juan et al., 2016; Suwannual et al., 2017). This is due to the fact that there have been many *M. oryzae* races that have been reported by various researchers.

Marker-assisted selection (MAS)

In conventional breeding, selection of traits of interest morphologically has been time-consuming and labour-intensive, coupled with the fact that this method of selection is subjective and as such subject to errors (Qi & Ma, 2020). Thus, marker-assisted selection which involves the use of molecular markers, such as DNA sequences or proteins to identify plants with specific characteristics (Ribaut & Hoisington, 1998) has been employed in modern-day breeding. Marker-assisted selection makes selection more effective, efficient and speedy (Acquaah, 2012). MAS has revolutionized plant breeding by accelerating the development of new varieties with improved traits that has ultimately led to increase in agricultural productivity and sustainability.

The application of MAS in plant breeding involves the following processes:

a) Identification of molecular makers. This involves researching the genome of the plant to identify DNA or protein sequences that are linked to the trait of interest.
b) Development of markers. After identifying sequences linked to the trait, the markers are developed usually through polymerase chain reaction (PCR) techniques.
c) Genotyping. This involves extracting DNA from target plants and using the markers developed to identify traits of interest in the samples.
d) Analysis of genotyping results. Results from the genotyping are analyzed to determine the presence or absence of traits of interest in the genotypes.

One advantage of the use of MAS in breeding is the fact that thousands of genotypes can be screened in the shortest possible time which is not feasible in conventional breeding (Ribaut & Hoisington, 1998). However, issues such as high cost and limited marker availability have made deployment of MAS problematic, especially in developing countries.

In practical breeding programmes, MAS can be employed in areas such as difficult-to-manage traits whose morphological characters are either time-consuming to collect using conventional means or traits that are under complex inheritance. MAS can also be employed to select for traits that are highly influenced by the environment or traits whose selection depends on specific environments (Miedaner & Korzun, 2012). MAS has been employed in many breeding programmes to stack multiple genes into one background through gene pyramiding, as well as breeding for many QTLs for a single disease resistance with complex inheritance (Miedaner & Korzun, 2012; Zheng et al., 2020)

Marker-assisted backcross breeding (MABB)

Backcross breeding involves the transfer of a major gene or a few genes into the background of an elite cultivar, such that the transferred gene or genes become(s) the only change in the cultivar (Acquaah, 2012). Marker-assisted backcross breeding involves the use of molecular markers to introgress a single locus or a few loci while retaining the essential characteristics of the recurrent parent (Collard & Mackill, 2008). MABB has been employed especially in the introgression of disease resistance genes into elite cultivars.

In breeding for resistance to diseases, such as rice blast, a lot of researchers have resorted to the use of marker-assisted backcross breeding as the most effective way to achieve this objective (Miah et al., 2015). This is because, most disease resistance genes are found in landraces or wild type varieties that tend to have many unfavourable genes except the disease resistance gene (Acquaah, 2012). Hence, the use of MABB as opposed to forward breeding has produced a lot of successes in breeding for resistance to most plant diseases.

Fortunately, many resistance genes have been mapped for rice blast and Rice yellow mottle diseases, and these have been employed in breeding for resistance to these two major rice diseases. According to Miah et al. (2015), the application of MABB can help achieve 99 % of the recurrent parent genome in just three backcross cycles, something that can only be achieved in six cycles when conventional backcross breeding is used. This does not only save time, but also save resources that could have been wasted if the conventional method of backcross breeding is used.

Marker-assisted selection (MAS) for disease resistance

Marker-assisted selection (MAS) is a popular breeding methodology that is mostly deployed in breeding for disease resistance (Acquaah, 2012; Arunakumari et al., 2016; Collard et al., 2017). Breeding for disease resistance, unlike breeding for yield and other morphological traits is entirely different in nature, in that, whiles the latter involves manipulating only one genetic system; that is plants, the former involves manipulation of two genetic systems, that is the pathogen and the host (Acquaah, 2012). This makes breeding for disease

resistance more technical, since there should be proper understanding of the two systems; that is the host plant and the disease pathogen. Since most disease resistance genes are recessive in nature (Jena & Mackill, 2008), the detection of resistance at the phenotypic level is difficult since recessive genes are usually undetectable in the phenotype at the heterozygous level. This makes deployment of MAS an indispensable tool in breeding for disease resistance.

In the case of rice blast disease, more than one hundred genes have been identified to confer resistance (Rai et al., 2011; Wu et al., 2017) and functional SNP markers for the identification of a majority of these have been developed. One fact must be stated, however, that reliance on major gene resistance is risky because such *R* genes can breakdown easily when new races of the pathogen emerge. Hence, partial resistance, which is under the control of many QTLs with minor effects is more durable and could therefore be deployed for disease resistance (Jena & Mackill, 2008).

There is a lot information available on molecular makers which are tightly linked to disease resistance genes (Wu et al., 2017). Since these molecular markers are more reliable than most phenotypic markers, deployment of the former makes selection for disease resistance in target germplasm easier than the use of conventional methods in achieving the same aim.

The introgression of *R* genes into susceptible cultivars to improve on their resistance is popular in the area of cereals breeding. In common wheat, eight QTLs for seven different traits were pyramided into the variety PBW343, this made the new genotypes resistant to wheat rust with improvement in grain yield (Gupta et al., 2010). Bacterial blight resistance genes Xa13 and Xa21 in rice, were introgressed into the cultivar PR106 through MAS. The same were

introgressed into the Indian rice cultivar MTU1010 and it showed durable resistance to bacterial blight disease (Zheng et al., 2020).

Use of KASP-SNP markers in marker-assisted breeding

Kompetitive Allele-Specific PCR (KASP) is seen as one of the most cost-effective, accurate SNP technologies in marker-assisted breeding (Devran et al., 2016; Tang et al., 2022). KASP-SNPs have been employed in most breeding programmes because of their high callability rate, low genotyping errors (0.7-1.6%) as compared to other SNP platforms, cost-effectiveness and high rate of transferability (Semagn et al., 2014; Steele et al., 2018). Designed by LGC genomics, KASP uses competitive allele-specific PCR that enables bi-allelic scoring of single nucleotide polymorphisms and InDels at specific loci (LGC Limited, 2021).

The KASP technology uses three primers, viz, two forward primers and a reverse primer. The allele-specific primers contain a special tail sequence that corresponds with a universal fluorescence resonant energy transfer (FRET) cassette that produces specific signals. The two forward primers are dyed with two different dyes. During the first round of PCR, one of the allele-specific primers binds the target SNP and elongates, attaching its tail sequence to the newly synthesized strand. A couple of rounds of PCR amplifies the newly synthesized strand so that the FRET cassette can bind to it to produce fluorescence signals, which can be read (Figure 2.7).

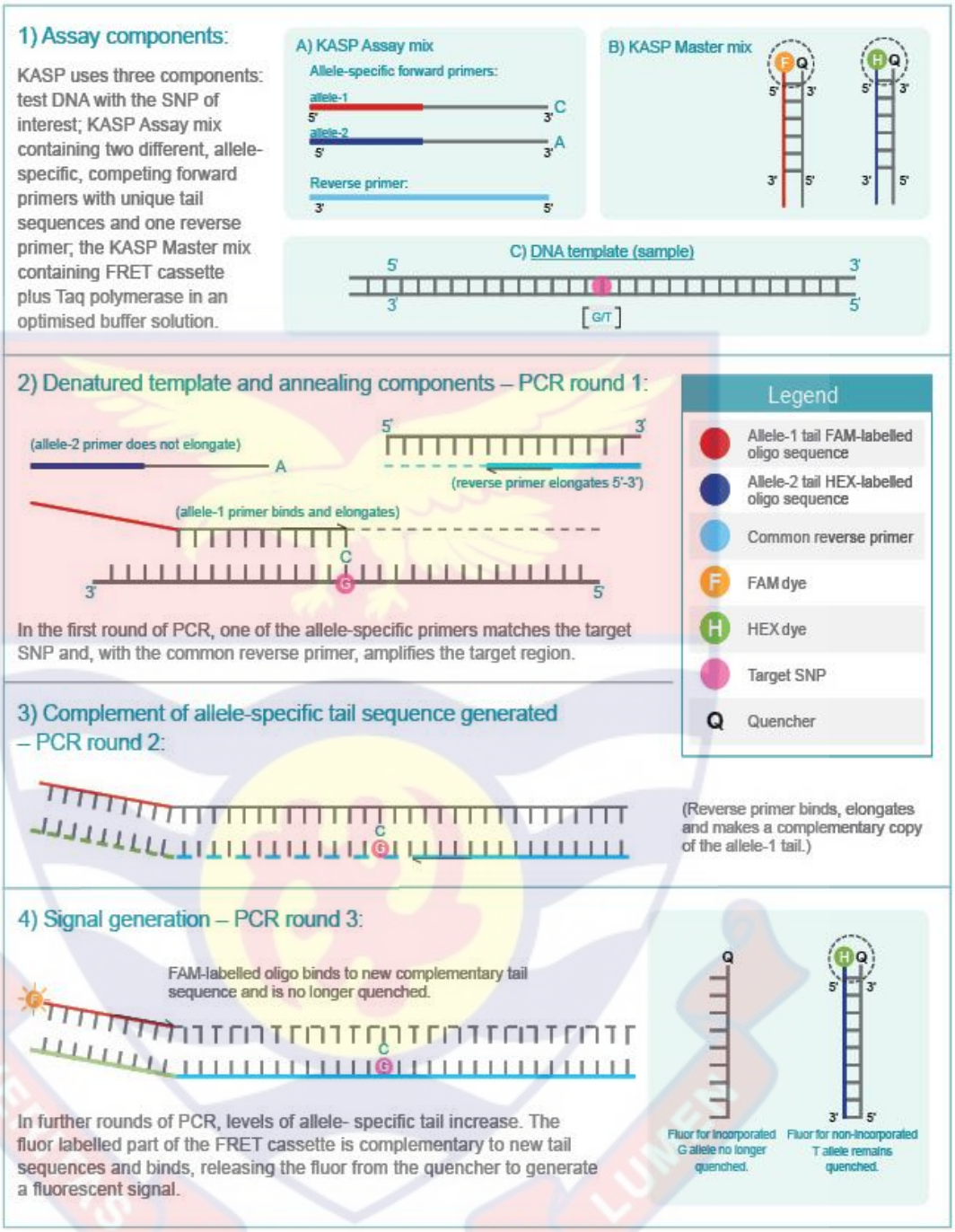

Figure 2.7: Step-by-step procedure on how the KASP technology works.

(Source: https://www.biosearchtech.com)

When the genotype is homozygous for the trait of interest, only one of the two fluorescent signals would be produced. However, if the genotype is heterozygous for the trait of interest, then a mixture of the two fluorescent signals would be produced (LGC Limited, 2021).

Diversity Array Technology (DArT) in marker-assisted breeding

Diversity array technology is a marker technology that provides a sequence independent high throughput, cost-effective whole-genome profiling (Yang et al., 2011). DArT was developed by Jaccoud et al. (2001) to address the limitations that are associated with existing technologies, such as RFLP, AFLP, SSR and SNP markers. Whereas these marker technologies could have at least one or many shortfalls, such as the inability to discover large number of polymorphic markers to cover whole genomes, use of gel electrophoresis, markers based on sequence information, high cost but low throughput, and high initial cost in marker discovery, DArT has sort to address these shortfalls (Wenzl et al., 2004).

In this method, the amount of specific DNA obtained from an organism or a group of organisms is assayed and compared to the DNA fragment derived from a representation of the total genomic DNA of the organism or a group of organisms (Jaccoud et al., 2001; Wenzl et al., 2004).

The DArT technology has three key features; a) independence on sequence information; b) the scope of the analysis is defined by the user and can be expanded; and c) high throughput but low cost (Wenzl et al., 2004). The technology is efficient in scanning the genome of organisms to identify diversity within the species. DArT has been employed in the whole genome scan for rice (Jaccoud et al., 2001; Xie et al., 2006), barley (Wenzl et al., 2004), wheat (Semagn et al., 2006), strawberry (Sánchez-sevilla et al., 2015) etc.

Yield and yield component traits in rice

Plant yield is a complex trait that is influenced by multiple quantitative agronomic traits (Jiaqin et al., 2009; Fentie et al., 2014). These traits that contribute positively to yield, if known, can be manipulated by breeders in order to indirectly select them to enhance yield, provided they have high heritability (Asante et al., 2019). In rice, grain yield is positively influenced by traits such as number of effective tillers (Asante et al., 2019; Demeke et al., 2023), panicle number (Asante et al., 2019; Demeke et al., 2023), panicle length (Asante et al., 2019), plant height (Asante et al., 2019), number of filled grains per panicle (Demeke et al., 2023), days to 50% flowering (Abdourasmane et al., 2016; Asante et al., 2019) and thousand grain weight (Demeke et al., 2023).

Agronomic traits such as plant height and days to maturity, are found to indirectly influence grain yield in rice through their influence on filled grain number per panicle, panicle number per unit area and 1000-grain weight (Sakamoto & Matsuoko, 2008). Li et al. (2019) have postulated that grain weight is controlled mainly by genetic factors whilst grain filling rate is influenced by environmental factors.

Some of the yield and yield component traits mentioned above have direct influence on yield whilst others have indirect influence on yield. According to Oladosu et al. (2018), tiller number, translated into panicle number, has the maximum indirect effect on grain yield. This assertion is supported by many researchers. The authors suggest that tiller number per plant, filled grains per panicle, and grain weight per hill could be employed as selection criteria to improve grain yield.

CHAPTER THREE

GENOTYPING OF SELECTED RICE ACCESSIONS FOR RESISTANCE TO BLAST AND RICE YELLOW MOTTLE DISEASES USING KASP-SNP MARKERS

Introduction

Rice is one of the most important staples in the world (Bodie et al., 2019). Over half of the world's population consume this staple, and it accounts for more than 19% of calorie intake (Yadav et al., 2017; Bazrkar-Khatibani et al., 2019; FAOSTAT, 2022;).

A lot of factors cause the low production of rice in sub-Saharan Africa. Key among these factors is biotic stress. Biotic stress alone can cause up to 100% yield losses (Baite et al., 2020; Neupane & Bhusal, 2020). In Ghana and sub-Saharan Africa in general, two main diseases limit rice production. These are Rice yellow mottle disease and rice blast disease (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a; Tawiah et al., 2021). *Rice yellow mottle virus* (RYMV) is the most devastating disease pathogen of rice in Africa. The disease can cause total crop failure, depending on the stage of infection, the variety infected and the type of management practice adopted to contain the disease (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a).

Rice yellow mottle virus disease originates from Kenya. The disease was discovered in 1966 in the Kisumu District near Lake Victoria (Bakker, 1970, 1971). From Kenya in East Africa, the disease has spread to almost all parts of the continent (Onasanya et al., 2006). RYMV has been reported in 29 countries in Africa (CABI, 2021). However, the disease is more prevalent in West Africa (Oludare et al., 2016).

Rice blast disease is seen as the second most important rice disease in Ghana after Rice yellow mottle disease, even though it is the most threatening rice disease in the world (Tanweer et al., 2015; Chen et al., 2018; Mao et al., 2018). The disease can affect many parts of the rice plant such as the nodes, panicles, grains, leaves, leaf sheaths and the collar (IRRI, 2013). Management of rice blast is more feasible, compared to the management of RYMV since the latter is a viral disease. Nonetheless, these two major diseases cause substantial yield losses.

Breeding for resistance is one of the most effective ways to mitigate the effect of plant diseases on crop production (Acquaah, 2012). Identification of resistant genes in the background of rice germplasm is key to finding the appropriate genes to introgress into the background of elite varieties.

A couple of markers have been developed for RYMV and blast resistances from SNP-marker technologies. SNP-marker technologies run on either uniplex or multiplex genotyping platforms that combine different detection methods and reaction formats (Semagn et al., 2014). Some popular SNP marker technologies commonly used these days are Kompetitive Allele-Specific PCR (KASP) from LGC Genomics; BeadXpress, GoldenGate and Infinium from Illumina; GeneChip and GeneFlex Tag array from Affimetrix; and TaqMan, SNaPshot from Applied Biosystems (Semagn et al., 2014; Devran et al., 2016). Each of these marker technologies have pros and cons. However, the KASP platform is preferred by most researchers because of its advantages such as cost-effectiveness, low genotyping errors and its transferability (Semagn et al., 2014).

The KASP technology runs on the uniplex genotyping platform. The technology uses fluorescent-based terminal readings to detect SNPs and InDels at specific sites within the chromosome (Yang et al., 2019). Two-colour fluorescents are used in this technology to detect products from different DNA samples extracted from organisms.

In this study, the KASP-SNP technology was used to detect resistances for both RYMV and blast diseases in the core rice germplasm at the CSIR-Crops Research Institute, Kumasi, Ghana. The aim was to identify resistant lines for RYMV and blast resistance genes in the germplasm.

Materials and methods

Plant materials and growing conditions

A total of 300 rice (*Oryza sativa* L.) germplasm, comprising 230 accessions from the core parental germplasm and advanced breeding lines of the Council for Scientific and Industrial Research (CSIR) - Crops Research Institute (CRI), Ghana was used for the experiment (Appendix 1). The experiment was conducted in a screen house at CSIR-CRI, Fumesua-Kumasi, Ghana. The study was conducted during the minor planting season of 2019. Sowing was done on 19th August 2019 and the last accession was harvested on 28th December 2019. The 300 rice genotypes were sown in plastic pots filled with 1.2 kg of sterilized sandy-loam soil. The pots were arranged in a randomized complete block design (RCBD) with three replications. Two seeds were sown per pot and were later thinned to one seedling per pot. The pots were arranged in such a way that a distance of 20cm was created between plants and 40cm between rows. Fertilizer

was applied at a recommended rate of 90 kg N: 60 kg P: 60 kg K per hectare. Weeds were controlled by hand-picking whenever necessary.

Leaf sampling

When the accessions were one month old, four discs of leaf samples per accession, 6 mm in diameter were collected in 96-well plates for DNA extraction and genotyping. The samples were oven-dried at 50º C for 24hrs in the plates. The leaf samples were packaged and shipped to Intertek (ScanBi Diagnostics AB, Alnarp-Sweden) for DNA extraction and KASP genotyping (Figure 3. 1).

Figure 3.1: Filling of 96-well plates with leaf samples (A). Dried samples ready for shipment to Intertek (B)

KASP-SNP low density genotyping

Nine KASP-SNP markers linked to known genes for disease resistance, namely blast and Rice yellow mottle diseases were used to genotype the 300 rice accessions to identify resistant genotypes. The names of the markers, the targeted genes associated with the traits, their chromosomal positions, as well as their favourable alleles are indicated in Table 3.1.

Table 3.1: List of rice KASP-SNP markers used for genotyping known genes for blast and Rice yellow mottle disease

Trait	Intertek SNP ID	Target QTL	Chromosome	Position	Allele 1 Favourable Allele	Allele 2 Unfavourable allele
Blast	snpOS00490	*Pik*	11	27984000	T	C
Blast	snpOS00488	*Pik*	11	27984000	G	A
Blast	snpOS00491	*Pik*	11	27987687	T	C
Blast	snpOS00499	*Pi54*	11	25263712	G	A
Blast	snpOS00451	*Pi9*	6	10389610	C	G
Blast	snpOS00006	*Pita*	12	10607554	C	A
RYMV	snpOS00435	*rymv1-2*	4	24948722	T	C
RYMV	snpOS00434	*rymv1-5*	4	24948702	-	INS
RYMV	snpOS00560	*RYMV3*	11	26377995	C	T

The KASP-SNP genotyping followed the genotyping protocol adopted by Intertek (ScanBi Diagnostics AB, Alnarp-Sweden). Results of the genotyping – in MS excel format – was then analyzed to determine the frequency of the population that possessed the various resistant genes of interest.

DArT mid-density genotyping

Mid density genotyping was performed for the 300 accessions using 1,094 markers from the DArT panel from Intertek (Agritech Lab, Australia). Four-leaf discs per accession were collected when the plants were 30 days old. Middle leaves that were not too young nor too old were collected. The samples were oven-dried at 50º C for 24 hours in 96-well plates and were later shipped to Intertek for the mid-density genotyping.

Statistical analysis

The genotyping results were analyzed using Microsoft Excel (version 2016) to create frequency a table from the number of accessions that possessed the resistant genes of interest. Flapjack- graphical genotype visualization software version 1.21.02.04 was used to visualize genotyping results and determine the allelic variations among the accessions. Statistical package R version 4.2.2 was used to perform cluster analysis and to generate phylogenetic tree from the mid-density genotyping results.

Results

Characteristics of the rice accessions

The current materials, which were genotyped belonged to different origins such as *indica*, *tropical japonica*, *temperate japonica*, and mixtures. The genotyping results revealed that the germplasm from CSIR-Crops Research Institute belonged to four main groups. This is indicated by the neighbor-joining tree produced from the genotyping results (Fig. 3.2).

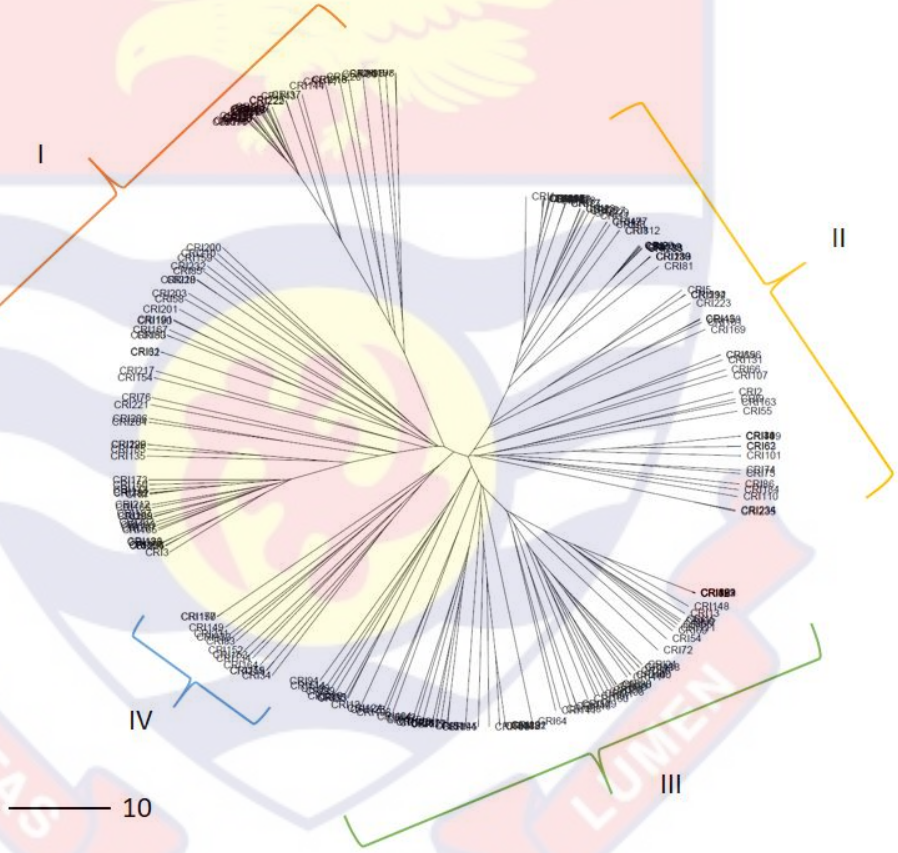

Figure 3.2: Phylogenetic tree generated from DArT genotyping using 1,094 SNPs. Branch lengths are equivalent to the number of nucleotide substitutions. The scale bar indicates 10 substitutions

The four main groups revealed by the phylogenetic tree from the genotyping results are *indica* lines, temperate *japonica* lines, tropical *japonicas* and a mixture of indica-japonica backgrounds. Cluster I contained germplasm that are tropical japonicas whilst cluster II contained temperate japonicas. Cluster III has lines comprising *indica* lines whilst the smallest cluster, cluster IV contains a mixture of indica-japonicas.

Resistance of the accessions to RYMV and blast

The low density genotyping results revealed different numbers in terms of the accessions' resistance to blast and RYMV diseases (Table 3.2). Two blasts genes, *Pi_54* and *Pita* showed high presence in the germplasm (24.33% and 33.00% respectively). Also, there was fair presence of the *RYMV1 (rymv1-2) R* gene in the population (3.33%). However, *RYMV1 (rymv1-5)* and *RYMV3* were not present in the population.

The other blast genes, *Pik* (snpOS00488, snpOS00490 and snpOS00490) and *Pi9* were among the lowest in terms of presence in the population (0.67% and 0.33% respectively).

Table 3.2: Genotyping results showing accessions within the germplasm that had resistant genes for blast and RYMV diseases

Trait	*Pik*	*Pik*	*Pik*	*Pi9*	*Pi54*	*Pita*	*RYMV1 (rymv1-5)*	*RYMV1 (rymv1-2)*	*RYMV3*
Intertek SNP ID	snpOS0 0488	snpOS0 0490	snpOS004 91	snpOS004 51	snpOS004 99	snpOS000 06	snpOS004 34	snpOS004 35	snpOS0056 0
No. of resistant accessions	2	2	2	1	73	99	0	10	0
Frequency (%)	0.67	0.67	0.67	0.33	24.33	33.00	0	3.33	0

Discussion

Breeding for resistance to diseases is one of the most effective ways of reducing the impact of biotic stresses on crop production. In the case of rice, Rice yellow mottle virus disease (RYMVD) and rice blast are the two main rice diseases in sub-Saharan Africa (Madhavi et al., 2016; Agnoun et al., 2019; Asante et al., 2020a). The two diseases combined can cause up to 100% yield loss in rice (Agnoun et al., 2019; Jamaloddin et al., 2020). This study aimed at looking for new sources of resistance to these two main diseases in the rice germplasm at the CSIR-Crops Research Institute. This should help in selecting parents when breeding for resistance to blast and RYMVD.

Disease resistance genes (*R* genes) can be obtained from landraces, most especially, or from improved varieties. It was therefore important to analyze the genomes of the 300 genotypes which serve as the core germplasm at CSIR-CRI in order to have a fair idea about the type of resistant genes that could be obtained from them.

The key part of this research was to find different sources of resistance for blast and RYMVD in the core germplasm at CSIR-Crops Research Institute. The results of the KASP-SNP genotyping (using nine markers linked to blast and RYMV) showed that some resistant genes for blast and RYMVD are found in some of the core germplasm. There are three major sources of resistance to RYMV (Pidon et al., 2017). These are *RYMV1*, *RYMV2* and *RYMV3*; with *RYMV1* having five allelic variants (Thiémélé et al., 2010; Pidon et al., 2017; Odongo et al., 2021). That is, *RYMV1 (Rymv1-1)* which is the susceptible version of the gene (Thiémélé et al., 2010), *RYMV1 (rymv1-2)*, *RYMV1 (rymv1-3)*, *RYMV1 (rymv1-4)* and *RYMV1 (rymv1-5)* which are the resistant versions of

RYMV1 (Pidon et al., 2017). The *rymv1-2* is the only RYMV resistant gene whose source is from *O. sativa* (Albar et al., 2006). All other RYMV-resistant genes mentioned above are from *O. glaberrima* sources (Thiémélé et al., 2010; Pidon et al., 2017; Odongo et al., 2021).

Even though SNP markers for *RYMV1 (rymv1-3), RYMV1 (rymv1-4)* and *RYMV2* were not available for this study, the results obtained for the other three resistant genes for RYMV revealed that only *RYMV1 (rymv1-2)* was present in the core rice germplasm at CSIR-CRI. This was represented by 3.33% of the germplasm genotyped. These results show that not enough sources of RYMV resistance are found in the core germplasm at CSIR-CRI due to the low presence of *RYMV1* (*rymv1-2*) and the absence of *RYMV1* (*rymv1-5*) and *RYMV3*.

Rice yellow mottle disease is one of the most important rice diseases affecting rice production in Ghana and, for that matter, sub-Saharan Africa. Thus, to combat this menace, it is imperative that variable sources of resistance are obtained so that they can be introgressed into varieties with high yields that are invariably susceptible to the disease.

The second resistance identified in the genotyping studies was resistance genes for rice blast. Being an equally important disease with over hundred resistance genes discovered (Sharma et al., 2012), it was imperative to know which of these resistance genes were available in the core germplasm at the CSIR-CRI. This would enable the selection of appropriate donors for improvement in resistance to this all-important disease. Six SNP markers were used to genotype for blast resistance in the lines. That is, *Pik* with three different

allelic variants located on chromosome 11, *Pi54*, *Pi9*, and *Pita* located on chromosomes 11, 6 and 12, respectively (Table 3.1).

The six blast genes considered here have broad spectrum resistance and can thus tolerate a wide range of *M. oryzae* races as compared to other known blast genes (Rai et al., 2011; Das et al., 2012; Kumari et al., 2013; Thakur et al., 2015). The genotyping results showed that there was high presence of *Pi54* and *Pita* in the core germplasm at the CSIR-Crops Research Institute (24.33% and 33% respectively). This shows that there is higher breeding potential for blast resistance than RYMV resistance within the rice germplasm at CSIR-CRI, Ghana.

However, unlike the two blast genes mentioned above, there was low presence for *Pik* and *Pi9* in the germplasm. Only 2 of the germplasm genotyped had the resistance gene *Pik*, representing 0.67% of the population, whilst 1 accession possessed the *R* gene for *Pi9*. Yadav et al. (2019) identified 24 blast resistance genes in 161 rice germplasm using 28 gene-specific markers. The results from their study indicated high presence of *Pikh* (*Pi54*) and *Pita* in their germplasm (73.29% and 54.0%, respectively). This is in line with the current research which also indicated high presence of *Pita* and *Pi54* in the germplasm at the CSIR-CRI. Similarly, Yadav et al., (2019) also recorded low presence of *Pi9* (15.52%). Their research, however, found very high presence of the *Pik* gene in their germplasm (161 out of 161 lines) contrary to the results of this research. This difference could be coming from the nature of the germplasm used for this study.

The genetic diversity analysis using the Diversity Array Technology (DArT) platform showed that the germplasm at the CSIR-CRI belonged to four main groups viz *indica*, tropical *japonica*, temperate *japonica* and a mixture of indica-japonicas.

In cluster I, which was mainly tropical japonicas, there was a branch from this that contained a few number of glaberrima and lines with glaberrima background. Even though the germplasm at CRI did not have a whole cluster containing *glaberrima*, there was a sub-cluster that contained glaberrima and glaberrima-derived lines. The other three clusters contained germplasm that are routinely used in the breeding pipeline at the CSIR-CRI. The genotyping results revealed significant diversity within the germplasm and could thus serve as a useful source in mining for other genes of importance.

Conclusion

The nature of germplasm used in every breeding programme is key since they determine the preferred traits available for selection. Based on the mid-density genotyping results, using 1,094 SNPs and InDels on the DArT platform, the core rice germplasm at the CSIR-Crops Research Institute belonged to four main groups, viz, *indica*, tropical *japonica*, temperate *japonica* and indica-japonica mixtures.

Out of 300 accessions genotyped, 10 (3.33%) possessed resistance genes for *RYMV1 (rymv1-2)* whilst none of the germplasm had resistance for *RYMV1 (rymv1-5)* and *RYMV3*. Thus, new resistance sources for *RYMV1 (rymv1-5)* and *RYMV3* must be introduced into the germplasm to provide more resistance sources for RYMV.

There are several resistance genes available for rice blast resistance. The core germplasm at the CSIR-CRI had enough sources for the blast *R* genes *Pi54* and *Pita* (24.33% and 33.0%, respectively). However, there was low presence for *Pik* and *Pi9* in the germplasm (0.67% and 0.33% respectively). Two genotypes, GR18 and Orylux6 were the only lines which possessed the three versions of the blast resistance gene *Pik*. Also, only GR18 possessed the *R* gene, *Pi9*. This makes Orylux6 and GR18 valuable accessions and must, therefore, be preserved well for pyramiding of blast resistance genes. For the germplasm analyzed in this study, there were more resistance sources for rice blast disease than RYMVD, reflecting the relative breeding efforts against the two diseases.

CHAPTER FOUR

INTROGRESSION OF BLAST RESISTANCE GENE *PI_54* AND *RYMV* RESISTANCE GENE *RYMV1* (*rymv1-2*) INTO FOUR POPULAR AROMATIC RICE VARIETIES IN GHANA

Introduction

Rice is seen as a food security crop. Globally, it is consumed by more than half of the population (Suvi et al., 2019). The world's population is expected to increase by 33% from 7.2 billion to 9.6 billion by 2050 (Nalley et al., 2017). This would mean the current food production must increase by about 50% in order the feed the increasing population. There are two ways to meet such demand. One is to increase the total area under cultivation. The second approach is to increase the current yield by half. Since there is scarcity of arable lands, using the latter approach seems more feasible (Soe et al., 2019; Asante et al., 2020a). This would require massive improvement in the current crop varieties in the hands of farmers.

Biotic and abiotic stressors cause substantial yield losses. Biotic stresses alone can lead to 100% yield loss (Baite et al., 2020; Neupane & Bhusal, 2020). Major diseases of rice such as rice blast and Rice yellow mottle disease affect the production of rice in Africa (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a; Tawiah et al., 2021). Globally, rice blast is the most important rice disease (Tanweer et al., 2015; Chen et al., 2018; Mao et al., 2018). The disease affects almost all parts of the rice plant, causing substantial yield losses and significant reduction in grain quality (IRRI, 2013; Kumari et al., 2013).

Several resistant genes have been discovered for both blast and RYMD. Chief among them are *RYMV1 (rymv1-2)* and *Pi54* (Albar et al., 2006; Traoré et al., 2010; Rai et al., 2011; Kumari et al., 2013; Thakur et al., 2015; Asante et al., 2020a). *RYMV1 (rymv1-2)* is a major resistant gene (*R* gene) that has its source from *O. sativa* (L). It confers resistance against different strains of RYMV disease (Ndjiondjop et al., 1999; Traore et al., 2015; Asante et al., 2020a). S1, S2, and S3 are the major strains of the virus in West Africa (Kouassi et al., 2005; Omiat et al., 2023). Over one hundred *R* genes have been discovered to confer resistance to rice blast (Sharma et al., 2012). Of these, *Pi54* located on chromosome 11 has been found to confer broad spectrum resistance to rice blast disease (Rai et al., 2011; Das et al., 2012; Kumari et al., 2013; Thakur et al., 2015).

Popular aromatic rice varieties in Ghana are either susceptible to RYMVD or rice blast disease or both. To date, no rice variety in Ghana has been bred specifically for resistance to blast and Rice yellow mottle disease (M. D. Asante, personal communication, September 12, 2022). The aim of this research was, therefore, to introgress resistance for RYMV and rice blast into four popular aromatic rice varieties in Ghana. This would enable the possible release of resistant versions of these popular aromatic rice varieties to farmers.

Materials and methods

Study area

The research was conducted at the Rice Breeding Nursery of CSIR- Crops Research Institute, Fumesua-Kumasi, Ghana. The area is characterized by a bimodal rainfall pattern. The major season starts at the beginning of April and ends by the end of July whilst the minor season starts by the beginning of September and ends by early to mid-November. The average annual rainfall is 1,397 mm and the temperature ranges from 20.6°C to 33.3°C (Weather Atlas, 2023).

Plant materials

The plant materials used for this study comprised a donor variety and four recurrent parents. The donor parent, Gigante is an *O. sativa* (L.) *indica* variety that originates from Mozambique (Sérémé et al., 2016). It contains the RYMV resistant gene *RYMV1 (rymv1-2)* and the blast resistance gene, *Pi_54*. The four recurrent varieties ware Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia. These are among the most popular aromatic rice varieties in Ghana (Asante, 2013; Asante et al., 2020a). However, these popular rice varieties are susceptible to blast and RYMV diseases.

Togo Marshall is an *indica* rice variety that is cultivated by Ghanaian farmers and is believed to have been introduced from Togo. It is predominantly grown by farmers in the Volta Region and parts of Greater Accra and Eastern regions. Jasmine 85 was introduced from the United States and released as Gbewaa Rice in 2009 (NVRRC, 2019). It is an *indica* variety with a potential yield of 6 t/ha. CRI-Agra Rice was introduced as IR841 and released in 2013.

It is grown in almost all rice growing areas in Ghana. Its potential yield is 8.0 t/ha. CRI-Amankwatia was also released in 2015 as a pure line selection from Togo Marshall. It is a lowland variety with a potential yield of 8.0 t/ha (NVRRC, 2019).

Before the introgression commenced, the four susceptible varieties and the donor parent, Gigante, were inoculated with *Rice yellow mottle virus* isolate from the CSIR-CRI rice field. Figure 4.1 shows the resistance levels of the four susceptible varieties and the donor parent after double inoculation with the virus.

Figure 4.1: RYMV resistance levels of Gigante (extreme right) and the four susceptible varieties, planted on 13/08/2021, inoculated on 13/09/2021 and captured on 08/10/2021 (25 dpi)

After double inoculation with the virus, Gigante showed strong resistance to the virus whilst the four varieties showed signs of the disease, which was an indication that the four varieties were actually susceptible to RYMV disease based on the RYMV symptoms they exhibited.

Breeding procedure

The donor parent, Gigante, having the RYMV resistant gene *RYMV1 (rymv1-2)* and the blast resistant gene *Pi54* was crossed to the four recurrent parents; Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia to produce F_1 generations in June 2019 (Fig. 4.2). The four different F_1 generations that were true hybrids were crossed back to their recurrent parents to produce BC_1F_1 lines by end of September 2019.

After genotyping the BC_1F_1 lines to identify lines that were heterozygous (hets) for *RYMV1 (rymv1-2)* and *Pi54*, they were crossed back to the recurrent parents to produce BC_2F_1 lines by January 2020. The BC_2F_1 lines were genotyped in May 2020 to identify lines that were hets for the two resistance genes. After selecting BC_2F_1 lines that were hets for *RYMV1 (rymv1-2)* and *Pi54*, the selected lines were crossed back to the four recurrent parents to produce BC_3F_1 lines by February 2021.

In October 2021, the BC_3F_1 lines were genotyped and seeds from lines that were heterozygous for the two resistance genes were selected and advanced to BC_3F_2 for further evaluation.

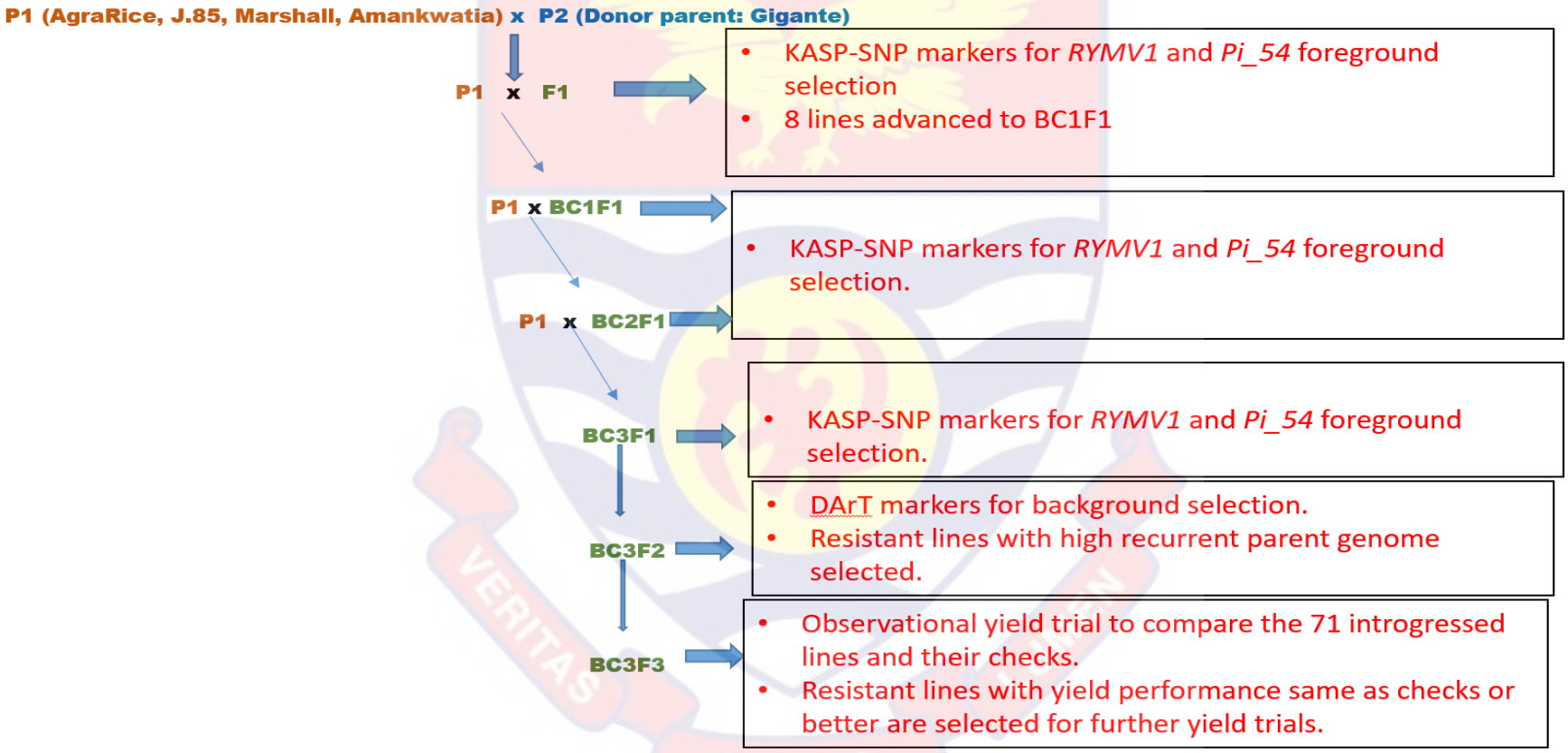

Figure 4.2: Schematic representation of the breeding scheme used to generate the RYMV and blast resistant lines

Generation of F1 lines and hybridity test

The donor parent, Gigante, was crossed to the four recurrent parents viz Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia (Table 4.2). They were coded as 01, 02, 03 and 04 respectively. A total of 34 F_1 seeds from the four crosses were grown and leaf samples were collected for genotyping to determine their hybridity (Table 4.2). Ten functional KASP-SNP makers including the RYMV resistance marker *RYMV1 (rymv1-2)*; Intertek SNP snpOS00435 and blast resistance marker *Pi54*; Intertek SNP snpOS00499 including 8 additional SNP markers were used for the hybridity test. Thirty out of the 34 lines that were genotyped were "true" F_1s (Table 4.1).

Foreground KASP-SNP genotyping

Two KASP-SNP markers, *RYMV1 (rymv1-2)* and *Pi54* were used for foreground selection. *RYMV1 (rymv1-2)* is one of the RYMV major resistance genes whose source is from *O. sativa* (Albar et al., 2006). The second resistance gene, *Pi54*, is a broad-spectrum blast resistant gene that confers resistance to a number of *M. oryzae* races (Rai et al., 2011; Das et al., 2012; Kumari et al., 2013; Thakur et al., 2015). Other grain quality and disease resistance makers were added to make a total of 10 markers for the foreground SNP genotyping (Table 4.1).

Table 4.1: List of rice KASP-SNP markers, chromosomal position and alleles used for foreground selection.

Trait	Intertek SNP ID	Target QTL	Chromosome	Position	Allele 1 Fav. Allele	Allele 2 Unfav. Allele
Blast	snpOS00499	*Pi54*	11	25263712	G	A
RYMV	snpOS00435	*rymv1-2*	4	24948722	T	C
Aroma	snpOS00022	*frg-1*	8	20382865	TATAT	AAAAGATTATGGC
Chalkiness	snpOS00024	*Chalk5*	5	3340295	G	A
Blast	snpOS00006	*Pita*	12	10607554	C	A
GT	snpOS00450	*Alk*	6	6752888	T	C
Waxy	snpOS00445	*Waxy_A*	6	1768724	C	T
Waxy	snpOS00037	*Wx(int)*	6	1768006	C	A
Drought	snpOS00400	*DTY1.1*	1	38081544	G	C
Drought	snpOS00402	*DTY1.1*	1	39014751	A	G

Leaf samples, which were about a month old, were collected in 96-well plates for DNA extraction and subsequent genotyping. The samples were oven-dried at 50º C for 24 hours in the plates. They were then packaged and shipped to Intertek (ScanBi Diagnostics AB, Alnarp-Sweden) for DNA extraction and genotyping.

DArT genotyping for background selection

The Diversity Array Technology (DArT) platform was used to genotype the BC_3F_2 lines that were generated from the crosses between the donor variety and the recurrent parents. A total of 1,094 KASP-SNPs comprising neutral markers and diagnostic markers were used.

Four leaf discs each of the BC_3F_2 lines were collected when the plants were 30 days old. The samples were oven-dried at 50º C for 24 hours in 96-well plates and were later shipped to Intertek (Agritech Lab, Australia) for the mid-density genotyping to identify lines with high recurrent parent genome.

Statistical analysis

Microsoft Excel 2016 was used to filter and count lines that were heterozygous for *RYMV1 (rymv1-2)* and *Pi54* for the F_1 to BC_3F_1 generations, as well as homozygous for the same traits in the BC_3F_2 generation.

Flapjack-graphical genotype visualization software version 1.21.02.04 was used to visualize genotyping results and determine the allelic variations among the accessions.

Results

F_1 hybridity test

The hybridity tests showed some F_1 lines were false F_1s, as seen from the entry, RYMV-02-4 and RYMV-03-8 in Table 4.2. All other F_1 lines that are in yellow colour are heterozygous for *rymv1-2* and *Pi54*. Also, the green colour shows the genotype has favorable allele for that trait and is thus in a homozygous condition whilst results in blue colour shows the genotype has unfavorable alleles for that trait in a homozygous condition.

Table 4.2: Hybridity test showing F_1 lines heterozygous for *rymv1-2* and *Pi54*.

Trait ID	Blast	Blast	RYMV	Aroma	Chalkiness	Amylose	Amylose	GT	Drought	Drought
Gene	**Pi54**	**Pita**	**rymv1-2**	**frg-1**	**Chalk5**	**Waxy**	**Waxy**	**Alk**	**DTY1.1**	**DTY1.1**
Sample Name	snpOS00499	snpOS00006	snpOS00435	snpOS00022	snpOS00024	snpOS00037	snpOS00445	snpOS00450	snpOS00400	snpOS00402
Parents										
CRI-Amankwatia	A:A	A:A	C:C	TATAT:TATAT	A:A	A:A	C:C	T:T	C:C	G:G
CRI-AgraRice	A:A	A:A	C:C	TATAT:TATAT	A:A	A:A	C:C	T:T	C:C	G:G
Jasmine 85- CRI	A:A	A:A	C:C	TATAT:TATAT	A:A	A:A	C:C	T:T	C:C	G:G
TogoMarshall	A:A	C:C	C:C	TATAT:TATAT	G:G	A:A	C:C	T:T	C:C	G:G
Gigante	G:G	A:A	T:T	AAAAGATTATGGC:AAAAGATTATGGC	A:A	A:A	C:C	C:C	G:G	G:G
TogoMarshall x Gigante (F1s)										
RYMV-01-1	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	G:A	A:A	C:C	T:C	C:G	G:G
RYMV-01-2	Unused	C:A	Unused	TATAT:AAAAGATTATGGC	G:A	Unused	C:C	C:C	C:G	Unused
RYMV-01-3	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	G:A	A:A	C:C	T:C	C:G	G:G
Jasmine 85 x Gigante (F1s)										
RYMV-02-1	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-02-2	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-02-3	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-02-4	A:A	A:A	C:C	TATAT:TATAT	A:A	A:A	C:C	T:T	C:C	G:G
RYMV-02-5	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
CRI-AgraRice x Gigante (F1s)										
RYMV-03-1	A:G	C:A	C:T	TATAT:AAAAGATTATGGC	G:A	A:A	C:C	T:C	C:G	G:G
RYMV-03-2	A:G	C:A	C:T	TATAT:AAAAGATTATGGC	G:A	A:A	C:C	T:C	C:G	G:G
RYMV-03-8	A:A	C:C	C:C	TATAT:TATAT	G:G	A:A	C:C	T:T	C:C	G:G
RYMV-03-9	A:G	C:A	C:T	TATAT:AAAAGATTATGGC	G:A	A:A	C:C	T:C	C:G	G:G
CRI-Amankwatia x Gigante (F1s)										
RYMV-04-1	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-04-3	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-04-6	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G
RYMV-04-7	A:G	A:A	C:T	TATAT:AAAAGATTATGGC	A:A	A:A	C:C	T:C	C:G	G:G

Generation of BC_1F_1, BC_2F_1 and BC_3F_1 populations

Two true F_1 lines from the four populations were selected and backcrossed to their recurrent parents to produce BC_1F_1 populations. A total of 361 BC_1F_1 plants were produced from the four crosses. The breakdown of the BC_1F_1 populations are shown in Table 4.3.

Table 4.3: BC_1F_1 populations derived from the four cross combinations for the introgression of *rymv1-2* and *Pi54*

S/No.	Cross Combination	No. of Entries
1	Togo Marshall x Gigante	62
2	Jasmine 85 x Gigante	87
3	CRI-Agra Rice x Gigante	81
4	CRI-Amankwatia x Gigante	131

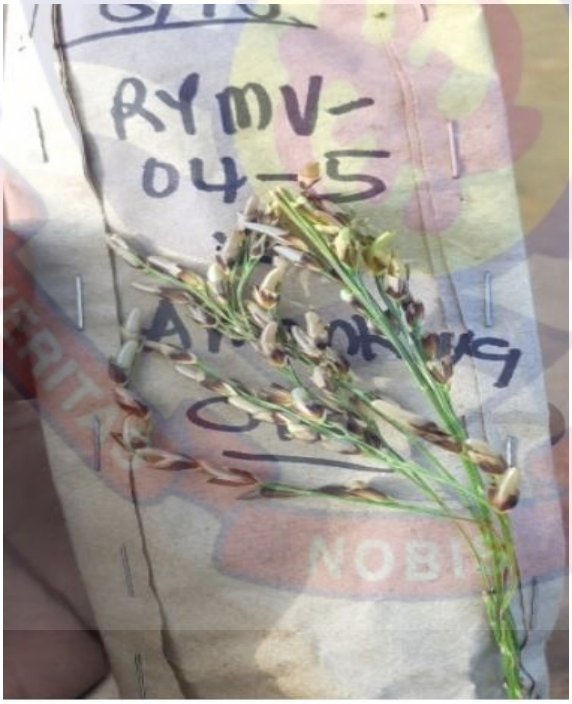

Figure 4.3: A sample of BC_1F_1 seeds produced from a cross between the F_1 line, RYMV-04-5 and its recurrent parent, CRI-Amankwatia

After genotyping the populations indicated in Table 4.3 to identify lines that were heterozygous for *rymv1-2* and *Pi54*, the results showed 157 lines in total were hets for *rymv1-2* whilst 176 lines were hets for *Pi54* (Table 4.4). Seventy-nine lines from the 157 BC_1F_1 lines were hets for both *rymv1-2* and *Pi54*. Eleven lines which were hets for both *rymv1-2* and *Pi54* from the four crosses were selected and backcrossed to the recurrent parents to produce BC_2F_1 lines. A total of 545 BC_2F_1 lines were produced from these crosses (Table 4.4). After genotyping these lines to identify hets for the two resistant genes, 269 lines out of these possessed *rymv1-2* whilst 242 lines possessed the *R* gene *Pi54*. Both genes were found in 122 lines (Table 4.4).

Twenty lines, heterozygous for both *rymv1-2* and *Pi54*, were selected from the BC_2F_1 population and were backcrossed to their respective recurrent parents to produce a BC_3F_1 population. A total of 239 lines were produced from the crosses between the selected BC_2F_1 lines and the recurrent parents (Table 4.4). When the 239 BC_3F_1 lines were genotyped, 129 lines were heterozygous for *rymv1-2* whilst 116 were hets for *Pi54*. The two resistance genes were found in 68 out of the 239 lines genotyped (Table 4.4). Sixteen lines out of the 68 lines heterozygous for both resistance genes were allowed to self to produce BC_3F_2 seeds.

Table 4.4: Generations of RYMV and blast-resistant lines obtained from crosses between donor parent and recurrent susceptible parents

Generation	Total Entries	*rymv1-2* (hets)	*Pi54* (hets)	*rymv1-2/ Pi54*	No. of lines for advance
F_1	34	30	30	30	8
BC_1F_1	361	157	176	79	11
BC_2F_1	545	269	242	122	20
BC_3F_1	239	129	116	68	15

hets: heterozygotes

Pedigree graphs for various lines was generated in the Breeding Management System (BMS). Figure 4.4 shows how a BC_3F_1 line, RYMV-B-01-6-37-1 with the two disease resistance genes was developed. The F_1 plant (RYMV-01) which resulted from a cross between Togo Marshall and Gigante was backcrossed to the recurrent parent, Togo Marshall, to produce the BC_1F_1 plants. Line 6 from the BC_1F_1s was selected and backcrossed to the recurrent parent to produce BC_2F_1s. Line 37 from the BC_2F_1 population was backcrossed to the recurrent parent to produce BC_3F_1 population (Figure 4.4). Line of this population was selfed to produce BC_3F_2 population. The result was generated from the Breeding Management System (BMS).

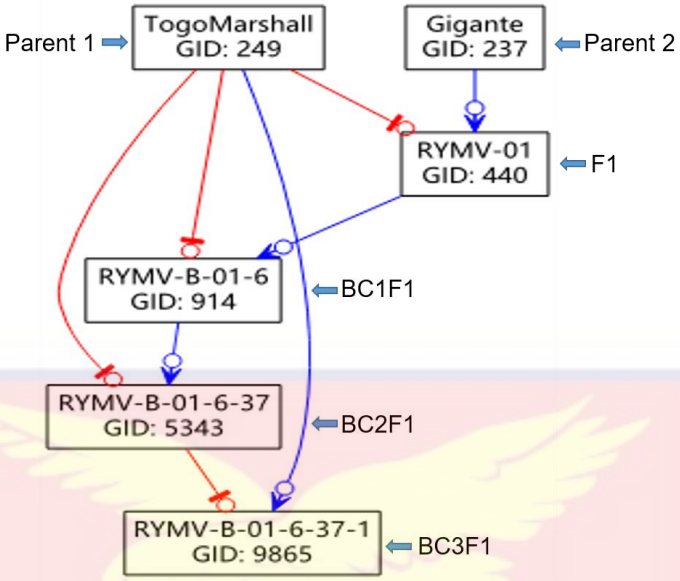

Figure 4.4: A pedigree graph generated from the Breeding Management System (BMS) showing how a BC_3F_1 line, RYMV-B-01-6-37-1 with the two resistance genes was developed

Foreground selection for *rymv1-2* and *Pi54* in a BC_3F_2 population

A population comprising 1,489 BC_3F_2 lines were generated from 16 BC_3F_1 lines that were hets for both *rymv1-2* and *Pi54*. After genotyping the population with 10 diagnostic markers, the results showed 372 and 350 lines were homozygous for *rymv1-2* and *Pi54*, respectively. Eighty-six lines were in a homozygous condition (fixed) for both *rymv1-2* and *Pi54* in the 1,489 BC_3F_2 population (Table 4.5).

Table 4.5: Progenies of foreground selection homozygous for *rymv1-2* alone, *Pi54* alone and *rymv1-2/Pi54* and the numbers used for advance

Generation	Entries	*rymv1-2* T:T	*Pi54* G:G	*rymv1-2/ Pi54* (T:T/G:G)	No. of lines for advance
BC_3F_2	1,489	372	350	86	71

The 71 lines which were advanced were fixed for *rymv1-2* and *Pi54* and comprised of combination of lines from the four recurrent parents. Breakdown of the 71 BC_3F_2 introgressed lines are as follows:

Togo Marshall x Gigante = 13 lines; Jasmine 85 x Gigante = 16 lines; CRI-Agra Rice x Gigante = 22 lines; CRI-Amankwatia x Gigante = 20 lines. Details of these BC_3F_2 derived lines are in Table 6.1 of Chapter 6 of this thesis.

Genetic control of the two disease resistance genes

Chi-square analysis was performed to find out whether the two disease resistance genes for rice blast and RYMV disease are controlled by a single gene, or that their inheritance is controlled by multiple genes. Based on the molecular results obtained from the BC_3F_2 population, a Chi-square value was computed and presented in Table 4.6.

The results show that the two genes are under single gene control. Thus, they follow simple Mendelian inheritance. The genotyping results indicated TT as homozygous for RYMV resistance and CC as not having the *rymv1-2* gene. For rice blast, GG showed resistance whilst AA showed the absence of the gene. From a population of 1,489 lines genotyped, 791 lines did not have resistance for both RYMV and rice blast. Two hundred and sixty-two lines had blast

resistance but not RYMV resistance, whilst 283 had *rymv1-2* but not *Pi54* (Table 4.6).

Table 4.6: Chi-square goodness of fit test for the inheritance of rymv1-2 and Pi54 in a BC_3F_2 population

Genotypes	C–: A–	C–:GG	TT:A–	TT:GG	Ratio	χ^2	P value
1,489	791	262	283	86	9:3:3:1	4.27	7.815

The Chi-square value was arrived at using the formula:

$$x^2 = \Sigma \frac{(Obs-Exp)2}{Exp}$$

$$= \Sigma \frac{(791-838)2}{838} + \frac{(262-279)2}{279} + \frac{(283-279)2}{279} + \frac{(86-93)2}{93}$$

$$= \Sigma \frac{(-47)2}{838} + \frac{(-17)2}{279} + \frac{(4)2}{279} + \frac{(-7)2}{93}$$

$$= \Sigma \frac{2{,}209}{838} + \frac{289}{279} + \frac{16}{279} + \frac{49}{93}$$

$$= 2.64 + 1.04 + 0.06 + 0.53$$

$$x^2 = 4.27$$

Since $x^2_{tab} > x^2_{cal}$ with 3 degrees of freedom at $\alpha=0.05$ the two disease resistance traits are under the control of single genes.

Background selection for BC_3F_2 derived lines using DArT markers

A total of 1,094 SNP makers were used to scan the background of the 71 BC_3F_2 derived lines to identify lines with high recurrent parental genome (RPG). The Diversity Array Technology (DArT) platform was used for the mid-density genotyping. The genotyping results showed 76% to 95% recurrent parental genome recovery in the RYMV-blast introgressed lines (Tables 4.7—4.10).

Thirteen BC_3F_2 lines derived from the cross between the donor parent Gigante and Togo Marshall produced the lowest RPG among the four crosses (76% to 86% RPG). Percentage heterozygosity ranged from 0.2% to 7.0% (Table 4.7). The genotyping results show three genotypes; RYMV-B-01-6-37-66, RYMV-B-01-6-37-4-10 and RYMV-B-01-31-12-12-75 have 87%, 85% and 86% of the recurrent parent genome, respectively. The rest of the genotypes had less that 85% of the recurrent parent genome.

Table 4.1: Genetic background of BC_3F_2 lines derived from cross between Togo Marshall and Gigante

SAMPLE NAME	NO. OF SNPS	% SNPS	HET COUNT	% HET	RPG TOTAL	RPG COVERAGE	DECISION
Togo Marshal	986	90.13	2	0.2	1.00	1	Recurrent parent
Gigante	994	90.86	3	0.3	0.00	1	Donor parent
RYMV-B-01-6-37-1-81	1002	91.59	16	1.6	0.83	1	No decision
RYMV-B-01-6-37-1-5	957	87.48	28	2.9	0.84	1	No decision
RYMV-B-01-6-37-66	1010	92.32	34	3.4	0.87	1	Select
RYMV-B-01-6-37-4-10	984	89.95	33	3.3	0.85	1	Select
RYMV-B-01-6-37-1-17	1007	92.05	34	3.4	0.82	1	No decision
RYMV-B-01-6-37-1-21	1004	91.77	20	2.0	0.80	1	No decision
RYMV-B-01-6-37-1-94	1002	91.59	70	7.0	0.78	1	No decision
RYMV-B-01-6-37-1-13	999	91.32	19	1.9	0.80	1	No decision
RYMV-B-01-31-12-12-69	1004	91.77	45	4.5	0.84	1	No decision
RYMV-B-01-6-37-1-27	1009	92.23	54	5.4	0.78	1	No decision
RYMV-B-01-6-37-1-91	995	90.95	36	3.6	0.76	1	No decision
RYMV-B-01-31-12-12-75	1002	91.59	17	1.7	0.86	1	Select
RYMV-B-01-6-37-1-37	1006	91.96	19	1.9	0.78	1	No decision

Sixteen genotypes were produced from the cross between Jasmine 85 and Gigante. These were also genotyped to identify lines with high RPG. Table 4.8 shows the results of the mid-density genotyping for this cross. Five genotypes contained more than 90% of the genome of the recurrent parent, Jasmine 85. Those are indicated as "selected" (Table 4.8). Percentage data count for this cross for the mid-density genotyping ranged from 90.8% to 93.0% whilst percentage heterozygosity ranged from 0.30% to 4.40% (Table 4.8).

Table 4.8: Genetic background of BC_3F_2 lines derived from cross between Jasmine 85 and Gigante

SAMPLE NAME	NO. OF SNPS	% SNPS	HET COUNT	% HET	RPG TOTAL	RPG COVERAGE	DECISION
Jasmine 85	993	90.80	3	0.30	1.00	1	Recurrent parent
Gigante	994	90.86	3	0.30	0.00	1	Donor parent
RYMV-B-02-20-24-13-53	1008	92.10	12	1.20	0.95	1	Select
RYMV-B-02-20-13-3-48	1003	91.70	4	0.40	0.88	1	No decision
RYMV-B-02-20-24-13-60	1015	92.80	11	1.10	0.93	1	Select
RYMV-B-02-20-13-3-50	1001	91.50	5	0.50	0.92	1	Select
RYMV-B-02-20-9-3-79	1000	91.40	34	3.40	0.86	1	No decision
RYMV-B-02-20-25-12-49	1005	91.90	13	1.30	0.87	1	No decision
RYMV-B-02-20-13-3-5	1017	93.00	12	1.20	0.90	1	No decision
RYMV-B-02-20-25-12-17	1012	92.50	29	2.90	0.90	1	No decision
RYMV-B-02-20-25-12-63	1008	92.10	28	2.80	0.89	1	No decision
RYMV-B-02-20-25-12-47	1004	91.80	30	3.00	0.88	1	No decision
RYMV-B-02-20-25-12-24	1004	91.80	33	3.30	0.88	1	No decision
RYMV-B-02-20-24-13-14	1008	92.10	19	1.90	0.93	1	Select
RYMV-B-02-20-9-3-91	1001	91.50	44	4.40	0.78	1	No decision
RYMV-B-02-20-25-12-30	1007	92.00	27	2.70	0.87	1	No decision
RYMV-B-02-20-13-3-19	1008	92.10	29	2.90	0.91	1	Select
RYMV-B-02-20-9-3-21	1006	92.00	21	2.10	0.83	1	No decision

Twenty-two BC_3F_2 lines derived from the cross between the donor parent Gigante and CRI-Agra Rice contained 78% to 92% of the recurrent parent genome (Table 4.9). Percentage data count for the mid-density genotyping for this cross ranged from 83.82% to 92.41%, whilst percentage heterozygosity ranged from 0.20% to 5.60%. The molecular results identified five BC_3F_2 which had more than 90% of the recurrent parent genome. These lines are marked as "selected" since they contain the highest RPG recovery (Table 4.9).

Table 4.2: Molecular results for background selection of BC_3F_2 lines derived from cross between CRI-Agra Rice and Gigante.

SAMPLE NAME	NO. OF SNPS	% SNPS	HET COUNT	% HET	RPG TOTAL	RPG COVERAGE	DECISION
CRI-Agra Rice	981	89.67	2	0.20	1.00	1	Recurrent parent
Gigante	994	90.86	3	0.30	0.00	1	Donor parent
RYMV-B-03-84-36-12-68	1006	91.96	18	1.80	0.84	1	No decision
RYMV-B-03-84-47-2-50	995	90.95	15	1.50	0.92	1	Select
RYMV-B-03-84-36-9-23	991	90.59	13	1.30	0.90	1	Select
RYMV-B-03-84-47-2-22	997	91.13	42	4.20	0.91	1	Select
RYMV-B-03-84-36-10-2	1009	92.30	7	0.70	0.91	1	Select
RYMV-B-03-84-36-10-33	1007	92.06	20	2.00	0.80	1	No decision
RYMV-B-03-84-36-12-15	1008	92.14	11	1.10	0.88	1	No decision
RYMV-B-03-84-36-12-71	1016	92.87	42	4.20	0.89	1	No decision
RYMV-B-03-84-36-9-51	1007	92.05	34	3.40	0.81	1	No decision
RYMV-B-03-84-47-2-96	994	90.86	14	1.40	0.90	1	No decision
RYMV-B-03-84-47-2-95	987	90.22	42	4.20	0.90	1	Select
RYMV-B-03-84-36-12-31	941	86.01	25	2.50	0.83	1	No decision
RYMV-B-03-84-36-9-38	997	91.13	15	1.50	0.81	1	No decision
RYMV-B-03-84-36-12-40	1013	92.60	13	1.30	0.81	1	No decision
RYMV-B-03-84-36-10-57	1011	92.41	34	3.40	0.87	1	No decision
RYMV-B-03-84-36-12-84	1007	92.05	41	4.10	0.79	1	No decision
RYMV-B-03-84-36-12-11	1010	92.32	56	5.60	0.86	1	No decision
RYMV-B-03-84-36-10-46	1010	92.32	47	4.70	0.78	1	No decision
RYMV-B-03-84-47-2-72	998	91.22	28	2.80	0.88	1	No decision
RYMV-B-03-84-36-10-71	917	83.82	34	3.40	0.83	1	No decision
RYMV-B-03-84-47-2-57	991	90.59	14	1.40	0.87	1	No decision
RYMV-B-03-84-36-12-76	998	91.23	21	2.10	0.85	1	No decision

The fourth cross was the cross between CRI-Amankwatia and Gigante to generate resistant lines for the two diseases. Twenty BC_3F_2 lines were generated from this cross. The result of the mid-density genotyping for background selection for these lines is indicated in Table 4.10. The mid-density genotyping results for the background selection for the introgressed BC_3F_2 lines with CRI-Amankwatia as the recurrent parent identified three genotypes that had 90% to 92% of the recurrent parent genome. Those are marked as "selected". Most of the genotypes had approximately 83% of the recurrent parent genome (Table 4.10). Effective SNP count for this cross ranged from 994 to 1,013 whilst percentage heterozygosity ranged from 0.20% to 5.00%.

Table 4.10: Genetic background of BC_3F_2 lines derived from cross between CRI-Amankwatia and Gigante

SAMPLE NAME	NO. OF SNPS	% SNPS	HET COUNT	% HET	RPG TOTAL	RPG COVERAGE	DECISION
CRI-Amankwatia	998	91.22	2	0.20	1.00	1	Recurrent parent
Gigante	994	90.86	3	0.30	0.00	1	Donor parent
RYMV-B-04-14-11-10-38	1008	92.14	5	0.50	0.79	1	No decision
RYMV-B-04-38-10-1-10	1005	91.86	17	1.70	0.84	1	No decision
RYMV-B-04-38-10-1-52	1010	92.32	25	2.50	0.92	1	Select
RYMV-B-04-38-10-13-7	1007	92.05	36	3.60	0.89	1	No decision
RYMV-B-04-14-11-10-74	1002	91.60	14	1.40	0.80	1	No decision
RYMV-B-04-38-10-13-78	1007	92.05	19	1.90	0.88	1	No decision
RYMV-B-04-38-10-1-8	1007	92.05	28	2.80	0.85	1	No decision
RYMV-B-04-14-11-10-86	1005	91.86	17	1.70	0.83	1	No decision
RYMV-B-04-38-10-13-93	1010	92.32	31	3.10	0.82	1	No decision
RYMV-B-04-38-10-1-18	1012	92.50	4	0.40	0.87	1	No decision
RYMV-B-04-38-10-13-82	1009	92.23	39	3.90	0.85	1	No decision
RYMV-B-04-14-11-10-22	1009	92.23	19	1.90	0.85	1	No decision
RYMV-B-04-38-10-1-25	1008	92.12	23	2.30	0.80	1	No decision
RYMV-B-04-38-10-13-12	995	90.95	22	2.20	0.83	1	No decision
RYMV-B-04-14-11-10-11	1000	91.41	17	1.70	0.81	1	No decision
RYMV-B-04-38-10-1-63	1007	92.05	19	1.90	0.82	1	No decision
RYMV-B-04-14-11-10-96	1010	92.32	26	2.60	0.90	1	Select
RYMV-B-04-38-10-1-64	1000	91.41	7	0.70	0.89	1	No decision
RYMV-B-04-38-10-13-80	1002	91.59	21	2.10	0.90	1	Select
RYMV-B-04-14-11-10-95	1013	92.60	50	5.00	0.86	1	No decision

Discussion

Breeding for resistance in crops is seen as one of the most effective ways in the control of plant diseases. It is less costly in the long-term as compared to the use of chemicals, and it has no adverse effect on the environment (Acquaah, 2012). Rice yellow mottle disease and rice blast, being the two most important rice diseases in sub-Saharan Africa (Madhavi et al., 2016; Agnoun et al., 2019; Asante et al., 2020a), cause significant yield losses that reduce rice productivity.

There are some released varieties that are tolerant to blast and RYMV diseases, though the impact of the two diseases is still noticeable on farmers' fields. More so, the most popular varieties in Ghana are susceptible to both diseases. Therefore, the successful introgression of resistant genes for RYMVD and blast into the four popular aromatic rice varieties in Ghana could lead to increased rice production.

The *R* gene *RYMV1 (rymv1-2)* is a single recessive gene that confers resistance to a number of isolates of the virus (Albar et al., 2006; Pidon et al., 2020). The blast resistance gene *Pi54*, on the other hand, is a dominant resistance gene that confers broad spectrum resistance to many isolates of *M. oryzae* (Kumari et al., 2013; Arunakumari et al., 2016). The four recurrent parents are aromatic, have comparatively higher yield potential (approximately 8 ton/ha), but are susceptible to these two major diseases. The choice of these four varieties was based on the fact that these varieties are among the most popular rice varieties grown in Ghana and also the fact that Ghanaian consumers prefer aromatic Jasmine-styled rice as compared to non-aromatic ones (Asante et al., 2015; Asante et al., 2020b).

The breeding scheme used to introgress the resistance genes into the background of the popular varieties is similar to the scheme adopted by Suwannual et al. (2017) and Xiao et al. (2016) when the authors pyramided two and four blast resistance genes respectively into selected susceptible rice backgrounds.

The F_1 hybridity test performed after the generation of the F_1 plants from the crosses between the donor variety, Gigante and the four susceptible varieties indicated that not all seeds produced from a cross between two parents are always "true" F_1s. According to Guo-Ling (2013), the purpose of hybridity test in marker-assisted selection is to eliminate false F_1s which would have erroneously been advanced to future generations. F_1 hybridity test is of immense importance because it saves the breeder from wasting resources to advance a line that is not a true hybrid. Also, the use of conversional methods to identify true hybrids is time-consuming and, in many cases, fail to identify true F_1s (Sundaram et al., 2008). Hence, DNA markers are highly preferred since they are accurate and precise (Pramanik et al., 2022). Thirty out of the 34 F_1 plants that were genotyped from the four crosses were true hybrids (Table 4.4). This gives high crossing (hybridity) accuracy of 88.24 %.

Crosses to generate BC_1F_1 lines for the four crosses were done after 8 true F_1s (two for each cross) were selected and backcrossed to their respective recurrent parents. This produced a total of 361 BC_1F_1 plants for genotyping to identify hets for both resistance genes (Table 4.4). The data produced from genotyping the BC_1F_1 population suggest that the two resistance genes (*rymv1-2* and *Pi54*) are under a single gene control since approximately 50% of the genotyped population were heterozygous for the two resistance genes (157 and

176 lines respectively). This assertion is confirmed from the Chi-square goodness of fit test that was performed using the molecular results from 1,489 BC_3F_2 populations (Table 4.6).

Crosses to generate the BC_2F_1 plants were done after selecting 11 true BC_1F_1 based on genotyping results. At the BC_2F_1 stage, most of the plants still looked tall and slender, with low tiller number, indicating that they were still having significant percentage of the donor parent's genome. The donor parent, Gigante, is characterized by slender culms, low tiller number and relatively tall culms. This meant that an additional backcross was needed in order to recover a larger percentage of the recurrent parents' genome. Theoretically, at BC_2F_1, 87.5% of the recurrent parental genome is expected to be in the backcross progeny (Acquaah, 2012). At BC_3F_1 this figure is expected to rise to 93.25% of the recurrent genome. This would make the introgressed lines close to the recurrent parents morphologically, but with an additional advantage of having the resistance genes for the two major diseases under study.

The BC_3F_1 lines generated from the crosses between the donor parent and the recurrent parents were relatively shorter than the BC_2F_1s and thus resembled the recurrent parents more than the previous generation. Agro-morphological data taken on these lines were not significantly different from the recurrent parents for most of the generated lines (data not shown). This gave the confidence that further selfing and subsequent background selection would produce lines that are morphologically and physiologically similar to the recurrent parents. This is in line with the work by Xiao et al. (2016) when the authors introgressed two blast resistance genes, *Pi46* and *Pita* into the background of an elite restorer line Hang-Hui-179 using marker-assisted

backcross breeding (MABB). Backcrossing the introgressed lines to BC_3F_2 enabled the authors recover over 92% of the recurrent parent genome.

The selfing that was carried to produce BC_3F_2 plants from the selected BC_3F_1 plants resulted in lines that were morphologically similar to their recurrent parents. The foreground selection that was performed produced a minimum of 13 and maximum of 22 introgressed lines per cross from the 1,489 BC_3F_2 lines genotyped (Table 4.5). Since the two resistance genes are monogenic in action based on the Chi-square goodness of fit test (Table 4.6), their expected genotypic ratio in the BC_3F_2 population is 1/16 (Acquaah, 2012). Thus, 93.06 out of the 1,489 BC_3F_2 were expected to carry the two resistance genes. After genotyping the lines, 86 out of the 1,489 lines carried the two *R* genes. This is closer to the expected number of 93.06. Seventy-one out of the 86 lines were evaluated for yield and yield-related traits. The rest were lost.

The mid-density genotyping that was performed using DArT markers to identify lines with high recurrent parent genome (RPG) gave varying results based on the recurrent parent type, but at the same time, served as an important exercise to identify the best lines for further evaluation. According to Miah et al. (2015), the adoption of marker-assisted backcrossing can aid in the recovery of up to 99% of the recurrent parent genome in just three backcrosses, whereas, if conventional methods are used, the same can only be achieved after six backcrosses. Thus, the use of MAS has helped shorten the breeding cycle to achieve high recurrent parental genome in just three backcrosses. Kim et al. (2021) recovered 97.4 – 99.1% of recurrent parent genome at BC_2F_1 when the authors used 386 genome-wide KASP-SNP markers for background selection of the recurrent parent Samgwang.

The current results for the background selection for the four recurrent parents gave varying results. For instance, the cross between Togo Marshall x Gigante produced BC_3F_2 lines that contained 76 – 86% of the genome of Togo Marshall (recurrent parent). This was the lowest recovery among the four crosses. The relatively low genome recovery recorded for this cross could be due to linkage drag associated with some traits from the donor parent in the backcross derivatives. Notwithstanding this issue of low genome recovery, three BC_3F_2 lines from this cross, viz, RYMV-B-01-6-37-66, RYMV-B-01-6-37-4-10 and RYMV-B-01-31-12-12-75 contained 87%, 85% and 86% of the recurrent parent genome, respectively. These three lines could be selected for further evaluation if they have comparatively high performance on the field.

The cross between Jasmine 85 and Gigante produced backcross derivatives that had higher RPG as compared to the derivatives from the cross between Togo Marshall and Gigante. The RPG percentage for this cross ranged from 78 – 95% with five genotypes recording RPG percentage ranging from 91 – 95% (Table 4.8). These five genotypes could be selected based on the mid-density genotyping results and further evaluated for their yield performance against the recurrent parent, Jasmine 85. The highest RPG recovery for this cross (95%) is encouraging, since it is higher than the theoretical RPG recovery of 93.25% at BC_3.

The third cross between CRI-Agra Rice and Gigante produced results similar to that of Jasmine 85 and Gigante. In that, the RPG recovery (78% to 92%) was higher than the cross between Togo Marshall and Gigante (Table 4.9). Five BC_3F_2 lines from this cross had RPG recovery ranging from 90 – 92%. These are marked as selected (Table 4.6) since they contain relatively higher

RPG from CRI-Agra Rice. These lines looked morphologically similar to AgraRice and could thus be evaluated further to check their performance against the check variety.

Similar results was also obtained between the last cross, CRI-Amankwatia and the donor parent, Gigante. The RPG recovery for this cross rangde from 79 – 92%. Three genotypes, RYMV-B-04-38-10-1-52, RYMV-B-04-14-11-10-96 and RYMV-B-04-38-10-13-80 had 92%, 90% and 90% of the RPG (Table 4.10). These were marked as selected since they had the highest RPG percentage recovery among the 20 BC_3F_2 lines which were genotyped for this cross. They could further be evaluated for their agronomic performance against the check, CRI-Amankwatia.

Conclusions

Breeding for resistance to diseases through marker-assisted backcross breeding is an effective way to introgress resistance into susceptible varieties. The use of molecular markers even helps eliminate false F_1s more effectively compared to the use of morphological markers since the latter is more subjective and could be influenced by the environment.

The F_1 hybridity test showed the need to verify F_1s before they are advanced to future generations. The results from this research showed 88.24% of the F_1 population were "true" F_1s whilst 11.76% were "false" F_1s. This test helped in the selection of true F_1s for further backcrosses.

The backcross results also showed that the two resistance genes *RYMV1 (rymv1-2)* and *Pi54* are monogenic in action based on the Chi-square goodness

of fit test performed. This validates earlier reports that *RYMV1 (rymv1-2)* and *Pi54* are under single gene control.

The mid-density genotyping performed to identify BC_3F_2 lines which had high recurrent parental genome recovery was important. The use of 1,094 SNPs for the background recovery helped identify genotypes that were similar to the recurrent parents and as such could be selected for further evaluation. The RPG for the genotypes ranged from 76 – 95% of among the derivatives of the four crosses. The highest RPG percentage was 95%. This figure is higher than the theoretical value of 93.25%. This was achieved in just three backcross cycles due of the use of MAS compared to conventional means, which would have taken six backcross cycles to achieve the same results.

The introgressed lines that have been selected based on the genotyping results could be further evaluated for their yield performance against the check varieties.

CHAPTER FIVE

SCREENING OF ELITE RICE GENOTYPES FOR REACTION TO *RICE YELLOW MOTTLE VIRUS* AND RICE BLAST DISEASES

Introduction

In sub-Saharan Africa, two major rice diseases, rice blast disease and Rice yellow mottle virus (RYMV) disease, contribute significantly to yield losses due to biotic stresses (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a). RYMV is particularly destructive, causing up to 100% yield losses depending on factors such as rice variety, infection timing, and disease management practices (Kouassi et al., 2005; Oludare et al., 2016; Agnoun et al., 2019). The symptoms of RYMV include stunted growth, yellow streaks on leaves, incomplete panicle emergence, and, in severe cases, plant death (IRRI, 2013).

RYMV is mainly spread by beetles of the *Chrysomelidae* family (Bakker, 1970). The disease is mainly prevalent in the transitional zone to the south of Ghana, though there have been recorded cases of RYMV in the northern parts of Ghana (Traore et al., 2015; Omiat et al., 2023)

Over one hundred blast resistance genes have been discovered and put into use in various breeding programmes (Sharma et al., 2012). Of these, *Pi54* has been found to confer single gene broad spectrum resistance to rice blast disease (Rai et al., 2011; Das et al., 2012; Kumari et al., 2013; Thakur et al., 2015).

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Rice blast disease starts by the development of lesions which comes about as a result of invasive hyphae in the tissue of the plant. Lesions develop in almost all parts of the rice plant, most especially, the leaves.

Breeding for disease resistance is one of the most effective ways to mitigate the effect of pathogens on crop plants in terms of yield because resistance genes are easy to deploy and they have no adverse effect on the environment (Acquaah, 2012). The purpose of this research was, therefore, to determine the reaction of lines that have been introgressed with resistance genes for Rice yellow mottle disease (*RYMV1,rymv1-2)* and rice blast disease (*Pi54*) to isolates of RYMV and *M. oryzae*.

Materials and methods

Study area

The research was conducted at the Rice Breeding Nursery (Screen house) of CSIR- Crops Research Institute, Fumesua-Kumasi, Ghana. The area is characterized by a bimodal rainfall pattern. The major season starts at the beginning of April and ends by the end of July whilst the minor season starts by the beginning of September and ends by early to mid-November. The average annual rainfall is 1,397 mm and the temperature ranges from 20.6°C to 33.3°C (Weather Atlas, 2023).

Plant materials

The plant materials used for the study consisted of 71 RYMV and blast resistant lines generated from crosses between Togo Marshall and Gigante, Jasmine 85 and Gigante, CRI-Agra Rice and Gigante, and, CRI-Amankwatia and Gigante.

Gigante has RYMV1 (rymv1-2) and Pi54 resistance genes (Sérémé et al., 2016). The four recurrent parents, Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia are among the most popular aromatic rice varieties in Ghana (Asante, 2013; Asante et al., 2020a). Even though these popular rice varieties are high-yielding, they are susceptible to blast and RYMV diseases.

Experimental design, seed planting and agronomic practices

The 71 resistant lines, their four recurrent parents, viz, Togo Marshall, Jasmine 85, CRI-Agra Rice and Amankwatia, as well as one susceptible check (Bouake 189), two resistant checks (Gigante and Tog7291) we nursed on 24th May, 2022 in horticulture plates. The experiment was laid in a 6 x 13 Alpha Lattice design with four replications. Rep 1 to 3 were inoculated whilst Rep 4 was used as the control (non-inoculated).

The seedlings were transplanted 14 days after sowing in the screen house of CSIR-CRI on 7th June, 2022. Irrigation water was supplied to make sure there was enough water in the soil at all times during the experiment. The recommended rates of fertilizer in Ghana for rice, which is 90 kg N: 60 kg P: 60 kg K, were applied.

Before transplanting, both pre-emergence and post-emergence herbicides were applied at recommended rates to make sure that the plots were free from weeds as well as rice seeds. Thereafter, weeds were controlled by hand-picking as and when they appeared.

RYMV inoculum preparation and inoculation

Isolates of RYMV were collected from the rice field at Sokwai in the Ashanti Region of Ghana where Omiat et al. (2023) detected the presence of the S2 strain of the virus. The authors also reported that the S2 strain is the most predominant strain of the virus in Ghana. The isolates were multiplied on the susceptible variety, Jasmine 85 to get enough inoculum to inoculate the test lines.

The infected Jasmine 85 leaves were collected and ground with cold distilled water at a ratio of 1:10 (w/v) using sterilized mortars and pestles as described by Thiémélé et al. (2010). After this, Carborundum powder was added to the preparation to aid in creating injury on the plant leaves to help in the penetration of the virus.

The first inoculum was applied at 21 days after sowing when the plants had achieved more than 3-leaf stage. The inoculum was applied by rubbing the leaves of the plant with the extract. One week after inoculation (28 DAP), the inoculum was applied again following the earlier procedure. This was done to ensure that there were no escapes during the first application.

Symptoms appearance and severity scores

Disease incidence and disease severity were recorded for each of the genotypes. Days to symptoms appearance was also recorded for each genotype. Appearance of symptoms and disease progression were recorded for the following days post-inoculation (dpi): 8dpi, 11dpi, 15pdi, 18dpi, 21dpi, 25dpi, 32dpi, 39dpi and 46dpi.

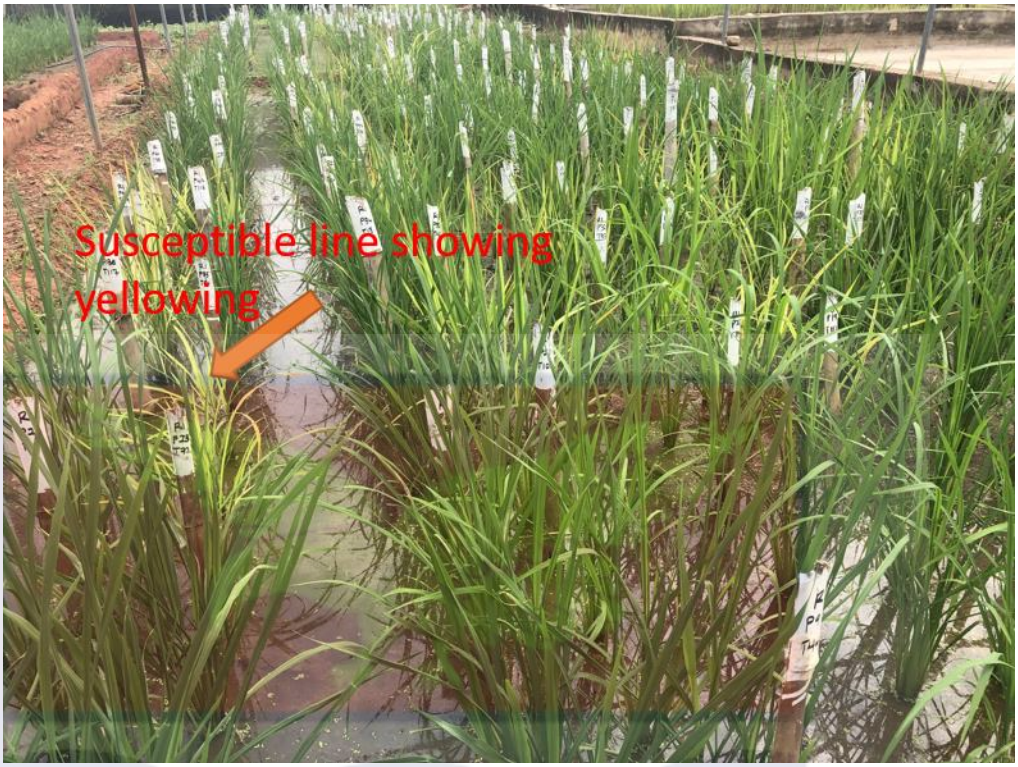

Figure 5.1: Inoculated field with susceptible lines showing signs of RYMV infection

Disease incidence was calculated following the formula used by Asante et al. (2020a):

$$I = \frac{PA}{PT} x100$$

Where I = disease incidence; PA = number of infected or dead plants; PT = total number of plants inoculated.

Disease severity scores were recorded for each genotype as indicated in the Standard Evaluation System for Rice (SES) where score 1= no symptom observed; 3= Leaves green but with sparse dots or streaks and a height reduction of less than 5%; 5= Leaves green or pale green with mottling and 6 – 25% height reduction and slight delay in flowering; 7= Pale yellow or yellow leaves with 26 – 75% height reduction, delayed flowering; 9= yellow or orange leaves with

more than 75% height reduction, no flowering or some dead plants (IRRI, 2013). The chart used for the disease scoring is shown in Figure 5.2.

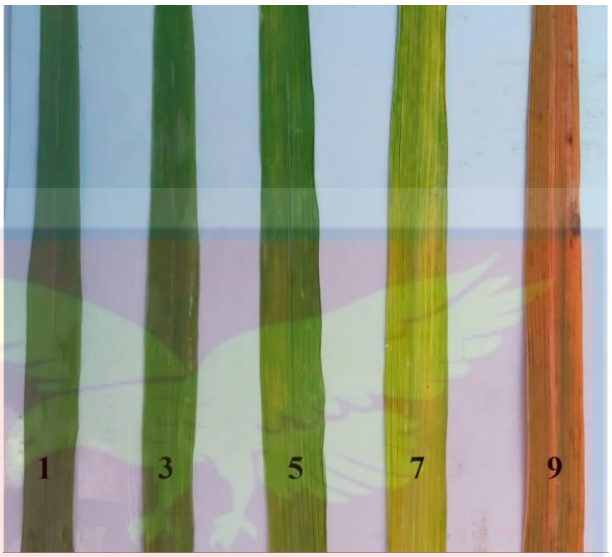

Figure 5.2: Disease severity scoring scale for RYMV. (Source: Asante et al., 2020a)

Disease severity was calculated for each genotype by following IRRI (2013) formula:

$$DI = \frac{n(3)+n(5)+n(7)+n(9)}{tn}$$

Where DI= disease index; n (3), n (5), n (7) and n (9) = number of plants that show reaction in a scale of 3, 5, 7 and 9, respectively; tn = total number of plants scored.

Serological assay

Double Antibody Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA) was performed to confirm the visual observations recorded for the various genotypes in reference to their reaction to the *Rice yellow mottle virus*. Polyclonal antibody that reacts positively with all known strains of the virus in West and Central Africa was obtained from DSMZ, Germany. It was used as

the coating antibody. The coating antibody was coupled with alkaline phosphate and used as the conjugate.

The following buffers were prepared and used in the ELISA test:

1. Coating buffer (pH 9.6); 2. Phosphate buffered saline (PBS, pH 7.4); 3. PBS-Tween (PBST); 4. Sample extraction buffer (pH 7.4); 5. Conjugate buffer; and 6. Substrate buffer. The buffers were prepared following the protocol by DSMZ, Germany, with reference to Clark and Adams (1977). The sample extraction and DAS-ELISA test were conducted at the Virology Lab of CSIR- Crops Research Institute, Kumasi, Ghana.

Leaf samples from all 78 entries, comprising 71 introgressed lines, 4 recurrent parents, the donor parent, one susceptible check and one resistant check were collected for the three replications which were inoculated. The leaf samples were collected at 28 dpi where viral activity was at its peak (Kouassi et al., 2005; Odongo et al., 2021).

The DAS-ELISA test was performed by first diluting 200 µl of the polyclonal antibody in 200 ml buffer at a recommended dilution rate of 1: 1000. After this, 200 µl was added to each well of the microliter plate. The plates were then covered and incubated at 37º C for 4hrs. The plates were then washed with PBS-Tween using a wash bottle. They were then soaked for a five minutes and washed was repeated two times. The plates were blotted by tapping them upside down on a tissue paper.

The samples were then extracted in the extraction buffer at the ratio of 1:20 (w/v). A volume of 200 µl of the aliquots of the test samples were added to duplicate wells. The plates were then covered and incubated overnight at 4º C. After this, the plates were washed three times using PBS-Tween. A volume

of 200 µl of the enzyme conjugate was then added to a conjugate buffer. The plates were then covered and incubated at 37º C for 4 hrs. After this, the plates were washed three times using PBS-Tween.

A volume of 200 µl aliquot of freshly prepared substrate was then added to each well. The plates were then covered and incubated at 37º C for 60 minutes. The reactions were assessed by measuring the spectrophotometric absorbance at 405 nm.

Scoring for rice blast disease

The screen house of the Rice Breeding Nursery is a hotspot for rice blast disease since a lot of blast screening has occurred there for the last five years. Thus, even though the genotypes were not inoculated with any race(s) of *M. oryzae*, the same was scored for the genotypes to at least give a preliminary view of the resistance of the genotypes to rice blast.

Data collection on yield and yield component traits

Data on yield component traits, such as tiller number, plant height and panicle number, were collected at maturity as described in the Standard Evaluation System for Rice (IRRI, 2013). Days to 50% flowering, days to maturity, yield per plant, fresh and dry biomass, grain length, grain width and seed colour were also measured for both the inoculated and non-inoculated plants. Grain yield was measured at 14% moisture content. An adjustment was made when the measured moisture content was not 14%.

The impact of the disease on the yield and yield component traits was calculated following the formula by Michel et al. (2008):

$$Im\ (\%) = \frac{(Ni-I)}{Ni} x100$$

Where Im = percentage disease impact; Ni = mean values of non-inoculated plants; I = mean values of inoculated plants.

Statistical analyses

Microsoft Excel version 2016 was used to collate the RYMV and blast disease scores after which severity scores were determined for each genotype using the same software. The same method was followed for scoring for blast. Disease impact was calculated using the same software based on the formula described by Michel et al. (2008).

Results

RYMV symptoms appearance and severity

One of the recurrent parents, Jasmine 85 and the susceptible check, Bouake 189 were the first to show symptoms of RYMV. It took just 8 days for these lines to show symptoms of RYMV (Table 5.1). The other three recurrent parents, CRI-Agra Rice, Togo Marshall, and CRI- Amankwatia, all took 11 days to show symptoms of RYMV infection (Table 5.1). Typical RYMV symptoms observed were yellowing with mottling, stunted growth of plants, orange-coloured leaves in highly susceptible plants and in some occasions, dead plants.

Figure 5.3: Newly developed RYMV resistant lines grown alongside susceptible recurrent parents Togo Marshall (A), Jasmine 85 (B), CRI-Agra Rice (C), CRI-Amankwatia (D). ***Image was captured 25 dpi.***

All the 71 introgressed lines, the donor parent, Gigante, and the resistant check, Tog7291, did not show any symptoms of RYMV throughout the scoring period which lasted for 46 days post-inoculation (dpi).

Serological assay

Results from the DAS-ELISA test showed the presence of the virus in some of the samples whilst others did not contain the virus or had very low viral loads in the leaf tissue. All the 71 RYMV-blast introgressed lines had ELISA test scores from 0.270 to 0.284. The buffer value was 0.270 whilst the water only value was 0.292 (Table 5.1). The value for the positive control was 0.456. The recurrent parent (Jasmine 85) had an ELISA test score of 0.463, above the positive control. Similar value was recorded for Togo Marshall (0.460). For CRI-Agra Rice and CRI-Amankwatia, the value was 0.459 (Table 5.1). The susceptible check (Bouake 189) was positive for ELISA with a score of 0.464.

Table 5.1: Reaction of 71 introgression lines and their checks to RYMV and blast diseases

Genotype	RYMV SEVERITY (1-9)	CLASS	ELISA SCORE (+0.456, buffer=0.270, H_2O=0.292)	ELISA REACTION	CLASS	DTSA	RYMV INCIDENCE	LEAF BLAST SEVERITY (0-9)
RYMV-B-01-6-37-1-21	1	HR	0.282	-	HR	NS	0.00%	0.11
RYMV-B-04-38-10-1-52	1	HR	0.271	-	HR	NS	0.00%	0.22
RYMV-B-04-38-10-13-7	1	HR	0.273	-	HR	NS	0.00%	0.22
RYMV-B-03-84-36-10-2	1	HR	0.284	-	HR	NS	0.00%	0.11
RYMV-B-03-84-36-12-76	1	HR	0.271	-	HR	NS	0.00%	1.11
RYMV-B-01-6-37-1-81	1	HR	0.273	-	HR	NS	0.00%	0.89
RYMV-B-03-84-36-10-57	1	HR	0.270	-	HR	NS	0.00%	0.22
RYMV-B-03-84-36-12-15	1	HR	0.274	-	HR	NS	0.00%	0.11
RYMV-B-03-84-36-12-40	1	HR	0.272	-	HR	NS	0.00%	0.13
RYMV-B-04-38-10-13-78	1	HR	0.273	-	HR	NS	0.00%	0.78
RYMV-B-03-84-36-9-51	1	HR	0.270	-	HR	NS	0.00%	0.22
RYMV-B-02-20-13-3-48	1	HR	0.275	-	HR	NS	0.00%	0.11
RYMV-B-04-38-10-13-82	1	HR	0.273	-	HR	NS	0.00%	0.78
RYMV-B-01-31-12-12-69	1	HR	0.274	-	HR	NS	0.00%	0.25
Tog7291	1	HR	0.274	-	HR	NS	0.00%	0.98
RYMV-B-04-38-10-13-12	1	HR	0.271	-	HR	NS	0.00%	0.43
Togo Marshall	6.66	S	0.460	++	S	11	100.00%	5.42
RYMV-B-03-84-36-12-31	1	HR	0.273	-	HR	NS	0.00%	0.15
RYMV-B-01-6-37-1-91	1	HR	0.272	-	HR	NS	0.00%	1.33
RYMV-B-01-6-37-1-94	1	HR	0.271	-	HR	NS	0.00%	0.44
RYMV-B-03-84-36-9-38	1	HR	0.271	-	HR	NS	0.00%	0.11

Genotype	RYMV SEVERITY (1-9)	CLASS	ELISA SCORE (+0.456, buffer=0.270, H_2O=0.292)	ELISA REACTION	CLASS	DTSA	RYMV INCIDENCE	LEAF BLAST SEVERITY (0-9)
RYMV-B-01-6-37-1-37	1	HR	0.274	-	HR	NS	0.00%	0.15
RYMV-B-04-38-10-1-18	1	HR	0.273	-	HR	NS	0.00%	0.52
RYMV-B-03-84-36-12-68	1	HR	0.275	-	HR	NS	0.00%	0.61
RYMV-B-04-38-10-1-63	1	HR	0.275	-	HR	NS	0.00%	0.42
RYMV-B-04-14-11-10-38	1	HR	0.277	-	HR	NS	0.00%	0.30
Jasmine 85 (CRI)	6.75	S	0.463	+++	HS	8	100.00%	4.71
RYMV-B-03-84-47-2-95	1	HR	0.278	-	HR	NS	0.00%	0.22
RYMV-B-02-20-25-12-24	1	HR	0.271	-	HR	NS	0.00%	0.40
RYMV-B-03-84-36-9-23	1	HR	0.278	-	HR	NS	0.00%	1.24
RYMV-B-01-6-37-1-17	1	HR	0.281	-	HR	NS	0.00%	0.21
RYMV-B-02-20-25-12-49	1	HR	0.280	-	HR	NS	0.00%	1.33
RYMV-B-03-84-36-10-33	1	HR	0.276	-	HR	NS	0.00%	0.61
RYMV-B-04-38-10-1-64	1	HR	0.275	-	HR	NS	0.00%	0.44
RYMV-B-03-84-47-2-96	1	HR	0.275	-	HR	NS	0.00%	0.11
RYMV-B-02-20-13-3-5	1	HR	0.279	-	HR	NS	0.00%	0.11
RYMV-B-04-14-11-10-74	1	HR	0.274	-	HR	NS	0.00%	0.22
RYMV-B-01-6-37-1-13	1	HR	0.272	-	HR	NS	0.00%	0.33
RYMV-B-04-14-11-10-11	1	HR	0.272	-	HR	NS	0.00%	0.26
RYMV-B-04-38-10-1-8	1	HR	0.281	-	HR	NS	0.00%	0.13
RYMV-B-03-84-47-2-57	1	HR	0.279	-	HR	NS	0.00%	0.70
RYMV-B-01-6-37-4-10	1	HR	0.277	-	HR	NS	0.00%	0.22
RYMV-B-02-20-24-13-60	1	HR	0.273	-	HR	NS	0.00%	0.44

Genotype	RYMV SEVERITY (1-9)	CLASS	ELISA SCORE (+0.456, buffer=0.270, H_2O=0.292)	ELISA REACTION	CLASS	DTSA	RYMV INCIDENCE	LEAF BLAST SEVERITY (0-9)
RYMV-B-02-20-13-3-50	1	HR	0.272	-	HR	NS	0.00%	0.11
RYMV-B-02-20-25-12-30	1	HR	0.270	-	HR	NS	0.00%	0.22
RYMV-B-04-14-11-10-22	1	HR	0.273	-	HR	NS	0.00%	0
CRI-Amankwatia	5.54	MR	0.459	++	S	11	100.00%	4.53
RYMV-B-03-84-36-10-46	1	HR	0.277	-	HR	NS	0.00%	0.11
RYMV-B-04-38-10-1-10	1	HR	0.278	-	HR	NS	0.00%	0.21
RYMV-B-01-6-37-66	1	HR	0.278	-	HR	NS	0.00%	0.33
RYMV-B-03-84-36-12-11	1	HR	0.277	-	HR	NS	0.00%	0.22
RYMV-B-04-38-10-1-25	1	HR	0.279	-	HR	NS	0.00%	0.33
RYMV-B-01-31-12-12-75	1	HR	0.278	-	HR	NS	0.00%	0.44
RYMV-B-02-20-25-12-17	1	HR	0.271	-	HR	NS	0.00%	0.17
RYMV-B-02-20-13-3-19	1	HR	0.276	-	HR	NS	0.00%	0.11
RYMV-B-02-20-24-13-53	1	HR	0.274	-	HR	NS	0.00%	0.22
RYMV-B-04-14-11-10-95	1	HR	0.273	-	HR	NS	0.00%	0.22
RYMV-B-03-84-47-2-50	1	HR	0.273	-	HR	NS	0.00%	0.11
RYMV-B-03-84-36-12-71	1	HR	0.278	-	HR	NS	0.00%	0.22
CRI-Agra Rice	5.79	MR	0.459	++	S	11	100.00%	4.56
Gigante	1	HR	0.275	-	HR	NS	0.00%	0.11
RYMV-B-01-6-37-1-5	1	HR	0.276	-	HR	NS	0.00%	0.22
RYMV-B-04-38-10-13-80	1	HR	0.279	-	HR	NS	0.00%	0.55
RYMV-B-03-84-47-2-72	1	HR	0.275	-	HR	NS	0.00%	0.33
RYMV-B-03-84-36-10-71	1	HR	0.281	-	HR	NS	0.00%	0.67

Genotype	RYMV SEVERITY (1-9)	CLASS	ELISA SCORE (+0.456, buffer=0.270, H_2O=0.292)	ELISA REACTION	CLASS	DTSA	RYMV INCIDENCE	LEAF BLAST SEVERITY (0-9)
RYMV-B-03-84-36-12-84	1	HR	0.274	-	HR	NS	0.00%	0.11
RYMV-B-02-20-9-3-91	1	HR	0.273	-	HR	NS	0.00%	0.23
RYMV-B-02-20-9-3-21	1	HR	0.272	-	HR	NS	0.00%	0.11
RYMV-B-04-14-11-10-86	1	HR	0.272	-	HR	NS	0.00%	0.56
RYMV-B-02-20-25-12-47	1	HR	0.278	-	HR	NS	0.00%	0.33
RYMV-B-02-20-24-13-14	1	HR	0.273	-	HR	NS	0.00%	0.11
RYMV-B-04-14-11-10-96	1	HR	0.278	-	HR	NS	0.00%	0.56
RYMV-B-02-20-25-12-63	1	HR	0.281	-	HR	NS	0.00%	1.00
RYMV-B-03-84-47-2-22	1	HR	0.280	-	HR	NS	0.00%	0.56
RYMV-B-01-6-37-1-27	1	HR	0.272	-	HR	NS	0.00%	0.11
RYMV-B-04-38-10-13-93	1	HR	0.273	-	HR	NS	0.00%	0.33
RYMV-B-02-20-9-3-79	1	HR	0.271	-	HR	NS	0.00%	0.11
BOUAKE 189	6.94	S	0.464	+++	HS	8	100.00%	5.33
Mean	**1.32**	**N/A**	**0.287**	**N/A**	**N/A**	**0.63**	**N/A**	**0.66**
Min	**1**	**N/A**	**0.270**	**N/A**	**N/A**	**0**	**0.00**	**0**
Max	**6.75**	**N/A**	**0.464**	**N/A**	**N/A**	**11**	**100.00**	**5.42**
S.E	**0.14**	**N/A**	**0.005**	**N/A**	**N/A**	**0.28**	**0.028**	**0.13**

DTSA = Days to Symptom Appearance, "-"= Highly resistant, "+"= Moderately resistant, "++"= Susceptible, "+++"= Highly susceptible

Rice leaf blast severity scores

The screen house where the RYMV screening was done is also a hotspot for rice blast disease. Thus, in addition to the screening for RYMV, scoring for rice blast disease was done for the 71 introgression lines, their four checks, the donor parent, and two other checks.

All 71 resistant lines had leaf blast severity scores ranging from 0 to 1.33 (Table 5.1). The donor parent (Gigante) and the resistant check (Tog7291) had severity scores of 0.11 and 0.98, respectively. The recurrent parents (Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia) had comparatively higher severity scores. That is, 5.42, 4.71, 4.56 and 4.53, respectively (Table 5.1).

Impact of RYMV on yield and yield-related traits

The impact of the disease on eight yield and yield-related traits were recorded post-inoculation. The data were taken when the plants had reached maturity as defined in the Standard Evaluation System for Rice (IRRI, 2013). In the case of the susceptible varieties, the disease had significant impact on all yield and yield-related traits measured (Table 5.2).

For tiller number, the minimum impact was -34.21% which was recorded for the introgression line RYMV-B-01-6-37-1-94, whilst the greatest impact was 91.67% recorded for the recurrent parent, Togo Marshall. All four recurrent parents had impacts ranging from 33.33% to 91.67% (Table 5.2). Similar trend was found in reference to panicle number. The lowest impact was

-28.79% recorded for the resistant line Tog7291, whilst the greatest impact was 96.40% recorded for Togo Marshall. The impact on the recurrent parents ranged from 33.33% to 96.40% (Table 5.2).

For dry biomass, the introgression line RYMV-B-02-20-25-12-63 had the least impact (-21.60%). The highest impact of the disease on dry biomass was recorded for the recurrent parent Togo Marshall. That is, 92.86% dry weight reduction (Table 5.2).

On yield per plant measured in grams, the disease had the greatest impact on the recurrent parent Togo Marshall (99.53% reduction in yield), whilst the least impact on yield was recorded for the introgressed line, RYMV-B-03-84-47-2-50 (-37.99% impact on yield). All the RYMV resistant lines had yield reduction from -37.99% to 29.20% (Table 5.2).

Table 5.2: Impact of RYMV on yield and yield-related traits of the 71 introgression lines and their checks measured as percentage reduction

GENOTYPE	TILLER NO.	PANICLE NO.	PLANT HEIGHT	PANICLE LENGTH	50%_FLW	MAT	DRY_BIO	YLD_PLT
RYMV-B-01-6-37-1-81	-8.11±0.76	-9.80±1.26	-1.72±0.51	5.06±0.23	6.26±0.71	4.61±0.48	3.03±0.63	6.40±0.24
RYMV-B-01-6-37-1-5	13.81±1.74	20.77±2.22	0.67±0.24	-4.20±0.82	1.79±0.20	6.54±0.70	10.82±0.25	10.85±0.74
RYMV-B-01-6-37-66	-7.81±0.73	-9.14±1.19	1.63±0.13	4.61±0.18	-1.45±0.17	-0.17±0.07	-0.36±1.02	-17.92±2.54
RYMV-B-01-6-37-4-10	0.71±0.24	2.96±0.19	1.79±0.11	6.00±0.34	2.63±0.30	3.33±0.33	-1.15±1.11	4.14±0.02
RYMV-B-01-6-37-1-17	-9.22±0.89	-2.17±0.39	-7.08±1.12	7.58±0.52	-3.60±0.41	-3.24±0.42	13.49±0.56	-10.58±1.70
RYMV-B-01-6-37-1-21	-12.00±1.20	-4.35±0.64	-0.65±0.39	-6.29±1.06	-1.45±0.17	-0.50±0.10	2.11±0.74	-8.26±1.43
RYMV-B-01-6-37-1-94	-34.21±3.73	-23.68±2.84	0.32±0.28	-6.23±1.05	0.59±0.06	-0.78±0.14	4.28±0.49	-6.58±1.24
RYMV-B-01-6-37-1-13	10.87±1.40	14.81±1.54	-1.87±0.52	-9.24±1.40	0.44±0.05	1.38±0.11	-4.69±1.51	8.33±0.46
RYMV-B-01-31-12-12-69	-12.18±1.22	-8.33±1.09	-3.95±0.76	-4.42±0.85	4.42±0.50	1.08±0.08	-0.28±1.01	29.20±2.83
RYMV-B-01-6-37-1-27	-16.67±1.74	-15.69±1.93	0.82±0.22	2.44±0.07	-1.44±0.17	-2.00±0.28	1.93±0.76	-10.85±1.73
RYMV-B-01-6-37-1-91	3.76±0.59	6.67±0.62	-1.58±0.49	-6.97±1.14	-0.70±0.08	2.40±0.23	4.63±0.45	2.25±0.24
RYMV-B-01-31-12-12-75	-7.69±0.71	-6.31±0.86	5.27±0.29	-1.87±0.56	4.05±0.46	4.18±0.43	9.86±0.14	13.98±1.10
RYMV-B-01-6-37-1-37	27.69±3.32	22.40±2.41	-2.13±0.56	-3.81±0.78	3.13±0.35	2.32±0.22	8.21±0.04	-3.96±0.95
RYMV-B-02-20-24-13-53	3.97±0.62	0.85±0.05	-4.74±0.85	-0.76±0.43	0.32±0.03	-1.96±0.27	-0.91±1.08	1.56±0.32
RYMV-B-02-20-13-3-48	-10.26±1.00	-3.85±0.58	-4.44±0.82	1.75±0.15	-1.12±0.13	-2.61±0.35	2.48±0.70	11.47±0.81
RYMV-B-02-20-24-13-60	-1.67±0.03	0.88±0.04	1.09±0.19	0.38±0.30	1.90±0.21	0.33±0.01	-0.67±1.05	5.01±0.81
RYMV-B-02-20-13-3-50	0.00±0.16	23.61±2.55	-5.61±0.95	2.04±0.11	-0.80±0.09	0.00±0.05	0.33±0.94	8.33±0.46
RYMV-B-02-20-9-3-79	5.56±0.80	10.00±1.00	5.94±0.36	-0.52±0.40	-0.33±0.04	0.17±0.03	-0.93±1.09	-8.16±1.42
RYMV-B-02-20-25-12-49	-4.88±0.39	-0.85±0.24	3.80±0.12	2.49±0.06	2.74±0.31	5.30±0.56	-0.23±1.01	-6.50±1.23
RYMV-B-02-20-13-3-5	-3.70±0.26	2.47±0.14	-8.59±1.29	0.62±0.27	2.12±0.24	0.73±0.03	0.26±0.95	8.59±0.49
RYMV-B-02-20-25-12-17	0.00±0.16	-4.04±0.60	6.67±0.45	6.60±0.41	0.79±0.09	0.53±0.01	-7.67±1.85	-6.27±1.21
RYMV-B-02-20-25-12-63	8.08±1.08	6.25±0.57	1.05±0.19	-2.96±0.68	-0.21±0.03	0.23±0.02	-21.60±3.44	16.80±1.42
RYMV-B-02-20-25-12-47	-9.26±0.89	-6.86±0.93	-2.08±0.55	10.68±0.87	-1.16±0.13	-2.00±0.28	25.47±1.92	4.64±0.03
RYMV-B-02-20-25-12-24	-5.56±0.47	-9.09±1.18	-9.54±1.40	-0.20±0.37	1.01±0.11	1.22±0.09	-7.67±1.85	-17.92±2.54
RYMV-B-02-20-24-13-14	-18.75±1.97	-15.05±1.86	-7.47±1.16	-0.45±0.40	1.46±0.16	1.40±0.11	-12.77±2.43	-29.70±3.88
RYMV-B-02-20-9-3-91	-5.71±0.49	-2.86±0.47	-10.71±1.53	6.18±0.36	1.82±0.20	3.28±0.33	24.07±1.76	-9.71±1.60

GENOTYPE	TILLER NO.	PANICLE NO.	PLANT HEIGHT	PANICLE LENGTH	50%_FLW	MAT	DRY_BIO	YLD_PLT
RYMV-B-02-20-25-12-30	-13.64±1.39	-3.17±0.51	5.00±0.26	1.43±0.18	-3.74±0.43	-0.87±0.15	4.03±0.52	0.00±0.49
RYMV-B-02-20-13-3-19	5.19±0.76	1.59±0.04	0.56±0.25	12.98±1.13	0.53±0.06	1.41±0.11	-1.34±1.13	-10.48±1.69
RYMV-B-02-20-9-3-21	0.00±0.16	1.01±0.03	2.57±0.02	2.91±0.01	-0.51±0.06	-3.76±0.48	4.21±0.50	-2.67±0.80
RYMV-B-03-84-36-12-68	-3.92±0.28	-1.33±0.30	-1.28±0.46	10.29±0.83	1.89±0.21	0.47±0.01	7.14±0.16	23.54±2.19
RYMV-B-03-84-47-2-50	-25.49±2.74	-22.22±2.68	2.85±0.01	-3.84±0.78	-2.01±0.23	0.00±0.05	-3.44±1.37	-37.99±4.82
RYMV-B-03-84-36-9-23	-11.46±1.14	2.08±0.09	-22.10±2.83	-3.04±0.69	0.11±0.01	1.52±0.13	-0.32±1.01	-10.86±1.73
RYMV-B-03-84-47-2-22	-10.75±1.06	-7.78±1.03	0.99±0.20	1.39±0.19	1.38±0.15	-2.21±0.30	-0.89±1.08	-9.60±1.59
RYMV-B-03-84-36-10-2	-32.41±3.53	-27.62±3.29	-1.86±0.52	7.94±0.56	3.47±0.39	5.32±0.56	11.87±0.37	-22.69±3.08
RYMV-B-03-84-36-10-33	-12.20±1.23	-6.67±0.90	8.88±0.70	2.95±0.01	-0.43±0.05	-2.19±0.30	-20.24±3.29	11.66±0.83
RYMV-B-03-84-36-12-15	-19.70±2.08	-15.15±1.87	2.54±0.02	-0.01±0.35	-0.33±0.04	3.52±0.35	8.21±0.04	-9.38±1.56
RYMV-B-03-84-36-12-71	-6.48±0.57	0.00±0.14	-3.40±0.70	1.84±0.14	3.15±0.36	2.37±0.22	4.84±0.43	14.56±1.17
RYMV-B-03-84-36-9-51	-10.00±0.98	-13.48±1.68	0.23±0.29	-2.64±0.65	3.76±0.43	3.19±0.32	-1.41±1.14	-18.62±2.62
RYMV-B-03-84-47-2-96	9.17±1.21	10.81±1.09	2.37±0.04	5.81±0.32	2.49±0.28	0.48±0.01	7.20±0.16	11.86±0.86
RYMV-B-03-84-47-2-95	-19.30±2.04	-5.26±0.74	5.88±0.36	8.86±0.66	1.78±0.20	0.48±0.01	-1.87±1.19	-3.07±0.84
RYMV-B-03-84-36-12-31	2.67±0.47	5.33±0.46	-7.35±1.15	-6.63±1.10	3.62±0.41	5.28±0.55	12.55±0.45	-0.95±0.60
RYMV-B-03-84-36-9-38	2.22±0.42	8.89±0.87	-2.16±0.56	14.13±1.26	1.59±0.18	1.54±0.13	4.27±0.49	5.13±0.09
RYMV-B-03-84-36-12-40	26.28±3.16	26.80±2.91	-9.93±1.44	-1.59±0.53	1.64±0.19	2.76±0.27	16.51±0.90	7.78±0.39
RYMV-B-03-84-36-10-57	-8.11±0.76	-4.63±0.67	11.38±0.98	6.44±0.39	1.59±0.18	0.00±0.05	18.91±1.18	-7.94±1.40
RYMV-B-03-84-36-12-84	-8.33±0.79	-9.65±1.24	17.49±1.68	11.30±0.94	-2.68±0.31	-0.41±0.09	11.29±0.31	-7.25±1.32
RYMV-B-03-84-36-12-11	17.02±2.10	18.12±1.92	4.18±0.16	-1.71±0.54	-2.53±0.29	-2.23±0.30	20.33±1.34	-8.92±1.51
RYMV-B-03-84-36-10-46	3.17±0.53	4.88±0.41	-3.08±0.66	0.68±0.27	-3.00±0.34	-2.08±0.28	8.09±0.06	11.24±0.79
RYMV-B-03-84-47-2-72	6.25±0.88	5.56±0.49	-1.03±0.43	7.58±0.52	5.64±0.64	4.02±0.41	12.12±0.40	5.50±0.13
RYMV-B-03-84-36-10-71	-18.80±1.98	-13.16±1.64	-2.35±0.58	-2.10±0.58	2.30±0.26	0.16±0.03	-5.19±1.57	-9.14±1.54
RYMV-B-03-84-47-2-57	-1.96±0.06	-6.25±0.86	-1.61±0.50	4.64±0.18	-1.37±0.16	3.24±0.32	-4.94±1.54	5.52±0.14
RYMV-B-03-84-36-12-76	-13.89±1.42	-12.38±1.55	-4.82±0.86	0.07±0.34	-3.77±0.43	-3.73±0.47	38.62±3.42	-18.96±2.65
RYMV-B-04-14-11-10-38	-5.88±0.51	-2.94±0.48	-9.88±1.44	2.45±0.07	-1.84±0.21	-0.79±0.14	-1.57±1.16	4.07±0.03
RYMV-B-04-38-10-1-10	-25.00±2.68	-18.10±2.21	6.95±0.48	-1.80±0.55	3.60±0.41	3.17±0.31	-4.67±1.51	-2.74±0.81
RYMV-B-04-38-10-1-52	5.05±0.74	4.30±0.35	11.17±0.96	3.95±0.10	1.59±0.18	1.27±0.10	-2.42±1.26	-4.76±1.04
RYMV-B-04-38-10-13-7	6.98±0.96	11.63±1.18	6.11±0.38	3.83±0.09	0.43±0.05	1.22±0.09	9.78±0.14	-0.79±0.58

GENOTYPE	TILLER NO.	PANICLE NO.	PLANT HEIGHT	PANICLE LENGTH	50%_FLW	MAT	DRY_BIO	YLD_PLT
RYMV-B-04-14-11-10-74	0.81±0.26	4.88±0.41	1.38±0.15	0.39±0.30	3.82±0.43	0.00±0.05	13.02±0.50	-4.65±1.02
RYMV-B-04-38-10-13-78	-15.43±1.59	-18.95±2.30	0.38±0.27	-8.53±1.32	4.02±0.46	0.00±0.05	-4.63±1.51	-7.31±1.33
RYMV-B-04-38-10-1-8	-7.41±0.68	-5.66±0.79	-0.84±0.41	-9.82±1.46	0.62±0.07	0.00±0.05	-0.53±1.12	-2.09±0.73
RYMV-B-04-14-11-10-86	0.93±0.27	1.90±0.07	-0.37±0.35	-6.45±1.08	1.33±0.15	0.50±0.01	-1.27±1.12	-5.46±1.12
RYMV-B-04-38-10-13-93	2.28±0.42	3.29±0.23	1.09±0.19	-14.79±2.03	5.82±0.66	4.38±0.45	3.61±0.57	0.39±0.45
RYMV-B-04-38-10-1-18	-11.46±1.14	-2.08±0.38	5.52±0.32	2.94±0.01	-1.23±0.14	0.08±0.04	2.44±0.70	0.96±0.38
RYMV-B-04-38-10-13-82	0.00±0.16	-1.04±0.26	-0.63±0.38	4.04±0.11	-4.71±0.54	0.51±0.01	-9.23±2.03	-17.65±2.50
RYMV-B-04-14-11-10-22	16.00±1.99	15.97±1.68	-8.86±1.32	-0.90±0.45	4.14±0.47	3.12±0.31	0.81±0.89	-1.94±0.71
RYMV-B-04-38-10-1-25	-22.22±2.37	-17.17±2.10	0.00±0.31	6.04±0.34	-0.42±0.05	2.56±0.24	1.49±0.81	-4.11±0.96
RYMV-B-04-38-10-13-12	-9.38±0.90	-12.64±1.58	-3.69±0.73	0.87±0.25	2.24±0.25	2.76±0.27	3.21±0.61	11.52±0.82
RYMV-B-04-14-11-10-11	-6.25±0.55	-4.17±0.62	-7.81±1.20	2.95±0.01	-2.52±0.29	-0.76±0.13	6.39±0.25	-1.46±0.66
RYMV-B-04-38-10-1-63	-22.52±2.40	-22.55±2.71	-5.08±0.89	-3.70±0.77	4.18±0.47	1.45±0.12	4.48±0.47	-8.13±1.42
RYMV-B-04-14-11-10-96	-8.33±0.79	-10.10±1.29	4.94±0.25	0.12±0.33	0.94±0.10	-0.40±0.09	1.36±0.82	11.19±0.78
RYMV-B-04-38-10-1-64	5.56±0.80	6.67±0.62	0.53±0.25	9.71±0.76	-0.73±0.09	3.05±0.30	5.20±0.39	-10.51±1.69
RYMV-B-04-38-10-13-80	2.96±0.50	2.33±0.12	4.23±0.17	-3.71±0.77	-0.11±0.02	-0.58±0.11	0.00±0.98	-4.76±1.04
RYMV-B-04-14-11-10-95	-30.77±3.34	-23.42±2.81	-0.76±0.40	-0.74±0.43	-5.08±0.58	-3.10±0.40	3.94±0.53	-8.23±1.43
Togo Marshall	91.67±10.61	96.40±10.84	86.75±9.57	67.60±7.36	-16.72±1.91	-14.21±1.67	92.86±9.60	99.53±10.85
Jasmine 85 (CRI)	35.19±4.17	60.19±6.72	43.39±4.63	32.59±3.37	-13.38±1.53	-2.33±0.31	91.36±9.43	96.73±10.53
CRI-Agra Rice	33.33±3.96	33.33±3.66	34.41±3.61	21.41±2.09	-8.25±0.94	-1.85±0.26	82.35±8.41	91.24±9.90
CRI-AMANKWATIA	58.70±6.85	66.67±7.45	40.17±4.27	17.94±1.70	-5.76±0.66	-4.63±0.58	89.86±9.26	97.62±10.63
Gigante	-12.58±1.27	-14.58±1.81	-1.29±0.46	-4.70±0.88	0.14±0.01	-1.46±0.21	12.56±0.45	4.29±0.00
BOUAKE 189	41.23±4.86	47.37±5.25	42.42±4.52	25.64±2.58	-8.52±0.97	-5.15±0.63	66.67±6.62	92.46±10.04
Tog7291	1.83±0.37	-28.79±3.42	2.53±0.02	0.55±0.28	0.34±0.04	0.06±0.04	0.24±0.95	-0.69±0.57
Min	**-34.21**	**-28.79**	**-22.10**	**-14.79**	**6.26**	**6.54**	**-21.60**	**-37.99**
Max	**91.67**	**96.40**	**86.75**	**67.60**	**-16.72**	**-14.21**	**92.86**	**99.53**
MSD	**68.02**	**63.34**	**24.36**	**19.67**	**9.46**	**8.85**	**27.20**	**57.52**

DRY BIO: Dry biomass; MSD: Mean Standard Deviation.

Discussion

Breeding for disease resistance is one of the most effective ways of increasing crop resistance to biotic stresses (Odongo et al., 2019). However, lines that have been introgressed with *R* genes must be tested to see how they would react in the presence of the pathogen. This would determine the effectiveness or otherwise on the introgressed genes. This research was therefore aimed at screening 71 lines that have been bred for resistance to RYMVD and blast for their reaction to the two diseases. Specifically, the lines were introgressed with *RYMV1 (rymv1-2)* and *Pi54*, which are both popular *R* genes for RYMV and blast resistances, respectively.

Scoring for RYMV was done on nine different occasions post-inoculation. All the 71 introgressed lines and their resistant checks (Gigante and Tog7291) did not show any symptoms of the disease. The resistance level displayed by the resistant checks is line with the findings of Sérémé et al. (2016), Asante et al. (2020a) and Anato et al. (2021), when their lines were subjected to different isolates of RYMV. Since the 71 introgression lines had their RYMV resistance gene from Gigante, it was not strange that they were also highly resistant to the disease just like their donor parent, which did not show any symptoms of RYMV up 46 dpi (Table 5.1).On the other hand, the four recurrent parents (Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia) showed susceptibility at various levels to the RYMV isolate. Jasmine 85, was the first genotype that showed symptoms of the disease at 8 dpi (Table 5.1). This is in agreement with an earlier study which reported that Jasmine 85 shows symptoms to RYMV 8 days post-inoculation (Asante et al. 2020a). Jasmine 85 had a severity score of 6.75 and it was thus confirmed as "susceptible"

according to the Standard Evaluation System for Rice (IRRI, 2013). Asante et al. (2020a) also found this variety to be susceptible to RYMV.

The three other recurrent parents, viz, Togo Marshall, CRI-Agra Rice and CRI-Amankwatia, had severity scores of 6.66, 5.79 and 5.54, respectively, and started showing symptoms of RYMV at 11 dpi (Table 5.1). These scores indicate that Togo Marshall is susceptible whilst CRI-Agra Rice and CRI-Amankwatia are moderately resistant. Asante et al. (2020a) also found CRI-Amankwatia as being moderately resistant to RYMV. The susceptible check, Bouake 189 also showed symptoms of RYMV at 8 dpi just like Jasmine 85 (Table 5.1). This genotype had a severity score of 6.94, confirming its susceptibility to RYMV. Various authors, such as Onasanya et al. (2006) and Michel et al. (2008), have also reported that Bouake 189 is susceptible to RYMV disease.

The ELISA test showed that Gigante and Tog7291, as well as the 71 introgressed lines were negative for the *Rice yellow mottle virus* (Table 5.1). Sérémé et al. (2016) and Asante et al. (2020a) found Gigante and Tog7291 to be negative for ELISA. Since the 71 introgression lines had the resistant gene *RYMV1 (rymv1-2)* from Gigante, this gene conferred resistance to the 71 resistant progenies. Hence, they turned out to be negative for *RYMV* in the ELISA test just like the donor parent. However, the vertical resistance found in Gigante has been reported to breakdown in the presence of some isolates of RYMV (Fargette et al., 2002; Traoré et al., 2006). Some of the isolates of RYMV from Ghana are known to break down the resistance in Gigante. Hence, there is the need to pyramid other RYMV genes into popular varieties to make the resistance more durable.

The ELISA test result confirmed that the four recurrent parents, Togo Marshall, Jasmine 85, CRI-Agra Rice, CRI-Amankwatia and the susceptible check, Bouake 189 were positive for *RYMV*. This is in line with the symptoms observed and scored in those lines (Table 5.1). The test result classified Jasmine 85 and Bouake 189 as "Highly susceptible", and the rest as "Susceptible". Asante et al. (2020a) rather found Jasmine 85 as "Susceptible" and CRI-Amankwatia as "Moderately resistant" when they performed ELISA test on these genotypes. The differences in the scoring here could be due to the different growing conditions of these lines as well as the nutrient content of the soil. However, Odongo et al. (2019) found Bouake 189 to be highly susceptible to *RYMV* when they performed ELISA test on this variety, which has been confirmed by the current findings.

One important part of this research was to evaluate the impact of the disease on yield and yield component traits. RYMV has been found as a disease that causes reduction in plant height, making the rice plant stunted, delays flowering and maturity, causes incomplete emergence of panicles, and in some cases, even death of plants (IRRI, 2013). It was therefore important to find out how the disease impacted on these characters of the progenies identified as resistant.

For tillering, the highest impact of the disease on the 71 introgression lines was 27.6% recorded for the genotype RYMV-B-01-6-37-1-37 compared to 91.67% recorded for the recurrent parent, Togo Marshall (Table 5.2). The same trend run through with the other yield-related traits. The impact of the disease was not significant for the 71 newly developed lines and their resistant checks, but was significant for the susceptible checks (Table 5.2).

For days to flowering, the greatest impact should be negative whilst the minimum impact should be positive since the disease causes delay in flowering (IRRI, 2013) and, as such, the control (non-inoculated) should flower earlier than the inoculated, if the disease was to have any effect on the plants. The same principle applies to maturity date. The results showed that the minimum impact was 6.26% for the introgression line RYMV-B-01-6-37-1-81 whilst the maximum impact was -16.72% recorded for Togo Marshall (Table 5.2). Togo Marshall again had the maximum impact of the disease on maturity date (-14.21%) whilst the introgression line RYMV-B-01-6-37-1-5 had the lowest impact (6.54%). This implies that, the disease had negative impact on flowering date and maturity dates on the recurrent parents. However, the disease did not have impact on the introgression lines in relation to days to flowering as well as days to maturity.

Besides the yield-related traits, Rice yellow mottle disease has negative impact on rice yield itself (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a). Thus, it was important to find out if the yield of the introgressed lines would be affected negatively by the presence of the disease. Percentage yield losses recorded for the 71 introgression lines ranged from -37.99% to 29.20%. Thus, none of the introgressed lines had more than 30% yield losses. Since the virus was not found in any of the 71 introgression lines when the ELISA test was performed, the 29.20% yield loss recorded for RYMV-B-01-31-12-12-69 could be due to nutrient gradient in the soil and not necessarily the effect of the virus. However, there was significant yield losses recorded in the four recurrent parents. The losses ranged from 91.24% to 99.53% (Table 5.2). This result shows that RYMV has significant impact on the yield of Togo

Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia as well as the susceptible check, Bouake 189.

The second resistance gene introgressed into the lines was the blast resistance gene, *Pi54*. Since the screen house, where the RYMV screening was done, was also a hotspot for rice blast disease, scoring was done for blast. Few lines from the 71 introgression lines showed some symptoms for blast. The lesions were few and the average scoring did not go beyond 2.0 for these lines, which was an indication of some level of resistance to blast in these lines (Table 5.2). However, the four susceptible recurrent parents had more lesions due to blast compared to than the resistant lines, with Togo Marshall having the highest severity score of 5.42. The other three recurrent parents, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia had severity scores of 4.71, 4.56 and 4.53, respectively. However, these lines should be inoculated with selected races of the fungus in order to determine their actual reaction to the fungal disease.

Conclusions

The 71 introgression lines for RYMV and blast resistances were highly resistant to Rice yellow mottle disease when inoculated with the RYMV isolate collected from a hotspot in the Ashanti Region of Ghana. However, the four recurrent popular aromatic rice varieties were either susceptible or moderately susceptible to the disease.

The recurrent parent, Togo Marshall, and the susceptible check, Bouake 189, were the first to show signs of RYMV infection at 8 dpi. The rest of the three recurrent parents showed symptoms of the disease at 11 dpi.

The DAS-ELISA test revealed that the donor parent and the 71 introgression lines did not contain the virus in their tissues. This was indicated by the negative test results obtained from these lines. However, all the four recurrent parents and the susceptible check, Bouake 189 showed presence of the virus in their leaf tissues. Based on viral loads, Togo Marshall, Jasmine 85 and Bouake 189 were "Susceptible" whilst CRI-Agra Rice and CRI-Amankwatia were "Moderately resistant".

The disease impacted negatively on the four recurrent parents and the susceptible check when 8 agronomic characters were assessed. Chief among these characters was plant yield. Here, the disease caused 91.24% to 99.53% yield losses among the four recurrent parents. The disease however did not cause any significant impact on the yield of the 71 introgression lines. The 71 introgression lines also showed resistance to the rice blast disease.

CHAPTER SIX

PRELIMINARY YIELD ASSESSMENT AND GENETIC VARIATION AMONGST ELITE RICE LINES RESISTANT TO BLAST AND RICE YELLOW MOTTLE DISEASES

Introduction

Rice has become a major staple food in Ghana (Asante et al., 2019; Tawiah et al., 2021). The per capita consumption has increased from 36 kg in 2018 (FAO, 2018) to 45 kg in 2021 (MoFA, 2021), and this expected to rise in the coming years. However, production has not increased that much to meet the increasing demand. While the total area under cultivation has increased by about 50% from 2013 to 2023, the average yield on farmers' fields has only increased by 34.6% in the same period. Thus, Ghana is still a net importer of rice (USDA, 2023). The constraints to achieving self-sufficiency include policy, socioeconomic factors, production factors, processing factors and marketing factors.

Chief among the production factors are biotic stresses which can cause up to 100% yield losses (Baite et al., 2020; Neupane & Bhusal, 2020). In Ghana, and sub-Saharan Africa in general, RYMD and rice blast disease are the two main diseases that limit the production of rice (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a; Tawiah et al., 2021). *Rice yellow mottle virus* (RYMV) is the most devastating disease pathogen of rice in Africa. The disease causes significant damage to rice. Crops losses can range from 25 to 100% , depending on the stage of infection, the variety infected and the type of management practice(s) adopted to contain the disease (Kouassi et al., 2005; Agnoun et al., 2019; Asante et al., 2020a;).

Rice yellow mottle virus disease was first discovered in 1966 in Kenya (Bakker, 1970, 1971). RYMV has been reported in 29 countries in Africa (CABI, 2021). However, the disease is more concentrated in West Africa compared to other parts of the African continent (Oludare et al., 2016).

Rice blast disease is another major rice disease that is not only found in Ghana but found throughout the world (Tanweer et al., 2015; Chen et al., 2018; Mao et al., 2018). Rice blast disease is invasive, such that the disease can affect many parts of the rice plant, such as the nodes, panicles, grains, leaves, leaf sheaths and the collar (IRRI, 2013).

One way to increase rice production in Africa, particularly in West Africa, is to breed for lines that are resistant to these biotic stresses. Breeding for disease resistance is one of the most effective ways to increase productivity of crops (Acquaah, 2012). In fact, research has shown that breeding for *R* genes into the background of crops can substantially increase yield. Breeding for disease resistance is more preferable since resistance genes are easy to deploy and have no adverse effect on the environment (Acquaah, 2012). It thus becomes the most effective way to manage diseases that affect crop plants.

In the case of rice blast disease, management practices such as reduction in the application of nitrogen fertilizers, good farm sanitation, wider spacing to reduce humidity in the farm and to allow proper air circulation, burning before planting, application of recommended fungicides, both as seed treatment or during the time of infection, are some of the ways to manage the disease. However, management of RYMV is challenging and less effective due to the impracticality of treating viral diseases. While farmers have attempted to control the disease by eliminating the main vector (beetles) and burning or burying

infected rice plants, these measures have been ineffective in controlling RYMV. In this situation, breeding for resistance remains the most effective way to control these two important diseases (Acquaah, 2012).

In Ghana, popular aromatic rice varieties, such as CRI-Agra Rice, Jasmine 85, CRI-Amankwatia and Togo Marshall, are susceptible or moderately susceptible to RYMV and blast diseases (Asante et al., 2020a; Tawiah et al., 2021). Rice consumers in Ghana prefer long grain aromatic rice (Asante, 2013; Asante et al., 2020b), thus, breeding for resistance to these two major diseases in the genetic background of these four popular aromatic rice varieties would go a long way to increasing rice production in the country. The objective of this research was to evaluate the yield performance and genetic variance of 71 newly developed lines resistant to blast and RYMV diseases.

Materials and Methods

Study area

The research was conducted at the research fields of CSIR- Crops Research Institute, Fumesua-Kumasi, Ghana. This area is found in the forest zone of Ghana, characterized by a bimodal rainfall pattern. The major season starts from April and ends by the end of July whilst the minor season starts from September and ends by November. The average annual rainfall is 1,397 mm and the temperature ranges from 20.6° C to 33.3° C. The average humidity is 67% (Weather Atlas, 2023).

Plant materials

The plant materials comprised newly developed 71 lines resistant to rice blast and Rice yellow mottle disease developed through the introgression of *RYMV1 (rymv1-2)* and *Pi54* genes through marker-assisted breeding. Details of these lines are given in Table 6.1.

Table 6.1: Newly developed rice lines with resistance to RYMV and blast plus checks

TRT_No.	GENOTYPE	SOURCE
1	RYMV-B-01-6-37-1-81	TOGO MARSHAL X GIGANTE
2	RYMV-B-01-6-37-1-5	TOGO MARSHAL X GIGANTE
3	RYMV-B-01-6-37-66	TOGO MARSHAL X GIGANTE
4	RYMV-B-01-6-37-4-10	TOGO MARSHAL X GIGANTE
5	RYMV-B-01-6-37-1-17	TOGO MARSHAL X GIGANTE
6	RYMV-B-01-6-37-1-21	TOGO MARSHAL X GIGANTE
7	RYMV-B-01-6-37-1-94	TOGO MARSHAL X GIGANTE
8	RYMV-B-01-6-37-1-13	TOGO MARSHAL X GIGANTE
9	RYMV-B-01-31-12-12-69	TOGO MARSHAL X GIGANTE
10	RYMV-B-01-6-37-1-27	TOGO MARSHAL X GIGANTE
11	RYMV-B-01-6-37-1-91	TOGO MARSHAL X GIGANTE
12	RYMV-B-01-31-12-12-75	TOGO MARSHAL X GIGANTE
13	RYMV-B-01-6-37-1-37	TOGO MARSHAL X GIGANTE
14	RYMV-B-02-20-24-13-53	JASMINE 85 X GIGANTE
15	RYMV-B-02-20-13-3-48	JASMINE 85 X GIGANTE
16	RYMV-B-02-20-24-13-60	JASMINE 85 X GIGANTE
17	RYMV-B-02-20-13-3-50	JASMINE 85 X GIGANTE
18	RYMV-B-02-20-9-3-79	JASMINE 85 X GIGANTE
19	RYMV-B-02-20-25-12-49	JASMINE 85 X GIGANTE
20	RYMV-B-02-20-13-3-5	JASMINE 85 X GIGANTE
21	RYMV-B-02-20-25-12-17	JASMINE 85 X GIGANTE
22	RYMV-B-02-20-25-12-63	JASMINE 85 X GIGANTE
23	RYMV-B-02-20-25-12-47	JASMINE 85 X GIGANTE
24	RYMV-B-02-20-25-12-24	JASMINE 85 X GIGANTE
25	RYMV-B-02-20-24-13-14	JASMINE 85 X GIGANTE
26	RYMV-B-02-20-9-3-91	JASMINE 85 X GIGANTE
27	RYMV-B-02-20-25-12-30	JASMINE 85 X GIGANTE
28	RYMV-B-02-20-13-3-19	JASMINE 85 X GIGANTE
29	RYMV-B-02-20-9-3-21	JASMINE 85 X GIGANTE
30	RYMV-B-03-84-36-12-68	CRI-AGRA RICE X GIGANTE
31	RYMV-B-03-84-47-2-50	CRI-AGRA RICE X GIGANTE
32	RYMV-B-03-84-36-9-23	CRI-AGRA RICE X GIGANTE
33	RYMV-B-03-84-47-2-22	CRI-AGRA RICE X GIGANTE
34	RYMV-B-03-84-36-10-2	CRI-AGRA RICE X GIGANTE

TRT_No.	GENOTYPE	SOURCE
35	RYMV-B-03-84-36-10-33	CRI-AGRA RICE X GIGANTE
36	RYMV-B-03-84-36-12-15	CRI-AGRA RICE X GIGANTE
37	RYMV-B-03-84-36-12-71	CRI-AGRA RICE X GIGANTE
38	RYMV-B-03-84-36-9-51	CRI-AGRA RICE X GIGANTE
39	RYMV-B-03-84-47-2-96	CRI-AGRA RICE X GIGANTE
40	RYMV-B-03-84-47-2-95	CRI-AGRA RICE X GIGANTE
41	RYMV-B-03-84-36-12-31	CRI-AGRA RICE X GIGANTE
42	RYMV-B-03-84-36-9-38	CRI-AGRA RICE X GIGANTE
43	RYMV-B-03-84-36-12-40	CRI-AGRA RICE X GIGANTE
44	RYMV-B-03-84-36-10-57	CRI-AGRA RICE X GIGANTE
45	RYMV-B-03-84-36-12-84	CRI-AGRA RICE X GIGANTE
46	RYMV-B-03-84-36-12-11	CRI-AGRA RICE X GIGANTE
47	RYMV-B-03-84-36-10-46	CRI-AGRA RICE X GIGANTE
48	RYMV-B-03-84-47-2-72	CRI-AGRA RICE X GIGANTE
49	RYMV-B-03-84-36-10-71	CRI-AGRA RICE X GIGANTE
50	RYMV-B-03-84-47-2-57	CRI-AGRA RICE X GIGANTE
51	RYMV-B-03-84-36-12-76	CRI-AGRA RICE X GIGANTE
52	RYMV-B-04-14-11-10-38	CRI-AMANKWATIA X GIGANTE
53	RYMV-B-04-38-10-1-10	CRI-AMANKWATIA X GIGANTE
54	RYMV-B-04-38-10-1-52	CRI-AMANKWATIA X GIGANTE
55	RYMV-B-04-38-10-13-7	CRI-AMANKWATIA X GIGANTE
56	RYMV-B-04-14-11-10-74	CRI-AMANKWATIA X GIGANTE
57	RYMV-B-04-38-10-13-78	CRI-AMANKWATIA X GIGANTE
58	RYMV-B-04-38-10-1-8	CRI-AMANKWATIA X GIGANTE
59	RYMV-B-04-14-11-10-86	CRI-AMANKWATIA X GIGANTE
60	RYMV-B-04-38-10-13-93	CRI-AMANKWATIA X GIGANTE
61	RYMV-B-04-38-10-1-18	CRI-AMANKWATIA X GIGANTE
62	RYMV-B-04-38-10-13-82	CRI-AMANKWATIA X GIGANTE
63	RYMV-B-04-14-11-10-22	CRI-AMANKWATIA X GIGANTE
64	RYMV-B-04-38-10-1-25	CRI-AMANKWATIA X GIGANTE
65	RYMV-B-04-38-10-13-12	CRI-AMANKWATIA X GIGANTE
66	RYMV-B-04-14-11-10-11	CRI-AMANKWATIA X GIGANTE
67	RYMV-B-04-38-10-1-63	CRI-AMANKWATIA X GIGANTE
68	RYMV-B-04-14-11-10-96	CRI-AMANKWATIA X GIGANTE
69	RYMV-B-04-38-10-1-64	CRI-AMANKWATIA X GIGANTE
70	RYMV-B-04-38-10-13-80	CRI-AMANKWATIA X GIGANTE
71	RYMV-B-04-14-11-10-95	CRI-AMANKWATIA X GIGANTE
-1	TOGO MARSHAL	RECURRENT PARENT (CHECK)
-2	JASMINE 85	RECURRENT PARENT (CHECK)
-3	CRI-AGRA RICE	RECURRENT PARENT (CHECK)
-4	CRI-AMANKWATIA	RECURRENT PARENT (CHECK)
-5	LEGON 1	ADDITIONAL CHECK

Experimental design and agronomic practices

The experiment was laid in an augmented design with four replications. The 71 developed lines were unreplicated whilst the four recurrent parents, Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia, as well as an aromatic variety- Legon 1- served as the five replicated checks. The seedlings were transplanted after 21 days of sowing. The plot size was 1 m x 1 m each with plant spacing of 20 cm x 20 cm.

N.P.K (15:15:15) and urea were applied at the recommended rate of 90 kg N: 60 kg P: 60 kg K in two splits. NPK was applied after transplanting and Urea was applied as top-dress at booting stage.

Weeds were controlled by hand-picking and insecticide (Imidacloprid) was applied at a rate of 30 ml/16L of water twice during the growing period to control insects, especially, stem borers.

Data collection

Five plants were selected per plot for determination of plant height (cm), number of effective tillers per plant, panicle number, panicle length (cm), grain length (cm), grain width (cm), and grain length-width ratio. Days to 50% flowering per genotype was recorded when 50% of the genotypes on the plot had headed. Days to maturity was calculated as the number of days from sowing to maturity where 85% of the grains had turned straw-brown (IRRI, 2013).

Thousand grain weight was measured by counting 1,000 random grains using DATA COUNT 25^{+} (Data Technologies), after which the grains were weighed using an electronic balance. Grain yield was calculated as weight of grains per plot at 14% moisture content and extrapolated to tons per hectare.

Data analyses

The data were subjected to one-way Analysis of Variance (ANOVA) using the aov() and lm() functions in R statistical software version 4.2.2. Genetic parameters considered in the analyses were genotypic variance (σ^2_g), phenotypic variance (σ^2_p) and environmental variance (σ^2_e). They were determined using the formulae described by Johnson et al. (1955):

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where σ^2_g = (MSG-MSE)/r

σ^2_e = MS Error

MSG = Mean square of the genotypes, MSE = Mean square of error and r = number of replications.

Genotypic coefficient of variation (GCV), environmental coefficient variation (ECV) and phenotypic coefficient of variation (PCV) were determined for the various agronomic traits using the formulae by Burton (1952) as follows:

$$GCV = \sqrt{\sigma^2_g} / \text{mean} \times 100$$

$$ECV = \sqrt{\sigma^2_e} / \text{mean} \times 100$$

$$PCV = \sqrt{\sigma^2_p} / \text{mean} \times 100$$

Classification of GCV and PCV were based on the classification described by Sivasubramanian and Menon (1973) as:

Low = Less than 10%

Medium = 10 – 20%

High = Greater than 20%.

Broad-sense heritability (H^2) was estimated for the various agronomic traits. They were grouped using the categorization method by Stanfield (1983) as "high ($0.50 < H^2 \leq 1.0$), moderate ($0.20 < H^2 < 0.50$) and low ($H^2 < 0.20$)".

Genetic advance, and genetic advance as a percentage of mean were determined following Singh and Chaudhary (1985) as follows:

$$GA = k.\sqrt{\sigma^2_p}\ (H^2)$$

Where k= constant, is selection deferential which was 2.06 at 5% selection intensity, σ^2_p = phenotypic variation, and H^2= broad sense heritability.

Genetic advance as a percentage of mean ($GAM_{\%}$) was calculated as:

$$GA_{\%} = (GA/\ mean) \times 100$$

$GAM_{\%}$ was classified as low (0 - 10%), moderate (10 – 20%) or high (>20%).

Pearson's correlation was performed to determine the relationship among the traits, especially with yield. Cluster analysis was also performed to determine the relationship among the genotypes in reference to the various agronomic traits measured.

A dendrogram was created using the NBClust package in R statistical software based on ten agro-morphological traits.

Principal component analysis was performed to determine the contribution of each component to the overall performance of the genotypes. The data were first standardized using the formula $Z = \frac{(x-mean)}{sd}$

Where *Z*= transformed value, *x*= trait value and *sd*= standard deviation.

Results

Yield and yield component traits

The introgressed lines derived from the cross between the donor parent and the four popular aromatic rice varieties were assessed in terms of yield and yield components. The results show that the highest average number of tillers (17.2) was in the genotype RYMV-B-01-6-37-1-5 whilst the lowest number of tillers (6.2) was in the genotype RYMV-B-04-38-10-13-7 (Table 6.2).

Plant height ranged from 94 to 172 cm. The lowest plant height was in genotype RYMV-B-01-31-12-12-69 whilst the highest was in genotype RYMV-B-01-6-37-1-81. Days to 50% flowering ranged from 87 to 110 days with 100 days as the mean of days to 50% flowering. Genotype RYMV-B-03-84-36-9-23 had the least number of days to 50% flowering whilst genotype RYMV-B-04-38-10-1-63 had the highest number of days to 50% flowering. Grain length (GL) ranged from 6.51 to 8.12 cm. The least grain length was in genotype RYMV-B-04-14-11-10-86 whilst the greatest was in genotype RYMV-B-01-6-37-1-94. Grain width (GW) ranged from 2.01 to 2.56 cm with the least found in genotype RYMV-B-03-84-36-12-31 whilst the greatest was found in genotype RYMV-B-03-84-36-10-57.

Grain yield, the most important parameter, ranged from 3.15 to 7.26 ton/ha. The highest yield was obtained in the genotype RYMV-B-04-38-10-13-78. The four recurrent parental lines (Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia) had grain yields of 6.92, 6.58, 7.09 and 6.77 ton/ha, respectively (Table 6.2).

Table 6.2: Yield and yield component traits for the 71 introgressed lines and their checks

TRT	GENOTYPE	PH	TN	PN	PL	GL	GW	GL/GW	50% FLW	TGWT	YLD (T/Ha)
64	RYMV-B-04-38-10-1-25	113±2.0	11.0±0.3	9.0±0.2	25.2±0.2	7.60±0.04	2.50±0.02	3.04±0.03	100±1	29.0±0.14	5.76±0.14
65	RYMV-B-04-38-10-13-12	96±1.9	6.8±0.1	5.6±0.1	24.6±0.2	7.44±0.04	2.43±0.02	3.06±0.03	96±1	29.0±0.14	5.56±0.14
44	RYMV-B-03-84-36-10-57	141±2.4	12.0±0.3	11.0±0.2	25.4±0.2	7.24±0.04	2.56±0.02	2.83±0.02	104±2	30.0±0.14	7.25±0.15
35	RYMV-B-03-84-36-10-33	137±2.2	10.0±0.2	9.0±0.2	26.4±0.3	7.48±0.04	2.22±0.01	3.37±0.03	108±2	29.0±0.14	7.02±0.15
-3	CRI-Agra Rice	116±2.0	11.4±0.2	10.4±0.2	25.3±0.2	7.12±0.03	2.37±0.01	3.02±0.03	105±2	27.5±0.13	7.09±0.15
-1	Togo Marshall	111±1.9	11.5±0.2	10.4±0.2	22.5±0.1	7.61±0.04	2.25±0.01	3.38±0.03	101±1	27.8±0.13	6.92±0.14
17	RYMV-B-02-20-13-3-50	111±1.9	6.8±0.1	5.4±0.1	25.6±0.3	7.05±0.03	2.28±0.01	3.08±0.03	105±2	27.0±0.13	3.54±0.11
42	RYMV-B-03-84-36-9-38	159±2.5	8.6±0.2	7.8±0.1	27.6±0.3	7.24±0.04	2.27±0.01	3.19±0.03	102±1	28.0±0.13	6.41±0.14
20	RYMV-B-02-20-13-3-5	108±2.0	7.2±0.1	6.1±0.1	25.1±0.2	7.51±0.04	2.43±0.02	3.09±0.03	103±1	27.0±0.13	4.27±0.12
-5	Legon 1	103±2.0	11.6±0.2	10.5±0.2	22.0±0.1	7.30±0.04	2.20±0.01	3.32±0.03	103±1	26.8±0.12	6.78±0.14
-4	CRI-Amankwatia	113±2.1	9.5±0.1	8.6±0.2	21.7±0.1	7.21±0.04	2.31±0.01	3.13±0.03	99±1	27.3±0.13	6.77±0.14
19	RYMV-B-02-20-25-12-49	105±2.0	9.0±0.1	7.6±0.2	23.9±0.2	7.00±0.03	2.34±0.01	2.99±0.02	102±1	28.0±0.13	4.76±0.12
29	RYMV-B-02-20-9-3-21	158±2.4	9.4±0.1	7.8±0.2	26.5±0.3	7.07±0.03	2.30±0.01	3.07±0.03	100±1	28.0±0.13	7.09±0.14
51	RYMV-B-03-84-36-12-76	163±2.5	13.4±0.3	12.8±0.3	27.4±0.3	7.60±0.04	2.33±0.01	3.26±0.03	106±1	30.0±0.14	6.26±0.14
56	RYMV-B-04-14-11-10-74	107±2.1	6.2±0.1	5.2±0.1	26.1±0.3	7.03±0.03	2.17±0.01	3.23±0.03	100±1	28.0±0.13	5.31±0.14
43	RYMV-B-03-84-36-12-40	151±2.4	10.0±0.2	9.0±0.2	26.1±0.3	6.62±0.03	2.38±0.01	2.78±0.02	98±1	28.0±0.13	6.29±0.14
36	RYMV-B-03-84-36-12-15	155±2.5	10.4±0.2	9.0±0.2	27.9±0.3	7.15±0.03	2.08±0.01	3.44±0.03	102±1	30.0±0.14	7.04±0.14
66	RYMV-B-04-14-11-10-11	104±1.9	6.2±0.1	5.8±0.1	23.7±0.2	6.72±0.03	2.28±0.01	2.94±0.02	96±1	29.0±0.14	3.95±0.11
46	RYMV-B-03-84-36-12-11	105±1.9	7.0±0.1	6.0±0.1	26.7±0.3	6.74±0.03	2.31±0.01	2.92±0.02	108±2	28.0±0.13	4.02±0.12
33	RYMV-B-03-84-47-2-22	114±2.1	10.0±0.2	9.4±0.2	24.7±0.2	7.24±0.04	2.35±0.01	3.09±0.03	92±1	27.0±0.13	6.00±0.14
1	RYMV-B-01-6-37-1-81	172±2.6	9.0±0.2	8.0±0.2	27.8±0.3	7.03±0.03	2.33±0.01	3.02±0.03	99±1	26.0±0.12	4.70±0.12

TRT	GENOTYPE	PH	TN	PN	PL	GL	GW	GL/GW	50% FLW	TGWT	YLD (T/Ha)
10	RYMV-B-01-6-37-1-27	165±2.4	8.6±0.2	7.6±0.2	24.6±0.2	7.19±0.04	2.06±0.01	3.49±0.03	92±1	28.0±0.13	4.64±0.12
8	RYMV-B-01-6-37-1-13	168±2.5	7.2±0.1	6.8±0.1	26.4±0.3	7.55±0.04	2.27±0.01	3.33±0.03	102±1	27.0±0.13	4.10±0.12
55	RYMV-B-04-38-10-13-7	118±2.1	6.2±0.1	5.8±0.1	25.2±0.3	7.65±0.04	2.21±0.01	3.46±0.03	105±2	29.0±0.14	3.15±0.11
9	RYMV-B-01-31-12-12-69	94±1.9	7.4±0.1	6.2±0.1	21.9±0.1	7.36±0.04	2.38±0.01	3.09±0.03	102±1	29.0±0.14	3.63±0.11
-2	Jasmine 85 (CRI)	118±2.2	9.8±0.2	9.3±0.2	24.0±0.2	7.11±0.03	2.34±0.01	3.04±0.03	103±1	27.5±0.13	6.58±0.14
39	RYMV-B-03-84-47-2-96	114±2.1	7.6±0.1	7.4±0.1	25.5±0.3	7.22±0.04	2.19±0.01	3.29±0.03	102±1	28.0±0.13	4.68±0.12
16	RYMV-B-02-20-24-13-60	106±1.9	7.3±0.1	6.6±0.1	25.9±0.3	6.99±0.03	2.34±0.01	2.99±0.02	107±2	28.0±0.13	4.41±0.12
28	RYMV-B-02-20-13-3-19	104±1.9	7.6±0.2	7.2±0.1	25.6±0.2	7.31±0.04	2.35±0.01	3.11±0.03	102±1	28.0±0.13	5.24±0.14
30	RYMV-B-03-84-36-12-68	110±2.0	7.2±0.1	6.8±0.1	24.5±0.2	7.25±0.04	2.32±0.01	3.12±0.03	104±2	29.0±0.14	6.64±0.14
40	RYMV-B-03-84-47-2-95	115±2.0	10.6±0.2	9.6±0.2	25.8±0.3	7.28±0.04	2.28±0.01	3.19±0.03	102±1	29.0±0.14	7.10±0.15
5	RYMV-B-01-6-37-1-17	96±1.9	10.6±0.2	10.2±0.2	24.7±0.2	6.89±0.03	2.09±0.01	3.30±0.03	99±1	26.0±0.12	6.77±0.14
59	RYMV-B-04-14-11-10-86	110±2.0	10.6±0.2	9.4±0.2	23.3±0.2	6.51±0.03	2.34±0.01	2.78±0.02	100±1	27.0±0.13	6.84±0.14
25	RYMV-B-02-20-24-13-14	110±2.0	8.4±0.2	8.2±0.2	25.8±0.3	7.04±0.03	2.37±0.01	2.98±0.02	101±1	28.0±0.13	5.52±0.14
26	RYMV-B-02-20-9-3-91	153±2.4	9.8±0.2	8.8±0.2	25.8±0.3	7.06±0.03	2.41±0.02	2.94±0.02	101±1	28.0±0.13	6.74±0.14
54	RYMV-B-04-38-10-1-52	106±1.9	8.0±0.1	7.0±0.1	22.3±0.2	7.37±0.04	2.35±0.01	3.14±0.03	104±2	28.0±0.13	4.22±0.12
50	RYMV-B-03-84-47-2-57	105±1.9	8.8±0.2	8.8±0.2	25.2±0.2	7.27±0.04	2.44±0.02	2.98±0.02	101±1	27.0±0.13	4.38±0.12
22	RYMV-B-02-20-25-12-63	113±2.0	8.4±0.2	8.0±0.2	25.6±0.3	6.55±0.03	2.34±0.01	2.80±0.02	102±1	27.0±0.13	4.04±0.12
4	RYMV-B-01-6-37-4-10	111±2.0	9.2±0.2	8.4±0.2	25.7±0.3	8.04±0.05	2.24±0.01	3.58±0.03	109±2	28.0±0.13	6.39±0.14
70	RYMV-B-04-38-10-13-80	112±2.0	9.4±0.2	9.2±0.2	22.8±0.2	7.19±0.04	2.42±0.02	2.98±0.02	95±1	29.0±0.14	6.94±0.14
52	RYMV-B-04-14-11-10-38	102±1.9	8.2±0.2	7.8±0.2	23.5±0.2	6.68±0.03	2.32±0.01	2.88±0.02	95±1	29.0±0.14	5.23±0.14
32	RYMV-B-03-84-36-9-23	162±2.5	7.2±0.1	6.5±0.1	23.3±0.2	6.72±0.03	2.21±0.01	3.04±0.03	87±1	27.0±0.13	5.07±0.14
12	RYMV-B-01-31-12-12-75	104±1.9	8.4±0.2	7.3±0.2	23.3±0.2	6.94±0.03	2.34±0.01	2.97±0.02	97±1	30.0±0.14	5.44±0.14

TRT	GENOTYPE	PH	TN	PN	PL	GL	GW	GL/GW	50% FLW	TGWT	YLD (T/Ha)
15	RYMV-B-02-20-13-3-48	99±1.8	6.6±0.1	6.4±0.1	26.0±0.3	7.78±0.04	2.47±0.02	3.15±0.03	103±1	31.0±0.14	5.70±0.14
49	RYMV-B-03-84-36-10-71	100±1.9	7.8±0.1	7.4±0.2	23.3±0.2	7.21±0.04	2.32±0.01	3.11±0.03	102±1	29.0±0.14	6.71±0.14
27	RYMV-B-02-20-25-12-30	104±1.9	6.8±0.1	6.0±0.1	23.2±0.2	7.43±0.04	2.20±0.01	3.38±0.03	102±1	28.0±0.13	4.29±0.12
18	RYMV-B-02-20-9-3-79	144±2.4	7.2±0.1	6.7±0.1	25.9±0.3	6.68±0.03	2.30±0.01	2.90±0.02	97±1	28.0±0.13	4.21±0.12
24	RYMV-B-02-20-25-12-24	113±2.0	8.2±0.2	8.2±0.2	26.0±0.3	7.47±0.04	2.34±0.01	3.19±0.03	99±1	29.0±0.14	5.36±0.14
37	RYMV-B-03-84-36-12-71	133±2.2	10.8±0.2	10.0±0.2	28.3±0.3	7.08±0.03	2.19±0.01	3.24±0.03	105±2	30.0±0.14	7.00±0.15
68	RYMV-B-04-14-11-10-96	120±2.1	8.4±0.1	7.0±0.1	24.0±0.2	7.05±0.03	2.35±0.01	3.00±0.03	108±2	28.0±0.13	5.81±0.14
63	RYMV-B-04-14-11-10-22	108±1.9	9.6±0.2	9.4±0.2	22.9±0.2	6.81±0.03	2.16±0.01	3.15±0.03	96±1	28.0±0.13	7.10±0.15
23	RYMV-B-02-20-25-12-47	105±1.9	9.4±0.2	8.6±0.2	24.3±0.2	6.72±0.03	2.36±0.01	2.85±0.02	92±1	26.0±0.12	6.36±0.14
71	RYMV-B-04-14-11-10-95	108±1.9	10.2±0.2	9.4±0.2	23.9±0.2	7.27±0.04	2.41±0.02	3.01±0.03	102±1	27.0±0.13	7.17±0.15
47	RYMV-B-03-84-36-10-46	157±2.5	12.0±0.3	11.8±0.3	25.7±0.3	6.71±0.03	2.03±0.01	3.31±0.03	102±1	27.0±0.13	7.12±0.15
61	RYMV-B-04-38-10-1-18	109±1.9	6.8±0.1	6.6±0.1	22.4±0.2	8.04±0.05	2.35±0.01	3.42±0.03	94±1	28.0±0.13	4.24±0.12
7	RYMV-B-01-6-37-1-94	138±2.1	12.0±0.3	11.8±0.3	23.6±0.2	8.12±0.05	2.28±0.01	3.57±0.03	94±1	29.0±0.14	6.70±0.14
6	RYMV-B-01-6-37-1-21	112±2.0	13.8±0.3	13.0±0.3	24.5±0.2	7.4±0.04	2.07±0.01	3.57±0.03	100±1	27.2±0.13	6.90±0.14
67	RYMV-B-04-38-10-1-63	111±2.0	12.6±0.3	10.6±0.2	25.3±0.3	7.28±0.04	2.26±0.01	3.22±0.03	110±2	29.0±0.14	6.92±0.14
21	RYMV-B-02-20-25-12-17	122±2.2	10.2±0.2	9.4±0.2	24.1±0.2	6.77±0.03	2.34±0.01	2.89±0.02	105±2	27.0±0.13	6.74±0.14
41	RYMV-B-03-84-36-12-31	162±2.6	11.2±0.2	10.5±0.2	25.7±0.3	7.07±0.03	2.01±0.01	3.51±0.03	99±1	28.0±0.13	7.15±0.15
57	RYMV-B-04-38-10-13-78	117±2.1	11.4±0.2	10.8±0.2	23.3±0.2	7.53±0.04	2.34±0.01	3.22±0.03	100±1	29.0±0.14	7.26±0.15
60	RYMV-B-04-38-10-13-93	116±2.0	10.0±0.2	9.0±0.2	24.4±0.2	7.21±0.04	2.36±0.01	3.05±0.03	100±1	31.0±0.14	7.17±0.15
14	RYMV-B-02-20-24-13-53	101±1.9	9.2±0.2	8.6±0.2	23.6±0.2	6.98±0.03	2.48±0.02	2.82±0.02	103±1	28.0±0.13	5.50±0.14
3	RYMV-B-01-6-37-66	136±2.3	8.6±0.2	7.5±0.2	23.8±0.2	7.04±0.03	2.11±0.01	3.34±0.03	98±1	29.0±0.14	7.09±0.15
48	RYMV-B-03-84-47-2-72	101±1.9	7.6±0.1	7.2±0.1	22.5±0.2	7.19±0.04	2.31±0.01	3.12±0.03	98±1	31.0±0.14	5.00±0.14

TRT	GENOTYPE	PH	TN	PN	PL	GL	GW	GL/GW	50% FLW	TGWT	YLD (T/Ha)
69	RYMV-B-04-38-10-1-64	107±2.0	8.0±0.2	7.2±0.1	23.7±0.2	8.03±0.05	2.45±0.02	3.28±0.03	100±1	28.0±0.13	6.03±0.14
13	RYMV-B-01-6-37-1-37	146±2.4	8.6±0.2	8.0±0.2	26.3±0.3	7.03±0.03	2.35±0.01	2.99±0.02	96±1	27.0±0.13	6.23±0.14
2	RYMV-B-01-6-37-1-5	101±1.9	17.2±0.4	17.2±0.4	24.6±0.2	6.90±0.03	2.43±0.02	2.84±0.02	97±1	28.0±0.13	7.13±0.15
53	RYMV-B-04-38-10-1-10	116±2.0	10.2±0.2	10.2±0.2	24.0±0.2	7.40±0.04	2.41±0.02	3.08±0.03	90±1	28.0±0.13	6.72±0.14
31	RYMV-B-03-84-47-2-50	108±2.0	9.4±0.2	9.0±0.2	25.8±0.3	7.32±0.04	2.30±0.01	3.18±0.03	102±1	28.0±0.13	7.14±0.15
62	RYMV-B-04-38-10-13-82	129±2.1	10.0±0.2	9.6±0.2	24.1±0.2	7.15±0.04	2.40±0.02	2.98±0.02	102±1	28.0±0.13	6.86±0.14
34	RYMV-B-03-84-36-10-2	105±1.9	9.2±0.2	8.2±0.2	24.8±0.2	7.31±0.04	2.49±0.02	2.94±0.02	100±1	27.0±0.13	6.76±0.14
11	RYMV-B-01-6-37-1-91	150±2.5	13.0±0.3	10.8±0.2	26.2±0.3	7.70±0.04	2.42±0.02	3.18±0.03	96±1	28.0±0.13	7.23±0.15
38	RYMV-B-03-84-36-9-51	144±2.4	9.2±0.2	8.2±0.2	23.8±0.2	7.86±0.04	2.32±0.01	3.39±0.03	90±1	27.6±0.13	7.11±0.15
58	RYMV-B-04-38-10-1-8	123±2.2	10.2±0.2	9.2±0.2	24.7±0.2	7.07±0.03	2.44±0.02	2.90±0.02	102±1	28.0±0.13	7.07±0.15
45	RYMV-B-03-84-36-12-84	146±2.4	11.6±0.2	10.6±0.2	26.1±0.3	7.12±0.03	2.43±0.02	2.93±0.02	101±1	28.0±0.13	7.08±0.15
	Max	**172**	**17.2**	**17.2**	**28.3**	**8.12**	**2.56**	**3.58**	**110**	**31.0**	**7.26**
	Min	**93.6**	**6.2**	**5.2**	**21.7**	**6.51**	**2.01**	**2.78**	**87**	**26.0**	**3.15**
	Mean	**121.4**	**9.4**	**8.6**	**24.8**	**7.21**	**2.31**	**3.12**	**100**	**28.15**	**5.97**

Agro-morphological characters

The analysis of variance showed significant differences among the genotypes for some of the agronomic characters studied. Plant height (PH) and grain yield (GY) differed significantly among the genotypes at 1% ($P < 0.01$). Panicle number (PN) and grain length were significant at 5% ($P < 0.05$). However, traits such as days to 50% flowering (DF), days to maturity (MD), grain length to width ratio (GL: GW), tiller number (TN), panicle length (PL), grain width (GW) and 1000 grain weight (TGW) did not differ significantly ($P > 0.05$) among the genotypes (Table 6.3).

Table 6.3: Analysis of variance (mean squares) for 10 agronomic traits measured for the 71 introgression lines and their checks

TRAIT	d.f	PH	TN	PN	PL	GL	GW	DF	MD	TGW	GY
Rep	3	405.71**	0.73	2.38	15.42**	0.04	0.008	33.76	14.32	2.83	2.89**
Genotype	75	461.19**	4.73	4.45*	2.48	0.13*	0.013	21.34	20.15	0.22	1.43**
Residual	12	41.73	2.31	1.79	1.05	0.02	0.002	2.24	3.17	0.38	0.22

PH: plant height; TN: tiller number; PN: panicle number; PL: panicle length; GL: grain length; GW: grain width; DF: days to 50% flowering;
MD: days to maturity; TGW: thousand grain weight; GY: grain yield/ ha; **significance at 1% probability; *significance at 5% probability.

Heritability, variance components and genetic advance for selected agronomic characters

Means, variances, heritability and genetic advance were determined for the various agronomic characters. Plant height ranged from 93.60 to 172 cm. There was a high environmental variance for plant height (41.73). Environmental coefficient of variation (ECV) for the various characters ranged from 2.95% to 16.16% with grain length (GL) and number of tillers (TN) having the lowest and highest ECV, respectively (Table 6.4).

Heritability (broad sense, H^2) was calculated for the major traits studied. Plant height (PH) had the highest heritability value (71.53%) whilst number of tillers (TN) had the lowest heritability (20.78%). Genetic advance as a percentage of mean ($GA_{\%}$) for the various characters studied ranged from 2.11% to 14.88% with 1000 grain weight (GW_{1000}) and plant height (PH) having the lowest and highest $GA_{\%}$ respectively.

Mean grain length (GL) for the genotypes ranged from 6.51 to 8.12 cm whilst mean grain width (GW) ranged from 2.01 to 2.56 cm. The average days to 50% flowering for the genotypes was 100.58 days whilst the average days to maturity was 130.41 days (Table 6.4).

Table 6.4: Estimates of genetic parameters for yield and yield component traits in rice lines

Trait	Mean	Range		Σ^2_g	σ^2_p	σ^2_e	GCV	ECV	PCV	H^2	GA	GA%
		Min	Max							(%)		
PH	119.92±3.23	93.60	172.00	104.87	146.60	41.73	8.54	5.39	10.10	71.53	17.84	14.88
TN	9.40±0.76	5.20	17.20	0.61	2.92	2.31	8.28	16.16	18.16	20.78	0.73	7.77
PN	8.60±0.67	5.20	17.20	0.67	2.46	1.79	9.49	15.53	18.20	27.20	0.88	10.20
PL	24.51±0.51	20.56	28.32	0.36	1.41	1.05	2.44	4.19	4.85	25.53	0.62	2.52
GL	7.22±0.11	6.51	8.12	0.03	0.05	0.02	2.05	2.95	3.59	60.00	0.28	3.88
GW	2.31±0.04	2.01	2.56	0.003	0.005	0.002	1.79	3.59	4.02	60.00	0.09	3.90
DF	100.58±1.82	87.00	110.00	4.77	7.01	2.24	1.41	3.62	3.88	67.95	3.71	3.69
MD	130.41±2.07	115.00	139.00	4.25	7.42	3.17	0.66	3.18	3.25	57.28	3.21	2.46
TGW	28.02±0.47	26.00	31.00	0.22	0.59	0.38	1.07	3.34	3.51	37.29	0.59	2.11
GY	6.10±0.23	3.15	7.59	0.30	0.52	0.22	9.03	7.66	11.84	57.69	0.86	14.10

PH: plant height; TN: tiller number; PN: panicle number; PL: panicle length; GL: grain length; GW: grain width; DF: days to 50% flowering; MD: days to maturity; TGW: thousand grain weight; GY: grain yield/ ha.

Association between agronomic characters

There was positive as well as negative correlations among the agronomic characters studied (Fig. 6.1). There was highly positive correlation ($P < 0.001$) between number of tillers and panicle number ($r = 0.97$). Number of tillers again was positively correlated with yield ($r = 0.71$). Panicle number also had a highly positive correlation ($P < 0.001$) with yield ($r = 0.69$). Plant height was positively correlated with panicle length ($r = 0.51$). Flowering date was also positively correlated with maturity time ($r = 0.58$). Grain length and grain length to width ratio had a positive correlation ($r = 0.64$, Figure 6.1).

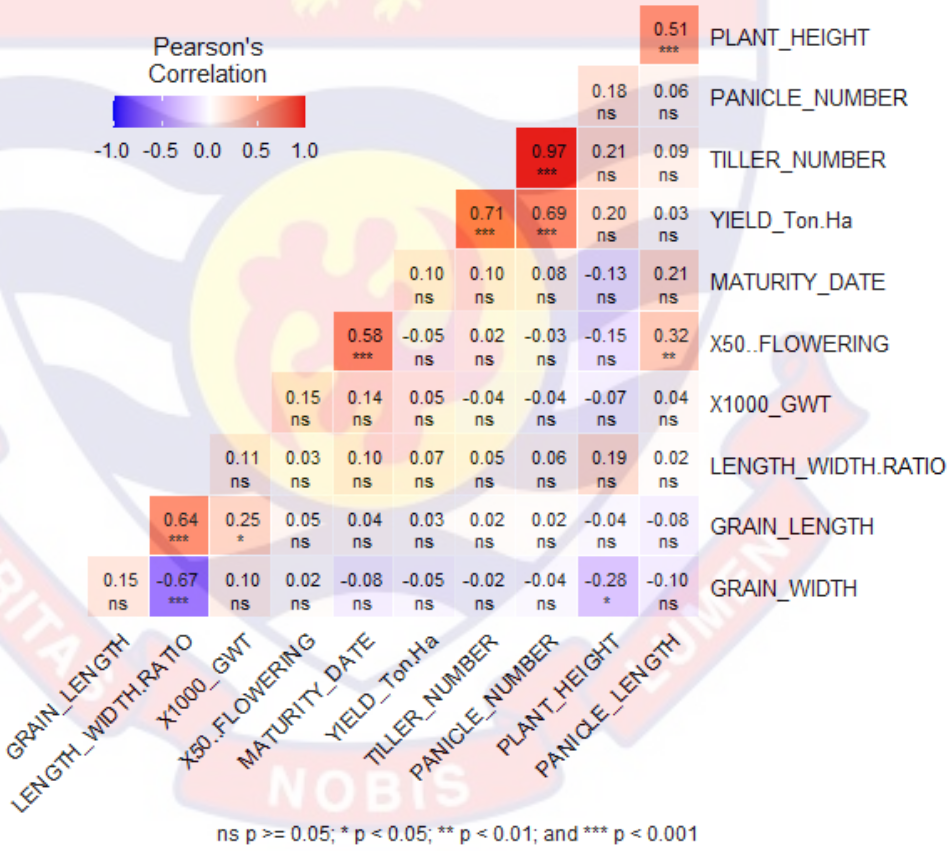

Figure 6.1: Pearson's correlation coefficients among 10 agronomic characters in rice lines and checks

On the other hand, grain width and grain length to width ratio had a highly negative correlation (r = -0.67). Plant height and grain width also had a negative correlation (r = -0.28) at 5% probability level.

There was no significant correlation between panicle length and number of panicles, plant height and number of panicles, days to maturity and 1000 grain weight, number of tillers and panicle length, grain length and yield, even though the correlation among these characters was positive. Also, there was non-significant negative correlation between plant height and 1000 grain weight, grain yield and days to 50% flowering, panicle length and grain length as well as grain width and grain yield (Figure 6.1).

Cluster analysis

Cluster analysis was performed to confirm the optimum number of clusters the 71 introgressed lines and their checks belonged to using ten agro-morphological traits. The results grouped the lines into four clusters using eleven indices. Clusters I, II, III, and IV consisted of 20, 23, 12, and 21 genotypes, respectively. Thus, the group with the largest cluster was cluster II (23 genotypes) whilst the smallest cluster was cluster III (12 genotypes, Figure 6.2). The inter-cluster values ranged from 5.72 to 9.06.

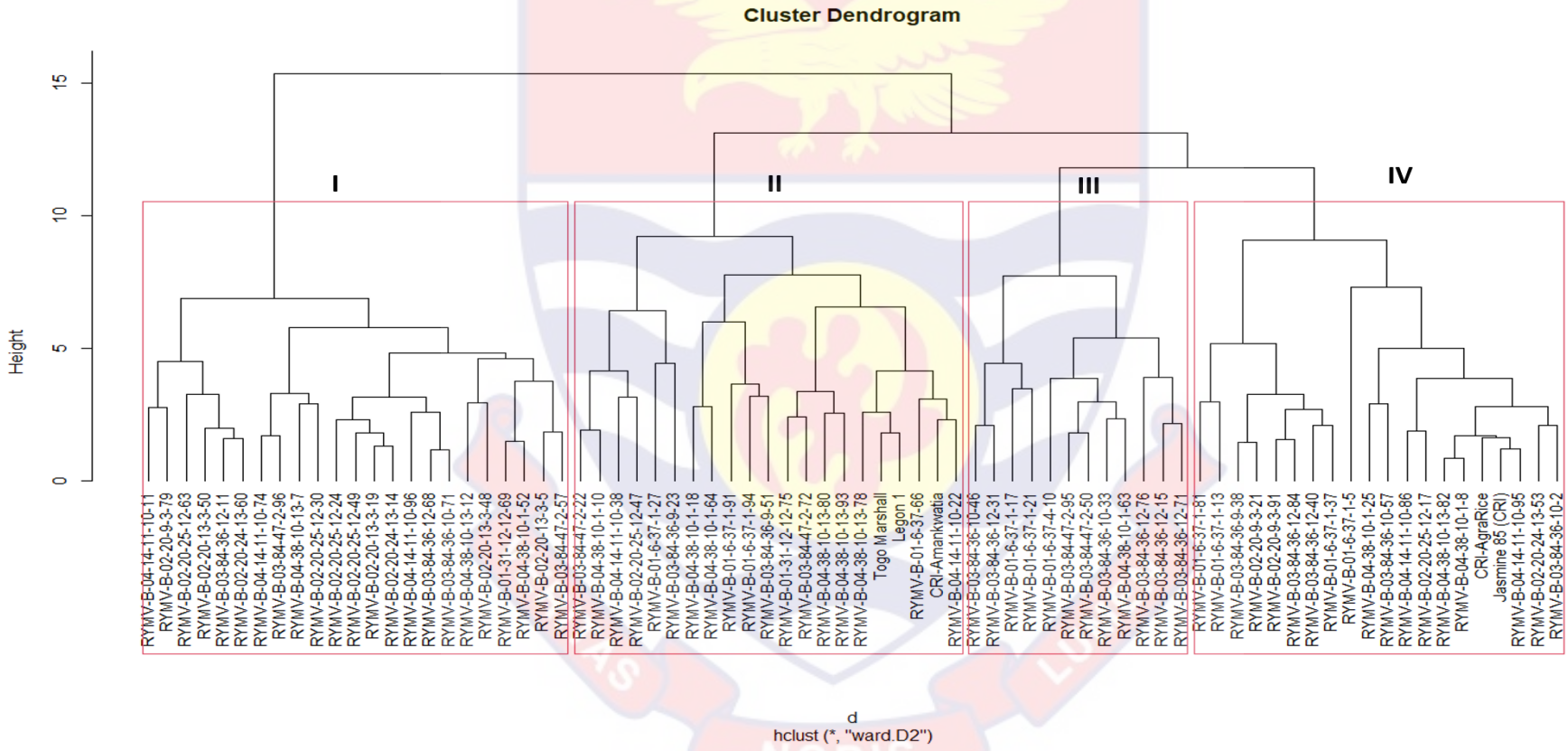

Figure 6.2: Dendrogram of rice lines evaluated for grain yield and yield-related traits in the minor season of 2022 at Fumesua-Kumasi

Principal component analysis and biplot for the genotypes studied

Principal component analysis (PCA) was performed to determine which traits contributed most to the variations in the genotypes and also to find out which of the agronomic traits contributed positively to grain yield.

The first three components explained 58.62% of the total variation in the genotypes (Appendix 2). Principal component 1 (PC1) contributed 25.03% of the total variation and the highest contributing characters to the total variation were tiller number (55.53%), panicle number (54.54%), plant height (25.28%) and grain yield (48.93%).

The PCA biplot indicated in Figure 6.3 shows the distribution of the traits considered, how they correlate with each other, and the performance of the various genotypes in relation to the traits studied. Just as there was highly positive correlation between tiller number, panicle number and yield, the PC biplot shows a similar fashion as the vectors for these three traits point in the same direction.

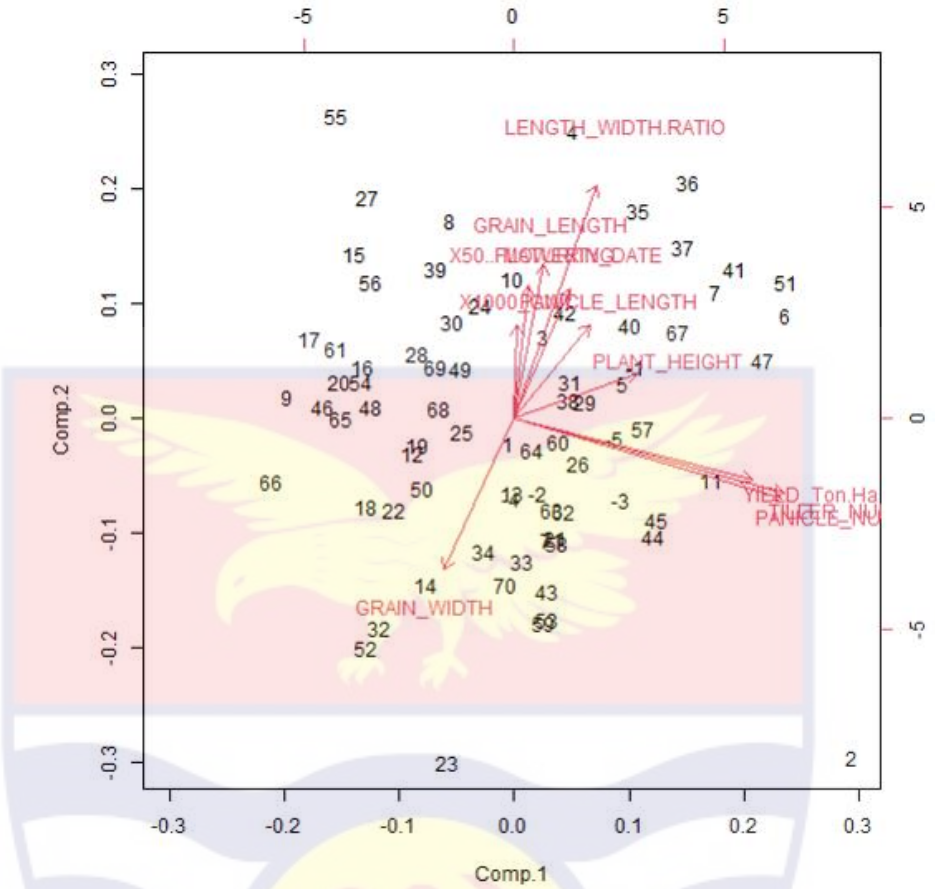

Figure 6.3: Biplot of eleven agro-morphological traits in rice lines evaluated during the minor season of 2022 in Kumasi

The performance of the genotypes in the biplot shows that genotypes 11, 44, 45 and 47 were the highest yielding genotypes. The yield data confirms this since the highest yield came from genotype 44 (7.25 ton/ha). Genotype 11, 47, and 45 followed with 7.23 ton/ha, 7.12 ton/ha and 7.08 ton/ha respectively.

Discussion

Crop yield holds much importance to farmers due to its direct impact on their livelihoods (Lobell & Gourdji, 2012). It is thus imperative that varieties that are earmarked for release should be able to perform well on farmers' fields. Traits that contribute positively to yield are also of interest to the breeder since these can be manipulated to the breeder's advantage in order to increase crop yield (Acquaah, 2012).

The 71 introgression lines resistant to *Rice yellow mottle virus* (RYMV) and rice blast were developed from four recurrent aromatic rice parents and a donor parent. The recurrent parents, Togo Marshall, Jasmine 85, CRI-Agra Rice and CRI-Amankwatia, are all susceptible or moderately susceptible to the two diseases (Asante et al., 2020a; Omiat et al., 2023). The donor parent, Gigante donated both *RYMV1 (rymv1-2)* and *Pi_54*. The objective of this study was to evaluate the performance of these newly developed RYMV and blast resistant lines in a preliminary yield trial.

The results of the preliminary yield trial showed that some of the introgression lines had yields equal to or higher than the highest recurrent parent (CRI-Agra Rice). This is an indication that not only was resistance obtained in the introgression lines, but they are equally good in performance related to yield and could, therefore, be evaluated further for possible release.

The analysis of variance showed significant differences among the genotypes for some of the characters studied. For instance, there were highly significant differences among the genotypes in terms of plant height. The 71 genotypes came from four different parents, therefore, high variation among the genotypes was expected, and for plant height is highly influenced by the

environment. Similar findings were obtained by Islam et al. (2015), Asante et al. (2019), Tiwari et al. (2019), Faysal et al. (2022) and Demeke et al. (2023) when the researchers assessed the genetic variability among selected rice genotypes.

There were highly significant differences among the genotypes in terms of grain yield (ton/ha). This was expected because the genotypes were recombinants from four rice crosses.

Two other traits from the results of the preliminary yield trial that differed significantly ($P<0.005$) were number of panicles (PN) and grain length (GL). For grain length, Nirmaladevi et al. (2015) and Asante et al. (2019) found highly significant differences among the genotypes they studied. This slight difference could be due to the fact that the genotypes they studied came from a more diverse background than the genotypes reported here which came from only four backgrounds.

The ANOVA revealed no significant differences amongst the genotypes in terms of days to 50% flowering (DF), days to maturity (MD), number of tillers (TN) and grain length to width ratio (GL:GW, Table 6.2). However, for days to 50% flowering and days to maturity, Abdourasmane et al. (2016), Tadesse et al. (2018), Asante et al. (2019), Tiwari et al. (2019), Faysal et al. (2022) and Demeke et al. (2023) found significant differences among the genotypes they studied. In the case of these introgression lines, the non-significance seen in reference to DF and MD could be due the fact that the lines came from parents with similar genetic backgrounds. As such, wide genetic differences may not be obvious.

Just like DF and MD, there were no significant differences among the genotypes in terms of number of tillers (TN). This finding is in agreement with the findings of Asante et al. (2019). However, this contradicts the findings of Tadesse et al. (2018), Faysal et al. (2022), and Demeke et al. (2023) who found significant differences in the number of tillers among the genotypes they studied. The similarity of the current findings with that reported by Asante et al. (2019) could be due to the fact that the two trials were performed in the same environment- Fumesua, Kumasi. Another reason for the non-significance in terms of number of tillers could be due to the fact that the genotypes studied came from similar genetic backgrounds (similar recurrent parents), as such, they exhibited less variation in terms of number of number of tillers.

Some traits in this study exhibited high genotypic and phenotypic variation, while others showed low variation (Table 6.3). According to the classification by Sivasubramanian and Menon (1973), four traits exhibited medium to near high phenotypic coefficient of variation (PCV). They are, plant height (10.10%), number of tillers (18.16%), number of panicles (18.20%) and grain yield (11.84%). Similar PCVs were obtained by Tadesse et al. (2018), Asante et al. (2019), Faysal et al. (2022) and Demeke et al. (2023). In this study, genotypic coefficients of variation (GCV) were lower than the phenotypic coefficient of variation (PCV) for all traits measured. Traits with high PCV values are largely influenced by the environment and, as such, difficult to breed for. Tadesse et al. (2018) and Asante et al. (2019) had slightly higher PCVs for yield - one trait that is largely influenced by the environment.

There were low PCV values for days to maturity (MD), grain length (GL), grain width (GW), days to 50% flowering (DF) and panicle length (PL). Faysal et al. (2022) also reported low PCV values for days to 50% flowering and panicle length. Asante et al. (2019) reported low PCV values for kennel length and kennel width whilst Tadesse et al. (2018) recorded lower PCV values for panicle length. Demeke et al. (2023) reported lower PCV values for days to maturity and panicle length. Thus, the current results are in agreement with earlier findings by various researchers. Low PCV values for a particular trait is an indication that the trait is not largely influenced by the environment and, as such, can be inherited in the next generation easily (Acquaah, 2012). This implies that breeders can select traits with low PCV values but high genetic advance for improvement in their breeding programmes.

Generally, broad sense heritabilty (H^2) values obtained for the various traits in the current study were relatively low compared to those obtained by Akbar et al. (2019), Asante et al. (2019) and Demeke et al. (2023). However, heritability values for plant height, grain length and grain width, days to 50% flowering and days to maturity were relatively high for the ten traits studied. High broad sense heritability combined with high genetic advance is an indication that such traits would be highly heritable.

Number of tillers, panicle length and grain yield had the lowest heritability values. This indicates that such traits are highly influenced by the environment and as such are relatively difficult to breed for, since they are controlled by many genes with minor effects (Acquaah, 2012). These findings are in agreement with the findings of Akbar et al. (2019); Asante et al. (2019)

and Demeke et al. (2023). The recurrent selection method can be used to improve such traits in rice.

Moderate genetic advance as a percentage of mean ($GA_{\%}$) was recorded for plant height (14.88%), number of panicles (10.20%) and grain yield (14.10%). These findings are in agreement with the findings of Tadesse et al. (2018), Asante et al. (2019), and Faysal et al. (2022). Moderate to high $GA_{\%}$ combined with high heritability values indicates that the trait in question can easily be improved upon through phenotypic selection (Sumanth et al., 2017). However, low $GA_{\%}$ were recorded for grain length (3.88%), panicle length (2.52%), grain width (3.90%), days to maturity (2.46%), 1,000 grain weight (2.11%) and number of tillers (7.77%). Even though grain length (GL), grain width (GW) and days to maturity (MD) had high heritability; 60%, 60% and 57.28%, respectively, their relatively low $GA_{\%}$ suggests that these traits are governed by both additive and non-additive gene actions (Abebe et al., 2017). Similar findings were reported by Asante et al. (2019), who found high heritability for kennel width (50%) but low $GA_{\%}$ (7.6%).

Number of tillers (TN), panicle length (PL) and 1,000 grain weight (TGW) had low heritability and low genetic advance. This indicates that there is the presence of non-additive gene action and large environmental influence as far as the inheritance of these traits is concerned. These findings are similar to the findings of Abdourasmane et al. (2016), Tadesse et al. (2018) Asante, et al. (2019), and, Tiwari et al. (2019). Since these traits are controlled by non-additive gene actions and are largely influenced by the environment, heterosis breeding would be the best method for breeding for such traits (Abebe et al., 2017).

Association between traits, especially with grain yield were either positive or negative (Fig 6.1). There was highly positive correlation between number of tillers and yield (r=0.71), as well as number of paniclesr and grain yield (r=0.69). Similar findings were obtained by Tadesse et al. (2018), Tiwari et al. (2019) and Faysal et al. (2022), who observed positive significant correlation between tiller number and yield. Asante et al. (2019) also found that number of tillers grain yield in some rice germplasm were positively correlated.

Plant height, panicle length, days to maturity and 1,000-grain weight were not significantly correlated with yield, though their association with yield was positive. These findings are in agreement with the findings of Tiwari et al. (2019) who reported positive non-significant association between plant height and 1,000-grain weight with yield, though they reported positive significant association between days to 50% flowering and days to maturity with yield, contrary to the findings of the current study. Other researchers have also reported different findings from the association between the traits mentioned above and yield. Asante et al. (2019) and Faysal et al. (2022) reported positive significant associations between plant height and yield, whilst Tadesse et al. (2018) reported negative significant association between the same traits. Whereas Asante et al. (2019) and Faysal et al. (2022) reported positive significant association between panicle length and yield, Tadesse et al. (2018) reported otherwise. The different reports given by the different researchers could be due to the fact that most of these traits are highly influenced by the environment and, as such, may give different values under different circumstances.

The highly positive correlation between number of tillers and yield suggests that number of tillers could be selected indirectly for yield. Thus, improvement in this trait would invariably improve yield. Since direct selection of yield is difficult because yield is a complex trait, number of tillers can be used to indirectly select for yield.

Negative association existed between days to 50% flowering and yield as well as grain width and yield, though they were not significant. As a result, early maturing rice varieties can be selected without significant yield reduction. Similar result was obtained by Faysal et al. (2022) for days to 50% flowering and yield, though Tadesse et al. (2018), Asante et al. (2019) and Tiwari et al. (2019) reported significant positive association between the same trait and yield.

Cluster analysis was performed to determine how diverse the genotypes were, using 10 agro-morphological traits. The results grouped the genotypes into four main clusters (Figure 6.2). Cluster I comprised 20 genotypes. Cluster II contained 23 genotypes. Cluster III was made up of 12 genotypes whilst cluster IV contained 21 genotypes. The genotypes clustered in four probably because they came from four different backgrounds though the four parents clustered differently. Cluster I contained the highest yielders with the highest number of tillers and panicles. Cluster II contained genotypes that were early in terms of flowering and maturity but had the lowest yield. Same characteristics from cluster II were observed in cluster III, though genotypes in cluster III were taller than genotypes in cluster II. Genotypes in cluster IV were also tall, had long panicles and long grains but lower yield compared to genotypes in clusters I and II.

The results from the cluster analysis can serve as a guide in selecting lines even as parents for crosses since genotypes in a particular cluster exhibit similar characteristics. Similar observations were made by Tadesse et al. (2018) in a study of diversity among hundred rice germplasm.

Principal component analysis (PCA) and the biplot generated from this study using the 11 agronomic traits determined the variations among the genotypes as well as the determination of the trait(s) that contributed to such variations observed in the genotypes. Eleven PCs were generated from the analysis (Appendix 2). The first three components explained 58.62% of total variations in the genotypes. PC1 was the most important component. In this component, number of tillers, number of panicles, plant height and yield contributed 55.53%, 54.54%, 25.28% and 48.93% of total variations respectively. In this component all the traits studied were positively associated with yield, except for grain width.

Grain length, grain length to width ratio, days to maturity and days to 50% flowering contributed most to the variations in PC2. These observations could be a good guide in selecting candidate lines for further evaluation. Similar findings were made by Asante et al. (2019) and Faysal et al. (2022).

The biplot obtained from PC1 and PC2 indicated the performance of the genotypes in relation to the 11 agronomic traits studied. The biplot revealed both positive and negative relationship among the traits and genotypes studied. For instance, there was positive association between grain yield, tiller number and panicle number. Genotypes 11, 44, 45, 47 and 6 were in the same direction as the vectors for yield and yield related traits mentioned above. Thus, these genotypes had the highest number of tillers, panicles as well as yield. The results

from the biplot also showed how the vectors for grain length and grain width moved in opposite directions, indicating a negative association between these two traits. Similar results were obtained by Asante et al. (2019) and Faysal et al. (2022) when they studied the behavior of a set of germplasm in relation to yield and yield related traits.

Conclusions

The genotypes evaluated varied from each other with reference to some specific traits such as plant height, panicle number and grain yield. There was high heritability for traits such as plant height (71.53%), grain length (60.00%), grain width (60.00%) and days to 50% flowering (67.95%). However, number of tillers, panicle length and 1,000-grain weight had low heritability (20.78%, 27.20%, 25.53% and 37.29%, respectively).

There was highly positive correlation between grain yield and number of tillers (71%), and number of panicles and yield (69%). The highly positive correlation between tiller number and yield suggests that number of tillers can be indirectly selected for improvement in yield.

The principal component analysis indicated that the highest variation within the genotypes came from number of tillers, number of panicles and yield. The biplot showed a positive association between grain yield, number of tiller and number of panicles.

Some of the 71 introgression lines performed very well as against the checks. CRI-Agra Rice produced the highest yield among the checks (7.09 ton/ha), whereas genotypes 44 (RYMV-B-03-84-36-10-57), 11 (RYMV-B-01-6-37-1-91) and 47 (RYMV-B-03-84-36-10-46) produced 7.25 ton/ha, 7.23

ton/ha, and 7.12 ton/ha, respectively, beating the highest yielding check (CRI-Agra Rice). The comparatively higher performance of these genotypes suggest that they are promising lines and should further be evaluated for possible release.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

General Discussion

Rice is the staple food for more than half of the world's population who depend on it for their daily calorie needs (Yadav et al., 2017; Bazrkar-Khatibani et al., 2019; FAOSTAT, 2022). It is also a food security crop for rural populations. It is the fastest growing food in Ghana with per capita consumption with current per capita consumption of 45kg/ person/year (MOFA 2021). However, about 50% of the 1.5 million MT consumed every year is imported (MOFA 2021). Thus, there is an urgent need to increase rice production to bridge the gap between supply and demand.

Rice yield in Ghana is adversely affected by biotic stresses including blast and RYMV diseases, which are the two main diseases in SSA. There is thus the need to tackle these two diseases in order to reduce yield losses and produce enough to bridge the demand-supply gap.

According to Acquaah (2012), resistant breeding is the most cost-effective way to reduce the impact of diseases on crop yield. Thus, this study aimed to breed resistance for rice blast and Rice yellow mottle disease into four popular aromatic rice varieties in Ghana.

The first part of this research was to genotype 300 selected rice germplasm from the CSIR-Crops Research Institute in order to identify resistant varieties for rice blast and Rice yellow mottle disease in the germplasm. The genotyping results showed interesting findings. For instance, the results showed that the germplasm at the CSIR-CRI did not contain any resistant varieties for

RYMV1 (rymv1-5) and *RYMV3*. This indicates that exotic germplasm containing these two resistance genes must be introduced into the germplasm at the CSIR-CRI. However, 10 out of the 300 germplasm representing 3.33% contained the resistance gene *RYMV1 (rymv1-2).* This shows that there are options for selection for *RYMV1 (rymv1-2)* and, thus, good donors could be selected for this trait in future crosses.

In the case of rice blast resistance, the germplasm had high representation for the blast genes *Pi54* (24.33%) and *Pita* (33.0%). This indicates that genes for resistance to blast, particularly *Pi54* and *Pita*, could easily be obtained from the germplasm. Only two out of the 300 genotypes contained the blast resistance gene, *Pik*. With reference to *Pi9,* only one of the germplasm contained this resistance gene. Thus, more sources of resistance especially in elite background, should be developed in-country or introduced into Ghana.

The most important part of this study was the introgression of rice blast and Rice yellow mottle resistances into the four popular aromatic rice varieties. The two resistance genes were obtained from the donor parent, Gigante, which has the resistance genes *RYMV1 (rymv1-2)* and *Pi54*. The crosses were made to produce four different F_1 populations representing the four recurrent parents. At BC_3F_2, 1,489 lines were genotyped, out of which 86 lines were found to be homozygous for these two resistance genes. Seventy-one out of the 86 lines were evaluated for yield performance. The mid-density genotyping which was performed to find lines with high recurrent parent genome showed some of the crosses had more than 87% of the recurrent parent genome (RPG). The use of molecular markers helped recover most of the RPG in just three backcrosses.

Miah et al. (2015) reported that the use of marker-assisted breeding helps cut breeding time, such that what can be achieved in just three backcrosses would only be achieved in up to 6 backcrosses if markers are not used and only conventional breeding methods are applied.

To check the resistance level of the blast and RYMV introgression lines, the genotypes were subjected to screening for *RYMV* through inoculation with virus isolate from a hotspot in the Ashanti Region of Ghana. All the 71 lines with the introgressed genes showed resistance for *RYMV* with very low severity scores. This was expected because the donor parent, Gigante, was previously reported to be highly resistant to *RYMV* isolates in Ghana. The lines were also found to resistant to rice blast. Hence, improving the resistance of the popular rice varieties to the two diseases is possible through marker-assisted breeding.

One important aspect of this study was to compare the performance of the introgression lines with their recurrent parents in terms of yield and yield-related traits. The results obtained from the preliminary yield trial showed that some of these lines were same or better than their recurrent parents in terms of yield. This implies that these lines have good potential and should be further evaluated for possible release.

The genotypes evaluated varied from each other with reference to some specific traits, such as plant height, panicle number and grain yield. There was high heritability for traits, such as plant height, grain length, grain width and days to 50% flowering. This implies that, such traits with high heritability would be easier to breed, compared to the other traits with low heritability.

There was highly positive correlation between grain yield and number of tillers (71%), and number of panicles and yield (69%). This suggests that number of tillers can be indirectly selected to improve grain yield.

Conclusions

The results of the genotyping of the 300 rice lines from CSIR-CRI germplasm showed high presence for two blast genes, *Pita* and *Pi54*. However, there was low presence of *Pik* and *Pi9* in the germplasm. For RYMV, 10 genotypes, representing 3.33% of the population, contained the *RYMV1 (rymv1-2)* gene. *RYMV3* and *RYMV1 (rymv1-5)* were however absent in the germplasm. There is therefore the need to introduce more resistance for RYMV in the germplasm at the CSIR-CRI.

The introgression of RYMV resistance gene, *RYMV1 (rymv1-2)* and the rice blast gene, *Pi54*, from the donor parent, Gigante, produced 86 plants fixed for these two genes at BC_3F_2. Seventy-one of these lines were screened for resistance to *Rice yellow mottle virus* and also scored for rice blast disease. All the 71 introgressed lines were found to be highly resistant to *RYMV* just like their donor parent. Also, the scores for blast shows the lines were resistant to rice blast disease. This indicates that the introgression of the resistance genes to these two major diseases was successful.

The introgression lines were evaluated for yield and yield-related traits. The highest yield recorded from the four recurrent parents was 7.09 ton/ha, and this was obtained from CRI-Agra Rice. However, the genotypes RYMV-B-03-84-36-10-57, RYMV-B-01-6-37-1-91 and RYMV-B-03-84-36-10-46 produced 7.25 ton/ha, 7.23 ton/ha, and 7.12 ton/ha, respectively, which was significantly

higher ($P < 0.05$) than the best check, CRI-Agra Rice. This indicates that the above-mentioned genotypes have high potential because, not only are they resistant to RYMV and blast due to the introgression, but they were also high yielding, compared to their recurrent parents.

Recommendations

Based on the findings of this research, the following recommendations are made:

i. There is the need to introduce more sources of RYMV resistance, especially *RYMV1 (rymv1-5)* and *RYMV3* into the Rice Breeding Programme at the CSIR-Crops Research Institute.

ii. The 71 introgressed lines should be artificially inoculated for rice blast disease to validate their resistance levels.

iii. The newly developed blast and RYMV resistant lines with high yield potential should be evaluated further in Advanced Yield Trials (AYT) to for release as commercial rice varieties.

iv. Gene pyramiding using other *R*-genes for blast and RYMV should be incorporated into the best yielding lines in order to develop more durable resistance.

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APPENDICES

Appendix 1: List of the 300 core germplasm at the CSIR-CRI used for genotyping for blast and RYMV resistance genes.

S/No.	GENOTYPE
1.	CRI-AMANKWATIA
2.	CRI-Agra Rice
3.	Obolo
4.	Jasmine 85-CRI
5.	Toxx3377
6.	ARICA 3
7.	Digang
8.	SA51-SARI
9.	IDSA85
10.	Togo Marshall
11.	CRI-Dartey
12.	CRI-Kantinka
13.	CRI-Oboafo
14.	CRI-Emopa
15.	CRI-Mpuntuo
16.	Enapa
17.	AGRA-CRI-LOL-2-7
18.	SA54-SARI
19.	AGRA-CRI-LOL-1-11
20.	AGRA-CRI-LOL-1-21
21.	CRI-1-21-5-12
22.	SA25-SARI
23.	CRI-1-11-19-12
24.	SAHEL 177
25.	SR35250-1-23-2-1
26.	SR35266-2-4-4-1
27.	SA35-SARI
28.	SR34590-HB 3433-5-1-1
29.	SR35266-2-16-1-1
30.	SR35266-2-18-2-1
31.	SR35266-2-12-4-1
32.	SR34053(#5-52)-1-4-2-10-1-2
33.	Jasmine 85-SARI
34.	ART143-150-B-1-B-B
35.	ART216-133-B-1-B-B
36.	ART216-149-B-1-B-B
37.	ART216-173-B-1-B-B
38.	ART216-187-B-1-B-B
39.	ART216-212-B-1-B-B
40.	ART263-11-B-1-B-B
41.	SA29-SARI
42.	ART314-14-B-1-B-B

43.	SA7-SARI
44.	ART387-2-1-1-1-0
45.	ART387-9-1-1-1-B
46.	ART397-12-1-1-1-B
47.	SA57-SARI
48.	ART397-3-2-1-1-B
49.	ART430-16-1-1-1-5
50.	SA59-SARI
51.	SA30-SARI
52.	ART1005-21-1-1-1-B
53.	SA26-SARI
54.	ART478-13-8-1-1-B
55.	ART478-8-1-1-1-B
56.	SA28-SARI
57.	ART483-10-1-1-1-B
58.	ART484-19-1-1-1-B
59.	ART493-16-1-1-1-B
60.	SA39-SARI
61.	ART132-35-1-1-B-B
62.	SA41-SARI
63.	SA52-SARI
64.	ART245-1-40-1-B-B
65.	SA50-SARI
66.	SA36-SARI
67.	ART152-3-1-1-B-B
68.	ART174-2-1-1-B-B
69.	ART245-1-16-1-B-B
70.	ART248-1-97-1-B-B
71.	ART75-30-1-1-B-B
72.	ART58-5-1-1-B-B
73.	ART58-46-1-1-B-B
74.	SA38-SARI
75.	SA10-SARI
76.	SA9-SARI
77.	ART64-31-1-1-B-B
78.	ART64-32-1-1-B-B
79.	SA60-SARI
80.	ART64-55-1-1-B-B
81.	ART68-12-1-1-B-B
82.	ART75-8-1-2-B-B
83.	ART75-14-1-2-B-B
84.	ART75-33-1-1-B-B
85.	ART75-56-1-2-B-B
86.	ART75-57-1-1-B-B
87.	ART79-12-1-1-B-B
88.	ART90-9-1-1-B-B
89.	ART90-12-1-1-B-B
90.	ART90-46-1-1-B-B

91.	ART105-3-1-2-B-B
92.	ART120-26-1-1-B-B
93.	ART125-26-1-1-B-B
94.	SA62-SARI
95.	ART73-69-1-2-B-B
96.	ART84-35-1-1-B-B
97.	SR35278-1-9-3-3
98.	ART67-17-1-1-B-B
99.	ART67-21-1-2-B-B
100.	ART98-4-1-1-B-B
101.	ART98-147-1-1-B-B
102.	SA21-SARI
103.	SA20-SARI
104.	ART100-56-1-1-B-B
105.	ART100-57-1-2-B-B
106.	ART101-99-1-2-B-B
107.	SA11-SARI
108.	ART112-74-1-1-B-B
109.	ART112-85-1-1-B-B
110.	ART71-2-1-1-B-B
111.	ART71-96-1-1-B-B
112.	SA56-SARI
113.	ART85-48-1-1-B-B
114.	ART93-112-1-1-B-B
115.	SA58-SARI
116.	ART350:10-2-1-B
117.	ART350:2-4-3-B
118.	ART350:2-6-1-B
119.	WAB 2101-WAC4-1-TGR1-WAT3-5-TGR2
120.	ART108-2-1-1-B-B
121.	NERICA-L 19
122.	IR 84105-B-B-B-TGR4
123.	WAB 2085-TGR2-WAT4-1-1
124.	WAB 2135-WAC B-2-TGR3-WAT8-3
125.	WAB 2138-WAC B-2-TGR2-WAT5-1
126.	WAB 2099-WAC1.FKR3-1-TGR1-2
127.	AGRA-CRI-LOL-2-27
128.	AGRA-CRI-LOL-2-29
129.	NERICA-L 41
130.	SA61-SARI
131.	CRI-1-11-15-5
132.	AGRA-CRI-LOL-1-7
133.	CRI-1-11-15-21
134.	CRI-1-21-5-12
135.	SA64-SARI
136.	Viwornor short
137.	KAF53
138.	KAF143

139.	BOUKE 189
140.	N1
141.	BETIA
142.	IDS 85
143.	TV2
144.	CK3
145.	KE40
146.	KE53
147.	ARICA 2
148.	SA53-SARI
149.	UPL 87
150.	UPL 39
151.	SA55-SARI
152.	AFRICARICE 6
153.	SA14-SARI
154.	UPL 44
155.	SA12-SARI
156.	GR18-SARI
157.	SA7-SARI
158.	UPL 30
159.	SA22-SARI
160.	AFRICARICE 4
161.	Khao Hlanon (AfricaRice)
162.	TV1
163.	644 Gold
164.	UPL 32
165.	Awarema
166.	Oreire
167.	228
168.	80 DAYS-SARI
169.	Gigante-SARI
170.	AGRA-SARI
171.	WAIQ1-SARI
172.	SA2-SARI
173.	Ex-Baika (Asutware)
174.	SA63-SARI
175.	SA65-SARI
176.	SR33705F2-60-1-1HV-1-1
177.	SA4-SARI
178.	SA15-SARI
179.	SA8-SARI
180.	SA66-SARI
181.	SR34590-HB 3433-8-3-1
182.	SR35266-2-11-1-1
183.	SR34590-HB3433-8-2-1
184.	SR35266-2-11-4-1
185.	SA31-SARI
186.	SR35266-2-12-1-1

187.	SR35266-2-12-2-1
188.	SR35266-2-17-1-1
189.	SR35266-2-12-4-1
190.	SR35266-2-17-2-1
191.	SA33-SARI
192.	SR35266-2-16-1-1
193.	UPL 6
194.	SR35266-2-20-1-1
195.	SR35266-2-18-1-1
196.	SR35266-2-18-2-1
197.	SR35266-2-18-3-1
198.	SR35266-2-19-1-1
199.	SR35250-2-4-2-3
200.	SR34053(#5-52)-1-4-2-10-3-1
201.	SR35250-2-3-1-1
202.	SR34053(#5-52)-1-4-2-10-3-3
203.	SA68-SARI
204.	SA69-SARI
205.	SA17-SARI
206.	SA13-SARI
207.	SR35278-2-10-1-3
208.	SR35285-2-8-4-1
209.	SR35250-1-15-1-1
210.	SR35266-2-20-3-1
211.	SR23364-128-1907-1-HV-1-1
212.	SR35266-3-1-5-1
213.	SR35266-3-1-3-1
214.	SR23364-133-171-1-HV-1-1
215.	SA18-SARI
216.	SR34590-HB3433-1-1-1
217.	SR34590-HB3433-1-3-1
218.	SR23364-128-1835-1-HV-1-1
219.	SR23364-133-17-1-HV-1-2
220.	SR34590-HB3433-2-1-1
221.	SR34590-HB3433-7-2-1
222.	SR35266-3-3-5-1
223.	SR34590-HB3433-7-3-1
224.	SR35266-3-2-3-1
225.	SA24-SARI
226.	SR35266-3-2-4-1
227.	SR34590-HB3433-6-2-1
228.	SR34590-HB3433-6-1-1
229.	SAHEL 134
230.	SAHEL 210
231.	SA19-SARI
232.	SR34796-1-4-6-3-2-1
233.	SR34590-HB3433-5-1-1
234.	SR34590-HB3433-3-1-1

235.	SR34034F3-71-2-1-1-3
236.	SA1-SARI
237.	SA3-SARI
238.	SR34042F3-22-1-1-1-3
239.	SR34042F3-22-1-1-5-3
240.	SR35300-1-HV-1-2
241.	SR33705F2-60-1-2-HV-1-2
242.	SR35230-2-9-2-2
243.	SA34-SARI
244.	SR3305F2-60-2-2-HV-1-1
245.	SR35266-2-16-2-1
246.	JKE99-32-D
247.	JKE99-26-D
248.	SR35278-1-7-2-2
249.	SR35278-2-10-1-2
250.	SR35278-1-9-3-1
251.	SR35278-1-9-2-1
252.	SR33705F2-61-3-2-HV-1-1
253.	SR33705F2-61-1-3-HV-1-1
254.	SR33705F2-67-1-1-HV-1-1
255.	SR34590-HB-3433-7-1-1
256.	SR35278-1-7-3-2
257.	SR35266-2-20-3-1-1
258.	SR34598-HB-16-HV-1-1
259.	SR35276-2-4-3-1-1
260.	SR35266-2-11-4-1-1
261.	SR34590-HB3433-1-3-1-1
262.	SR35266-2-11-1-1-1
263.	HR32080-HB3567-4
264.	HR32086-HB3569-37
265.	SR35311-HB3497-4
266.	JKE56-3-D
267.	SR35311-HB3497-88
268.	SR35329-HB3509-91
269.	JKE56-12-D
270.	HR32046F1-2-26-1
271.	HR32054F1-1-29-1
272.	HR32066F1-4-19-1
273.	HR32066F1-4-16-1
274.	HR32067F1-2-14-1
275.	HR32067F1-3-5-1
276.	HR32069F1-1-3-1
277.	HR32069F1-1-13-1
278.	HR32069F1-1-18-1
279.	HR32069F1-2-4-1
280.	JKE56-30-D
281.	HR32068F1-3-10-1
282.	HR32068F1-4-11-1

283.	Japonica 1
284.	Gigante
285.	Tog7291
286.	DigJas-3
287.	DigJas-61
288.	DigJas-78
289.	AgKE99-32
290.	AgKE99-26
291.	AgKE99-56
292.	JKE56-3
293.	JKE56-12
294.	JKE56-30
295.	KBR 2
296.	FKR 62N
297.	KBR 12
298.	KBR V4
299.	KBR 10
300.	ORYLUX 6

Appendix 2: Results of Principal Components Analysis (PCA) in relation to yield and yield component traits of the 71 introgressed lines and their checks.

Component loadings:

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11
GRAIN_LENGTH	0.061	0.373	0.076	0.518	0.333	0.453	0.073	0.0003	0.028	0.012	0.510
GRAIN_WIDTH	-0.144	-0.367	-0.369	0.230	0.483	0.340	0.149	0.0173	-0.077	0.009	-0.527
GL/GW RATIO	0.169	0.564	0.342	0.210	-0.123	0.077	-0.065	-0.003	0.073	-0.015	-0.679
MATURITY_DATE	0.113	0.315	-0.507	-0.062	-0.297	0.002	0.614	0.397	-0.060	-0.010	0.0026
PANICLE_LENGTH	0.156	0.225	-0.153	-0.550	0.406	0.133	-0.139	0.108	0.621	-0.021	-0.0003
PANICLE_NUMBER	0.545	-0.194	-0.055	0.112	-0.058	0.038	-0.263	0.320	-0.042	0.686	-0.0006
PLANT_HEIGHT	0.253	0.109	0.303	-0.449	0.403	0.031	0.271	-0.051	-0.624	0.049	0.003
TILLER_NUMBER	0.555	-0.182	-0.078	0.094	-0.037	0.055	-0.244	0.215	-0.114	-0.721	0.015
50%_FLOWERING	0.030	0.320	-0.560	-0.126	-0.129	0.154	-0.430	-0.478	-0.333	0.060	-0.00009
1000_GWT	0.006	0.226	-0.210	0.274	0.451	-0.778	-0.104	0.086	-0.058	-0.006	0.0011
YIELD_(ton/ha)	0.489	-0.149	-0.040	0.124	-0.017	-0.146	0.417	-0.666	0.284	0.040	-0.004

Component variances:

Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11
2.753	1.965	1.730	1.566	1.101	0.783	0.441	0.346	0.292	0.023	0.0008

Importance of components:

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11
SD	1.659	1.402	1.315	1.251	1.049	0.885	0.664	0.588	0.540	0.150	0.030
Variance	0.250	0.179	0.157	0.142	0.100	0.071	0.040	0.031	0.027	0.002	0.00008
Cum. proportion	0.250	0.430	0.586	0.729	0.829	0.900	0.940	0.971	0.998	0.999	1.000

SD: Standard deviation. Cum: Cumulative