

**INTEGRATING GENETIC RESISTANCE WITH BIOCONTROL  
AGAINST  
RICE BLAST AND DROUGHT**

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**A thesis submitted in partial fulfilment of the requirements for the degree of  
Doctor of Philosophy (PhD) in Plant Breeding**

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June 2015

## THESIS ABSTRACT

Annual demand for rice in Liberia far outstrips local production, mainly as a result of rice blast and drought. The use of resistant cultivars has been widely considered as the most economical means of controlling the disease and mitigating the impact of drought stress (DS) on rice (*Oryza sativa* L.). However, despite the high variability of the rice blast pathogen (*Pyricularia oryzae* Cavara), efforts aimed at genetically managing the disease have focused mainly on the development of cultivars with vertical resistance, which has repeatedly failed soon after the release of new cultivars because new pathotypes evolved with matching virulence genes. The current research was consequently undertaken to devise breeding strategies aimed at durably controlling rice blast and mitigating the impact of DS on rice by integrating host plant resistance with biocontrol agents. Towards these objectives, separate studies were undertaken to appraise local perspectives of rice production in Liberia and to identify key rice varietal traits preferred by farmers, to characterize upland rice locally cultivated in Liberia for resistance to rice blast and tolerance to drought, to determine suitable environments for screening rice for drought tolerance and quantitative resistance to rice blast, to determine the gene action and combining ability for the inheritance of key rice blast resistance traits, to determine the effects of a combination of ethephon and gibberellic acid (GA3) on the hybridization of rice, and to investigate the effects of two strains of *Trichoderma harzianum* on blast resistance and drought tolerance of rice.

Results of both factor and conjoint analyses of farmers' selection criteria and trait preferences revealed that farmers considered a combination of specific agronomic and morphological characteristics, including grain yield, grain quality, stress resistance and post-harvest traits when selecting rice varieties for cultivation. Farmers expressed a strong preference for hardy, early maturing varieties with intermediate stature, which generate soft but discrete (loose) grains when boiled.

Pathogenicity tests involving 38 upland genotypes, inoculated with three pathotypes of *P. oryzae* under controlled environment (CE) conditions, revealed that the blast resistance of selected varieties of upland rice from Liberia was characterized by the occurrence of both race-specific and race-non-specific patterns of resistance. Additive main effects and multiplicative interaction analysis revealed significant ( $p < 0.05$ ) genetic variability among the genotypes for the number and size of sporulating lesions per plant. Rice plants subjected to intermittent DS both under CE and field conditions exhibited larger and more lesions with sporulating centres than those grown under well-watered conditions, thereby

indicating the value of managed DS for evaluating rice for horizontal resistance to blast disease.

Studies on drought tolerance of 22 of the upland rice genotypes showed that drought imposed for 21 days during the booting-to-grain-filling stage of development severely diminished grain yield (GY) and its components, and that the difference in grain yield between plants subjected to DS during the tillering stage and those grown under continuously well-watered conditions was not significant ( $p = 0.05$ ). This study identified the booting-to-grain-filling stage as the primary phenological stage for improving drought tolerance of upland rice from Liberia. Principal component analysis showed that the 22 genotypes exhibited the greatest variation for GY, tiller productivity (TP) and chlorophyll content index (CCI) under well-watered conditions, and for leaf rolling, GY, CCI, spikelet fertility (SF), and TP, under drought imposed during the booting-to-grain-filling stage. Simple correlation and path analyses identified high levels of biomass at heading (BM), stomatal conductance (SC) and SF as important selection criteria for achieving high GY under well-watered conditions, and TP, BM and 1000 grain mass as the key criteria for selecting high yielding genotypes under DS occurring around the booting-to-grain-filling stage.

Field screening for drought tolerance of rice over two seasons, using up to 24 genotypes, revealed that a novel technique using a plastic mulch soil cover system, designed at the University of KwaZulu-Natal, was effective for excluding rainfall from the plants, and is therefore a valuable tool for facilitating controlled drought screening of rice plants during a season with normal rainfall levels.

Evaluation of eight blast susceptible genotypes and their F<sub>2</sub> progenies derived from a full diallel mating system indicated that both additive and non-additive gene actions were involved in the inheritance of LN, LS and area under the disease progress curve (AUDPC), which are three key parameters measured to quantify the levels of horizontal resistance against rice blast. However, the genes with additive effects were more important, since the level of general combining ability (GCA) for each trait was higher than that of specific combining ability (SCA). These results indicated that future breeding of Liberian rice varieties aimed at increasing the level and durability of rice blast resistance should emphasize recurrent selection for reduced LS and lower LN and AUDPC, using genotypes that exhibit negative GCA effects for these traits as progenitors.

Greenhouse studies with plant growth hormones showed that application of ethephon at 4000 to 6000  $\mu\text{L L}^{-1}$  during microsporogenesis, combined with gibberellic acid applied at 90 or 150  $\mu\text{L L}^{-1}$  during anthesis can provide for reliable male sterilization of flowers without diminishing female fertility. This can be used to facilitate the large scale cross-pollination of

rice genotypes and their progeny, which is essential for recurrent selection breeding programmes.

The results of both CE and field studies demonstrated that two biocontrol products, Eco-77® and Eco-T®, containing different strains of *T. harzianum*, were useful for managing rice blast and the effects of drought on rice, respectively. Notably, the impact of the biocontrol treatments tended to increase with the levels of host plant resistance.

Overall, the results of this research show that new high yielding cultivars with durable resistance to rice blast and tolerance to drought can be bred in Liberia by adopting a recurrent selection scheme, using locally adapted genotypes as progenitors, and evaluating breeding lines under managed drought stress and well-watered conditions. Integrating the current biocontrol agents with improved host plant resistance will be important for increasing rice production in Liberia and other regions where rice suffers from rice blast and drought.

## DECLARATION

I, Quaqua S. Mulbah, hereby declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research;
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Quaqua S. Mulbah (**Candidate**)

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

Signed: ..... Date: .....

**Prof. Mark D. Laing (Co-Supervisor)**

Signed: ..... Date: .....

**Prof. Hussein A. Shimelis (Principal Supervisor)**

## ACKNOWLEDGEMENTS

I wish to express my sincere thanks and appreciation to my supervisor, Professor Mark Laing, for inspiring me to undertake the research reported in this thesis. With his enthusiasm, patience and dedication, he steadily guided me in every aspect of my research, and taught me many important concepts along the way. My co-supervisor, Professor Hussein Shimelis, also made plant breeding and PhD work so much easier and more fulfilling for me. I am sincerely grateful for his sustained interest, guidance, encouragement and helpful criticisms. Many thanks to the Government of the Republic of Liberia and the African Development Bank for generously funding this study.

My special and sincere thanks also go to the following:

- Professors Albert T. Modi and Isa Bertling for giving me access to their equipment (plant efficiency analysers, leaf chlorophyll meter and leaf porometer) for data collection.
- Mrs Susan Van der Meyer, Mr Ian Doidge and their work crew, and Messrs Richard Burgdorf and Philemon Zondi for their generous contributions towards the success of my experiments.
- All the Liberian farmers who provided seeds of their local varieties, and the people of Gibi District in Liberia for facilitating the PRA study.
- Dr Tafadzwananshe Mabhaudhi and all of my colleagues and friends of UKZN for all their support and encouragement.

To my wife, Nuwoh, and my children, Ma Yamah, Garmai, Lesay and David, I say thank you for your love, patience and encouragement. Finally, I give the ultimate thanks and glory to my God and Creator for His grace, which enabled me to reach this far.

## TABLE OF CONTENTS

THESIS ABSTRACT .....	ii
DECLARATION.....	v
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
THESIS INTRODUCTION.....	1
1. Background.....	1
2. Rationale for research focus .....	6
3. Research objectives.....	7
4. Thesis outline .....	8
5. References.....	9
Chapter 1: LITERATURE REVIEW.....	12
1.1 Taxonomy of the rice plant .....	12
1.2 Rice in Liberia.....	12
1.3 Blast disease of rice .....	15
1.3.1 Occurrence and epidemiology.....	15
1.3.2 Effects of blast on rice physiology and yield .....	16
1.4 The rice blast pathogen .....	17
1.4.1 Nomenclature and host range .....	17
1.4.2 Pathogenic stability and variability of blast pathogen .....	18
1.5 Genetics of rice blast resistance.....	19
1.6 Durability and specificity of horizontal resistance.....	20
1.7 Breeding for horizontal resistance .....	21
1.8 Characteristics of drought stress and drought tolerance in rice .....	22
1.8.1 Effects of drought stress on rice.....	22
1.9 Drought tolerance.....	24
1.10 Breeding for drought tolerance.....	25
1.10.1 Breeding targets for drought tolerance .....	25
1.11 Screening for drought tolerance .....	26
1.12 Integrating host plant resistance with biocontrol .....	27
1.13 Conclusion.....	27
1.14 References .....	28
CHAPTER 2: FARMER-PREFERRED TRAITS AND CRITERIA FOR SELECTING RICE VARIETIES IN THE GIBI DISTRICT OF LIBERIA.....	39

<b>2.1 Abstract</b> .....	39
<b>2.2 Introduction</b> .....	40
<b>2.3 Research Methodology</b> .....	41
2.3.1 <i>Description of the area and design of the study</i> .....	41
2.3.2 <i>Survey and sampling procedures</i> .....	42
2.3.3 <i>Data structure and analyses</i> .....	43
<b>2.4 Results</b> .....	45
2.4.1 <i>General aspects of rice production and utilization in Gibi District</i> .....	45
2.4.2 <i>Farmers' perception of rice production system and cropping pattern</i> .....	45
2.4.3 <i>Varieties and sources of rice seed grown in Gibi District</i> .....	47
2.4.4 <i>Farmers' perception of current rice varieties</i> .....	48
2.4.5 <i>Farmers' criteria for discriminating between rice varieties</i> .....	48
2.4.6 <i>Farmers' preferences and priorities for varietal traits</i> .....	49
2.4.7 <i>Farmers' perception of production problems</i> .....	51
<b>2.5 Discussion</b> .....	52
2.5.1 <i>Demographic aspects of rice production and utilization</i> .....	52
2.5.2 <i>Farmers' perception of rice production and varieties</i> .....	53
2.5.3 <i>Analysis of farmers' selection criteria</i> .....	54
2.5.4 <i>Farmer-preferred varieties</i> .....	54
2.5.5 <i>Farmers' assessment of rice production problems</i> .....	55
<b>2.6 Conclusion</b> .....	56
<b>2.7 References</b> .....	56
<b>CHAPTER 3: BLAST RESISTANCE OF SELECTED UPLAND RICE FROM LIBERIA</b> .	60
<b>3.1 Abstract</b> .....	60
<b>3.2 Introduction</b> .....	61
<b>3.3 Materials and methods</b> .....	62
3.3.1 <i>Plant materials</i> .....	62
3.3.2 <i>Sources of infection</i> .....	62
3.3.3 <i>Description of study sites</i> .....	63
3.3.4 <i>Experimental design and treatments</i> .....	63
3.3.5 <i>Inoculation of test plants</i> .....	64
3.3.6 <i>Disease evaluation and data analyses</i> .....	64
<b>3.4 Results</b> .....	65
3.4.1 <i>Qualitative reactions of genotypes to rice blast</i> .....	65
3.4.2 <i>Quantitative reaction of genotypes to rice blast</i> .....	68
3.4.3 <i>Effects of genotype and water regime on blast disease severity</i> .....	70



<b>3.5 Discussion</b> .....	75
3.5.1 <i>Effects of pathotype on rice blast resistance</i> .....	75
3.5.2 <i>Genetic variation for qualitative and quantitative blast resistance</i> .....	75
3.5.3 <i>Effects of environment on expression of quantitative traits</i> .....	78
<b>3.6 Conclusion</b> .....	78
<b>3.7 References</b> .....	79
<b>CHAPTER 4: DROUGHT TOLERANCE OF SELECTED UPLAND RICE GENOTYPES FROM LIBERIA</b> .....	82
<b>4.1 Abstract</b> .....	82
<b>4.2 Introduction</b> .....	83
<b>4.3 Materials and methods</b> .....	84
4.3.1 <i>Plant materials</i> .....	84
4.3.2 <i>Controlled environment study: Experimental design and treatments</i> .....	85
4.3.3 <i>Field experiments: Experimental design and treatments</i> .....	85
4.3.4 <i>Measurements and data analyses</i> .....	87
<b>4.4 Results</b> .....	88
4.4.1 <i>Effects of water regime on seedling emergence and height</i> .....	88
4.4.2 <i>Influence of water regime on plant performance under field conditions</i> .....	89
4.4.3 <i>Performance of genotypes under well-watered conditions</i> .....	92
4.4.4 <i>Responses of rice genotypes to drought stress imposed during the booting-to-grain-filling stage of development</i> .....	95
4.4.5 <i>Association between grain yield and other traits in response to drought stress imposed during the booting-to-grain-filling stage of rice development</i> .....	97
<b>4.5 Discussion</b> .....	97
4.5.1 <i>Growth-stage-specific vulnerabilities of genotypes to drought stress</i> .....	98
4.5.2 <i>Responses of genotypes to drought stress during the establishment, tillering and booting-to-grain-filling stages of development</i> .....	99
4.5.3 <i>Importance of traits for selection</i> .....	99
<b>4.6 Conclusion</b> .....	101
<b>4.7 References</b> .....	101
<b>CHAPTER 5: SCREENING RICE (ORYZA SATIVA L.) FOR TOLERANCE TO DROUGHT USING A PLASTIC MULCH SOIL COVER SYSTEM</b> .....	105
<b>5.1 Abstract</b> .....	105
<b>5.2 Introduction</b> .....	106
<b>5.3 Materials and methods</b> .....	107
5.3.1 <i>Description of study site</i> .....	107
5.3.2 <i>Experimental design and treatments</i> .....	108

5.3.3	<i>Layout of the plastic mulch soil cover facility</i> .....	108
5.3.4	<i>Water supply, soil water assessment and drought imposition</i> .....	109
5.3.5	<i>Data collection and analyses</i> .....	109
<b>5.4</b>	<b>Results</b> .....	110
5.4.1	<i>Soil water status of plots</i> .....	110
5.4.2	<i>Effect of rainfall on soil water status</i> .....	110
5.4.3	<i>Effects of the PMSC on selected physiological and agronomic responses of rice genotypes</i> .....	112
<b>5.5</b>	<b>Discussion</b> .....	114
5.5.1	<i>Soil water potential and plant growth responses</i> .....	114
5.5.2	<i>Impact of rainfall on drought treatment under the PMSC</i> .....	115
5.5.3	<i>Cost analysis</i> .....	115
<b>5.6</b>	<b>Conclusion</b> .....	116
<b>5.7</b>	<b>References</b> .....	116
<b>CHAPTER 6: COMBINING ABILITY AND GENE ACTION OF THREE COMPONENTS OF HORIZONTAL RESISTANCE AGAINST RICE BLAST</b> .....		119
<b>6.1</b>	<b>Abstract</b> .....	119
<b>6.2</b>	<b>Introduction</b> .....	120
<b>6.3</b>	<b>Materials and methods</b> .....	121
6.3.1	<i>Plant materials and experimental design</i> .....	121
6.3.2	<i>Data analyses</i> .....	121
<b>6.4</b>	<b>Results</b> .....	122
6.4.1	<i>Genotypic means and variances</i> .....	122
6.4.2	<i>Combining ability</i> .....	124
6.4.3	<i>Estimates of genetic parameters for LS, LN and AUDPC</i> .....	125
6.4.4	<i>Graphical analysis of genetic components</i> .....	126
<b>6.5</b>	<b>Discussion</b> .....	128
6.5.1	<i>Combining abilities of rice parents and their crosses for LN, LS and AUDPC</i> .....	128
6.5.2	<i>Gene actions for LN, LS and AUDPC in the parental genotypes</i> .....	129
6.5.3	<i>Graphical assessment of genetic components</i> .....	130
6.5.4	<i>Heritability for LN, LS and AUDPC</i> .....	131
<b>6.6</b>	<b>Conclusion</b> .....	131
<b>6.7</b>	<b>References</b> .....	132
<b>CHAPTER 7: INVESTIGATION OF ETHEPHON AND GIBBERELIC ACID AS A COMBINED CHEMICAL HYBRIDIZING AGENT FOR RICE</b> .....		135
<b>7.1</b>	<b>Abstract</b> .....	135
<b>7.2</b>	<b>Introduction</b> .....	136

<b>7.3 Materials and methods</b> .....	137
7.3.1 <i>Study sites, experimental design and trial establishment</i> .....	137
7.3.2 <i>Measurements and data analyses</i> .....	138
<b>7.4 Results</b> .....	139
7.4.1 <i>Effect of ethephon on pollen viability</i> .....	139
7.4.2 <i>Effects of Ethephon and GA3 on spikelet fertility</i> .....	140
7.4.3 <i>Effects of ethephon and GA3 on panicle exertion</i> .....	140
7.4.4 <i>Effects of ethephon and GA3 on plant height</i> .....	141
7.4.5 <i>Effects of ethephon and GA on outcrossing</i> .....	141
<b>7.5 Discussion</b> .....	143
7.5.1 <i>Effect of ethephon on the induction of male sterility</i> .....	143
7.5.2 <i>Effect of growth stage on efficacy of ethephon for male sterility induction</i> .....	144
7.5.3 <i>Impacts of ethephon and GA on spikelet fertility and outcrossing</i> .....	145
7.5.4 <i>Effects of ethephon and GA3 on panicle exertion and plant height</i> .....	146
7.5.5 <i>Influence of genotype on efficacies of Ethephon and GA3</i> .....	147
<b>7.6 Conclusion</b> .....	147
<b>7.7 References</b> .....	148
<b>CHAPTER 8: EFFECTS OF <i>TRICHODERMA HARZIANUM</i> ON THE RESPONSES OF RICE (<i>ORYZA SATIVA</i> L.) TO BLAST DISEASE AND DROUGHT STRESS</b> .....	153
<b>8.1 Abstract</b> .....	153
<b>8.2 Introduction</b> .....	154
<b>8.3 Materials and methods</b> .....	155
8.3.1 <i>Plant materials</i> .....	155
8.3.2 <i>Description of study sites</i> .....	155
8.3.3 <i>Experimental design and treatments</i> .....	156
8.3.4 <i>Inoculation of test plants</i> .....	157
8.3.5 <i>Sampling and data analyses</i> .....	157
<b>8.4 Results</b> .....	159
8.4.1 <i>Effect of Trichoderma on the number of sporulating lesions per plant</i> .....	159
8.4.2 <i>Effect of Trichoderma on size of sporulating lesions</i> .....	160
8.4.3 <i>Effects of Trichoderma on disease severity and progression</i> .....	161
8.4.4 <i>Maximum disease severity</i> .....	163
8.4.5 <i>Area under the disease progress curve</i> .....	164
8.4.6 <i>Percent reduction in disease severity</i> .....	165
8.4.7 <i>Effects of Trichoderma on pre- and post-infection drought tolerance of rice</i> ...	166
8.4.8 <i>Effect of Trichoderma on biomass accumulation of rice</i> .....	166

<b>8.5 Discussion</b> .....	167
8.5.1 <i>Effects of Trichoderma on the number of sporulating lesions and lesion size inflicted by P. oryzae on rice</i> .....	168
8.5.2 <i>Effect of Trichoderma on drought tolerance of rice</i> .....	168
8.5.3 <i>Effects of Trichoderma on growth and yield of rice</i> .....	169
8.5.4 <i>Genotypic influences on the effectiveness of Trichoderma harzianum</i> .....	169
8.5.5 <i>Effect of blast disease on drought tolerance of rice plants</i> .....	169
<b>8.6 Conclusion</b> .....	170
<b>8.7 References</b> .....	170
<b>THESIS OVERVIEW</b> .....	174

# THESIS INTRODUCTION

## 1. Background

Rice (*Oryza sativa* L.) is the most important source of food and calories for more than one-half of the human race (Partnership, 2013). The crop provides employment and livelihood for close to a billion households in Africa, Asia and the Americas (Diouf, 2003). Rice has been ranked consistently, since 2008, as the second most important food and agricultural commodity in the world, with a mean trade value of \$182 billion per annum (FAO, 2013).

In Africa, rice is a major part of the diets of millions of people (Oteng and Sant'Anna, 1999). According to Africa Rice Centre (AfricaRice, 2010), the demand for rice on the continent continues to increase. The current import costs of rice to African countries is at least \$4 billion per year for approximately 10 million tonnes of rice (AfricaRice, 2010), indicating the economic importance of rice in the lives of many Africans.

In Liberia, the primary target region, rice is the most important food crop, annually accounting for at least 80% of the total land area under annual staple food production (GOL, 2009). The nation has one of the highest per capita consumption (120 kg p.a.) of rice in the world (GOL, 2010; IRRI, 2014). This makes rice a major food security issue, as any change in its availability and price can impact the poor directly.

Like many other nations in Africa, Liberia largely depends on rice imports to augment its recurring domestic production deficit (Table 1). This failure to be self-reliant in rice production is largely caused by the low annual production as a result of low yields per unit area (Table 1). Whereas the global mean yield of rice is about 4.2 t ha<sup>-1</sup> (FAO, 2011), yields in Liberia are only 1.4 t ha<sup>-1</sup>. The poor yield and production of rice in Liberia are caused by a combination of environmental and socio-economic factors.

Apart from the socio-economic limitations, rice blast and drought stress are the most important constraints limiting the yield and production of rice in Liberia (Maclean *et al.*, 2002). Reversing the yield reduction caused by rice blast and drought would not only improve food security and reduce national trade deficits, but also increase the income and livelihood of resource poor farmers, particularly if the interventions are sustainable and affordable.

Rice blast and drought are also common problems affecting most rice-producing countries around the world (O'Toole, 2004), many of which depend on foreign imports to satisfy domestic demands. As the global food crisis of 2005 to 2008 clearly highlighted, developing countries cannot allow themselves to remain reliant upon foreign food imports (Shah, 2008;

Headey and Fan, 2010). Hence, there is a great need to develop tools to reduce the impact of rice blast and drought on rice yield and production in order to avert future food crises.

Table 1: Liberia rice production, yield and trade trends (Data source: Diagne *et al.* (2008); GOL (2010); MOA-Liberia (2012))

Production / trade	1961- 1970	1971- 1980	1981- 1990	1991- 2000	2001- 2010
Paddy production (1000 t)	141.30	234.90	278.75	123.32	204.25
Yield (t ha <sup>-1</sup> )	0.83	1.23	1.24	1.15	1.22
Harvested area (1000 ha)	186.90	190.40	224.74	105.49	164.70
Milled rice consumption (1000 t)	115.19	186.52	251.64	134.20	350.71
Per capita consumption (kg yr <sup>-1</sup> )	93.97	113.61	118.04	58.20	106.30
Milled rice imports (1000 t)	36.54	51.78	91.18	44.48	170.97
Import value (1,000,000 US\$)	6.31	17.39	35.65	13.66	90.98
Self-sufficiency ratio (%)	83.00	84.99	73.99	63.91	39.10

### ***Rice blast and drought in the target environment***

Rice blast, caused by *Pyricularia oryzae* Cavara, is amongst the most important rice diseases in the West African sub-region and globally. The disease causes annual yield losses of up to 50% in most rice growing areas (Scardaci *et al.*, 1997; Wallwork, 2009), and annually destroys more than 150 million tonnes of rice around the world, enough to feed about 60 million people (Zeigler *et al.*, 1994). In Liberia, yield losses resulting from blast infections have been reported to be as high as 77% in experimental trials (Zeigler and Correa, 2000). Farmers generally lack access to any form of agro-chemicals to control diseases and pests, or to enhance rice growth, leaving the use of resistant varieties as the only available option for controlling rice blast.

Like blast, drought stress is a global constraint to rice production, especially in rainfed farming systems. Production losses resulting from drought stress have been estimated at 44-71% in major rice producing countries in Asia (Pandey *et al.*, 2007). In sub-Saharan Africa, where farmers depend solely on rain for irrigation, drought accounts for about 20% of their annual yield losses (Serraj and Atlin, 2008). Globally, the average reduction in annual rice production due to drought is estimated at 18 million tonnes (O'Toole, 2004). In Liberia, drought essentially limits rice production to not more than one crop per year, even though all other environmental factors remain favourable for year-round production. Even during the regular season, some farmers often fail to plant and consequently lose entire crops due to erratic rainfall distribution. The lack of capital to invest in irrigation structures

and equipment makes the use of drought tolerant varieties as the only option for managing the effects of drought on the growth and productivity of upland rice for farmers in Liberia.

Acting together, these two factors, rice blast and drought, pose a serious threat to food security, livelihood earnings, as well as peace and stability, in Liberia and in most parts of the developing world, with significant implications for global peace and commerce. Furthermore, as reduced production leads to increased prices, given constant demand, nations like Liberia that depend on foreign imports will have to increase production in order to meet domestic demand at an affordable cost.

### ***Nature of the relationship between the target environment and the problems of rice blast and drought***

The problems of rice blast and drought, associated with rice production in Liberia, are closely linked to the climate of the region and the farming practices of the growers. Developing a thorough understanding of these variables is an important pre-requisite for formulating appropriate interventions to adequately address the problems.

Historical weather data show that the climate of Liberia has consistently proved conducive for the development and outbreak of pests and diseases at almost any time of the year (Figures 1 and 2). The climate is tropical and humid, presenting uniform high temperatures throughout the year, with a daily mean of 27°C. The daily temperature rarely falls below 20°C, and humidity typically averages around 82%. Under the prevailing climatic conditions of Liberia, the rice blast fungus is able to reproduce and cause new infections throughout the year (TeBeest *et al.*, 2007). Furthermore, the pathogenicity of *P. oryzae* is usually enhanced by aerobic conditions such as those that characterize upland rice production, in contrast to paddy rice (Scardaci *et al.*, 1997; IRRI, 2010).

Apart from its conduciveness to pest and disease outbreaks, the climate of Liberia is characterized by rainfall patterns that also hinder rice production in the country. Liberia is considered to be a high rainfall country, recording annual rainfall that range from 3500 to 4600 mm along the coast in the south, to between 1500 to 2500 mm in the highlands of the north (Figure 1). Compared to low rainfall countries like South Africa and others that are arid or semi-arid, the high rainfall received annually in Liberia may give the impression that drought should be a rare event impacting on agriculture in the country. However, rainfall from November to April of each year is barely sufficient to support upland rice production, using the current varieties, as most parts of the country experience a largely unimodal rainfall pattern occurring between May and October (Figure 2). The uneven distribution and intensity of rainfall in Liberia impacts on the potential for large scale production of rice at

any given time of the year. In the wet season, the intense rainfall results in flooding in the lowlands. In the dry season, the level of rainfall is not adequate to sustain reliable rice production (van Staten, 2005).

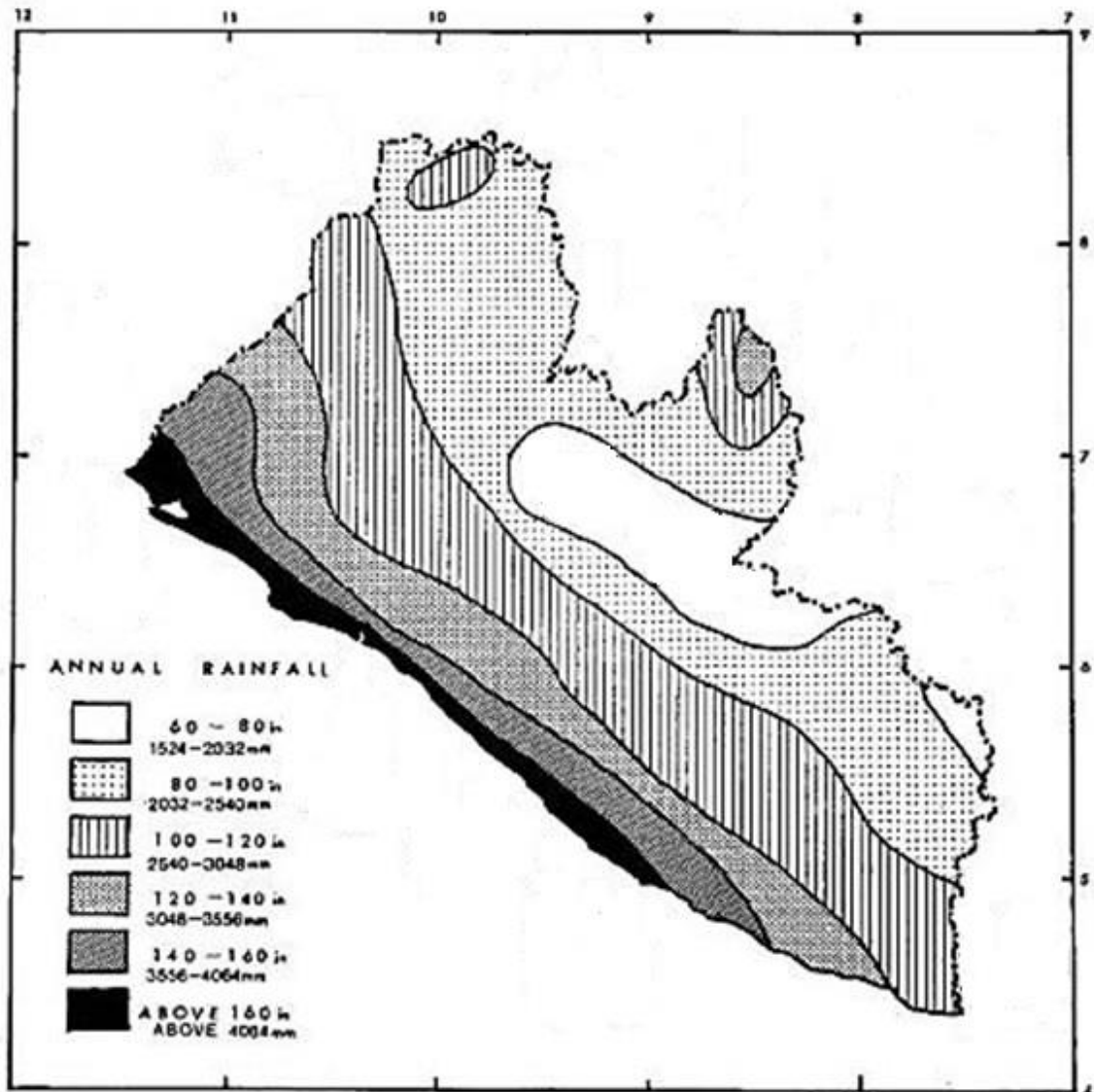


Figure 1: Rainfall map of Liberia (MOA-Liberia, 2007)

Furthermore, in addition to the global phenomenon of climate change, the historical weather data of Liberia clearly points to alternating wet and dry periods during successive decades (McSweeney *et al.*, 2010a; b). Whereas the early 1970's and 1980's were relatively dry, and 2005 and 2006 were extremely dry, the 1960's and late 1970's were particularly wet. These variable climatic patterns have the power to undermine rice production and threaten food security and livelihood earnings in resource poor communities, where crops are produced under rainfed conditions. The seasonality of rainfall and its erratic nature even in the rainy season, therefore renders drought as the single most important limitation to the intensification of rice production in Liberia by double cropping.



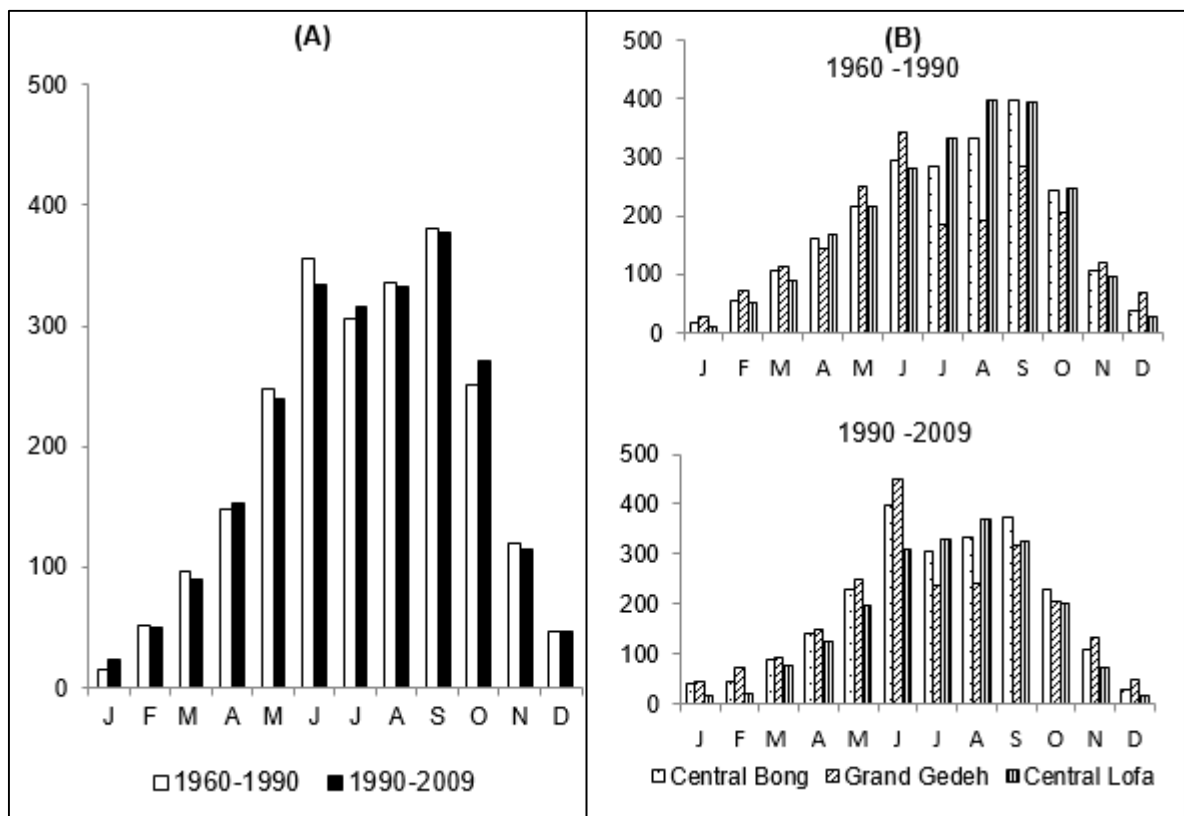


Figure 2: Monthly distribution of rainfall (A) nation-wide and (B) across three regions of Liberia over two time periods. Data are means of the total rainfall for each month. Data source: McSweeney *et al.* (2010a; b).

***Effect of the farming system on the incidence and severity of rice blast and drought in Liberia***

The scale of problems posed by rice blast and drought are exacerbated by the agronomic practices adopted by rice farmers in Liberia. Rice in Liberia is grown almost exclusively under rainfed conditions either in the uplands, which account for 90% of the total rice production area (Lancon and Erenstein, 2002; WARDA, 2007), or in wetland paddies. The production system is largely subsistence-based, characterized by traditional shifting cultivation practices, which also explains the low productivity of the crop. This production system is a high risk to vulnerable small scale farmers because rainfall is the only source of irrigation for the crop. The occurrence of drought can adversely affect production and lead to loss of income and increased hunger. Also, the production system based on shifting cultivation on lands under tribal tenure is not sustainable in the long term because population growth intensifies competition between farmers for superior lands, and between rice and other crops for the limited land resources. Since this system has failed to provide

sufficient food for the nation, an intensive production system that integrates improved agricultural techniques that both improve crop growth and maintain soil quality has been advocated (Otenga, 1999). Furthermore, environmental concerns over natural forest depletion, coupled with the shrinking area of forest land available to support shifting cultivation will lead to demand for more intensive use of land for rice production and the option of two or more upland crops per year will become increasingly important to sustain rice production in the country. A change in land tenure will be predicated by competition for land, and will also impact on future rice production.

## **2. Rationale for research focus**

From the foregoing, it is clear that there are many challenges to increasing rice yield and production in Liberia. Effective methods of controlling blast in this environment are needed to limit the impact of the disease on crop performance, and interventions that limit the effects of drought stress on rice are also needed to improve yield and production.

Rice blast, like most other fungal diseases of plants, may be controlled by applying fungicides, implementing sanitation to eliminate sources of inoculum, and other crop management procedures that reduce susceptibility of the crop to the disease, or by using resistant varieties. For drought stress, its impact can be diminished or eliminated by either providing irrigation, or by using drought tolerant cultivars that either reduce water use or escape probable periods of drought. Of all the potential approaches, cultivation of blast resistant and drought tolerant cultivars remains the simplest and most economical means of managing rice blast and drought stress (Lenne, 2000). Hence, providing farmers with rice blast resistant cultivars that are drought tolerant will largely help reduce the threat posed by the disease and drought in Liberia.

Breeding for resistance against rice blast and tolerance to drought, like any other genetic trait, may be achieved by identifying and appropriately manipulating sources of resistance to produce better varieties. Effective exploitation of sources of resistance for any trait is possible only if the genetics underlying the expression of the trait are known. Current understanding of resistance against rice blast suggests that the trait can be either simply inherited or quantitatively inherited (Koide *et al.*, 2009); whilst drought tolerance is thought to be quantitatively inherited (Chang *et al.*, 1982; Mitra, 2001). By simple inheritance, it is implied that the trait is qualitative: it is either effective and exhibited as complete resistance, or ineffective and exhibited as complete susceptibility. Quantitatively inherited resistance is partially effective and is expressed on a continuous scale, ranging from poor to excellent resistance, without offering complete resistance. Rice blast resistance conferred by simple inheritance has consistently become ineffective in a relatively short time, mainly in cropping

environments infested with multiple races of the pathogen due to the evolution of new, virulent races of the pathogen. This is a serious problem for rice breeders, as several hundred races of blast have been identified by their virulence against major gene resistance in rice (Ou, 1985; Khush and Jena, 2009). Cultivars with quantitative resistance are believed to be capable of proffering stable resistance against multiple races of a pathogen, thereby expressing more durability of resistance than that provided by simply inherited genes.

The agro-ecology of Liberia is interconnected with much of West Africa's. Studies of the virulence spectrum of rice blast in the sub-region from 1996 to 2002 have found a range of 11 to 28 *P. oryzae* pathotypes in each of four countries surveyed. This indicates both a high degree of pathotype diversity, and a high density of pathogen virulence within the sub-region (Sreenivasaprasad *et al.*; Chipili *et al.*, 2001). The occurrence of such diversity in the blast pathogen populations in the region clearly dictates quantitative resistance as the most viable option for genetically protecting rice from blast disease in the region.

Since durable resistance against multiple races of the blast pathogen and drought tolerance are both inherited quantitatively, it may be possible to concurrently improve both traits. However, environmental factors often influence the expression of quantitative traits, thereby requiring special consideration for choosing screening environments suitable for evaluation of the quantitative traits.

Breeding to improve quantitative traits may also require the creation of large numbers of crosses and progenies for evaluation. Such a goal could be difficult to achieve with a self-pollinating crop such as rice. Effective means of overcoming that difficulty and facilitating mass emasculation of rice florets will therefore be required for enhancing breeding for quantitative blast resistance and drought tolerance. Additionally, the expression of quantitative resistance and drought tolerance is usually partial. The integration of host plant resistance with other disease and stress management tools can also be useful for increasing and sustaining rice production in both blast-infested and drought-prone environments.

### **3. Research objectives**

The following studies were therefore undertaken to increase yields of upland rice varieties for Liberia, primarily by breeding for improved quantitative resistance to rice blast and for enhanced drought tolerance. The specific objectives of these studies were to:

- a. Assess local farmers' needs and identify their preferences for various rice varietal traits;

- b. Evaluate and characterize upland rice varieties from Liberia for reaction to rice blast and drought stress;
- c. Determine suitable environments for screening drought and quantitative resistance against rice blast;
- d. Determine the gene action and combining ability for the inheritance of rice blast resistance traits;
- e. Determine the efficacy of ethephon and gibberellic acid at facilitating mass emasculation in rice; and
- f. Investigate the effect of *Trichoderma harzianum* on blast resistance and drought tolerance of rice.

#### 4. Thesis outline

This thesis consists of eight distinct chapters (Table 2) reflecting a number of activities related to the above-mentioned objectives. Chapters 2 to 8 are written in the form of discrete research chapters, each following the format of a stand-alone research paper. The referencing system used in the chapters of this thesis is based on the Journal of Crop Science system. This is the most recommended thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters. Chapter 6 has been accepted for publication in *Euphytica*, while Chapter 8 is currently being considered for possible publication in the *Journal of Biocontrol Science and Technology*.

Table 2: Thesis structure

Chapter	Title
-	Thesis introduction
1	Literature review
2	Farmer-preferred traits and criteria for selecting rice varieties in the Gibi District of Liberia
3	Blast resistance of selected upland rice from Liberia
4	Drought tolerance of selected upland rice <i>genotypes</i> from Liberia
5	Screening rice ( <i>Oryza sativa</i> L.) for tolerance to drought using a plastic mulch soil cover system
6	Combining ability and gene action of three components of horizontal resistance against rice blast
7	Investigation of ethephon and gibberellic acid as a combined chemical hybridizing agent for rice
8	Effects of <i>Trichoderma harzianum</i> on the responses of rice ( <i>Oryza sativa</i> L.) to blast disease and drought stress
-	Thesis overview

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# CHAPTER 1

## LITERATURE REVIEW

### 1.1 Taxonomy of the rice plant

The rice plant belongs to the grass family *Poaceae* (*Gramineae*) and consists of two cultivated (*Oryza sativa* L. and *Oryza glaberrima* Steud.) and 22 wild species (Aggarwal *et al.*, 1999; Chopra and Shyam, 2002). *Oryza sativa*, also known as Asian rice, consists of two main types: *Japonica* and *Indica*, which originated in the tropical and sub-tropical / temperate parts of Asia, respectively (Glaszmann, 1987; Garris *et al.*, 2005). The *O. glaberrima* species (African rice) has been found to also consist of two main types, namely a “floating” photosensitive ecotype and a “non-floating” early erect ecotype (Ghesquiere *et al.*, 1997; Sarla and Mallikarjuna, 2005). Both rice species show parallel variation for nearly all traits, and it is often difficult to distinguish *O. glaberrima* from *O. sativa* (Oka *et al.*, 1978; Richards, 1996). However, *O. glaberrima* varieties can be identified by their usual short round ligule shapes, lack of panicle branching, glabrous leaves and spikelets, small seeds and red caryopses (Nayar, 1973).

The African rice has been cultivated as a crop only in West Africa, where it originated more than 2000 to 3000 years ago (Porteres, 1956; Nayar, 2010). *Oryza sativa* originated in Asia, at about the same time as *O. glaberrima* (Porteres, 1956) and is cultivated in all rice-producing regions of the world, including West Africa, where it has largely displaced the African rice, due to its higher yield potential (Wopereis *et al.*, 2009). Subsistence farmers in West Africa have traditionally grown varieties of the two cultivated species of rice in the same field, partly as a mean of preserving diversity and minimizing risk in the heterogeneous environments, where they grow the crop with little or no modern input (Ghesquiere *et al.*, 1997; Semon *et al.*, 2005). Probably, because of the poor yield characteristics of *O. glaberrima*, the limitation of funding for research, and the need to urgently address the perennial problem of food shortage on the African continent, it has received little attention for breeding, compared to *O. sativa*. However, the recent work at AfricaRice, leading to the creation of NERICA rice, a hybrid obtained from crosses between the two cultivated species of rice (Jones *et al.*, 1997; Sie *et al.*, 2005; Somado *et al.*, 2008) has highlighted the significance of exploiting *O. glaberrima* for rice improvement in Africa.

### 1.2 Rice in Liberia

Rice cultivation is indigenous to Liberia. Before its creation as an independent state, the land of present day Liberia was known as one of the areas of intensive rice production in



West Africa (Carney, 2001; Dunn *et al.*, 2001). In the 15<sup>th</sup> century a French navigator reported that rice was a staple food for the people in that region, with *O. glaberrima* varieties being the species of rice grown (Voeks and Rashford, 2013).

Up until the 1960's, farmers in Liberia relied mainly on traditional varieties developed and maintained mostly by women farmers. Thomasson (2010) reported the systematic maintenance of a collection of more than 112 traditional varieties, with specific adaptability to local conditions of that farming community, in one village alone in the 1980s.

Modern efforts aimed at improving rice in Liberia have been consolidated through a national programme body - presently known as the Central Agricultural Research Institute (CARI) - in collaboration with international programmes such as the West African Rice Development Association (WARDA, now AfricaRice), the International Institute for Tropical Agriculture (IITA) and the International Rice Research Institute (IRRI). Rice breeding in Liberia was first initiated in the late 1960's at the Liberia Agriculture Company (LAC), leading to the release in 1967-68 of LAC 23, which was basically a selection from a local variety (Gupta and O'Toole, 1986).

Through the collaborative efforts, several *O. sativa* varieties were tested and a number of them, including Suakoko 8, 9, 10 and 12 were released between 1977 and 1979, for cultivation in various agroecologies (Dalrymple, 1986). Suakoko 8, a selection from a cross between Siam 25 and Malunja 3, Suakoko 10 (an improved Mashuri) and Suakoko 12 (an introduction from IRRI, IR1416-131-5) were either rainfed or irrigated lowland varieties, while Suakoko 9 (a selection from LAC 23) was developed for the upland agroecology (Virmani *et al.*, 1978; Dalrymple, 1986).

By 1989 about 14 *O. sativa* varieties had been released, formally or informally, for cultivation in the upland agroecology (Table 1.1). By then, the national programme had assembled more than 3 000 rice accessions in its gene bank, all of which were lost during the civil war which lasted from 1989 to 2003. Consequently, information describing farmers' adoption and use of released varieties has been lacking. It is possible, though, that some or all of the varieties are being grown by farmers; but, their names and genetic characteristics have certainly changed, as farmers rely on their own standards to name and maintain their varieties under the prevailing informal seed system.

Since 2006, several introductions, mainly New Rice for Africa (NERICA) varieties from AfricaRice have been evaluated at CARI for possible release as the relevant procedures and laws are being put in place (MOA, 2008). Efforts to introduce F1 hybrid rice to Liberia are also being pursued by partners from the People's Republic of China. So far, LAC 23

(both red and white), remains the only recognized “improved” upland variety ever adopted by farmers in Liberia. LAC 23 is still being promoted by the government and praised by farmers (FAO, 2014).

Table 1.1: Rice varieties released in Liberia (Dalrymple, 1986; Chaudhary *et al.*, 1998; FAO, 2002).

Variety name	Ecology	Time to maturity (days)
IR5	Rainfed lowland	135-140
ITA 212	Rainfed lowland	130-135
ITA 222	Rainfed lowland	130-135
ITA 306	Rainfed lowland	110-115
Kuati Kundor	Rainfed lowland	NA
Mahsuri	Rainfed lowland	125
ROK 10	Rainfed lowland	140-150
ROK 14	Rainfed lowland	
ROK 3	Rainfed lowland	125-135
Suakoko 12	Rainfed lowland	
Suakoko 8	Rainfed lowland	140-145
ROK 5	Tidal wetland	140-145
IDESSA 6	Upland	110-120
IRAT 133	Upland	115-125
LAC 23	Upland	135-140
Morobekan	Upland	145
OS 6	Upland	130-135
WAB 32 80	Upland	NA
WAB 56 125	Upland	NA
WAB 56 50	Upland	NA
WAB 56 104	Upland	NA
WAB 96-1-1	Upland	NA
WAB 56-50	Upland	NA
WABIS 550	Upland	NA
WABIS 18	Upland	NA
WABSOKA	Upland	NA
Suakoko 9	Upland	135-140
IR 5	Upland	135-145

Essentially, varietal improvement of upland rice in Liberia has been mainly concentrated on the introduction of exotic varieties or application of mass selection to the traditional stock of germplasm. So far, the new introductions have failed to impact on production because they were not adopted by the farmers, who preferred their landraces. The lack of records on the impact of released varieties other than LAC 23 and its relative Suakoko 9, suggests that adoption by farmers has been negligible. Non-adoption of the varieties could have been as the result of their low comparative advantage over the traditional varieties, or the result of non-preference by farmers. Determination of farmers’ preferences could therefore help shape future breeding objectives, if any new varieties are to be widely adopted.

## 1.3 Blast disease of rice

### 1.3.1 Occurrence and epidemiology

Rice blast, caused by *Pyricularia oryzae* Cavara, is the most important disease of rice worldwide. The disease was first recorded in China (1637) and later in Japan (1704), Italy (1828), USA (1876), India (1918) and in more than 80 other countries since then (Ou, 1987). In Africa, rice blast was reported for the first time in 1930 (Feakin, 1976). Today, the disease affects all rice growing areas around the world (Khush and Jena, 2009). Blast is known to affect rice in all agroecologies, but rice grown under dryland conditions is known to be more susceptible to the disease (Zeigler *et al.*, 1994).

The epidemiology of rice blast disease is polycyclic. In the field, primary inoculum arises from infected plant materials such as seeds and straw left on the soil surface, whereas the secondary inoculum is generated from sporulating lesions produced on infected plants during the cropping period. The numbers of infection cycles and spores produced per lesion, and consequently, disease severity, tend to depend largely on the conduciveness of the environment and the level of host plant resistance. Environmental conditions that favour the outbreak and spread of blast disease are mainly moderate temperatures (24 - 28°C), periods of high humidity, and high levels of available nitrogen (TeBeest *et al.*, 2007).

The process of *P. oryzae* infection of rice plants may be summarized into five basic steps, as described by Hamer *et al.* (1988): (1) conidiation and conidial dispersal; (2) landing and attachment of a conidium on a host surface; (3) appressorium formation; (4) appressorium penetration; and (5) invasive hyphal growth (Figure 1.1). Conidia from sporulating lesions of *P. oryzae* are disseminated to new hosts by splash or wind dispersal. Upon landing, a dispersed conidium releases from its apex compartment a sticky mucilage that enables it to adhere firmly to the surface of the rice plant. Usually, within 1.5 h the conidium germinates, and produces a germ tube that differentiates into a specialized infection structure known as appressorium, from which an infection peg penetrates the host tissue (Parker *et al.*, 2008).

Conidiation and factors that pre-dispose plants to infection are key to understanding epidemics of rice blast and consequently formulating actions for controlling the disease, or for evaluating host resistance. In both field and controlled environments, moderately high temperatures and high humidity are known to influence sporulation and conidia dispersal (Manibhushanrao and Krishnana, 1991). Alternating light and dark photoperiods of 16/8 hr, and various growth media (including potato dextrose, corn meal, oatmeal and rice polish agars) have been successfully used to culture isolates of *P. oryzae* and to produce conidia

for pathogenicity assays (Latterell and Rossi, 1986; Hosseini-Moghaddam and Soltani, 2013; Lodhi *et al.*, 2013).

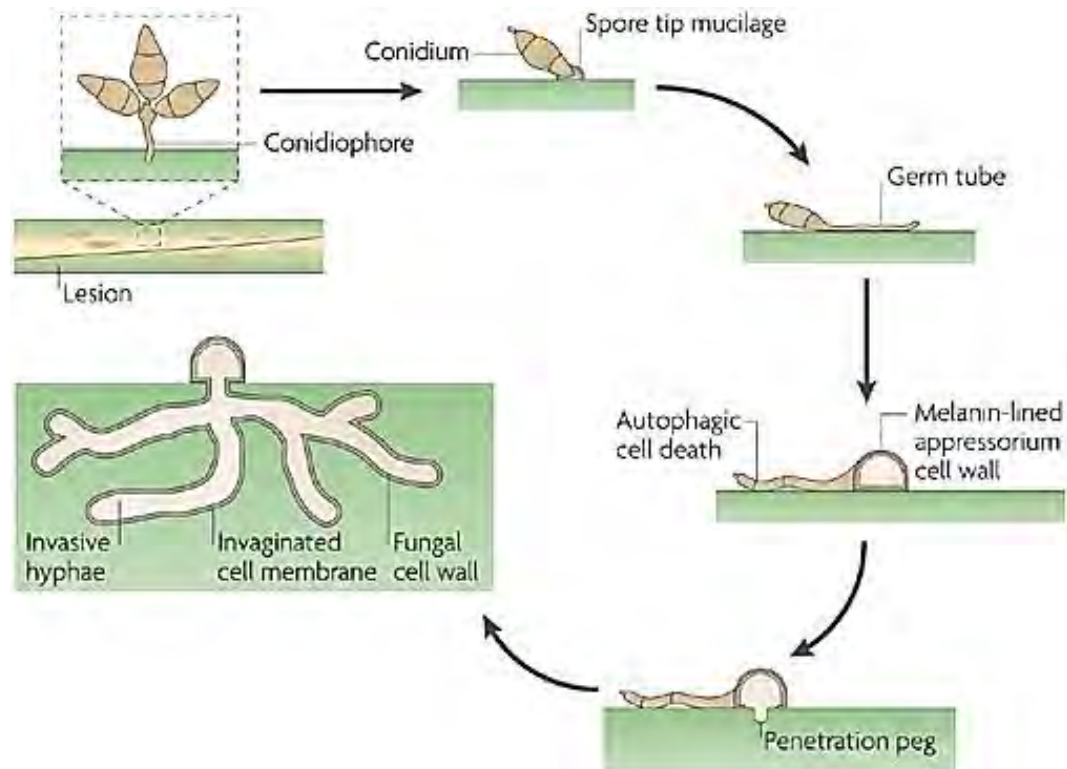


Figure 1.1: Disease cycle of rice blast. Source: Wilson and Talbot (2009).

### 1.3.2 Effects of blast on rice physiology and yield

Nearly all parts of the rice plant are susceptible to blast. Blast infection leads to direct destruction of cells, resulting in the disruption of vascular translocation of water and minerals in the affected and neighbouring tissues of the plant, thereby negatively affecting yield. Blast lesions tend to reduce the rates of leaf photosynthesis and respiration, and to reduce overall canopy photosynthesis, the primary effect being a reduction of carbohydrate production (Bastiaans and Kropff, 1993). Lesions produced on heavily infected leaves during the early growth stages usually lead to premature senescence. Leaf blast effectively reduces net radiation interception, crop growth and leaf area formation during the vegetative stage of crop growth, leaving less green leaf area during the reproductive phase. The net effect is a reduction in source capacity to fill the developing grain sink, leading to a net reduction in grain yield (Bastiaans, 1993). Lesions appearing on the collar can lead to death of an entire leaf. Blast attacking the nodes often causes the stem to break. Blast may also attack the panicle and rot the neck, thereby leading to loss of the entire panicle or prevention

of grain filling and maturation (Wilson and Talbot, 2009; FAO, 2013). Seeds may also be contaminated and become sources of new infection.

## **1.4 The rice blast pathogen**

Based on scientific and economic relevance, the rice blast fungus, *P. oryzae*, is regarded as the most important fungal pathogen in the world (Dean *et al.*, 2012). One area of scientific importance of the pathogen lies in its use as a model organism for elucidating the molecular basis of plant fungal diseases (Ebbole, 2007). Members of the genus parasitize more than 50 species of grass plants worldwide (Ou, 1985; Beckerman *et al.*, 1997), as well as *Arabidopsis*, which is a dicotyledonous plant (Park *et al.*, 2009).

### **1.4.1 Nomenclature and host range**

The rice blast pathogen is a polymorphic fungus belonging to the class ascomycete. Both the anamorph (*Pyricularia*) and teliomorph (*Magnaporthe*) are heterothallic and filamentous (Talbot, 2003). The teliomorph is the sexually reproducing form, consisting of two mating types. Only fertile strains of opposite mating types can be paired in a sexual cross leading to the formation of perithecia containing ascospores contained in uninucleate asci (Talbot, 2003). According to Nelson (1996) and Kronstad and Staben (1997) mating in this bipolar system is controlled by two different alleles at a single locus, and the genes are believed to encode master regulators of sexual development that regulate the expression of mating type-specific genes, such as pheromones, receptor signal transduction and self-non-self recognition. The asexual form (anamorph) produces three-celled conidia that are borne on the apex of specialized hyphae known as conidiopores. The teliomorph is rarely found under field conditions, but can be propagated in the laboratory. Since the anamorph is the form relevant to this study, its name shall be used throughout this thesis to refer to the pathogen, as advised by Rossman *et al.* (1990).

Due to unresolved confusion over classification, the blast pathogen (its anamorph) has interchangeably been referred to in the literature as *Pyricularia grisea* Sacc. or *P. oryzae*. However, Couch and Kohn (2002) proposed that *P. oryzae* should be recognized as a distinct species, separated from *P. grisea*. Using dendrograms generated from the DNA sequences of the genes for actin, beta-tubulin, and calmodulin, and from the analysis of mating experiments, these researchers showed that the two taxa differed molecularly by several base substitutions in each of three loci, and that mating between the two species did not occur. Hence, the name *P. grisea* remains consistent with strains isolated from crab grass (*Digitaria ciliaris* (Retz.) Koeler), while the name of strains isolated from rice and other

grasses was confirmed as *P. oryzae* (Ou, 1987; Rossman *et al.*, 1990). These authors also noted that the morphs of the two species are very similar morphologically.

#### **1.4.2 Pathogenic stability and variability of blast pathogen**

Due to the remarkable ability of *P. oryzae* to overcome the defences of rice plants, questions about host specificity or cross infection by isolates between rice and other grass hosts, including the stability of *P. oryzae* populations are important. Cross inoculation studies have revealed that some isolates from rice plants are capable of infecting other grasses, and some isolates from non-rice grass hosts are able to infect rice plants (Mackill and Bonman, 1986; Choi *et al.*, 2013). Other studies have concluded that populations of *P. oryzae* were strongly delimited by host range (Valent *et al.*, 1984; Hamer *et al.*, 1989; Borromeo *et al.*, 1993), although failing to completely rule out the possibility that some strains are capable of infecting both rice and other grasses. On the basis of restricted fragment length polymorphism (RFLP) data, Borromeo *et al.* (1993) found that *P. oryzae* populations infecting rice and rice-field weeds in the Philippines shared a common ancestry, but that isolates from grass weeds in rice fields could not infect the rice plants.

Earlier studies reviewed by Ou (1985) reported the recovery of multiple pathotypes from not only single lesions, but also from monoclonally derived cultures, and concluded that *P. oryzae* pathotypes were highly polymorphic and pathogenically unstable or continuously changing. This view was later opposed by Latterell and Rossi (1986) who, on the basis of 30 years' research involving repeated testing of more than 2,000 blast isolates, concluded that blast pathotypes were basically stable. They noted, however, that induced or natural mutation usually resulting in increased sporulation or broader host range did occur, but quite rarely. Several researchers have contended that estimates of the diversity and variability of pathotypes could be inflated due to difficulties in standardizing testing procedures and interpreting lesion types (Latterell, 1975; Bonman *et al.*, 1987). Levy *et al.* (1991) used *Magnaporthe grisea* repeat (MGR)-DNA fingerprints to accurately identify the pathotypes of several isolates collected over a 30 year period and to delineate the organization of clonal lineages between and within the groups of isolates, demonstrating the efficiency of MGR probes for resolving pathotype diversity.

The occurrence of many pathotypes of *P. oryzae* has been reported in most rice growing areas, and new races are reported regularly. New races are thought to emerge as the result of virulent mutations that occur at the rate of one in  $10^3$  to  $10^5$  (Kiyosawa, 1976). Chumley and Valent (1990) attributed these virulent mutations to deletions, appearing at an estimated rate of 5% in some isolates. Marchetti (1994) further argued that owing to the huge number

of propagules produced in the field, spontaneous mutations would give rise to thousands of pathogen variants, many of which would disappear immediately because they offered no advantage in fitness. Hence, according to Marchetti (1994), the discovery of new races follows when pathotypes capable of infecting a new pathodeme increase, over time, to the point where they become noticeable.

## **1.5 Genetics of rice blast resistance**

Resistance against rice blast has been extensively studied, leading to the discovery of at least 85 major (R) genes and 347 quantitative trait loci (QTLs) (Ballini *et al.*, 2008). However, several of the genes may be identical to each other or are tightly linked, leading to similar genes being called by different names (Kinoshita *et al.*, 1994). The major genes and QTLs with large effects governing rice blast resistance and their putative locations on rice chromosomes have been reviewed by Koide *et al.* (2009).

Generally, resistance against plant diseases can be classified into two main categories: vertical and horizontal resistance. Both types of resistance are known to occur in rice. The terms were first introduced in the genetics of pest and disease resistance by Vanderplank (1963). Accordingly, vertical resistance (VR), sometimes called hypersensitivity resistance, is often complete and produces a resistant infection type (IT) because it fully inhibits pathogen reproduction on a genotype possessing it, where the pathogen race is avirulent. It is usually race-specific (effective against all races of the pathogen except novel, virulent races), monogenic, controlled by R genes, and largely insensitive to environmental effects. The hypersensitive response to pathogen attack is exhibited when cell death occurs at the site of an attempted infection by the pathogen (Morel and Dangl, 1997), thereby preventing the spread of the disease and reinforcing plant defences by triggering systemic acquired resistance, and inducing lignification and synthesis of antimicrobial compounds (Kombrink and Somssich, 1995; Pontier *et al.*, 1998). Rice blast lesions appearing as tiny brown specks on leaves are normally scored as a resistant infection type, since these lesions are typical of a hypersensitive response (IRRI, 2006).

Unlike VR, horizontal resistance (HR) is usually expressed as partial resistance, in a gradient that ranges from low to high, depending on the environment, host genotype, and the aggressiveness of the pathogen isolate. It is often described as partial resistance or quantitative resistance. Parlevliet and van Ommeren (1975) defined a partial-resistance genotype as one that allows limited but significantly reduced pathogen reproduction when compared with a genotype that is susceptible. Horizontal resistance is considered a quantitative trait, as the mechanisms underlying its expression are believed to be complex

and involve multiple genes or alleles, each of which contributes additively to the expression of the trait. This principle of additive action of the polygenes accounting for horizontal resistance contrasts with the principle that vertical resistance is controlled by mono- or oligogenic resistance genes.

### **1.6 Durability and specificity of horizontal resistance**

The resistance spectrum of HR has largely been described as broad; that is, it is effective against all races of the same pathogen species, although the aggressiveness of pathogen isolates may vary (Wisser *et al.*, 2005). Race-specificity of partial resistance has been reported by Bonman *et al.* (1989), Talukder *et al.* (2004) and Zenbayashi *et al.* (2002) amongst others, suggesting that HR genes function similarly as complete resistance genes. However, Vanderplank (1984) pointed out that partial vertical resistance can also occur, where the resistance expressed is initially good but partial, but this is later matched by a new virulent race, rendering it ineffective. Li *et al.* (1999) earlier suggested that HR genes could in fact be R genes, having shown that rice cultivars carrying a major resistance gene against bacterial blight, *Xa4*, exhibited residual levels of resistance against other virulent strains. Defence genes have been implicated in the expression of HR, when markers derived from such genes were used to enhance selection for HR against rice blast in separate studies (Liu *et al.*, 2004; Wu *et al.*, 2004). Vergne *et al.* (2010) also concluded that constitutive, rather than induced, expression of defence-related genes could be responsible for a significant portion of HR against rice blast.

Cloning of an HR gene, *pi21*, revealed that the mechanisms controlling HR are quite different from that controlling VR (Fukuoka *et al.*, 2009). Devanna *et al.* (2014) reported that at least 19 of the 23 vertical blast resistance genes cloned and characterized contained nucleotide-binding sites and leucine-rich domains, which showed race-specific interactions with avirulence gene products of the pathogen (DeYoung and Innes, 2006; McHale *et al.*, 2006). These are unlike *pi21*, which has a heavy-metal-binding leucine rich domain that is functionally dissimilar to other proteins of known function (Fukuoka *et al.*, 2007; Fukuoka *et al.*, 2009).

From the above studies, it is clear that HR differs from VR. The polygenic nature of HR suggest that the mechanisms controlling HR are not confined to the action of any single gene, such as *pi21*. Rather, HR results from the combined action of additive genes to provide protection to a plant. Epistatic interactions between the various genes could also account for the effects of environment on the expression of HR.



## 1.7 Breeding for horizontal resistance

The use of resistant cultivars has been long recognized as the most economical means of controlling rice blast. It is easy to manage by farmers, and is more environmentally-friendly than the use of fungicides, which pose risks to users and the environment. Genetic resistance against rice blast can be bred by either pedigree or population breeding, or a combination of the two methods. However, pedigree breeding is largely used to introgress major genes with qualitative effects. The literature is replete with studies where newly released rice cultivars protected by novel major genes, or novel major gene combinations, abruptly succumb to blast only a few years after their release, creating a “boom-and-bust” cycle. The need is for a more durable form of genetic resistance. In order to breed for durable resistance to blast, Roh *et al.* (2009) enumerated the potential approaches, including the accumulation of minor genes, pyramiding of major genes and the breeding of a combination of minor and major genes. The multiline approach involves synthesizing as many lines as possible with different R genes, in order to provide an array of planting materials, each capable of resisting different races of the pathogen. The limitation of this approach may lie in the difficulty presented in choosing which variety to plant at a given time because it is not possible to predict which virulent race will be present a priori. It is also an extremely expensive approach in terms of breeding resources.

Several rice cultivars (Moroberekan, LAC-23, ROK 16, OS 6, and Tetep) have been observed to demonstrate durable resistance to blast in their areas of cultivation (Ahn, 1994; Fomba and Taylor, 1994). Bonman (1992) associated the durable resistance in those cultivars with polygenic horizontal resistance, and found no evidence of race specificity. Genetic studies have uncovered at least two major genes and QTLs in the genetic background of those cultivars (Chen *et al.*, 2000; Chen *et al.*, 2009), suggesting that the complementary effects of those genes and QTLs could contribute to the observed broad-spectrum and durable resistance.

When aiming to increase horizontal resistance to rice blast, it would first be necessary to eliminate vertical resistance genes present in the parent population, in order to avoid the “vertifolia” effect (Vanderplank, 1984), whereby the presence of one or more effective major genes obstruct the phenotypic selection for horizontal resistance genes. One efficient technique to do so is the “one-pathotype” technique (Robinson, 1987). A single pathotype is used to screen the parent population, and all subsequent progeny. Any cultivar expressing a high level of resistance to the pathotype is eliminated, and only susceptible parents are used. Subsequently, the same pathotype is used to screen the progeny, to ensure that any residual vertical resistance genes are rendered ineffective in each

generation. Cultivars carrying resistance QTLs, such as the *Pi21* locus, have been reported to maintain resistance for up to a century of cultivation, but the co-introduction of this gene with the undesirable gene for taste has since hindered efforts to transfer this QTL to other varieties (Fukuoka *et al.*, 2009).

## **1.8 Characteristics of drought stress and drought tolerance in rice**

The requirement for consistent supplies of water is recognized universally as one of the most important constraints for agriculture. In agronomic terms, drought may be defined as the occurrence of sustained water deficit resulting from the insufficiency of water to support crop growth and production (McKee *et al.*, 1993; Wilhite, 1993). Water stress ensues when soil water has diminished, or when its uptake is interrupted, to the extent that plant roots cannot absorb enough water to meet transpirational demands by the foliage.

In the target environment, Liberia, about 90% of the soils are highly weathered and consequently classified as Oxisols (Latosol), according to the USDA soil classification system (Reed, 1951; De Datta and Feuer, 1975). Besides being low in natural fertility due to the dominance of clay particles by oxides of iron and aluminium, the Oxisols of Liberia, as in other parts of the world, are soils prone to drought due to their low capacity to retain water (Beinroth *et al.*, 1996). Plants grown in these soils often suffer from water stress much earlier than those grown in other soils, thereby rendering regular supplies of water as an essential input, in addition to nutrient minerals, for healthy crop growth and production. However, Liberia experiences seasonal droughts, and erratic rainfall in the rainy season. Understanding the nature of drought stress and its effect on rice, including the intricacies involved in breeding for the trait are therefore important for the success of any study aimed at improving drought tolerance of the crop.

### **1.8.1 Effects of drought stress on rice**

Water is a major component of soils, and is the solvent for translocating minerals from the soil and dissolved substances in the plant. Additionally, water constitutes 80 – 90% of herbaceous plants (Kramer and Boyer, 1995). Water plays a number of essential roles, such as the maintenance of cell turgidity, and is an essential substrate in photosynthesis and a reactant or ligand in numerous biochemical reactions (Rand, 1992; Lambers *et al.*, 2008).

The impact of water shortage on plant growth has been well studied. It reduces plant growth and vigour, resulting in wilting, the deficiency of essential nutrients and eventual plant mortality (Lahiri *et al.*, 1973; Peet, 2005). The ability of plants to absorb essential nutrients

from the soil is largely diminished by the lack of soil water. Lack of water in plants inhibits cell expansion and development, thereby limiting plant growth. The rates of transpiration and photosynthesis may diminish under drought stress as stomata close in response to water stress. Leaf rolling, caused by drought, essentially reduces the effective leaf area for the interception of solar radiation, and leads to reduced canopy photosynthesis and enhanced leaf senescence (Wopereis *et al.*, 1996).

Studies by Yamauchi *et al.* (1994) also showed that drought reduces root growth in rice. In their investigation of rooting pattern and soil water extraction of four rice cultivars in response to water deficit, Lilley and Fukai (1994a) found that root length and density was larger in the surface soils and declined with depth, and that root growth virtually ceased when a water deficit was imposed at either the vegetative or reproductive stage. They concluded that selecting for deeper roots and large root density could prove useful for developing more drought-tolerant upland cultivars, since these traits enabled the extraction of more water.

Drought can cause injury to plants at all stages of the production cycle, and the magnitude of injury increases with the intensity and duration of exposure. Even during the normal production season, delayed or erratic rainfall early during the season can adversely impact crop establishment, and can lead to additional costs in terms of seeds and labour for replanting, or low yields as a result of poor crop stands. Drought may reduce plant height, the number of tillers and the subsequent number of panicles when it occurs during the vegetative stage (Bouman and Tuong, 2001); however the effect of reduced tiller and panicle number per hill can be compensated for either by an increase in the number of grains per panicle or an increase in grain mass or both (Bouman *et al.*, 2007).

When drought occurs late during the season, such that it coincides with the reproductive stage of crop development, yield can be severely diminished. Drought can be especially damaging during the period from three weeks before flowering up to a week after anthesis. Drought occurring during the early stages of panicle development may delay flowering in rice (Puckridge and O'Toole, 1980; Inthapan and Fukai, 1988), and the magnitude of delay is often proportional to the duration (Tsuda and Takami, 1991) and intensity (Lilley and Fukai, 1994b) of the stress. The occurrence of drought between panicle initiation and flowering can also lead to marked reduction in spikelet fertility, and the number of spikelets per panicle, thereby resulting in decreased number of grains per panicle and / or poor grain filling (Saini and Westgate, 1999). The negative effect of drought on spikelet fertility can arise from poor anther dehiscence, pollen shedding and germination (Ekanayake *et al.*, 1990; Rang *et al.*, 2011).

Some of the negative effects of drought on yield may be reversed or mitigated upon relief of drought; however, others such as enhanced leaf senescence, spikelet sterility and reduction in the number of spikelets, are often irreversible. A thorough knowledge of the nature of drought and factors that influence drought susceptibility or resistance of plants, particularly rice, is therefore necessary for informing selection and other breeding decisions that may lead to the improvement of the crop.

## **1.9 Drought tolerance**

Response to drought is often expressed by plants in the form of escape, avoidance or tolerance (Levitt, 1972). Understanding the characteristics of drought tolerance could be useful for defining useful targets for breeding improved varieties.

According to Mitra (2001), drought escape is inherently structured to ensure that the life cycle of a plant is completed before a period of acute water deficit. Drought avoidance operates by ensuring that the water status of plant tissues remains relatively high under conditions of water deficit. Unlike escape and avoidance, drought tolerance is expressed when a plant remains viable even when the water potential of its tissues becomes low. Hence, drought tolerance is clearly defined in terms of stable grain yield under stress, following from the maintenance of growth processes and normal development (Serraj *et al.*, 2009).

Biological mechanisms underlying favourable drought responses under stress, and the alleles or loci underlying the biological mechanisms of drought responses have been found to be extremely complex (Bidinger, 2002). One of the mechanisms that has been associated with high dry matter accumulation in drought tolerant rice cultivars is the superior ability to access soil water, and this feature is believed to be largely endowed by a deep rooting ability (Lilley and Fukai, 1994b; Fujii and Horie, 2001). Other traits that have been observed to play roles in the complex expression of drought tolerance of rice are early vigour, leaf rolling, leaf drying, plant recovery ability, anther dehiscence, grain mass, osmotic adjustment and leaf water potential (Chang *et al.*, 1982; Jongdee *et al.*, 2002) and canopy temperature (Garrity and O'Toole, 1995).

Plant traits associated with each of the three mechanisms of drought tolerance are outlined and described according to Price *et al.* (2002). Traits conferring drought escape are usually short growth duration, developmental plasticity or variation in flowering time and maturity, and remobilization of pre-anthesis assimilates to grain. Traits typical of drought avoidance are usually of two types: those that reduce water loss from plant tissues, and others that specifically maintain turgor. Traits responsible for reducing water use from plant tissues

include low surface and stomatal conductance, leaf rolling and smaller leaf area, whilst an efficient root system and high hydraulic conductance are two specific traits that maintain turgor. High protoplasmic resistance tends to increase desiccation tolerance, as high osmotic adjustment and cell elasticity and small cell size tend to increase turgor. Such traits are typical drought tolerance traits.

However, most of these traits are difficult to measure, and require sophisticated and costly equipment, in most cases. Furthermore, some of the mechanisms by which plants resist drought by regulating their internal water status (leaf rolling, stomata closure) can negatively affect other processes (such as solar radiation interception and carbon dioxide uptake) that lead to reduced biomass accumulation and yield attainment. Therefore, mechanisms advancing earliness may diminish yield. Consequently, traits associated with reduced water use should not be discounted as long as the extent of their impact on economic yield is minimal. Plants usually exhibit more than one form of adaptation to drought in any given environment of water deficit (Turner, 1981). Furthermore, drought tolerance is generally a complex trait that integrates both direct actions and interactions of several morphological, biochemical and physiological characters. Therefore the screening and selection of parental materials and progenies should be aimed at combining a number of traits that integrate the various modes of drought tolerance.

## **1.10 Breeding for drought tolerance**

When breeding for tolerance to drought, the major factors to consider are usually, but not limited to, the target environment, the sources of resistance, and the screening methodologies adopted to enhance selection.

### **1.10.1 Breeding targets for drought tolerance**

Precise characterization of drought occurring in a target environment, the region of future cultivation of an improved variety, could facilitate the identification of appropriate traits for drought and assist breeding efforts. Kamoshita *et al.* (2008) classified the types of drought occurring in rice production regions by ecosystem, and by severity and timing of the drought, in relation to the crop developmental stage. They noted that the upland rice environment was characterized by frequent incidences of mild stress, which may become severe between major rainfall events; whereas lowland rice typically encountered drought when standing water in the field dried up during rainless periods.

Drought may also be classified on the basis of the time at which it occurs; hence, there may be a vegetative stage, intermittent or terminal drought event. In the upland ecology of Liberia

where the rainfall pattern is largely unimodal, rice grown during periods of below average rainfall, early in the wet season, is prone to vegetative stage drought; whereas crops grown late during the season may suffer terminal drought. Hence, the optimum planting time is when it becomes certain that rainfall will be frequent enough to support a crop from sowing to maturity. In Liberia this means that under rainfed, upland conditions only one crop per annum is possible. Whilst the end of the rainy season may be predicted fairly accurately the start of consistent rainfall in the rainy season is often difficult to predict. Practical breeding targets for such an agroecology could therefore be for drought escape, to enable the rice to grow to maturity before the rains recede, and drought tolerance to keep plants alive early during the season until the wet season becomes fully established. Drought tolerance could also be useful for achieving double cropping, which would be possible if a level of supplementary irrigation was available to farmers.

Various approaches, both conventional and molecular, are being explored around the world to breed and improve rice for drought tolerance. At AfricaRice the conventional approach has been mainly aimed at developing early maturing cultivars that are capable of escaping late season drought, while their molecular breeding approaches are currently focused on the application of marker assisted selection to combine traits for deep root penetration and osmotic adjustment in high yielding genotypes (Sie *et al.*, 2008). Research at IRRI and its collaborators has primarily focused on the development of two types of germplasm for drought tolerance: (1) aerobic varieties, for well-drained unbanded uplands and banded fields in the upper most toposequence; and (2) varieties for drought prone lowlands (Atlin *et al.*, 2008). Direct selection for yield under stress is widely cited as a key strategy for breeding these materials (Venuprasad *et al.*, 2007; Atlin *et al.*, 2008).

### **1.11 Screening for drought tolerance**

Procedures for screening for drought tolerance should be fully capable of identifying genetic variations that are heritable in the target environment. The screening procedure also has to reliably provide drought stress events with the timing, severity and duration that are characteristic of the target environment (Atlin *et al.*, 2008). In order to adequately quantify and articulate the nature of drought stress in the target environment, long term climatic data have to be carefully analysed.

Screening for drought tolerance is usually designed or aimed to target a yield-based definition of tolerance or basic stress response mechanisms. According to Bidinger (2002), following a yield based definition often requires taking into account all other factors that affect yield (such as day length, temperature, and disease) in the target environment, when selecting the screening environment, to ensure that none of these factors vary significantly

enough as to affect the phenology of the crop, since drought escape plays a major role in determining yield under stress. Otherwise, drought escape may operate differently between the target and screening environments. In their review of drought tolerance traits in rice and their QTLs, Kamoshita *et al.* (2008) concluded that, in addition to selecting for specific traits or genomic regions, screening for yield as a main trait, followed by low leaf roll scores, low spikelet sterility and high drought tolerance index (DTI) in managed drought environments appeared to be the most useful traits to screen for when breeding for drought tolerant cultivars.

### **1.12 Integrating host plant resistance with biocontrol**

Integrated approaches to pest and stress management have been advocated as a means of more effectively controlling the problem posed by the stress factors. In essence, it is a means of intensifying efforts to control a disease or problem, using a combination of resources, which in most cases are not totally effective on their own, with the intention that the resources will complement each other, thereby yielding a better result. The key to success may lie in the ability of the factors to act additively or synergistically, rather than antagonistically in any way. The foregoing discussions reveals that the levels of expression of quantitative resistance against rice blast and drought can vary mainly with the intensity of the problem. Given that quantitative resistance is largely incomplete, inputs that enhance its effectiveness would be useful to reduce the impact of blast on rice yields (Shyamala and Sivakumaar, 2012; Spence *et al.* 2014).

### **1.13 Conclusion**

Mono- and oligogenic resistance to rice blast does not have a history of providing stable resistance to blast due to the rapid evolution of virulent races in conducive environments. The long life of preferred rice varieties in Liberia could be the result of either the farming system, the presence of stable horizontal resistance in these cultivars, or a combination of the two. The shifting cultivation farming system that has allowed farmers in Liberia to cut and burn new forest land every year for rice cultivation is fast giving way to fixed fields, as competition for unclaimed land increases. The widening gap between local production and demand for rice could be reduced by intensifying rice production. However, the use of fixed fields, and more intensive rice culture in the uplands will likely result in increased incidence and severity of pests and diseases. Erratic and low rainfall will also continue to threaten upland rice intensification. More resilient varieties will be needed to effectively manage the increased threats. As populations of the rice blast pathogen are highly variable, polygenic

resistance would be more useful for protecting varieties against blast and other diseases. Breeding methods that improve polygenic resistance against rice blast, and concurrently allow for the selection of enhanced drought tolerance, would be best for addressing the two most important problems limiting upland rice production in Liberia and most other parts of the world.

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## CHAPTER 2

### FARMER-PREFERRED TRAITS AND CRITERIA FOR SELECTING RICE VARIETIES IN THE GIBI DISTRICT OF LIBERIA

#### 2.1 Abstract

Upland rice varieties grown by farmers in Liberia are largely low yielding, traditional varieties that have not been systematically bred for enhanced performance. The rate of adoption of new varieties by farmers in the country and several parts of the world has been low because many of the new varieties lack key traits required by farmers. Understanding farmers' varietal preferences, priorities and trait preferences are crucial for the outcome of a successful breeding project, which is the widespread adoption of the new varieties by farmers. This study was undertaken to develop a clear understanding of the systems of rice production and rice utilization in Liberia, and hence to determine the most important selection criteria of farmers and their criteria for choosing rice varieties. Two hundred eighty farmers from four village clusters in the Gibi District of Liberia were selected and interviewed individually. Focus group discussions were also held. The results confirmed that the current array of rice varieties grown by farmers in the study area were old and that farmers still exclusively practiced shifting cultivation for producing rice. Factor analysis of 23 phenotypic traits revealed that farmers select varieties based on a combination of yield, grain quality, disease and pest resistance and post-harvest traits when deciding which rice varieties to grow. In addition to high grain yield, conjoint analysis of farmers' preference ranking of nine model varieties revealed that early maturity, intermediate plant height, and cooking quality (a tender, non-sticky texture) were the main traits that influenced farmers' preferences for a new variety. Numerous problems, ranging from the prevalence of birds and rodents to the paucity of farming implements and a perceived increasing frequency of poor early rains, and rice blast, were cited by farmers as the major problems limiting rice production in their communities. It is therefore concluded that any breeding effort aimed at developing more resilient rice varieties for the region should also involve selection for high yield, early maturity, intermediate plant height, and good grain cooking quality.

Keywords: cooked grain texture, farmers' perception, grain yield, participatory rural appraisal, rice

## 2.2 Introduction

Farmers have played an active role in the domestication and subsequent improvement and maintenance of many crop species, leading to constantly evolving landraces (Scialabba *et al.*, 2002). However, with the evolution of modern agriculture, advances in plant breeding have led to the development of better and more productive varieties, and in the process, often negated the role of small-scale producers in many plant breeding decisions (Atlin *et al.*, 2001). As a result, the improved varieties developed by centralized breeding have seldom benefited resource-poor farmers, since the varieties are normally developed for optimum production environments with inputs such as fertilizers and water, or to meet the requirements of clients with access to resources (Ceccarelli and Stefania, 2005). Reasons for the non-adoption of new or otherwise improved varieties by subsistence farmers have been widely studied (Brush, 1991; Almekinders and Elings, 2001; Weltzien *et al.*, 2005; Witcombe *et al.*, 2006). Besides the lack of seeds, or information on a new variety, the major reason cited for farmers declining a new variety has been related to its inability to meet their needs (IRRI, 2006).

In Liberia, rice is the primary staple food and is the major source of calories in the diets of the indigenous population. As the primary food crop, rice has been produced in Liberia largely by subsistence farmers, predominantly under dry land conditions, using traditional shifting cultivation and a “slash and burn” technology. The crop accounts for up to 80% of the land cultivated for staple food production (GOL, 2009; GOL, 2010) and is a major source of income for thousands of rural inhabitants in the country. However, the rice seed system in Liberia is largely informal (Mwah, 2012), forcing farmers to obtain seeds from unregulated sources. Local initiatives based on indigenous knowledge systems aimed at selecting, maintaining and preserving traditional varieties (Thomasson, 2001) virtually disappeared as a result of the civil war which ravaged Liberia for over 14 years. This debilitated the local rice production system further.

As post-war national agricultural services to farmers resumed after 2005, crop improvement has become a key priority. Towards this end, interactions with rural communities have focused mainly on assessments of food security and vulnerability, and farmer mobilization for the transfer of improved agricultural technologies, including new varieties. During the early stages of disseminating some of the newly introduced varieties, no attempt was made to confirm the merits of these varieties for the targeted communities, often leading to failure of adoption by the farmers. In one region, Grand Gedeh County, for example, many farmers objected to growing the variety FKR-19 because they found that it headed too soon after transplanting and that the mature plants were not tall enough to facilitate harvest by the

traditional panicle reaping method. Subsequent attempts in 2011 to disseminate variety WITTA-4 in the same region had to be accompanied by an intensive marketing campaign, with varying responses from the locals. Some communities rejected the variety on the grounds that they already had better cultivars, whilst others accepted it but often with circumspection. These cases suggest that farmers value their own experience and priorities, and will not gamble with unknown varieties. There is a lack of solid research into documenting rice farmers' experiences and needs, in part because of the prolonged absence of agricultural research and extension activities in the country.

Investigations into farmers' needs and priorities are needed to accurately assist the planning of crop improvement and other agricultural service-oriented programmes. Recognizing the short-comings of many of the past and current interventions, this study sought to involve farmers in crop improvement decisions, starting from the beginning, in the planning stage, thereby establishing a scientific basis for breeding new rice varieties. If they are bred specifically to meet the expressed needs of Liberian farmers, then these new varieties may be adopted by rice farmers. Hence, a consultative analysis of the current status of the local production system was undertaken to assess farmers' perceptions of, and preferences for rice varieties and traits. Data on farmers' perception of various aspects of the current farming system, along with their desired standards for new varieties are presented and discussed within the context of their implications for breeding, using basic participatory rural appraisal (PRA) tools, as described by Biggs (1989).

## **2.3 Research Methodology**

The research mainly involved a survey to assess the knowledge, thoughts, opinions, and feelings of rice farmers about aspects of rice production in a farming district in Liberia.

### ***2.3.1 Description of the area and design of the study***

The survey was conducted in four clusters of villages in the Gibi District of Margibi County, Liberia (Figure 2.1) during the rice pre-planting season from January to February of 2012. The communities are ordinarily rural, but are accessible by motor roads for most of the year. The district was chosen because it has consistently been a rich source of locally produced ("country") rice, but has received little attention for research and development of the crop. The population of the four village clusters varied from 2 000 – 2 800 households (LEGIS, 2009). The region is part of the upper highland tropical forest zone, which comprises the major agricultural belt of Liberia (CAAS-Lib, 2007). The rainfall during the nearly six month long wet season is bimodal and ranges from 1 200 – 2 900 mm per annum. The soils are predominantly Latosols with loam to sandy clay loam textures (Reed, 1951), and are usually

acidic and low in minerals, as a result of the high leaching of essential nutrients that occurs under conditions of high rainfall and limited soil cover (Beinroth *et al.*, 1996). Farmers in the study area mainly practice mixed cropping, with rice as the primary crop.



Figure 2.1: Location of study area. \* Villages grouped into four clusters (1 – 4)

### **2.3.2 Survey and sampling procedures**

Both structured and semi-structured interviews were used to solicit farmers' responses during the survey. A total of 280 households were randomly selected in the villages for the structured interviews (Table 2.1). Four groups, one for each village cluster, comprising of equal numbers of males and females, were set up for the group interviews. Each group, containing 12 or 10 members, was identified and selected with the aid of facilitators who also resided in the respective communities. The characteristics of each group were kept as

homogenous as possible in order to minimize biases and promote freedom of expression amongst the participants, as advised by Krueger and Casey (2000).

Table 2.1: Number of farmers interviewed in PRA survey in Gibi District, Liberia

Type of interview	Cluster	Name of Village / Town	Male	Female	Total
Individual	1	Whorn, Joe Vahn	28	42	70
	2	Bawfeng, Isaac Town, Molim	33	37	70
	3	Payeta, Petereta,	35	35	70
	4	Bakuoima, Ginda, Togbapolu	31	39	70
	Total		125	155	280
Group	1	Whorn, Joe Vahn	6	6	12
	2	Bawfeng, Isaac Town, Molim	5	5	10
	3	Payeta, Petereta,	5	5	10
	4	Bakuoima, Ginda, Togbapolu	5	5	10
	Total		21	21	42

For the structured interviews, information was gathered through a questionnaire administered to individual heads of households with the aid of trained facilitators. The questionnaire was pre-tested in a pilot survey involving 15 farmer staff of the Central Agricultural Research Institute of Liberia, and subsequently fine-tuned before the actual survey. The order and wording of the questionnaire items were carefully standardized and scheduled in order to minimize any context effect, to ensure reliability of both the aggregated responses and comparison between the respondents, as advised by Phellas *et al.* (2011).

The semi-structured interviews and group discussions that were held in each village cluster were largely open-ended to allow the participants to freely convey their thoughts and ideas (Grandstaff and Grandstaff, 1985). Furthermore, participants of the group discussions were listened to carefully not only for content, but also for possible emotions, ironies, contradictions and tensions in order to both confirm the facts and the meaning behind the facts, as suggested by Grudens-Schuck *et al.* (2004).

### **2.3.3 Data structure and analyses**

Data on demographic aspects of rice production and utilization, including farm size, cropping pattern, sources of seeds, and other information were recorded from the structured interviews, using the Likert scale (Likert, 1932) and dichotomous questions. These two types of questions were also used to determine farmers' perception of the current production system and varieties. Farmers' assessments of their most important production problems were measured by pair-wise ranking.

A set of potential selection criteria for 23 trait characteristics were proposed by leading farmers who were consulted. These criteria were scored on a scale of 0 – 5 (0 meaning not

important and 5 meaning highly important). The scores obtained were then subjected to factor analysis using the principal components method to determine the most important rice varietal traits that influenced farmers' decisions to grow a variety.

The influence of grain yield and quality, in addition to plant height and growth duration, and their attributes, on farmers' preferences for future rice varieties were also determined using conjoint analysis procedures. Thirteen model varieties (9 and 4 as experimental and holdout cases, respectively) were constructed for the experiment using the SPSS (IBM Statistics) orthogonal design facility, with above average and average as levels of grain yield, and tender, flaky and soft-sticky as categories of grain quality. Categories of plant height were tall, intermediate and short (dwarf), whilst periods of growth were early (90 – 110 days), mid (120 – 140 days) and late (above 150 days). All the model varieties were scored on a 0 – 10 scale (0 meaning undesirable, and 10 highly desirable) by individual participants of the group discussion and ranked later by all the members of the group.

The scoring system utilized throughout the study was the full scoring approach described by Maxwell and Bart (1995), and all rankings were accomplished by pair-wise comparisons. The scoring system required participants to give each item to be scored any value within the prescribed ranges, so that the respondents were free to allocate the same score to different items, as they deemed fit. With the pair-wise comparisons, matrices were used to match the alternatives provided two at a time, as described by Russell (1997). The alternatives were subsequently ranked by the number of times they appeared in a matrix. Where there was a tie, the alternative allocated the higher rank was that which won when both alternatives were compared.

All numerical data from the interviews were analysed using SPSS version 19 (IBM Statistics). Data on the demographic aspects of rice production and utilization, as well as those on farmers' perception of the current production system, varieties and problems were subjected to analysis of variance to determine the average responses of the farmers.

Scores of both the experimental and holdout cases for the model varieties were analysed to determine the relative importance of each trait and utility of their attributes, using the conjoint analysis syntax, specifying grain quality as a discreet factor and grain yield, plant height and growth duration as linear factors. Group ranks and the ranked frequency scores of the individuals for the experimental cases were compared using the Chi-square test for goodness of fit to determine how well group consensus compared with individual choices. Patterns and themes arising from the group discussions were also summarized and analysed by logic to determine communal consensus on the various questions and key issues.



## **2.4 Results**

### **2.4.1 General aspects of rice production and utilization in Gibi District**

Data on cultivation practices, land holdings and use, and utilization of rice in the study area are presented in Table 2.2. The data show that lands for rice production in Gibi District are mainly owned communally. Only 6.1% of the respondents indicated personal ownership of the land used to grow their rice. Farmers' estimates of their annual plot sizes ranged from 0.3 to 1.8 ha. All the respondents indicated that they had not planted rice in or around the same plot in the last three years, and that they regularly grew other crops in addition to rice. Twelve percent (12%) of the farmers declared that they grew other crops instead of rice sometimes, but not always. The farmers ranked rice as the most important crop, followed by cassava (*Manihot esculenta* Crantz.), and vegetables. Maize (*Zea mays* L.) and sweet potato (*Ipomoea batatas* L.) were jointly ranked in fourth place, followed by taro (cocoyam) (*Colocasia esculenta* L. Schott) and yam (*Dioscorea* spp.) and other crops. From the individual and group interviews, farmers indicated that they grew either upland rice alone, or both upland and lowland rice depending on the availability of suitable land (Table 2.2).

The utilization pattern of rice produced varied significantly with farmer. Most farmers indicated that they grew rice mainly to feed their households. However, 72% of the farmers indicated that they had sold rice in the past year, and the quantity sold was up to 25% of their production.

In the group interviews, some farmers said that they only sold the surplus from their rice production, whilst others sometimes had to sell some of their production when they found no other means of accessing cash to meet unavoidable expenses.

### **2.4.2 Farmers' perception of rice production system and cropping pattern**

In the group discussions, farmers agreed that lowland rice could produce a high yield, when properly sown and managed. However, they recorded their preference for upland rice production because of its lower requirements for inputs such as labour for sowing, weeding and fertilizing. On the system of land use, farmers indicated that each year they had to find a new piece of land to grow upland rice, but that the length of the fallow period had become shorter and shorter. Their solution to the shortage of fallow land for farming in their communities was to migrate to a new community or to travel further away from the village to find suitable communal lands that were unclaimed. Panellists in the focus group discussions expressed a fear that land use was changing fast, and their communal crop lands were increasingly being planted to perennial crops, particularly rubber (*Hevea*

*brasiliensis* Mull. Arg.), thereby leading to increased competition for an increasingly limited area of farm land for annual crops.

Table 2.2: Rice production practices and utilization in Gibi District, Liberia

Production practices	Descriptions	Village cluster and response (%)				Mean (%)
		1	2	3	4	
Land tenure	Personal	2.9	11.4	4.3	5.7	6.1
	Communal	92.9	87.1	94.3	94.3	92.2
	Rental	4.3	1.4	1.4	0.0	1.8
Shifting cultivation	Yes	100.0	100.0	100.0	100.0	100.0
	No	0.0	0.0	0.0	0.0	0.0
Estimated plot size	< 0.5 ha	5.7	8.6	1.4	2.9	4.7
	0.6 – 1.0 ha	94.3	85.7	98.6	97.1	93.9
	> 1.0 ha	1	5.7	0.0	0.0	1.7
Number of rice crops last year	1	77.1	85.7	67.1	90.0	80.0
	2	22.9	12.9	32.9	7.1	19.0
	3	0.0	1.4	0.0	2.9	1.1
	> 3	0.0	0.0	0.0	0.0	0.0
Type of rice crop produced last year	Upland	82.6	86.7	86.1	87.5	85.7
	Lowland	2.9	1.4	1.4	1.4	1.8
	Up- / lowland	14.5	11.9	12.5	11.1	12.5
Usual number of varieties grown / field	1	2.9	10.0	10.0	5.7	7.2
	> 1	97.1	90.0	90.0	94.3	92.8
Sold rice?	Yes	70.5	64.7	77.6	75.2	72.0
	No	29.5	35.3	22.4	24.8	28.0
Amount of rice sold?	75 – 100%	0.0	0.0	0.0	0.0	0.0
	50 – 74%	0.0	0.0	0.0	0.0	0.0
	25 – 49%	0.0	0.0	0.0	0.0	0.0
	1 – 24%	100.0	100.0	100.0	100.0	100.0
Purchased rice?	Y	100.0	100.0	100.0	100.0	100.0
	N	0.0	0.0	0.0	0.0	0.0
Goal of production	Self-use	90.0	87.1	100.0	90.0	91.8
	Sale only	0.0	0.0	0.0	0.0	0.0
	Self-use & sale	10.0	12.9	0.0	10.0	8.2
Estimated rice purchase	1 – 25 %	55.7	90.0	75.7	77.1	74.6
	26 – 50 %	35.7	5.7	21.4	18.6	20.4
	51- 75%	8.6	5.7	0.0	1.4	3.9
	76 – 100%	0.0	0.0	1.4	2.9	1.1

See Table 2.1 for description of village clusters

For the pattern of rice cropping, the interviews revealed that farmers grew only one rice crop on any given piece of land per season; or more than one crop, but each on different pieces

of land. Only 20% of the farmers indicated that they had produced more than one rice crop during the past season (Table 2.2).

### **2.4.3 Varieties and sources of rice seed grown in Gibi District**

In both the structured and semi-structured interviews, farmers indicated that they mainly grew traditional varieties of upland rice, which they referred to as “country rice”. All the farmers indicated they had not grown any new variety provided by government employees since 1997. Farmers who maintained their own varieties indicated that they kept up to 7 upland rice varieties in their personal collections (Table 2.3). A common theme documented across the four discussion groups was that certain varieties were unique to collections kept by specific farmers. Farmers listed the most common varieties in the district as LAC 23, Mahn, Jia, and Sodiadu.

The farmers affirmed that the seeds they cultivated were sourced from their personal collections, from other farmers, or bought from unofficial sources. Seventy-nine (79%) of the respondents planted seeds from their own stock, 5% had received seeds from other farmers, and the rest bought the seeds that they had planted (Table 2.3). From the focus group discussions, farmers disclosed that they normally acquired new varieties from their neighbours, but under a customary contract that made the donor the joint owner of the first crop produced from the seed. From the group discussions, farmers also revealed that they had lost several of their varieties during the civil war, but that they had recovered most of them, often from distant communities. Most farmers indicated that they grew more than one variety in a single field during the past year (Table 2.3). They also indicated that they regularly dedicated a small portion of their fields to testing new varieties, and to multiplying seeds acquired from other farmers.

Table 2.3: Types and number of rice varieties and sources of seeds grown by farmers in Gibi District, Liberia

Varieties	Descriptions	Village cluster and response (%)				Mean
		1	2	3	4	
Type of variety grown since 1997	New only	0.0	0.0	0.0	0.0	0.0
	Traditional only	100.0	100.0	100.0	100.0	100.0
	New & traditional	0.0	0.0	0.0	0.0	0.0
Number of varieties kept by farmer*	–	5.5	6.0	10.3	7.1	7.2
Major seed source for last season	Own	92.9	74.3	61.4	88.6	79.3
	Neighbour	1.4	12.9	4.3	1.4	5.0
	Market	5.7	12.9	35.7	10	16.1

See Table 2.1 for description of village clusters. \* Values for the number of varieties kept by farmers are counts, not percentages.

#### ***2.4.4 Farmers' perception of current rice varieties***

The respondents indicated that most of the varieties they grew had been grown for as long as they could remember. From the focus group discussions, it was learned that farmers have only heard of the availability of new varieties from the one agricultural research station, but were never given the opportunity to test them. They overwhelmingly indicated their willingness to grow new varieties, provided they had the opportunity to independently evaluate their performances. The respondents strongly agreed that their current collections of varieties were quite old, and that although they treasured them, they remained open to increasing their collections by adopting new varieties that met their criteria.

#### ***2.4.5 Farmers' criteria for discriminating between rice varieties***

Farmers indicated that they mainly relied on easily distinguishable morphological traits such as paddy colour, paddy or caryopsis shape (grain length and grain length-width ratio), caryopsis colour, and awn length and colour to differentiate between their varieties. They also relied on peculiar agronomic or quality traits such as growth duration, growth habit, yield potential, pest resistance, or grain quality.

Factor analysis of up to 23 traits showed that farmers usually considered a combination of morphological, agronomic, and grain quality traits when selecting their varieties. The major morphological, agronomic and grain quality traits used by farmers to select varieties were plant height, grain yield and growth duration, and cooked grain texture, respectively (Table 2.4). Panicle mass, followed by the number of productive panicles, number of grains per panicle and seed set rate were highly correlated with Factor 1. These traits are basically components of grain yield; hence, Factor 1 was largely a measure of the components of grain yield, which on average were 79% correlated with the factor. Factors 2, 3 and 4, respectively, explained grain quality, pest and disease resistance, and post-harvest traits.

Cooked grain texture, followed by grain taste, received the highest scores for grain quality traits used by farmers to select varieties. Host plant resistance traits given high scores by farmers were disease resistance and weed competitiveness, followed by lodging resistance. Milling recovery, followed by ease of milling and threshability were the key post-harvest traits given high scores by the farmers.

Table 2.4: Factor loadings of various traits used by farmers as selection criteria for new rice varieties. Values in bold face are highly correlated with the given factor.

Rice traits	Factor 1 Grain yield / Agronomic	Factor 2 Grain Quality	Factor 3 Stress resistance	Factor 4 Post- harvest
Grain taste	-0.008	0.487	-0.174	0.137
Grain colour	0.088	0.116	-0.164	0.068
Grain size	0.503	0.437	0.585	0.101
Cooked grain texture	0.064	<b>0.844</b>	0.131	0.016
Ease of cooking	0.183	0.182	0.090	0.111
Milled grain appearance	0.092	0.149	-0.004	<b>0.542</b>
Milling recovery	-0.155	-0.014	-0.209	<b>0.836</b>
Panicle weight	<b>0.803</b>	0.086	-0.098	-0.498
Number of grains / panicle	<b>0.652</b>	0.042	0.251	0.343
Seed set rate	<b>0.648</b>	0.291	-0.072	-0.163
Number productive of panicles	<b>0.757</b>	0.283	0.126	0.070
Number of tillers	0.588	0.349	0.066	-0.007
Yield stability	0.419	0.029	0.089	0.000
Plant height	0.428	-0.014	<b>0.583</b>	0.190
Lodging resistance	0.065	0.290	0.320	-0.075
Disease resistance	0.124	0.083	<b>0.860</b>	0.293
Insect resistance	0.098	0.011	0.203	-0.063
Drought resistance	0.081	0.007	0.462	0.126
Earliness	0.406	0.124	-0.067	0.273
Threshability	0.292	-0.039	-0.491	<b>0.551</b>
Ease of milling	0.114	-0.105	0.012	<b>0.686</b>
Weed competitiveness	0.205	0.120	<b>0.626</b>	0.024
Grain shattering	0.239	0.117	-0.480	-0.057

#### **2.4.6 Farmers' preferences and priorities for varietal traits**

Conjoint analysis to determine farmers' choices based on hypothetical models showed that the ideal variety preferred by farmers would be one with a high grain yield, tender grain texture after cooking, early maturity and intermediate plant height. In the event that such a variety was not available, farmers' preferences and choices would follow the pattern displayed in Table 2.5.

Table 2.5: Farmers' ranking of nine model varieties created to reflect a mixture of traits

Variety	Traits and attributes				Village cluster				Mean Rank
	Grain yield	Grain quality	Plant height	Growth duration	1	2	3	4	
1	above average	soft sticky	short	mid	6	5	6	6	6
2	above average	tender	tall	late	4	4	4	5	4
3	average	flaky	short	late	9	9	9	9	9
4	average	soft-sticky	tall	early	1	2	2	2	2
5	average	tender	intermediate	mid	5	6	5	4	5
6	above average	soft-sticky	intermediate	late	7	7	7	7	7
7	above average	tender	short	early	2	1	1	1	1
8	above average	flaky	tall	mid	8	8	8	8	8
9	above average	flaky	intermediate	early	3	3	3	3	3

Accordingly, the lowest preference was for short-statured, late maturing varieties, with an average yield and flaky grains. The relative importance and path worth (utility scores) of the four cardinal traits and their respective attributes to farmers in the study area are presented in Table 2.6. Grain texture received the highest score for relative importance, followed by grain yield. Flaky and soft-sticky grains, and late maturity received negative utility scores, whilst the rest of the traits gained positive estimates. The Chi-square test of goodness of fit indicated that individual choice of model varieties could be reliably estimated ( $p < 0.01$ ) by group consensus.

Table 2.6: Relative importance and utility of traits and their descriptions that farmers use to choose new varieties

Trait	Description	Utility estimate	Standard error	Relative importance
Grain yield	above average	1.2	0.21	32.6
	average	2.1	0.21	
Grain texture	tender	5.3	0.19	27.4
	flaky	-3.2	0.19	
	soft & sticky	-1.1	0.19	
Plant height	tall	1.3	0.48	8.9
	intermediate	4.2	0.25	
	short	0.9	0.48	
Maturity	early	3.8	0.47	15.2
	mid	2.8	1.11	
	late	-1.5	1.11	
Constant		13.3	1.42	

#### **2.4.7 Farmers' perception of production problems**

Farmers indicated the prevalence of several problems affecting rice production in their district. The problems posed by the various factors are presented in Table 2.7. Birds, rodents and poor farm implements were considered to be the most serious problems for the farmers across the four village clusters. Rice blast was identified as the most important disease problem they encountered, and ranked as the 8<sup>th</sup> most important problem that limited production of their rice crops. Poor rains during the early part of their cropping season was ranked as the 4<sup>th</sup> most important production problem.

Table 2.7: Relative importance of constraints to rice production in Gibi District, Liberia. Scores are the means of the number of times each constraint appeared in the pair-wise comparison matrix.

Production constraints	Village Cluster				Mean	Rank
	1	2	3	4		
Birds	27.5	30.2	23.6	14.7	24.0	1
Rodents	19.2	15.4	17.5	17.3	17.4	2
Poor farm implements	10.5	17.6	14.3	21.7	16.0	3
Poor early rains	17.9	13.3	11.1	18.2	15.1	4
Weeds	11.6	16.7	15.7	9.5	13.4	5
Low soil fertility	9.2	13.9	11.5	15	12.4	6
Shortage of high bush	11.7	8.2	13.2	12.3	11.4	7
Rice blast	10.6	7.4	13.3	8.5	10.0	8
Termites	13.2	8.7	10.5	7.7	10.0	8
Excessive rains	4.1	7.8	8.3	1.1	5.3	9
Brown spot	4.8	3.3	2.7	8.2	4.8	10
Stem borers	6.2	4.3	3.3	4.5	4.6	11
Leaf feeders	0.1	1.1	2.7	4.8	2.2	12
Lack of seeds	0.1	2.7	1.1	4.7	2.2	12
Lack of suitable cultivars	2.7	1.1	1.3	1.1	1.6	13
Sheath rot	1.1	1.6	0.1	2.6	1.4	14
Smut	2.6	1.1	0.0	1.1	1.2	15
Uncontrolled livestock	0.2	0	2.7	0	0.7	16

See Table 2.1 for descriptions of village clusters

## 2.5 Discussion

### *2.5.1 Demographic aspects of rice production and utilization*

Both individual and group interviews revealed that farmers largely grew traditional varieties, and often, more than one variety in the same field, sometimes according to land contour and soil characteristics. This shows that farmers still regard diversity as their major safeguard against crop failure and food vulnerability, and would like to increase their pool of germplasm. Indeed, the farmers' collections of varieties were old, partly due to the low rate of cultivar adoption amongst them, and because they are resource poor and farm only on small fields. Up until the eruption of civil war in 1990, the pool of improved cultivars was mainly dominated by LAC-23 (both red and white), which had only a marginal yield advantage over traditional varieties (IFAD, 1989). In effect, diffusion of improved cultivars into the upland production system of Liberia has been poor and no other bred cultivar, apart from LAC 23, can be identified as being in circulation, despite the long history of rice research in the country. The need for genuinely superior upland rice varieties remains high in Liberia.



Rice production and utilization are mainly influenced by the socio-economic conditions of farm households, and the consumption behaviour of urban dwellers. Respondents to the interviews clearly indicated that they produced rice mainly for their own consumption (Table 2.2), a pattern of rice harvest utilization that has not changed over the years. However, one startling revelation from this study is that none of the respondents were able to produce sufficient rice to feed their own household, let alone produce a surplus to sell. Accordingly, they only sold portions of their limited harvest in desperate situations, and resorted to other crops like cassava to supplement their calorie intakes. Similar responses were obtained in a food security and vulnerability study held earlier in other parts of the country (CAAS-Lib, 2007). This confirms the need to increase the productivity of rice farmers in Liberia.

### **2.5.2 Farmers' perception of rice production and varieties**

In the late 1970's a number of high yielding rice varieties, mainly from the International Rice Research Institute (IRRI), were introduced to Liberia through the efforts of AfricaRice (then WARDA) in collaboration with the country's Ministry of Agriculture (MOA). Several were released as cultivars. However, none but LAC 23 and Suakoko 8 remain in cultivation today. Those varieties are only available in pure form at the nation's one agricultural research station. Some respondents noted that where novel varieties were locally adopted by farmers, they had evolved to appear different in characteristics to what they were like when first released. This could be due to further development of those materials owing to the mass selection commonly practiced by farmers in the informal seed sector, as characterized by seed saving for cultivation purposes during the succeeding season. The limited adoption or rapid degeneration of new varieties further show the weakness of the national research programme, as it seemed to have neglected the establishment of a strong outreach programme for the dissemination of new varieties. The establishment of a national programme dedicated to the promotion, development and maintenance of new varieties will therefore be important for boosting rice production in Liberia.

Group discussions on farmers' perceptions of the rice production system revealed that the rice production system is still dominated by dryland cultivation (Table 2.2). Essentially, rice is produced in Liberia on both dry and wet lands, with the former alone accounting for at least 85% of the area amongst rice producing households in the Gibi District of Liberia. Similar estimates have been reported for other parts of the country (GOL, 2006). Farmers have traditionally preferred upland production over lowland production in paddies, despite its lower yield potential (Hughes *et al.*, 1989), mainly because the cultivation of upland rice is less demanding in terms of labour for planting and weeding (Parthasarathi *et al.*, 2012),

and it is more amenable to intercropping with other crops that ensure additional income and which contribute to food security (Gupta and O'Toole, 1986; Prajitno, 2007). It is also not surprising that the farming system, dominated by shifting cultivation, has remained basically unchanged over the years.

Farmers confirmed that the availability of land, mainly communally owned, for farming is shrinking rapidly. The issue of sustainability becomes more worrying because it is difficult to change farmers' practices. However, with a growing population, available communal land is diminishing. Solving the issue of land tenure is an even bigger challenge. Intensive research, especially in collaboration with farmers, will be needed to improve and sustain rice production in Liberia. With shorter fallow periods and an increasing shift towards fixed farming, pest and disease problems will increase, further challenging farmers to seek more resilient varieties. Constant crop improvement and cultivar development will therefore be needed to address such problems and needs.

### ***2.5.3 Analysis of farmers' selection criteria***

Factor analysis of farmers' selection criteria showed that a farmer's decision to grow a new variety may be based on a combination of grain yield, agronomic, grain quality, pest resistance and post-harvest traits. The high correlations between yield components and Factor 1, grain quality traits and Factor 2, pest and disease resistance and Factor 3, and post-harvest traits and Factor 4 are a good indication of how well farmers understood rice varietal traits and their contribution to the selection of varieties. Farmers understood that as panicle mass increases, so does the number of productive panicles, and to a lesser extent, the number of grains per panicle and the rate of seed set.

The texture and taste of cooked grains, and host plant resistance and post-harvest characteristics, were important to farmers when deciding which varieties to plant. This analysis emphasizes the fact that farmers are extremely knowledgeable about the crop they grow, and their input on decisions to develop new varieties could prove valuable to the eventual success of any breeding programme.

### ***2.5.4 Farmer-preferred varieties***

A conjoint analysis of the farmers' preferences was undertaken to create model varieties that could be used to predict and determine farmers' preferred varieties for the future. It was established that the varieties farmers would like to grow should present a combination of high yield, tender cooked grain texture, early maturity and intermediate plant height.

Grain quality, particularly eating quality, remains important when evaluating varieties. This partly explains why farmers have stuck to growing traditional upland rice than lowland rice,

in spite of the higher yield potential of the latter. Earlier studies on the adoption of lowland rice varieties in Liberia, for example, found that farmers preferred a variety (Suakoko 8) because the taste and eating quality of its grains were perceived as similar to their best upland varieties (ECC, 1987).

In addition to grain yield and quality, plant height and grain yield were key components of farmers' preference criteria for choosing any new variety. Intermediate height and early maturity were regarded by farmers as essential attributes of the respective traits. The importance of these traits were not unique to farmers in the study area. Using a hedonic price model to evaluate rice trait preferences of West African rice producers, Dalton (2004) found that non-yield production characteristics including plant height, growth duration, grain colour, plate size and tenderness of cooked grains, were more important to farmers than yield.

Panicle reaping is the method traditionally used by farmers to harvest their rice crops. Farmers complained that it is painful stooping low to harvest dwarf cultivars. Plants of intermediate height (110 – 130 cm) were thus preferred. Farmers were clear that they preferred to work with tall plants compared to dwarf ones. According to them, to harvest very tall plants, they simply break the straw by trampling the stems, thus lowering the panicles to a position that they can conveniently reach for reaping. The dwarf plant types, however, provide the significant advantage of lodging resistance, compared to tall plants. Farmers could be supported to adopt an improved harvesting technique, specifically the faster and more efficient sickle reaping method, which would allow for selection of shorter varieties, resistant to lodging.

### ***2.5.5 Farmers' assessment of rice production problems***

Low yield and production of rice in Liberia are the direct result of various problems acting singly or with each other. The most important yield limiting factors in the study communities were birds, rodents, poor farm implements and poor rains early in the production season. The problem of birds could be overcome by synchronizing cultivation and expanding aggregate production across regions so that the impact of the problem becomes less pronounced for any one farmer. Farmers largely attributed the problems of rodents and weeds to the use of low vegetation areas, which usually border the main habitat of the groundhog (*Thryonomys swinderianus*), for rice production. These problems cannot, however, justify the continual depletion of the high forest reserves for shifting cultivation of rice.

The issue of poor farming implements has been one of the prominent features of slash-and-burn methods employed in shifting cultivation. More farmer education and empowerment will be required to move them away from subsistence to commercial, albeit small-scale, farming. This could potentially stimulate national or private investment in equipment and machinery for land preparation, cultivation and other production activities. But this will require the establishment of private tenure for rice farmers.

## 2.6 Conclusion

Studying farmers' perceptions and preferences is an integral part of any process aimed at finding sustainable solutions to important food security problems in any region. This study relied on various rural participatory appraisal methods to identify the essential traits of rice varieties that influence farmers' decisions to adopt new rice varieties in Liberia. It was found that besides high grain yield, farmers highly valued grain quality traits, especially, the texture of cooked grains. Plant height, and earliness of varieties were two other traits that farmers regarded as important to their decisions for selecting varieties to plant. As the need to develop more resilient varieties is clear, it is important that breeding efforts aimed at accomplishing that goal also incorporate the traits of grain quality, plant height and crop duration, as desired by farmers in the target regions. This will increase the level and speed of adoption of any future variety. Information on the demographic aspects of rice production and utilization, as well as perceived problems in the study area, should also be useful to plant breeders and other providers of agricultural services to Liberia.

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## CHAPTER 3

### BLAST RESISTANCE OF SELECTED UPLAND RICE GENOTYPES FROM LIBERIA

#### 3.1 Abstract

Eliminating the confounding influence of vertical resistance and creating reliable screening environments are two essential prerequisites in breeding for horizontal resistance against rice blast. Two greenhouse trials and a field study were conducted to evaluate up to 38 upland rice genotypes for breeding to enhance quantitative resistance against rice blast disease caused by *Pyricularia oryzae*, and to identify suitable growth conditions for discriminating between rice genotypes for quantitative resistance against blast. The first greenhouse study revealed that the reactions of the 38 rice genotypes to three distinct races of *P. oryzae* were characterized by the occurrence of both race-specific and race-non-specific resistance patterns, even though the susceptible genotypes were not killed by the disease. Up to 34% of the genotypes exhibited complete resistance to all three pathotypes tested, whereas 26% were susceptible to all three pathotypes. Variation for two quantitative resistance traits (the number of sporulating lesions per plant and the number of leaves with sporulating lesions per plant) was mainly due to genotypic differences, as determined by AMMI analysis. Twenty-two genotypes produced susceptible reaction types against SIK-111, the most virulent of the three pathotypes. These were further evaluated for levels of quantitative resistance using spreader plants under greenhouse and field conditions. Up to 10 of the 22 genotypes (LR-2, 6, 8, 10, 11, 14, 24-2, 25 and 26) were scored for smaller numbers or sizes of lesions with sporulating centres and / or disease severity and AUDPC scores, suggesting that they possessed higher levels of horizontal resistance. Plants subjected to intermittent drought stress developed larger and more lesions with sporulating centres than those grown under well-watered conditions, both in the greenhouse and in the field. Field testing did not seem suitable for evaluating quantitative resistance against rice blast in South Africa as values recorded for components of the trait were lower than captured in the greenhouse. In order to advance breeding for quantitative blast resistance using the current set of genotypes, it would therefore be important to recombine genotypes showing lower values of lesion number (LN), lesion size (LS), and / or AUDPC, and to evaluate their progeny against SIK-111 under controlled environmental conditions and managed drought stress, or field conditions more conducive to rice blast.

Keywords: AUDPC, *Pyricularia oryzae*, race-non-specific, resistance breeding, rice



## 3.2 Introduction

Rice blast, caused by *Pyricularia oryzae* Cavara, is a serious constraint to rice (*Oryza sativa* L.) production in Liberia and other rice growing countries. Annually, the disease destroys enough rice to feed about 60 million people (Zeigler *et al.*, 1994) and causes yield losses of up to 77% in Liberia. As such, breeding for blast resistance is a major objective of most rice breeding programmes because the use of resistant varieties would be the safest and cheapest way of controlling the disease. However, populations of *P. oryzae* are usually highly variable genetically (Ou, 1985). The result is that rice cultivars containing single major (R) genes conferring resistance against many races of the pathogen frequently become susceptible as one or more virulent races become prominent (Kiyosawa, 1982; Bonman *et al.*, 1992; Han *et al.*, 1995). This phenomenon has been a major impediment to genetically controlling the disease, especially in such blast-prone agro-ecologies that exist in the target environments (TeBeest *et al.*, 2007; Khush and Jena, 2009). Since it is impossible to limit pathogen variability, horizontal resistance could be exploited, instead of race-specific or vertical resistance, to control rice blast.

Horizontal resistance, also known as partial resistance, is usually conferred by polygenes whose actions are additive in nature (Vanderplank, 1984; Stoskopf *et al.*, 1993). Knowledge of population genetics has revealed that the combined action of the polygenes tends to protect plants against multiple races of a pathogen (Khush, 1977; Parlevliet, 1985; Agrios, 1997). Despite these merits, breeding horizontal resistance has been seen as an unattractive breeding goal because of the difficulty involved in assessing the often numerous components associated with the trait, and the difficulties in conducting recurrent selection in rice. As with other quantitative traits, the performance of a cultivar for horizontal resistance against a disease is usually only a partial reflection of its genetic value due to environmental influences (Semagn *et al.*, 2010). Choosing non-adapted donors for the improvement of such a trait could potentially delay progress as additional trials would be required to first establish their adaptability. Hence, improving rice for horizontal resistance within a specific target environment would require the use of parents which should be adapted, as much as possible, to the agro-ecological conditions of that environment. Conditions that favour epidemics of the disease would also be required to establish the true resistance potential of a variety. Furthermore, research shows that detection of and breeding for horizontal resistance is often difficult in the presence of vertical resistance, which may mask the expression of horizontal resistance genes (Landeo and Turkensteen, 1989; Robinson, 2004). Excluding or inactivating the vertical resistance genes is important to guarantee measurable progress in breeding programmes aimed at increasing levels of horizontal resistance to blast (Robinson, 2006), for example, by using a single pathotype

approach to eliminate the expression of vertical resistance genes in the parents and their progeny.

In order to identify potential rice genotypes, with adaptation to the target environment, for future hybridization, a collection of upland rice genotypes from Liberia were characterized for both qualitative and quantitative blast resistance. In the process, different pathotypes of *P. oryzae* were also evaluated for their capacity to produce susceptible lesions on the genotypes. The value of the various genotypes, as well as different growth environments, for improving horizontal resistance of rice are analysed and discussed.

### **3.3 Materials and methods**

Two controlled environment (CE) experiments and a field study were conducted to characterize blast resistance of upland rice from Liberia. The first CE study investigated race specificity of three rice blast pathotypes against all the varieties; the second CE experiment and field study were subsequently carried out to confirm the interaction of the most aggressive pathotype with only the genotypes on which it caused susceptible infection types in the previous study.

#### **3.3.1 Plant materials**

A total of 40 rice genotypes, including 38 traditional varieties and an improved cultivar from Liberia, and a Japonica variety from IRRI, were used in the studies. The traditional varieties were collected from 5 regions (Gibi [6.6, -10.0], Fuamah [6.78, -10.39], Suakoko [6.99, -9.58], Belle Fassama [7.52, -9.99] and Kilibay [6.97, -9.98]), sorted by glume characteristics and grain colour, and kept true-to-type through selection at the Central Agricultural Research Institute (CARI) of Liberia. The improved cultivar (LAC 23) and a *Japonica* variety, Lijiangxintuanheigu (LTH), were used as resistant and susceptible checks, respectively in all the trials. LAC 23, a selection from traditional upland rice varieties in Liberia, is the oldest and best improved cultivar grown in the country. Lijiangxintuanheigu is a universally susceptible genotype that is often used as a susceptible check in rice blast differential varieties.

#### **3.3.2 Sources of infection**

Pathotypes used for challenging the test plants were mainly sourced from AfricaRice in Cotonou, Benin. Based on preliminary observations (data not presented), three of the pathotypes (SIK111, EDOZ and GH-8) were selected for their sporulating capacity and virulence to LTH and were utilized for the experiments.

### 3.3.3 Description of study sites

The CE and field studies were conducted at the research facilities of University of KwaZulu-Natal in Pietermaritzburg, South Africa. The first CE study was carried out in a glasshouse (30 / 25°C; 80% RH) and the second conducted in a greenhouse tunnel, with day and night time temperature varying between 30 to 20°C and 65-95% RH. Humidity and leaf wetness in the glasshouse were increased by covering plants with thin sheets of transparent plastics overnight and by misting with fine sprays of sterile water three times daily for up to 7 days after inoculation.

Field trials were conducted at the university's Ukulinga Research Farm (29° 40' S, 30° 24' E; 806 meters above sea level [m.a.s.l.]). Based on annual means of long term climatic data, Ukulinga has a mean annual temperature and rainfall of 18°C and 738 mm, respectively. Climatic data for the duration of the field experiment are presented in Table 3.1.

Table 3.1: Monthly climatic data during the field trial (November 2012 – May 2013) at Ukulinga Research Farm. Data source: Agricultural Research Council – Institute for Soil, Climate and Water (ARC–ISCW), South Africa.

Month	Minimum temperature (°C)	Maximum temperature (°C)	Solar radiation (MJ m <sup>-2</sup> )	Reference Evapotranspiration (mm)
November	9.62	32.90	15.92	91.17
December	14.12	31.50	20.71	124.90
January	14.75	38.40	18.28	113.60
February	13.90	33.80	18.84	105.00
March	12.96	32.00	15.43	93.98
April	9.28	35.10	14.12	86.96
Mean	12.44	33.95	17.22	615.61*

\* Values for reference evapotranspiration are totals, not averages.

### 3.3.4 Experimental design and treatments

All studies were laid out in a randomized complete blocks design with three replications. Treatments for the first CE experiment consisted of 40 genotypes (38 traditional and two check varieties), and three *P. oryzae* pathotypes, replicated at different time periods. Plants were established by direct seeding of pre-germinated seeds in 30 X 30 cm seedling trays, with 15 plants per genotype X pathotype X replication.

The second CE and field studies comprised a factorial arrangement of 24 genotypes (LR 2, 6, 8, 9, 11, 14, 19, 21, 24-1, 24-2, 25, 26, 27, 29, 32, 36, 38, LAC 23 and LTH) and two water regimes (well-watered and intermittent drought stress). Test plants were grown in 4.8 L pots at 2 hills per pot. Soils in well-watered pots were kept continuously moist throughout the experiment, or subjected to intermittent drought stress, commencing at 14 days after

emergence (DAE), by withholding water and only irrigated when plants showed symptoms of leaf rolling.

Treatments for the field experiment were the same as for the second CE study. Fifteen plants of each genotype per water regime per replication were grown in single row plots. Spaces within and between rows were 15.0 cm. For both the second CE and field studies, the uniform blast nursery procedure, using SIK-111, was used to develop the disease epidemic.

For each experiment, soils were amended appropriately to ensure a proper balance of nutrients. Nitrogen was supplied prior to sowing at the rate of 90 kg ha<sup>-1</sup>, and at two week intervals thereafter beginning at 14 days after sowing at the rate of 30 kg ha<sup>-1</sup>, in order to stimulate vigorous vegetative growth.

### **3.3.5 Inoculation of test plants**

For the first CE trial, disease was initiated by spraying plants with solutions containing 1 X 10<sup>5</sup> spores of each of the *P. oryzae* pathotypes. The inoculants were grown on oat meal agar, amended with chlorotetracycline (25 mg L<sup>-1</sup>), at 20 to 25°C under 16 / 8hrs alternating light and dark periods in a laboratory growth chamber (Labcon, South Africa). Spore solutions were prepared by scraping off and washing mycelia in sterile water containing 0.2% gelatine. The gelatine was used to increase the osmotic concentration of the spore solution to prevent lysis, and to increase adhesion of the sprayed solution to the surface of the plants.

Following the uniform blast nursery procedure (IRRI, 2006) for the second CE and field studies, LTH seedlings, pre-infected in the glasshouse, were used as disease spreaders to initiate disease on the experimental plants. The spreader plants were established in single alternating rows before and after each genotype. Spreader plants were prepared in polyethylene bags and transferred with intact growth media to their designated rows to minimize transplanting shock. Each epidemic was considered ended when the spreader plants completely succumbed to the disease.

### **3.3.6 Disease evaluation and data analyses**

For the first CE, the predominant lesion type (LT), considered for determining qualitative resistance, was assessed at 7 days after inoculation (DAI) using the 0 – 9 scale developed by IRRI (2006). The number of lesions with sporulating centres per plant (LN) and the number of leaves with sporulating lesions per plant (NLL) were also determined at 7 DAI for the first CE study. For the second CE and field studies, LN was determined at 10 days after transplanting the spreaders. The sizes of sporulating lesions (LS) were estimated at 10 days

after transplanting the spreaders, using the keys created by Roumen (1992). The predominant lesion on the older leaves were chosen for lesion size measurement. Data on LT, LN and NLL were recorded for 10 plants per experimental plot.

During the second CE and field studies, disease severity on all fully expanded leaves per plant was estimated visually for 10 plants per plot, five times at 3 day intervals using a diagrammatic scale (depicting 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 82% of diseased leaf area), as developed by Notteghem (1981). Disease severity scores were used to calculate area under the disease progress curve (AUDPC) based on the formula developed by Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{N-1} \frac{(Y_i + 1 + Y_{i+1})}{2} (X_{i+1} - X_i),$$

where  $Y_i$  = the percent of diseased leaf area on the  $i$ th day of assessment,  $X_i$  = time of the  $i$ th assessment in days from that of the first assessment, and  $N$  = total number of times disease was assessed.

Data on LT was averaged per genotype to determine qualitative resistance against rice blast, and also subjected to ranks overall ANOVA to further evaluate differences between the genotypes and pathotypes, using IRRI's statistical software CropStat Version 7 (IRRI, 2007). CropStat Version 7 was also used to analyse data on LN and NLL from the first CE study, based on the additive main effects and multiplicative interaction (AMMI) statistical model. All other data were subjected to analyses of variance using the statistical package GenStat® Version 14 (VSN, International). The means of factors showing significant difference were separated by Fisher's least significant difference (LSD) test at the 5% probability level.

## **3.4 Results**

### ***3.4.1 Qualitative reactions of genotypes to rice blast***

In order to check for qualitative resistance to rice blast, genotypes were evaluated for the type of lesion produced in response to inoculation with different rice blast pathotypes. The genotypes varied significantly by LT, both between and within pathotypes (Table 3.2).

Table 3.2: ANOVA for rice blast resistance traits assessed on spray inoculated plants in a controlled environment facility.

Source of variation	Df	Mean square		
		LT	LN	NLL
Genotype	39	56239.0***	587.6***	9.7**
Pathotype	2	130804.0***	1344.4**	19.6**
Genotype X Pathotype	78	13008.2 ***	160. 6**	2.5**
Replication	2	869.0ns	63.0ns	0.9ns
Genotype X replication	78	1142.4ns	71.2ns	1.3ns
Pathotype X replication	4	1064.2ns	68.7ns	1.2ns
Genotype X pathotype X replication	156	917.7 ns	65.8ns	1.1ns
Error	3231	586.2	38.41	0.5

\*\*\*( $p < 0.001$ ), \*\*( $p < 0.01$ ), ns ( $p > 0.05$ ); Df = degrees of freedom; LT = Lesion type; LN = number of sporulating lesions per plant; NLL = number of leaves with sporulating lesions per plant.

Each cultivar was classified as resistant or susceptible, based on the score of the LT exhibited (Table 3.3). A total of 27 genotypes showed susceptible reactions to at least one of the pathotypes. Ten genotypes, including LR 2, 6, 8, 10, 11, 14, 24-1, 24-2, 25 and 26, in addition to both check varieties, showed compatible reactions to all three of the pathotypes, whilst 13 (LR- 1, 7, 12, 13, 16, 17, 19, 20, 31, 33, 34, 35, and 37) exhibited equally incompatible reactions to all three of the pathotypes. SIK-111 produced susceptible LT reactions on more genotypes than the other two pathotypes.

Table 3.3: Qualitative classification of rice genotypes resistance to three *P. oryzae* pathotypes. Scores are means of predominant lesion type; compatible or incompatible reactions are presented as R or S, respectively.

Genotypes	Pathotypes					
	EDOZ		GH-8		SIK 111	
	Score	Class	Score	Class	Score	Class
LR 1	0.0	R	0.7	R	0.3	R
LR 2	5.7	S	5.7	S	5.0	S
LR 3	0.3	R	0.3	R	5.7	S
LR 4	2.3	R	0.0	R	5.0	S
LR 5	3.0	R	0.7	R	5.7	S
LR 6	7.0	S	5.7	S	5.0	S
LR 7	0.0	R	0.0	R	1.0	R
LR 8	5.7	S	5.0	S	6.3	S
LR 9	7.3	S	0.0	R	1.0	R
LR 10	7.3	S	5.0	S	7.0	S
LR 11	7.0	S	6.3	S	6.3	S
LR 12	0.3	R	0.3	R	1.0	R
LR 13	3.0	S	0.0	R	0.0	R
LR 14	5.0	S	5.0	S	6.3	S
LR 15	1.7	R	0.0	R	8.3	S
LR 16	1.0	R	0.7	R	0.0	R
LR 17	0.3	R	1.0	R	2.3	R
LR 18	7.0	S	2.3	R	7.7	S
LR 19	0.0	R	0.3	R	1.7	R
LR 20	0.0	R	2.3	R	1.7	R
LR 21	3.0	R	5.7	S	7.7	S
LR 22	0.0	R	0.0	R	5.0	S
LR 23	6.3	S	3.0	R	7.0	S
LR 24-1	7.0	S	5.7	S	7.0	S
LR 24-2	5.0	S	5.7	S	7.0	S
LR 25	7.0	S	5.0	S	5.7	S
LR 26	5.7	S	5.7	S	5.0	S
LR 27	2.3	R	0.0	R	6.3	S
LR 28	0.3	R	8.3	S	7.0	S
LR 29	6.3	S	0.0	R	3.0	R
LR 31	0.0	R	0.0	R	0.0	R
LR 32	0.0	R	0.0	R	5.0	S
LR 33	0.0	R	0.0	R	0.0	R
LR 34	1.0	R	0.0	R	0.0	R
LR 35	0.0	R	1.7	R	0.7	R
LR 36	1.0	R	6.3	S	6.3	S
LR 37	1.0	R	0.7	R	0.0	R
LR 38	0.0	R	0.0	R	5.0	S
LAC 23	5.0	S	5.3	S	5.0	S
LTH	9.0	S	9.0	S	9.0	S
Mean	2.9		2.4		4.2	
	SEM					
Genotypes	0.32					
Pathotypes	0.09			CV (%) = 9.7		

SEM= standard error of the means; CV = coefficient of variation.

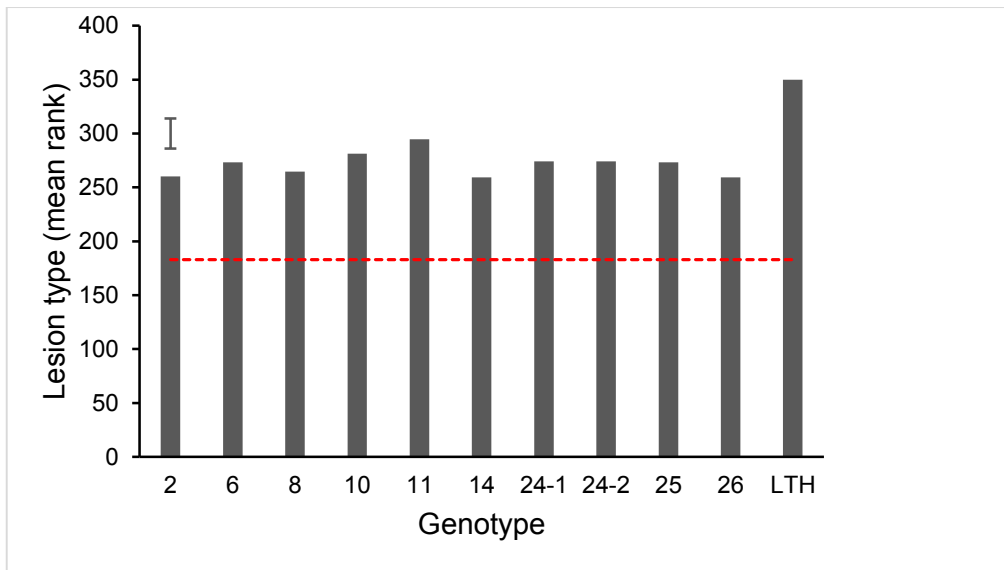


Figure 3.1: Mean ranking of blast susceptible genotypes. Bars represent only genotypes that showed compatible interactions with all of three pathotypes. Error bar represents LSD ( $P = 0.05$ ), trend line represents mean rank for 40 genotypes.

### 3.4.2 Quantitative reaction of genotypes to rice blast

Averaged over the three blast pathotypes across the first CE study, the mean responses of the 40 genotypes for the number of sporulating lesions and infected leaves per plant respectively ranged from 0 to 29, and 0 to 3.6. Significant interactions between genotypes and pathotypes were also observed. Additive main effects and multiplicative interaction (AMMI) analysis revealed that 9 genotypes (LR- 1, 12, 16, 19, 31, 33, 34, 35 and 37) expressed the highest levels of quantitative resistance to all three pathotypes, whilst four (LR- 21, 23, 24-1 and LTH) showed the lowest, as indicated by the number of sporulating lesions (Figure 3.2a) and leaves infected (Figure 3.2b) recorded per plant. Genotypes LR- 3, 4, 17, 20, 22, 27 and 32 appeared quite unstable for these traits. SIK-111 induced the highest number of sporulating lesions on the genotypes, and GH-8 appeared least capable of inflicting a large number of sporulating lesions on the plants or infecting a large number of leaves.



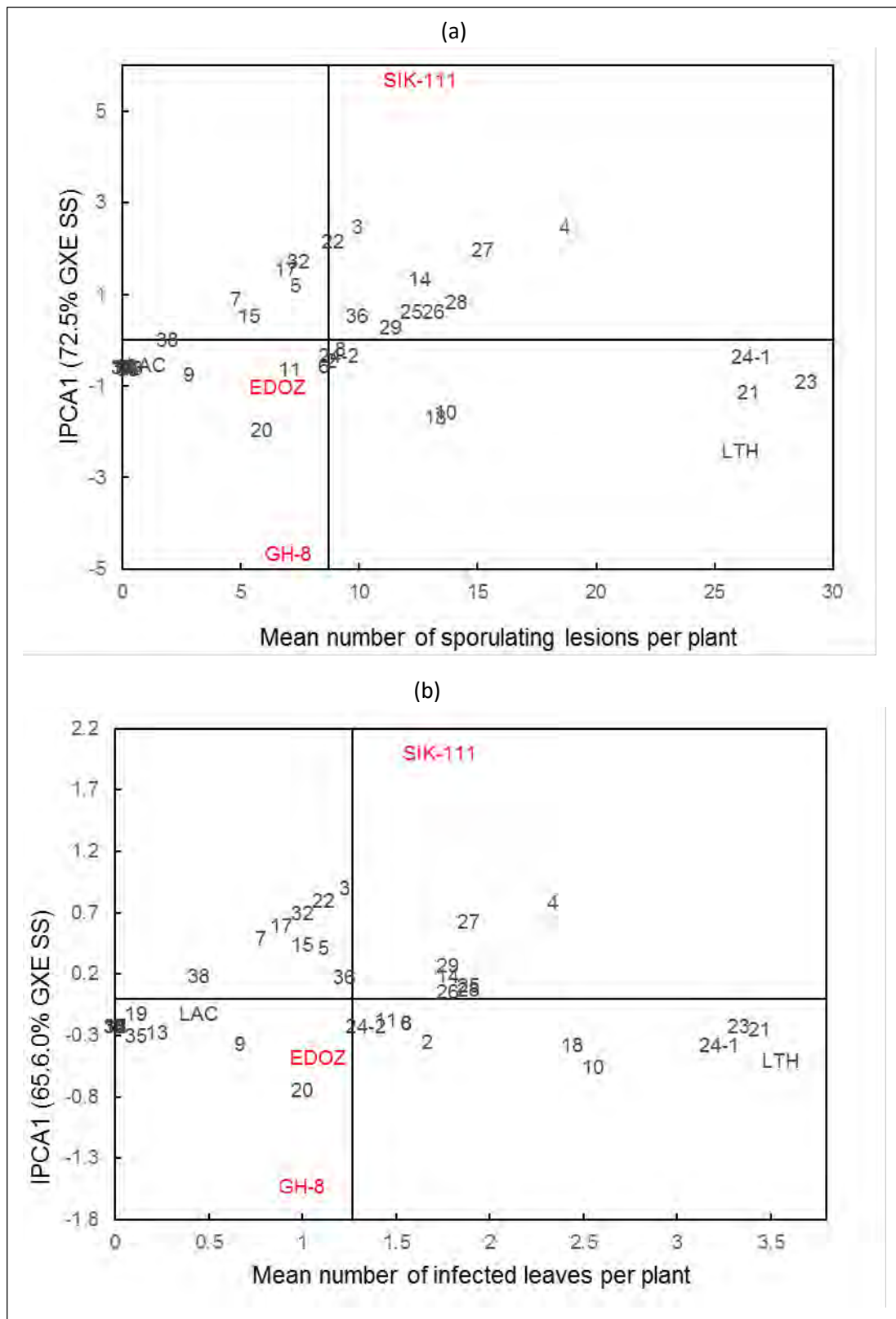


Figure 3.2: Mean and interaction effects of 40 rice genotypes and three *P. oryzae* pathotypes for the number of rice blast sporulating lesions (a) and number of infected leaves per plant (b)

The AMMI ANOVA (Table 3.4) revealed that the genotypes, followed by the pathotypes, accounted for most of the variation in the numbers of sporulating lesions / infected leaves observed in this study. The variation due to interaction of genotype and pathotype was markedly small. Of the interaction variance (SS), IPCA 1 accounted for 72.5% (for the total number of sporulating lesions produced per plant) and 65.6% (for the number of infected leaves per plant), while the second IPCA explained only 27.5 and 34.4% of the interaction SS for the respective traits (Table 3.4).

Table 3.4: AMMI ANOVA for the number of sporulating lesions and infected leaves per plant. (\*) indicates significant variance ratio ( $p < 0.01$ ).

Source of variation	Number of sporulating lesions per plant				No of infected leaves per plant			
	D.f.	S.S.	M.S.	F Ratio	D.f.	S.S.	M.S.	F Ratio
Treatments	119	38131.00	320.40	8.23	119	612.20	5.15	9.58*
Genotypes	39	22919.00	587.70	15.10	39	377.30	9.68	18.01*
Environments	2	2689.00	1344.40	50.09	2	39.20	19.62	39.24*
Block	6	161.00	26.80	0.69	6	3.00	0.50	0.93
Interactions	78	12524.00	160.60	4.13	78	195.70	2.51	4.67*
IPCA1	40	9078.00	227.00	5.83	40	128.40	3.21	5.98*
IPCA2	38	3446.00	90.70	2.33	38	67.30	1.77	3.30*
Error	234	9106.00	38.90		234	125.70	0.54	
Total	359	47398.00	132.00		359	740.90	2.06	

D.f.= degree of freedom; S.S.= sum of squares; M.S.= mean squares.

### **3.4.3 Effects of genotype and water regime on blast disease severity**

#### **3.4.3.1 Lesion size and lesion number**

The quantitative responses of the genotypes to rice blast were assessed in both the second CE and field studies. The size of sporulating lesions 10 days after deployment of disease spreaders varied significantly amongst the genotypes grown under greenhouse or field conditions (Table 3.5). The mean difference in the size of sporulating lesions between the two water regimes during this period was negligible under greenhouse conditions; but in the field, intermittent drought stress (before and during blast incidence) tended to significantly increase lesion size. Genotype by water regime interaction was also significant as some genotypes developed larger lesions under well-watered conditions, contrary to the general pattern of lesions being larger under intermittent drought stress. On average, lesion size recorded in the field was slightly larger than that recorded in the greenhouse.

The number of lesions with sporulating centres was markedly smaller in the genotypes from Liberia, compared to the susceptible check, LTH (Table 5) both in the CE and field trials. The number of lesions developed on 8 of the genotypes (LR- 2, 6, 8, 10, 24-2, 25, 26 and

38) were highly comparable to that observed on the resistant check variety, LAC 23. The number of sporulating lesions on plants subjected to drought stress was significantly higher ( $p < 0.01$ ) than those observed on plants grown under well-watered conditions, both in the field and in the greenhouse. Genotype by water regime interaction was not significant ( $p = 0.05$ ) for the trait in either growing environments.

Table 3.5: Effects of growing conditions on the number and size of sporulating lesions developed on different rice genotypes.

Genotype	Sporulating lesion size (mm <sup>2</sup> )				Lesion number			
	Greenhouse		Field trial		Greenhouse		Field trial	
	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered
LR-2	3.2	2.7	3.4	3.2	16.0	10.0	3.1	2.3
LR-3	3.6	4.2	4.2	3.9	33.0	23.3	3.2	3.8
LR-4	1.9	4.6	3.7	2.4	42.0	32.0	6.8	6.0
LR-5	2.8	2.3	2.8	3.0	22.7	24.3	3.4	3.0
LR-6	4.4	2.2	3.6	3.8	10.0	6.7	3.5	2.8
LR-7	3.9	2.3	3.4	3.2	35.0	25.0	3.8	2.3
LR-8	2.1	2.6	2.6	2.1	12.7	12.7	2.9	1.6
LR-10	2.1	2.3	1.6	2.8	8.0	12.0	5.4	4.6
LR-11	5.6	2.2	4.2	4.9	20.0	16.0	3.2	3.0
LR-14	3.2	3.8	3.8	3.5	24.0	21.0	5.2	4.3
LR-15	4.5	3.1	4.5	4.9	19.0	16.7	1.2	2.4
LR-21	3.2	3.2	2.8	3.5	32.0	23.3	8.1	11.0
LR-22	3.0	5.5	4.7	3.1	34.0	28.0	4.7	2.9
LR-23	7.0	4.4	6.0	6.7	36.3	33.0	13.7	10.6
LR-24-1	3.2	2.0	2.8	2.6	30.0	34.0	11.8	12.0
LR-24-2	2.8	4.2	3.8	3.1	8.0	4.0	3.5	3.0
LR-25	3.2	1.9	2.5	3.4	14.3	10.7	4.5	3.2
LR-26	2.8	2.9	3.1	2.7	16.3	8.0	7.8	6.7
LR-27	4.1	3.1	3.3	4.5	29.5	33.2	9.4	8.0
LR-32	2.7	5.3	4.0	1.9	19.0	26.0	2.9	2.0
LR-36	2.6	4.4	3.3	1.8	27.8	33.0	2.4	2.6
LR-38	3.9	3.1	3.8	4.2	9.0	6.3	1.6	0.5
LAC 23	2.6	3.2	3.5	3.0	7.3	6.3	1.4	0.3
LTH	6.1	6.3	6.2	6.7	51.7	49.0	18.6	13.0
Mean	3.5	3.4	3.7	3.5	23.2	20.6	5.5	4.7
	Sporulating lesion size (mm <sup>2</sup> )				Lesion number			
	Greenhouse		Field		Greenhouse		Field	
LSD <sub>(p = 0.05)</sub>	Genotype	0.6	0.7	8.2	2.1			
	Water regime	0.2	0.2	2.4	0.7			
	Genotype x Water regime	0.8	0.9	11.6	4.9			
CV (%)	14.1	27.8	33.0	36.1				

### 3.4.3.2 Area under disease progress curve

Area under the disease progress curve values ranged from 75.4 to 505, and differed significantly ( $p < 0.001$ ) between the 24 genotypes exposed to conidia of *P. oryzae* from spreader plants in the greenhouse (Table 3.6).

Table 3.6: Mean greenhouse and field blast severity scores for 24 rice genotypes grown under different water regimes.

Genotype	AUDPC				Maximum diseased leaf area (%)			
	Greenhouse		Field trial		Greenhouse		Field trial	
	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered
LR-2	252	132	48	40.9	17.2	15.5	8.6	3.9
LR-3	339	99	48	30.7	62.4	28.9	21.2	13.1
LR-4	369	171	84.9	53	72.9	17.8	18.5	9.6
LR-5	573	150	91.8	46.5	80.6	14.9	15.3	18.2
LR-6	157.5	147	31.8	48.8	6.3	7.1	3.2	1.4
LR-7	300	273	69.5	84.6	58.2	40.0	9.1	13.2
LR-8	222	132	41.1	40.9	18.6	8.2	4.3	6.2
LR-10	195	219	54.9	67.9	8.8	14.1	2.4	2.0
LR-11	144.8	157.5	30.3	48.8	11.8	9.4	3.9	2.7
LR-14	171	99	33.3	30.7	9.6	12.2	2.8	2.2
LR-15	121.5	417	27.9	59.3	8.5	82.6	2.3	1.9
LR-21	243	205.5	57.9	63.7	26.1	22.2	19.1	5.9
LR-22	417	207	85.9	64.2	81.6	67.7	20.8	18.5
LR-23	241.5	246	65.5	76.3	33.3	42.0	10.7	7.6
LR-24-1	219	339.8	77.2	67.9	53.7	40.6	16.9	12.2
LR-24-2	168	102	36.5	52.1	6.5	13.2	2.3	1.5
LR-25	217.5	147	43.8	67.4	7.1	15.5	3.6	1.6
LR-26	243	168.8	45.9	52.3	19.7	11.2	4.9	4.5
LR-27	414	114	95.2	35.3	79.7	18.3	19.9	13.1
LR-32	312	273	62.8	84.6	65.7	29.9	16.9	9.9
LR-36	240.8	217.5	55.4	67.4	23.0	36.7	8.5	5.2
LR-38	78	72.8	16.9	21.6	4.4	4.5	2.2	1.0
LAC23	170.5	131.2	39.2	40.7	7.6	6.2	3.5	1.7
LTH	524	486	132.4	149.7	82.0	82.0	41.0	58.6
Mean	263.9	196.1	57.3	58.1	35.2	26.7	10.9	9.0
			AUDPC		Max. Diseased leaf area (%)			
			Greenhouse	Field	Greenhouse	Field		
	Genotype		21.9	4.3	17.2	13.3		
	Water regime		6.3	1.6	4.9	2.2		
LSD <sub>(p = 0.05)</sub>	Genotype x Water regime		31.0	7.2	24.3	15.9		
CV (%)			8.3	28.9	26.8	31.7		

All the traditional varieties displayed lower AUDPC values than the susceptible check, indicating lower levels of susceptibility to leaf blast. In the field, the AUDPC values varied significantly with genotype ( $p < 0.001$ ), but not with water regime ( $p = 0.05$ ). For both water regimes, AUDPC recorded in the field was markedly lower than values measured in the controlled environment facility. Maximum disease severity measured on plants in the greenhouse and the field followed a similar trend as measurements for AUDPC (Table 3.6).

### 3.4.3.3 Plant mortality and grain yield

Plant mortality was low in all genotypes, except the susceptible check in both the greenhouse and the field (Table 3.7). In the field, mortality was significantly higher amongst plants subjected to drought stress ( $p < 0.001$ ). Grain yield per plant varied significantly with genotype ( $p < 0.001$ ), and tended to decrease with drought stress, both in the greenhouse and the field.

Table 3.7: Effects of environment on mortality and yield of rice blast affected plants

Genotype	Mortality (%)				Grain yield (g plant <sup>-1</sup> )			
	Greenhouse		Field trial		Greenhouse		Field trial	
	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered	drought stressed	well watered
LR-2	0.0	0.0	0.0	0.0	5.2	9.7	6.5	12.0
LR-3	0.1	0.0	0.2	0.0	1.3	2.4	2.7	4.8
LR-4	0.0	0.0	0.1	0.0	5.3	6.0	6.7	8.3
LR-5	0.0	0.0	0.1	0.0	4.7	4.8	6.0	6.8
LR-6	0.0	0.0	0.0	0.0	2.2	16.0	3.5	18.3
LR-7	0.0	0.0	0.1	0.0	4.7	4.8	6.4	7.1
LR-8	0.0	0.0	0.0	0.0	5.0	7.6	6.4	9.9
LR-10	0.0	0.0	0.0	0.0	3.3	4.3	4.6	6.6
LR-11	0.0	0.0	0.0	0.0	2.2	7.3	3.6	9.6
LR-14	0.0	0.0	0.0	0.0	9.9	8.6	11.2	10.9
LR-15	0.1	0.0	0.1	0.0	0.8	3.8	2.2	6.1
LR-21	0.0	0.0	0.0	0.0	3.5	7.8	4.9	10.1
LR-22	0.0	0.0	0.0	0.0	2.4	6.0	3.8	8.3
LR-23	0.0	0.0	0.0	0.0	1.6	2.2	3.0	4.6
LR-24-1	0.1	0.0	0.2	0.0	0.9	5.0	2.2	7.3
LR-24-2	0.0	0.0	0.0	0.0	6.2	6.3	8.2	8.7
LR-25	0.0	0.0	0.0	0.0	4.0	9.9	5.4	12.3
LR-26	0.0	0.0	0.0	0.0	3.5	11.6	4.9	13.9
LR-27	0.0	0.0	0.0	0.0	1.5	2.6	2.8	4.9
LR-32	0.0	0.0	0.0	0.0	2.0	3.1	3.4	5.5
LR-36	0.0	0.0	0.0	0.0	3.8	5.5	5.1	7.9
LR-38	0.0	0.0	0.0	0.0	3.2	4.1	4.5	6.5
LAC23	0.0	0.0	0.0	0.0	5.2	6.1	6.5	8.4
LTH	100.0	100.0	92.0	81.0	0.0	0.0	0.9	1.5
Mean	4.2	4.2	3.9	3.4	3.4	6.1	4.8	8.4
			Mortality (%)		Grain yield (g plant <sup>-1</sup> )			
			Greenhouse	Field	Greenhouse		Field	
LSD <sub>(p = 0.05)</sub>	Genotype		0.05	1.66	4.5		5.3	
	Water regime		0.02	0.48	1.5		2.2	
	Genotype x Water regime		0.08	2.35	6.4		8.1	
CV (%)			2.7	40.0	17.2		23.6	

## **3.5 Discussion**

### **3.5.1 Effects of pathotype on rice blast resistance**

Host specificity is a common characteristic of the rice blast pathogen (Couch *et al.*, 2005). Selection of virulent pathotypes would therefore be required for differentiating blast resistance of various host genotypes. SIK-111 was the most virulent of the three pathotypes as it showed a compatible interaction with at least twice as many genotypes as the least aggressive pathotype, GH-8. Its interaction pattern with the genotypes for both the number of sporulating lesions and infected leaves per plant was quite distinct from those of the other two pathotypes, which appeared to be similar (Figure 3.2). SIK-111 would therefore be useful for further evaluating horizontal resistance of the genotypes with which it showed a compatible interaction.

### **3.5.2 Genetic variation for qualitative and quantitative blast resistance**

The infection type produced by a blast pathotype is often used as a key criteria to establish the susceptibility or resistance of a rice plant against rice blast; while indices relating to disease severity are often used to determine quantitative resistance (Ahn *et al.*, 1998).

#### **3.5.2.1 Qualitative resistance in the rice population**

In these studies, expression of LT was used as an index to determine the presence or absence of qualitative (vertical) resistance in the genotypes. From the artificial inoculation trial conducted in the greenhouse, it was observed that differences between the genotypes that developed susceptible LTs against all three pathotypes, and the susceptible check variety were far larger than that between those cultivars and the resistant check (Figure 3.1). In that this group, however, differences in the main effects of LR- 2, 8, 11, 14 and 26 were negligibly small, indicating that the combined virulence effect of the pathotypes on these genotypes was essentially the same, and that the genotypes possessed similar levels of resistance. In disease assessment trials, entries that showed consistent LT ratings ranging from 4 to 6, with mean scores of not more than 5.5 were regarded as having good levels of horizontal resistance (IRRI, 2006). In these studies, 3 genotypes (LR- 2, 14 and 26) exhibited similar LT profiles, suggesting the possible existence of several genotypes in the parent population with useful levels of horizontal resistance.

Adaptation of the genotypes to different rice blast pathotypes could be a major characteristic of Liberia's upland rice genetic resources, as observed from the significant genotype by pathotype interaction for LT. Traditionally, subsistence farmers have always maintained several varieties, growing them more than one at a time per cropping cycle or alternating them from time to time, as a mean of minimizing the risks of crop loss to unfavourable

environmental factors (Smith and Lenhart, 1996), including disease incidence. The presence of different vertical resistant genes in different varieties is a key property of the multi-line approach to increasing the durability of resistance in a farming system (Dabholkar, 2006; Skamnioti and Gurr, 2009). But such an approach may not limit loss during severe blast outbreaks as much as a good level of horizontal resistance.

### **3.5.2.2 Quantitative resistance in the rice population**

Measurements on the numbers of sporulating lesions and infected leaves were used to assess quantitative (horizontal) resistance of the genotypes under disease pressure initiated by both spray and simulated natural inoculation. Genotypes capable of minimizing both the number and size of sporulating lesions are better suited to tolerate a blast epidemic and to slow the spread of the disease once it starts (Villareal *et al.*, 1981; Yeh and Bonman, 1986). In the first glasshouse experiment, LR 9, 13, 19, 35, and 38 developed lower numbers of sporulating lesions and had fewer leaves with sporulating lesions. Sporulating lesions produced on those genotypes were rather small and could be controlled by a single R gene with a large effect (Roumen, 1993). These genotypes, like those on which the pathotypes were unable to develop sporulating lesions (LR 1, 12, 16, 33, 34, and 37), may not be suitable for breeding for horizontal resistance using any of the pathotypes used in these studies because of the race-specific resistance exhibited against the pathotypes. Intermediate numbers of sporulating lesions or lesions of intermediate sizes observed in all other genotypes (except LR 21, 23, and 24-1) may likely be attributed to horizontal resistance genes. Bonman *et al.* (1991) and Parlevliet (1992) have similarly classified reduced lesion size and numbers as rate-reducing or partial resistance.

Leaves with sporulating lesions, besides having the potential to further spread the disease also suffer impaired capacity to photosynthesize and translocate carbohydrates needed by the plant (Bastiaans, 1991; Bastiaans and Roumen, 1993). Susceptible plants with fewer lesion-affected leaves could greatly mitigate the physiological effect of rice blast as long as the lesion-free leaves continue to function normally with little interference from any lesion. Hence, the number of sporulating lesions could be a useful index for quantifying horizontal resistance. The additive effects of fewer and smaller lesions can drastically diminish sporulation potential (Yeh and Bonman, 1986), and therefore the magnitude of secondary infections.

From the spray inoculation experiment, it was further determined that the genotypes were more diverse than the pathotypes for both the number of sporulating lesions and number of infected leaves as portrayed by the large proportion of (48% / 51%) sum of squares



calculated in the analysis of variance (Table 3.4). The SS of the pathotypes was markedly small (5.7% / 5.3%), revealing that most of the variation in pathogenicity of the pathotypes as well as the GXI interaction were due to the large differences between the genotypes. This further suggests that considerable variation exists for horizontal resistance against rice blast in Liberia's upland rice population. The ANOVA table further shows that the first two IPCAs were enough for highlighting the GXI interaction for these traits as they could predict the interaction of the 40 genotypes and 3 pathotypes used in the study.

A significant interaction was observed between genotype and pathotype for the number of leaves in plants bearing at least one sporulating lesion. Genotypes LR- 2, 6, 8, 11, 14, 24-2, 25 and 26 exhibited low to moderate main effects and interactions with all three of the pathotypes, and tended to differ progressively along the interaction and main effect gradients for the number of sporulating lesions per plant (Figure 3.1). It would therefore be useful to use these genotypes to breed for improvement of horizontal resistance, using SIK-111 as the designated pathotype, since the genotypes produced susceptible infection types against that pathotype. Even though fewer sporulating lesions is desirable, other genotypes (LR- 1, 12, 16, 19, 31, 33, 34, 35 and 37) that showed higher levels of quantitative resistance to all the three pathotypes, may not be useful for breeding for horizontal resistance using SIK-111 as the designated pathotype, because the genotypes exhibited incompatible interactions with it. Nonetheless, when a pathotype that is capable of producing susceptible lesions on those genotypes is found, it could then become useful for hybridization using the one pathotype technique to eliminate the effects of vertical resistance carried in these genotypes (Robinson, 2006). As the means of both checks were rather distant and distinct from those of the above (Figure 3.1), these varieties could also be useful for a genetic study into the pattern of inheritance of horizontal resistance against the disease.

It was observed that the AMMI biplots displayed a high similarity between the number of infected leaves and the number of sporulating lesions per plant. The close association of these traits ( $R^2 = 0.87$ ,  $t_{pr} < 0.001$ ) was also confirmed by correlation and regression analysis. It may therefore be possible that selecting for the number of leaves with sporulating lesions could largely simplify screening in large populations when the number of sporulating lesions per plant are regarded as a measure of horizontal resistance.

The number of sporulating lesions, lesion number and AUDPC corresponded well with the levels of grain yield obtained in the greenhouse study. This observation corresponds with assertions that plants with horizontal resistance tend to show reduced disease severity and therefore produce higher yields under disease pressure than others that are susceptible or possess lower levels of resistance (Crill *et al.*, 1982).

### **3.5.3 Effects of environment on expression of quantitative traits**

As observed in the spray inoculation trial, significant genotypic differences for lesion size was recorded when the blast epidemic was initiated by spreader plants in the greenhouse or in the field, indicating that either type of the inoculation protocol may be adapted for differentiating rice plants for blast resistance. The mean lesion size of the genotypes was larger under soil moisture conditions defined by intermittent drought stress, indicating that this water regime is more useful for determining the minimum limits of rice blast lesions and screening for blast resistance. This conclusion was supported by earlier studies, which showed that during periods of rapid leaf growth, drought stress tends to increase the size of rice blast lesions (Bonman *et al.*, 1988; Gill and Bonman, 1988).

The pattern of infection was quite distinct between drought stressed and well-watered plants. During the initial stages of the epidemics, the older leaves of plants subjected to pre-infection drought stress were more infected and developed more lesions than younger leaves. The trend was the opposite for plants grown under well watered conditions. However, as the epidemic progressed, the young leaves of the drought stressed plants became even more severely infected than those grown under stress-free conditions. This appeared as a form of secondary susceptibility, in that there were two prominent infection events: initially older leaves were almost exclusively infected, and only later (about a week later), upper leaves became severely infected. In a related experiment, Gill and Bonman (1988) reported a longer period of infection when plants were subjected to drought stress prior to their exposure to the blast pathogen. Variation in patterns of leaf infection with the level of water deficit was not apparent.

The number of sporulating lesions developed on the 22 genotypes showing susceptibility to SIK-111 were more in the uniform blast nursery in the greenhouse than in the spray inoculated trial. In the field, the numbers were much lower. The means of the genotypes for the number of sporulating lesions per plant measured in the three different environments were significantly correlated ( $t_{pr} < 0.01$ ), suggesting very little or no GXE interaction was associated with the trait. Hence, conditions that tend to increase plant responses for this trait would be best for evaluating plants if good progress is to be attained when breeding for horizontal resistance using the number of sporulating lesions per plant as a selection criterion.

## **3.6 Conclusion**

From the three experiments conducted, it was found that different rice accessions from Liberia responded differently to three pathotypes of *P. oryzae* used to infect them. Most

genotypes that developed susceptible LTs expressed partial resistance to the disease, unlike the susceptible check. Blast resistance in upland rice from Liberia was characterized by both race and race non-specific interactions. Since LR- 2, 6, 8, 11, 14, 24-2, 25 and 26 expressed low to moderate counts of sporulating lesions, and measures of lesion size and disease severity, they should be further tested for gene action to determine their suitability as parents for breeding new varieties with high levels of horizontal resistance against blast. Field conditions were not ideal for studying blast resistance in the genotypes because the disease pressure under field conditions was lower than that experienced under controlled environment conditions. As the key conditions that influence blast epidemics can be more effectively controlled in the greenhouse than in the field, the greenhouse should be considered for future assessment of rice plants for blast resistance if reliable estimates are to be obtained. Furthermore, since disease severity was more pronounced under conditions of intermittent drought stress, it would also be better to screen rice under such conditions in order to increase the likelihood of identifying truly resistant genotypes. Finally, rice blast pathotypes, such as SIK-111, capable of inducing susceptible lesion types on the genotypes recommended above, should be the designated pathotype for future studies of horizontal resistance.

### 3.7 References

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## CHAPTER 4

### DROUGHT TOLERANCE OF SELECTED UPLAND RICE GENOTYPES FROM LIBERIA

#### 4.1 Abstract

Studies on the drought tolerance (DT) status of varieties of rice (*Oryza sativa* L.) from Liberia are limited, although a significant portion of the national crop is produced under drought-prone, upland conditions. Both controlled environment and field experiments were conducted to evaluate 24 rice genotypes for their reaction to drought stress (DS) imposed during the establishment, tillering and booting-to-grain-filling phenological stages of development. All genotypes emerged earlier in soils with a water content of 75% or 50% of field capacity than in continuously well-watered soils, and remained viable until the relief of DS at 28 days after sowing. Unlike plants subjected to DS around the time of booting-to-grain-filling, the grain yield (GY) and its components of plants subjected to DS during the tillering growth stage did not vary significantly from the control plants grown in well-watered plots. The genotypes LR 24-2, followed by LR 32, 8, and 11 produced the highest GY in the plots subjected to DS during the booting-to-grain-filling growth stage, whilst LR 8, followed by LR 2 and LAC 23 expressed the highest yield potential under well-watered conditions. Principal component analysis revealed that the 24 genotypes exhibited the greatest variation for GY, tiller productivity (TP) and chlorophyll content index (CCI) under well-watered conditions, and for leaf rolling, GY, CCI, spikelet fertility (SF), and TP, under DS applied during the booting-to-grain-filling stage. Simple correlation and path analyses revealed that high levels of biomass at heading (BM), stomatal conductance and SF were the traits most strongly associated with high GY under well-watered conditions, whereas TP, BM and 1000 grain mass were the traits that most influenced GY in the genotypes subjected to DS at the stage of booting-to-grain-filling. These results showed that the stage of booting-to-grain-filling of crop development should be targeted for improving DT in the current population of rice germplasm, using combinations of LR 24-2, 32, 11, 8, and 2, and LAC 23 as the primary progenitors. The results also showed that yield potential and DT can be improved by directly selecting for GY under all field conditions, and indirectly for high levels of BM, SC and SF under well-watered conditions, and TP, BM and 1000 grain mass under DS applied during the booting-to-grain-filling stage.

Key words: booting-to-grain-filling, drought stress, rice, tillering, traits, well-watered conditions

## 4.2 Introduction

Drought is a major constraint to the production of rice (*Oryza sativa* L.), particularly upland rice, which is the predominant form of rice production in Liberia and other parts of sub-Saharan Africa (Balasubramanian *et al.*, 2007). In these agro-ecologies, drought occurs intermittently at various times during the cropping cycle, leading to water deficits, as a result of poor or irregular rainfall, which is the only source of water for the crop (De Datta, 1981). A low frequency or intensity of rainfall, coupled with poor moisture holding capacity of the soil, can lead to water deficits, which if they occur even for short periods, can reduce crop yields. Rainfall in Liberia is distinctly seasonal, being most abundant from April to September, and is sparsely distributed during the rest of the year (McSweeney *et al.*, 2010a; 2010b). The prolonged spells of water deficits, annually occurring between October and March, makes drought the single most important factor stopping year-round production of rice in Liberia, and most of West Africa.

Drought influences an array of physiological processes, ranging from photosynthesis, to growth, yield and quality of crops. The effect of drought stress on rice tends to increase with the intensity of water deficit, which is a combination of the duration and level of drought stress. Intermittent drought is recognized as a major cause of poor yields under upland rice culture (De Datta and Vergara, 1975; Pandey *et al.*, 2007; Pandey and Bhandari, 2009).

In addition to intensity, the timing of drought may also determine its level of impact on a rice crop. During the establishment phase of rice production, drought stress may limit germination and emergence, retard seedling growth, and result in poor crop stands (Pandey and Bhandari, 2009). The potential number of panicles and biomass accumulation may be reduced when drought occurs during the tillering stage of development (Bouman *et al.*, 2007). Drought occurring during the reproductive and ripening stages of development can lead to direct reductions in grain yields (Inthapan and Fukai, 1988; Saini and Westgate, 1999). Acute drought stress at any time during the cropping cycle can lead to plant mortality and complete crop failure.

Yield potential, which is the maximum yield obtainable in stress-free environments, may be incompatible with superior drought tolerance (Laing and Fischer, 1977; Fischer and Maurer, 1978; Blum, 2005) because most cultivars that produce high yields in well-watered environments perform poorly under drought stress, relative to other cultivars with lower yield potentials. The traditional breeding approach, which emphasizes yield as a selection criterion for drought tolerance (Richards, 1982), clearly suggests that the yield realized in a drought-affected environment is a function of drought tolerance (Atlin *et al.*, 2008). Therefore, it is likely that the genetic mechanisms controlling yield potential and drought

tolerance, determined as yield realized in a stress-afflicted environment, may be dissimilar in one or more respects. Genotypes that combine both traits will be desirable for increasing rice production under high risk, rainfed conditions.

Constitutive traits that enable genotypes to maintain a high internal water status during extended periods of water deficit could be important for indirectly selecting cultivars with improved tolerance to drought. However, research has demonstrated that the value of a secondary trait usually depends on the level of its association with grain yield (Lafitte *et al.*, 2003), implying the need for direct or indirect selection of these traits.

In the current study, the growth and yield performances of 24 rice genotypes from Liberia were investigated under both well-watered and water-limited conditions, in order to identify potential parents in order to breed high yielding, drought tolerant cultivars for upland rice ecologies, and to identify yield responsive traits that could be useful for indirectly selecting genotypes with superior yield potential and drought tolerance. The responses of the genotypes to drought occurring at different stages of plant development were also assessed in order to determine the optimum screening patterns for drought tolerance breeding in upland rice for Liberia.

### **4.3 Materials and methods**

Both controlled environment (CE) and field trials were conducted at the research facilities of the University of KwaZulu-Natal (UKZN) in Pietermaritzburg, South Africa to determine the responses of selected upland rice from Liberia to drought imposed for 21 days during the establishment, tillering and booting-to-grain-filling stages of development. The plant establishment stage consisted of the period commencing from sowing up to 28 days after sowing (DAS). The tillering stage was considered to be the period from the emergence of the first tillers to the commencement of stem elongation. The period from 10 days after emergence of the flag leaf to the onset of grain ripening was considered to be the booting-to-grain-filling stage, as described by De Datta (1981) and Yoshida (1981).

#### **4.3.1 Plant materials**

Twenty-four rice genotypes from Liberia were evaluated. The genotypes included 22 traditional upland varieties, which had shown compatible reactions to a virulent pathotype of *Pyricularia oryzae*, SIK-111 (Chapter 3), and two improved cultivars, LAC 23 and FKR 19. LAC 23 and FKR 19 were used as resistant and susceptible checks, respectively. LAC 23, a selection from a traditional upland variety in Liberia, is the oldest and best performing improved cultivar grown in the country, and is known to show high levels of drought tolerance under severe drought stress in the vegetative stage, and under moderate drought



stress in the reproductive stage (De Datta and Seshu, 1982). FKR-19 is an improved rice variety from Burkina Faso, developed mainly for wetland conditions, with little drought tolerance.

#### **4.3.2 Controlled environment study: Experimental design and treatments**

The CE experiment was conducted in a glasshouse (day / night temperature, 30 / 20°C; 65% RH) situated on the Life Sciences Campus of UKZN. The experiment was laid out in a completely randomized design with four replications. The treatments included three water regimes: 100% field capacity (FC), 75% FC, and 50% FC, representing well-watered, moderate drought stress and acute drought stress, respectively. Twenty-five seeds of each genotype per replication were sown 3.0 cm apart, 1.0 cm deep in 4.8 L plastic pots (27.0 cm high, with an upper diameter of 30.0 cm), utilizing a local topsoil as the growing medium. Each pot represented a single replication of each genotype per water regime. At sowing, all pots were saturated with water until free drainage occurred. Thereafter, the water-limiting treatments were imposed and maintained by regularly weighing the pots, and only replacing lost water up to the desired FC level, when necessary. The 100% FC (well watered) pots were watered once or twice daily until drainage occurred.

#### **4.3.3 Field experiments: Experimental design and treatments**

The field experiments were conducted under two contrasting environmental conditions (bare soil, or soil covered with a plastic mulch to exclude rainfall), over two seasons at UKZN's Ukulinga Research Farm (29° 40' S, 30° 24' E; 806 m above sea level), from early November to end of April in 2012/2013 and 2013/2014. Based on annual averages of long term climatic data, Ukulinga has a mean annual temperature and rainfall of 18°C and 738 mm, respectively. Weather data for the period of the field trials are presented in Table 4.1. During the first year, plants were evaluated separately for reaction to drought imposed at both the tillering and the booting-to-grain-filling stages of development, and only for drought stress imposed at the booting-to-grain-filling stage during the second year.

The experimental design for each environment consisted of a split plot arrangement, with water regime as the main plot, and genotype as the sub-plot. Each treatment combination was randomly assigned in three blocks. Genotypes were established by transplanting two-week old seedlings of each genotype at one seedling per hill in single row plots measuring 15.0 m for the first year, and 30.0 m for the second year. Intra- and inter-row spacing was 15.0 X 50.0 cm.

Table 4.2: Monthly minimum and maximum temperatures, solar radiation, and evapotranspiration at Ukulinga research site during the experiments. Data source: Agricultural Research Council (ARC), SA.

Year	Month	Temperature		Solar radiation (MJ m <sup>-2</sup> )	Evapotranspiration (mm)
		Minimum (°C)	Maximum (°C)		
2012/ 2013	November	9.62	32.90	15.92	91.17
	December	14.12	31.50	20.71	124.90
	January	14.75	38.40	18.28	113.60
	February	13.90	33.80	18.84	105.00
	March	12.96	32.00	15.43	93.98
	April	9.28	35.10	14.12	86.96
	Mean	12.44	33.95	17.22	615.61*
2013/ 2014	November	14.88	25.00	19.00	112.30
	December	15.93	23.40	16.78	100.50
	January	18.10	28.00	20.46	131.10
	February	18.20	28.20	20.83	116.10
	March	17.13	26.20	16.30	101.70
	April	13.62	24.40	15.48	87.49
	Mean	16.31	25.87	18.14	649.19*

\* Values for Reference evapotranspiration are totals, not means.

The well-watered treatment was maintained by irrigating regularly, ensuring that the soil water tension at 30 cm depth of the profile remained at levels below -15 kPa, throughout each experiment. Drought stress was imposed by withholding irrigation supply to the intended plots for 21 days beginning at 4 weeks after transplanting, or at booting. MPS-2 dielectric water potential sensors (Decagon Devices, USA) were used to monitor moisture tension in the soil. Drip irrigation, supplied by perforated plastic pipes ([www.netafim.co.za](http://www.netafim.co.za)) was used to deliver water to the plots, both covered and uncovered. The irrigation tubes for the plastic-covered plots were buried beneath the plastic mulch, such that water flow was directed upward at the plastic mulch, in order to prevent erosion due to water pressure. Delivery and control of irrigation water to the plots were managed with the aid of solenoids and an automatic irrigation scheduling unit (Hunter Industries, USA).

The trials were laid out on a well-drained, slightly sloping land. The upper levels of the slope were allocated to the drought stress treatment plots, and the lower level to the control treatment, in order to reduce seepage (interplot interference), and to improve drainage of the stress treatment plots.

Since the genotypes differed in days to heading, plants were planted at different time intervals in order to achieve synchronization of booting for all the plants in the reproductive and ripening stage drought stress treatment plots.

#### **4.3.4 Measurements and data analyses**

Data collected from the CE experiment included emergence and seedling plant height. Seedlings were counted daily for up to 14 days to determine the number of days to 50% emergence ( $D_{50E}$ ) and final emergence (FE). Seedling heights (PH) were measured at 28 days after sowing (DAS) by determining the length of each main culm, from the surface of the soil to the tip of the longest leaf.

During the field experiments the following data were collected: leaf rolling (Lr), leaf death (Ld), days to heading (DDH), flowering delay (FD), spikelet fertility (SF), leaf chlorophyll content index (CCI), and stomatal conductance (SC), in addition to above ground biomass at heading (BM), tiller productivity (TP), grain yield (GY), and 1000 grain mass (GM). Leaf rolling, CCI and Sd were measured 14 days after withholding irrigation (DAWI), and Ld at 21 DAWI by visually integrating the symptoms exhibited by five plants per plot, according to the standard evaluation system for rice (IRRI, 2006).

Leaf rolling was scored during the morning hours using the scale 0, 1, 3, 5, 7 or 9, indicating leaves were healthy, leaves just started to fold (shallow), leaves folded and showed deep V-shape, leaves fully cupped (U-shape), leaves folded with margins touching (o-shape), or leaves tightly rolled, respectively. Based on the total leaf area lost to desiccation, leaf death was scored on a scale of zero (no senescence) to 5 (leaves completely dried). Drought recovery, after relieving tillering stage drought stress, was assessed 10 days after reinstatement of irrigation. Leaf chlorophyll content index and SC were measured around 10 am to 12 noon at each occasion, using a portable leaf chlorophyll meter (SPAD-502 Plus, Konica Minolta) and a leaf porometer (SC-1, Decagon Devices), respectively. The top most fully developed leaf on each of five plants, chosen randomly per plot, was selected for the measurements.

The days to flowering was recorded as the number of days from transplanting to the time when 50% of the plants per plot headed. Flowering delay for each genotype was measured as the difference between the days to flowering in the stress-applied plots and the days to flowering in the well-watered (control) plots. Above ground biomass was determined at heading, by averaging the dry masses of five sampled and tagged plants per plot, after oven drying at 70°C for 48 hr. Spikelet fertility, TP and 1000 GM per plant were determined by harvesting the five plants per plot at maturity. Spikelet fertility was measured according to the method proposed by Lafitte *et al.* (2003). A sample of 6 to 12 panicles were taken from the harvested plants, threshed by hand, and the filled and empty spikelets separated and weighed. Thereafter, the mass of 200 spikelets from each of the two subsamples were obtained and used to calculate spikelet fertility as follows:

Spikelet fertility (%) =  $100 \times (\text{number of filled grains in the sample}) / (\text{number of filled grains} + \text{number of unfilled spikelets})$ , where the number of filled grains or empty spikelets was the quotient of the total mass of filled grains or empty spikelets and the average mass of 200 filled grains or empty spikelets. Tiller productivity was determined as the percentage of tillers that developed grain-bearing panicles.

All data were summarized by analysis of variance, using the statistical software GenStat® Version 14 (VSN, International) to determine the mean performances of the genotypes for each trait. Fisher's LSD test was applied to separate the means of the levels of treatments showing significant differences for  $D_{50E}$ , FE and PH. Since several different traits were measured on different scales during the field trials, principal components analyses, based on correlations, were applied to determine the linear combinations of the traits that contained most of the variation between the genotypes. Simple correlation and path analysis were calculated to determine the associations between the measured traits, as well as their usefulness for predicting grain yield under both well-watered conditions and drought stress imposed during the booting-to-grain-filling stage.

## **4.4 Results**

### ***4.4.1 Effects of water regime on seedling emergence and height***

Data on final emergence, the days to 50% emergence and seedling height at 28 DAS are presented in Table 4.2. The genotypes varied significantly ( $p < 0.01$ ) for all three traits under the three water regimes. The mean number of days to 50% emergence was lower for seeds sown under soil water conditions of 75% and 50% FC than for those sown under well-watered conditions. Except for FKR 19, the genotypes emerged relatively earlier in soils with lower water status than those kept continuously well-watered. The levels of final emergence were statistically similar between the three water regimes.

At 28 DAS, seedling height followed an increasing trend, with increasing levels of water in the soil, except for LR 26, 5, 38, 15 and LAC 23 whose heights were statistically similar under the two water limited conditions. LR 26 exhibited the lowest mean decline in seedling height due to low soil water status, whilst LR 24-1 exhibited the highest decline in seedling height.

#### **4.4.2 Influence of water regime on plant performance under field conditions**

The mean effects of water regime on the expression of various traits during the 2012 / 2013 cropping season are presented in Table 4.3. Drought stress imposed during the booting-to-grain-filling stage of the plants' development significantly reduced the performances of the plants for all the traits, and increased DDH by a mean of 13 days. Except for CCI, Lr, Ld, SC, and TP during the periods of drought stress, differences in the expression of traits between the well-watered plots and those subjected to drought stress during the tillering stage of development were not significant ( $p = 0.05$ ). Drought imposed during the tillering stage led to a significant increase in tiller productivity ( $p < 0.01$ ). The chlorophyll content index, Lr, Ld and SC of the genotypes measured during tillering stage drought stress did not differ significantly from those obtained when drought was imposed during the booting-to-grain-filling stage. Of all the genotypes, FKR-19 suffered the greatest injury, as exhibited by its yield performance under tillering stage drought stress (Figure 4.1).

As reported in Chapter 5, differences between plants grown on bare soil and those grown on soil covered with plastic mulch were not significant for all the traits, except CCI, Lr, SD and Ld, in the plots subjected to drought during both the tillering and reproductive stages of development. Significant differences were also found between the two environments for TP and GY of the plants subjected to drought during the booting-to-grain-filling stage of development.

Table 4.2: Plant height and emergence characteristics of rice genotypes grown under three levels of soil water content

Genotype	Plant height at 28 DAS (cm)			Days to 50% emergence			Final emergence (%)		
	100%	75%	50%	100%	75%	50%	100%	75%	50%
LR 2	43.3	25.3	21.5	5.0	4.0	5.0	100.0	100.0	100.0
LR 3	44.3	31.3	20.7	5.0	5.0	5.0	96.0	100.0	76.0
LR 4	50.2	28.5	19.3	5.0	4.0	5.0	100.0	100.0	100.0
LR 5	45.2	25.0	24.3	5.0	4.0	5.0	96.0	100.0	100.0
LR 6	43.8	26.0	21.2	6.0	4.0	5.0	100.0	100.0	96.0
LR 7	41.4	23.9	18.4	6.0	4.0	6.0	92.0	96.0	100.0
LR 8	46.8	31.7	23.3	7.0	4.0	5.0	100.0	100.0	100.0
LR 10	46.9	28.2	23.8	6.0	5.0	5.0	96.0	100.0	100.0
LR 11	51.7	29.3	23.3	5.0	4.0	4.0	100.0	100.0	96.0
LR 14	45.7	31.8	23.7	5.0	4.0	6.0	80.0	80.0	100.0
LR 15	42.0	23.4	17.7	5.0	4.0	4.0	100.0	100.0	100.0
LR 21	44.5	29.7	23.0	5.0	5.0	5.0	96.0	100.0	100.0
LR 22	43.0	28.3	21.4	6.0	4.0	4.0	100.0	100.0	100.0
LR 23	47.8	31.5	25.1	5.0	5.0	5.0	96.0	100.0	100.0
LR 24-1	49.3	29.6	23.5	5.0	4.0	5.0	96.0	100.0	100.0
LR 24-2	45.8	30.7	25.5	5.0	5.0	5.0	100.0	100.0	100.0
LR 25	46.3	28.3	23.5	5.0	4.0	5.0	100.0	100.0	100.0
LR 26	41.1	27.2	23.9	5.0	5.0	5.0	100.0	100.0	96.0
LR 27	45.9	29.8	19.9	5.0	5.0	5.0	100.0	100.0	100.0
LR-32	47.3	27.4	21.8	5.0	4.0	5.0	100.0	100.0	96.0
LR 36	44.4	26.0	21.8	5.0	5.0	5.0	92.0	100.0	100.0
LR 38	43.2	24.2	20.7	5.0	4.0	5.0	96.0	100.0	100.0
LAC 23	49.2	32.9	27.6	5.0	4.0	4.0	100.0	100.0	100.0
FKR 19	39.4	22.3	21.8	5.0	6.0	6.0	100.0	76.0	76.0
Mean	45.4	28.0	22.4	5.3	4.4	5.0	97.3	98.0	97.3
LSD( $p = 0.05$ )	2.1 <sup>a</sup>	0.8 <sup>b</sup>	3.6 <sup>c</sup>	0.3 <sup>a</sup>	0.1 <sup>b</sup>	0.5 <sup>c</sup>	1.2 <sup>a</sup>	0.5 <sup>b</sup>	2.0 <sup>c</sup>
CV (%)			7.3			5.7			4.3

<sup>a</sup> LSD( $p = 0.05$ ) for genotype; <sup>b</sup> LSD( $p = 0.05$ ) for water regime; <sup>c</sup> LSD( $p = 0.05$ ) for genotype X water regime

Table 4.3: Effect of water regimes on the expression of various traits

Trait	Water regime			LSD <sub>(p = 0.05)</sub>
	Well watered	Drought at tillering stage	Drought at booting-to-grain-filling stage	
1000 grain mass (g)	29.19	28.46	15.74	6.55
Biomass (g plant <sup>-1</sup> )	77.93	68.25	59.34	12.26
Chlorophyll content index	27.33	11.72	12.08	4.00
Days to heading	120.08	120.45	132.91	6.02
Flowering delay (day)	-	0.00	12.83	-
Grain yield (g plant <sup>-1</sup> )	31.65	30.48	11.44	7.82
Leaf death	-	3.64	3.15	0.91
Leaf rolling	-	6.77	6.79	0.32
Spikelet fertility (%)	84.56	81.95	22.53	11.33
Stomatal conductance	393.64	30.20	29.67	25.60
Tiller productivity (%)	80.99	84.81	5.32	2.04

Data are means for two environments (bare soil and soil covered with plastic mulch) during the 2012 / 2013 cropping season.

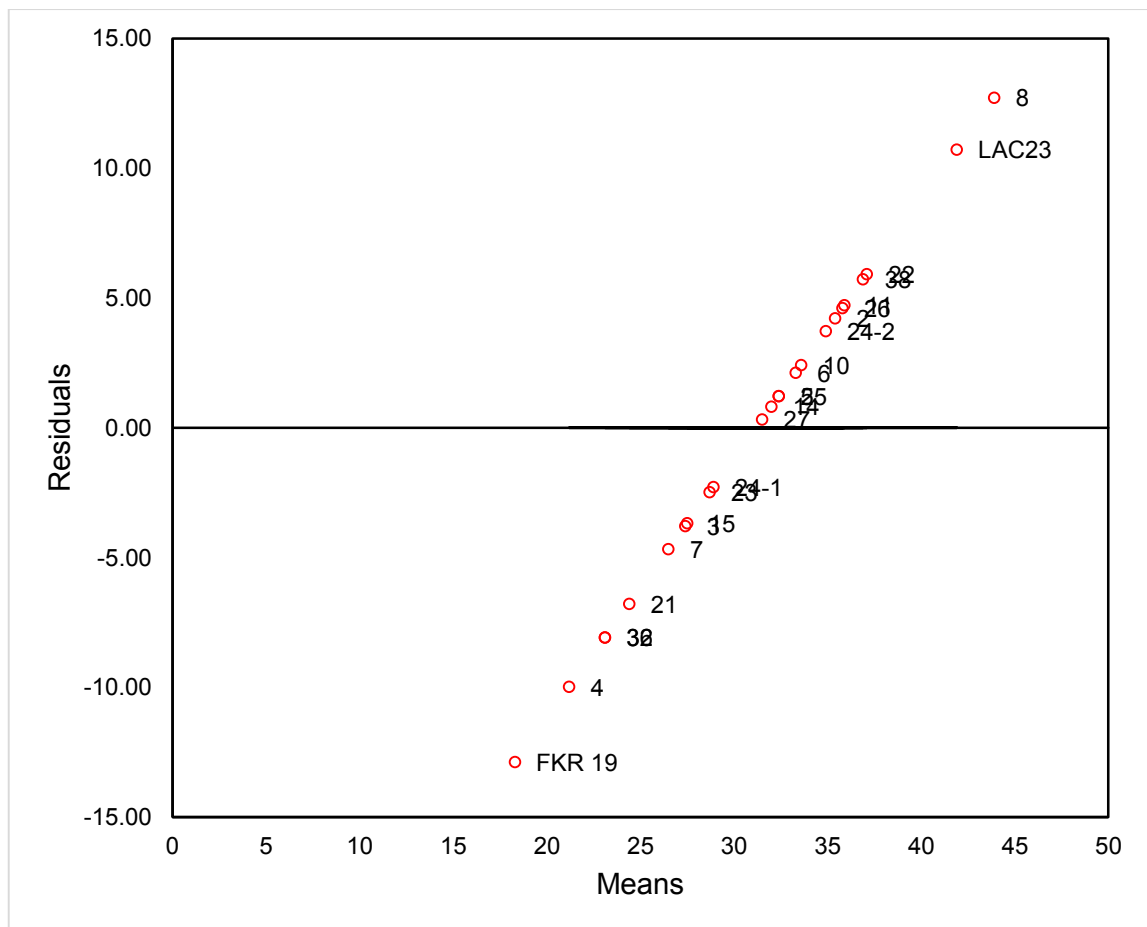


Figure 4.1: Pattern of yield responses of 24 rice genotypes subjected to drought stress during the tillering stage of development

### 4.4.3 Performance of genotypes under well-watered conditions

Mean squares of various sources of variation for traits expressed by the plants grown under well-watered conditions are presented in Table 4.4. Significant levels of variation were recorded amongst the genotypes for all the traits measured, except CCI. Differences between the growing environments (bare soil and soil covered with plastic mulch) was significant only for BM and CCI (Table 4.4).

Table 4.4: Mean squares and significant tests of eight traits expressed by 24 rice genotypes grown under well-watered conditions

Component	Sources of Variation							
	Genotype	Soil cover	Year	Genotype x Soil cover	Genotype x Year	Soil cover x Year	Genotype x Soil cover x Year	Error
DF	23	1	1	23	23	1	23	190
1000 GM	47.10***	0.11	0	0.01	0.01	0.02***	0.01	0.04
BM	2248.00***	10.81*	10.35	68.28	3.12	0.61	3.39	269.00
CCI	174.71	102.82**	0.61	10.19	14.85	6.30	14.33	22.00
DDH	1356.53**	28.13	80.22	15.76	11.95	19.01	13.70	156.03
GY	537.49***	91.46	3.32	37.41	0.48	0.00	0.76	17.00
SC	14100.90**	8562.70	1357.20	2294.90	2781.50	3523.80	2693.10	3225.00
SF	132.67**	21.62	30.23	17.91	3.86	0.26	4.38	7.00
TP	659.42	0.50	21.78	26.45	2.76	0.00	4.52	14.20

DF, GM, BM, CCI, DDH, GY, SC, SF and TP represent degrees of freedom, 1000 grain mass, above ground biomass at heading, chlorophyll content index, days to heading, grain yield, stomatal conductance spikelet fertility and tiller productivity, respectively. \*\* and \*\*\* represent level of significant value at  $p < 0.01$  and  $0.001$ , respectively.

The influence of year on the responses of the genotypes was not significant. Compared to the other traits, minimal variability between the genotypes were recorded for 1000 GM, SF and CCI.

Principal components analysis of the eight traits measured revealed that most of the variation (74%) between the genotypes under well-watered conditions occurred in the direction of GY, TP, and CCI (Table 4.5). The first principal component (PC1) accounted for 35.2% of the variation, and was primarily a measure of GY. It also showed that the higher yielding genotypes were those that accumulated more BM by the time of heading. The second PC (PC2) was mainly correlated with TP (0.55), and showed that the genotypes with higher levels of TP also tended to exhibit higher values for 1000 GM (0.51). The third PC accounted for 10.06% of the total variation, and was negatively related to CCI (-0.64). The genotypes that showed lower levels of CCI and SC also tended to have poor grain filling characteristics, leading to lower values for 1000 GM. The late maturing genotypes tended to exhibit increased greenness (CCI) and higher levels of SF, and these traits exhibited the highest level of correlation with the fourth PC.



Table 4.5: Latent vectors showing eight traits of 24 rice genotypes evaluated under well-watered conditions in two environments over two seasons

Trait	Principal component			
	PC1	PC2	PC3	PC4
1000 grain mass	-0.1097	<b>0.5175</b>	-0.4247	0.3221
Biomass	<b>0.5254</b>	-0.1169	0.1266	-0.0782
Chlorophyll content index	0.0247	-0.3276	<b>-0.6567</b>	0.3662
Days to heading	0.2096	<b>-0.4709</b>	0.2482	<b>0.6037</b>
Grain yield	<b>0.5468</b>	0.0654	0.0133	-0.2457
Stomatal conductance	0.3981	0.0916	<b>-0.4621</b>	-0.2972
Spikelet fertility	0.4447	0.2002	-0.0144	0.3493
Tiller productivity	0.1098	<b>0.5803</b>	0.3112	0.3477
Percentage of variation	35.2100	21.47300	16.9600	10.0600
Cumulative variation	35.2100	56.9400	73.9000	83.9600

Based on the percentage of the variances accounted for by the PCA biplot (57%) (Figure 4.2), it reflects the responses of the genotypes for each trait, as well as the association between each pair of traits. The genotypes were well dispersed in all sections of the PCA biplot. LR 8 appeared more similar to the resistant check and LR 2, whereas LR 6 and LR 21 were similar to FKR-19 for most traits. LR-8, followed by LR-2, produced the highest grain yield, with a marginal advantage over the elite LAC 23 genotype. The latest maturing genotypes were LR 23 and 26, whilst LR 7 and 38, which also exhibited the lowest CCI, were the earliest to mature. Traits with vectors pointing in the same direction were positively correlated, and those in opposite direction, were negatively correlated. Stomatal conductance, SF and BM were more closely associated with GY than were the rest of the traits.

Simple correlation analysis showed that BM at heading, SC and SF were highly correlated ( $P < 0.01$ ) with grain yield under well-watered conditions (Table 4.6). The direct and indirect paths leading to high grain yields for the current population under well-watered conditions, as determined by path analysis of the data on correlation between the various traits are also presented in Table 4.6. The days to heading emerged as the direct path leading to high grain yield, indirectly through BM and CCI.

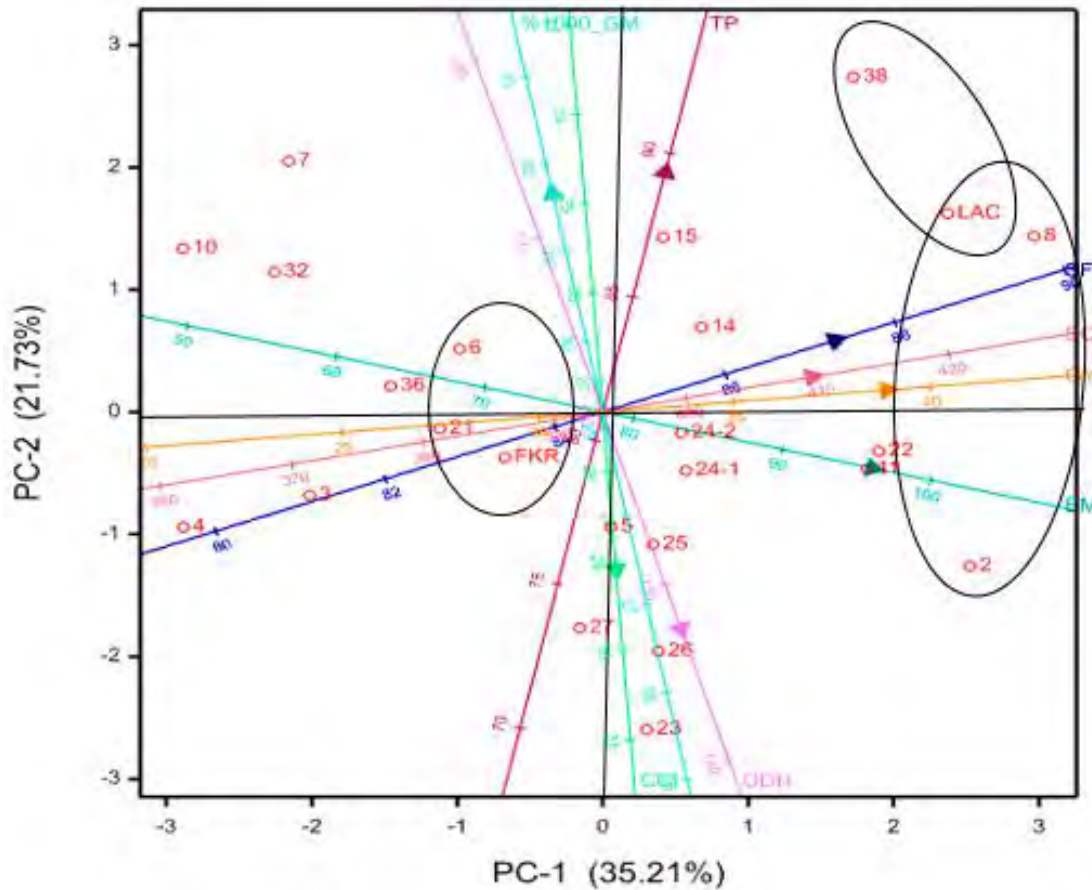


Figure 4.2: Association amongst 24 rice genotypes and 8 traits assessed under well-watered conditions

Table 4.6: Associations between grain yield and yield components of 24 rice genotypes grown under well-watered conditions. The bold-numerals arranged diagonally are direct path values; the remaining numerals are indirect contributors to grain yield. Numerals in column labelled GY are correlation coefficients.

Trait	Path coefficient							GY
	1000 GM	BM	CCI	DDH	SC	SF	TP	
1000 GM	<b>0.05</b>	0.01	0.00	-0.07	0.01	0.00	0.00	<b>-0.12</b>
BM	-0.02	<b>-0.04</b>	0.00	0.05	0.02	-0.02	0.00	<b>0.85**</b>
CCI	0.00	0.00	<b>-0.06</b>	0.04	0.01	0.00	0.00	<b>-0.07</b>
DDH	-0.02	-0.01	-0.01	<b>0.17</b>	-0.01	-0.01	0.00	<b>0.11</b>
SC	0.01	-0.02	-0.01	-0.02	<b>0.06</b>	-0.02	0.00	<b>0.62**</b>
SF	0.00	-0.02	0.00	0.03	0.03	<b>-0.03</b>	0.00	<b>0.57**</b>
TP	0.02	0.00	0.02	-0.02	-0.02	-0.01	<b>0.00</b>	<b>0.16</b>

DDH, CCI, BM, GM, SC, SF, TP, and GY represent days to heading, chlorophyll content index, straw dry mass, grain mass, stomatal conductance, spikelet fertility, tiller productivity and grain yield per plant, respectively. \*\* represents  $p < 0.01$ .

#### 4.4.4 Responses of rice genotypes to drought stress imposed during the booting-to-grain-filling stage of development

The levels of the variation for several traits amongst the plants subjected to drought stress during the booting-to-grain-filling stage of rice development are presented in Table 4.7. Variation for each of the traits was due mainly to genotypic effects, and less to the year or growing environment. Interactions between the different combinations of factors were not significant, except for 1000 GM, which showed a significant soil cover by year interaction.

Table 4.7: Mean squares of 10 traits expressed by 24 rice genotypes in response to drought stress occurring during the booting-to-grain-filling stage of development

	Sources of Variation							
	Genotype	Soil cover	Year	Genotype x Soil cover	Genotype x Year	Soil cover x Year	Genotype x Soil cover x Year	Error
DF	23	1	1	23	23	1	23	190
1000 GM	47.10***	0.11***	0.00	0.01	0.01	0.02***	0.01	0.04
BM	616.40***	288.60	9.90	108.90	203.40	8.10	141.60	207.90
CCI	12.67**	0.05	0.01	0.00	0.00	0.00	0.02	1.01
FD	177.04***	0.10	0.04	0.18	0.19	0.00	0.21	0.22
GY	75.36***	0.08	0.00	0.19	0.17	0.00	0.19	0.23
Ld	7.64**	0.14	0.00	0.19	0.19	0.00	0.23	0.27
Lr	17.81**	0.09	0.00	0.19	0.20	0.00	0.18	0.25
SC	24.73**	0.13	0.03	0.00	0.01	0.01	0.02	0.98
SF	2197.62***	0.11	0.00	0.19	0.18	0.00	0.24	0.24
TP	178.03***	0.12	0.00	0.19	0.21	0.00	0.20	0.21

DF, GM, BM, CCI, FD, GY, Ld, Lr, SC, SF and TP represent degrees of freedom, 1000 grain mass, above ground biomass at heading, chlorophyll content index, flowering delay, grain yield, leaf death, leaf rolling, stomatal conductance, spikelet fertility and tiller productivity, respectively.

Principal component analysis revealed that the variation between the genotypes subjected to drought stress during the booting-to-grain-filling stage of development can be largely explained by their patterns of Lr, GY, CCI, SF, and TP (Table 4.8). The fitted values of the genotypes for each trait are depicted in Figure 4.3, as the orthogonal projection of their respective points to the vector of each trait. LR 24-2, followed by LR 32, 8, and 11 produced the highest GY, whilst the susceptible check (FKR 19) and LR 23 produced the least. LR 10, 2 and 11 exhibited the lowest leaf rolling characteristics, whilst LR 36, followed by LR 8, 32 and 25 portrayed the highest values of CCI. Spikelet fertility, which showed a negative correlation with the third PC, was highest in LR 6, LR 8 and LAC 23. Flowering delay was shortest in LR 11, followed by the resistant check variety, and LR 10 and LR 6. Genotypes LR 38, 7 and 15 were the earliest maturing varieties, with similar FD profiles as LR 11, LAC 23, LR 10 and LR 6.

Table 4.8: Latent vectors of the directions in which 24 rice genotypes exhibited the greatest variations for a combination of 10 traits, when subjected to drought stress during booting-to-grain-filling in two environments, over two seasons

Trait	Principal component			
	1	2	3	4
1000 grain mass	<b>0.4577</b>	-0.3002	0.0256	-0.0612
Biomass	0.1623	0.3790	0.3043	0.3864
Chlorophyll content index	0.0335	0.3015	<b>0.6485</b>	-0.0756
Flowering delay	-0.2319	0.3817	-0.4632	-0.2484
Grain yield	0.2584	<b>0.5018</b>	-0.0837	0.3155
Leaf death	-0.3937	-0.1029	0.0095	0.4236
Leaf rolling	<b>-0.4950</b>	0.2741	0.0650	0.0558
Stomatal conductance	0.4074	0.0455	-0.0269	0.0857
Spikelet fertility	0.2498	0.2283	<b>0.5077</b>	0.2625
Tiller productivity	0.1193	0.3714	0.0427	<b>0.6489</b>
Percentage of variation	33.3500	17.6500	12.8600	11.1000
Cumulative variation	33.3500	51.0000	63.8600	74.9600

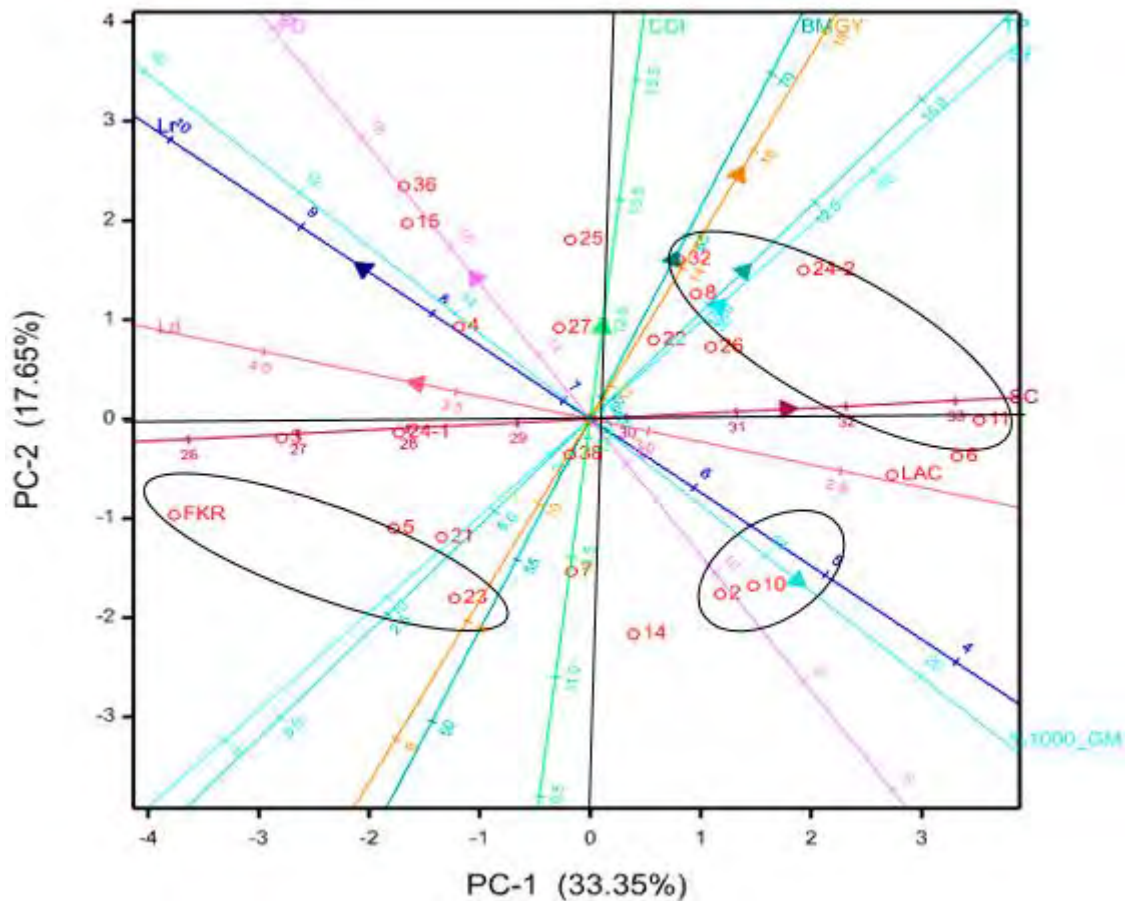


Figure 4.3: Association amongst 24 rice genotypes and 10 traits assessed under drought stress applied during the booting-to-grain-filling stage of development

#### 4.4.5 Association between grain yield and other traits in response to drought stress imposed during the booting-to-grain-filling stage of rice development

Table 4.9 depicts both the direct and indirect contributions of each trait to GY, as well as the levels of correlation between the traits and GY, as derived from the assessment of the rice plants subjected to drought stress during the booting-to-grain-filling stage of development. Tiller productivity and BM were the most important traits associated with high GY. Thousand grain mass directly influenced grain yield, via lower leaf rolling characteristics; whereas, high leaf rolling characteristics directly influenced grain yield negatively.

Table 4.9: Direct and indirect contributions of various traits to grain yield, as exhibited by 24 rice genotypes subjected to drought stress during the booting-to-grain-filling stage of development. The bold-numerals arranged diagonally are direct path values; the remaining numerals are indirect contributors to grain yield. Numerals in column labelled GY are correlation coefficients. Numerals in column labelled GY are correlation coefficients.

Trait	Path coefficient									GY
	1000 GM	BM	CCI	FD	Ld	Lr	SC	SF	TP	
1000 GM	<b>0.82</b>	-0.01	0.00	0.02	-0.02	-0.68	-0.01	0.02	0.00	<b>0.15</b>
BM	0.07	<b>-0.06</b>	0.01	0.01	-0.01	-0.05	0.00	0.03	0.00	<b>0.45**</b>
CCI	-0.09	-0.01	<b>0.03</b>	0.01	0.00	0.10	-0.01	-0.03	0.00	<b>0.14</b>
FD	-0.33	0.01	0.00	<b>-0.06</b>	0.00	0.36	0.01	0.02	0.00	<b>0.12</b>
Ld	-0.41	0.01	0.00	0.00	<b>0.03</b>	0.40	0.01	-0.04	0.00	<b>-0.22</b>
Lr	-0.72	0.00	0.00	-0.03	0.02	<b>0.77</b>	0.02	-0.07	0.00	<b>-0.13</b>
SC	0.34	0.00	0.00	0.02	-0.01	-0.39	<b>-0.03</b>	0.07	0.00	<b>0.41</b>
SF	0.09	-0.01	0.00	-0.01	-0.01	-0.26	-0.01	<b>0.20</b>	0.00	<b>0.36**</b>
TP	0.14	-0.01	0.00	0.01	0.00	-0.19	-0.01	0.06	<b>-0.01</b>	<b>0.62**</b>

DF, GM, BM, CCI, FD, GY, Ld, Lr, SC, SF and TP represent degrees of freedom, grain mass, above ground biomass at heading, chlorophyll content index, flowering delay, grain yield, leaf death, leaf rolling, stomatal conductance, spikelet fertility and tiller productivity, respectively. \*\* represents  $p < 0.01$ .

#### 4.5 Discussion

Identifying potential progenitors, specific breeding targets and effective selection criteria are essential requirements for developing resilient varieties for drought-prone environments. Results of many previous studies suggest that yield stability across the expected range of variability in moisture regimes of such environments may be achieved by cultivating varieties that yield relatively higher, both under stress-free and drought-stressed conditions (Ramezanil and Torabil, 2011; Raman *et al.*, 2012). In the current studies, the performances of a collection of rice genotypes from Liberia were assessed for various traits under well-watered or under drought stress conditions imposed during the establishment (seedling), tillering or booting-to-grain-filling stages of development.

#### **4.5.1 Growth-stage-specific vulnerabilities of genotypes to drought stress**

Drought stress due to the limitation of water during plant establishment mainly resulted in a significant reduction in seedling growth, as the genotypes grown in the pots with soil water contents of 50% or 75% FC were more stunted than those grown in the well-watered pots. Despite this negative impact of limited soil water content, no seedling mortality was observed. These results show that the traditional genotypes possessed good levels of tolerance to drought occurring at the establishment phase of development, a trend that is indicative of their specific adaptability to production under dry land conditions.

Drought stress imposed during the tillering stage of growth, by withholding irrigation for up to 21 days, did not significantly alter the mean yields of the genotypes from the pattern observed under well-watered conditions, although the yield of the susceptible check was significantly lower than the mean. The lack of differences in yields and most of its components was perhaps the result of a drought that was not severe enough as to cause plant mortality and to diminish crop stand. Earlier research has shown that yield reduction arising from drought stress occurring during the vegetative stage of rice growth were mainly attributed to poor plant recovery following relief of the stress (Herve and Serraj, 2009; Serraj *et al.*, 2009).

Despite the limited impact of drought stress on yield and its components, when imposed during the tillering stage of development, drought stress induced leaf rolling and leaf death, and led to significant decreases in CCI and SD. The lack of significant differences in key traits (including BM, GY, DDH, SF and 1000 GM) between genotypes grown under well-watered conditions and those subjected to drought stress during the tillering growth phase indicates that drought stress occurring around mid-season may not threaten the production of upland rice genotypes from Liberia, as long as it does not continue indefinitely or become intense enough to cause significant mortality. A close relationship between yield under moderate drought stress and yield under well-watered conditions was earlier reported by Seetharama *et al.* (1982), suggesting that mild drought stress is usually insufficient to induce reactions that can clearly discriminate between genotypes for drought tolerance.

The levels of leaf rolling, leaf death, CCI and SD recorded under vegetative phase drought stress was not statistically different to those observed when the genotypes were subjected to drought stress during the booting-to-grain-filling stage of development. Early screening for these traits could therefore be sufficient for charactering genotypes of the present population for these traits.

The grain yield and yield components of all the genotypes significantly diminished as a result of withholding water during the booting-to-grain-filling stage of development, showing

that this growth phase is the critical growing period or phenological stage to consider when breeding to improve drought tolerance of rice. Studies have shown that rice is particularly sensitive to drought stress occurring at the reproductive stage of development, even if the drought occurs for some limited period that coincides with the period of grain formation and grain filling (Ekanayake *et al.*, 1989; Saini and Westgate, 1999).

#### **4.5.2 Responses of genotypes to drought stress during the establishment, tillering and booting-to-grain-filling stages of development**

During the establishment phase of development, drought was imposed by limiting soil water content to levels not exceeding 50% or 75% FC, following the sowing of seeds. All the genotypes, except FKR 19, emerged more evenly and faster under those water-limiting conditions. Although the genotypes varied significantly for each of the three traits (final emergence, days to 50% emergence and PH at 28 DAS) measured, the differences for these traits were small and were not sufficient to exploit for crop improvement purposes.

As during plant establishment, drought imposed during the tillering stage of development did not seem sufficient to clearly differentiate the upland genotypes for drought tolerance. However, all of the upland genotypes appeared to possess superior levels of drought tolerance relative to the susceptible check (FKR 19).

When drought was imposed during the booting-to-grain-filling stage of development, significant differences were observed between the genotypes for all traits measured. LR 24, 32, 11 and 8 emerged as the least affected genotypes. Generally, at least two traditional upland genotypes appeared better than the resistant check (LAC 23) for every trait measured, whilst all appeared better than FKR-19 for every trait. These results indicate that several of the upland genotypes could be useful as parents when breeding to improve drought tolerance of rice from Liberia.

#### **4.5.3 Importance of traits for selection**

The traditional approach to breeding crops for drought tolerance has mostly focused on yield as the criterion for selection (Richards, 1982). However, in situations of severe drought stress, yield may be eliminated, rendering it impossible to apply selection upon the genotypes: they all score zero for yield. It may therefore become necessary to rely on secondary traits to determine drought tolerance of breeding lines. The value of each secondary trait will therefore depend on the level of its association with grain yield under drought stress in the target environment (Lafitte *et al.*, 2003). Principal components, simple correlation and path analyses were therefore applied to explore the association between the various traits in order to identify traits that could be used as secondary selection criteria

for determining both drought tolerance and yield potential within the germplasm under study.

Under well-watered conditions, the correlation between the various traits and the first two principal components revealed that high SF, SC and BM accumulation were important for achieving high grain yield, whilst the second cluster showed that the late maturing genotypes tended to display higher levels of CCI. Tremblay *et al.* (2011) showed that leaf chlorophyll content is directly associated with plant nitrogen status, which is essential for the healthy growth and yield of crops (Prasertsak and Fukai, 1997; Adhikari *et al.*, 1999). However, the higher CCI of the late maturing genotypes did not relate closely with high GY, as revealed by simple correlation and path analyses (Table 4.6). Hence, selection for shorter DDH can be important for enhancing the capacity of genotypes to escape terminal drought, thereby improving GY in drought-prone environments.

The third cluster of genotypes projected by the PC analysis revealed that the 1000 GM of the genotypes tended to increase with improved levels of TP. Hence, selecting for improved TP can be useful for grain quality assurance because ineffective tillers are likely to contribute immature grains, which may inadvertently lower head rice yield, as well as the eating and cooking qualities of grains (Counce *et al.*, 1996; Wang *et al.*, 2007).

Simple correlation analysis also revealed BM at heading and spikelet fertility as direct selection criteria for high grain yield under well-watered conditions. But two of these traits can only be assessed towards or at the end of the production cycle, rendering stomatal conductance as a key trait to use for the prediction of high grain yield early during the season.

Path analysis, which is a statistical procedure used to explore cause and effect relationships in a system of correlated variables (Lleras, 2005), identified DDH as the most important criterion for improving grain yield than other traits; however, this may be achieved by indirect selection for high biomass at heading, and for high spikelet fertility. Path coefficient analysis revealed that when breeding rice for tolerance to drought occurring at the booting-to-grain-filling stage of development, 1000 GM and Lr can be important for directly influencing high grain yield, using progenitors from the current population. Hence, by directly selecting for high 1000 GM and low leaf rolling scores, the breeder could inadvertently be selecting for high tiller productivity and low leaf death tendencies, respectively. In the event of intense drought stress, leading to very low grain yield, 1000 GM could be useful for further differentiating genotypes for levels of drought tolerance. However, leaf rolling may be a more useful index for determining drought tolerance of genotypes in the event of complete yield loss, or when screening is to be done early during the growing period.



## 4.6 Conclusion

The upland genotypes exhibited the ability to emerge faster, and in greater numbers under limited soil water conditions of 50% to 75% FC than under continuously wet conditions of 100% FC. These varieties also had the capacity to recover from drought stress imposed at the tillering phase of growth, although tillering was relatively lower in plants subjected to stress. All the plants were able to recover, progress to maturity, and yield almost equally as the well-watered controls. These results showed that the traditional upland rice varieties from Liberia were less vulnerable to drought stress for short periods during the establishment and tillering stages of development, as the ultimate impact of drought stress occurring at those times had relatively little effect on the final grain yield of the plants. However, drought occurring at the booting-to-grain-filling stage of development significantly diminished spikelet fertility, tiller productivity and grain yield of all the genotypes. Hence, the booting-to-grain-filling stage should be the most important growth stage to consider for improving upland rice from Liberia for tolerance to drought stress. The Liberian varieties LR 2, LR 8, LR 11, LR 24-2, and LR 32 should be considered as possible progenitors for improving drought tolerance and yield potential of upland rice from Liberia, since they were the best performing genotypes under both well-watered and drought conditions occurring around the booting-to-grain-filling stage of development. Tiller productivity, above ground biomass at heading and 1000 grain mass appeared to be the most important drought responsive traits because they directly influenced grain yield in the population, as shown by the high levels of correlation with grain yield under reproductive stage drought stress. These traits should be used as secondary criteria for further selection of varieties with improved tolerance to drought, as the breeding cycle progresses. In addition, since field screening for drought tolerance is usually unreliable in a high rainfall country like Liberia, a more precise phenotyping methodology for such environments need to be developed.

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## CHAPTER 5

### SCREENING RICE (*ORYZA SATIVA* L.) FOR TOLERANCE TO DROUGHT USING A PLASTIC MULCH SOIL COVER SYSTEM

#### 5.1 Abstract

Screening for drought tolerance during a normal cropping season in most target environments is problematic in the absence of a reliable water control mechanism such as a rainout shelter. The feasibility of using a plastic mulch soil cover (PMSC) facility to stimulate drought stress in rice (*Oryza sativa*) during periods of normal rainfall was investigated under field conditions and over two seasons. Twenty-four genotypes were grown either on bare soil, or on soil covered entirely with a 130  $\mu\text{m}$ -thick polyethylene sheet, and subjected to well-watered or drought stressed conditions during the tillering or the booting-to-grain filling stages of development. Soil water potential and temperature at depths of 30 and 60 cm in the soil profile, stomatal conductance (SC), chlorophyll content index (CCI), chlorophyll fluorescence (CF), leaf rolling, leaf death, tiller productivity and grain yield of the plants were measured to determine the usefulness of the PMSC facility for drought screening. Measured soil water potential at depths of 30 and 60 cm during periods of controlled drought revealed that the PMSC facility largely excluded water when rainfall occurred. Leaf rolling and leaf death scores of plants subjected to drought stress in the PMSC facility were significantly higher ( $p < 0.05$ ) than those subjected to drought stress in the bare soil. Also, when subjected to drought stress during the booting-to-grain-filling stage, plants grown in soil covered with plastic mulch showed significantly lower ( $p < 0.01$ ) levels of tiller productivity and grain yield, compared to those grown in bare soil. The plastic mulch did not significantly alter ( $p = 0.05$ ) soil temperature. Data on SC, CCI and CF revealed that plants grown in the well-watered plots of the PMSC photosynthesized and developed normally, as shown by high values of SC and CF, relative to the plants in simulated drought plots. This suggested that the plastic covering of the soil had no adverse effect on plant growth and development. The PMSC system was effective for enhancing managed drought stress in this study, and is therefore recommended for screening rice during periods of normal rainfall. The PMSC system is cheaper than a rain-out shelter facility, and is scalable for use on large plots and at diverse locations where rain-out shelters are neither affordable nor feasible.

Keywords: drought screening, drought tolerance, plastic mulch soil cover, rice, soil water potential

## 5.2 Introduction

In rainfed agriculture, uneven and low rainfall often exposes plants to drought stress, which is a major constraint to crop production and yield stability world-wide (Farooq *et al.*, 2012). The ultimate impact of drought stress on crop growth and development depends on the severity of drought, which is determined by its timing, duration and intensity (Serraj *et al.*, 2005).

Drought, mild or severe, poses a constant threat to rice production under rainfed conditions (Garrity *et al.*, 1986). Drought stress occurring at various stages of crop development may slow crop growth rates, limit the absorption and translocation of minerals, and reduce tillering and biomass accumulation (Bouman *et al.*, 2007). When drought occurs at the reproductive stage of rice development, it can impair grain formation and yield by disrupting panicle development, spikelet fertility and grain filling (Saini and Westgate, 1999). Reducing the impact of drought stress on rice production is therefore critical to avoiding losses and to enhancing productivity of the crop.

Water saving production technologies, including the cultivation of drought tolerant and aerobic varieties, have been recommended to reduce water use in rice production without sacrificing yield (Tuong and Bouman, 2003). However, identification and development of such varieties requires field screening procedures capable of resolving small differences in the levels of drought tolerance of the selected entries. In situations where other elements of the environment, such as sub-optimal temperatures, tend to influence and confound results of screening during off-season periods of limited rainfall, test materials may have to be evaluated during a normal cropping season, which may be characterized by abundant rainfall. Also, during so-called dry seasons, unanticipated rainfall events may interfere with managed drought treatments during critical stages of assessment. When screening is to be conducted during a normal season, a reliable, accurate drought simulation technique may be needed to eliminate interference from rainfall and to impose drought of the desired intensity and duration at the right growth stages, if reliable results are to be obtained (Clarke and Townley-Smith, 1984; Subbarao *et al.*, 1995).

Traditionally, rain-out shelters have been used for drought simulation studies during normal cropping seasons (Blum, 2010). Unfortunately, the high cost of constructing a durable rain-out shelter facility has largely limited its use to a few localities, and usually for small scale applications. Furthermore, their size is always quite limited (Saxena and O'Toole, 2002). As the problem of water scarcity intensifies around the world due to climate change, more research will be needed to develop efficient water-saving technologies, including the breeding of more drought tolerant varieties of all crops, particularly in emerging and

developing economies where the problem of drought is often acute. In a quest to create a technology that combines the flexibility of low cost and adaptability to multiple locations and various scales of operation, the present study was undertaken. The goal was to investigate the usefulness of a plastic mulch soil cover (PMSC) system for imposing an experimentally controlled drought stress, aimed at screening rice genotypes for drought tolerance during periods of normal to high rainfall.

### 5.3 Materials and methods

Plants were grown either on soil covered with polyethylene sheets, or on bare soil, and were subjected to either of two water regimes during the tillering stage of development from November to April in 2012/13, or during the booting-to-grain-filling stage during the same months in 2013/14.

#### 5.3.1 Description of study site

The experiments were conducted at the Ukulinga farm (29°40' S, 30°24' E, 806 m above sea level) of the University of KwaZulu-Natal in Pietermaritzburg, South Africa. The prevailing temperatures, solar radiation and reference evapotranspiration measured during the trials are presented in Table 5.1.

Table 5.3: Measured monthly climatic data during the field trial at Ukulinga, Pietermaritzburg (2012/13 & 2013/14). Data source: Agricultural Research Council (ARC) – Institute for Soil, Climate and Water (ISCW), SA.

Years	Months	Temperature (°C)		Solar radiation (MJ m <sup>-2</sup> )	Evapo-transpiration (mm)
		Minimum	Maximum		
2012/13	November	9.62	32.90	15.92	91.17
	December	14.12	31.50	20.71	124.90
	January	14.75	38.40	18.28	113.60
	February	13.90	33.80	18.84	105.00
	March	12.96	32.00	15.43	93.98
	April	9.28	35.10	14.12	86.96
	<b>Mean</b>		<b>12.44</b>	<b>33.95</b>	<b>17.22</b>
2013/14	November	14.88	25.00	19.00	112.30
	December	15.93	23.40	16.78	100.50
	January	18.10	28.00	20.46	131.10
	February	18.20	28.20	20.83	116.10
	March	17.13	26.20	16.30	101.70
	April	13.62	24.40	15.48	87.49
	<b>Mean</b>		<b>16.31</b>	<b>25.87</b>	<b>18.14</b>

\* Values for reference evapotranspiration are totals, not averages.

### **5.3.2 Experimental design and treatments**

The design of the experiment, under each ground cover environment during both seasons, consisted of a split plot arrangement. Two water regimes (well-watered and drought stressed) were administered as the main plot factor, and twenty-four rice genotypes as the sub-pot treatment, replicated across three blocks. The genotypes (sub-plots) were randomly assigned to each water regime.

The well-watered treatment was maintained by regularly watering to keep water potential at the 30 cm depth of the soil at -15 kPa throughout the trial. Drought stress was imposed by completely withdrawing water supply to the target plots for 21 days during the tillering or booting-to-grain-filling stages of plant growth.

The genotypes included 22 rice varieties traditionally grown under rainfed upland conditions in Liberia, and two check varieties (LAC 23 and FKR 19). LAC 23 and FKR 19 were used as tolerant and susceptible checks, respectively. Plants were established in single row plots by transplanting one three-week-old seedling per hill, and establishing them under well-watered conditions before subjecting them to drought stress at 4 or 16 to 22 weeks after transplanting (WAT). Within and between row spacings in each ground cover environment were 15.0 and 50.0 cm, respectively.

### **5.3.3 Layout of the plastic mulch soil cover facility**

The basic components of the plastic mulch soil cover (PMSC) facility were polyethylene sheets (130  $\mu\text{m}$  thick) and underground drip irrigation pipes. Plots were established on slightly sloping land to permit utilization of the lower levels of the slope for the well-watered (control) plots. After ploughing, ridges (10.2 m long) were prepared 50.0 cm apart to facilitate run-off of water from the plastic sheeting. Thereafter, each block of plots consisting of 12 ridges was completely covered with a single layer of the polyethylene sheet. The extreme ends of the sheets were buried in trenches in the ground to a minimum depth of 1.0 m, in order to prevent lateral flow of water into the experimental plots from the ground bordering the plastic covered plots. The vegetation cover of the surrounding soil was also maintained to enhance evapotranspiration and water removal from the soil. Directly above the apex of the ridges, the plastic was perforated at 15.0 cm intervals, making circular holes (with diameters of 2.5 cm) to accommodate individual plants.



#### **5.3.4 Water supply, soil water assessment and drought imposition**

Water to the plastic-covered plots was provided through a network of pressure-compensated dripper lines (Netafim, Israel), buried beneath the plastic mulch, such that water flow was directed upward at the plastic mulch, in order to prevent erosion due to water pressure. Delivery and control of irrigation water to the plots were managed with the aid of solenoids and an automatic irrigation scheduling unit (Hunter Industries, USA). Water to the bare-soil plots was delivered by overhead sprinklers (Rainbird, USA). A 2.0 m barrier of plastic sheeting was used to prevent water delivered by the sprinklers from reaching plots subjected to drought stress.

#### **5.3.5 Data collection and analyses**

Soil water potential was monitored daily to determine the water status of the plots. Daily water potential and temperature of the soil at depths of 30 and 60 cm were calculated from hourly measurements obtained with the aid of dielectric water potential sensors (MPS-2, Decagon Devices, USA) that were connected to a data logger (EM50, Decagon Devices, USA). Chlorophyll fluorescence, leaf chlorophyll content index, and stomatal conductance of the test plants were recorded after withholding water and compared to those of plants grown on bare ground in order to ascertain the effect of the plastic mulch covering on plant performance. A portable plant efficiency analyser (Pocket PEA, Hansatech Instruments), was used to measure CF, and a leaf porometer (SC-1, Decagon Devices) to measure SD. The chlorophyll content index was measured with the aid of a chlorophyll meter (SPAD-502Plus, Konica Minolta). Measurements were taken between 10 am and noon, on the top-most, fully developed leaf of each plant, and averaged over five plants per treatment combination. Leaf rolling and leaf death scores were recorded by visually integrating the respective symptoms per plot, according to a standard evaluation system for rice (IRRI, 2006). Leaf rolling was scored during the morning hours on the following scale of 0, 1, 3, 5, 7 or 9: indicating leaves that were healthy; leaves just started to fold (shallow); leaves folded and showed deep V-shape; leaves fully cupped (U-shape); leaves folded with margins touching (O-shape); or leaves tightly rolled. Based on the total leaf area lost to desiccation, leaf death was scored on a zero (no senescence) – 5 (leaves completely dried) scale.

Rainfall data was monitored and captured daily by an automatic weather station. Soil water potential data and plant growth responses during the imposed drought periods were subjected to analysis of variance and compared to determine the effectiveness of the PMSC system for simulating drought.

## **5.4 Results**

### ***5.4.1 Soil water status of plots***

Data on soil water status and rainfall measured during the trial are presented in Figure 5.1. Water potential (30 cm depth) in the control plots remained well below -15 kPa, indicating that the plots were well watered and free of drought stress throughout the experiment. Soil water potential (at 30 and 60 cm depths) in the simulated drought plots decreased (became more negative) following the withdrawal of irrigation; soil water potential approached limits below field capacity (-33.3 kPa) after 3 days of withholding irrigation, during both the tillering and booting-to-grain-filling stages of development of the plants. Changes in soil water potential were more pronounced at the 30 cm depth of the profile, where soil water potential declined to levels below -500 kPa, the effective range of the sensors, after 10 - 19 days of withholding water. During both the tillering and booting-to-grain-filling stages of development, the soil water potential between the 30 and 60 cm profile depths varied significantly ( $P < 0.001$ ) in all plots, except the control, where the mean soil water potential ranged from -12.6 kPa during the booting-to-grain-filling stage to -12.7 kPa during the tillering stage.

### ***5.4.2 Effect of rainfall on soil water status***

The effect of rainfall on the water status of the soil during the managed drought period varied significantly between the PMSC and bare soil treatments (Figure 5.1). No change was detected in soil water potential at depths of 30 or 60 cm under the PMSC during or following a rainfall event. Under bare soil, water potential in the soil tended to decline on most days of rainfall, including the following one to two days following heavy rainfall events.

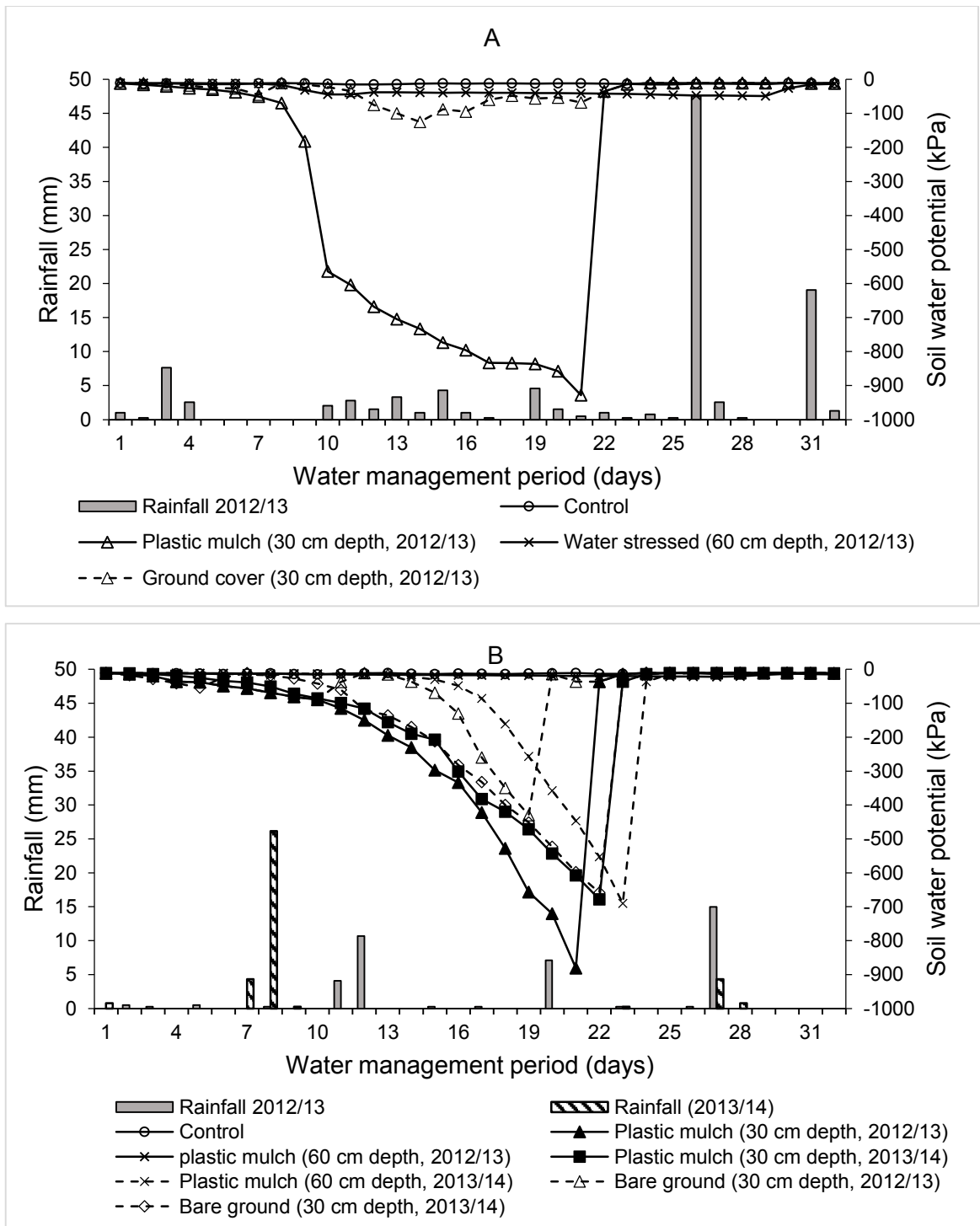


Figure 5.1: Effect of managed drought treatment and rainfall on soil water potential during both the tillering (A) and booting-to-grain-filling (B) stages of growth.

### **5.4.3 Effects of the PMSC on selected physiological and agronomic responses of rice genotypes**

The PMSC caused no adverse effect on the test genotypes, apart from causing them to exhibit signs and symptoms of drought stress when drought was intentionally imposed (Figure 5.2). The values of all the traits measured (CCI, SC, CF, tiller productivity and grain yield) for the plants grown under well-watered conditions in the PMSC plots did not differ significantly from those of plants grown under well-watered conditions in plots without plastic cover (Tables 5.2 and 5.3). No leaf rolling, nor leaf death, was observed for all the plants grown under well-watered conditions, irrespective of soil cover type. The temperature of the soil at both 30 and 60 cm depths ranged from 20 to 24°C (Table 5.2), and did not significantly differ ( $p = 0.05$ ) with soil cover.



Figure 5.2. Using a plastic mulch soil cover system to facilitate drought screening of rice under field conditions during a normal rainfall season. Note: nets were used to prevent bird damage.

Table 5.2: Soil temperature and physiological responses of rice genotypes grown in soil with or without plastic mulch soil cover. Data are means for two seasons for booting-to-grain-filling stage drought stress, and means for one season under tillering stage drought stress.

Parameters	Tillering stage				Booting-to-grain-filling stage			
	Plastic covered soil	Bare soil	LSD (p=0.05)	CV (%)	Plastic covered soil	Bare soil	LSD (p=0.05)	CV (%)
<b>Soil Temperature (°C)</b>								
Well watered	23.69	24.21			20.22	23.25		
Drought stressed	24.37	22.86			20.64	21.05		
			1.92	13.80			3.33	15.67
<b>Chlorophyll fluorescence (f<sub>v</sub>/f<sub>m</sub>)</b>								
Well watered	0.79	0.78			0.79	0.79		
Drought stressed	0.65	0.72			0.58	0.71		
			0.07	10.82			0.09	13.25
<b>Chlorophyll content index (SPAD value)</b>								
Well watered	45.33	42.6			41.72	38.50		
Drought stressed	18.21	26.7			22.6	19.57		
			2.25	19.67			2.30	17.50
<b>Stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>)</b>								
Well watered	0.40	0.39			0.38	0.39		
Drought stressed	0.22	0.24			0.25	0.23		
			0.08	14.7			0.12	11.11
<b>Leaf roll</b>								
Well watered	0.00	0.00			0.00	0.00		
Drought stressed	7.80	6.50			8.20	8.20		
			0.68	12.20			1.22	9.82
<b>Leaf death</b>								
Well watered	0.00	0.00			0.00	0.00		
Drought stressed	4.10	3.10			4.30	3.50		
			0.06	13.98			0.05	13.33

Table 5.3 Tiller productivity and grain yield of rice plants grown in soil either covered or not with a plastic mulch. Data are means for two seasons for booting-to-grain-filling stage drought stress, and means for one season under tillering stage drought stress.

Parameters	Tillering stage				Booting-to-grain-filling stage			
	Plastic covered soil	Bare soil	LSD (p=0.05)	CV (%)	Plastic covered soil	Bare soil	LSD (p=0.05)	CV (%)
<b>Tiller productivity (%)</b>								
Well watered	81.04	80.12			81.04	80.12		
Drought stressed	88.59	86.42			5.3	4.7		
			3.84	21.33			7.62	17.80
<b>Grain yield (g plant<sup>-1</sup>)</b>								
Well watered	31.09	28.17			31.09	32.17		
Drought stressed	29.09	34.72			8.43	6.02		
			3.24	15.22			5.67	10.96

## 5.5 Discussion

Water is held largely in the soil by matric forces, both adsorptive and capillary, which plants must overcome to absorb water from the soil (Hillel, 1971; Lal and Shukla, 2004). Matric potential constitutes the major component of total soil water potential, which is normally equal to the former in non-saline environments (Campbell, 1988). Soil water potential corresponding to the upper and lower limits of available water (i.e., field capacity and permanent wilting point) in fine textured soils, as the one used for the current experiment, are typically estimated at -33 and -1500 kPa, respectively (O'Geen, 2012). Monitoring the status of water in the soil is not only an essential practice for sustaining crop production, but is also a critical component of studies involving plant water relations (Ram *et al.*, 1996; Torres *et al.*, 2002; Munoz-Carpena, 2004). Therefore, in this study soil water potential was logged regularly to determine the status of water availability in the plots during the course of the experiments.

### 5.5.1 Soil water potential and plant growth responses

Throughout the study, soil water potential in control plots was maintained at levels below -15 kPa, which is an indication that the plants were well watered and free of drought stress. As expected, following the withdrawal of irrigation to the target plots, the water potential at both depths of 30 and 60 cm became increasingly negative, leading to drought stress for the plants. Plants began showing signs of wilting less than 5 days after cessation of irrigation; however, assessments of drought sensitivity of the test plants were only done 7 to 21 days after withdrawal of irrigation. The difference between plants grown in the well-

watered and drought applied plots were obvious as shown in Figure 5.2 and Tables 5.2 and 5.3.

The water potential of the soil at 30 cm remained significantly lower than that at 60 cm (Figure 5.1), most likely as a result of higher extraction of water from the upper zone by the plants, as rice is characteristically a shallow rooted plant (Kondo *et al.*, 1999). Studies have shown that at least 90% of the root lengths of most upland and lowland rice cultivars remain concentrated within the upper 40 cm of the soil profile (Beyrouthy *et al.*, 1997; Crusciol *et al.*, 2013). The relatively few strands of roots that extended further down to 60 cm did not absorb much water from this zone.

### **5.5.2 Impact of rainfall on drought treatment under the PMSC**

The impact of rainfall on the drought treatment plots was closely monitored during the course of the trials. Leaf roll scores were recorded two to three days after single or consecutive rainfall events, and compared with the scores recorded on the previous day. Soil water status on every day of rainfall and the days thereafter was also noted. The results of leaf roll scores and soil water data showed that rainfall had no significant influence on the efficacy of the system (Figure 5.1). Soil water potential did not increase after rainfall events. Wilted plants remained wilted even after several consecutive days of rainfall.

Soil water potential data also showed that water intercepted by the leaves or perforations in the plastic was not significant enough to trigger any significant shift in soil matric potential, at least at the 30 cm depth (Figure 5.1). This demonstrates the value of using a PMSC facility to exclude rainfall water during a drought screening experiment.

Drought sensitivity (particularly leaf rolling and leaf drying) data recorded from drought imposed plots showed that the PMSC was highly effective at excluding water from simulated drought plots, leading to stress on the plants. These were further validated by readings from the soil water sensors, which ranged from -10 kPa before the withholding of water, to well over -500 kPa two to three weeks later. Differences between the drought imposed and well-watered plots under the plastic cover were quite clear, as seen from the responses of plants (Figure 5.2).

### **5.5.3 Cost analysis**

The plastic sheeting used in the experiment was standard silage plastic (LDPE) sheeting with a thickness of 130 microns. Silage plastic sheeting is manufactured to various specifications of length, width, thickness, strength and colour (Visqueen, 2014). Weather resistant sheets of high strength and good resistance to tearing are recommended. A roll of

a 18 m wide, standard gauge (125 -130 microns) would cost around R6.41 (\$0.59) m<sup>-2</sup> (ObalCentrum, 2014), putting the cost of setting up the facility (sheets, irrigation system and construction) at less than R10.86 (\$1.00) m<sup>-2</sup> of plot area, compared to a minimum of R140.00 – R2533.30 (\$13.00 – \$235.00) m<sup>-2</sup> for a rainout shelter (Ries and Zachmeier, 1985; Chauhan *et al.*, 1997).

## 5.6 Conclusion

Screening for drought tolerance during the regular cropping season is essential to reduce the influence of genotype by environment interaction on the usefulness of results. The capacity of the current technology, using plastic mulch cover, to exclude rainfall and facilitate the imposition of drought of desired duration and intensity, renders it a useful tool for intensive drought tolerance and screening studies. Because the technology is relatively cheap, compared to the cost of erecting a sound rain-out shelter, it can be scaled up to accommodate as many entries as needed, and can be a useful tool for researchers in developing countries, where research funds are often scarce. This plastic mulch soil cover methodology should therefore be widely disseminated to agricultural research institutions in Africa in order to enhance their capacity in drought research.

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## CHAPTER 6

### COMBINING ABILITY AND GENE ACTION OF THREE COMPONENTS OF HORIZONTAL RESISTANCE AGAINST RICE BLAST

#### 6.1 Abstract

Major gene resistance to rice blast, caused by *Pyricularia oryzae* Cavara, has not provided stable resistance in many countries. The alternative approach is to accumulate minor genes for resistance, an approach that holds great potential for protecting rice against multiple races of the pathogen. However, its exploitation is limited by the lack of understanding of the inheritance pattern in specific rice genetic resources. Eight pure line rice varieties from Liberia, along with their F2 segregants derived from a complete diallel mating design were consequently evaluated under controlled environment conditions to determine combining ability and gene actions for three components of horizontal resistance (HR) (number of sporulating lesions per plant (LN), size of sporulating lesions (LS) and area under the disease progress curve (AUDPC)), in order to develop breeding strategies for future improvement of HR to blast. Genetic analysis of general combining ability (GCA) and specific combining ability (SCA) based on the fixed effects model of Griffing's Method 1, showed that both additive and non-additive gene actions were involved in the inheritance of LN, LS and AUDPC in this study. However, the high ratio of GCA to SCA found for the traits suggested that genes with additive effects were more important. P1 was adjudged to be the worst combiner for LN, LS and AUDPC since it expressed the highest positive GCA effects for the traits. P6 emerged as the best combiner for LS, whilst P2 proved to be the best for both LN and AUDPC, as they showed the highest negative GCA effects for the respective traits. Graphical analysis of the covariance and variances of the traits ruled out epistasis as a contributor to the limited non-additive gene action revealed for LN, LS and AUDPC. The graphical analysis further showed that dominance gene action detected in the parents was rather partial. The overall results revealed that a population breeding approach should be used to improve HR in Liberian rice, using LS, LN and AUDPC as selection criteria, and that all of the parents but P1 would be good donors. High estimates of heritability and phenotypic correlation were found for all three traits, which suggested that selection for the traits under controlled environment conditions would be effective in a recurrent selection programme.

**Key words:** combining ability, component of resistance, gene action, resistance breeding, rice blast.

## 6.2 Introduction

Rice blast, caused by *Pyricularia oryzae* Cavara, is a devastating disease of rice (*Oryza sativa* L.), which is a staple food for more than one-half of the world's population (Talbot, 2003; Bouman *et al.*, 2007). The disease occurs almost everywhere rice is grown, and accounts for annual losses of 10 – 30% in rice production (Skamnioti and Gurr, 2009). Reducing the impact of the disease on rice production is therefore necessary in order to ensure food security. As with many other disease and pest problems, the use of resistant varieties remains the safest and most economical means of managing rice blast. However, successful use of resistant varieties containing major genes that confer resistance against the pathogen have been limited by the rapid evolution of virulent races globally (TeBeest *et al.*, 2007; Khush and Jena, 2009). Such varieties become susceptible as new virulent races emerge (Ou, 1985). An alternative form of host plant resistance is therefore needed to adequately control the disease genetically.

In many plant-pathosystems, horizontal resistance mechanisms have proved capable of increasing the production life of cultivars by providing protection against multiple races of the pathogen (Devasahayam and Henry, 2009; Keane, 2012). Improving the levels of horizontal resistance in rice against blast is a better long term option for genetically limiting the scale of crop losses caused by the disease. However, the genetic mechanisms controlling the inheritance of important horizontal resistance traits for the disease are not well understood, and this limits its full exploitation for protecting rice against the disease. The number of sporulating lesions per plant (LN), the size of sporulating lesions (LS), and area under the disease progress curve (AUDPC) are known as important traits that contribute to horizontal resistance against rice blast (Bonman, 1992; Wang *et al.*, 1994; Mohapatra *et al.*, 2008). Undertaking rice breeding to improve these traits would therefore require a thorough understanding of the genetic basis for their expression in rice.

Insight into the genetic mechanisms controlling the expression of traits can be obtained by evaluating breeding lines and their hybrids for combining ability and gene action. Knowledge of combining ability and gene action is important for selection of suitable parents for hybridization, as well as identification of promising recombinants for the breeding programme (Sprague and Tatum, 1942; Falconer *et al.*, 1996). Breeders can also utilize such information to determine the most appropriate breeding procedure for improving specific traits of interest.

In order to understand the combining ability and gene actions for LS, LN, and AUDPC, the present study determined genetic components of variation for each trait in an array of eight rice varieties and their F<sub>2</sub> segregants derived from a complete diallel mating design. This

paper presents and discusses the breeding values of the parents and the performances of their hybrids, and suggests a strategy for developing new varieties with improved horizontal resistance based on advancing the traits studied.

## 6.3 Materials and methods

### 6.3.1 Plant materials and experimental design

Plant materials used in this study consisted of eight pure line rice varieties (Table 6.1) and their F<sub>2</sub> progenies. The 8 parents were crossed in a full diallel mating scheme in a greenhouse situated on the Life Sciences campus of the University of KwaZulu-Natal in South Africa, during the summer of 2012. The few seeds of the 56 F<sub>1</sub> hybrids produced were later grown in a glasshouse to generate sufficient F<sub>2</sub> seeds for the current study.

Table 6.1: Eight rice pure lines used for the diallel crosses

Code	Name	Origin	Blast resistance
P1	LTH	IRRI	Highly susceptible
P2	LAC 23	Liberia	Highly resistant
P3	Kuwaylikulay	Liberia	Moderately resistant
P4	Laiefeh	Liberia	Moderately resistant
P5	Cammueta	Liberia	Moderately resistant
P6	Paylaieto	Liberia	Moderately resistant
P7	Molonliagie	Liberia	Moderately resistant
P8	Wennie	Liberia	Moderately resistant

The F<sub>2</sub> segregants and the parents were evaluated in a simulated uniform blast nursery in a greenhouse. The experiment was laid out in a randomized complete blocks design, and replicated three times between November 2013 and May 2014. Up to 60 plants of each genotype per replication were grown in seedling trays and 10 were randomly selected and tagged for measurements.

A blast epidemic was initiated by exposing the test plants to disease spreader plants. Plants of LTH were used as spreaders and were inoculated with conidia harvested from mycelia of a *P. oryzae* isolate, SIK-111, obtained from AfricaRice.

### 6.3.2 Data analyses

The number and size of sporulating lesions were recorded 10 days after exposure to the diseased spreaders. The percentage of diseased leaf area was estimated at three-day

intervals, using a diagrammatic scale developed for rice blast (Notteghem, 1981), and used to calculate the area under the disease progress curve (AUDPC). The following equation developed by Shaner and Finney (1977) was used to calculate AUDPC:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(Y_i + 1 + Y_{i+1})}{2} (X_{i+1} - X_i) \quad \text{Equation 6.1,}$$

where  $Y_i$  = the percent of diseased leaf area on the  $i$ th day of assessment,  $X_i$  = time of the  $i$ th assessment in days from that of the first assessment, and  $n$  = total number of times disease was assessed.

Differences in LN, LS and AUDPC values between the 64 genotypes (parents and hybrids) were determined by ANOVA using Genstat Version 16 (VSN, International). General combining ability (GCA) of the parents and specific combining ability (SCA) of the hybrids were analysed according to (Griffing, 1956), following the fixed effects model of Method 1, using the statistical software DIAL 98 (Ukai, 2006). DIAL 98 was also used to estimate other genetic parameters, including additive and dominance variances, and broad- and narrow-sense heritability over the three replications following the methods of (Hayman, 1954a; Hayman, 1954b). Graphical analysis of covariances / variances ( $W_r$ - $V_r$ ) were performed following the methods of Hayman (1954b) and Jinks (1954) to further explain gene action in the parental genotypes.

## 6.4 Results

### 6.4.1 Genotypic means and variances

The mean of each trait varied significantly with genotype for each trial (Table 6.2). The number of sporulating lesions per plant ranged from a high of 55 on P1 to a low of 7 on P2. Crosses P2xP7 and P1xP4 yielded the highest and lowest numbers of sporulating lesions per plant, respectively. The largest and smallest lesions were recorded on P1 and P5, respectively. The values of AUDPC were comparatively low for P7 and cross P2xP4. The highest AUDPC values were recorded from P1 and cross P1xP5.

Table 6.2: Mean sporulating lesions, lesion size and AUDPC of eight inbred rice genotypes and their F2 segregants

Genotypes	Sporulating lesions plant <sup>-1</sup>			Lesion size (mm <sup>2</sup> )			AUDPC		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Parents									
P1	52.0	54.0	60.0	6.7	14.3	20.0	530.0	541.0	524.0
P2	7.3	6.4	8.1	3.5	4.2	3.2	170.0	170.0	178.0
P3	17.0	17.8	22.0	3.4	3.5	3.3	252.0	250.0	255.0
P4	11.0	10.5	9.8	3.8	3.9	4.0	160.0	157.0	152.0
P5	12.7	12.0	14.0	2.6	4.2	2.8	222.0	210.0	229.0
P6	24.0	23.0	17.0	3.8	2.9	3.7	175.0	175.0	171.0
P7	8.0	9.0	7.2	4.2	4.0	4.8	165.0	170.0	168.0
P8	16.3	16.0	17.2	3.1	3.9	3.9	243.0	250.0	239.0
Crosses									
P1xP2	27.0	17.5	42.6	4.1	9.6	13.5	292.5	343.5	273.5
P1xP3	38.0	24.5	41.4	3.5	11.3	10.0	350.0	375.0	403.0
P1xP4	27.0	45.0	32.6	6.0	8.2	17.1	277.5	345.0	281.5
P1xP5	33.5	38.5	28.1	5.1	11.0	12.5	350.0	367.5	391.0
P1xP6	45.1	28.5	37.5	6.2	7.5	8.5	289.0	341.0	257.0
P1xP7	39.6	17.5	42.1	6.0	13.7	11.5	292.0	345.0	333.0
P1xP8	23.7	20.5	25.0	5.9	11.5	13.1	363.0	377.5	409.5
P2xP3	10.0	8.5	7.5	4.3	4.1	3.1	195.5	207.5	213.0
P2xP4	9.6	7.5	7.3	3.6	4.1	3.8	163.5	165.0	157.0
P2xP5	9.8	7.5	8.1	3.3	4.0	3.3	205.0	294.5	162.5
P2xP6	14.3	8.1	8.0	3.7	3.4	3.1	167.8	162.5	190.5
P2xP7	6.4	7.3	6.3	4.1	4.1	3.9	167.0	169.5	164.0
P2xP8	12.0	13.5	12.1	3.4	4.0	3.4	177.5	185.0	194.5
P3xP4	13.2	14.1	14.8	3.7	3.6	3.8	172.0	205.0	192.0
P3xP5	14.5	13.1	13.1	3.2	3.5	3.3	222.5	237.5	226.0
P3xP6	15.5	17.2	16.9	3.4	3.4	3.5	237.5	212.5	205.0
P3xP7	13.2	12.3	12.9	3.9	3.9	4.2	238.0	207.5	195.0
P3xP8	13.7	10.5	15.0	3.3	3.5	3.4	250.0	242.5	252.5
P4xP5	11.1	10.8	12.6	3.6	4.2	3.8	200.0	202.5	165.5
P4xP6	13.0	17.1	22.3	3.8	3.7	4.0	166.0	265.0	164.5
P4xP7	11.1	9.5	8.2	4.1	3.7	4.2	164.5	267.0	160.0
P4xP8	14.3	12.8	15.5	3.7	3.8	3.8	230.0	197.5	227.5
P5xP6	16.0	17.1	23.4	3.4	4.1	3.7	207.5	195.5	205.0
P5xP7	9.0	9.5	7.1	3.8	4.1	4.4	198.0	195.0	196.5
P5xP8	15.4	14.5	17.0	3.1	4.1	2.9	237.5	229.0	235.0
P6xP7	16.5	12.0	14.4	4.0	4.0	3.8	169.8	168.5	171.0
P6xP8	12.1	15.1	22.2	3.4	3.8	3.7	162.5	202.5	222.5
P7xP8	9.5	10.6	12.8	3.4	4.0	4.3	195.0	204.5	211.0
Mean	17.8	16.4	18.9	4.0	5.4	5.8	229.4	245.4	232.6
CV (%)	22.2	26.7	24.9	9.3	4.1	3.6	12.5	17.2	13.3
LSD	5.8	6.1	7.6	2.2	2.6	3.2	90.4	95.2	97.6

AUDPC = area under the disease progress curve; CV = coefficient of variation; LSD = least significance difference. See codes of genotypes in Table 1.

### 6.4.2 Combining ability

The variances due to GCA and SCA were both highly significant for LN and LS; but for AUDPC, only the GCA variance was significant (Table 6.3). The ratio of GCA to SCA for the three traits were quite high and ranged from 43% for LN, to 54% for AUDPC. No reciprocal effect was observed for the inheritance of any of the three traits.

Table 6.3: Analysis of variance for combining ability in the F2 population of an 8 X 8 complete diallel cross in rice.

Source of variation	Df	Mean square		
		Lesion number	Lesion size (mm <sup>2</sup> )	AUDPC
Trial	2	269.73 **	51.74 **	6454.00 **
GCA	7	1927.71 **	145.72 **	93778.23 **
SCA	20	45.14 *	1.90 <sup>ns</sup>	1751.05 **
Reciprocal	28	2.17 <sup>ns</sup>	0.46 <sup>ns</sup>	55.71 <sup>ns</sup>
Error	110	24.21	3.89	701.79
GCA : SCA (%)	-	42.71	76.69	53.56

\*  $p < 0.05$ , \*\*  $p < 0.01$ , <sup>ns</sup>  $p = 0.05$ ; Df = degrees of freedom; AUDPC = area under the disease progress curve.

General and specific combining ability effects for LN, LS and AUDPC are shown in Table 6.4. For all the traits measured, P1 displayed the highest positive GCA effect essentially for susceptibility. P2 showed the highest negative effect for both LN and AUDPC, which is in a desirable direction for resistance breeding. The highest negative GCA effect for LS was exhibited by P6. All the crosses derived from P1 showed high positive SCA effects for LN, as P2xP8 exhibited the highest negative SCA effect for the trait. Positive SCA effects for LN, LS and AUDPC were exhibited by P1xP8. The lowest negative SCA effects for LS and AUDPC were recorded from the crosses between P1 and P3 and P1 and P6, respectively. Crosses involving P1 and all the other parents, but P5, showed increasing negative trends of SCA.



Table 6.4: General combining ability effects of parents and specific combining ability effects of crosses for three components of horizontal resistance against rice blast in the F2 generation of an 8 X 8 diallel population of rice

Parents	Lesion number	Lesion size (mm <sup>2</sup> )	AUDPC
General combining ability effects			
P1	17.22***	4.93***	118.30***
P2	-6.36**	-0.77	-37.58**
P3	-1.41	-1.00*	6.22
P4	-2.01*	-0.43	-31.06**
P5	-1.99*	-0.74	-0.23
P6	1.50	-1.09*	-31.37**
P7	-4.31**	-0.26	-28.66**
P8	-2.64*	-0.65	4.38
Specific combining ability effects			
P1xP2	0.77	-0.22*	-12.20
P1xP3	1.44	-0.79**	16.84**
P1xP4	2.27*	-0.78**	-20.55
P1xP5	0.73	0.22	16.78
P1xP6	0.91	-1.56**	-25.91***
P1xP7	2.76*	0.61	-0.95
P1xP8	8.89***	0.95	26.00***
P2xP3	-0.97	0.46	2.05
P2xP4	-0.90	-0.11	-4.18
P2xP5	-0.59	-0.08	23.82***
P2xP6	-2.41*	0.13	7.88
P2xP7	-0.04	-0.06	-1.58
P2xP8	-4.14	-0.12*	-15.75
P3xP4	-0.04	-0.03	-20.13**
P3xP5	-0.41	-0.07	-11.79
P3xP6	-0.95	0.39	8.84
P3xP7	1.11	0.10	1.30
P3xP8	-0.26	-0.07	3.09
P4xP5	-1.94*	-0.11	-14.02
P4xP6	0.58	0.20	24.28***
P4xP7	-1.49*	-0.45	22.24***
P4xP8	1.44	-0.31	10.37*
P5xP6	1.92	0.44	-0.38
P5xP7	-2.57*	-0.04	-9.26
P5xP8	2.85*	-0.36*	-4.97
P6xP7	-0.27	0.17	-4.87
P6xP8	0.23	0.23*	-11.83*
P7xP8	0.51	-0.32*	-6.87

\* p < 0.05, \*\* p < 0.01; \*\*\* p < 0.001; AUDPC = area under the disease progress curve.

#### 6.4.3 Estimates of genetic parameters for LS, LN and AUDPC

Important genetic parameters estimated for each trait expressed by the 8 parental lines and their progenies are presented in Table 6.5. The additive genetic variance accounted for a

large proportion of the gene action expressed for all the three traits studied. The proportions of dominant to recessive genes in the parents were less than zero for LN and LS, but slightly above 1 for AUDPC. The degree of dominance for all traits were quite small and largely in the negative direction for LN and AUDPC, or mildly positive for LS. Both the broad and narrow sense heritability were high for all the traits, and ranged from 84 to 94 %.

Means of the crosses were generally less than those of the parents for LS and AUDPC. The converse was, however, true for LN. Variances of the parents were markedly larger than the variances of either their hybrids or of the environment.

Table 6.5: Estimated genetic parameters and standard errors for three blast resistance traits in rice lines and their hybrids evaluated in three greenhouse environments.

Parameter	Lesion number	Lesion size (mm <sup>2</sup> )	AUDPC
Additive variance	235.46 ± 32.29	11.31 ± 3.24	144483.77 ± 1354.06
Dominance variance 1	9.24 ± 10.39	-2.47 ± 1.41	707.26 ± 398.90
Dominance variance 2	10.88 ± 8.10	-1.59 ± 0.99	606.25 ± 280.52
Additive X dominance	15.90 ± 29.28	-4.43 ± 2.78	3243.48 ± 1345.09
Average degree of dominance	0.20 ± 0.06	0.00 ± 0.15	0.22 ± 0.40
Proportion of dominant genes	0.59 ± 0.08	0.00 ± 0.37	0.75 ± 0.06
Average direction of dominance	-2.56 ± 1.82	0.35 ± 0.77	-6.52 ± 9.19
Heritability (broad sense)	0.94 ± 0.01	0.84 ± 0.2	0.96 ± 0.01
Heritability (narrow sense)	0.916 ± 0.02	0.89 ± 0.3	0.94 ± 0.01
Mean of parents	18.85 ± 0.97	4.90 ± 0.4	238.38 ± 4.87
Mean of crosses	17.38 ± 0.36	5.10 ± 0.16	234.65 ± 1.97
Mean of whole diallel	17.57 ± 0.34	5.08 ± 0.15	235.11 ± 1.81
Variance of parents	242.78 ± 32.28	12.65 ± 3.24	15692.27 ± 1354.57
Variance of crosses	87.25 ± 6.56	6.41 ± 0.82	4190.72 ± 251.58
Variance of whole diallel	103.39 ± 6.86	7.01 ± 0.80	5292.58 ± 262.51
Environmental variance	7.32 ± 0.88	1.34 ± 0.16	208.49 ± 25.60

AUDPC = area under the disease progress curve.

#### **6.4.4 Graphical analysis of genetic components**

The coefficients of the regression of Vr on Wr for LN, LS and AUDPC were 1.15, 0.98, and 1.06, respectively, and did not differ significantly from unity. For LN, P1 and P2 were far from the regression line, unlike the rest of the parental array (Figure 6.1). All of the parents, except P1, also tended to cluster along the regression line for LS. For AUDPC, the entire array of parents tended to cluster close to the regression line. The parents, however, maintained distinct positions along the regression line. For all the traits, the regression line intercepted the Wr axis well above the origin (Figure 6.1).

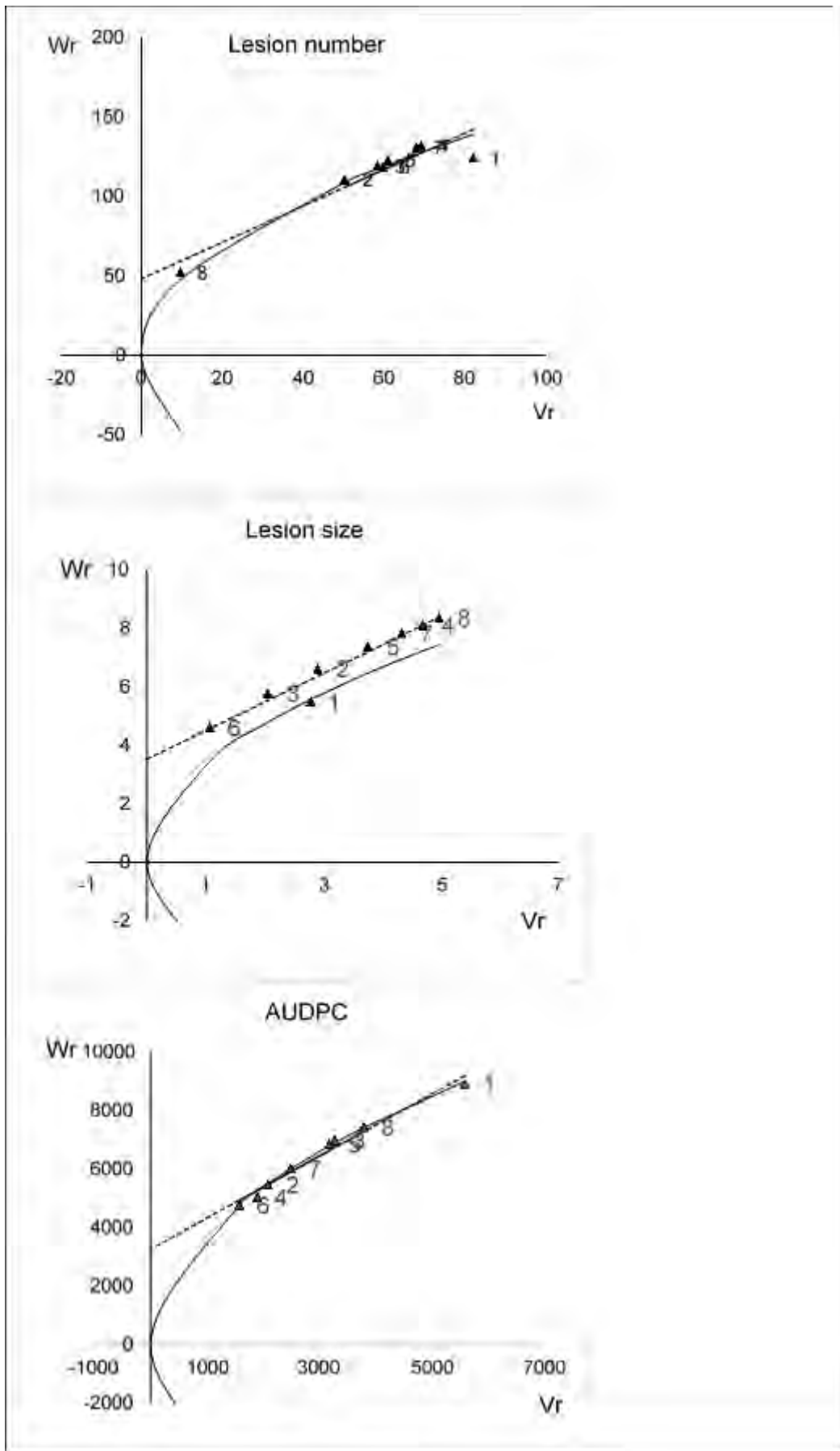


Figure 6.1: Covariance ( $W_r$ ) / variance ( $V_r$ ) graph for rice blast resistance traits.

## 6.5 Discussion

### 6.5.1 Combining abilities of rice parents and their crosses for LN, LS and AUDPC

Biometrical procedures developed by Griffing (1956) to study the combining ability of genotypes and their relatives are commonly used by plant breeders to assist selection of parents for hybridization (Shattuck *et al.*, 1993). A full diallel crossing system, in which parents are mated in all possible combinations to produce hybrids, was used to determine and compare the performances of 8 pure line rice varieties and their F<sub>2</sub> hybrids for LN, LS and AUDPC in the current study. As defined by Sprague and Tatum (1942), the average performance of a parent in a hybrid combination is termed GCA, whilst the deviation of the performance of a hybrid from the expectation based, on the average GCA effects of the lines that produced the hybrid is termed the SCA.

According to Falconer *et al.* (1996), the GCA and SCA variances, respectively correspond to the variances of additive and dominance effects, assuming the absence of epistasis. In the current study, the sum of squares due to GCA and SCA were highly significant for LN and AUDPC, and therefore indicated that both additive and non-additive gene actions were important in the mechanisms governing the inheritance of those traits. Variation for LS, owing to SCA of the crosses, was non-significant, thereby suggesting that non-additive effects were not important for the inheritance of the trait. It would therefore be useful to ensure that genotypes inherently showing smaller sporulating lesions are involved in crosses aimed at generating progenies with smaller lesions. However, a study by Jeanguyot (1984) suggested that smaller sporulating lesions can be the result of race-specific interactions. To minimize the risk of race-specificity for smaller lesion size, genotypes which exhibit a combination of declining lesion size in older leaves and larger lesions in the younger leaves could be targeted for selection (Roumen, 1992).

In their review on hybrid breeding of autogamous crops, Reif *et al.* (2007) affirmed that in the absence of epistasis, the ratio of the GCA to SCA variances tend to increase as the relevance of dominance effects decreases. The high ratios of GCA to SCA obtained for all the traits therefore, suggest that the three components of host resistance studied here were largely under additive genetic control. These results are similar to those reported from studies with other rice populations. In tests involving two sets of F<sub>3</sub> lines derived from two parents, Ali *et al.* (2004) reported the occurrence of additive gene action for lesion number and size in one set, and possible epistasis for one gene, in addition to additive gene action, in the other. Veillet *et al.* (1996) also observed both additive and non-additive effects for

lesion size and lesion density from a genetic study involving a population of upland rice from Brazil. The predominance of GCA variance is not only advantageous for identifying promising hybrids based on predictions from parents, but also for ensuring consistent gain from recurrent selection (Duvick *et al.*, 2004; Gordillo and Geiger, 2008). A recurrent selection programme could therefore be useful for improving the three traits for horizontal resistance to blast studied in these trials.

The parents exhibited various levels of GCA effects for each of the traits studied. P1 showed the highest positive effects for LN, LS and AUDPC, and consequently proved to be the worst combiner, since positive effects for these traits would mean higher levels of disease susceptibility. The best combiner for LS was P6, whilst P2 emerged as the best combiner for LN and AUDPC, as indicated by the high negative effects they expressed for the respective traits. The P2 and P6 varieties would therefore be important for producing progenies with increased quantitative resistance against rice blast.

Crosses involving P1 exhibited high SCA effects for LN. This observation strongly supports the role of P1 as a poor combiner in this study. The high susceptibility of P1 to blast may largely lie in its weakness to sustain large numbers of sporulating lesions, which quickly coalesce and become much larger. The negative SCA effects for LS observed when P1 was crossed with the other parents suggest that dominance gene action played a role in the non-additive effects that govern the expression of the trait.

### **6.5.2 Gene actions for LN, LS and AUDPC in the parental genotypes**

Concepts of the proportion of dominant and recessive genes occurring in a group of parents, as well as the degree and direction of dominance, has been clearly explained by Jinks and Hayman (1953) and Viana *et al.* (2001). The estimated ratios of dominant to recessive genes in the parents used in this study were characteristically similar for LN and LS, as they both fell below zero, thereby indicating that the recessive genes in the varieties were more numerous than dominant genes. For AUDPC, the dominant genes were more numerous than recessive genes in the parents, as reflected by the proportion of dominant to recessive genes, which exceeded 1.

The negative direction of the dominance occurring in the polygenic system under review for LN and AUDPC shows that dominance alleles present could have also contributed to the reduction in the values of these traits in the hybrids. The direction of dominance for LS was positive, contributing to the tendency for increased lesion size in the hybrids; if dominance gene action were largely responsible for increasing lesion size, it would be more difficult to

increase blast resistance by introgressing alleles for smaller lesions. Since this is not the case, evidence of the predominance of additive genetic effects for the inheritance of these traits, as indicated by the high ratio of GCA to SCA, remains strong. Since additive genes are largely fixable, unlike to those with non-additive effects (Dabholkar, 2006), the best combiners found in this study should be useful for developing new rice varieties with higher levels of horizontal resistance against blast.

The diallel was largely effective for increasing rice blast resistance in the F<sub>2</sub>, especially when LN and AUDPC are taken as the index. This was demonstrated by the average reduction in the value of these traits, compared with those of the parents. Selecting the best progenies as parents for the next generation would likely lead to future gains. The variation observed in the experiment for all the traits were mostly due to the parents (Table 6.3). The environmental variance was smaller, indicating the potential of such environments for reliably screening for blast resistance based on the traits under reference.

### **6.5.3 Graphical assessment of genetic components**

Graphical analysis of the covariance and variances of LN, LS and AUDPC measured in this study showed that the regression coefficient for each trait did not statistically differ from unity. This showed that epistasis cannot be considered to have played a role in influencing the expression of any of the traits measured. In order for gene actions and components of variance estimated from the analysis of a diallel to be valid, the following assumptions for the additive / dominance genetic model listed by Hayman (1954b) must be present:

- a. Diploid segregation of chromosomes
- b. Homozygosity of parents
- c. Independent effect of non-allelic genes
- d. Independent distribution of genes between parents and no multiple allelism.

From the graphical analysis, epistasis can be ruled out from playing any role in the inheritance of LN, LS and AUDPC in this study. Secondly, the rice plant is diploid ( $2n = 24$ ), and self-fertilization prevails as the primary mode of reproduction of the species. All these satisfy the above assumptions, and consequently uphold the merit of the diallel procedure employed to study the inheritance of the three traits under reference.

The parental lines used in this study did not scatter widely away from the regression line which highlighted the association of  $W_r$  and  $V_r$  for the traits measured in this study. This may indicate that the parents did not vary very much for the traits. However, it is noted that since all of the other parents showed lower values for these traits than P<sub>1</sub>, these parents

can be used as donors for a breeding programme aimed at developing blast resistance in high yielding rice varieties.

For all the traits, the regression line was found to intercept the  $W_r$  axis at points above the origin, indicating that any dominance present in the parental lines was partial for the traits measured. P6 carried the maximum number of dominant alleles for LS and AUDPC, whilst P8 carried the maximum for LN, as indicated by their close proximity to the origin (Figure 6.1). On the other hand, P1 tended to harbour the maximum number of recessive alleles for LN and AUDPC, and P8 harboured the maximum for LS (Figure 6.1). The occurrence of partial dominance ( $[H1/D]^{1/2}$ ) for the traits, as assessed graphically, is consistent with the calculated result, which shows that the mean degree of dominance for the traits were less than unity (Table 6.5).

#### **6.5.4 Heritability for LN, LS and AUDPC**

Heritability, both in the broad and narrow sense, for all traits were quite high in the environment in which the genotypes were evaluated. This underscores the value of screening under controlled conditions that both influence plant susceptibility and pathogen aggressiveness. It also showed that selection for all three traits is likely to be effective in increasing blast resistance. The high levels of heritability estimated in this study was derived from the limited variability in growth conditions imposed in the greenhouse, even though the experiment was replicated across three distinct time periods. Under field conditions, estimates of heritability would probably be lower because environmental variables such as temperature, humidity, moisture and soil heterogeneity are often uneven.

### **6.6 Conclusion**

Since not much was known about the mode of action of genes controlling LN, LS and AUDPC, undertaking a genetic study of parents and their F<sub>2</sub> progenies derived from a diallel mating scheme was important for determining an appropriate procedure for improving Liberian rice varieties for horizontal resistance against blast when considering those traits as criteria for selection. The analysis of general and specific combining abilities was useful for showing that the traits were mainly controlled by additive genes, although non-additive genes with partial dominance effects were also present. The occurrence of additive gene effects with partial dominance for the three traits showed that recurrent selection methods that emphasize GCA would be the best strategy for breeding new rice varieties with improved blast resistance. Varieties such as P2 and P6, which showed high GCAs, would be useful for recombining genes with the expectation that favourable recombinants would

be incrementally recovered as the selection cycle progressed. The likelihood of obtaining consistent results when screening breeding lines for horizontal resistance against blast under controlled environment conditions that favour the disease was highlighted by the high heritability scores for the traits. If the natural levels of disease conducive factors in the field can be appropriately mimicked in a greenhouse, then screening in the greenhouse can enhance the selection process and the responses to selection.

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## CHAPTER 7

### INVESTIGATION OF ETHEPHON AND GIBBERELLIC ACID AS A COMBINED CHEMICAL HYBRIDIZING AGENT FOR RICE

#### 7.1 Abstract

Crop improvement and genetic analyses of traits require the making of large numbers of pair-wise crosses and progenies. In rice (*Oryza sativa* L.), hand emasculation is slow and tedious, limiting the number of controlled cross pollination events that can be completed in a single season. Chemical hybridizing agents (CHAs) may facilitate emasculation when appropriately applied on growing plants; but their use as a breeding tool for rice has been limited, partly due to genotypic differences, and a lack of information on effective chemicals, doses, and timing of applications. In this study, ethephon and gibberellic acid were evaluated at various doses and growth stages of rice to determine their utility for breeding tropical upland rice. Five upland rice genotypes of Liberia were grown in a controlled environment, and at the phenological stages of flag leaf collar formation and microsporogenesis they were sprayed with ethephon at 0, 1 000, 2 000, 4 000, or 6 000  $\mu\text{L L}^{-1}$ . At heading, gibberellic acid was applied onto these plants once or twice as a foliar spray at 0, 90 or 150  $\mu\text{L L}^{-1}$ . Treatment combinations were laid out in a randomized complete blocks design, with four replications. Pollen viability, panicle exertion, plant height, seed set and outcrossing rates were assessed at various times from anthesis to post-harvest. Ethephon and subsequent gibberellic acid applications induced significant changes in all the traits measured. Pollen viability decreased with increasing doses of ethephon. Ethephon significantly reduced ( $p < 0.001$ ) panicle exertion and the rate of seed set in all genotypes. Gibberellic acid significantly increased panicle exertion, thereby enhancing rates of seed set and outcrossing in ethephon-treated plants. Unlike gibberellic acid, efficacy of ethephon depended on panicle developmental stage, as microsporogenesis proved to be the most sensitive stage for effecting physiological male sterility in the plants. The levels of male sterility induced by ethephon were acceptable given the level of outcrossing achieved, when aided by gibberellic acid application. The combination of both of compounds could be useful as an effective CHA for breeding rice.

Keywords: microsporogenesis, outcrossing, panicle exertion, pollen viability, spikelet fertility

## 7.2 Introduction

Recurrent selection is often identified as being the most appropriate selection method when breeding crops to improve complex traits in plants (Ali *et al.*, 2004; Runge *et al.*, 2004), including horizontal resistance against rice blast. This may require the execution of controlled crosses and polycrosses in order to increase the likelihood of obtaining desirable recombinants, as the recurrent selection cycles progress (Hallauer and Darrah, 1985; Pandey and Gardner, 1992). Hand emasculating and generating large numbers of crosses in rice (*Oryza sativa* L.) may be a difficult and time-consuming procedure because of the structure and biology of the rice florets. Owing to the cleistogamous nature of the rice flower, self-pollination naturally prevails as the predominant mode of reproduction (Taillebois and Guimaraes, 1988; Tripathi *et al.*, 2011), therefore efficient emasculating is required to allow for cross-pollination to take place.

Cross-pollination in rice may be enhanced either by mechanically removing anthers from the florets (Acquaah, 2007), by using a cytoplasmic male sterility system (Chan and Cheah, 1983), or by using chemical hybridizing agents (CHAs) to effect emasculating (Brabosa *et al.*, 1987; Acquaah, 2012). Utilizing mechanical or hand emasculating to facilitate large-scale hybridization of rice may be both labour-intensive and time-consuming, owing to the difficulties involved in removing the anthers from each of the many tiny rice florets (Tripathi *et al.*, 2011). Although potentially cheaper and more convenient to utilize, cytoplasmic male sterility (CMS) systems are only amenable to a limited pool of rice genetic resources (Brabosa *et al.*, 1987). Chemical hybridizing agents, on the other hand, have the potential of combining the advantages of those other two means of promoting rice hybridization: they require less labour and time for deployment as with CMS, but they can be effective on a wider range of rice germplasm, as with hand emasculating. Given these advantages, CHAs are an attractive option for controlling pollination and facilitating rice breeding based on recurrent selection, or for genetic analyses.

Essentially, CHAs are gametocides or compounds used to effect male sterility with the aim of facilitating cross-pollination between chosen parents. Various chemicals, including auxins, halogenated aliphatic acids, gibberellins, ethephon [2-chloroethyl phosphonic acid], arsenates, dalapon, oxanilates and some patented compounds (RH-531, RH-532, N-312, and HAC-123) have been tested and developed as gametocides for various crops (Tu and Banga, 1998; Virmani *et al.*, 2003a; Sharma and Sharma, 2005). Many of these compounds are, however, either highly toxic to users and the environment (Thakur and Rao, 1988), or difficult to obtain (Virmani *et al.*, 2003a), except for a few such as ethephon and gibberellic

acid (GA). Investigating the gametocidal properties of these compounds could have value to rice breeding involving large numbers of crosses.

Ethephon, widely available under the trade name Ethrel<sup>®</sup>, spontaneously releases ethylene in aqueous solution, and has shown potential as a CHA causing male sterility in crops such as oats (Pinto *et al.*, 1988), pearl millet (Thakur and Rao, 1988) and wheat (Brabosa *et al.*, 1987). Its value as a CHA is very dependent on the dosage and timing of application. It can also sterilize the ovaries of flowers, rendering flowers both male and female sterile. However, its efficacy as a CHA on rice remains poorly understood. Also, the capacity of ethephon to impair the elongation of the upper internodes of plants (Tu and Banga, 1998) could potentially limit its usefulness for breeding rice, in the absence of a suitable mitigating factor. Beek (1986) solved these problems by treating wheat plants with gibberellic acid (GA) to counter the negative effects of ethephon, achieving male sterility, combined with female fertility and plant vigour.

The present study was therefore undertaken to determine the level and timing of ethephon for inducing male sterility in rice, and to investigate the use of a second compound, GA, mainly for its ability to mitigate the negative side-effects of ethephon.

## **7.3 Materials and methods**

### **7.3.1 Study sites, experimental design and trial establishment**

The experiment was conducted in a greenhouse tunnel at the Life Sciences campus of the University of KwaZulu-Natal during the summer of 2012/13 with day and night temperatures of 30/20°C, and 65% relative humidity. A randomized complete blocks design was used in a factorial experiment consisting of five upland rice genotypes, five doses of ethephon and three doses of gibberellic acid, replicated three times.

The test plants consisted of five upland genotypes (LR-1, 8, 10, 18 and 19) that differed mainly by hull colour, but with similar basal leaf sheath colour (green). Plants were grown in 4.8 L plastic pots (with height and upper diameter of 27 cm and 30 cm, respectively) with three plants per pot, per genotype.

Ethephon was applied at 0, 1 000, 2 000, 4 000 or 6 000  $\mu\text{L L}^{-1}$  at the phenological stages of panicle differentiation or at microsporogenesis. The stages of panicle differentiation and microsporogenesis were determined according to the rice plant growth staging system described by Counce *et al.* (2000). Penultimate leaf collar formation, which coincides with the latter stages of panicle differentiation, was used as a marker for panicle differentiation. The time at which the flag leaf collar formed and aligned horizontally with the collar of the

penultimate leaf was considered to be concurrent with the stage of microsporogenesis (Counce *et al.*, 2000; Williams *et al.*, 2010).

Gibberellic acid-3 (GA3) was applied once at 0, 90 or 150  $\mu\text{L L}^{-1}$  at anthesis, when the heads of the main culm started to emerge, or twice at 0, 45 or 75  $\mu\text{L L}^{-1}$  at anthesis and at 2 days after the onset of anthesis. Both ethephon and GA3 were applied as foliar sprays onto whole plants until plants were completely drenched with the spray solution.

### **7.3.2 Measurements and data analyses**

Pollen viability, panicle exertion, spikelet fertility, plant height and outcrossing success were the main parameters measured. Spikelet fertility and outcrossing success were assessed as measures of female fertility, whilst plant height and panicle exertion were considered as attributes of the levels of elongation of the uppermost internodes of the plants. Prior to GA application, 10 intact florets (which had not shed pollen) were randomly taken from each panicle and immediately fixed in 70% ethanol for microscopic examination of pollen viability. Three of the six anthers were extracted from a random subsample of five florets, ruptured and stained with a 1% iodine potassium iodide (IKI) solution, and the pollen examined under a stereo microscope (Stemi 2000 c, Carl Zeiss Microscopy). Data on pollen count were recorded for three microscopic fields. Only darkly stained, round pollen grains were considered viable and therefore, fertile. Unstained (withered or spherical) and partially stained round pollen grains were considered to be sterile. The percentage of viable pollen was calculated as the sum of fertile pollen divided by the total number of pollen counted and multiplied by 100.

Panicle exertion was assessed at 10 days after anthesis by recording the extent of coverage of each panicle by the flag leaf sheath, using a rating scale developed by IRRRI (2006), where 1, 3, 5, 7 and 9, represent 0, 1 -10, 11 – 25, 26 – 40, and > 40% of the panicle enclosed in the flag leaf sheath, respectively.

The level of seed set at maturity was used to estimate spikelet fertility. Empty or unfilled spikelets were regarded as sterile, and any spikelet containing a caryopsis was considered fertile. Spikelet sterility was rated using a similar scale as for pollen sterility. Plant height was determined at 10 days after the beginning of anthesis. The length of the main culm, measured from the base of the plant to the apex of the panicle was determined as plant height and expressed in cm. Anthesis was the period when the spikelets on the main culms began opening to effect pollination.

During anthesis, supplementary pollination was carried out by shaking the panicles of a blooming genotype aligned parallel to the test plants in order to enhance possible outcrossing. The supplementary male parent was distinguished from the test genotypes mainly by its purple coloured basal leaf sheath. Outcrossing was determined at 45 days after harvest by sowing to 100 mature seeds obtained from each experimental unit, and counting the number of seedlings that showed pigmented basal leaf sheaths.

All measurements were taken and averaged over three panicles per plant, and the data subjected to analysis of variance using GenStat Version 14 (VSN International). The means of factors showing differences at the 0.05 level of significance were separated using Fisher's LSD test procedure.

## 7.4 Results

### 7.4.1 Effect of ethephon on pollen viability

Data on the effect of ethephon on pollen viability at anthesis are presented in Table 7.1. The magnitude of pollen viability was significantly lower ( $p < 0.01$ ) in plants treated with ethephon at microsporogenesis than in those plants treated at panicle differentiation. Pollen viability decreased consistently with increasing doses of ethephon, irrespective of when the plants were treated with ethephon. On average, pollen viability ranged from 94% in the control to 16% in plants treated with 6 000  $\mu\text{L L}^{-1}$  ethephon at microsporogenesis. Differences in sensitivity to ethephon was negligible amongst the rice genotypes ( $p = 0.05$ ).

Table 7.1: Effect of ethephon on the viability (%) of rice pollen.

Treatment		Genotype					
Growth stage	Ethephon ( $\mu\text{L L}^{-1}$ )	LR 1	LR 8	LR 10	LR 18	LR 19	Mean
Panicle differentiation	0	96.67	97.21	94.87	98.21	95.91	96.57
	1000	91.39	86.53	79.5	80.47	88.54	85.29
	2000	72.33	78.66	80.16	82.85	80.75	78.95
	4000	50.92	58.8	44.4	51.57	58.27	52.79
	6000	44.94	46.59	47.45	40.94	60.32	48.05
	mean	71.25	73.56	69.28	70.81	76.76	72.33
Microsporo-genesis	0	94.62	95.49	91.72	97.11	93.4	94.47
	1000	86.1	78.25	66.92	68.48	81.51	76.25
	2000	55.33	65.56	67.98	72.32	68.93	66.02
	4000	20.79	33.5	10.27	21.83	32.65	23.81
	6000	11.13	13.8	15.19	4.67	35.96	16.15
	mean	53.6	57.32	50.41	52.88	62.49	55.34
Grand mean		62.42	65.44	59.85	61.84	69.62	63.84

LSD( $p = 0.05$ ) = [Genotype = 11.21; Ethephon = 7.94; Growth stage = 9.62]; CV = 17.2%

### 7.4.2 Effects of Ethephon and GA3 on spikelet fertility

The effect of ethephon and GA3 on spikelet fertility of the genotypes differed significantly with application dose (Figure 7.1). On average, spikelet sterility was 41 to 44% lower in all plants of every genotype treated with high doses of ethephon. Differences between the genotypes for spikelet fertility were not significant ( $p = 0.05$ ).

Gibberellic acid significantly increased ( $p < 0.01$ ) spikelet fertility, particularly in plants treated with high levels of ethephon (Figure 7.1). The levels of spikelet fertility recorded did not differ between the 90 and 150  $\mu\text{L L}^{-1}$  doses of GA3, nor between the full and split applications ( $p = 0.05$ ).

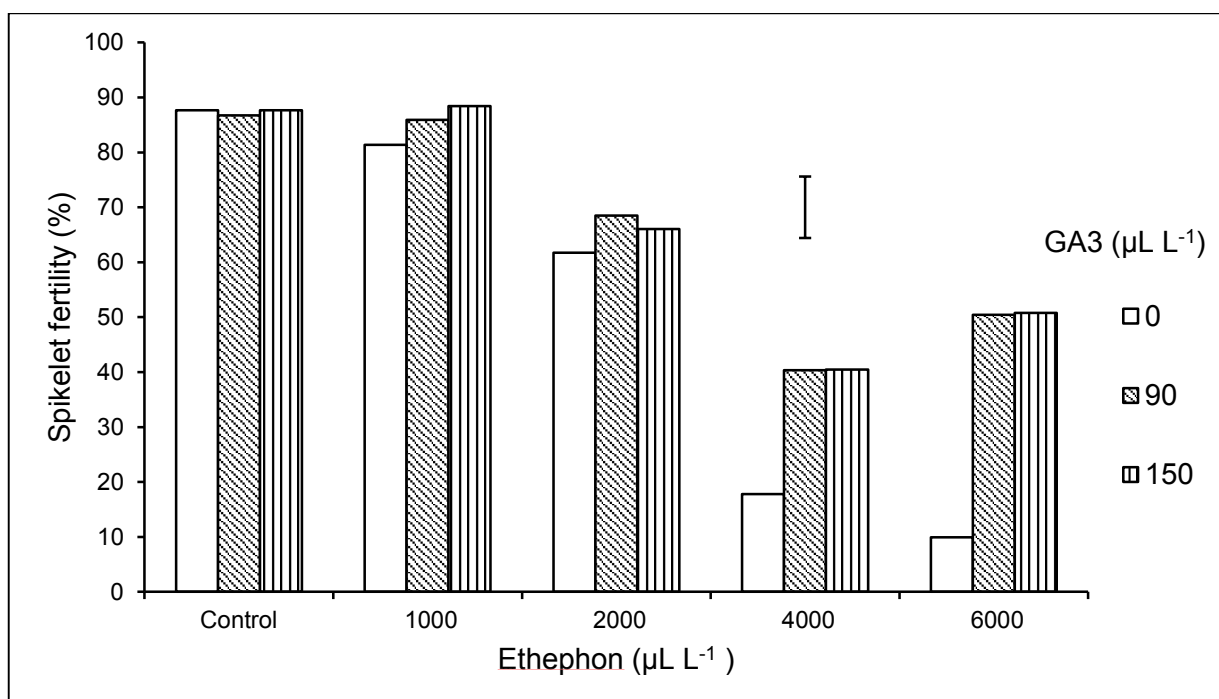


Figure 7.1: Effect of gibberellic acid (GA3) on spikelet fertility of rice plants treated with ethephon at the microsporogenesis stage of development. Error bar indicates  $\text{LSD}_{(p=0.05)}$ .

### 7.4.3 Effects of ethephon and GA3 on panicle exertion

The panicles of all the plants of every genotype treated with ethephon exerted poorly from the flag leaf sheath, with more than 25 to 40% of the lengths of panicles remaining enclosed in flag leaf sheaths (Figure 7.2). The proportion of the panicles covered by the flag leaf increased steadily as the dosage of ethephon increased. Differences in panicle exertion resulting from treatment with ethephon was negligible between the genotypes.



Gibberellic acid significantly increased ( $p < 0.001$ ) the rate of panicle exertion in all the cultivars, effectively countering the negative effects of ethephon. However, application of GA3 in split doses produced no more effect on panicle exertion than a single application. Also, the difference between the 90 and 150 ( $\mu\text{L L}^{-1}$ ) application levels of GA3 for influencing panicle exertion was not significant ( $p = 0.05$ ).

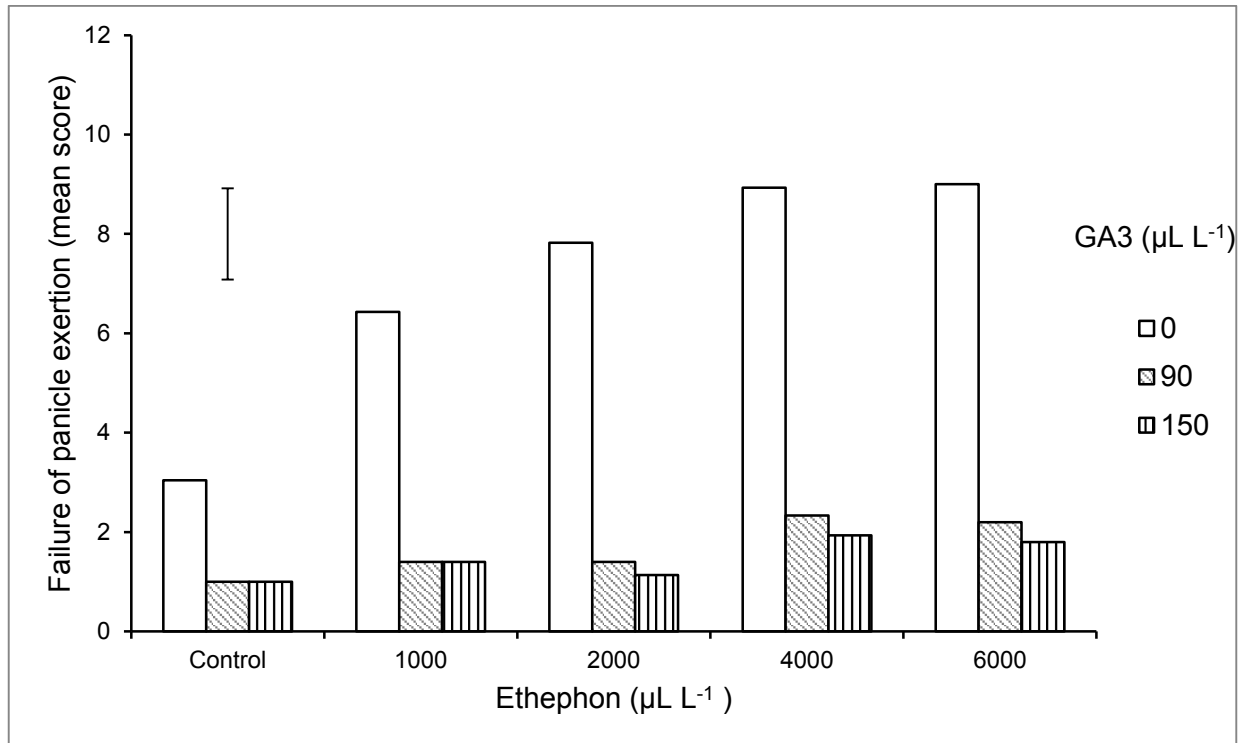


Figure 7.2: Effects of ethephon and GA3 on the level of panicle enclosure by the flag leaf sheaths of rice plants (failure of exertion). Error bar indicates  $\text{LSD}_{(p = 0.05)}$ .

#### **7.4.4 Effects of ethephon and GA3 on plant height**

Plant height showed a linear decline with increasing dosage of ethephon (Figure 7.3). The genotypes significantly differed ( $p < 0.001$ ) for plant height, but the trend of differences was neither affected by ethephon, nor by GA. Differences in plant height resulting from single or split application of GA were also non-significant ( $p = 0.05$ ), irrespective of the dosage of GA applied. GA applied at 150 ppm, however, led to excessive increase in plant height (Figure 7.4).

#### **7.4.5 Effects of ethephon and GA on outcrossing**

Increased levels ( $p < 0.01$ ) of outcrossing (cross pollination) were observed in ethephon treated plants amended with GA3 (Figure 7.5). The highest rates of outcrossing (up to 25%) were recorded for plants treated with a combination of 4 000 or 6 000  $\mu\text{L L}^{-1}$  ethephon and

90 or 150  $\mu\text{L L}^{-1}$  GA3. Differences between the single and split doses of GA3 were not significant ( $p = 0.05$ ) for the rates of outcrossing measured.

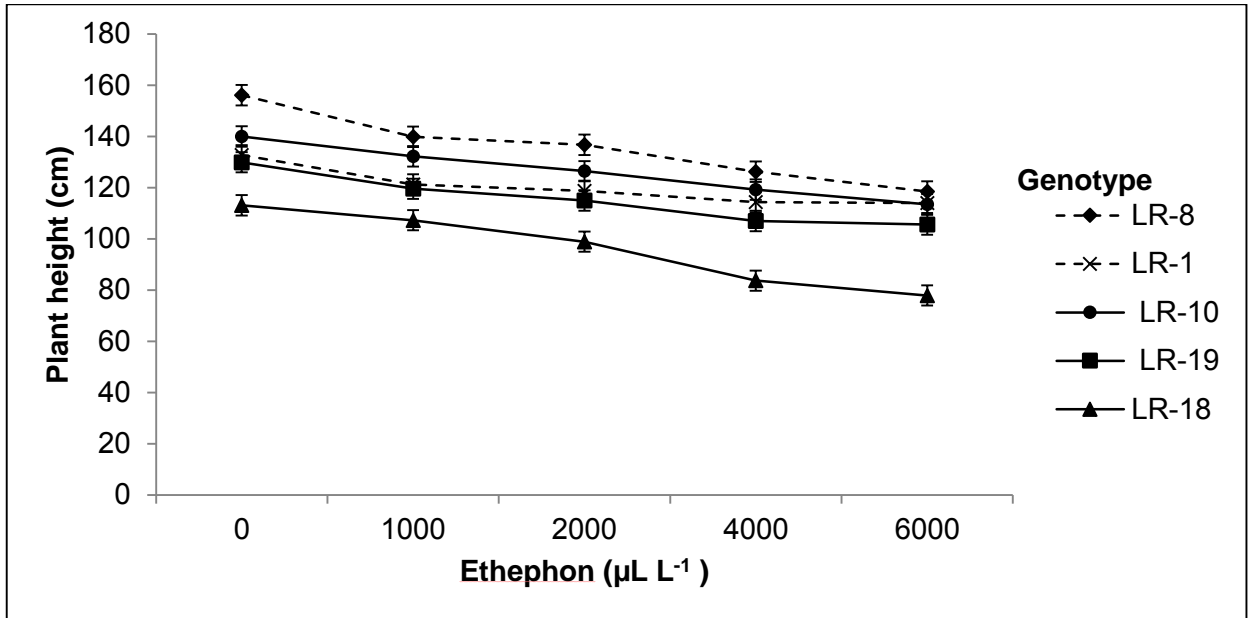


Figure 7.3: Effect of ethephon on plant height at 10 days after anthesis. Error bars represent  $\pm 3.97$  SEM.

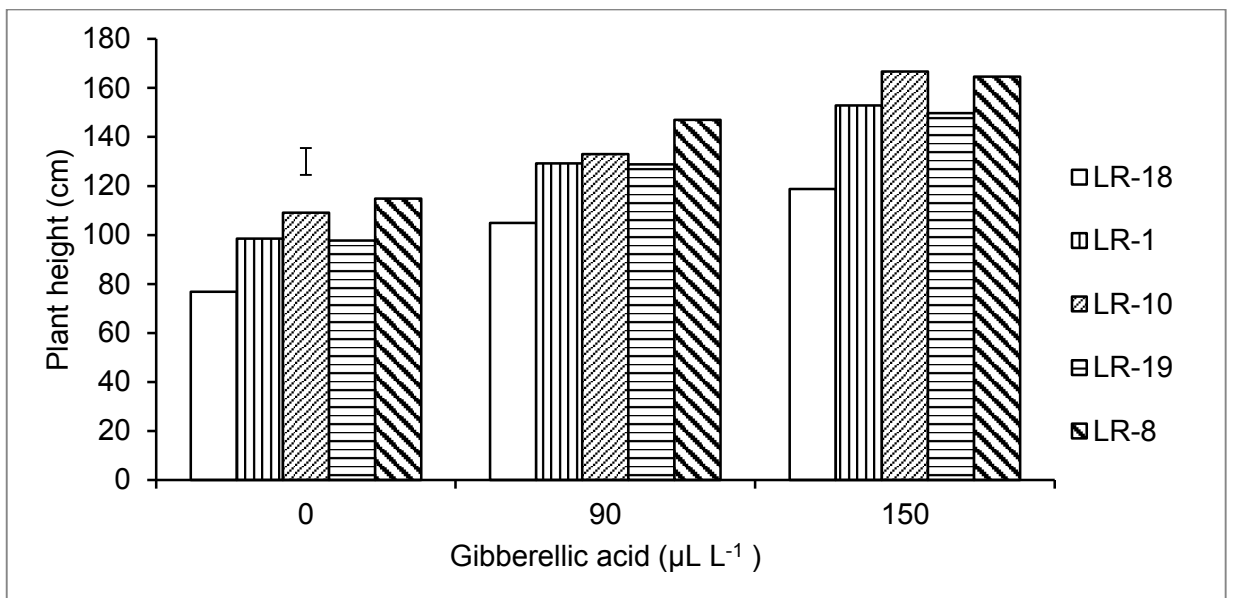


Figure 7.4: Effect of GA3 on stature of rice plants at 10 days after anthesis. Error bar indicates  $\text{LSD}_{(p = 0.05)}$ .

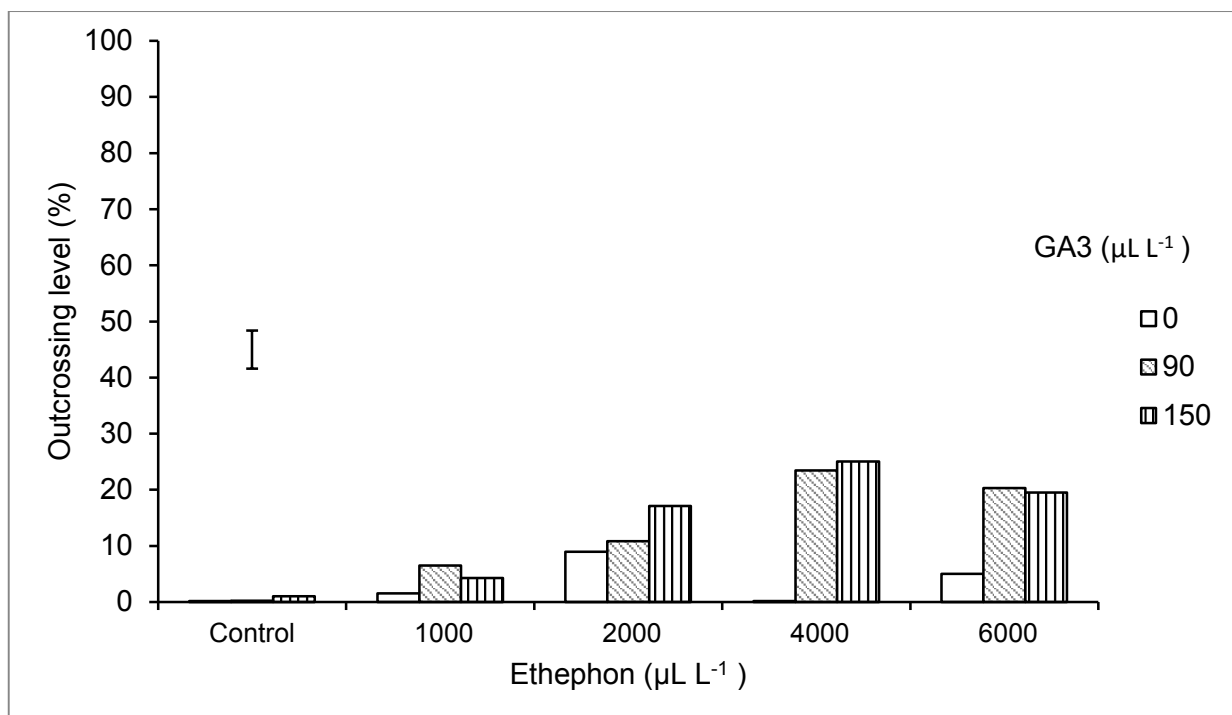


Figure 7.5: The effect of GA3 on the levels of outcrossing in the spikelets of rice plants treated with various doses of ethephon. Error bar indicates  $LSD_{(p = 0.05)}$ .

## 7.5 Discussion

Ethephon and GA are well known plant growth regulators used in agriculture for various applications, including crop hybridization. As a result of its availability and safety, ethephon was selected and evaluated in this study for its capacity to facilitate cross pollination in rice by inducing male sterility of designated female plants. However, ethephon has been reported to suppress plant height and limit female fertility in plants (Brown and Earley, 1973; Kaul, 1988; Kurepin *et al.*, 2013); hence, GA was tested for its ability to reverse the negative effects of ethephon on rice (Beek, 1986).

### 7.5.1 Effect of ethephon on the induction of male sterility

Male sterility, the failure of male gametes to function, may result from one or more of the following events: disruption of meiosis in pollen mother cells, leading to the arrest of anther development; reduced deposition of starch and poor vacuole formation in the microspores, resulting in the failure of pollen germination or cessation of pollen tube elongation and therefore, the failure of fertilization; and delayed dehiscence or indehiscence of normal anthers (Sharma and Sharma, 2005).

In this experiment, ethephon induced male sterility in test genotypes by significantly reducing pollen viability, or conversely, increasing pollen sterility. The recorded loss of

pollen viability could be attributed to the disruption of pollen development resulting from shock suffered by plants upon exposure to ethephon. Plants appeared physically stressed within 2 – 7 days after application of ethephon, and symptoms of pitting appeared on the leaves and sheaths of the treated plants (data not shown). Parmar *et al.* (1979) reported similar symptoms on rice treated with high doses of ethephon, ranging from 2000 – 10000 ppm. The symptoms of pitting observed in the current study followed an increasing trend with increasing levels of ethephon.

Ethephon is a recognized releaser of ethylene, which when exogenously applied during critical periods of pollen formation, inhibits starch accumulation and / or the formation of sperm nuclei in the pollen grains (Bennett and Hughes, 1972; Tu and Banga, 1998). From experiments with *Petunia hybrid*, Kovaleva *et al.* (2007) showed that between the period of meiosis and early microspore stage, endogenous ethylene induced pollen abortion by causing degeneration of the tapetum. Abdullah *et al.* (2001) also found that in rice, stress during the critical period of spikelet development led to pollen sterility by inhibiting starch synthase activity, thereby limiting the translocation of soluble carbohydrates in the developing spikelets. Stress has also been reported to cause defects in the lipids components of pollens, leading to pollen sterility in *Arabidopsis* (Aarts *et al.*, 1995; Fiebig *et al.*, 2000).

### **7.5.2 Effect of growth stage on efficacy of ethephon for male sterility induction**

All of the genotypes were more sensitive to ethephon at microsporogenesis than at panicle differentiation, as indicated by the levels of pollen sterility induced by ethephon application at each stage. Microsporogenesis is the period when the pollen mother cells are undergoing meiosis, at which time they are extremely sensitive to stress. Stresses such as drought (Yue *et al.*, 2013) and low (Nishiyama, 1984; Gothandam *et al.*, 2007) or high (Matsui and Omasa, 2002; Rang *et al.*, 2011) air temperatures have been associated with pollen abortion during this period. Yoshida (1981) described panicle differentiation as the time when the panicle branches, and spikelets are formed. Any effect of stress on plants at this time can result in the loss of entire panicle branches and spikelets, as observed in this study. Hence, in order to ensure high levels of pollen sterility, application of ethephon should be timed to coincide with the period around microsporogenesis.

### **7.5.3 Impacts of ethephon and GA on spikelet fertility and outcrossing**

Spikelet fertility, or the level of seed set, was evaluated as a measure of pistil fertility in this study. Low levels of spikelet fertility and outcrossing were observed in plants treated with ethephon alone, suggesting that ethephon negatively affected the pistils of the rice florets. Similar negative effects by ethephon on female fertility of rice has also been reported by Xi *et al.* (1981).

Overall, pollen sterility measured in this study was not complete. The viable pollen within the spikelets most likely out-competed the pollen from the designated male parent, accounting for the low levels of outcrossing recorded. The relative positions of the male and female parts of the rice flower naturally tends to limit the crossing of two different plants. At anthesis, dehiscence of rice anthers tends to occur concurrently with glume opening, at which time the stigmas are also receptive (Matsui *et al.*, 1999; Matsui *et al.*, 2000), thereby leading to the promotion of self-pollination rather than cross-pollination. In genotypes whose anthers have large basal pores, self-pollination occurs even more efficiently (Matsui and Kagata, 2003). In such genotypes, the basal pores are usually situated above the stigma, and open during glume opening, so that the pollen grains fall directly onto the stigma (Morinaga and Kuriyama, 1944). High pollen loads from the male parents could therefore be required to increase the levels of outcrossing in partially male sterile female plants.

While ethephon proved capable of causing some gametocidal effects in the rice plants, GA3 appeared to counter, or mitigate the accompanying negative effects of ethephon. Higher levels of spikelet fertility and outcrossing were observed in GA3 treated plants, compared with those treated with ethephon alone. A minimum of a two-fold increase in spikelet fertility was recorded when plants treated with high levels of ethephon were supplementally treated with GA3 (Figure 7.5).

Essentially, rice is a self-pollinating crop. Although natural outcrossing occurs in the species, it is usually less than 1% (Taillebois and Guimaraes, 1988; Rong *et al.*, 2004). The increase in fertility facilitated by GA3 could be due to GA3 directly increasing the vitality of the pistil, by enhancing stigma exertion. Gibberellic acid has been reported to play a major role in increasing stigma exertion and receptivity in rice, leading to significantly higher rates of outcrossing in male sterile rice plants (Virmani and Sharma, 1993; Gavino *et al.*, 2008a), as stigmas protruding out of the glume are more easily accessible by pollen from other blooming panicles. While Beek (1986) reported the use of GA3 to enhance female fertility of wheat plants treated with ethephon, Colombo and Favret (1996) showed that GA3 increased pollen sterility in bread wheat when applied successively at high concentrations.

It is, therefore, unlikely that GA3 influenced the increase in spikelet fertility by reviving the viability of the pollen previously suppressed by ethephon.

#### **7.5.4 Effects of ethephon and GA3 on panicle exertion and plant height**

Besides inducing pollen sterility, ethephon suppressed the growth of the uppermost internode of all genotypes by causing the failure of panicles to extend out of the flag leaf sheath. Similar effects of ethephon on panicle exertion and plant height have been reported for millet (Thakur and Rao, 1988), rice (Chan, 1983) and wheat (Kaul, 1988). Poor panicle exertion, as with spikelet sterility, has also been reported in rice as a result of drought and other stresses occurring during the reproductive stage of crop development (Ahmed *et al.*, 1996), which can lead to early senescence.

The effect of ethephon on panicle exertion and plant height was phytotoxic, and these effects are undesirable. The failure of panicles to extend out of the flag leaf sheaths, or for plant height to increase any further was certainly equivalent to a net cessation of growth. In effect, ethylene released by ethephon is a plant growth hormone that also accelerates senescence. It was therefore to be expected that the growth of plants ceased or slowed substantially following the application of ethephon sprays.

Gibberellic acid significantly increased panicle exertion and plant height in all genotypes; hence, it effectively countered the negative effects of ethephon on plants by fully extending the panicles out of the flag leaf sheaths, as was anticipated. Gibberellic acid has been similarly used in hybrid rice seed production to enhance panicle exertion of female CMS lines (Yuan *et al.*, 2003). Essentially, GA3 is a plant growth regulator that is involved in several growth and developmental processes, including the regulation of stem elongation (Spielmeyer *et al.*, 2002; Sun, 2010), of plants (Naeem *et al.*, 2001; Emongor, 2007). GA has been found to increase growth of cells by mainly stimulating the production mRNA molecules responsible for coding for hydrolytic enzymes, which eventually increases plant growth (Solaimalai *et al.*, 2001).

Differences in panicle exertion and plant height, as influenced by either split or single dose of GA3, were negligible, implying that a single application of GA3 is adequate to reverse the negative effects of ethephon for breeding purposes. Split application of GA3, however, has been used to increase seed yield, not only by increasing panicle exertion and seed set, but also by increasing the number of effective tillers (Gavino *et al.*, 2008b; IRRI, 2009). Application of GA3 at 90 or 150  $\mu\text{L L}^{-1}$  was equally effective for increasing panicle exertion; however, the higher dose excessively increased plant height, especially in plants treated with lower doses of ethephon. Appropriate control of GA3 dosage could be important for

plant height adjustment in a large scale breeding project. In the event that the male parents become shorter than the female, small doses of GA3 could be used to adjust their heights, in order to optimize pollen dispersion onto the females (Sindhua and Kumar, 2003; Virmani *et al.*, 2003b).

#### **7.5.5 Influence of genotype on efficacies of Ethephon and GA3**

The capacity of ethephon to reduce pollen viability and consequently cause male sterility was largely the same for all genotypes tested. Also, the rice genotype did not seem to influence the effect of ethephon on panicle exertion and plant height, although genotypes inherently differed for these traits. Hence, it is likely that ethephon + GA3 can be used as a male gametocide for a wide range of rice genotypes.

### **7.6 Conclusion**

In a rice breeding programme where large numbers of crosses are required, a male sterility technology is needed, not only to accelerate progress, but also to minimize the costs involved in hand emasculation. The use of male gametocides to effect mass emasculations needed to facilitate large numbers of crosses may be advantageous for application in recurrent selection programmes that often require several cycles of random polycrosses. Using chemicals to effect male sterility in the chosen female parents of rice plants could be useful for ensuring cross pollination of large numbers of plants quickly and cheaply. In this study, ethephon was used as a male gametocide; it induced acceptable levels of pollen sterility in all the genotypes tested. However, its efficacy as a CHA by itself would be limited because it also tended to diminish seed set and outcrossing, mostly as the result of its negative effects on panicle exertion. Gibberellic acid applied at 90 or 150  $\mu\text{L L}^{-1}$  reversed the negative effects of ethephon by stimulating the extension of the panicles out of the flag leaf sheaths, thereby increasing the levels of seed set and outcrossing in the treated plants. The application of a combination of ethephon and GA3 can be useful as a chemical tool for breeding rice. The best outcome resulted when ethephon was applied at 4 000  $\mu\text{L L}^{-1}$  at the microsporogenesis stage of rice development, followed by application of GA3 at 90  $\mu\text{L L}^{-1}$  at anthesis. Although results from these preliminary studies indicated the possibility of effecting random polycrosses in these genotypes with the aid of ethephon and GA3, further studies may be required to further test the usefulness of this CHA treatment under field conditions. Hence, when perfected, this CHA treatment has potential in rice hybrid development as well. Testing of other compounds will be needed in order to find a compound capable of inducing higher levels of male sterility, ideally 100% sterility, with no loss of female fertility and seed development.

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## CHAPTER 8

### EFFECTS OF *TRICHODERMA HARZIANUM* ON THE RESPONSES OF RICE (*ORYZA SATIVA* L.) TO BLAST DISEASE AND DROUGHT STRESS

#### 8.1 Abstract

Integrating host plant resistance with cost-effective methods of control could enhance the management of blast disease and drought stress affecting rice (*Oryza sativa* L.). Both greenhouse and field experiments were conducted to evaluate two biological control products (Eco-77® and Eco-T®), containing different strains of *Trichoderma harzianum*, for their capacity to mitigate the effects of rice blast and drought stress on rice, and to determine the effect of rice blast infection on drought tolerance. Five rice genotypes were treated with Eco-77® and Eco-T®. Eco-77®, applied twice as a foliar spray onto rice infected with blast, significantly reduced ( $p < 0.01$ ) disease severity by limiting the size of sporulating lesions, resulting in a reduced area under the disease progress curve (AUDPC). The efficacy of Eco-77® at limiting the severity of rice blast tended to increase when combined with Eco-T®, applied as seed dressing or as root drench, prior to the sowing or transplanting of rice. Eco-77® was not effective at reducing disease severity on the universally susceptible genotype, LTH, compared to the other genotypes, which seemed to display various levels of host resistance. Eco-T® significantly increased drought tolerance by reducing the rate of plant mortality, and increasing root mass density, shoot dry mass and grain yield per unit area of the plants subjected to post-blast infection drought stress under field conditions. The mortality rate of rice blast-infected plants subjected to drought stress, by 62 days after sowing, was significantly higher than that recorded in the blast-free plants. The mortality rate, root mass density, shoot dry mass and grain yield of rice plants subjected to pre-blast-infection drought stress were significantly higher than those of the plants subjected to post-blast-infection drought stress. These results indicate that the development of blast disease, followed by drought stress, is more damaging to rice productivity and yield, than the impact of drought stress succeeded by blast disease. Because the *Trichoderma* treatments were more effective in reducing the effects of rice blast in plants that showed higher levels of blast resistance, integrating *Trichoderma* with host plant resistance is a good strategy to reduce the impact of blast and drought on rice crops.

Keywords: biological control agents, disease severity, drought stress, Eco-T®, Eco-77®, rice

## 8.2 Introduction

Rice blast, caused by *Pyricularia oryzae* Cavara and drought are serious constraints to rice production in all rice growing regions of the world (Serraj *et al.*, 2008; Khush and Jena, 2009). These problems are especially acute in developing countries, such as Liberia, where the crop is predominantly grown by subsistence farmers, under rainfed conditions in upland fields. Host plant resistance has remained the most important tool for managing plant diseases. However, the frequent failure of vertical resistance used to control rice blast (Bonman, 1992; Han *et al.*, 1995) has often resulted in severe crop losses, reflecting a clear need to develop new varieties with effective levels of horizontal resistance. Control of rice blast using minor genes however, is usually only partial resistance, although it is useful for reducing losses to the disease (Bonman *et al.*, 1992; Wang *et al.*, 1994). Like resistance to rice blast, drought tolerance, currently conferred by plant breeding, is often inadequate to protect rice plants against yield losses caused by drought stress. Augmenting the levels of control proffered by varieties with horizontal resistance to blast, and increasing tolerance to drought would therefore be valuable to ensure reliable yields from rice crops in Liberia or similar agro-ecologies.

Biological control agents (BCAs) are widely known to offer a wide range of benefits to plants exposed to suboptimum conditions, ameliorating both biotic and abiotic stresses, and can be useful when integrated with host plant resistance (Folli-Pereira *et al.*, 2013). *Trichoderma harzianum* Rifai, neo typified as *T. harzianum* s.l. (Gams and Meyer, 1998), is recognized as an important BCA against several agriculturally important root and foliar pathogen species (Inbar *et al.*, 1994; Grondona *et al.*, 1997; Reino *et al.*, 2008; Yobo *et al.*, 2013; Zafar *et al.*, 2013). *Trichoderma harzianum* may control diseases of plants by either direct mycoparasitism (Mukherjee *et al.*, 1995), or by antibiosis through its production of secondary metabolites (Ordentlich *et al.*, 1992; Scarselletti and Faull, 1994). *Trichoderma harzianum* may also act by lysing pathogenic cells (Elad *et al.*, 1982), by inducing host plant resistance (Zimand *et al.*, 1996; De Meyer *et al.*, 1998), or by combining two or more of these mechanisms.

Some strains of *T. harzianum* have also proven effective for mitigating the harmful effects of abiotic stresses such as salinity, cold, and drought (Bjorkman *et al.*, 1998; Yildirim *et al.*, 2006; Mastouri *et al.*, 2010). The capacity of *T. harzianum* to mitigate the effect of abiotic stresses may be mediated by its ability to enhance the growth of crops under adverse conditions (Chang *et al.*, 1986; Yedidia *et al.*, 2001; Mwangil *et al.*, 2011).

Despite the potential of *T. harzianum* for the management of agriculturally important stresses, research on its usefulness for managing the effects of rice blast and drought stress

in rice, has received little attention. The present studies were therefore carried out, under both greenhouse and field conditions, to determine the usefulness of two strains of *T. harzianum* (Eco-77® and Eco-T® from Plant Health Products (Pty) Ltd, South Africa) for managing rice blast infections, as well as the effects of pre- and post-blast infection drought stress on rice.

### **8.3 Materials and methods**

Both controlled environment (CE) and field experiments were conducted to evaluate the capacity of two strains of *T. harzianum* to manage the effects of rice blast and drought stress on rice. Concurrently, experiments were conducted to determine the effect of rice blast infection on the response of rice to drought stress.

#### **8.3.1 Plant materials**

Five rice genotypes, including two landraces (LR 8 and 18) and an improved cultivar (LAC 23) from Liberia, one improved cultivar from Burkina Faso (FKR 19), and Lijiangxintuanheigu (LTH), a traditional variety from Japan, were used in the experiments. The genotypes from Liberia were upland varieties; FKR is mainly grown in irrigated paddies; and LTH is a universal blast-susceptible genotype widely used as a susceptible check for rice blast differential variety studies. Lijiangxintuanheigu and FKR 19 were used as susceptible checks for rice blast and drought stress, respectively. LAC 23 was used as a resistant check for both rice blast and drought stress.

#### **8.3.2 Description of the study sites**

The CE and field studies were conducted at the research facilities of University of KwaZulu-Natal in Pietermaritzburg, South Africa. The CE experiment was carried out in a greenhouse tunnel (25 / 30 °C; 65% RH) from August to December of 2012, whilst the field trial was conducted at the university's Ukulinga Research Farm (29° 40' S, 30° 24' E; 806 m above sea level) from November 2013 to April 2014. Based on annual means of long term climatic data, Ukulinga has a mean annual temperature and rainfall of 18°C and 738 mm, respectively. Climatic data for the duration of the field experiment are presented in Table 8.1.

Table 8.4: Monthly climatic data during the field trial (November 2013 – April 2014). Data source: Agricultural Research Council – Institute for Soil, Climate and Water (ARC–ISCW), South Africa.

Months	Temperature (°C)		Solar radiation (MJ m <sup>-2</sup> )	Reference Evapo-transpiration (mm)
	Minimum	Maximum		
November	14.88	25.00	19.00	112.30
December	15.93	23.40	16.78	100.50
January	18.10	28.00	20.46	131.10
February	18.20	28.20	20.83	116.10
March	17.13	26.20	16.30	101.70
April	13.62	24.40	15.48	87.49
<b>Mean</b>	<b>16.31</b>	<b>25.87</b>	<b>18.14</b>	<b>649.19<sup>a</sup></b>

<sup>a</sup> Values for Reference evapotranspiration are totals, not averages.

### 8.3.3 Experimental design and treatments

The CE study was laid out in a randomized complete block design with four replications, comprising a factorial arrangement of the five rice genotypes, four factors of *Trichoderma harzianum* (Control, Eco-T®, Eco-77®, and Eco-T® plus Eco-77®) and two water regimes (well watered and drought stressed). A split-split plot design was utilized for the field experiment, using the same sets of treatments tested in the CE. The main plots were the water regimes, and the subplots the *Trichoderma* factors. The genotypes were the sub-sub plots, randomly assigned to each subplot.

Test plants for the CE study were grown in 4.8 L plastic pots with 15 plants per pot, whilst plants in the field were grown in 1.5 m X 3.0 m plots, with intra-row and inter-row spacings of 0.15 m X 0.15 m. Soils in well-watered pots and plots were kept continuously moist throughout the experiment. Drought stress was imposed prior to, or after plant infection by rice blast. The pre-infection drought stress was applied beginning at 10 days before plant exposure to disease spreader plants, by withholding water and only irrigating on the day of exposure to disease spreaders. For the post-infection drought stress treatment, a water deficit was applied intermittently, beginning at 44 days after sowing or transplanting (DAS / DAT), and temporarily restored when all of the plants showed symptoms of complete leaf rolling, scored as 9 according to the International Rice Research Institute's (IRRI) drought evaluation system for rice (IRRI, 2006).

Eco-T® and Eco-77® were obtained as wettable powder formulations of *T. harzianum* strains KD and B77, respectively; each containing 2 X 10<sup>9</sup> conidia g<sup>-1</sup>. Eco-T® was applied as a seed dressing at 2.0 g kg<sup>-1</sup> of seed, or as seedling root drench prior to sowing or



transplanting at 0.25 g L<sup>-1</sup>. Eco-77® was applied as a conidial suspension with 0.5 g L<sup>-1</sup> of sterile water, applied twice a foliar spray: when blast symptoms appeared as tiny specks or light green water-soaked spots, and at 5 days after the first application. The control seeds or plants were treated with a placebo containing a sterile mixture of each *Trichoderma* formulation and water. The foliage of plants treated with Eco-T® were also sprayed with a placebo in order to exclude any effect due to the spraying.

For each experiment, soils were amended in keeping with the results of soil analyses. Phosphorus was applied as basal application at 20 kg ha<sup>-1</sup>; and nitrogen was applied prior to sowing at a rate of 110 kg ha<sup>-1</sup>, in order to stimulate vigorous vegetative growth, and thereafter, at 30 kg ha<sup>-1</sup> at 62 DAT.

#### **8.3.4 Inoculation of test plants**

An isolate of *P. oryzae* (SIK-111), sourced from the AfricaRice Centre in Cotonou, Benin, was used to infect the test plants with rice blast. A uniform blast nursery procedure was applied to initiate the disease cycle, using pre-infected LTH seedlings as disease spreaders. The test plants were exposed to the disease spreaders at 21 DAS in the CE, and at 21 DAT in the field. The spreader plants were established in single alternating rows before and after each genotype, both in the CE and in the field. In the field, the disease spreaders were prepared in polyethylene bags and transferred with intact growth media to their designated rows to minimize transplanting shock.

#### **8.3.5 Sampling and data analyses**

The number of lesions with sporulating centres per plant (LN) were determined at 10 days after exposing plants to the disease spreaders (DAEP) in the CE and at 10 and 14 DAEP in the field. The size of lesions with sporulating centres (LS) was estimated at 10 DAEP, both in the CE and in the field, using the keys adapted by Roumen (1992) (Figure 8.1). The predominant lesion on the older leaves were chosen for LS measurement. Data on LN and LS were recorded from 10 randomly chosen plants per pot or plot.

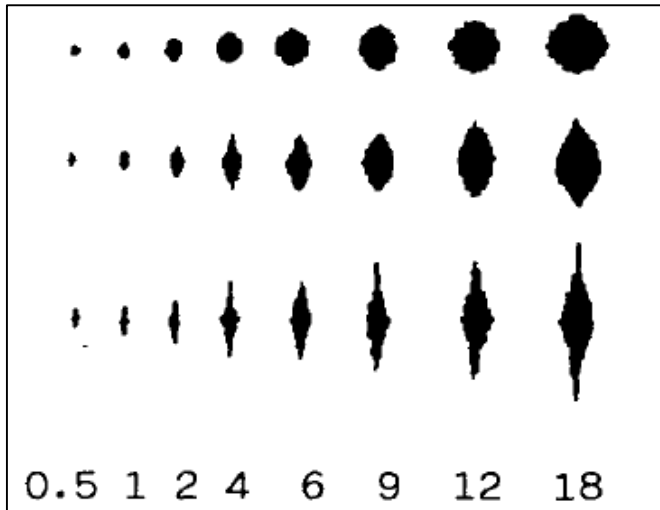


Figure 8.1: Assessment key for estimating the size of sporulating rice blast lesions. Source: Roumen (1992).

Disease severity on all fully expanded leaves per plant was estimated five times at three day intervals (31, 34, 37, 40 and 43 DAS), beginning at 8 – 10 days after exposure of plants to the inoculum source. Ten plants per pot in the CE, and all but the border plants of each plot in the field, were visually scored for disease severity, using a diagrammatic scale (depicting 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 82% of diseased leaf area) developed by Notteghem (1981). Leaf area was considered 100% affected when entire leaves died from lesions on the leaf collars. The sequential disease severity scores were used to calculate the area under the disease progress curve (AUDPC), in order to compare the effectiveness of the treatments and genotypes, based on the formula developed by Shaner and Finney (1977):

$$\text{AUDPC} = \sum N_i = 1((Y_i + 1 + Y_{i+1})/2)(X_{i+1} - X_i) \quad (8.1),$$

where  $Y_i$  = the percent of diseased leaf area on the  $i$ th day of assessment,  $X_i$  = time of the  $i$ th assessment in days from that of the first assessment, and  $N$  is the total number of times disease was assessed. The highest diseased leaf area during disease progress was recorded as maximum disease severity.

Ten days following relief of post-blast infection drought stress, drought recovery of the plants was assessed by counting the number of surviving plants. Differences in the mortality rates between blast-infected and blast-free plants under CE conditions were analysed to determine the effect of rice blast infection on rice responses to drought stress.

At heading, the field grown plants were sampled from an area of 1.0 m<sup>2</sup> per plot to determine dry shoot mass and root mass density. Root samples were collected from the 0 to 30 cm

depths of the soil profile, with the aid of a core sampler, according to the procedures outlined by Henry *et al.* (2012). Three soil cores were collected from each plot from the centre of four hills and sorted in plastic bags. Roots were subsequently separated from the soil by flotation, following the protocol described by Bohm (1979). Samples were soaked in, and mixed with sufficient water, and the floating roots isolated by sieving. At maturity, an area of 1.0 m<sup>2</sup> per plot was harvested to measure grain yield. Root and shoot dry masses were determined after oven drying the samples at 70°C for 48 hr. Root mass density was expressed as the mass of root dry matter per volume of the soil core sampled. Grain yield was determined after reduction of grain moisture content to 14% by drying the seeds at ambient temperature.

All data were subjected to analyses of variance using the statistical package GenStat® Version 14 (VSN, International). The means of factors showing significant difference were separated by Fisher's least significant difference (LSD) test at the 5% probability level.

## **8.4 Results**

### ***8.4.1 Effect of Trichoderma on the number of sporulating lesions per plant***

The number of sporulating lesions developed on plants at 10 days after exposure to *P. oryzae* were not affected by treatment with either *Trichoderma*, nor by the water regime, under both CE and field conditions. However, in both the CE and field, the number of sporulating lesions per plant differed significantly with genotype ( $p < 0.001$ ) (Figure 8.2). The susceptible check, followed by LR 8, developed the highest number of sporulating lesions per plant. The number of sporulating lesions recorded per plant were lower under field conditions than CE conditions. The pattern of differential susceptibility between the genotypes for the number of sporulating lesions per plant observed in the CE was similar to that observed under field conditions. No significant interaction effects were found between the treatments. At 14 days after exposure to disease spreaders, plants treated with Eco-77® alone, or with Eco-77® plus Eco-T®, displayed significantly lower numbers of sporulating lesions on the top of most fully expanded leaves (Figure 8.3).

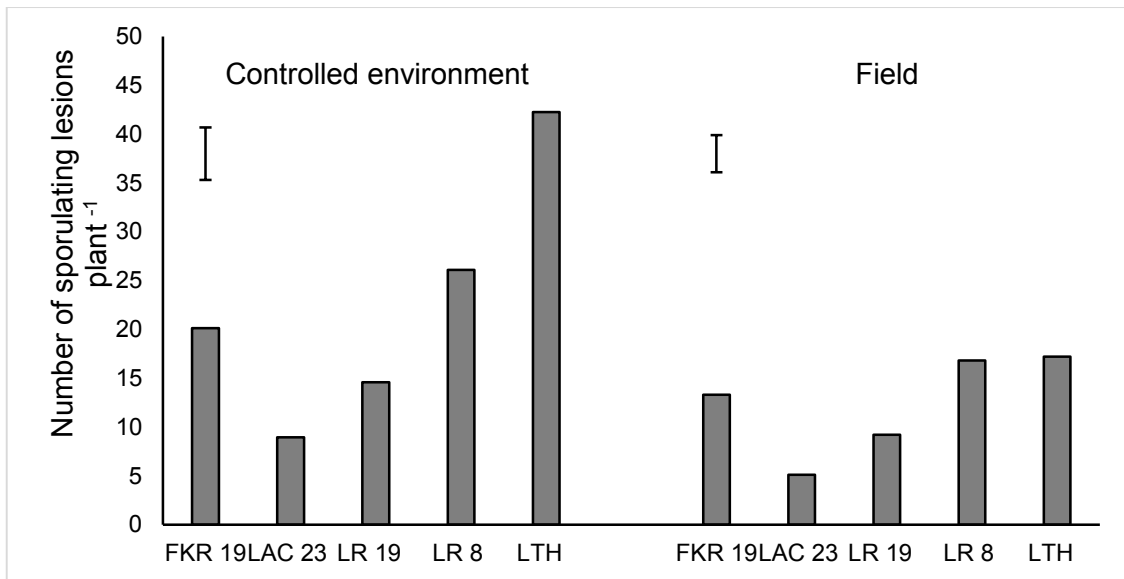


Figure 8.2: Number of sporulating lesions developed by five rice genotypes under controlled environment and field conditions at 10 days after exposure to disease spreaders. The error bars represent  $LSD_{(p = 0.05)}$ .

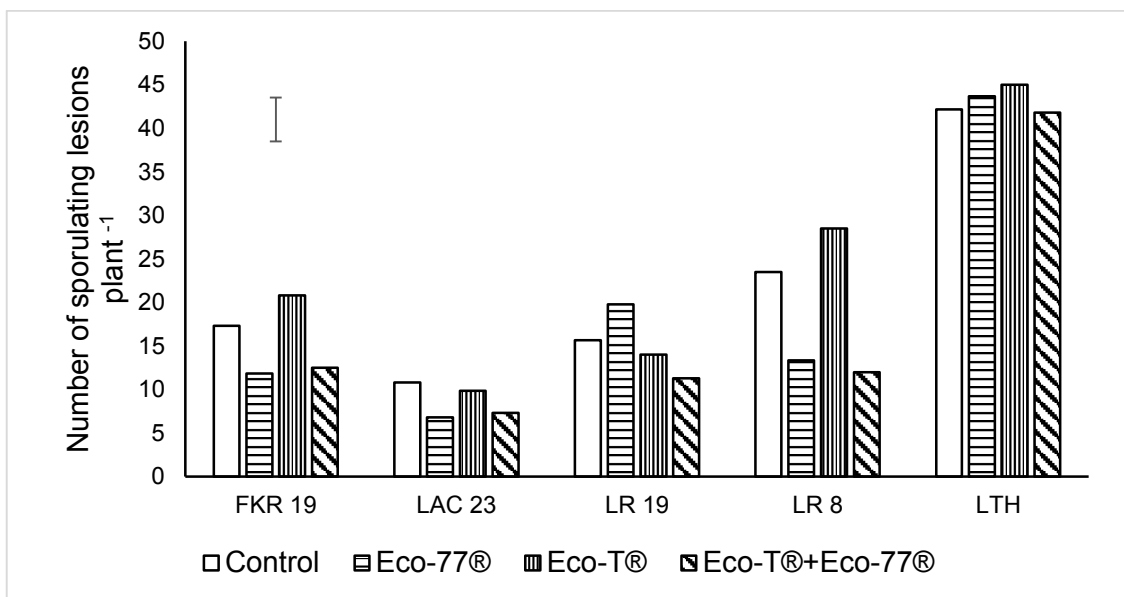


Figure 8.3: Numbers of sporulating lesions developed on five rice genotypes under field conditions at 14 days after exposure to disease spreaders, following treatment with *T. Harzianum* strains. The error bar represents  $LSD_{(p = 0.05)}$ .

#### 8.4.2 Effect of *Trichoderma* on size of sporulating lesions

Data on the size of lesions developed on the plants are presented in Figure 8.4. Eco-77® alone, or in combination with Eco-T®, significantly reduced the size of sporulating lesions ( $p < 0.01$ ) that developed on the genotypes. Eco-T® alone had no influence on LS measured in this study. The pattern of reaction for LS of the *Trichoderma* treatments and varieties did not differ between measurements obtained from the CE and the field trials. On

average, the LS measured on plants in the field were slightly larger than the measurements obtained from the CE; however, differences between the two were negligible ( $p = 0.05$ ).

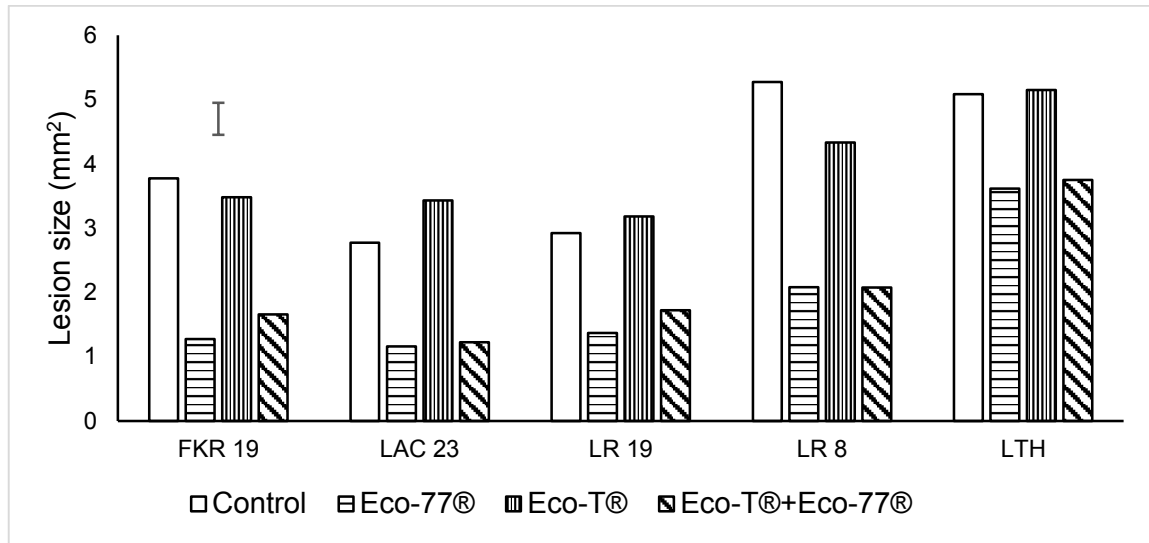


Figure 8.4: Effect of two strains of *T. harzianum* on the size of rice blast lesions on five rice genotypes. The error bar represents  $LSD_{(p=0.05)}$ .

#### 8.4.3 Effects of *Trichoderma* on disease severity and progression

Disease severity (percentage of leaf area with sporulating lesions) differed significantly over time with genotype, *Trichoderma* treatment and with pre-infection water regime, both in the CE and in the field trial (Table 8.2). Disease progressed significantly faster on susceptible genotypes than the resistant genotypes. Eco-77® and Eco-77® plus Eco-T® significantly slowed the progress of disease, under both well-watered and water stressed conditions, both in the CE and in the field (Figure 8.5). Overall, disease progress was faster in drought stressed than in well-watered plants.

Table 8.2: ANOVA for rice blast severity in the greenhouse and field

Greenhouse Trial						
Source of variation	Mean squares					
	D.f.	31 DAE	34 DAE	37 DAE	40 DAE	43DAE
Genotype	4	653.0***	2185.5***	4488.5***	5593.4***	9094.8***
<i>Trichoderma</i>	3	64.1*	891.2***	5142.7***	9422.8***	7312.4***
Water regime	1	99.0*	1763.3***	1717.6***	56.0	76.5
Genotype x <i>Trichoderma</i>	12	15.2	111.2	234.0	304.8	216.0
Genotype x water regime	4	86.2*	311.3	264.1	520.6	1054.5**
<i>Trichoderma</i> x water regime	3	20.8	1243.1	835.3	167.3*	128.7
Genotype x <i>Trichoderma</i> x water regime	12	24.5	1073.6	4163.2	1720.2	2413.3
Error	78	20.0	136.5	157.1	190.0	229.6

Field Trial						
Genotype	4	715.2***	1890.0***	5054.1***	6321.3***	8922.2***
<i>Trichoderma</i>	3	69.8*	736.8**	5431.5***	9501.2***	6895.0***
Water regime	1	97.6*	1688.4***	2011.0***	62.7	105.5
Genotype x <i>Trichoderma</i>	12	17.0	97.9	265.8	317.4	372.2
Genotype x water regime	4	91.0*	295.0	304.5	543.0	982.8**
<i>Trichoderma</i> x water regime	3	22.5	1111.7	888.5	176.7*	117.5
Genotype x <i>Trichoderma</i> x water regime	12	25.9	1021.1	4420.0	1781.0	1954.2
Error	78	22.8	122.3	177.5	195.6	190.0

D.f = degree of freedom; \*\*\*, \*\*, \* = significance levels of  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , respectively.

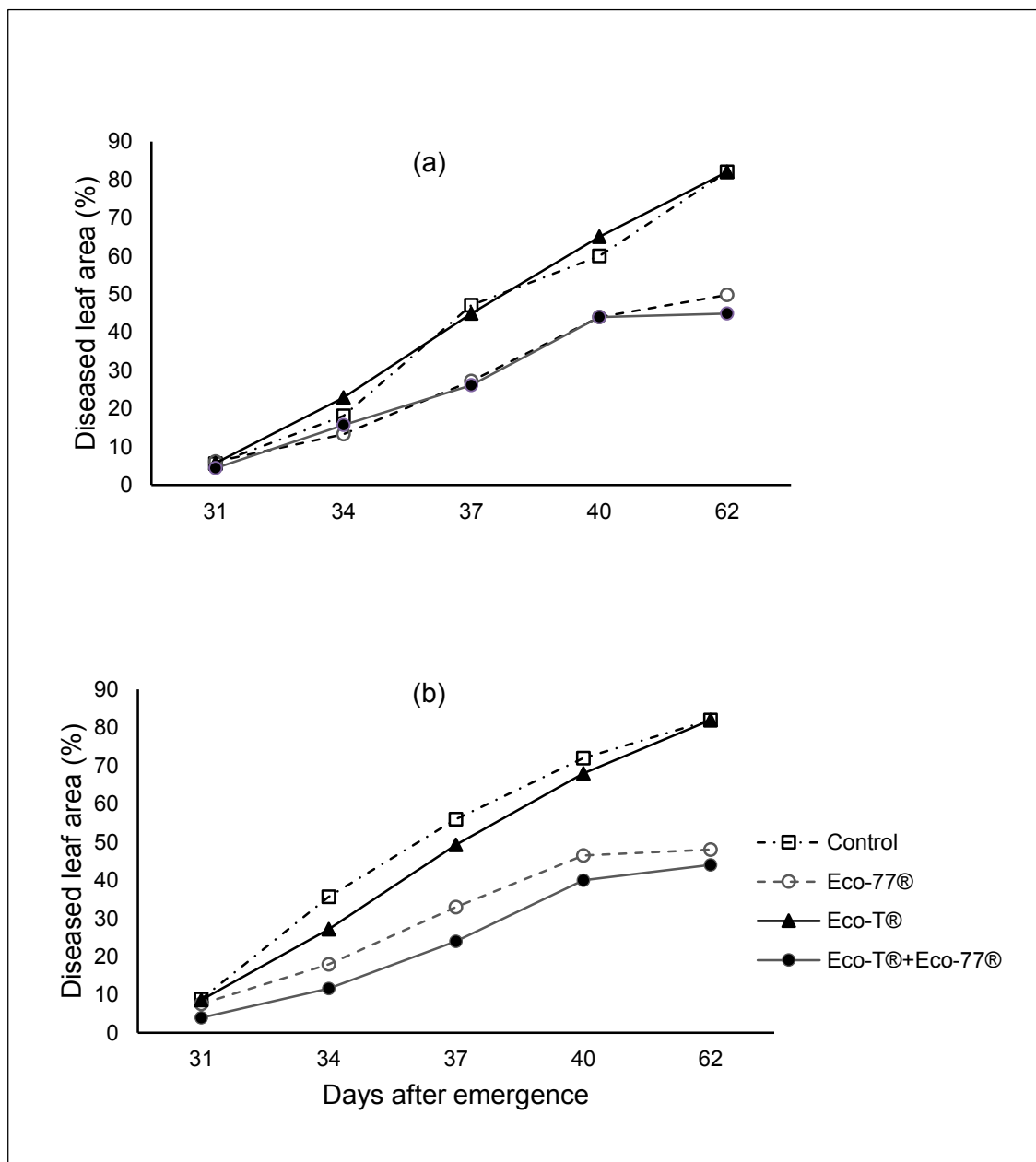


Figure 8.5: Effect of *Trichoderma harzianum* on the progress of rice blast severity on plants grown under well-watered (a) and drought stressed conditions

#### 8.4.4 Maximum disease severity

Maximum disease severity measurements taken under CE conditions are displayed in Figure 8.6. The *Trichoderma* treatments and genotypes differed significantly ( $p < 0.001$ ) for their effects on the maximum area of leaves infected by rice blast, both in the CE and in the field trials. Maximum disease severity was significantly lower in plants treated with Eco-77® alone or with Eco-77® plus Eco-T®. The water regime tended to have no significant influence on maximum disease severity; however, a significant interaction ( $p < 0.05$ ) was

observed between water regime and genotype for maximum disease severity. Disease severity was more pronounced in LR 8 when grown under well-watered than under drought stressed conditions.

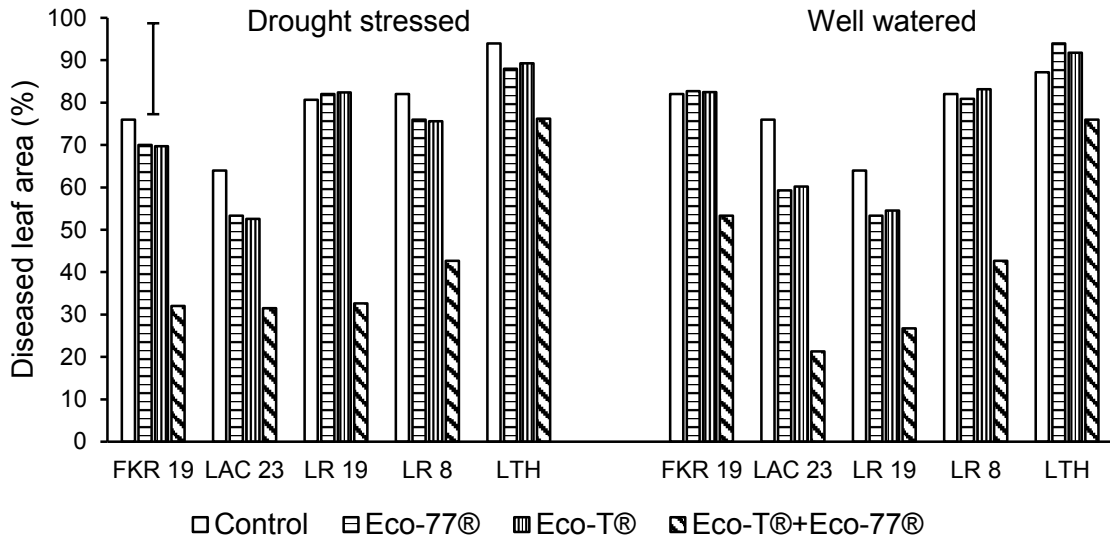


Figure 8.6: Maximum disease severity in five rice genotypes treated with or without *Trichoderma harzianum* treatments. The error bar represents  $LSD_{(p = 0.05)}$ .

#### 8.4.5 Area under the disease progress curve

The area under the disease progress curve varied significantly with genotype, *Trichoderma* treatment, and with water regime ( $p < 0.001$ ), as portrayed in Figure 8.7. Treatments with Eco-77® showed the lowest values of AUDPC with all the genotypes. The AUDPC values were significantly lower in every other genotype than the blast susceptible check, LTH, irrespective of water regime, both in the field and in the CE trials. On average, plants subjected to pre-blast infection drought stress developed significantly higher AUDPC values than those grown under well-watered conditions prior to infection by rice blast. Interactions between either combinations of the three factors were not significant, except for that between genotype and water regime ( $p < 0.05$ ). This was only observed in the CE trial in which the AUDPC values were higher under drought stressed conditions than under well-watered conditions for LTH, LR 8 and 18; and the opposite was true for LAC 23 and FKR-19 (Figure 8.7).



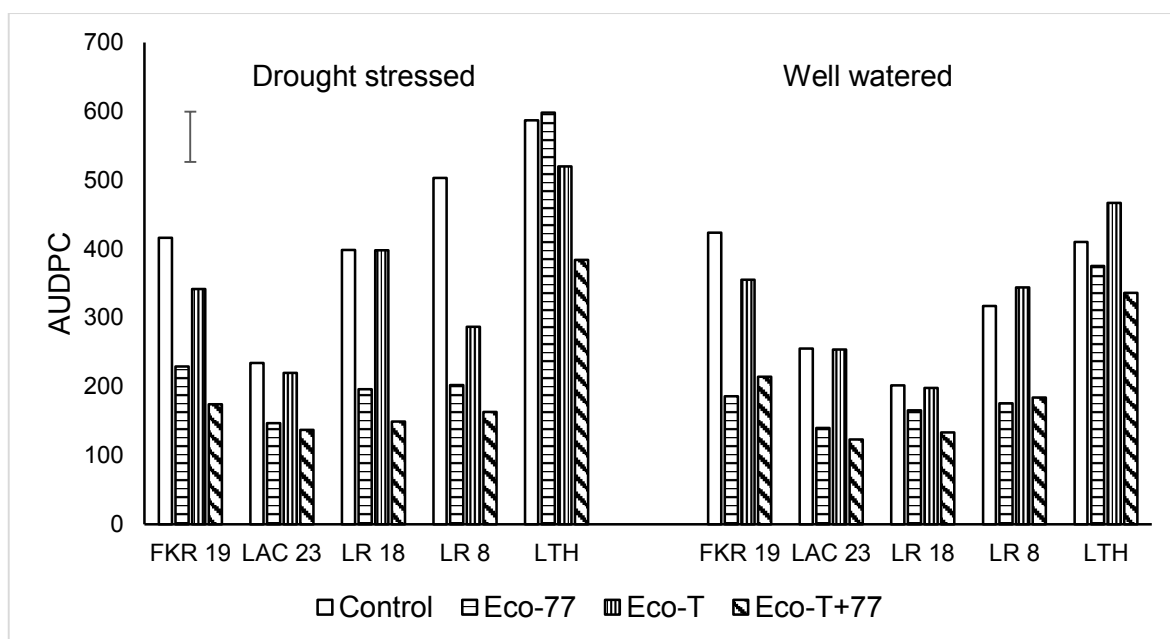


Figure 8.7: The effect of *T. harzianum* treatments on rice blast intensity under contrasting soil water conditions, when applied to five rice genotypes. The error bar represents  $LSD_{(p = 0.05)}$ .

#### 8.4.6 Percent reduction in disease severity

Table 8.3 shows the level of reduction in disease severity caused by the *Trichoderma* treatments. The level of reduction in disease severity by *Trichoderma* varied significantly with genotype. The susceptible check, LTH, displayed the least reduction in disease severity. The combination of Eco-77® and Eco-T® achieved the greatest reduction in disease severity under both well-watered and intermittent drought stressed conditions. The difference in disease reduction between the two soil water regimes was not significant ( $p = 0.05$ )

Table 8.3: Reduction in the severity of rice blast developed by five rice cultivars as a result of different *Trichoderma* treatments. Values are percentages.

Genotype	Drought stressed				Well watered				
	ECO77	ECOT	ECOT+77	Mean	ECO77	ECOT	ECOT+77	Mean	
LAC 23	42.7	10.7	32.0	28.5	44.0	16.7	54.7	38.5c	
LR 19	38.7	0.0	50.0	29.6	21.3	10.7	37.3	23.1bc	
LR 8	44.7	6.0	39.3	30.0	28.0	0.0	39.3	22.4b	
FKR	33.3	6.0	44.0	27.8	34.0	-0.7	28.7	20.7b	
LTH	6.0	6.0	18.0	10.0	6.0	-6.0	12.0	4.0a	
Mean	33.1	5.7	36.7	25.2	26.7	4.1	34.4	21.7	
LSD									15.8
CV (%)									6.3

Means with the same letters are not significantly different at  $p = 0.05$ .

### 8.4.7 Effects of *Trichoderma* on pre- and post-infection drought tolerance of rice

Rice blast infected plants subjected to drought stress two weeks after blast incidence (62 WAS) showed significantly high rates of mortality ( $p < 0.001$ ), ranging from 53 to 90% (Figure 8.8). No mortality was observed in rice-blast-free plants subjected to water stress by 62 DAS. *Trichoderma* applications significantly reduced the mortality rate of the blast infected plants of all the varieties subjected to drought stress, with Eco-T® accounting for the highest reduction in the level of plant mortality (Figure 8.8). The level of mortality also varied significantly with genotype; LR 18 exhibited the lowest mortality, followed by LAC 23. Differences in plant mortality between the well-watered plants and those subjected to pre-infection drought stress were not significant ( $p = 0.05$ ).

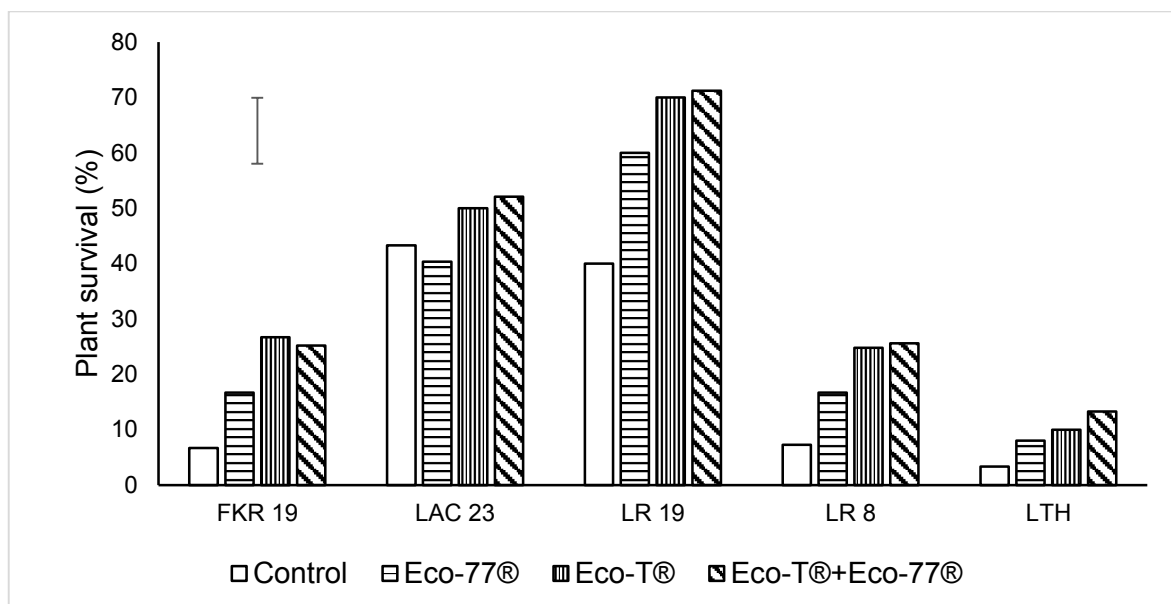


Figure 8.8: Effect of *Trichoderma* on survival of rice blast-infected plants of five rice genotypes subjected to drought stress. The error bar represents  $LSD_{(p = 0.05)}$ .

### 8.4.8 Effect of *Trichoderma* on biomass accumulation of rice

Under post-blast infection water stress, plants treated with Eco-T® or with a combination of Eco-T® and Eco-77® developed significantly higher shoot dry mass and grain yield per unit area, and higher root mass density than those left untreated, or treated with Eco-77® alone (Table 8.4). Shoot dry mass and grain yield of plants did not vary significantly with *Trichoderma* treatment for the plants grown under well water conditions. However, the dry mass of roots varied with *Trichoderma* treatment under well-watered conditions, with the plants treated with Eco-T® and Eco-T® plus Eco-77® being significantly heavier than the other plants.

Table 8.4: Effects of *Trichoderma harzianum* on growth and yield of five rice genotypes under different post-blast-infection water regimes

Trichoderma	Genotype	Drought stressed			Well watered		
		RMD (mg cm <sup>-3</sup> )	SDM (g m <sup>-2</sup> )	GY (g m <sup>-2</sup> )	RMD (mg cm <sup>-3</sup> )	SDM (g m <sup>-2</sup> )	GY (g m <sup>-2</sup> )
Control	LAC 23	3.6	370.8	162.5	6.2	479.2	184.0
	LR 19	3.7	256.9	112.5	5.4	371.0	162.5
	LR 8	3.3	363.8	160.0	5.6	562.7	197.5
	FKR	2.9	172.4	88.6	6.0	233.9	128.6
	LTH	0.4	47.0	22.5	0.4	78.4	37.5
	Mean	2.8 <sup>b</sup>	242.2 <sup>b</sup>	109.2 <sup>b</sup>	4.7 <sup>a</sup>	345.0 <sup>ab</sup>	142.0 <sup>a</sup>
Eco-T®	LAC 23	6.4	395.2	178.8	5.8	455.0	182.6
	LR 19	5.9	266.1	124.6	5.3	405.2	166.4
	LR 8	6.2	368.5	181.3	6.8	498.9	201.3
	FKR	5.7	190.4	113.6	5.3	355.7	133.4
	LTH	0.4	47.9	22.7	0.5	62.5	32.5
	Mean	4.9 <sup>a</sup>	253.6 <sup>a</sup>	124.2 <sup>a</sup>	4.7 <sup>a</sup>	355.5 <sup>a</sup>	143.2 <sup>a</sup>
Eco-77®	LAC 23	3.4	368.2	158.2	5.5	384.8	179.1
	LR 19	4.1	255.5	114.6	6.1	419.4	157.6
	LR 8	3.1	358.6	166.1	6.7	515.4	185.4
	FKR	2.8	180.0	96.5	5.4	230.4	112.8
	LTH	0.5	45.2	23.5	0.4	73.4	24.0
	Mean	2.8 <sup>b</sup>	241.5 <sup>b</sup>	111.8 <sup>b</sup>	4.8 <sup>a</sup>	324.7 <sup>a</sup>	131.8 <sup>a</sup>
Eco-T® + Eco-77®	LAC 23	6.6	403.3	174.4	6.2	432.8	193.2
	LR 19	5.8	278.0	121.7	6.6	389.5	176.0
	LR 8	6.6	372.1	173.2	5.9	566.4	199.5
	FKR	3.1	183.5	117.0	4.6	214.0	124.5
	LTH	0.5	42.6	17.8	0.4	58.6	30.0
	Mean	4.5 <sup>a</sup>	255.9 <sup>a</sup>	120.8 <sup>a</sup>	4.7 <sup>a</sup>	332.3 <sup>b</sup>	144.6 <sup>a</sup>
Grand Mean		3.8	248.3	116.5	4.8	339.4	140.4
CV (%)		37.2	16.4	22.8	37.2	16.4	22.8

RMD =root mass density; SDM = shoot dry mass; GY = grain yield; CV = coefficient of variation. Means in the same column followed by different letters differ significantly ( $p = 0.05$ ).

## 8.5 Discussion

Understanding the influence of *Trichoderma harzianum* on blast disease and drought stress responses of rice could be useful for devising more effective strategies for managing two of the most important problems limiting rice production in many parts of the world. Also, further insights into the nature of the relationship between rice blast and drought stress would help producers more effectively address the risks posed by these problems.

### **8.5.1 Effects of *Trichoderma* on the number of sporulating lesions and lesion size inflicted by *P. oryzae* on rice**

The capacity of *T. harzianum* to control rice blast in these studies depended largely on the strain applied to control the pathogen. In both the CE and field experiments, Eco-77® was effective at controlling blast when applied at the time plants began showing symptoms of the disease. Conidia of the pathogen were likely to be established on the plants by then, and the differences between the *Trichoderma*-treated and control plants were not significant at 10 days after exposure to the disease spreaders. However, at 14 days after plant exposure to disease spreaders under field conditions, a marked reduction in the number of sporulating lesions on new leaves was observed on plants treated with Eco-77® alone, or with Eco-77® plus Eco-T®, suggesting that if applied earlier, Eco-77® could be more effective.

Eco-77® also significantly reduced the size of sporulating lesions and overall disease severity in plants, as measured by AUDPC and maximum disease severity levels. The capacity of Eco-77® to reduce disease severity in these studies was likely mediated through the mechanism of parasitism. Studies have shown that *T. harzianum* can disrupt the pathogenic activity of other fungi by coiling around their hyphae and producing enzymes that dissolve the cells walls of the pathogen (Elad et al., 1982; Mukherjee et al., 1995). Benhamou and Chet (1997) showed that chitinase is a common enzyme produced through gene transcription by *T. harzianum* to parasitize *Pythium ultimum*. Eco-T®, applied as a seed coating or as a seedling root drench, did not control rice blast in these studies. However, studies by Singh *et al.* (2012) have shown that seed treatment with a strain of *T. harzianum* reduced rice blast severity in lowland rice plants by 17 to 25%. It is likely that Eco-T® may be more adapted for managing rhizosphere-related problems, as specifically indicated by the manufacturer.

### **8.5.2 Effect of *Trichoderma* on drought tolerance of rice**

*Trichoderma*-treated plants subjected to post-blast infection drought stress displayed an enhanced ability to recover from the effect of drought stress. Eco-T® was particularly effective for reducing plant mortality, as a significantly higher number of plants treated with this formulation were able to survive, compared to the other treatments. Disease-challenged plants treated with *T. harzianum* have been found to exhibit characteristics of tolerance to various abiotic stresses, including drought, when applied as a seed coating (Mastouri *et al.*, 2010) or as a soil drench (Yildirim *et al.*, 2006).

### **8.5.3 Effects of *Trichoderma* on growth and yield of rice**

Shoot and root dry matter and grain yield of plants were measured to determine the effect of the various *Trichoderma* treatments on the growth and yield performance of rice. Eco-T® caused a significant increase in shoot and root biomass at heading, and consequently increased grain yield per unit area. The capacity of some strains of *T. harzianum* to improve crop growth and productivity has been demonstrated in several studies (Bjorkman *et al.*, 1998; Bal and Altintas, 2006; Chiurase *et al.* 2015; Shores and Harman, 2008; Zhang *et al.*, 2013).

The increase in root biomass, as mediated by Eco-T® under the well-watered conditions, did not correspond to any increase in shoot biomass or grain yield. A larger root volume may be expected to lead to increased extraction and utilization of nutrients from the soil, which could translate into higher above ground biomass and yield, if the plant was under stress. However, under ideal conditions, extra root biomass is unlikely to affect yield as it confers no advantage to the plants.

### **8.5.4 Genotypic influences on the effectiveness of *Trichoderma harzianum***

Genotypic differences in plant mortality was observed, irrespective of *Trichoderma* treatment, when plants were subjected to post-blast-infection drought stress. This was probably due to differences in the levels of their genetic resistance to the disease. It was also observed that the more effective *Trichoderma* treatment (Eco-77®) did not significantly reduce disease severity in the universally blast susceptible genotype (LTH), as it did the other cultivars, which possessed various levels of host plant resistance against rice blast (Chapter 3). This suggests that some minimum level of host resistance may be required to delay disease progress, if the potential of Eco-77® for controlling rice blast is to be realized.

### **8.5.5 Effect of blast disease on drought tolerance of rice plants**

Comparison of drought sensitivity and disease severity (reflecting percentage of diseased leaf area) data showed that disease severity was fairly constant within treatments and genotypes, whereas blast-infected plants subjected to water stress showed poor recovery (or high mortality), irrespective of genotype and the strain of *Trichoderma* with which they were treated. Both rice blast and drought stress contribute to plant mortality, and consequently, reduction in rice yield and productivity. But the trends recorded in these studies suggest that, although water stress may increase rice susceptibility to blast, its ultimate impact on yield and productivity of the crop may be far less pronounced than the impact of drought stress that may affect rice blast afflicted plants. When plants are infected

with rice blast in farmers' fields, prioritizing proper management of water resources would, therefore, be important to prevent further losses of both crop and grain yield.

## 8.6 Conclusions

Integration of two or more methods of control can be more effective for managing several pests and diseases of crops. In this study, it was found that strains of the BCA, *T. harzianum*, were highly effective at reducing the severity of rice blast and mitigating the adverse effects of drought stress on blast affected plants. The effectiveness of the *Trichoderma* treatments for reducing the severity of rice blast and enhancing drought tolerance of the blast susceptible LTH were however, nominal. The Japanese cultivar, LTH, is not known to possess any blast resistance genes, and was therefore unable to contribute towards limiting the effect of the disease on it, compared to the other genotypes which displayed various levels of partial resistance, as determined in previous studies. Integrating *T. harzianum* with genotypes possessing good levels of drought tolerance will also be important for reducing the ultimate impact of drought stress on rice blast-infected plants, since it was also found that the impact of drought stress on rice blast-infected plants can be far more pronounced than the impact of rice blast incidence on drought stressed rice plants. Because the commercially available *T. harzianum* formulations studied in this experiment are relatively inexpensive and have other plant growth-enhancing potentials, they should be recommended to smallholder farmers to enhance production in their fields.

## 8.7 References

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## THESIS OVERVIEW

The focus of this study was to develop strategies for improving rice blast resistance and drought tolerance of rice, which are two of the most important factors limiting rice production in Liberia and other parts of the world, where the crop is cultivated mainly under rainfed conditions. In this overview, the major findings of the research are presented and discussed within the context of their implications for future research and development.

Since farmers are the primary users of most agricultural innovations, the first of the seven studies conducted comprised a comprehensive survey, conducted in the Gibi District of Liberia, aimed at appraising the local farming systems and determining the key varietal traits valued and preferred by farmers of that district of Liberia. It was important to solicit farmers' input on breeding decisions in order to ensure that farmers' needs and preferences were considered from the very beginning of the breeding programme. This would facilitate early adoption of any new cultivar arising from the breeding effort. In that study, factor analysis of the pair-wise comparisons of 23 traits revealed that the farmers of Liberia considered a combination of grain yield (GY), grain quality, stress resistance and post-harvest traits when deciding which variety to plant. These findings showed that the farmers had a sound knowledge of the crop they grew, and their contributions to breeding decisions will be important for the success of any rice improvement programme in that country. Conjoint analysis of the ranked preference data from the study also showed that apart from high GY, farmers would be more inclined to adopt new varieties that are early maturing, and have intermediate plant heights, and produce grains that cook to a tender state, but where the cooked grains remain separated. The farmers further identified the lack of improved varieties with blast resistance and drought tolerance as the key problems that they faced in producing the crop. This confirmed that the farmers were cognizant of the limitations of their current stock of cultivars and would be prepared to adopt new ones, if the new varieties met their selection criteria, and surpassed current varieties in these traits, without any critical deficiencies.

The second study was aimed at characterizing up to 38 rice genotypes, currently grown by farmers in Liberia, for their reaction to rice blast, in order to determine their potential as parents for breeding new cultivars with improved resistance to the disease. Only 26% of the genotypes developed susceptible infection type (IT) lesions against all the three pathotypes tested, and about 40% displayed either susceptible or resistant ITs against one or two of the pathotypes (i.e., typical of major gene resistance, which is matched by virulent pathotypes). Interestingly, only fewer than 2% of the genotypes that developed susceptible ITs against either or all of the pathotypes were completely killed by the disease, i.e., 98%

expressed partial vertical resistance. These results showed that the rice blast resistance expressed by the test members of Liberia's rice population is characterized by the occurrence of both race specific and race non-specific patterns of resistance. Further controlled environment and field evaluations of the 22 genotypes that developed susceptible ITs against SIK-111, the most virulent of the three pathotypes, revealed that most of the genotypes possessed very low levels of horizontal resistance. This finding clearly highlighted the need for further research that should focus on population improvement for higher levels of blast resistance for the agro-ecology of Liberia based on stable, quantitative resistance.

Additive main effect and multiplicative interaction (AMMI) analysis of the number and size of sporulating lesions revealed significant levels of variability for quantitative resistance to rice blast in the Liberian rice population, which was due mainly to genotypic effects and less to environment. These results suggested that there are useful levels of diversity in the upland rice germplasm of Liberia, which could be exploited for the improvement of blast resistance.

The blast screening study also showed that rice plants subjected to intermittent drought stress, both in the controlled environment and field, developed larger and more lesions with sporulating centres. Similar effects of water deficit on rice blast development in the field was reported by Bonman *et al.* (1988), and Gill and Bonman (1988). The present study showed the value of using managed drought stress conditions in future blast screening research.

The drought tolerance experiments revealed that, as with most rice germplasm (Ekanayake *et al.*, 1989; Boonjung and Fukai, 1996; Bouman *et al.*, 2007), rice genotypes from Liberia were particularly sensitive to drought occurring around the booting-to-grain-filling stage of development. The levels of spikelet fertility (SF) and GY of plants of all the genotypes subjected to drought stress for 21 days, beginning at booting, were significantly low, compared with others subjected to drought stress for a similar length of time during the tillering growth stage. On the other hand, the best genotypes showed the ability to recover from drought imposed during both the seedling and tillering stages of growth, with little effect on final yield. The seedlings also showed a higher emergence pattern under limited soil water conditions. These results demonstrated that improvement of upland rice germplasm of Liberia should focus more on enhancing tolerance to drought occurring during the reproductive stage of development. Because genotypes with high yield potentials may not necessarily possess superior drought tolerance (Fischer and Maurer, 1978; Blum, 2005), it was desirable to identify traits that predicted the capacity of genotypes to combine higher levels of both traits, with the aim of devising breeding strategies that could increase yield

stability in the population. Using simple correlation and path coefficient analyses, the current studies revealed that high levels of biomass and stomatal conductance at heading, and high levels of SF tended to be associated with genotypes expressing a high GY when grown under well-watered conditions. In contrast, for plants subjected to drought stress during the booting-to-grain-filling stage, their GY was highly associated with their levels of tiller productivity (TP), biomass at heading, and their 1000 grain mass. Future application of these selection criteria will be important for concurrently increasing both yield potential and drought tolerance in upland rice. Variability within the present population was mainly explained by GY, TP, CCI and SF, as determined by principal components analysis. The analysis of variance revealed significant genotypic differences for these traits. This showed that the genotypes that developed the highest values could be used for breeding purposes. It was also noted that the days-to-heading of at least 90% the genotypes were particularly long. This trait was also poorly correlated with grain yield. Although a key strategy in breeding for drought tolerance is to increase the effectiveness of mechanisms that reduce water use and enhance stress tolerance (Richards, 2006; Cattivelli *et al.*, 2008), mechanism that enhance the chances of genotypes escaping drought should also be sought by breeding for varieties with fewer days-to-harvest. Incorporating genes for earliness could therefore be important for sustaining upland rice production, especially in Liberia where the wet season is becoming shorter and less reliable (McSweeney *et al.*, 2010a, 2010b).

During the drought screening study, the genotypes were grown either in bare soil or in soil covered with plastic sheets, in order to also determine the utility of a plastic mulch soil cover (PMSC) system for simulating drought during periods of rainfall. The basic components of the system comprised 130  $\mu\text{m}$ -thick polyethylene sheeting, drip irrigation pipes, solenoids, an automatic irrigation controller, and soil moisture sensors. The experiments showed that the PMSC facility was effective at excluding water from rainfall because the water potential at both the 30 and 60 cm depths of soil did not increase (become less negative) during or after periods of rainfall, as observed at similar depths in the control plots. Tiller productivity, GY, and both leaf rolling and leaf death scores of plants grown in the control plots (bare soil) were also significantly higher than those grown in the PMSC environment due to interference of rainfall during critical periods of assessment and stress management. This clearly highlighted the importance of an effective water exclusion mechanism as a key pre-requisite for executing managed drought trials in the field, as emphasized by Seetharama *et al.* (1982) and Atlin *et al.* (2008). Cost analysis showed that the PMSC facility can be about 13 to 235 times cheaper to set up than a rain-out shelter. Due to its low cost and scalability to cover large areas of land and to use diverse locations, the technology of PMSC

could be useful for further drought research in developing countries where funding is often limited.

As revealed in Chapter 3, twenty-two of the Liberian upland cultivars produced susceptible infection types against SIK-111, which proved to be the most virulent of the three pathotypes tested. Six of those cultivars were consequently chosen and recombined, along with the universally susceptible variety, LTH, and a resistant check, LAC 23, in a full diallel mating scheme. The F<sub>2</sub> progenies and their parents were subsequently evaluated for the size and number of sporulating lesions per plant and the area under the disease progress curve (AUDPC), in order to determine the gene action underlying the inheritance of these three basic component traits of quantitative (horizontal) resistance to rice blast. This study showed that both additive and non-additive gene actions were involved in the inheritance of these traits. However, the genes with additive effects were more important, since the ratio of the general combining ability (GCA) to specific combining ability (SCA) was higher. Previous studies have shown that the predominance of GCA effects in a genetic system normally leads to consistent gains in a recurrent selection programme (Duvick *et al.*, 2004; Gordillo and Geiger, 2008). Hence, future works aimed at increasing the level and durability of rice blast resistance should emphasize recurrent selection for reduced number and size of sporulating lesion per plant, and lower AUDPC. Heritability of sporulating lesion number per plant, sporulating lesion size per plant and AUDPC were particularly high in the study, indicating a high degree of consistency in the screening environment. The importance of a conducive screening environment was also highlighted in the screening study involving the parental population, as stated above. Future studies should therefore prioritize conditions that assure high and consistent disease pressure when screening for horizontal resistance to rice blast.

When recurrent selection is adopted for improving rice in Liberia for durable resistance against blast disease the one pathotype technique, as proposed by Robinson (2004, 2006), will be used to ensure the elimination of major gene effects, and the accumulation of additive genes for horizontal resistance as the breeding programme progresses. Using the best crosses of the current segregating population, SIK-111 would be useful as the designated pathotype because it worked well at matching all vertical resistance genes present in the Liberian germplasm tested in the current research.

Large numbers of crosses are normally required when aiming to improve complex traits in crop genotypes using a recurrent selection scheme (Hallauer and Darrah, 1985; Pandey and Gardner, 1992). The difficulty of generating large number of crosses in a cleistogamous plant, such as rice, can limit the application of recurrent selection. From the study on gene

action and combining ability of horizontal resistance against rice blast (Chapter 6), it was shown that GCA was more important than SCA, and hence that recurrent selection is the most appropriate breeding scheme for improving the current population of rice genotypes from Liberia for increased yield, blast resistance and drought tolerance. Therefore, an additional study was undertaken to determine the usefulness of a combination of ethephon and gibberellic acid (GA3) for overcoming that limitation. That study showed that application of ethephon at 4000 to 6000  $\mu\text{L L}^{-1}$  at the microsporogenesis stage of rice panicle development can be effective for inducing male sterility in the Liberian rice cultivars on which it was tested. Gibberellic acid, applied at 90 or 150  $\mu\text{L L}^{-1}$  during anthesis, was effective for reversing the negative effect of the ethephon on treated plants, which was manifested as poor panicle exertion, high pistil sterility and reduced plant height. These results demonstrate that a combination of ethephon and GA3 can be an effective chemical hybridizing treatment for promoting cross pollination in rice. In turn, this makes it feasible to make the large numbers of crosses needed in a recurrent selection programme. A rice breeding programme could be undertaken using recurrent selection, based this male gametocide treatment, or novel male gametocides such as ethyl 4'-fluorooxanilate 6-BA-benzyladenine (Ghebrehiwot *et al.* 2015). This programme would use the best of the Liberian germplasm identified in this study as the initial parent population. Screening would be to select for the traits valued by Liberian farmers, including resistance to drought and blast, cooking quality and for high yields.

The final study investigated the effects of strains of a biocontrol agent (BCA) *Trichoderma harzianum* on the responses of rice to blast disease and drought stress. A commercial biocontrol product, Eco-T®, containing *T. harzianum* Strain KD, was effective at reducing the rate of plant mortality and increasing root mass density, shoot dry mass and GY of plants subjected to drought stress when applied as seed dressing or root drench. Another *T. harzianum* product, based on Strain B77 (commercially available as Eco-77®), significantly reduced the size of sporulating lesions and the AUDPC of rice blast infected plants when applied twice as a foliar spray. Similar stress mitigating and growth enhancing effects by *T. harzianum* on other crops have been reported by Elad *et al.* (1980), Datnoff *et al.* (1995) and Bal and Altintas (2006). More importantly, this research clearly highlighted the value of integrating host plant resistance with biocontrol, as plants with that expressed higher levels of host resistance were more responsive to the *T. harzianum* treatments, compared to highly susceptible varieties. Integrating host plant resistance with biocontrol could therefore be important to stabilizing rice production in blast-infested and drought-prone agroecologies.

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