Evaluation of rice genotypes for grain yield and resistance to bacterial leaf blight (*Xanthomonas oryzae* pv.*oryzae)* disease

By

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food crop in many African countries including Tanzania. However, both regional and national rice production have failed to meet demand due to several constraints, among which is the bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae*. Moreover, attempts to increase rice production through the introduction of modern cultivars has motivated farmers to leave local landraces for high yielding, but often susceptible varieties. The overall goal of this study was to increase and strengthen rice production in Tanzania through development of high yielding and BLB resistant varieties. The specific objectives were: to i) analyse genotype x environment interaction (GEI) effects for reaction to bacterial leaf blight under natural infection and rice grain yield performance across different environments in Tanzania ;ii) assess the heritability, variability and efficiency of indirect selection using secondary traits for grain yield improvement among rice genotypes; and iii) assess relationship among traits using correlation, path coefficients and genotype-by-trait associations in rice. The study was conducted at three sites namely Katrin, Igurusi and Kyela, all in Tanzania. Thirty rice genotypes, which include two checks, Txd 306 (susceptible check) and IR- 24 (resistant check), were evaluated. The experimental design was a 6 x 5 alpha lattice design with three replications. Data was collected on early vigour, days to early flowering, plant height (cm), panicle length(cm), number of tillers per hill, dead heart, bacterial leaf blight scoring, lodging percent, days to maturity, dry straw weight (kg), spikelets per panicle, grain length (mm), grain width (mm), 1000-grain weight (g), harvest index (%) and yield per plot (kg). Data were analysed using SAS version 9.4 and GenStat 17th edition. ANOVA was used to detect the significance of GEI. The Additive Main Effect and Multiplicative Interaction (AMMI) and the Genotype plus Genotype by Environment Interaction (GGE) biplot models were used for further analysis of GEI and stability. From the results, genotypes NERICA 4 followed by IR-24were the most resistant to BLB while Supa India was the most susceptible. Dakawa 83 was the most resistant at Katrin while NERICA 4 was the most resistant at Igurusi and Kyela. Genotypes NERICA 2 and LOWLAND NERICA 6 were the most stable across environments for BLB resistance, while IR54 and Txd 306 were the most unstable. Based on the GGE biplot analysis, the three environments fell into two mega environments where as at Kyela, NERICA 4 and IR-24were identified as the most resistant genotypes while at Katrin Dakawa 83 and NERICA 1 were identified as the most resistant genotypes. Genotype by Environment Interaction effect for grain yield was not significant and as a result, genotype comparison for the same trait was based solely on mean performance across all the environments. The best three genotypes for grain yield were Txd 306, Txd 88 and WITA 10, but in contrast, NERICA 4, Supa India and Mwanza were the worst performers for the same trait. As for broad sense heritability estimates, days to early flowering had the

highest estimate of 99.67%, indicating less influence of the environment, while lodging% had 0.00% heritability indicating high influence of the environment. For variability, the phenotypic coefficient of variation (PCV %) values were higher than the genotypic coefficient of variation (GCV %) for all the traits. The highest PCV(%) was for lodging percent (5325.463) followed by number of spikelets per panicles (1005.352)and the lowest was for grain width (1.197) followed by grain length(2.406).The GCV (%) was highest for number of spikelets per panicle (419.902) followed by plant height (97.843) and the lowest was for lodging percent (0.000) followed by grain yield (0.314), genetic advance (GA) was highest for spikelets per panicles (66.79) and lowest for lodging percent (0.000), while for genetic advance as a percentage of mean (GAM %) the highest was for yield per plot (104.13) followed by dry straw weight (92.11) and the lowest was for lodging percent (0.00) followed by panicle length (8.89).Not all the traits under consideration could be used for indirect selection for yield per plot since none of them had a relative selection efficiency equal to or greater than unity. Regarding diversity assessment, cluster analysis based on Euclidian distance indices revealed that Txd 88 and SATO IX were the most similar pair, followed by IR-56 and IR54, which were also similar to each other, and the most divergent genotypes were Txd 306 and Wahiwahi followed by Wahiwahi and Txd 85. Diverse genotypes can be targeted for hybridization since progenies of diverse parents are often more heterotic than those of related parents.

The assessment of relationship among traits using correlation and path analysis the traits which were positive and highly significantly correlated to grain yield were harvest index (0.77***) followed by dry straw weight (0.46***), while negative significant correlations were observed for early vigour (-0.22*). Direct effects on grain yield were positive for harvest index (0.80) and dry straw weight (0.51), while indirect effects were highest for days to maturity through harvest index (0.25) followed by number of tillers per hill through harvest index (0.23). For genotype-by-trait associations, genotypes NERICA 1, NERICA 2, NERICA 4, WAB 450-12-12-BL1- and IR-24 were associated with BLB resistance; on the other hand Txd 306, WITA 10, Txd 88, Txd 85, and SATO I were associated with high yield, although Txd 306 was also associated with susceptibility to BLB, whereby WITA 10 was high yield and resistance to bacterial leaf blight.

Moreover this study provided information on the presence of genotype by environment interaction in Tanzanian rice growing environment, valuable blight resistance and high yielding genotypes such as WITA 10 and moderate BLB resistance with high yield for genotypes such as Kalalu, Txd (88) and Txd (85), which could be used in rice breeding improvement and conservation efforts of rice.

DECLARATION

I, **Claudia. A. Tesha**, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons‘ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
a. Their words have been re-written but the general information attributed to them has been referenced. b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
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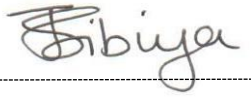
Claudia. A. Tesha

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



Dr. Julia Sibiya (Main Supervisor)

Dr Cousin Musvosvi (Co-Supervisor)

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DEDICATION

I dedicate this dissertation to my parents and my family.

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ABBREVIATIONS

AMMI	Additive main effects and multiplicative interaction
ANNOVA	Analysis of variance
BLB	Bacterial leaf blight
CV	Coefficient of variation
DF	Degree of freedom
DM	Days to maturity
DSTRW	Dry straw weight
DTEF	Days to early flowering
EV	Early vigour
G	Genotype
GC	Genotype code
GEI	Genotype by environment interaction
GGE	Genotype main effects and genotype by environment interaction
GL	Grain length
GW	Grain width
HI	Harvest index
IRRI	International Rice Research Institute
KATRIN	Kilombero Agricultural Training and Research Institute
LOD%	Lodging %
NERICA	New Rice for Africa
PCA	Principal component analysis
PH	Plant height
PL	Panicle length
REML	Residual maximum likelihood
SS	Sum of squares
TGWT	Thousand grain weight
TH	Tillers per hill
WARDA	West African Rice Development Authority
YP	Yield per plot

INTRODUCTION

1 Background

Rice (*Oryza* spp.),is grown in many countries across the globe covering a total area of about 163 million ha with a global production of about 740 million tonnes and an average yield of about 4,539 kg/ha (FAOSTAT, 2012). The Asian continent ranks first with over 90.1% of the world production, followed by the American continent (5.1%), African continent (4.2%), Europe (0.5%) and Oceania (0.1%). The major producing countries are China (206.5 million tonnes), India (157.2 million tonnes), Indonesia (70.8 million tonnes), Bangladesh (52.2 million tonnes) and Vietnam with 44.9 million tonnes (FAOSTAT, 2012).

Worldwide, rice was ranked second in cultivated acreage in 2012 after wheat (AfricaRice, 2013). Other crops included in the top ten were maize, soybean, barley, sorghum, millet, cotton (seed), rapeseed and dry beans. In Africa, rice is grown and consumed in more than 40 countries. Its production has increased from 3.3 million tonnes in 2000 to 11.6 million tonnes in 2015.More than 20 million farmers in Africa are engaged in rice production and about 100 million people are dependent on it directly for their livelihood (Nwanze et al.,2006). In addition, the rice agricultural sector in Africa is an important contributor to economic development and reduction of life-threatening poverty and ensures food security to many families (AfricaRice, 2012). In East Africa, Tanzania is the second largest producer of rice after Madagascar with 720,000 ha under rice production and small-scale farmers owning 0.5–3.0 ha of land produce 90% of it.

2 Importance of rice and its improvement in Tanzania

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is a staple food crop for more than half of the world's human population. Rice grain contains 75 to 80% starch, 12% water and 7% protein (Oko et al., 2012). Minerals like calcium, magnesium and phosphorus are present along with some traces of iron, copper, zinc and manganese. In addition, rice is a good source of niacin, thiamine and riboflavin (Oko et al., 2012)

In Tanzania rice is the second most important staple and commercial crop after maize and thus it is a good source of employment, food security and income for farming households (RLDC, 2009). About 18% of Tanzanian farming households grow rice contributing to 2.66% of the total GDP. The leading rice producing regions of Tanzania are Morogoro, Shinyanga, Tabora, Mwanza, Mbeya, Rukwa, and Arusha. The production regions fall under three main ecologies: lowland cultivation (72% of rice hectares), upland (about 21%) and irrigation (less than 10%) (FAO, 2011).

Production of rice has increased immensely in Tanzania from 330,000 tonnes in 1997 to 662,000 tonnes milled rice in 2010. In the last 20 years, there has been a small yield increase from 1.32 t/ha in 1995, 1.66 t/ha in 2005 and 2.21 t/ha in 2014 (CRP, 2016) with an average of 0.045t/ha annual increase. The productivity of 2.21 t/ha is about half the global average of 4.40 tonnes/ha (Li et al., 2014). The national production has had an overall positive growth for about a decade from 1998 to 2007 although characterized by large variabilities from year to year with an average gain of 0.017t/ha per year (RLDC, 2009).

Regardless of the importance of rice in Tanzania, production is faced with many challenges including insect/bird pests, diseases, poor field management, use of old genetics methods such as crossing the varieties which are not improved, and lack of rice varieties that can tolerate unfavourable biotic and abiotic conditions (EUCORD, 2012).In strategies to feed the projected population of 9.4 billion people (Koksharova, 2010) by 2050, a focus on improving important agronomic traits of important crops including rice is needed. Thus, to meet present and projected demand and attain rice self-sufficiency, plant breeders have to develop high yielding cultivars with resistant traits and desirable agronomic traits for different environments.

The development of new genotypes requires knowledge on the variability present in the germplasm to build an effective breeding programme. The knowledge about genetic variability can provide information on whether the variations are heritable or non-heritable. The degree of variation due to heritable components is very important as it guides the breeder in selection of parents for crop improvement (Dudley et al., 1969). Therefore, in order to effect selection for high yield, this study focused on obtaining information on genetic variability, correlations, path coefficients, genotype by environment interactions and genotype by trait associations.

3 Taxonomy and diversity of the genus *Oryza*

Rice (*Oryza* spp.), a member of the family Gramineae is widely grown in tropical, subtropical and temperate regions (Ezuka and Kaku, 2000).The genus *Oryza* contains approximately 22 species, 20 of which are wild and two are cultivated, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) (Vaughan, 1994). About fourteen wild species are diploid, having 24 chromosomes (2n = 24), whereas eight wild species are tetraploids with 48 chromosomes (2n = 48). *Oryza sativa* is the most widely grown of the two cultivated species. It is grown worldwide, including Asia, North and South America, European Union, Middle East and Africa. *Oryza glaberrima,O. sativa* and *glaberrima-sativa* hybrids are replacing *O. glaberrima* in many parts of Africa due to higher yields (Linares, 2002). Recently, the West African Rice Development Association (WARDA) developed inter-specific varieties known as New Rice for

Africa (NERICA), from crosses between *O. sativa* and *O. glaberrima*. These varieties have been widely released in Africa (Africa Rice Center-WARDA., 2008).

4 Rice diseases and production constrains in Tanzania

Rice production is constrained by bacterial, fungal, and viral diseases. Amongst these, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* (Xoo) can be devastating (Swings et al., 1990). Bacterial leaf blight is widespread in several rice growing areas covering both tropical and temperate countries (Mew, 1987; Mew et al., 1993; Gnanamanickam et al., 1999; Séré et al., 2005). The BLB disease has been reported to occur all over the world including different areas of Asia, USA, Africa, and northern Australia (Adhikari et al., 1995; Onasanya *et al.*, 2009).The presence of *X. oryzae pv. oryzae* has also been confirmed in Tanzania (Ashura et al., 1999). The disease normally affects filling of the grains and emergence of panicles, causing yield losses between 20 to 50%, with a range from 50 to 90% loss reported in some other areas (Onasanya et al., 2013). A latest assessment of rice diseases in Tanzania reported the occurrence of bacterial leaf blight in some parts of rice growing areas in Mara, Mbeya, Iringa and Morogoro (Habarurema et al., 2012).However, little is known about the variability of local *Xanthomonas oryzae pv.oryzae* pathogen populations (Africa Rice Center-WARDA., 2008).

Factors that contribute to low yields in Tanzania include use of low yielding varieties, drought, low soil fertility, incidence of pests and diseases, little supply of fertilizer and weed invasion (Mghase, et al., 2010) (URT, 2009). Among the weeds that affect rice fields, *Oryza longistaminata* and *O. punctate* are important production constraints in southern and eastern parts of Tanzania. A study that centred on farmers' opinion and preferences showed that in Morogoro region, the major rice production constraints were lack of improved varieties, disease susceptibility, inadequate seed supply for planting, drought and high production costs (Bucheyeki et al., 2011). Apart from the above constraints, salinity was also reported as one of the challenging factor for irrigated lowland rice in the north-coast of Tanzania (Kashenge-Killenga et al.,2012). Moreover, many lowland rice ecologies face severe shortage of water, parasitic weeds and to some extent, diseases such as rice yellow mottle virus, rice blast and bacterial leaf blight (URT, 2009).

5 Problem statement

Despite the economic importance of rice, production has remained low due to a number of biotic, abiotic and socio-economic constraints (Feldmann & Alford, 2009). Biotic stresses such as insect pests, diseases and abiotic stresses such as high temperature, salinity, drought,

acidity, and iron toxicity are prevalent across the production regions. Bacterial leaf blight disease is one of the constraints, which causes both yield and grain quality losses (Savary et al., 2000). Farmers in Tanzania have confirmed BLB as the second most important disease of upland, lowland and irrigated rice after rice blast (Wilfred, 2006). In 2011, the disease was ranked as the third most important disease after *rice yellow mottle virus* (RYMV) disease and leaf blast (Hashim et al*.*, 2018). Moreover, severely infested grains due to BLB are not suitable for human consumption (Barnwal et al., 2013). The pathogen normally blights the leaves and damages the photosynthetic activities, ultimately killing the leaf. The reduction in yield can be as high as 90% under severe infection and 20% under moderate infection (T. B. Adhikari et al., 1995; Vasudevan & Kavitha, 2014).The BLB disease infection in rice cultivated under aerobic surroundings results in 30% lower yields than in rice cultivated under flooded environments (Yaqoob et al., 2012). Seedling infection can result in 20-50% seedling death (Yaqoob et al., 2012).

Small-scale farmers in Tanzania fail to recognize bacterial disease because they are not aware of it and the symptoms can be mistakenly attributed to other diseases, nutrient deficiencies and climatic effects, particularly drought. Only about 2.5% of the rice growers in Tanzania are familiar with bacterial leaf blight as a production constraint, while most farmers associate the low yields with other diseases (Ashura et al*.*, 1999). Consequently, the misdiagnosis has led to the use of unsuitable chemicals, resulting in the increase of the pathogen (Atiqur et al*.*, 2017).

In Tanzania, the use of bactericides, biological control, cultural practices and pest resistant varieties to reduce crop losses in management of BLB have been used on a small-scale and often now and then due to low level of awareness (IRRI, 2002). According to (Suh et al*.*, 2013), only 20% of rice producers are using bactericides when growing rice. Bactericides are expensive and not readily available to small-scale farmers. Nevertheless, pre-plant soil fumigants such as methyl bromide (bromomethane) that have a broad spectrum of activity have been used widely to protect high-value crops from pathogens (Atiqur et al*.*, 2017), but due to their hazardous effects on the environment and human beings, they have been discontinued. Bactericides that have been used to control bacteria with outstanding results but have been found to be toxic include paushamycin and Bion chemical (Brenner et al., 2006).These bactericides result in environmental hazards and reduced economic benefits. Therefore, management practices that will ensure no danger to the natural ecosystem and target crop are needed. These can be used in integrated pest management programmes

(IPM). The management practice should also be cheaper and readily available to the farmers, making use of disease resistant rice varieties the preferred control measure.

The use of resistant varieties is suitable for resource poor farmers because it does not require additional costs and is environment-friendly (Khoury & Makkouk, 2015). Globally, BLB-resistant rice cultivars have been developed (Nino-Liu et al., 2006). However, pathogenic races of the bacterium are highly variable and differ amongst regions, sites and even fields within a site (Jagjeet et al., 2010). More than 31 races of the pathogen *X. oryzae* pv.*oryzae* have been reported in several countries (Adhikari et al., 1999; Noda et al., 2001) (Nino-Liu et al., 2006).Correspondingly, as many as thirty-one resistant (R) genes against the bacterial blight pathogen have been identified in rice (Sudarsanam & Sabbu, 2016) and selected in a series from *Xa1* to *Xa31* (Banik & Jambhulkar, 2007). Thus, it is important inbreeding for resistance to BLB to screen germplasm in different locations.

6 Research objectives

The overall goal of this study was to increase and strengthen rice production in Tanzania through development of high yielding and BLB resistant varieties. The specific objectives were to:

1) Analyse genotype x environment interaction effects for reaction to bacterial leaf blight under natural infestation and grain yield performance in rice *(Oryza sativa L*) across multi-environments in Tanzania;

2) Assess the heritability, variability and efficiency of indirect selection using secondary traits for grain yield improvement among rice genotypes; and

3) Assess relationship among traits using correlation, path coefficients and genotype-by-trait analysis in rice (*Oryza sativa .L*).

7 Dissertation outline

This dissertation consists of five separate chapters reflecting the number of activities related to the above-mentioned objectives. The referencing system used in the chapters of this dissertation is based on the Crop Science journal. This is one of the recommended formats by the University of KwaZulu-Natal. The structure of the dissertation is given below.

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CHAPTER: 1

LITERATURE REVIEW

1.1 Introduction

This chapter presents topics relevant to the research focus to provide the theoretical base for the research. The review covers aspects on evaluation of rice genotypes for resistance to bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*) disease and yield components. The topics reviewed also include the ecology and taxonomy of bacterial leaf blight, its occurrence and distribution. The economic importance of the disease and yield losses, symptoms and characteristics of the pathogen are also highlighted. Aspects on disease cycle and epidemiology and pathogen survival are discussed. The host-pathogen relationship is given, highlighting the susceptible host stages and disease management to create an important frame of reference for the research study.

1.2 Ecology and taxonomy of bacterial leaf blight

Bacterial leaf blight disease was believed to be mainly due to soil acidity (Ezuka and Kaku, 2000). This is because the diseased leaves were first reported from fields applied with ammonium sulphate, where they exuded dew drops with an acidic reaction, while the drops from healthy leaves in the same field were not acidic(Nishida, 1909).Ashura et al.(1999) also stated that acidic soils were one of the factors favouring the occurrence of the disease. Takaishi (1909), while studying the effect of acidic soils in disease development observed that diseased leaves formed yellow bacterial masses when dried. Inoculation of healthy leaves with these bacterial masses resulted in the infection of the leaves. Bokura (1911) isolated and reported that the bacterium (*Bacillus oryzae*) came from the leaves and not from the acidic soil. According to reports by Tangani and Mizukani (1962) from Japan, the bacterial leaf blight of rice was believed to be a physiological reaction resulting from soil acidity.

Based on the study of its morphology and physiology, the bacterium was first named *Bacillus oryzae* Hori and Bokura (Rao et al., 2007). However, a study by Ishiyama (1933) identified the bacterium as *Pseudomonas oryzae* Uyeda and Ishima. Later, it was renamed *Bacterium oryzae*. The name *Xanthomonas oryzae* was later reviewed to *Xanthomonas campestris pv. oryzae* (Ishiyama, 1933) in the list of pathovars presented by the committee on Taxonomy of Phytopathogenics in Bacteria of the International Society for Plant Pathology. Swings et al. (1990) recently considered the bacterium to be a distinct species from *Xanthomonas campestris* on the basis of phenotypic, genotypic and chemotaxonomic data which shows that colonies of *Xanthomonas oryzae.pv. oryzae (Xoo)* are circular, convex, whitish yellow, with

smooth surface, entire margin and opaque against transmitted light, and thus proposed the name *Xanthomonas oryzae.pv. oryzae.* Researchers of bacterial blight of rice are now using this name widely.

Swing et al. (1990) reported that the overwintering of the bacterium occurs in two forms. The first being the dry form where it is found in the vascular vessels and xylem parenchyma of dried plants. If they are moistened by rainwater in winter, these dry form bacteria gradually die. The second is the growth form bacterial cells found on stubble and in root system of perennial wild plants, especially *Leersia* spp. The pathogen survives in an inactive stage. The dry form can be activated and turn into the growth form after receiving moisture under favourable conditions. The pathogen can survive on rice stubbles, straw and weed hosts. The BLB is vascular and spreads through xylem vessels. Lesions usually begin at the margin a few centimetres from the tip, as water stripes. It can occur at all stages of growth and development of the rice plant.

1.3 Occurrence and distribution of BLB

The bacterial blight is one of the most serious and oldest recorded rice diseases. The disease has been known in various localities of southern Japan since 1881 as white crushing disease (Nishida, 1909). Tangami and Mizukani (1962) reported that bacterial leaf blight was first seen by farmers in the Fukuoka area of Kyushu Island in Japan in 1884-1985 and was distributed from central to south western parts of Japan from 1908 to 1910. It has been commonly observed in the southwest of Japan since 1926 and has also been recorded in the northeast.

The disease increased after 1950 and by 1960 it was known to occur in all parts of Japan, except the northern island of Hokkaido. Its bacterial nature was established and the causal bacterium described by Ishiyama (1922). Occurrence of BLB has now been reported from Japan (Nishida, 1909),Korea (Takeuchi, 1930),U.S.S.R (Vzoroff, 1938), Indonesia (Reitsuma and Schure, 1950), Taiwan (Hashioka, 1951), China (Siang, 1952), Mexico (Dickson, 1956), Thailand (Jalavich arana 1958), India (Maharashtra), (Srinivasan et al., 1959), Sri Lanka (Pieris, 1962), Vietnam (Anon., 1963), the Philippines (Goto 1964), Bangladesh (Alim, 1967), Australia (Buddenhagen et al., 1969), Malaysia Purushothaman, (1974), Cambodia (Anon., 1970), Latin America (Ou, 1977) and the United States of America (Jones et al., 1989). In Africa, it was first reported in 1979 in Mali (Buddenhagen et al., 1979) and has since been reported in many other African countries. These countries include Senegal (Trung, 2011), Cameroon (Notteghem and Baudin, 1981), Niger (Reckhaus, 1983), Madagascar and Nigeria (Buddenhagen, 1985), Burkina Faso (Séré and Nacro, 1992), Tanzania (Ashura et al., 1999),

Benin, Guinea, the Gambia, Mozambique, Rwanda and Uganda (Onasanya et al., 2009; El-Namaky, 2011).

1.4 Economic importance of the disease

The bacterium causes economic yield losses as high as 80%. Table 1.1 shows yield losses due to BLB as reported from various countries.

Table 1. 1 Report of yield losses due to rice bacterial leaf blight disease

No	Country/state/region	Yield losses	Author
1	Japan	20-30%	Mizukani and Wakimoto (1969)
2	Korea	50%	Lee (1975)
3	Phillipines	8-25.87%	Lapis and Liansuthsakon (1975)
4	Phillipines	50%	Mew et al. (1993)
India			
5	A.P	6-60%	Srivastava et al.(1967)
6	Pantnagar Nainital	6-74%	Ahmed and Singh (1975)
7	Gujarat	70-80%	Joshi (1977)
8	India	38-40%	Mohiuddin et al.(1977)
9	India	10-56%	Rao and Kauffman (1977)
10	Faizabad,U.P	14.7-81.3%	Singh et al. (1977)
11	Hyderabad	25-72.7%	Reddy et al. (1978)
12	Punjab	60-70%	Raina et al. (1981)
13	Haryana	6.3-36.8%	Srivastava and Kapoor (1982)
14	Haryana	1.9-33.6%	Sunder et al. (2004)
Africa			
15	West Africa	2.7-41%	Lapis and Liansuthsakon (1975)
16	East Africa	20-50%	Chaudhary et al. 2012

1.5 Symptoms of BLB

According to Tangani and Mizukani (1962), as the disease advances on seedlings, small water soaked spots are observable on lower leaf margins. The lesions develop from leaf tips as water soaked lesions (Mizukami, 196l). The lesions gradually enlarge in size (both in length and in width), turn yellow or orange and a narrow water soaked area appears between healthy and diseased area of leaf blade, which is usually demarcated by wavy margins. The lesions are formed at one or both edges of the leaf blades with or without wavy margins. On susceptible varieties, the lesions extend from leaf blades to leaf sheath. The symptoms in the field appear at maximum tillering stage.

At the tillering and reproductive stages, the symptoms are known as leaf blight, a systemic infection that produces tannish grey to white lesions along the veins. If the plant produces panicles, the sterility percentage and number of immature grains will increase. Grain from diseased plants are easily broken during milling. In severely diseased fields, infected grain appears on the glumes as discoloured spots surrounded by water-soaked areas (Rao et al., 2002).

Bacterial blight is avascular bundle disease, and it has three common kinds of symptoms. Firstly, leaf blight type lesions are formed on either side of the leaf blade, which are slightly wavy in margin, the lesions extend from the tip to leaf towards the leaf base along the margin. The infection also progresses towards the mid rib forming 'v' shape blight. A severely infected field gives light brown appearance from a distance. The second type of symptom is wilting (Kresek) which occurs immediately after transplanting and the third type is withering which results in death of the entire plant (Yoshimura et al., 1959). Kresek is a more severe form of the disease that develops if roots or leaves are damaged and infected during transplanting at the seedling stage. Infection at this stage usually results in seedling death, 1-6 weeks after transplanting. The symptoms on the leaves are sometimes difficult to distinguish from those of various other leaf diseases, both physiological and parasitic, and the kresek symptoms are not easily separated from rice stem borer damage. A few simple methods to identify the disease have been described by Srivastava and Rao (1966), Srivastava (1972), Joshi (1977) and Ou (1985).

1.6 Characteristics of the pathogen

Morphologically, according to Ishiyama (1933), *Xanthomonas oryzae* are rod-shaped, gram-negative bacteria with a round ends and tare. There are variations in length of individual cells from approximately 0.7 μm to 2.0 μm and width ranges from 0.4 μm to 0.7 μm. The bacterial cells move using a single polar flagellum 6-8μm. The bacterium does not form spores, is aerobic and colonies on solid media that contain glucose appear as round, convex, mucoid and yellow in colour due to production of the pigment xanthomonadin characteristic of the genus (Bradbury, 1984). *Xanthomonas oryzae pv. oryzae* cells produce copious capsular extra cellular polysaccharides, which are important in the formation of droplets or strands of bacterial exudates from infected leaves providing protection from desiccation and aiding in wind and rain-borne dispersal (Swings et al., 1990).

To isolate the bacterium, sections of leaf tissues are surface-sterilized and macerated in distilled water, and the resulting suspension is streaked on 1% dextrose nutrient agar or Wakimoto agar and incubated at 25-28°C. (Reddy and Ou, 1974). Colonies of *X. oryzae pv.*

oryzae are slow-growing, mucoid and straw to yellow in colour. The isolated bacteria stain pink-red and show thin viscid mucoid strand indicating positive for KOH solubility test and gram-negative nature of the bacteria. A clear zone of hydrolysis is formed around the bacterial colonies when the plates are flooded with Lugol's iodine. Hence, the bacterium indicates positive for starch hydrolysis. Inoculated Tween 80 agar plates show the presence of white precipitate around the colonies of the bacteria, indicating a positive reaction for lipase activity. In addition, the *Xoo* isolates show liquefaction of gelatin and acid production from glucose Kaur and Thind (2002) states that necrosis is observed in tobacco plants indicating positive for hypersensitive reaction and positive for pathogenicity tests (Bradbury, 1984).

1.7 Disease cycle and epidemiology

The development of bacterial leaf blight depends on many factors, which include presence of rice stubbles and ratoons of infected plants, presence of bacteria in the rice and irrigation channels, warm temperature, high humidity, rain and deep water, over fertilization and handling of seedlings at transplantation (Singh and Paroda, 1994).Infected seed and plant debris perpetuate the disease from one season to another season. Other potential sources of inoculum are volunteer rice plants, infected chaff and weed host (Eswamurthy, 1993).

The bacteria are usually found in the glumes. *X. oryzae pv. oryzae* enters the rice leaf through hydathodes at the leaf tip and leaf margin (Ou, 1985). *X. oryzae pv. oryzae* also penetrates the leaf through stomata and multiplies in the sub-stomatal cavity where it colonizes the intercellular spaces of parenchyma. Within a few days, the bacterial cells and exopolysaccharides fill the xylem and oozes out from the hydathodes forming beads or strands of exudates on the leaf surface, a characteristic sign of the disease and a source of secondary inoculum. *X. oryzae pv. oryzae* may also gain access to the xylem through wounds or openings caused by emerging roots at the base of leaf sheath within the xylem. The bacterium presumably interacts with xylem parenchyma but may also penetrate into the endosperm (Eswamurthy, 1993; Shen et al., 2002).

Murthy and Devdath (1981) reported transmission of *X. c.* pv*. oryzae* by leaf hopper and grasshopper contamination of the mouth parts/body at the time of feeding on diseased plants. Pandey and Basu (1989) indicated that the grasshoppers may help indirectly by providing wounds on the plant tissue through their feeding, thus additional avenues for the pathogen to enter the host. The transmission of the pathogen is favoured by intense wind driven rainfall that facilitates bacterial entry into plant tissue through wounded leaf edges. Bacteria may also be disseminated in irrigation water as well as by humans, insects and birds (Liu et al., 2006). Cells on the leaf surface may become suspended in guttation fluid as it exudes at night and

enters the plant by swimming movement or passively as fluid is withdrawn into the leaf in the morning.

The bacterium multiplies in the intercellular spaces of the underlying epithem cells then enters and spreads into the plant through xylem (Noda and Kaku, 1999; Liu et al., 2006). *X.oryzae pv. oryzae* can survive in rhizosphere of weeds of genera *Leersia* and *Zizania* as well as in the base of the stem and the roots of rice stubble. *X. oryzae pv. oryzae* can also survive in the soil for 1-3 months depending on the soil moisture and acidity. In the tropics high temperature of 25-34°C, humidity of over 70% and abundance of host plants typically allow *X. oryzae pv. oryzae* to persist throughout the year (Liu et al., 2006). Severe epidemics often occur through the wind-blown rain that disperse bacteria. Bacterial leaf blight is more severe in highly managed systems such as irrigated paddy fields or with high nitrogen fertilizer application where the disease is aggravated by warm humid and wet conditions (Vzoroff, 1938). Once inside the vascular system, the bacterium multiplies and moves in both directions. Spread takes place in wind and rain, but primarily in flood and irrigation water (Sido and Basso 2005).

1.8 Studies on survival of pathogen in water from different sources

Singh (1971) reported that bacteria can survive only for 15 and 38 days in raw field water and raw pond water, respectively and for more than 12 months in sterilized tap water and sterilized distilled water. Thus, these sources can be used as inexpensive, reliable and practical medium for the preservation of the pathogen without any loss of viability and virulence. On the other hand, Chauhan (1973) reported that bacteria can survive only for 12 and 20 days in paddy field water and tap water, respectively and for more than 12 months in sterilized tap water and distilled sterilized water. Reddy and Reddy (1992) stated that *X. campestris* pv. *oryzae* did not survive long in field water and declined rapidly within ten days at all the three pH (6.0, 7.0 and 8.0) levels. In the absence of competitive microflora (in sterilized distilled and field water), the bacterium could survive for 75 days at 26°C and 210days at 2-4°C, whereas, at 26°C the bacterium was noticed up to seven days only in unsterilized field water.

1.9 Susceptible host stage to infection by Xoo

The susceptibility of rice to vascular infection by *X. c.* pv. *Oryzae* is known to decrease with plant age (IRRI, 1963). Mahmood and Singh (1970) observed that the bacterial blight infection increased significantly with increasing age of seedlings, where 60 days old seedlings gave significantly higher infection than younger seedlings (30, 20 and 15 days). Chauhan (1973) reported that the rice plant was susceptible to infection at all stages of its growth, but severity of disease decreased with the increasing age. Maximum numbers of plants were infected in the 40-60 days age group. However, Srinivasan (1982) observed that younger seedlings of

12-40 days old were more vulnerable to infection by *Xanthomonas campestris pv oryzae* than 45 and 50 day old seedlings, leading to development of the wilt phase.

Qi and Mew (1985) reported that the disease severity on some cultivars gradually decreased from seedling to flag leaf, whereas in others, it showed a distinct change to a resistant reaction on a certain leaf. The adult plant resistance of rice BLB appears to be race specific. Goel and Gupta (1990) reported the effect of host age on the expression of resistance to seven isolates of *X. c.* pv. *oryzae* in nine rice cultivars/lines. The growth stage of the plant when adult plant resistance became operative ranged from maximum tillering (50 DAS) to the booting stage (70 DAS) in different cultivars/isolate combinations. The disease severity in some cultivar/isolate combinations gradually decreased from seedling stage to boot stage, whereas in others an unexpected decline in disease severity was noticed at/or after maximum tillering (Goel and Gupta, 1990).

Koch and Mew (1991) observed that the fastest increase of disease occurred between 30 and 50 days after sowing, while Mazzola etal. (1993) showed that cultivarsIR-BB10, IR-BB21 and IR24 were susceptible at the seedling stage but on adult plants IR-BB21 had significantly shorter lesion length than those observed on susceptible IR24. Younger plants (less than 21-days-old) are most susceptible (Mew et al., 1993).

1.10 Disease Management

1.10.1 Reaction of rice genotypes to BLB

The long-term disease management strategies include use of disease resistant varieties. This is considered as the best alternative to reduce crop losses, being most effective, cheap and eco-friendly. Several rice genotypes and breeding lines from different countries have been identified, which may prove effective in combating the disease and for breeding resistance cultivar (Rao et al., 2007).

Breeding and the development of resistant cultivars carrying major resistance (R) genes have been the most effective and economical strategy to controlling BLB disease (Huang et al., 1997; Jena and Mackill.2008; Singh et al., 2001). Qualitative resistance, which confers major gene-specific resistance against some pathogen races, is the easiest to incorporate into breeding programmes and is usually considered a gene-for-gene type of resistance. For many pathogens and insects, this type of qualitative resistance is not often durable because of rapid changes in the virulence of the pathogen or biotype of the population (Leach et al., 2007). As a result, increasing attention has focused on the accumulation of major disease resistance genes in crop plants. Pyramided lines carrying two, three or four bacterial blight resistance

genes showed broad-spectrum and higher resistance than the lines with a single resistance gene (Suh et al., 2009). However, conventional breeding methods to improve rice cultivars for BLB resistance have not been that successful (Shin et al., 2011).

To date, at least 38 BLB resistance genes conferring host resistance against various strains of *Xoo* have been identified (Bhasin et al., 2012; Natrajkumar et al., 2012). All these resistance genes follow Mendelian pattern of major gene inheritance and express resistance to a diverse group of *Xoo* pathogens (Sun et al., 2004). Several of these genes have already been incorporated into rice cultivars, which are now widely cultivated in many countries (Sundaram et al.2008). Of these 38 R genes, six have been physically mapped (*Xa2, Xa4, Xa7, Xa30, Xa33* and *Xa38*) and six have been cloned (*Xa1, xa5, xa13, Xa21*, *Xa26* = *Xa3* and *Xa27*) (Liu et al., 2006; Natrajkumar et al., 2012). BLB resistance gene *Xa4* is one of the most widely exploited resistance genes in many rice breeding programmes and it confers durable resistance in many commercial rice cultivars (Mew et al., 1992; Sun et al., 2003).The *Xa21* gene was identified in the wild species *Oryza longistaminata* and is highly effective against BLB races of South and Southeast Asia (Khush et al., 1990). The *xa5* gene, which is naturally found only within the *Aus* subpopulation of rice (Garris et al., 2003), provides recessive resistance to several *Xoo* races of the Philippines.

Molecular markers can be used to identify and pyramid favourable and multiple alleles for biotic and abiotic stress resistance in a collection of diverse genotypes (Singh et al., 2001; Suh et al., 2009). Marker-assisted selection (MAS) for pyramiding important genes along with rapid background recovery of the recurrent parent, while maintaining the exquisite quality characteristics of rice, could be an effective approach for rice improvement (Sundaram et al., 2008;Xu and Crouch, 2008;Ye, 2010). Gene pyramiding is difficult using conventional breeding methods due to the dominance and epistasis effects of genes governing disease resistance. Moreover, genes with similar reactions to two or more races are difficult to identify and transfer through conventional approaches (Joseph et al., 2004; Rajpurohit et al., 2011; Sundaram et al., 2009). However, the availability of molecular markers closely linked to each of the resistance genes makes the identification of plants with two and three genes possible (Shanti et al., 2010; Sundaram et al., 2008). Three BLB resistance genes (*xa5, xa13* and *Xa21*) were pyramided in cultivar PR106 using MAS. Testing with 17 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates under artificial inoculation and field conditions showed that the combination of genes provided a wider spectrum of resistance to the pathogen populations prevalent in the region (Singh et al., 2001). In a previous study, the IR24 NILs (IRBB lines) containing *Xa4*, *xa5*, *Xa7* and *Xa21* genes and their combinations conferred different degrees of resistance to K1, K2, K3 and K3a races in a field inoculation experiment in Korea (Jeung et al., 2006; Suh et al., 2009). The resistance gene pyramid of *Xa4* + *xa5* + *Xa21*would be the

most effective strategy for improving Korean japonica cultivars for BLB resistance (Jeung et al., 2006; Kim et al., 2009). The identification of closely linked markers has also enabled pyramiding of *Xa4*, *xa5* and *Xa21* using MAB.

1.10.2 Screening and rating of BLB disease

Screening for resistance to BLB in rice has been done in either the field or greenhouse through visual assessment and use of molecular markers. In the field, screening for resistance has been conducted in hot spot areas where the disease is widespread (AfricaRice, 2010).Susceptible spreader-row plants have been planted between experimental plots to increase disease pressure in the field to ensure no disease escape (Kumar et al., 1992). Another way of increasing disease pressure is by direct inoculation through different methods such as clipping method, spraying method, needle-pricking and dipping method that are normally done at booting stage in the field or green-house (Kauffman, et al 1973).

Scoring system for evaluation of BLB disease severity in the greenhouse is based on lesion length measurement using a scale of0-9 or estimation of percent diseased leaf area. On other hand, in the field, disease severity is usually measured in percent diseased leaf area by scoring 1-50%>.

Resistance Genes Tagged with Molecular Markers in the application of molecular markers in breeding for BLB resistance resulted in mapping and tagging of some dominant and recessive genes, that is, *Xa4, xa5, Xa7*, *xa13*, and cloning of *Xa21*. These genes are being used as sources of disease resistance and in developing lines with single genes and pyramids with two, three, four and five bacterial blight resistance genes. Many genes for BLB have been identified in the breeding programme, and are currently available in monogenic and pyramid lines in IR24 background for use in developing or improving commercial varieties. The availability of molecular markers for these genes has made improving resistance to BLB more efficient.

1.10. 2 Eradication of pathogen from rice seed by hot water treatment

Pre-soaking of infected seed for 12 hours at room temperature in water solution of agrimycin (0.025%) + wet table ceresan (0.05) and later heat treatment in water at 52-54°C for 30 minutes was reported to eradicate the infection to the extent of 95 to 100%(Srivastava and Rao, 1964;Sinha and Nene,1967). The pre-soaking of seed can also be done in cold water, streptomycin sulphate (27 ppm) or ceresan (1000 ppm) for 8 hours, followed by hot water (54-55°C) for 20-30 minutes (Rajagopalan et al., 1968).

1.10.3 Chemical management

Chemical treatment of rice seeds has also been reported to be effective in eradicating the bacterium. Solanky (1988) treated infected rice seeds and seedlings with stable bleaching powder resulting in reduced disease incidence and plants that showed improved height and weight and increased grain and straw yields. Soil drenching with stable bleaching powder also reduced the disease index. It was concluded that stable bleaching powder control bacterial leaf blight. Natarajan (1988) tested different chemicals for the control of *Xanthomonas oryzae* pv.*oryzae*and bleaching powder was the most effective in reducing bacterial leaf blight followed by plantomycin, paushamycin + copper oxychloride, and paushamycin.

1.10.4 Organic management

Brar (1994) studied the effect of organic and inorganic sources of nutrients on the incidence of disease in rice. Nutrient source significantly influenced the occurrence of diseases in rice. NPK application through chemical fertilizer either in single or balance form without organic manure increased the severity of BLB of rice. Das et al. (1998) evaluated some natural products like fresh cowdung and antibiotics (plantomycin) against bacterial leaf blight of rice. The results showed that foliar spraying of fresh cow dung suspension at 50kg cow dung / ha reduced the incidence of BLB of rice significantly resulting in the lowest percentage of leaf area infected (18.53%) compared with 38.03% in the unsprayed control. Zaragoza,1959 reported that the control of rice bacterial leaf blight (*Xanthomonas oryzae*) using a new agricultural antibiotic, zongshengmycin (organic) was investigated when infected rice seeds were soaked in a 100mg/kg solution (at 58^0C cooling to room temperature) of the antibiotic for 48 hours, bacteria on the surface and inside the seeds were completely killed. This removed the source of infection. When rice seeds were soaked in a 50mg/kg solution of zhongshengmycin in water (at 55-60^0c, cooling down to air temperature) for 48hours before sowing in the field, disease severity was significantly reduced. However, when disease was severe, it was necessary to apply a spray of 15 mg/kg solution of the antibiotic (zongshengmycin).

1.10.5 Cultural and physical management

Cultural methods have also been reported to be effective in managing diseases such as BLB (Zhao et al., 2010). The important cultural methods include; timely sowing, that is sowing and transplanting should be done when the seedlings have attained 4 – 5 leaf stage, optimum plant densities by putting 2 – 3 seedlings per hill, proper management of water by avoiding flooding the field, application of moderate nitrogenous fertilizers not more than 80kg /ha and following instructions given by seed companies on amount of application in each variety. The

control of rice bacterial leaf blight can also be done by removing the weeds around the field to avoid spreading of the disease (Ahammed, 1992).

1.10.6 Biological control

Biological control of BLB has also been investigated. Sidhan et al.(1997) tested phylloplane organisms isolated from rice leaves and reported that *Pseudomonas acidovorus* was most effective for reducing lesion length when applied seven days before inoculation of BLB pathogen, followed by *Aspergillus ochraceus, Fusarium chlamydosporum,* and *Fusarium pallidoroseum*. The effectiveness of the antagonists was reduced when applied after inoculation with the pathogen. Lore (2004) tested the efficacy of control of bacterial blight in China with an avirulent mutant of the pathogen DU 728 strain in the greenhouse and field plots. The rate of control with one application of DU 728 spray (106 cfu/ml) was 48.5%. Higher dosages did not significantly increase control. When DU 728 was mixed with salicyclic acid (10µ/ml), control was increased to 60%.Babu et al. (2003) analysed rice (cv. IR50) leaves clip-inoculated with *Xanthomonas oryzae* for the accumulation of pathogenesis related proteins and observed a marked increase in activities of chitinase and beta-1-3 gluconase. Western blot analysis showed that a protein with a molecular mass of 35 Kda cross – that reacted with barley chitinase antibody was induced in rice in response to inoculation with *Xanthomonas oryzae*. The appearance of this chitinase was correlated with the increase in activity of this enzyme during the test period.

1.11 Genotype x environment interaction

Genotype x environment interaction (GEI) is the response of genotypes to environmental changes. It is expressed when the genotypic and environmental effects differ in accordance with the genotype and specific environment. Differential performance of genotypes is caused either by differential responses of the same set of genes to changes in the environment or by expression of different sets of genes in different environments (Cooper and Delacy, 1994; Crossa et al., 1995). The GEI reaction is manifested either as rank order change of the genotypes between environments (crossover GEI), or as alterations in the absolute differences between the genotypes without affecting the rank order (Crossa et al., 1995; Haji and hunt, 1999). The crossover interaction results in serious consequences on breeding progress (Cooper and Delacy, 1994; Crossa et al., 1995). For example, the same set of genes responsible for high yield under stress environment may be responsible for low yield potential. In this case, breeding progress is delayed due to changes in the composition of the selected and the rejected genotypes in each environment. This reduces heritability hence the breeding progress. In such cases, genotypes must be bred for specific adaptation to certain

environments. Under such circumstances, plant breeders desire to find stable genotypes that show little interaction with environments (Yan et al., 2007).

An appropriate stable cultivar is capable of using resources that are available in high yielding environments, while maintaining above average performance in all other environments (Finlay and Wilkinson, 1963). Methods for analyses and interpretation of GEI patterns include regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), principal component analysis (PCA) (Hill and Goodchild, 1981), additive main effects and multiplicative interaction (AMMI) (Gauch and Zobel, 1988) and genotype plus genotype by environment (GGE) analysis (Yan, 2001). Of these, AMMI and GGE biplot are widely used. The AMMI model integrates analysis of variance (ANOVA) and principal component analysis (PCA) into a combined approach that can be used to analyse multi-location trials (Crossa et al., 1995; Gauch and Zobel 1988; Zobel et al., 1988). In AMMI1 a biplot of main effects with interaction PCA1 (IPCA1) facilitates visualisation of correlation among environments and the response patterns of the genotypes and their interactions with the environments by using sign and magnitude of IPCA1 values (Yan and Hunt, 2001). In AMMI2 a biplot of IPCA1 and IPCA2 is constructed which visualises magnitude of interaction for each genotype and environment. The GGE biplot analysis on the other hand puts together genotypic main effects (G) and GEI to facilitate graphical visualisation of cultivar evaluation and mega environment identification (Yan et al., 2000, Yan, 2002).

Yan et al. (2007) compared GGE Biplot and AMMI analyses. They concluded that; (i) both GGE biplot analysis and AMMI analysis combine rather than separate G and GEI in mega-environment analysis and genotype evaluation, (ii) the GGE biplot is superior to the AMMI1 graph in mega-environment analysis and genotype evaluation because it explains more G+GEI and has the inner-product property of the biplot and helps identify cultivars that were adapted across locations and stability, and (iii) the discriminating power vs. representativeness view of the GGE biplot is effective in evaluating test environments, which is not possible in AMMI analysis. On the other hand, the “which-won-where” patterns are not always easy to visualize in the AMMI1 graph, particularly when many genotypes and test environments are involved (Ebdon and Gauch, 2002). This is because, in the AMMI1 graph, the environments can be labelled only along the abscissa rather than across the graph, and the genotypes are represented by straight lines rather than by dots. Therefore, the AMMI graph is better viewed as a tool for presenting conclusions rather than as a tool for discovering which-won-where patterns (Ebdon and Gauch, 2002); Gauch, 2006). Also Gauch et al. (2007) reviewed AMMI and GGE analyses, and concluded that the AMMI mega-environment graph incorporated more of the genotype main effect and captured more of GEI than did the GGE

biplot, and thereby displayed the "which-won-where" pattern more accurately for complex datasets. When the G x E interaction is captured well by one principal component, the AMMI graph of genotype nominal yields described winning genotypes and adaptive responses more simply and clearly than the GGE biplot. For genotype evaluation within a single mega-environment, a simple scatterplot of mean and stability was more straightforward than the mean x stability view of a GGE biplot.

Moreover, on the genotype by environment effects on reaction to disease, Baker (1988) states that many of the observed crossover genotype –environment interactions are manifestations of differences in disease resistance or some other highly heritable character. This suggests that when no such explanation can be offered, crossover interactions are to be regarded as random variables whose impact can be minimized by adequate sampling of the environments. However, there is limited information available on disease resistance and stability GGE biplot as indicated by Ouk et al. (2007).

1.12 Heritability, variability for grain yield and yield components

Heritability is the proportion of observed phenotypic variation in a progeny that is attributable to the effects of genes (Rahman and Hossain, 2014). It is a property of the trait, the population and the environment. Altering one of these factors results in different estimates of heritability (Acquaah, 2007). There are two different estimates of heritability; broad and narrow sense heritability, the latter, which is the degree of resemblance between relatives, is more useful to plant breeders as it determines response to selection. High narrow sense heritability estimates correspond to additive gene action while low heritability estimates show non-additive gene action. Moreover, a trait with a high heritability estimate indicates that the transmission of that trait from the parents to progeny is very high and that simple selection procedures may be employed to select for superior genotypes (Jayasudha and Deepak, 2010).

In rice, estimation of heritability for grain yield and other yield components have mostly been based on broad sense heritability. Several studies in India and Philippines have reported moderate to high broad sense heritability estimates for grain yield in very susceptible varieties (Karthikeyan et al., 2010; Ogunbayo et al., 2011). Mohan, 2011) reported that grain yield is a complex trait, quantitative in nature and a combined function of a number of constituent traits. Consequently, selection for yield may not be effective without taking into consideration yield component traits. Thus, positive correlations between yield and yield components are required for effective indirect selection for grain yield in rice (Ogunbayo et al., 2011). Therefore, it is important for plant breeders to understand the degree of correlation between yield and its components.

1.13 Multi-trait relationships

Grain yield is regarded as the primary character with the main breeding objective in all crops focusing on high yield. However, direct selection for yield is not sufficiently effective due to its low heritability. The use of morphological and physiological traits commonly known as secondary traits, for indirect selection for higher yields has often been suggested Badu-Apraku, al. (2007). Although correlation coefficients are very important in determining the relative contribution of each secondary trait to grain yield, they are insufficient in determining whether the trait affects grain yield directly or indirectly (Nandan et al., 2010). Through path analysis, the correlation coefficient may be partitioned into components due to direct effect of a predictor variable upon its response variable and due to indirect effects of a predictor variable on the response variable through another predictor variable (Dewey and Lu, 1959). Plant breeders use path analysis to identify traits that are useful as selection criteria to improve crop yield (Surek and Beser, 2003). In this study, correlation and path analysis were used to identify traits that had direct effects on grain yield in order to devise a multiple trait selection criteria for improvement of yield in rice.

1.14 Diversity among genotypes

Jayaman et al. (2007) studied seventy-five genotypes of rice, which grouped into ten clusters. Clustering pattern revealed that geographic diversity is not a reasonable index of diversity. The average inter cluster distance was maximum between cluster IX and X (66.58) followed by cluster VI and IX (62.59) and cluster IV and X (56.52) suggesting that these groups of genotypes were highly divergent from each other. The genotypes in clusters revealed substantial differences in the means for important yield contributing characters suggesting that the genotypes belong to these clusters, which form ideal pairs for initiating hybridization.

Chakravarthi, et al. (2010) observed divergence was an efficient tool for the selection of parents used in hybridization programme. In a study to identify diversity, fifty-three rice genotypes consisting of high yielding rice varieties/ cultures and IRRI germplasm lines were raised at Rice Research Station, Tirur during Sornavari, 2009. They were evaluated for eight yield and yield attributing characters using D2 analysis. Based on the analysis, the genotypes were grouped into 11 clusters. The maximum number of 16 and 15 genotypes were grouped under cluster XI and I respectively, while clusters II, IV, V, VI, VIII, IX and X had only two genotypes each and clusters III and VII consisted of 3 and 5 genotypes, respectively. Maximum inter cluster D2 value was observed between cluster I and X (32.96) followed by cluster I and IV (32.90). The distance between two clusters indicates the genetic diversity between genotypes. Therefore, the combined characters may be given importance during hybridization programme.

Gracia, et al. (2010) studied diversity among 39 local rice genotypes using Mahalanobis statistic. Based on genetic distance, these genotypes were grouped into eight clusters. Cluster VI was the largest, consisting of 21 genotypes, while clusters I, II, III, IV, and V contained two genotypes each and cluster VI and VIII contained four genotypes each. Grouping of genotypes in different clusters indicated the existence of significant amount of variability among the genotypes for the traits studied. High degree of divergence was recorded between cluster IV and VIII. Based on high mean performance of the traits studied, two clusters (IV & II) had local rice genotypes Biliya and Doddabatta.

Summary

From the literature review it was observed that BLB disease of rice is a major constraint to rice production under rainfed upland and lowland ecologies in East Africa, causing significant yield losses about 20%-50%. The disease is more severe under smallholder rice faming systems where low input agriculture is practiced. Cultural and chemical controls methods have been proposed to control BLB disease but may not be appropriate due to negative effects on the environment and lack of capital to purchase bactericides by smallholder farmers. Therefore, breeding for disease resistance varieties has been suggested as the most practical option to effectively address the problem of BLB.

The review also noted that under disease conditions, genotype x environment interactions (GEI) are common. Multi-locational trials, therefore, are needed to determine the magnitude of GEI and to assist in identification and recommendation of high resistance genotypes and stable genotypes that show little interaction with the environment or genotypes specifically adapted to certain environments.

Moreover, phenotypic traits can be used to discriminate varieties into clusters though they do not always reflect the genetic constitution in rice because of environmental influences but they can never be excluded in crop improvement. Few papers have reported on the use of phenotypic traits in discriminating varieties into clusters, hence there is need to evaluate these methods and see their effectiveness. Therefore, studying of phenotypic traits in this current study will help to improve hybridization of germplasm. Currently there is no literature regarding the levels of diversity in Tanzania, which has implications on the rice breeding. Therefore, there is need to study the diversity of widely grown varieties in Tanzania.

The literature review has shown that BLB resistance is the major challenge that needs to be addressed to achieve predicted high production levels of rice yields productions. The challenge is to identify sources of resistance to BLB with different genetic background and breed resistant cultivars adapted to the local conditions and with farmer preferred traits to improve adoption.

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CHAPTER 2

Genotype × environment interaction analysis for bacterial leaf blight disease infection and grain yield performance of rice (*Oryza sativa* L.) across multi-environments

Abstract

Bacterial leaf blight (BLB) of rice (*Oryzae sativa* L.), caused by *Xanthomonas oryzae* pv. *oryzae,(Xoo)* is a major constraint and is broadly spread in all irrigated and lowland rainfed rice producing areas of Tanzania. The pathogen is highly adaptable and its control is difficult. Development and deployment of host resistance is the most effective means of BLB management. The objective of this investigation was to evaluate genotype by environment interaction (GEI) and stability for reaction to bacterial leaf blight and grain yield among rice genotypes across environments in eastern and southern parts of Tanzania. Therefore, this study was conducted which comprised 30 rice genotypes inclusive of a resistant check (IR-24) and a susceptible check (Txd-306).The genotypes were evaluated in a 6 x 5 alpha lattice design with three replications at three locations; ARI-KATRIN, Igurusi and Kyela. Moreover disease score data were collected on the 30 rice genotypes 80 days after planting for the early maturing varieties by recording length of leaf showing symptoms of BLB and grain yield data was collected by weighing the total grains per plot. The additive main effects and multiplicative interaction (AMMI) analysis and genotype plus genotype x environment interaction (GGE) biplot analysis were used to assess the magnitude of GEI and stability of performance for each genotype. The genotype and environment main effects and their interactions were highly significant. Ranking of the genotypes changed across environments revealing a crossover type of GEI. The AMMI and GGE biplot analysis identified NERICA4, as the most resistant and stable genotype across environments. Other genotypes that were resistant and stable include NERICA 1, NERICA 2, LOW-LAND NERICA 6, Tule na bwana and the check IR-24. Regarding grain yield, genotype and environment main effects were whereas GEI effect was not significant. Genotypes Txd- 306, Txd-88, WITA-10 and Kalalu were the top yielders. High yielding genotypes that were resistant to BLB were also identified. The identified resistant and high yielding materials could be used in hybridization programmes to develop cultivars that are more desirable.

Keywords: Bacterial leaf blight, rice**,** GGE, AMMI, grain yield.

2.1 Introduction

Genotype x environment interaction (GEI) is the differential genotypic responses to environmental changes (Baker, 1988) ;Crossa, 1990; Romagosa and Fox, 1993). The genotypic main effects provide sufficient information about the performance of the genotypes across environments in the absence of GEI. However, with significant GEI, differences between genotypes vary widely among environments (Annicchiarico, 2002). Multi-environment trials are designed to measure the response of genotypes across environments and hence determine the extent of GEI and whether they can be used or misused in plant breeding programmes. The significances of phenotypic variation depend mostly on the environment. The variation is mostly because not all genotypes perform in a similar way to changes in the environment and no two environments are the same.

Moreover, GEI results in genotype rank changes from one environment to another, a difference in measure among environments, or a combination of these two conditions. If the performance of genotypes grown in different environments is different, then GEI becomes a major challenging factor to plant breeding. It is important for plant breeders to identify specific genotypes adapted to or stable in environment(s), thereby achieving quick genetic gain through screening of genotypes for high adaptation and stability under varying environmental conditions prior to release as a variety (Fox, 1997). However, most genotypes exhibit unstable resistance when grown in different locations or agro-ecological zones. This complicates demonstrating the superiority of a particular variety. To address this challenge, multi-environment yield trials and high adaption of the genotype are essential to identify adaptable high resistance and yield cultivars and discover sites that best represent the target environment (Yan et al., 2000).

Due to the differential responses of the genotypes, diseases have been identified as one of the contributory factor to GEI in rice (Fox et al., 1997). In addition, Gravois et al. (1990) state that many of the observed crossover GEI are expression of differences in disease resistance or some other highly heritable character suggesting that when no such explanation can be offered crossover interactions should be regarded as a random variable whose impact can be minimized by adequate sampling of the environment. Adaptability is the result of genotype, environment and GEI and generally falls into two classes: (1) the ability to perform at an acceptable level in a range of environments, referred to as general adaptability, and (2) the ability to perform well only in desirable environments, known as specific adaptability (Arshadfar & Utka, 2006). Combined analysis of variance can quantify GEI and describe the main effects but does not explain the interaction effect (Kaya, Palta, & Taner, 2002) ; Hill et al., 1981).

The fundamental reason Additive Main effects and Multiplicative Interactions (AMMI) is appropriate for agricultural research is that the ANOVA part of AMMI can separate the G and E main effects and the GEI effects (Gauch et al., 2008). Besides, its greatest advantage is its ability to extract interaction Principal Component Axis (PCA) along which there is a maximum variation, thereby indicating the number of components necessary to explain the pattern in the interaction residual (Flores, 1998). Additive Main Effect and Multiplicative Interaction model and genotype and GEI (GGE) biplot analysis are the most commonly used analytical and statistical tools to determine the pattern of genotypic responses across environments (Kaya et al., 2002; W Yan et al., 2000; Zobel, 1996). AMMI and GGE biplot for graphical display of data (Kaya et al., 2002; W Yan et al., 2000; Zobel, 1996) and (Eberhart & Russell, 1966) model are the most commonly used analytical and statistical tools to identify stable, high yielding and adaptable genotype(s) for wider and/or specific environments. Therefore, the objective of the present study was to evaluate GEI and stability of performance in respect of reaction to BLB and grain yield performance of rice genotypes including farmer varieties and the improved varieties from government institutions and international organisations.

2.2 Materials and Methods

2.2.1 Germplasm

Thirty rice genotypes (Table 2.1) comprising of 21 cultivars from ARI-KATRIN in Tanzania, three cultivars from International Rice Research Institute (IRRI) and six cultivars from AfricaRice were used in this study. Cultivars IR-24 from IRRI and Txd 306 (SARO 5) from ARI-KATRIN were used as resistant and susceptible checks, respectively.

Table 2. 1 Rice genotypes used in the study

Genotype code	Cultivar name	Ecology	Sub-species	Source
G1	NERICA 1	Upland	*Interspecific*	AfricaRice
G2	NERICA 2	Upland	*Interspecific*	AfricaRice
G3	NERICA 4	Upland	*Interspecific*	AfricaRice
G4	LOW LAND NERICA 6	Lowland rainfed	*Interspecific*	ARI-KATRIN
G5	WAB 450-12-12-BL1-DV 4	Upland variety	*Interspecific*	Africarice
G6	IR-56	Upland variety	*Sativa*	IRRI
G7	WITA 10	Upland variety	*Sativa*	Africarice
G8	WAB 450-12-4-BL1-DV1	Upland variety	*Sativa*	Africarice
G9	IR54	Upland	*Sativa*	IRRI
G10	Kalalu	Lowland	*Sativa*	ARI-KATRIN
G11	Katrin	Lowland	*Sativa*	ARI-KATRIN
G12	Dakawa 83	Lowland	*Sativa*	ARI-KATRIN
G13	Txd 85 (Improved)	Irrigated	*Sativa*	ARI-KATRIN
G14	Txd 88 (Improved)	Irrigated	*Sativa*	ARI-KATRIN
G15	Mwangaza	Irrigated	*Sativa*	ARI-KATRIN
G16	Tai	Lowland	*Sativa*	ARI-KATRIN
G17	SATO I	Lowland	*Sativa*	ARI-KATRIN
G18	Txd 307	Lowland	*Sativa*	ARI-KATRIN
G19	SATO IX	Lowland	*Sativa*	ARI-KATRIN
G20	Komboka	Lowland	*Sativa*	ARI-KATRIN
G21	Kalamata	Lowland	*Sativa*	ARI-KATRIN
G22	Supa India	Lowland rainfed	*Sativa*	ARI-KATRIN
G23	Mwanza	Lowland rainfed	*Sativa*	ARI-KATRIN
G24	Tule na bwana	Lowland	*Sativa*	ARI-KATRIN
G25	Sindano	Lowland	*Sativa*	ARI-KATRIN
G26	Zambia	Lowland	*Sativa*	ARI-KATRIN
G27	Kalundi	Lowland	*Sativa*	ARI-KATRIN
G28	Wahiwahi	Lowland	*Sativa*	ARI-KATRIN
G29	IR-24	Upland	*Sativa*	IRRI
G30	Txd 306 (Improved)	Lowland	*Sativa*	ARI-KATRIN

2.2.2 Trial locations

The experiment was carried out at three locations in different agro-ecological regions in Tanzania. Detailed information about the trial locations is presented in Table 2.2.

Table 2. 2 Features of the three environments used in the study

Location	Latitude	Longitude	Altitude (m)	Annual rainfall (mm)	Average temperature (°C)	Soil type
ARI-KATRIN	36°41'0E	8°6'0"S	2500	1418	27.9	Sandy loam and clay
Igurusi	33°51'0"E	8°51'0"S	1211	1235	21.3	Sand and clay
Kyela	33°55'0"E	9°34°60"S	495	2158	24.9	Sandy loam

2.2.3 Experimental design and management of trials

The 30 genotypes were laid out in a 6 x 5 alpha lattice design with three replications at each location. Each genotype occupied two 5m long rows. The space between the rows was 0.2m and space between plants in a row was 0.2m. Two seeds were placed per hill; however, thinning was done to one plant per hill at two weeks after planting. At planting, double ammonium phosphate fertilizer (ratio 18:46) was applied at a rate of 50kg N and 50 kg P_2O_5 per hectare. After thinning, the trials were top dressed with urea (46% N) at a rate of 20kg N per hectare.

2.2.4 Data collection

Disease reaction on the rice genotypes was recorded based on length of the leaf showing symptoms of BLB at crop maturity stage. The length of the BLB lesion was then classified in accordance with (IRRI, 1996) and Cottyn and Mew(2004).Data were collected on the 30 genotypes by recording disease score 80 days after planting for the early maturity varieties, using the IRRI standard scoring scale (IRRI, 1996). Table 2.3 and Figure 2.1 show the affected leaves with BLB. Grain yield data was collected by weighing the total grains per plot.

Table 2. 3 Standard Evaluation System (SES) for rice scale for BLB scoring (0-9) in the field

Scale	**Percentage of Diseased leaf area**	**Description**
1	1 -5	Resistant (R)
3	5 – 12	Medium resistant (MR)
5	13 – 25	Medium susceptible (MS)
7	26 – 50	Susceptible (S)
9	>50	Highly susceptible (HS)

Source: (IRRI, 1996) SES for Rice, Fourth Edition, Philippines

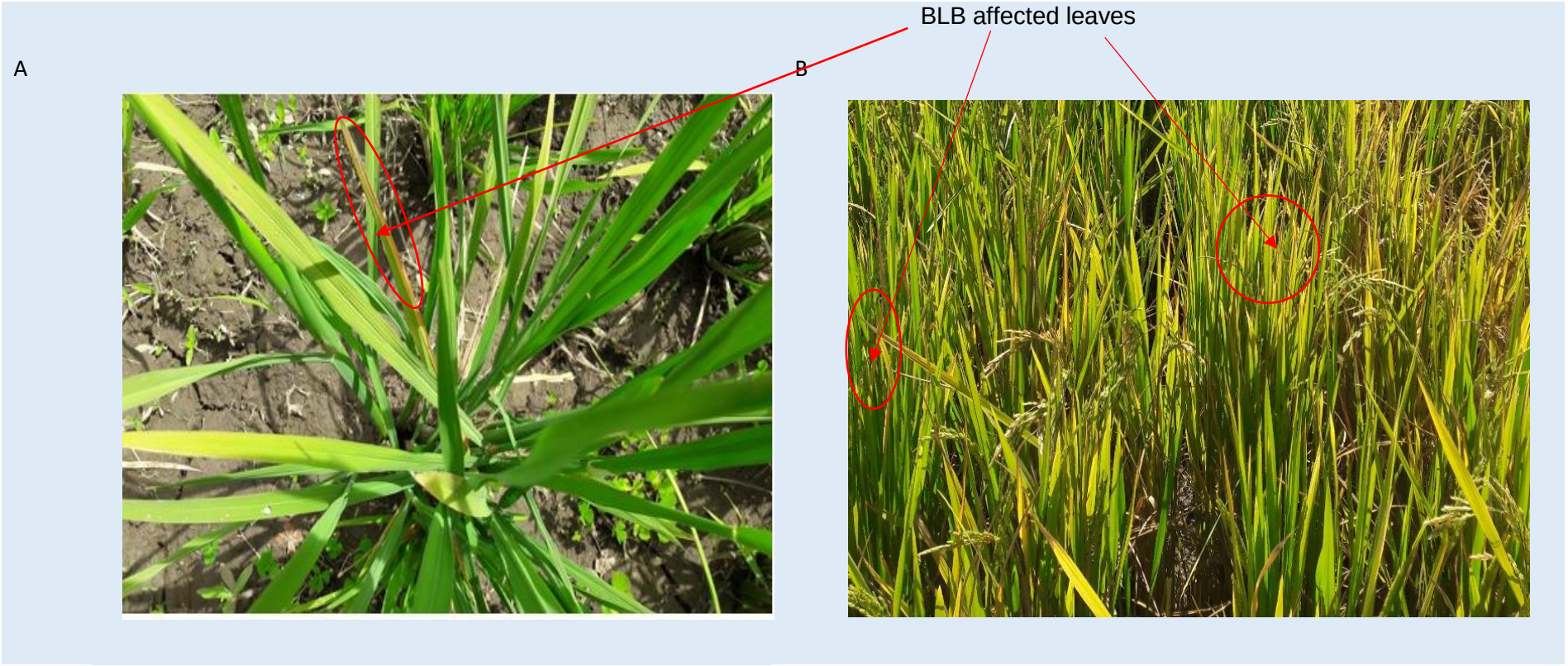

Figure 2. 1 BLB assessment field photographs. A and B represent BLB affected leaves

2.3 Statistical analysis

2.3.1 ANOVA at individual location and across locations

Analysis of variance (ANOVA) for each location was done separately, followed by combined ANOVA across locations for BLB resistance scores and grain yield. The ANOVA was performed using the PROC GLM of SAS version 9.4 (SAS Institute, 2014) and the TUKEY option was used for mean separation. The linear model used for the single location ANOVA was:

$$Y_{ij(l)} = \mu + R_i + \beta_{l(i)} + G_j + \varepsilon_{ijl}$$

Where:

$\mu, R_i, \beta_{l(i)}, G_j$ and ε_{ijl} represent the mean, replication effect, the incomplete block within replication effect, the genotype effect and random error, respectively.

For across location ANOVA, PROC GLM was performed and the TUKEY option was used for mean separation. The linear model used was as follows:

$$Y_{ijkl} = \mu + E_k + R_{i(k)} + \beta_{l(ik)} + G_j + GE_{jk} + \varepsilon_{ijkl}$$

Where;

Y_{ijkl} =is the response of the *j*th genotype *k*th environment and *i*th replication within environment and *l*th block within replication: μ is the grand mean of the experiment, E_k is the environment effect, G_j is the genotype effect, $\beta_{l(ik)}$ is the block within replication effect, GE_{jk} is the genotype x environment interaction effect and ε_{ijkl} is the random error.

2.3.2 AMMI analysis

Genotype stability for resistance to BLB disease was determined using the additive main effect and multiplicative interaction (AMMI) analysis in GENSTAT 17thEdition statistical software. Considering a yield trial with a two-way factorial design of *g* genotypes and *e* environments, with *r* replications, the AMMI model combines ANOVA with additive parameters and PCA with multiplicative parameters into a single analysis (Gauch and Zobel, 1997).

The AMMI model used is:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where, Y_{ge} = is the response of the genotype (g) in the environment (e), μ is the grand mean, α_g = genotype deviation, β_e = environment deviation γ_n = is the singular value for component n, γ_{gn} = is the eigenvector value for g, δ_{en} = is the eigenvector value for e and residual term is ρ_{ger}, ε_{ger} = the random error.

2.3.3 GGE biplot analysis

For GGE analysis, (Yan 2002) model was used in Genstat 17th edition and is presented as follows.

$$Yij - \mu - \beta j = \lambda 1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

Where: Y_{ij} = response of genotype i in environment j;

μ is the grand mean

β_j = main effect of the environment

j; λ_1 and λ_2 are singular values (SV) for the first and second principal components (PC1 and PC2), respectively.

ξ_{i1} and ξ_{i2} are eigen vectors of genotype i for PC1 and PC2, respectively

εij = is the residual associated with genotype i in environment j.

Biplots were constructed to visualize the performance of genotypes in individual environments, to compare genotypes concerning performance and stability, and to show relationships among environments.

2.4 Results

2.4.1 Analysis of variance for BLB scores

At individual locations, genotype had significant effect on reaction to BLB at two locations *viz*. ARI-KATRIN and Kyela, and there were no differences among genotypes at Igurusi; whereas. Across the locations, reaction to BLB was highly significant for environment, genotype main effects and GEI (Tables 2.4, 2.5 and 2.6).

Table 2. 4 Analysis of variance for BLB scores and grain yield per plot across three locations

Source of variation	Degrees of freedom	Bacterial Leaf Blight (scores)	Yield /Plot (kg)
Environment (E)	2	14.50***	0.18*
Replication (R) within E	6	2.60***	0.11*
Block (E*R)	45	0.96**	0.06
Genotype (G)	29	2.83***	0.89***
GxE	58	1.48***	0.02
Error	129	0.47	0.04

*, **, and *** = Significant at P<0.05, P<0.01 and P<0.001

2.4.2 Mean Bacterial leaf blight scores of genotypes

At ARI-KATRIN, genotypes had a significant (P<0.001) effect on reaction to BLB and the highest score was for IR-56 and IR-54 which recorded (4.98), followed by Kalalu, Txd 85,Txd 88 and Supa India which recorded (3.17), Mwanza (3.08) and Zambia,Kalundi and Wahiwahi which recorded (3.00). The mean BLB score was 1.98 which showed the disease was not extremely high at ARI-KATRIN. The maximum score for a disease was forIR-56 and IR-54 (4.98) while the least score was for Dakawa 83 (0.87).

At Igurusi the highest BLB score was for Komboka (3.66) followed by Mwanza (3.57), Supa India (3.46), Sindano (3.44) and Txd 306 (3.34) while the least score was for NERICA 4 (0.93). The mean disease score was 2.36 which showed moderate disease severity compared to ARI-KATRIN. The maximum score at Igurusi was 3.66 for Komboka and the minimum score was 0.93 for NERICA 4.At this location, genotype effect was not significant.

For Kyela, the highest disease rating score was for Txd 306 (5.68) followed by Supa India (5.59), Txd 307 (4.48), IR-56 (3.71) and Mwangaza (3.59) in that order. The least score was for NERICA 4 (0.84), followed by IR-24 (1.51). The mean score was 3.13 suggesting moderate disease severity, but a higher disease pressure compared to ARI-KATRIN and Igurusi. The minimum score was for NERICA 4 (0.84). In addition, there were significant differences among genotypes for reaction to BLB.

Results of the rice genotypes evaluated across three locations, revealed differences in genotypic reaction to BLB. Genotypes NERICA 1, NERICA 2, NERICA 4, LOWLAND NERICA 6, Kalamata, Tule na bwana and IR-24 showed resistance to the disease in all three locations while genotypes IR-56, IR-54 and Txd 88 showed moderate resistance to the disease in all

three locations. Moreover, the most resistance genotype was NERICA 4 which scored 0.93 while Supa India (4.07) was the most susceptible, while the mean BLB score was 2.46.

2.4.3 AMMI analysis

AMMI analysis of variance for reaction to BLB showed highly significant (P<0.001) treatments, genotype, environments and GEI effects. Genotypes, environments and GEI accounted for 28.09%, 14.83%, and 26.71% of the total sum of squares. The PCA axis (IPCA1) of the interaction were highly significant (P<0.001) and contributed 81.35% of the GEI sum of squares. The IPCA1 scores for each genotype are shown in Table 2.6 and those for environments are shown in Table 2.7.Genotypes NERICA 2, LOW LAND NERICA 6, and IR-24 had very low IPCA1 scores whereas Txd -306 and IR-54 had high IPCA1 scores.

Table 2. 5 Analysis of variance for AMMI model for bacterial leaf blight across the three locations

Source	Degrees of freedom	Sum of squares	Mean squares	Proportion of total variance explained (%)	G x E explained (%)
Total	269	421.501	1.567		
Treatments	89	293.531	3.297***	69.632	
Genotypes	29	118.412	4.081***	28.090	
Environments	2	62.543	31.244**	14.828	
Block	5	27.445	4.563***	6.501	
Genotype x environment	58	112.623	1.942***	26.720	
IPCA 1	30	91.609	3.054***		81.350
Residuals	28	21.000	0.751		18.650
Error	129	100.6	0.578	23.867	

, and * = Significant at P<0.01 and P<0.001

Table 2. 6 Mean BLB scores of the tested genotypes at individual locations and across locations, and IPCA1 scores for the genotypes

G	ARI-KATRIN		IGURUSI		KYELA		ACROSS		IPCA
1	0.92	R	2.12	R	2.50	R	1.85	R	0.36
2	1.66	R	2.12	R	2.88	R	2.23	R	0.07
3	1.01	R	0.93	R	0.84	R	0.93	R	-0.32
4	1.68	R	2.12	R	2.88	R	2.23	R	0.07
5	2.83	R	2.26	R	3.07	MR	2.72	R	-0.38
6	4.98	MR	3.28	MR	3.71	MR	3.99	MR	-0.76
7	1.01	R	1.74	R	3.22	MR	1.99	R	0.23
8	3.09	MR	1.66	R	3.22	MR	2.66	R	-0.44
9	4.98	MR	1.80	R	3.36	MR	3.38	MR	-1.05
10	3.17	MR	2.67	R	3.44	MR	3.09	MR	-0.38
11	0.94	R	2.81	R	3.53	MR	2.43	R	0.36
12	0.87	R	1.59	R	3.53	MR	2.00	R	0.23
13	3.17	MR	2.75	R	3.53	MR	3.15	MR	-0.38
14	3.17	MR	3.28	MR	3.54	MR	3.33	MR	-0.32
15	1.03	R	3.00	MR	3.59	MR	2.54	R	0.29
16	1.03	R	3.28	MR	3.46	MR	2.59	R	0.36
17	1.03	R	2.08	R	3.46	MR	2.19	R	0.23
18	1.05	R	3.19	MR	4.48	MR	2.91	R	0.62
19	1.05	R	2.52	R	3.46	MR	2.34	R	0.23
20	1.02	R	3.66	MR	2.81	R	2.50	R	0.36
21	1.02	R	2.90	R	2.81	R	2.24	R	0.23
22	3.17	MR	3.46	MR	5.59	MS	4.07	MR	0.33
23	3.08	MR	3.57	MR	2.62	R	3.09	MR	-0.38
24	1.02	R	2.77	R	2.62	R	2.14	R	0.23
25	1.01	R	3.44	MR	2.62	R	2.36	R	0.29
26	3.00	MR	2.89	R	2.66	R	2.85	R	-0.32
27	3.00	MR	2.52	R	2.66	R	2.73	R	-0.32
28	3.00	MR	2.16	R	2.67	R	2.61	R	-0.38
29	1.00	R	2.00	R	1.51	R	1.50	R	-0.09
30	1.00	R	3.34	MR	5.68	MS	3.34a	MR	1.01
Mean	1.98		2.36		3.13		2.49		
Min	0.87		0.93		0.84		0.93		
Max	4.98		3.66		5.68		4.07		
SE	0.05		0.20		0.07		0.12		
P.value	<.000		0.69		<.0001		<.0001		
C.V%	14.30		46.18		12.21		27.51		

R, = resistant, MR= moderately resistant, MS= moderate susceptible.

G1= NERICA 1,G2= NERICA 2, G3=NERICA 4, G4=LOW LAND NERICA 6, G5=WAB 450-12-BL1-DV4, G6=IR-56, G7=WITA 10, G8=WAB 450-12-4-BL1-DV1, G9=IR-54, G10=Kalalu, G11=Katrin, G12= Dakawa 83, G13=Txd 85, G14= Txd 88, G15=Mwangaza, G16= Tai, G17= SATO 1, G18=Txd 307, G20=Komboka, G21= Kalamata, G22= Supa India, G23= Mwanza, G24= Tule na bwana, G25= Sindano, G26= Zambia, G27= Kalundi, G28= Wahiwahi, G29=IR-24, G30= Txd 306.

The IPCA 1 scores for the environments are presented in Table 2.7. ARI- KATRIN had the highest magnitude IPCA1 score (-1.858), followed by Kyela (1.346) and lastly Igurusi (0.513).

Table 2. 7 Mean BLB scores and IPCA1 scores for individual locations

Environment	Number	Mean	IPCA1
ARI-KATRIN	1	1.978	-1.858
IGURUSI	2	2.356	0.513
KYELA	3	3.133	1.346

2.4.4 GGE biplot analysis

2.4.4.1 Relationship among test environments

The goodness of fit of the GGE biplot was 89.50%; PC1 contributed 51.62% while PC2 accounted for 37.88% of the total variation (Figure 2.2). At Kyela and Igurusi, G30 and G22 had the highest BLB scores. Considering the angles between vectors of environments, Kyela and Igurusi had a very small angle (acute) between them and both these environment vectors and that of ARI-KATRIN had wider angles between them. Based on this observation, Kyela was not correlated to Igurusi ecologies, hence Kyela was useful for selecting specifically adapted genotypes like G30 and G22.Thus, these environments may be good sites for selecting genotypes with general resistance to bacterial leaf blight disease. Moreover, Igurusi, which had the shortest vector, is less useful for selecting genotypes compared to the other two environments. ARI-KATRIN and Kyela had longer vectors from the origin, whereas Igurusi had the shortest vector compared to these two. ARI-KATRIN and Kyela had the same distance vector. On the other hand, ARI-KATRIN that had low severity of bacterial leaf blight occurrence is an average site for selecting specifically adapted genotypes.

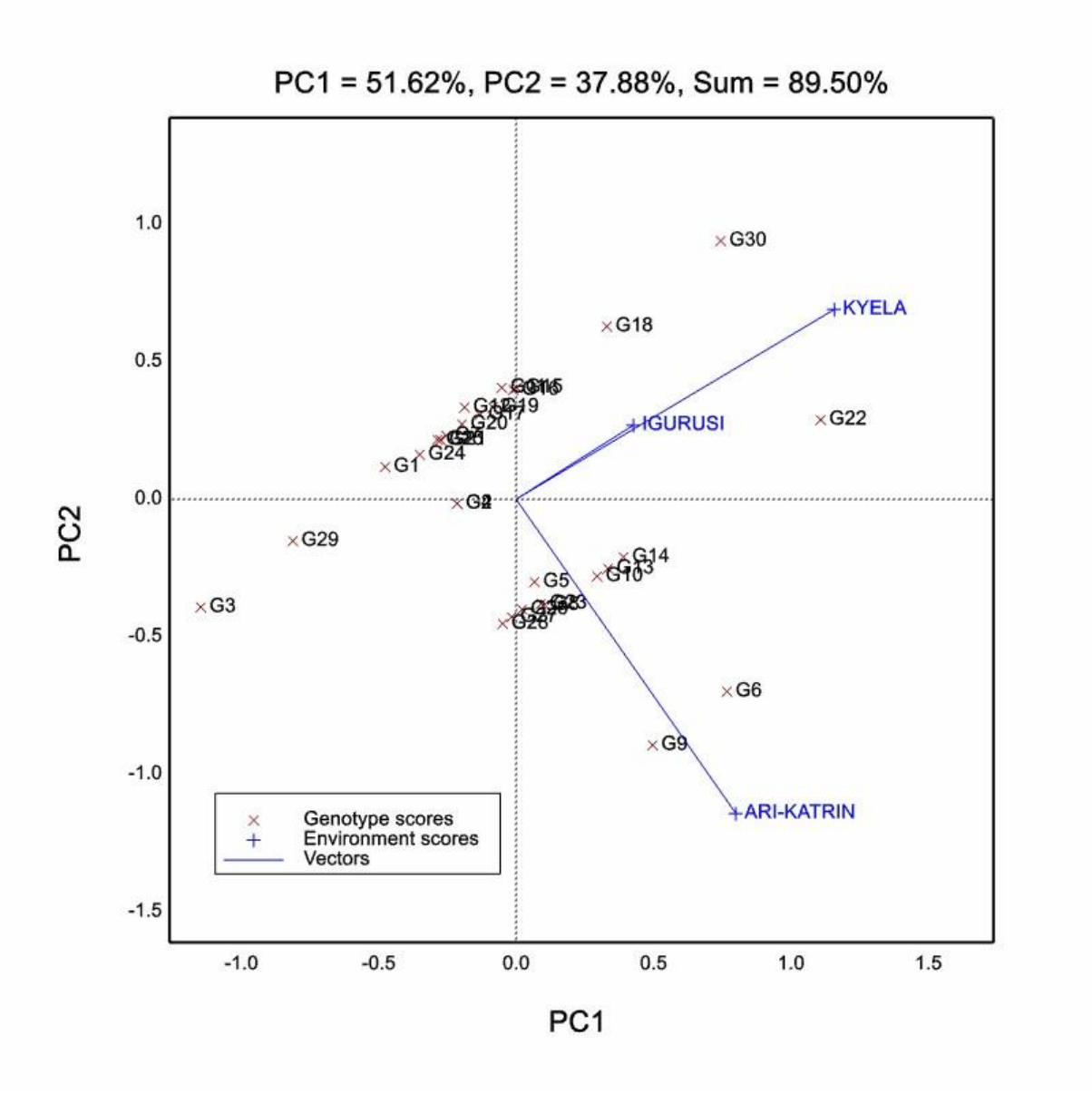

Figure 2. 2 Relationship among test environments

2.4.4.2 The Which-Won-Where polygon view

The polygon view of the GGE biplot (Figure 2.3) displays the “which won where” pattern of genotype by environment dataset. The radial lines originating from the centre of the biplot divided the polygon into seven sectors. The three environments fell into two sectors and there were two environments. The first environment consisted of Kyela and Igurusi with high disease pressure and the genotype with the highest disease rating score was Txd 306. The second environment was represented by ARI-KATRIN and the genotype, which had the highest disease rating score in this environment, was IR-54.

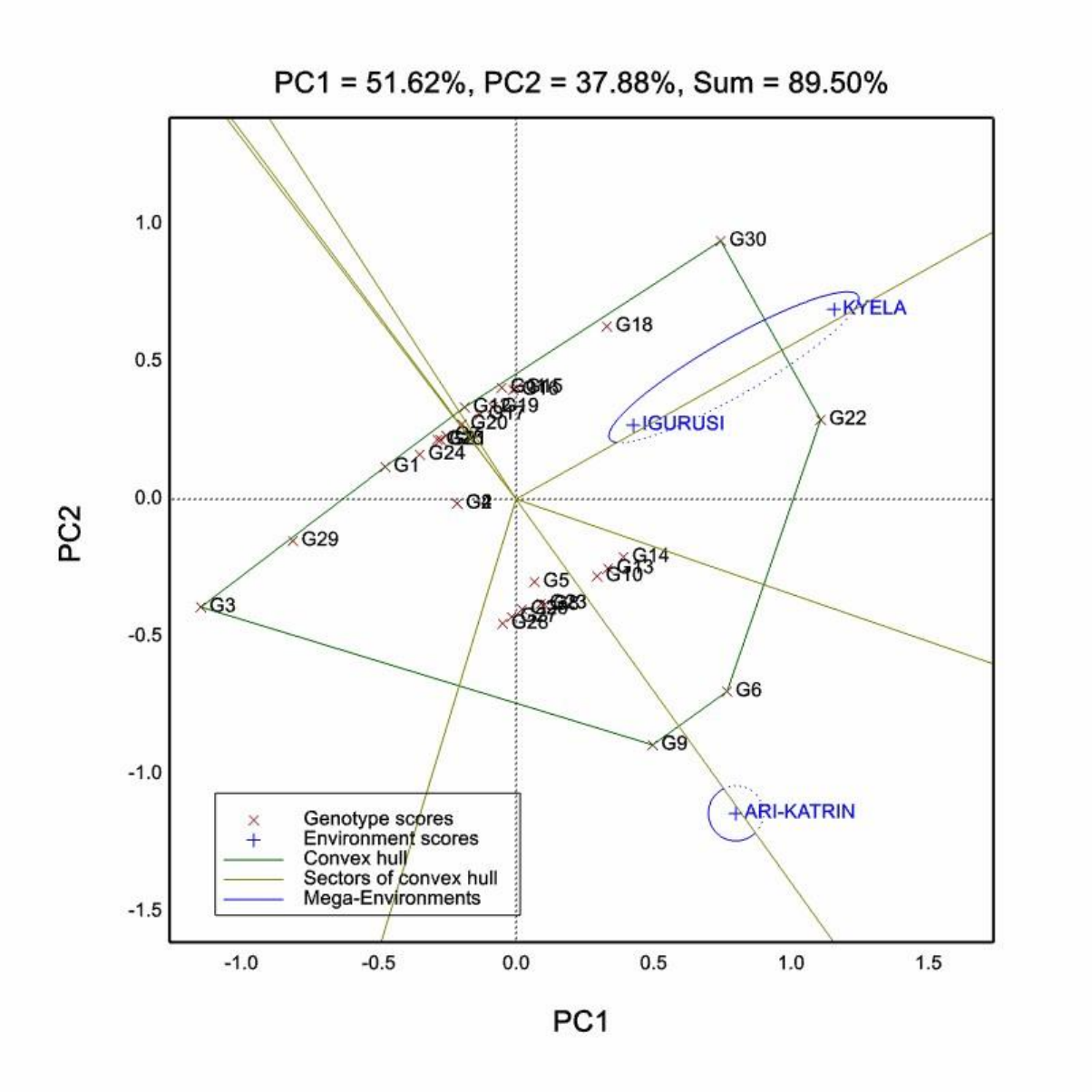

Figure 2. 3 Polygon view of the GGE biplot based on symmetric scaling

2.4.4.3 Comparing genotype resistance

Figure 2.4 shows comparison of genotype susceptibility. NERICA 4 had the lowest disease rating score and fell into the concentric ring of the biplot. Other genotypes with low disease rating scores and located in concentric rings of the biplot were IR-24, NERICA 2, NERICA 1, Tule na bwana, Komboka, Kalamata and WITA 10 which had moderate disease resistance, and performed below average in respect of disease rating scores. Moreover Genotypes NERICA 2, LOW LAND NERICA 6 and IR-24 had short perpendicular projections to the AEC axis. Whereby, genotypes with long perpendicular projections to the AEC axis were IR-54, Txd 306, IR-56 and Txd 307.

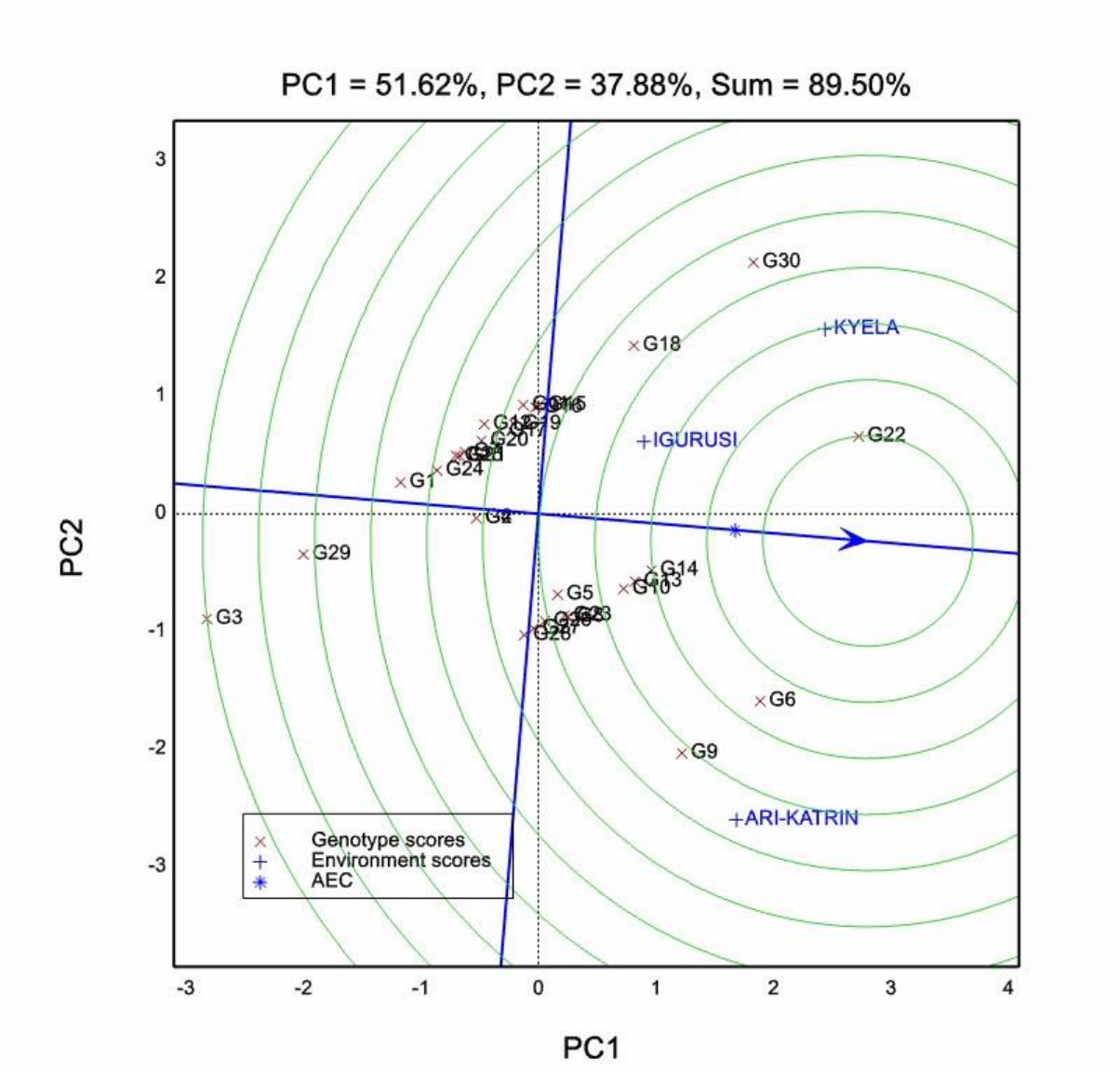

Figure 2. 4 Comparing genotypes with respect to reaction to BLB and consistency of performance

2.4.4.4The grain yield performance of 30 rice genotypes across three locations

The grain yield results of the 30 genotypes in each location and across all the three locations were as follows; At ARI-KATRIN the mean value was 4.02t $ha^{-1}$and the highest yield was from Txd 306 (8.29 t ha^{-1}) while the lowest was from NERICA 4 (0.84 t ha^{-1}). However, at Igurusi the mean value was 3.63 t $ha^{-1}$and the highest yield was for Txd 306 (7.07t ha^{-1}) and the lowest was from NERICA 4(0.87t ha^{-1}).At Kyela the mean value was 3.24$tha^{-1}$and the highest yield was from Txd 306(6.89tha^{-1}) and the lowest was from NERICA 4 (0.42tha^{-1}). Moreover, across the three locations the mean value was 3.64tha^{-1} and highest yield was for Txd 306 (7.42tha^{-}

[1]) and the lowest for NERICA 4 (0.74tha^{-1}). The genotype effect was highly significant (P= <0.0001) at all the three locations and across the locations.

Table 2. 8 Performance of the 30 rice genotypes in respect of grain yield (t ha^{-1}) at individual and across locations

Genotype	ARI-KATRIN	Igurusi	Kyela	Across Locations
NERICA 1	2.56	1.87	0.50	1.64
NERICA 2	1.83	2.27	1.29	1.79
NERICA 4	0.84	0.87	0.51	0.74
LOW LAND NERICA 6	5.99	5.42	4.27	5.23
WAB 450-12--12BL1-DV4	2.91	2.78	2.41	2.70
IR-56	1.00	3.20	2.80	2.33
WITA 10	6.41	6.16	6.20	6.26
WAB-450-12-4-BL1-DV1	1.72	3.11	2.72	2.52
IR-54	4.02	4.85	3.60	4.16
Kalalu	6.89	6.29	4.79	6.00
Katrin	3.53	3.63	1.56	2.91
Dakawa 83	4.91	5.09	3.36	4.45
Txd 85	6.09	5.13	3.92	5.05
Txd 88	7.21	6.62	5.62	6.48
Mwangaza	1.65	1.85	2.01	1.84
Tai	5.95	5.44	5.28	5.56
SATO 1	6.09	5.56	5.84	5.83
Txd 307	5.86	4.74	4.72	5.10
SATO IX	5.32	4.62	4.78	4.90
Komboka	3.78	3.55	3.25	3.52
Kalamata	4.51	4.63	4.48	4.54
Supa India	1.51	1.13	0.45	1.03
Mwanza	1.26	1.06	0.86	1.06
Tule na bwana	2.56	2.92	2.33	2.60
Sindano	0.91	1.68	1.34	1.31
Zambia	2.11	1.43	1.44	1.66
Kalundi	2.43	1.61	1.89	1.98
Wahiwahi	1.38	2.08	2.76	2.07
IR-24	3.13	3.18	2.53	2.95
Txd 306 (Improved)	8.29	7.07	6.90	7.42
Mean	4.021111	3.63	3.24	3.63
Minimum	0.84	0.87	0.45	0.74
Maximum	8.29	7.07	6.89	7.42
SE	0.19	0.2	0.88	0.18
P.value	<0.0001	<0.0001	<0.0001	<0.0001
C.V%	26.03027	29.68485	27.17	27.76

2.5 Discussion

2.5.1 ANOVA and AMMI analysis

The combined analysis of variance and AMMI analysis showed significant effects of genotype (P<0.001), environment (P<0.01) and GEI (P<0.001).The significance of genotype effect implies that there was adequate variation amongst the genotypes, which would permit selection for desirable genotypes. The significance of environments suggests that the environments were diverse, with large differences among environmental means causing variation in the disease reaction of genotypes across the environments. The highly significant GEI effect suggests that there was differential reaction of genotypes to BLB disease from one environment to another. In AMMI, genotype, environment and GEI contributed 69.63% of the total variation, while GEI alone captured 26.72% of the total sum of squares. The AMMI model demonstrated the presence of GEI, and this GEI variance was partitioned into the first interaction principal components axes (IPCA1) and residuals. The IPCA1 accounted for 91.6% of the GEI sum of squares (Badu Apraku et al (2011).

Genotypes NERICA 2 and LOW LAND NERICA 6, with IPCA1 score of 0.07 were the most stable across the three locations compared to other genotypes, followed by IR-24, which had IPCA1 score of -0.09. Genotype IR-54 was the most interactive therefore unstable (-1.05) followed by Txd 306 (1.01) which were unstable across the three environments. GenotypeIR-56, was moderately resistant but unstable with IPCA1 of -0.76, while genotype Txd 307, was resistant but unstable with IPCA1 score of 0.62. For the improvement of BLB resistance, the use of genotypes NERICA 2, LOW LAND NERICA 6 and IR-24, which combine high levels of resistance with stability of resistance, would be recommended (Sanni et al., 2009; Nassir, 2013).

2.5.2 Mean percentage of BLB lesions on the tested genotypes

For mean performance in respect of BLB scores, several genotypes were highly interactive implying that selection for stability across locations is useful. In this study, the identified genotypes with stable resistance for bacterial leaf blight were NERICA 2, LOW LAND NERICA 6 and IR-24. The resistance for bacterial leaf blight within these genotypes should be explored in other environments. However, with regard to mean performance, the mean at ARI-KATRIN was 1.98, Igurusi 2.36 and Kyela 3.13, while across the three locations it was 2.49. This implies that Kyela is the best location for evaluating BLB resistance compared to ARI-KATRIN and Igurusi. Results suggest that genotype Dakawa 83 is more resistant at ARI- KATRIN and can be useful in that location, however NERICA 4 is more resistant at Igurusi and Kyela so it could be useful in these two locations.

2.5.3 The GGE biplot analysis

Concerning relationship among the test environments, ARI-KATRIN and Kyela had longer and same vector length compared to Igurusi, which had a very short vector from the origin. This means that Kyela and ARI-KATRIN were more discriminative than Igurusi. Kyela, which is also known for high natural BLB incidence levels (hot spot) could be recommended for BLB screening, and this agrees with (Ashura et al., 1999).However, the angle between vectors for environments ARI-KATRIN and Kyela was wider than that between vectors of environments Kyela and Igurusi. The angle between ARI-KATRIN and Kyela (90°) suggests that the environments are uncorrelated. However, the positive association between Kyela and Igurusi was mostly due to favourable conditions for BLB thus suggesting they are correlated.

The GGE biplot classification of genotypes and environments revealed two environments; the first environment was for Kyela and Igurusi with positive PC2 scores, and the second, ARI-KATRIN with negative PC2 score. Genotypes NERICA 4, IR-24, NERICA 1, NERICA 2, LOW LAND NERICA 6, followed by Kalamata and LOW LAND NERICA 6 had below average BLB scores and had stable performance across environments. The most unstable but resistant genotype demonstrating a strong GEI was Txd 307, and the moderately resistant and unstable genotypes were IR-56 and IR-54. Genotype Dakawa 83 was specifically suitable for ARI-KATRIN, while NERICA 4 was suitable for Igurusi and Kyela hot spot locations.

Although the environment main effect may contribute up to 80% or more of the total variation, it is usually the genotype main effect and the genotype x environment interaction (GEI) that are relevant to cultivar evaluation (Yan, 2002). The use of GGE biplots has been appreciated by many researchers in rice and other crops (Hagos & Abay, 2013; Kivuva *et al.*, 2014; Lakew *et al.*, 2014) Muthoni et al., 2015) as it graphically displays general patterns of genotype responses across environments in multi-environmental trials data not usually covered in the general ANOVA. In this study, the GGE biplot results revealed that there was positive correlation between the two environments for the disease at Kyela and Igurusi. This was expected because the two environments were established in the same agro-ecology, the weather conditions in these two sites mainly favour high disease pressure, especially high humidity and high rainfall compared to ARI-KATRIN which has low humidity and rainfall. This also implies that there is a need for separate breeding programmes for the different locations to evaluate under different weather conditions.

The polygon view of GGE biplot is very useful for visualising the best genotypes in each environment and grouping environments for visualisation of possible crossover GEI and mega environments (Yan & Tinker, 2006). Different environments can fall into different sectors, which imply that there are different high resistant cultivars for those sectors and it shows

crossover GEI suggesting that the test environments could be divided into mega-environments (Yan et al., 2017). In this investigation, the environments fell into two sectors revealing the possibility of two mega environments and presence of crossover type of GEI. Kyela and Igurusi fell into the sector, which was directly opposite to the sector in which NERICA 4 was the vertex genotype, therefore in terms of resistance (low BLB scores) NERICA 4 was the winner for these environments. Likewise, ARI-KATRIN fell into the second sector, which was opposite to the sector in which Dakawa 83 was the winner there NERICA 4 won at ARI-KATRIN. Other researchers in Africa have also appreciated the use of the polygon view of GGE biplot in identification of the best genotypes in terms of yields in different environments and revealing of possible mega environments (Kivuva et al., 2014; Lakew et al., 2014) ; Muthoni et al., 2015.

2.6 Conclusion

The GGE biplot showed that the ranking of the genotypes changed across environments revealing a crossover type of genotype x environment interaction. Igurusi and Kyela had high disease pressure and ARI-KATRIN low disease pressure suggesting the need for separate breeding programmes for the high and low disease in Tanzania. Genotypes identified as resistant and susceptible differed in GGE biplot representations. The GGE biplot showed that G3 was specifically adapted to Kyela and Igurusi and G12 to ARI-KATRIN low disease pressure environment, while G30 followed by G14 and G10 were the highest yielding genotypes across the three environments. By comparing genotype resistance, the GGE biplot showed that G3, G4, G29, G1, G2, G21 and G24 performed below the average in respect of disease rating score, and were specifically adapted across the three test environments. Since the results of this study are based on one season data, more temporal and spatial environments will be needed to give meaningful recommendations. Moreover these results emphasize that the environment contributes to differential genotype reactions to BLB, and hence, to obtain true resistant genotypes there is a need for evaluating in multi-locations with several seasons of testing. There is a need to evaluate different isolates from each test environment to separate the effects of the physical environment from differences caused by differing pathotypes. This information could be applied in breeding programmes to develop rice cultivars with durable resistance to the BLB pathogen *Xoo*. Due to diverse agro-climatic rice growing zones as the case in the three sites, and the presence of a number of genetically distinct virulent *Xoo* strains in Tanzania, pyramiding of two or more effective genes in agronomically superior genotypes and search for new disease resistance genes in context of African origin from wild *Oryza spp* seems to be the most effective disease management strategy. In addition, the GGE biplots showed that most of the NERICA varieties were resistant and stable across three locations, e.g., NERICA 2 and LOW LAND NERICA 6.Therefore,

these NERICAs could be used as resistance donors in development of new BLB resistant varieties.

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CHAPTER 3

Genetic analysis and evaluation of secondary traits for use in indirect selection of grain yield improvement among rice genotypes

Abstract

Genetic based knowledge of different yield traits plays a major role in varietal improvement of rice. Genetic variation gives room for possibility of recombination, which is essential for the development of new varieties in any crop. The objective of this study was to estimate heritability, variability and diversity among genotypes and to evaluate efficiency of secondary traits for indirect selection of grain yield. Observations were recorded for 16 quantitative traits on 30 rice genotypes. The genotypes differed significantly for some of the characters and the genotypic and phenotypic coefficient of variation indicated the presence of favourable amount of variability. Regarding heritability estimates, the highest heritability was observed for days to early flowering (99.67%) followed by days to maturity (99.35%), grain length (98.21%), yield per plot (97.13%), and 1000 grain weight (89.48%)**.** Spikelets per panicles (66.79) had highest genetic advance and grain yield per plot exhibited the highest genetic advance as percent of mean (%) of 104.13, followed by dry straw weight (92.11%) and harvest index (66.36). Harvest index had the highest efficiency for indirect selection for grain yield; though the efficiency was less than unity, this trait should be given top priority in selection process. Grain yield per plot showed highly significant and positive genotypic correlation with harvest index, grain length, and dry straw weight per plot. Cluster analysis showed that the genotypes could be classified into eight distinct groups. Overall, results revealed adequate variability and diversity, which can be exploited in rice breeding.

Keywords: heritability, correlated responses, genetic variability.

3.1 Introduction

In development of a crop improvement programme, the degree of genetic variability for a specific trait in the population is very important (Ganapathy et al., 2011).However, variability alone will not indicate the degree of improvement through selection (Priyadharshini et al., 2011). Robinsion et al. (1949) emphasized that heritability of the character is a main concern to the breeder, since it indicates the possibility and extent to which improvement is possible through selection. It has been suggested that heritability together with genetic advance will bring out the genetic gain expected from selection (Johnson *et al.*, 1955). Estimates of broad-sense heritability (H^2), phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), genetic advance (GA) and genetic advance as percent of the mean (GAM), provide genetic information that indicate the possible progress that will be made through selection. The outstanding function of heritability is in expressing the reliability of the phenotypic value for a trait as a guide to the breeding value for that trait in a population (Falconer, 1960). In its broadest sense it specifies the proportion of the total phenotypic variability that is due to genetic causes (Allard, 1960). Traits with high percent heritability are less affected by the environment in their expression and quantitative traits usually have low heritability estimates due to their sensitivity to the environment (Allard, 1960). For effective selection, (Falconer, 1960) proposes using a combination of genetic parameters, genetic and phenotypic coefficients of variation, heritability and genetic advance.

In genetic diversity analysis, most breeders utilize morphological characteristics because they are inexpensive, rapid, and simple to score. The study of these characteristics does not require sophisticated equipment. In addition, this evaluation could be useful in developing reliable selection indices for important agronomic traits in rice by using genetic distances and cluster analysis to identify the groups of genotypes. An investigation into the nature and the degree of divergence is useful for an understanding of the course of evolution and for classifying population into groups based on diversity, particularly, when they are overlapping for one or more characters. This information may be important for the improvement of rice genotypes. However, the knowledge about the source of diversity for the different traits is of considerable importance, since the major aim of the plant breeder is to improve the yield and the quality by developing superior varieties.

Moreover, for designing an effective breeding programme, sufficient knowledge about the degree and direction of association between yield and its components traits, is of utmost significance to the breeders when they have to exercise selection for immediate improvement of more than one character. Genotypic correlation is a good measure of the degree of

association between traits. Yield being a complex trait may be improved by selecting an easier to measure secondary trait if the secondary trait is highly correlated with yield, and has a high heritability (Falconer, 1960). This would make indirect selection for yield via a secondary trait more efficient (giving better response in yield) relative to direct selection for yield *per se*. Therefore, the objective of the present study was to

(1) Estimate genetic variability and heritability of the traits;
(2) Assess efficiency of indirect selection of secondary traits.

3.2 Material and methods

3.2.1Germplasm

Thirty genotypes (Table 2.1) which were sourced from different organizations within Tanzania and from international organisations were used.

3.2.2 Environments, trial design and agronomic procedures

The 30 genotypes were evaluated in three environments of which two environments represented BLB hot spots and the other had moderate BLB incidence. At all three sites, the germplasms were planted in a 6x5 alpha lattice design with three replications. The germplasm were planted in two row plots, 5 m in length at inter-row and intra- row spacing of 20 cm. Three seeds were placed per hill by hand at a depth of 3cm. Plants were thinned two weeks after emergence to one plant per hill. At planting, double ammonium phosphate fertilizer (ratio 18:46) was applied at a rate of 50kg N and 50 kg P_2O_5 per hectare. After thinning, the trials were top dressed with urea (46% N) at a rate of 20kg N per hectare.

3.2.3 Data collection

Data were collected on 16 quantitative traits based on the descriptors for rice (IRRI, 1992) as presented in Table 3.1. Data were recorded from five randomly selected plants in each plot and the mean of the five plants were used for statistical analysis except for dry straw weight, grain yield per plot and BLB score, which were done on per plot basis.

Table 3. 1 Descriptions and measurements of the traits

Trait	Acronym	Description
Early vigour	EV	Scoring 1-very high, 3-high 5-intermediate, 7-low, 9-very low.
Days to early flowering 50%	DTEF	The number of days from cultivation to early flowering day
Plant height (cm)	PH	The average of height from the base to the tip of last leaf (Flag leaf).
Panicle length	PL	From the base (first node) to the tip of last spikelet of panicle.
No. of tillers per hill	TH	Counting of the tillers per hill.
Dead heart	DH	Scoring 1- very low 3- 3-low, 5- intermediate 7- high 9- very high.
Bacteria leaf blight scoring	BLB	1-9 scale: 1 = no infection; 3 = 6-12%; 5 = 13 - 25%; 7 = 26-50%; 9 = 50>% leaf area covered with lesions at heading stage.
Lodging %	LO	10%-very low 30%-low 50%-intermediate,70%-high 90%-veryhigh100%-extremely high
Days to maturity	DM	The number of days from cultivation to maturing day at 80%
Dry straw weight	DSTRW	The total weight of straw after threshing per plot was measured in kg.
Spikelet per panicle	SPP	At maturity counting of the total spikelets per panicle.
Grain length	GL	Measured as the distance from the base of the lowest glume to the tip.
Grain width	GW	Measured as the distance across the fertile lemma and palea at the widest point.
1000 grain weight	TGW	One thousand seeds were counted and weighed (g)
Harvest index	HI	For total biological yield the entire plant above the ground level was harvested, sun dried and weight at maturity. The value of harvest index was calculated from the following formula given below. Harvest index (%) =Economical yield /Biological yield× 100
Grain yield/plot	YP	Weighing the total grains per plot (two rows that had 5M).

Source: (UPOV, 2004)

3.3 Data analysis

Analyses included ANOVA across locations; variance components, heritability, and genetic advance estimation; genotypic correlations and relative selection efficiency estimation, and diversity analysis.

3.3.1 Analysis of variance across locations

For across location ANOVA, the PROC GLM of SAS version 9.4 (SAS Institute, 2014) was used and statistical analysis, for combined ANOVA and genotype was considered to be random factors, thus REML analysis was used. The linear model used was as follows:

$$Y_{ijkl} = \mu + E_k + R_{i(k)} + \beta_{l(ik)} + G_j + GE_{jk} + \varepsilon_{ijkl}$$

Where;

Y_{ijkl} is the response of the *j*th genotype, *k*th environment and *i*th replication within environment and *l*th block within replication: μ is the grand mean of the experiment, E_k is the environment effect, G_j is the genotype effect, $\beta_{l(ik)}$ is the block within replication effect, GE_{jk} is the genotype x environment interaction effect and ε_{ijkl} is the random error.

3.3.2 Variance components

The restricted maximum likelihood (REML) method of the MIXED procedure in SAS Version 9.4 (SAS Institute, 2014) was used to estimate variance components, where locations were fixed effects and genotypes where random effects.

The variance components including genotypic variance (σ_g^2), genotype by location variance (σ_{gl}^2) and error variance (σ_e^2) were estimated and obtained directly from the MIXED procedure output.

Phenotypic variance (σ_p^2) was calculated as follows:

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}$$

Where *l* = number of locations, *r* = number of replications within location

3.3.3 Phenotypic coefficient of variation

Phenotypic coefficient of variation (PCV) was estimated by the formula suggested by Bruton (1952).

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where

σ_p^2 = phenotypic variance

$\bar{x}$ = phenotypic trait population mean

3.3.4Genotypic coefficient of variation

Genotypic coefficient of variation (GCV) was estimated by formula suggested by Burton (1952).

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

Where by $\sigma_g^2 =$ genotypic variance and $\bar{x}$ = phenotypic trait population mean.

PCV and GCV were classified as follows (Robinson *et al.,* 1949)

Low = 0 to 10%

Moderate = 10-20%

High = > 20%

3.3.5 Heritability

Broad sense heritability (H^2) values were calculated based on entry mean basis as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}}$$

Where:

σ_g^2 =Genotypic variance

$\sigma_{gl}^2 =$ Variance due to genotype x location interaction

σ_e^2 = error variance

*l*and *r* are the numbers of environments and replications per environment, respectively. Heritability values were classified into low: 0-30%, medium: 31-60%, and -high:>61% (Robinson et al., 1966).

3.3.6 Genetic advance

The extent of genetic advance expected through selection for each character was calculated as per formula suggested by Johnson *et al.*, (1955).

$$GA = K \times H^2 \times \sigma_p$$

Where,

$GA =$ expected genetic advance,

$H^2 =$ heritability for the trait,

$\sigma_p =$ phenotypic standard deviation of the trait,

$K =$ Selection differential that is 2.06 at 5% selection intensity (Lush, 1949).

3.3.7Genetic advance as percent of mean

Genetic advance as percent of mean was calculated as follows

$$GAM = \frac{GA}{\bar{x}} \times 100$$

Where

$GAM =$ Genetic advance as percent of mean

$\bar{x} =$ phenotypic trait population mean.

The GAM values were classified as follows (Johnson et al., 1955):

Low = 0 to 10 %

Moderate = 10-20 %

High = > 20 %

3.3.8 Genotypic correlation

A multivariate model in PROC MIXED of SAS version 9.4 (SAS Institute, 2014) was used to compute genotypic correlations between grain yield and each of the other traits. The standard error of the genotypic correlation was determined using the DELTA method (Lynch and Walsh, 1998).

$$r_g = \frac{\sigma_{gxy}}{\sigma_{gx} \times \sigma_{gy}}$$

Where,

$r_g =$ genotypic correlation,

$\sigma_{gxy} =$ genotypic covariance of characters' x and y,

$\sigma_{gx} =$ square root of genotypic variance of character x,

$\sigma_{gy} =$ square root of genotypic variance of character y.

The genotypic correlations were considered significantly different from zero if their absolute value were greater than 1.96 times their standard error (Holland, 2004) ; Bhatt, 1970).

3.3.9 Efficiency of indirect selection for yield per plot via a secondary trait

The relative selection efficiency (*RSE*) of indirectly improving grain yield using a secondary trait was estimated according to (Falconer and Mackay, 1996) as follows:

$$RSE = r_g \times \frac{h_x}{h_y}$$

Where,

$r_g =$ absolute value of genotypic correlation

$h_x =$ square root of heritability of a secondary trait.

$h_y =$ square root of heritability of yield per plot

3.3.10 Diversity analysis

SAS version 9.4 (SAS Institute, 2014) was used for cluster analysis. The EUCLIDIAN method of PROC DISTANCE was used to calculate distance indices between each pair of genotypes. PROC TREE was implemented to construct a dendrogram using the Euclidian distance indices.

3.4 Results

3.4.1 Parameters of genetic variability

Range of variability, estimates of genotypic and phenotypic coefficients of variation, broad sense heritability (%) and genetic advance expressed as percentage of mean are presented in the Table 3.3.

3.4.2 Coefficients of variation

It was observed that the estimates for genotypic coefficients of variation (GCV) were lower than the phenotypic coefficients of variation (PCV) for all the characters. Lodging% exhibited the lowest GCV (0.000) but for the PCV it was the highest of 5325.463%. Whereas, the number of spikelets per panicle had the highest GCV (419.902%) and the PCV for the same was 1005.352%. It was followed by plant height (GCV= 97.843% and PCV=294.959%), days to early flowering (GCV=64.307% and PCV=66.251%), days to maturity (GCV=48.124% and PCV=50.562%), 1000 grain weight (GCV=38.146% and PCV=78.496%), yield per plot (GCV=19.198% and PCV=24.312%), number of tillers per hill (GCV=17.542% and PCV=73.346%), dry straw weight (GCV=14.087% and PCV=70.178%), early vigour (GCV=8.842% and PCV=36.944%), bacterial leaf blight (GCV=8.080% and PCV=46.406%) and harvest index (GCV=2.800% and PCV=4.577%). The PCV estimates were higher than GCV estimates for all the traits. The highest difference between GCV and PCV value was observed for lodging (5325%) followed by number of spikelet per panicle (585.45%), followed by plant height (197.116%). Grain length had the lowest difference between GCV and PCV estimate (0.339%).

3.4.3 Genetic advance and genetic advance as percent of means

For the 16 quantitative traits estimates of GA and GAM across locations were determined (Table 3.3). The GA estimates were relatively from highest to lowest for spikelets per panicles (66.79), plant height (22.34 cm), days to maturity (15.66) and days to early flowering (15.40). Other traits showed the lowest values of genetic gains. The lowest genetic advance was observed for lodging% (0.000) followed by dead heart (0.13) and harvest index (0.19). The genetic advance for grain yield per plot was low (3.78). However, when expressed as a percentage of mean value, the GAM for yield per plot was 104.13%. Other characters like harvest index (66.36), showed high genetic advance as percentage of mean and for the lowest was for lodging % (0.00) followed by panicle length (8.89).

Table 3. 2 Variance components, heritability estimates, coefficients of correlation, genetic advance and genetic advance mean of 30 rice genotypes evaluated across three locations

TRAIT	σ_g^2	σ_{gl}^2	σ_e^2	σ_p^2	$\frac{\sigma_{gl}^2}{l}$	$\frac{\sigma_e^2}{rl}$	$H^2(\%)$	Trait mean	$PCV(\%)$	$GCV(\%)$	GA	$GAM(\%)$
EV	0.279	0.356	0.531	1.166	0.119	0.059	61.095	3.16	36.944	8.842	1.36	43.01
DTEF	54.632	0.000	1.651	56.284	0.000	0.183	99.665	84.96	66.251	64.307	15.40	18.12
PH	83.566	90.112	78.240	251.918	30.037	8.693	68.331	85.41	294.959	97.843	22.34	26.15
PL	0.449	0.208	0.937	1.594	0.069	0.104	72.120	21.10	7.553	2.126	1.88	8.89
TH	3.145	0.000	10.005	13.151	0.000	1.112	73.885	17.93	73.346	17.542	5.51	30.73
DH	0.005	0.038	0.042	0.085	0.013	0.005	22.567	1.32	6.446	0.381	0.13	10.21
BLB	0.201	0.474	0.480	1.155	0.158	0.053	48.763	2.49	46.406	8.080	1.08	43.57
LOD	0.000	520.480	85.045	605.525	173.493	9.449	0.000	11.37	5325.463	0.000	0.00	0.00
DM	55.667	0.220	2.599	58.487	0.073	0.289	99.353	115.67	50.562	48.124	15.66	13.54
DSTR	0.239	0.000	0.950	1.189	0.000	0.106	69.329	1.69	70.178	14.087	1.56	92.11
SPP	645.420	166.980	732.900	1545.300	55.660	81.433	82.480	153.71	1005.352	419.902	66.79	43.45
GL	0.142	0.000	0.023	0.165	0.000	0.003	98.211	6.86	2.406	2.067	0.83	12.09
GW	0.005	0.000	0.014	0.019	0.000	0.002	76.170	1.55	1.197	0.314	0.22	14.17
TGW	9.240	0.000	9.774	19.014	0.000	1.086	89.483	24.22	78.496	38.146	8.04	33.18
YP	3.486	0.000	0.928	3.589	0.000	0.103	97.126	3.63	24.312	19.198	3.78	104.13
HI	0.008	0.000	0.005	0.013	0.000	0.001	93.414	0.29	4.577	2.800	0.19	66.36

EV= Early vigour, DTEF= Days to early flowering, PH= Plant height, PL= Panicle length, TH= Number of tillers per hill, DH= Dead heart, BLB= Bacterial leaf blight, LOD= Lodging %, DM= Days to maturity, DSTR= Dry straw weight, SPP= Number of spikelets per panicle, GL= Grain length, GW= Grain width, TGW= Thousand grain weight, YP= Yield per plot, HI= Harvest index

3.4.5 Heritability

According to Robinson's (1966) classification, broad sense heritability (%) can be described as high, moderate or low based on the percentage as follows: >60% (high), 30-60% (moderate), and 0-30% (low). In this study, moderate to high heritability estimates were observed for different traits. The lowest heritability was observed for lodging % (0.00%) followed by dead heart (22.567%), bacterial leaf blight which was moderate (48.763%), early vigour (61.095%), plant height (68.331%), dry straw weight (69.329%) and panicle length (72.120%). While days to early flowering showed the highest broad sense heritability (99.665%) followed by days to maturity (99.353%), grain length (98.221%), yield per plot (97.126%) and harvest index (93.414%).

3.4.6 Efficiency of indirect selection for Yield per plot via a secondary trait

Significant genotypic correlations r_g were observed for days to early flowering, panicle length, days to maturity, dry straw weight and harvest index (Table 3.4). The relative selection efficiency estimates for indirect selection for YP via a secondary trait (RSE) were as follows: for harvest index which was 0.83 was near to 1, days to maturity had a value of 0.59, days to early flowering(0.53), and 0.50 for dry straw weight, and for panicle length, the RSE was 0.34. For other traits such as early vigour, plant height, number of tillers per hill, dead heart, bacterial leaf blight, lodging %, spikelets per panicle, grain length, grain width and 1000 grain yield per plot, RSE was not calculated since the genotypic correlation coefficients were non- significant (Table 3.4).

Table 3. 3 Efficiency of indirect selection for Yield /Plot via secondary traits

TRAIT	$r_g \pm SE$	H_x	H_y	*RSE*
Early vigour	-0.23 ± 1.04	7.82	9.86	-
Days to early flowering	0.53 ± 0.06	9.98	9.86	0.53
Plant height(cm)	-0.44±0.37	8.27	9.86	-
Panicle length(cm)	0.39±0.03	8.49	9.86	0.34
Tillers per hill	1.00±0.77	8.60	9.86	-
Dead heart	0.23±1.07	4.75	9.86	-
Bacterial leaf blight	0.11±0.21	6.98	9.86	-
Lodging %	-0.99±1.12	0.00	9.86	-
Days of maturity	0.58±0.08	9.97	9.86	0.59
Dry straw weight(kg)	0.60±0.15	8.33	9.86	0.50
Spikelets per panicle	-0.62±1.16	9.08	9.86	-
Grain length(mm)	-0.12±0.26	9.91	9.86	-
Grain width(mm)	-0.24±0.27	8.73	9.86	-
1000 grain weight(g)	-0.01±0.25	9.46	9.86	-
Harvest index	0.85±0.83	9.67	9.86	0.83

- The RSE could not be determined because the r_g was not significantly different from zero.

3.4.7 Diversity among genotypes

Variations were observed among the 30 rice genotypes with respect to the 16 traits that were evaluated. The genetic distances that were determined for each pair of genotypes using morpho-physiological traits including early vigour, days to early maturity, plant height, panicle length, tillers per hill, dead heart, bacterial leaf blight, lodging%, days to maturity, dry straw weight, spikelets per panicle, grain length, grain width, 1000 grain weight, harvest index and yield per plot are presented in Table 3.5. Figure 3.1 shows the dendrogram that was constructed using genetic distances.

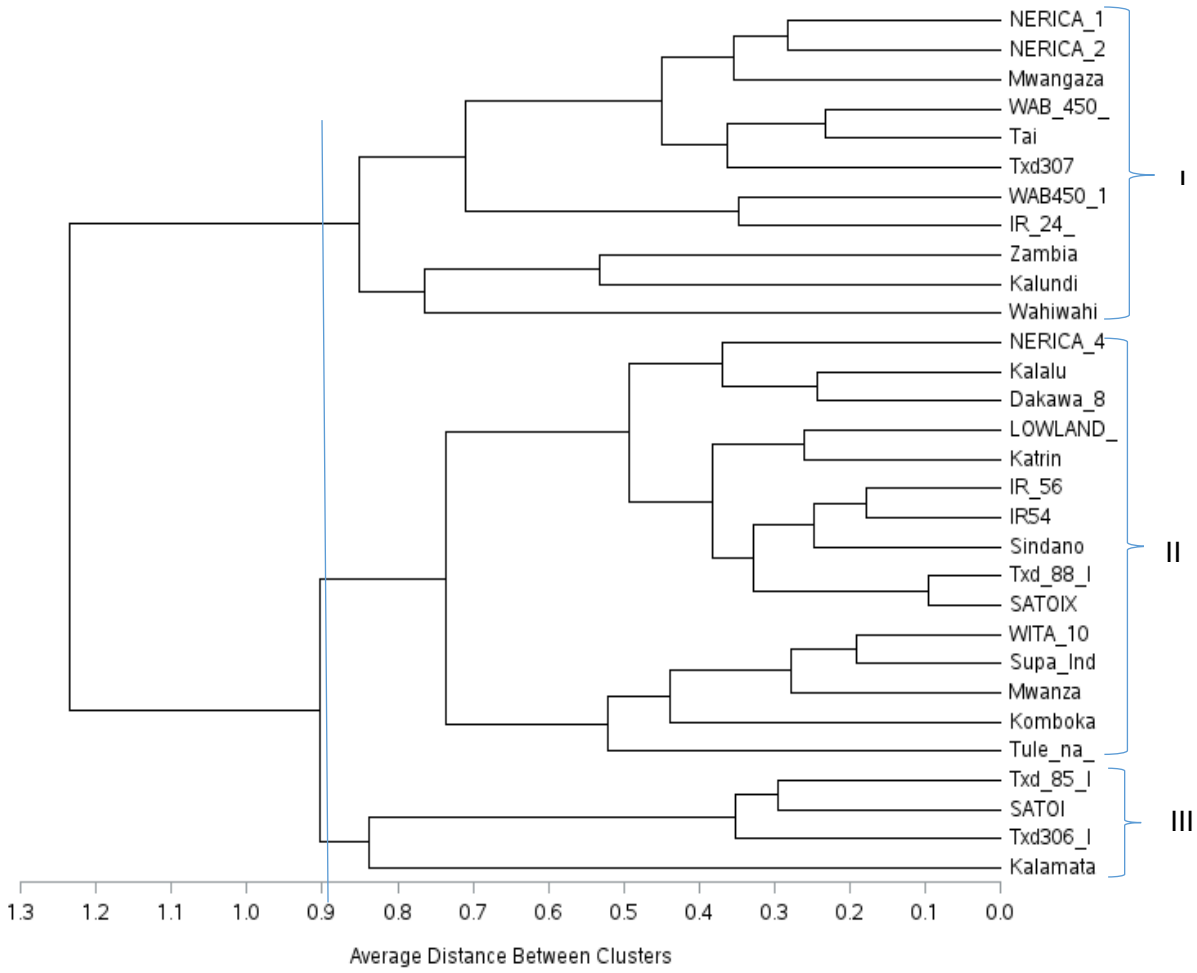

Figure 3. 1 Dendrogram for 30 rice genotypes derived from an UPGMA cluster analysis based on morpho-physiological traits

A dendrogram was constructed of the 30 rice genotypes. All 30 rice genotypes could be easily distinguished. The Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis led to the grouping of the 30 rice varieties into three major clusters, 11 rice genotypes formed cluster-1, cluster-2 respectively the largest cluster comprised of 15 rice genotypes and cluster-3 comprised of four rice genotypes. The maximum genetic distance was between Txd 306 and Wahiwahi (Figure 3.1), followed by Wahiwahi and Txd 85, Wahiwahi and Kalamata, IR-24 and Kalamata, and Txd 306 and IR-24. However, the lowest genetic distances were between SATO IX and Txd 88, followed by IR 54 and IR 56, Supa India and WITA 10, and between Sindano and IR 56. In this study, the dendrogram showed that the genotypes of genetically similar backgrounds clustered together thus giving a clear picture on which genotypes are similar to each other and which ones are not.

3.5. Discussion

3.5.1 Variability, Heritability and Genetic Advance

In this study, the extent of variability in the available germplasm was studied using variability parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (H^2), genetic advance (GA) and genetic advance of means (GAM). The estimation of these parameters helps breeders in achieving the required crop improvement by selection. The results on variability parameters obtained in the study are discussed below.

Number of spikelets per panicle, plant height, 1000 grain weight, and dry straw weight and days to early flowering showed higher estimates of GCV and PCV, indicating the presence of large variation among the genotypes for these characters. Therefore, simple selection can be practiced for further improvement of these characters. This was in conformity with the findings of Jayasudha and Deepak (2010) for plant height, Karthikeyan et al. (2010) for dry straw weight per plot, (Fiyaz & Chandrashekar, 2011) for spikelets per panicle, (Fukrei, Kumar, Tyagi, Rai, & Pattanayak, 2011) for days to early flowering and straw yield per plant, Lal and Chauhan (2011) for spikelets per panicle and plant height, (Devi & Kamireddy, 2015) for spikelets per panicle and Parikh et al. (2012) for 1000 grain yield.

Dead heart, grain width, grain length, panicle length and harvest index recorded low estimates of GCV and PCV, indicating low range of variation for these characters in the present genetic materials, thus offering narrow scope for further improvement of the characters. Sinha et al. (2004) reported similar findings for grain length and grain width, (Fukrei et al., 2011) for panicle length and Devi and Kamireddy, (2015) for dead heart and panicle length.

All traits registered higher estimates of PCV than GCV, indicating the variation is not only due to genotypes but also due to environmental effects and selection for these characters may be practised with caution.

Heritability and genetic advance are regarded as important selection parameters. Burton (1952) suggested that genetic variation along with heritability estimates would give a better idea about the efficiency of selection. Heritability is a good index of the transmission of hereditary values from parent to their offspring. The estimates of heritability help the plant breeder in selection of elite characters from diverse genetic populations. In this chapter, in general, high heritability values were recorded for all the characters. Days to 50% early flowering, days to maturity, plant height, productive tillers per plant, number of panicles per running meter, panicle length, spikelets per panicle, 1000 grain weight, grain yield per plot,

straw yield per plot, harvest index, dry straw weight, panicle length and plant height showed high heritability estimates indicating the least influence of environment on these characters.

These findings were in agreement with the reports made earlier in rice by (Okelola, Adebisi, Kehinde, & Oluwole, 2016) for days to 50% early flowering, plant height, grain yield per plot, Atanu and Sabesan (2010) for days to 50 percent early flowering, days to maturity, plant height, grain yield per plot, Jayasudha and Deepak (2010) for days to 50 percent early flowering, spikelets per panicles, grain yield per plot, and harvest index, Karthikeyan et al.(2010) for days to maturity, plant height, panicle length, 1000 grain weight, grain yield per plot, dry straw yield per plot and harvest index, Naresh et al*.* (2012) for days to 50 percent early flowering, days to maturity, plant height, 1000 grain weight and grain yield per plot and Devi and Kamireddy, (2015) for days to 50 percent early flowering, days to maturity, plant height, panicle length, spikelets per panicle, 1000 grain weight and harvest index. Heritability estimates are generally influenced by; the type of genetic material, sample size, method of sampling, the way the experiment is conducted, method of calculation and effect of linkage etc., therefore, their scope was restricted (Lal and Chauhan, 2011).

Heritability values coupled with genetic advance would be more reliable and useful in predicting the gain under selection than heritability estimates alone. The characters such as yield per plot, harvest index, days to maturity and days to early flowering exhibited high heritability coupled with high genetic advance, indicating that most likely heritability was due to additive gene effects and selection may be effective for these characters. Rita et al. (2009) and Jayasudha and Sharma, (2010) observed similar results for days to maturity, and Devi and Kamireddy, (2015)for harvest index and yield per plot. High heritability coupled with moderate genetic advance are normally classified as indicating that additive gene action were present. In this study all the characters which showed high heritability coupled with high genetic advance such as days to maturity, spikelets per panicles and days to early flowering indicated high genetic control. Since in self-pollinated crop homozygous lines are developed, the dominance component will not contribute to the phenotype of homozygous lines derived from a population. Consequently, in such cases additive and genetic variance are important for variation.

Earlier reports also indicated high genetic advance for days to maturity (Jayasudha & Sharma, 2010) and Karthikeyan et al*.,* 2010), 1000 grain weight (Subudhi and Dikshit, 2009; Karthikeyan et al*.,* 2010 and (Devi and Kamireddy, 2015) and grain yield per plot (Karthikeyan et al*.,* 2010 and (Vanniarajan *et al.*, 2012). High heritability coupled with high genetic advance as percent of mean was recorded for grain length, grain width, dry straw weight, and yield per plot and harvest index indicating that most likely the heritability is due to additive gene effects,

which might cause variations among varieties / genotypes, and selection may be effective for these characters. Similar kind of observations were reported by Sinha et al. (2004) for yield per plot, Karthikeyan et al. (2010) dry straw weight and harvest index, (Prajapati *et al.*, 2011) for grain length and grain width, Venkata et al. (2011) for harvest index, and Parikh et al. (2012) for yield per plot and dry straw weight.

The characters early vigour and bacterial leaf blight exhibited moderate heritability and moderate genetic advance as percent of mean suggesting that both additive and non-additive gene effects were involved for variations of these characters, so selection and heterosis breeding both may be effective for improvement of these traits. These results were in agreement with the earlier findings of Sinha et al. (2004) for early vigour, Karthikeyan et al. (2010) for early vigour.

3.5.2 Efficiency of indirect selection for grain yield

According to Falconer, (1960) RSE of greater than unity (1) would permit use of a secondary trait for indirect selection for a primary trait such as grain yield. In this study, no trait had an RSE greater than 1. However, harvest index had an RSE of 0.83, which is close to unity. This trait (harvest index) seems promising and should be given top priority during selection for grain yield improvement. Other traits influencing grain yield but with moderate RSE should not be ignored during selection for grain yield improvement; these traits include days to maturity (0.59), days to early flowering (0.53) and dry straw weight (0.50).

3.5.3 Diversity and grouping

The amount of diversity available in the crop decides the success of any crop improvement programme. Assemblage and assessment of diversity in the germplasm is thus essential to know. In the present investigation, 30 genotypes of rice were studied for their diversity with respect to 16 important quantitative characters.

The eight clusters that were observed in this study indicate the existence of high level of diversity among the genotypes. Cluster I consisted of six accessions, cluster II consisted of two genotypes, cluster III consisted of two genotypes, cluster IV had one genotype, cluster V consisted of 10 genotypes, cluster VI –five genotypes, cluster VII- three genotypes and cluster VIII- one genotype.The genetic distances and the dendrogram showed that G14 and G19were the most similar pair, followed by G6 and G9 which were also highly similar to each other; while the dissimilar genotypes were G30 and G28 followed by G28 and G13. The dissimilar genotypes can be used for hybridization to bring heterosis among the genotypes. Similar genotypes such as G14 and G16 could be sharing common parents in their pedigrees. A

breeding programme may be initiated in which individuals in different clusters are crossed and significant heterosis would be expected.

3.6. Conclusion

There was wide genetic variability for all the 16 characters studied. In general, the level of variability as determined by PCV and GCV was high among the genotypes. Grain yield had significant positive genotypic association with days to maturity, panicle length, number of tillers per hill, dry straw weight, spikelets per panicle, and days to maturity and harvest index indicating a significant contribution on grain yield per plot. Based on diversity, the 30 rice genotypes were grouped into 8 clusters. No trait had an RSE for indirect selection for grain yield of greater than or equal to unity; however, harvest index had an RSE of 0.83 which is close to 1 and this trait should be given top priority during selection for grain yield improvement. Traits with moderate RSE values such as days to maturity, days to early flowering and dry straw weight should also be considered for selection for grain yield improvement. Diversity analysis revealed considerable divergence among the 30 genotypes. Eight clusters were observed based on Euclidian distances, and a crossing programme could be designed involving individuals from different clusters with the anticipation of significant heterosis for grain yield and other traits.

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CHAPTER 4

Correlations, path coefficient analysis and genotype-by-trait associations in rice (*Oryza sativa* L.)

Abstract

The present investigation was undertaken to assess the relationship among traits of rice using correlation, path coefficient and genotype-by-trait associations among 30 rice (*Oryza sativa* L) genotypes that were received from the agriculture research station ARI-KATRIN and rice institution AfricaRice and the International Rice Research Institute. The 30 genotypes were evaluated during the wet season of 2017 in a 6x5 alpha lattice design with three replications. Correlation analysis showed that traits which were positively and highly significantly correlated to yield per plot were harvest index (0.77***), dry straw weight (0.46***) and days to early flowering (0.40***) which means they contributed substantially to grain yield. On the other hand, path coefficient analysis revealed that harvest index (0.080) had a positive direct effects on grain yield. Due consideration should be given on it while selecting for grain yield improvement in rice. However, there were other positive direct contributors such as dry straw weight (0.51), days to maturity (0.11) and grain width (0.11). It will be necessary to select simultaneously for these traits together with those with strong positive indirect effects on grain yield in order to improve grain yield in rice. The genotype-by-trait biplot analysis revealed that the genotypes NERICA 4, IRRI-24,NERICA 2, Mwangaza, Tule na bwana and Wahiwahi had superior performance for BLB resistance, while Txd 306 was superior for yield contributing traits, whereas, the genotype Wahiwahi showed superior performance for days to maturity and days to early flowering. Hence, crosses involving these three categories of genotypes may result in BLB resistant genotypes coupled with high grain yield contributing traits and early maturity. The genotypes NERICA 4 and IRRI-24 were identified as best cultivars as they combined BLB resistance and several other good traits and thus could serve as good parents for use in breeding for better cultivars that combine grain yield and bacterial leaf blight resistance.

Key words: correlations, path coefficients, genotype-by-trait association

4.1 Introduction

Multi-trait relationships and the associations between yield and other component traits are key consideration for all crop breeders. Observed and true associations between traits may be quantified in terms of simple phenotypic and genotypic correlation coefficients, respectively (Dewey and Lu, 1959). However, yield is a complex trait and is influenced directly as well as indirectly by its various components. Correlation coefficients alone do not explain the complexity of the associations between traits or how change in a trait affects an associated trait (Dabholker, 1992; Dewey and Lu, 1959).To address this deficiency, path coefficient analysis developed by (Ellett & Ericson, 1986) disaggregates the correlation coefficient into the direct and indirect effects of various variables (traits) on a dependent variable (Sivathanu et al., 2015) ;Sabaghnia et al., 2015). Direct effect is when a variable affects another without being influenced by other variables whereas indirect effects occur when the relationship between two variables is mediated by one or more variables (Patil & Sushir, 2011)

Knowledge of the associations between yield and its component traits and among the component traits themselves would allow for more effective selection for yield. In rice, grain yield has been reported to be highly directly associated with: panicle per hill and straw weight per plant (Akabari & Niranjana, 2015) productive tillers and 1000 weight (Sao et al., 2016; Tongoona et al., 2016) yield per plot, grain length and grain width (Chander *et al.*, 2017; Ponnaiah et al., 2018) and plant height, panicle length, days to maturity and harvest index (Reddy et al., 2001). Studies that have generated such information on rice in Tanzania are limited.

The genotype-by-trait (GT) biplot analysis proposed by Bernal et al., (2013) is another powerful statistical tool for studying relationships among traits, evaluating cultivars based on multiple traits and for identifying those that are superior in certain traits. These could be candidates for use as parents in a breeding programme or directly released for commercial production. The main and important feature of genotype-by-trait (GT) analysis is that it facilitates the graphic visualization of the genetic correlations among traits (Yan & Rajcan, 2002); Lee et., 2003) and relatively easy in identification and selection of genotypes based on multiple traits (Bernal et al., 2013; Yan & Rajcan, 2002). It also provides information that helps to detect less important (redundant) traits and identify those that are appropriate for indirect selection for a target trait. Genotype-by-trait analysis of rice cultivars based on multiple traits including yield components and disease resistance traits to obtain the aforementioned essential knowledge for use in breeding is rare in literature. Therefore, the objective of this investigation was to assess relationship among traits of rice using correlation and path

coefficients among traits and to study genotype-by-trait associations among local and introduced rice genotypes in Tanzania.

4.2 Materials and Methods

The present investigation was carried out during the wet season of2017 in the eastern and southern parts in Tanzania. ARI-KATRIN is in the east and two sites Igurusi and Kyela are located in the southern agro-climatic zone of Tanzania. Detailed information about climate and soil at each of the locations is presented in Table 2.2

4.2.1. Plant materials

The experimental material comprised of 30 diverse genotypes of rice. The materials were obtained from ARI- KATRIN (Kilombero Agricultural training and Research Institute), International Rice Research Institute (IRRI) and AfricaRice. The relevant features of these genotypes are presented in Table 2.1.

4.2.2 Trial design and crop management

The field was ploughed and harrowed twice until a fine layer of soil was obtained. The experiment was laid-out in an alpha lattice design with three replications. The crop was sown on 3rd March 2017 at Katrin, 6th March at Igurusi and 7th March in Kyela, and each genotype was sown in two rows of 5m length with a spacing of 20 cm between rows and 20 cm between plants within rows. Border rows were sown to avoid border effect and intrude damage. Thinning was done at seedling stage to leave single seedlings per hill. The crop was fertilized as presented in Section 3.2.2. Other crop management practices such as irrigation, weeding and plant protection measures were carried out as and when needed during the crop growth period**.**

4.3 Data collection

Bacterial leaf blight severity was rated at maturity stage: for early maturity varieties from 80 days and for late maturity from 100 days after planting using the 1-9 scale (IRRI, 1996) to describe the symptoms. The rating was; 1 = 1-5% leaf area affected, 3=5-12% leaf area affected, 5=13-25% leaf area affected, 7=26-50 leaf area affected and 9=50> leaf area affected and thirty plants were randomly selected and identified for data collection. On each plant, data were collected on early vigour (scoring 1-9), days to early flowering (by counting number of days from cultivation to early flowering day), plant height (by measuring average of height from base to the tip of last leaf), panicle length (by measuring from the base (first node) to the tip of last spikelet of panicle), number of tillers per hill (by counting of tillers per hill).

Dead heart (scoring 1-9), lodging% (by recording % from 10%-100%) and days to maturity (the number of days from cultivation to maturity day at 80%) were also recorded. Data on dry straw weight (the total weight of straw after threshing per plot was measured in kg), number of spikelets per panicle, grain length (by measuring distance from the base of the lowest glume to the tip), grain width (measured as the distance across the fertile lemma and weighed in g), 1000-grain weight (one thousand seeds were counted and weighed (g)), harvest index (for total biological yield the entire plant above the ground lever was harvested, sun dried and weight at maturity, then harvest index was calculated by harvest index (%) = economical yield / biological yield x 100 and yield per plot (by weighing the total grains per plot) were also collected.

4.3.1 Data analysis

4.3.2 Phenotypic correlation analysis

Simple Pearson correlation coefficients were calculated using mean values for all traits from all locations using PROC CORR of SAS version 9.4 (SAS, 2014).

The phenotypic correlation was determined as follows, according to Know and Torrie (1964).

$$r_p = \frac{\sigma_{pxy}}{\sigma_{px} \times \sigma_{py}}$$

Where, r_p = phenotypic correlation, σ_{pxy} = phenotypic covariance of x and y characters, σ_{px} = square root of phenotypic variance of x character, σ_{py} = square root of phenotypic variance of y character.

4.3.3 Path coefficient analysis

Correlation does not provide an exact picture of the relative importance of influence of each of the component characters, because it does not analyse the direct and indirect influence of characters on yield. The path coefficient analysis, a cause and effect relationship provides knowledge of relative importance of each of the component characters. Path coefficient analysis was done according to the procedure suggested by Dewey and Lu (1959).

If grain yield is the effect y and χ_1 is the cause, the path coefficient for the path from cause χ_1 to the effect y is $\frac{\sigma_{x1}}{\sigma_y}$

Direct and indirect effects were worked out using phenotypic correlations as follows.

Direct effect of χ_1 on $y = p\chi_1 y$

Where, px_1 is the path coefficient of χ_1 on y

Similarly, direct effects of other attributes on grain yield were worked out.

Indirect effect of χ_1 via χ_2 on $y = p\chi_2 y.r\chi_1\chi_2$

Where, $P\chi_2 y$ is the path coefficient of the component character χ_2 on y

$r\chi_1\chi_2$ is the phenotypic correlation between χ_1 and χ_2.

The path coefficient scales suggested by Kiani, (2012), where 0.00-0.09 is negligible, 0.10-0.19 low, 0.2 0-0.29 moderate, 0.30-0.99 high and >1.0 very high were used.

4.3.4 Genotype by trait model

From a genotype-by-environment-by-trait three-way table, genotype-by-trait tables across all environments or across a subset of the environments can be generated and visually studied using biplots. Biplot analysis of genotype by trait tables is a typical example of biplot analysis of multivariate data. The model for biplot analysis of genotype by trait data is SVD of trait-standardized two-way table, i.e., equation with s_j being the standard deviation for trait j. A genotype by trait biplot can help understand the relationships among traits (breeding objectives) and help identify traits that are positively or negatively associated, traits that are redundantly measured, and traits that can be used in indirect selection for another trait. It also helps to visualize the trait profiles (strength and weakness) of genotypes, which is important for parent as well as variety selection (Bernal *et al.*, 2013).

Adjusted mean values of the traits were used for the analysis of genotype by trait and trait associations. To display the genotype by trait two-way data in a biplot, the formula suggested by Yan & Rajcan, (2002) was used as follows:

$$\frac{T_{ij} - T_j}{sj} = \lambda_1 \zeta_{i1} \tau_{j1} + \lambda_2 \zeta_{i2} \tau_{j2} + \varepsilon ij$$

where, T_{ij} = is the average value of genotype i for trait j, T_j is the average value of trait j over all genotypes, sj is the standard deviation of trait j among the genotype averages; ζ_{i1} and ζ_{i2} are the first principal component (PC1) and the second principal component (PC2)

scores, respectively, for genotype i τ_{j1} and τ_{j2} are the PC1 and PC2 scores, respectively, for trait j, and εij is the residual of the model associated with the genotype i and trait j.

Equation is a principal component analysis of standardized data with two principal components. Because different traits use different units, the standardization is necessary to remove the units. PC1 and PC2 must be scaled so that the one value is symmetrically distributed between the genotype scores and the trait scores. A Genotype by trait biplot is constructed by plotting the PC1 scores against the PC2 scores for each genotype and each trait.

4.4 Results

4.4.1 Correlation

The phenotypic correlation coefficients between yield and its related components in all possible comparisons were presented in Table 4.1. Association with grain yield per plot was positive and significantly highest for harvest index (0.77***) only while the other traits were not reliable with grain yield per plot.

Table 4. 1 Phenotypic correlation coefficients between 16 quantitative traits evaluated across three locations

Trait	EV	DTEF	PH	PL	TH	DHTRI	BLB	LODI	DM	DSTR	SPP	GL	GW	TGW	HI	YP
EV	1.00	0.19**	-0.52***	-0.50***	0.02ns	0.42***	0.14*	-0.41***	0.19**	0.31***	0.39***	-0.52***	-0.59***	-0.28***	-0.03ns	-0.22*
DTEF		1.00	-0.35***	-0.17*	0.08ns	-0.26***	0.19**	-0.24***	0.90***	0.20**	0.12ns	-0.28***	-0.25***	-0.13*	0.31***	0.40***
PH(cm)			1.00	0.80***	-0.34***	-0.28***	-0.19**	0.72***	-0.44***	0.17*	0.17ns	0.41***	0.65***	0.31***	-0.11ns	0.00ns
PL(cm)				1.00	-0.21***	-0.42***	-0.32***	0.52***	-0.25***	0.24***	0.38***	0.45***	0.74***	0.29***	0.09ns	0.23**
TH					1.00	-0.13*	-0.01ns	-0.21*	0.09ns	0.08ns	0.15*	-0.03ns	-0.08ns	-0.10ns	0.29***	0.33***
DHTRI						1.00	0.25***	-0.27***	0.26***	-0.23**	0.49***	-0.58***	-0.62***	-0.22*	0.06ns	-0.08ns
BLB							1.00	-0.14*	0.20**	0.01ns	0.28***	-0.28***	-0.33***	-0.19**	-0.05ns	-0.05ns
LOD								1.00	-0.30***	0.20***	0.22***	0.39***	0.48***	0.28***	-0.17*	-0.03ns
DM									1.00	0.23ns	0.13*	-0.27***	-0.33***	-0.17ns	0.25***	0.38***
DSTR(kg)										1.00	0.35***	0.21***	0.26***	0.10ns	-0.12*	0.46***
SPP											1.00	0.43***	0.48***	0.16**	0.04ns	0.29***
GL(mm)												1.00	0.63***	0.44***	-0.06ns	0.11ns
GW(mm)													1.00	0.40***	-0.04ns	0.16ns
TGW(g)														1.00	-0.12*	-0.03ns
HI															1.00	0.77***
YP(kg)																1.00

***- Significant at P<0.001, **- Significant at P<0.01, *-Significant at P<0.05, ns-Non significant

EV= Early vigour, DTEF= Days to early flowering, PH= Plant height, PL= Panicle length, TH= Tillers per hill, DH= Dead heart, BLB= Bacterial leaf blight, LODI= Lodging percent, DM= Days to maturity, DSTR= Dry straw weight, SPP= Spikelet per panicle, GL= Grain length, GW= Grain width, TGW= Thousand grain weight, HI= Harvest index YP= Yield per plot.

4.4.3 Path coefficient analysis

For the 16 quantitative traits, determination of direct and indirect path coefficients were estimated (Table 4.2). It was evident from the results that grain yield per plot was the result of days to early flowering, days to maturity, tillers per hill, dry straw weight per plot and harvest index as they had significant effects on it. The highest and positive direct effect (0.80) was from harvest Index and others were dry straw weight (0.510), days to maturity (0.11), grain width (0.11). These traits should be given top priority during selection of grain yield improvements. Traits, which indirectly affect grain yield via other traits, should also be considered during selection. In this study significant indirect effects were observed for days to early flowering through harvest index (0.2487), panicle length via dry straw weight (0.12), tillers per hill through harvest index (0.23), dead heart via dry straw weight (-0.12), days to maturity through dry straw weight (0.12), number of spikelets per panicles through dry straw weight (0.18), grain length through dry straw weight (0.11), and grain width through dry straw weight (0.13).

Table 4. 2 Direct and Indirect effects of secondary traits on grain yield

TRAIT	EV	DTEF	PH	PL	TH	DH	BLB	LODI	DM	DSTR	SPP	GL	GW	TGW	HI
EV	**0.0197**	-0.0107	0.0152	-0.0151	0.0015	0.0370	0.0028	0.0033	0.0204	-0.1580	-0.0173	-0.0232	-0.0663	-0.0001	-0.0267
DTEF	0.0038	**-0.0554**	0.0102	-0.0053	0.0051	0.0226	0.0038	0.0019	0.0973	0.0997	0.0051	-0.0125	-0.0280	-0.0001	0.2487
PH (cm)	-0.0102	0.0192	**-0.0294**	0.0242	-0.0215	-0.0250	-0.0039	-0.0059	-0.0475	0.0890	0.0075	0.0183	0.0732	0.0002	-0.0851
PL(cm)	-0.0097	0.0096	-0.0234	**0.0304**	-0.0133	-0.0369	-0.0065	-0.0042	-0.0275	0.1214	0.0168	0.0198	0.0836	0.0001	0.0700
TH	0.0005	-0.0044	0.0100	-0.0064	**0.0633**	-0.0110	-0.0003	0.0017	0.0100	0.0424	0.0064	-0.0014	-0.0094	0.0000	0.2309
DH	0.0083	-0.0142	0.0083	-0.0127	-0.0079	**0.0882**	0.0050	0.0022	0.0286	-0.1173	-0.0215	-0.0256	-0.0702	-0.0001	0.0495
BLB	0.0027	-0.0106	0.0057	-0.0098	-0.0008	0.0222	**0.0200**	0.0011	0.0215	0.0040	-0.0121	-0.0123	-0.0369	-0.0001	-0.0402
LODI	-0.0080	0.0133	-0.0213	0.0157	-0.0135	-0.0234	-0.0027	**-0.0081**	-0.0321	0.1030	0.0098	0.0173	0.0544	0.0001	-0.1385
DM	0.0037	-0.0497	0.0129	-0.0077	0.0058	0.0233	0.0040	0.0024	**0.1085**	0.1170	0.0056	-0.0121	-0.0376	-0.0001	0.1992
DSTR(kg)	-0.0061	-0.0108	-0.0051	0.0072	0.0053	-0.0203	0.0002	-0.0016	0.0249	**0.5103**	0.0155	0.0093	0.0293	0.0000	-0.0962
SPP	-0.0077	-0.0064	-0.0050	0.0116	0.0092	-0.0430	-0.0055	-0.0018	0.0137	0.1796	**0.0441**	0.0190	0.0541	0.0001	0.0304
GL(mm)	-0.0103	0.0156	-0.0121	0.0136	-0.0020	-0.0508	-0.0055	-0.0031	-0.0297	0.1070	0.0189	**0.0444**	0.0712	0.0002	-0.0458
GW(mm)	-0.0116	0.0138	-0.0190	0.0225	-0.0053	-0.0548	-0.0065	-0.0039	-0.0361	0.1324	0.0211	0.0280	**0.1129**	0.0002	-0.0288
TGW(g)	-0.0055	0.0071	-0.0092	0.0090	-0.0062	-0.0197	-0.0038	-0.0023	-0.0181	0.0492	0.0072	0.0195	0.0446	**0.0005**	-0.0998
HI	-0.0007	-0.0172	0.0031	0.0027	0.0183	0.0055	-0.0010	0.0014	0.0270	-0.0614	0.0017	-0.0025	-0.0041	-0.0001	**0.8003**

EV= Early vigor, DTEF= days to early flowering, PH= Plant height, PL= plant length, PL= Panicle length, TH = Tillers per hill, DH = Dead heart, BLB = Bacterial leaf blight, LODI = lodging percent, DM = Days to maturity, DSTR = Dry straw weight, SPP = Spikelet per panicle, GL = Grain length, GW = Grain width, TGW = Thousands grain weight, HI = Harvest index.

4.4.4 Genotype by trait associations

4.4.4.1Genotype by traits biplot analysis of the performance of 30 rice genotypes based on multiple traits

A genotype-by-trait (GT) biplot analysis was carried out with nine important traits and 30 genotypes using data collected from across three environments. The biplot accounted for 87.78% of the total variation, of which PC1 explained 81.73% and PC2 explained 6.05%. Considering the furthest genotypes from the biplot origin, a line was first drawn to join these genotypes so that all other genotypes are within the biplot (Figure 4.1). Then perpendicular lines to each side of the biplot were drawn, starting from the biplot origin. The perpendicular lines are equality lines between adjacent genotypes on the polygon, which facilitate visual comparison (Yan and Tinker 2006). The equality lines divided the biplot into sectors, and the winning genotype for each sector is the one located on the respective vertex. The genotype which occupied vertex position in the biplot is known as vertex genotype. The vertex genotypes in each sector were regarded as the genotype with the highest value of the traits within the sector. These vertex genotypes could be exploited in hybridization programme as potential parents in the development of varieties, hybrids and populations that are outstanding in those traits.

From Figure 4.1 genotypes G3, G1, G2 and G28 were regarded as top genotypes, which exhibited superior performance for the bacterial leaf blight resistance traits allocated within the sector. For plant height, genotype G24 had the highest plant height, while G28 exhibited better performance for days to maturity and days to early flowering, and genotype G1 and G2 exhibited superior performance for early vigour. For the character dry straw weight, the genotypes G9, G7 and G30 showed better performance, whereas for the trait harvest index, the genotype G10 exhibited superior performance and for the trait yield per plot, the best performing genotype was G30 although it was a susceptible check for bacterial leaf blight. Even though the genotypes G3, G1, G2, and G28 showed better performance for high resistance to bacterial leaf blight, they were not the best for grain yield per plot. So these genotypes may be used for crosses with the intention to combine grain yield and bacterial leaf blight resistant traits in a single genotype. The suggested genotypes for crosses are NERICA4 with Txd 306. NERICA 4 was resistant to BLB but low yielding, while Txd 306 was susceptible to bacterial leaf blight but high yielding. This cross could result in a genotype that is resistant to BLB, but also high yielding. Genotype G22 exhibited poor performance for the lodging%, indicating that it could be improved by crossing with genotypes which had low lodging% in order to improve it. Figure 4.1 is the biplot showing the interactions between genotype and traits.

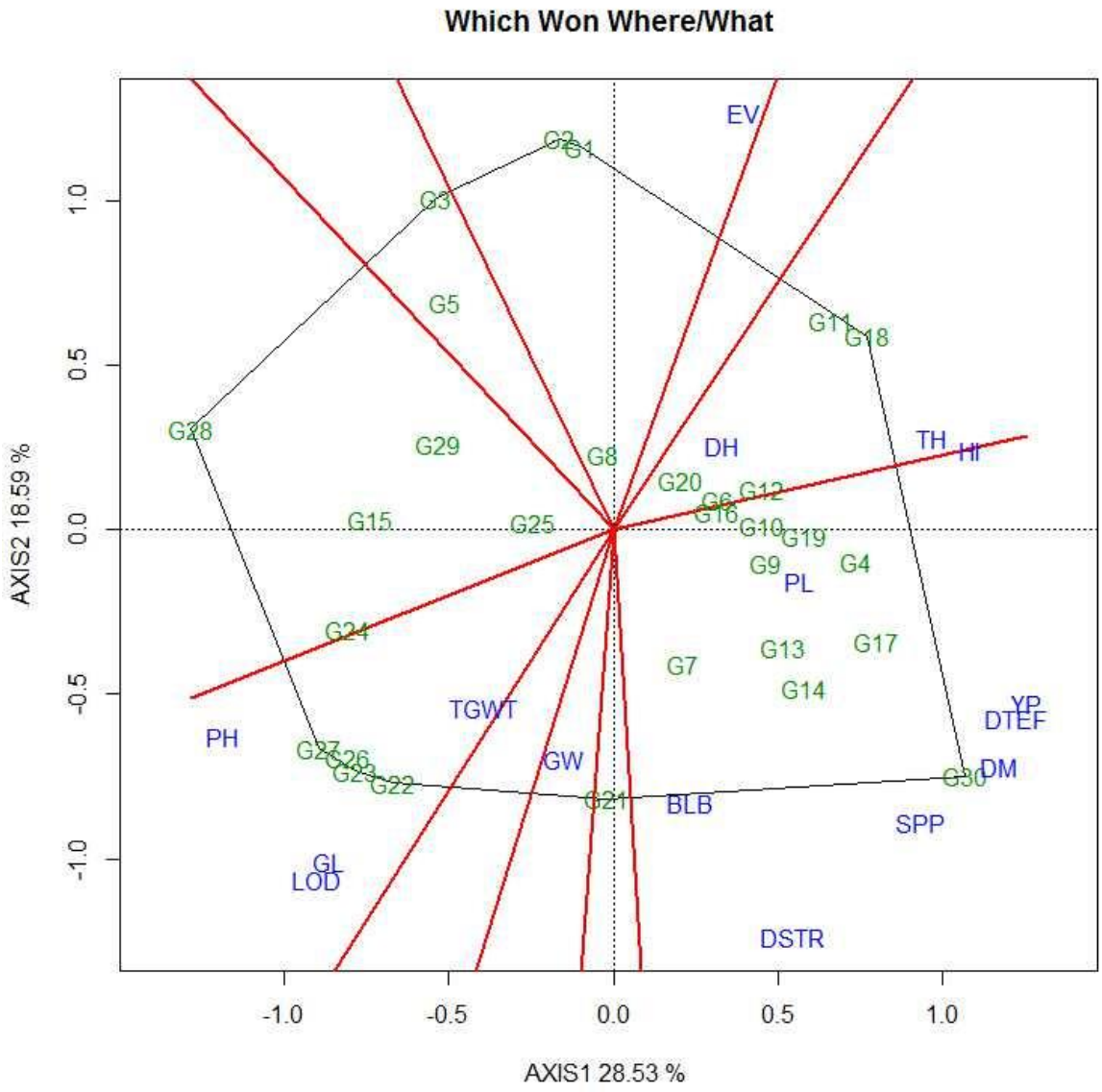

Figure 4.1 Biplot showing association between genotypes and traits

EV= Early vigour, DH= Dead heart, TH= Number of tillers per hill, HI= Harvest index, PL= Panicle length, YP= Yield per plot, DTEF= Days to early flowering, DM= Days to maturity, BLB= Bacterial leaf blight, SPP= Number spikelets per panicle, DSTR= Dry straw weight, TGWT= Thousand grain weight, PH= Plant height, GW= Grain width, GL= Grain length and LOD= Lodging %.

4.5 Discussion

4.5.1Correlation

Correlation studies provide better understanding of yield components that helps the plant breeder during selection (Alan et al., 2013; Haider & Kaku, 2012). The phenotype of a plant is the result of interaction of a large number of factors so the final yield is the sum total of the effects of several component characters. Yield is the final phenotypic performance of the plant, which is influenced by various factors such as genetic, environment and their interactions. This complex quantitative character is under the control of polygenes. Polygenes are highly sensitive to the environment. Hence, the selection of superior genotype based on yield alone may not be effective. For the rational approach towards the improvement of yield, selection has to be operated through associated characters. Traits like harvest index (0.77***), that showed highly positive correlation with grain yield per plot must be taken into consideration during selection and improving rice varieties, while negative correlation between like early vigour (-0.22*) and grain yield implies entries which exhibited poor vigour (high vigour scores) also had a poor yields.

Days to early flowering displayed significant positive association with days to maturity. This result was in agreement with the earlier findings of Agahi et al., (2007); Bhujel et al., (2018); Wattoo et al., (2010). Days to maturity was significantly correlated with dry straw weight. Bhujel *et al.*, (2018) reported a similar type of positive association of days to maturity with dry straw weight. Plant height exhibited significantly positive correlation with panicle length, 1000 grain weight and dry straw weight per plant. Similar results were observed by Basavaraja *et al.*, (2011); Bhujel *et al.*, (2018); Hajiaqatabar & Kiani, (2016); Kumar *et al.*, (2015); Madhubabu *et al.*, (2011), Jayasudha and Deepak (2010) for panicle length and Madhubabu *et al.*, (2011) for 1000 grain weight. Similarly, tillers per hill had significantly positive correlation with harvest index. These results were similar to the findings of Agahi *et al.*, (2007); Madhubabu *et al*., (2011); Sivasankar *et al.*, (2018) for harvest index. The character panicles per hill had positive correlation with straw yield per plot. Panicle length had significant positive association with spikelets per panicle. (Basavaraja et al., 2011; Kumar et al., 2015; Madhubabu et al., 2011; Rahman & Syed, 2012) observed similar associations. The character spikelets per panicle had positive correlation with dry straw weight per plot.

4.5.2 Path coefficients

The highest direct positive effects on grain yield were contributed by harvest index, dry straw weight, grain width and days to maturity. When compared to the path coefficient scales suggested by Lenka and Mishra (1973) where 0.00-0.09 is negligible, 0.10-0.19 low, 0.2 0-

0.29 moderate, 0.30-0.99 high and >1.0 very high, harvest index (0.80), dry straw weight (0.510), had high direct effects, whereas grain width (0.113), days to maturity (0.108) had low direct effects. This means that genotypes with high harvest index and dry straw weight give more grain yield per plot and the high yields can be achieved.

The characters, days to maturity, dry straw yield per plot, and harvest index exerted positive direct effect on grain yield per plot and correlation of these characters with grain yield was positively significant. Thus, direct selection for these traits could be rewarding for yield improvement. These findings are in agreement with reports of Agahi *et al.*, (2007); Kumar *et al.*, (2015); Mahmud *et al.*, (2007); Rashid *et al.*, (2017); Sivasankar *et al.*, (2018) for harvest index, Basavaraja *et al.*, (2011); Hajiaqatabar & Kiani, (2016); Kishore *et al.*, (2015); Kumar *et al.*, (2015); Lingaiah *et al.*, (2014); Rahman & Syed, (2012) for days to maturity, and Jayasudha and Deepak (2010); Kamireddy *et al.*, (2016); Madhubabu *et al.*, (2011) for harvest index.

4.5.4 Genotype by trait biplot

Yield improvement is the ultimate goal for most of the breeding programmes. Yield could be described as the sum total of all physiological and developmental processes that occur from sowing to maturity as conditioned by environmental factors prevalent during the growing season (Rubio et al., 2004). Yield being a complex trait controlled by polygenes, improvement in yield is difficult through direct selection and may be a lot easier through selection for component traits involved in the pathway (i.e. indirect selection). As a result, it has become a routine in breeding trials in which yield improvement is of prime importance, to gather data on multiple traits associated with grain yield. Furthermore, a cultivar gains wide acceptability based on a package of desirable traits and not just its yield potential.

In GGE biplot for yield data, the grain yield per plot and harvest index were closely and associated to each other. This suggests that high yielding genotypes in three locations depend much on their highest harvest index and therefore this trait may be referred to as the yield – related trait. However, plant height and lodging were positively associated but negatively associated with yield per plot. Therefore, these two traits are not closely related with yield per plot. BLB was closely related and positively associated with dry straw weight, suggesting that BLB occurrence can weaken the leaves and therefore affect the dry straw weight. Also yield per plot was negatively correlated with BLB indicating the crucial importance of resistance to BLB in these three locations.

Moreover, the genotypes which were resistant to BLB can be used in crosses with other genotypes which performed well in respect of yield especially Txd-306. This is because a genotype is more or less a complex biological system rather than a simple collection of independent traits, and an effective breeding programme requires the essential components of the system and the interrelationship among them (Bernal *et al.*, 2013). In addition, in an inclusive multi-trait selection process proposed by (Yan & Frégeau-reid, 2018), selection strategies are grouped into three categories: independent selection, independent culling, and index selection; so that all the aspects in a variety or parent line selection are taken into consideration. The GGE biplot helps to provide the information that assists in detecting less important traits and identifying those that are appropriate for indirect selection for a target trait.

4.6 Conclusion

In this study, path coefficient analysis indicated that grain yield was positively and significantly correlated with harvest index, dry straw weight, days to early flowering, days to maturity, tillers per hill and panicle length. Path coefficient analysis indicated that, among yield components, harvest index, dry straw weight and grain width had a positive direct effect on grain yield and therefore, may be considered as selection criteria for the improvement of grain yield. In addition, the genotype by trait (GT) biplot analysis was used to identify the best traits that are important in classifying resistant and high yielding genotypes and to know the relationships between traits and genotypes. The genotypes NERICA 4, IR-24,NERICA 2, NERICA 1, LOWLAND NERICA 6, WAB 450-12-12-BL1-DV4, Mwangaza, Tule na bwana and Wahiwahi showed superior performance for BLB resistant, whereas the genotypes Txd -306 showed superior performance for high yield. Hence, crosses involving these two categories of genotypes may result in the production of resistant genotypes coupled with high grain yield. Based on genotype-by-trait biplot analysis, it can be concluded that the traits plant height, days to maturity, lodging%, bacterial leaf blight scores, days to early flowering, early vigour, dry straw weight harvest index and yield per plot are important traits for yield as well as resistance and hence they could be considered as key components during the selection programme aimed at improvement for grain yield and BLB resistance. The genotypes NERICA 4, SATO 1 and IR-24 were identified as the best cultivars and therefore most desirable as they combined several good traits in their genetic composition and thus could serve as good genetic raw materials when crossed with the other high yielding and BLB resistant genotypes.

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Chapter 5

General overview of the research findings

5.1 Introduction

The aim of this study was to contribute to the increase and strengthening of rice production in Tanzania through development of high yielding and BLB resistant varieties. Selections were done to identify BLB resistant varieties and BLB susceptible genotypes endowed with other desirable traits, which can be used for improvement.

The objectives of this study were:

1. To analyse genotype x environment interaction effects for reaction to bacterial leaf blight under natural infestation and grain yield performance across environments in rice *(Oryza sativa L*)
2. To assess the heritability, variability and efficiency of indirect selection of secondary traits for grain yield improvement among rice genotypes.
3. To study correlation, path coefficients and genotype-by- trait associations in rice (*Oryza sativa .L*)

5.2 Research summary

5.2.1 Genotype × environment interaction analysis for reaction to bacterial leaf blight under natural infestation and grain yield performance across environments in rice (*Oryza sativa* L.)

The study showed a highly significant ($p<0.001$) genotype x environment interaction (GEI) effects for BLB. The ranking of the genotypes across environments revealed a crossover type of GEI whereby in three locations, the GGE biplot analysis identified NERICA 2 and LOWLAND NERICA 6 as resistant and the most stable genotypes across environments. For the 'which won where' in plot, the genotype which won in ARI-KATRIN was Dakawa 83,while in Igurusi and Kyela the genotype which won was NERICA 4.

5.2.3 Genetic analysis and evaluation of secondary traits for use in indirect selection of grain yield improvement among rice genotypes

High broad sense heritability estimates were observed for days to early flowering (99.665%), days to maturity (99.353%), grain length (98.211%) and for grain yield per plot (97.126). Broad sense heritability estimations were low for lodging % (0.00%) and the other such as dead heart (22.567%).The traits spikelets per panicles, 1000- grain weight, number of tillers per hill, panicle length and early vigour were important direct contributors to yield improvement. Regarding variability parameters, lodging % had the highest PCV% (5325.463%) followed by

spikelets per panicles (1005.352%) and plant height (294.959%); number of spikelets per panicle had the highest GCV% (419.902%) followed by plant height 97.843% and days to early flowering (64.307%). As for GA, the highest was for spikelets per panicles (66.79), followed by plant height (22.34) and days to maturity (15.66). Grain yield had the highest GAM of 104.13% followed by dry straw weight (92.11%) and harvest index (66%). In respect of efficiency of secondary traits for indirect selection for grain yield, harvest index (0.83) was the best, followed by days to maturity(0.59) and days to early flowering (0.53) these traits should be given top priority in selection for improvement of grain yield in rice.

5.2.4 Correlations, path coefficients and genotype-by-trait associations in rice (*Oryza sativa* L.)

The traits which were positively correlated with yield were harvest index (0.77***), dry straw weight (0.46***), days to early flowering (0.40***), days to maturity (0.38***), number of tillers per hill (0.33***) and spikelets per panicles (0.29***). Traits which had direct effect on grain yield are harvest index (0.800), dry straw weight (0.510) and grain width (0.113) and those that contributed indirectly were days to early flowering through harvest index (0.249), and panicle length through dry straw weight (0.121), number of tillers per hill through harvest index(0.231), dead heart through dry straw weight (-0.117), lodging percent through dry straw weight (0.103), lodging percent through harvest index (-0.1385), days to maturity through dry straw weight (0.117), days to maturity through harvest index (0.200), spikelets per panicles through dry straw weight (0.180),grain length through dry straw weight (0.107) and grain width through dry straw weight (0.132),so these are the traits which should be considered when a breeder needs to improve rice genotypes for grain yield. For genotype-by-trait associations, the superior genotype for early vigour was Kalamata, days to early flowering was Wahiwahi, plant height was Kalamata, and for BLB resistance, NERICA 4; days to maturity, Wahiwahi; dry straw weight, Txd 306; harvest index, SATO IX, and grain yield per plot Txd 306.Therefore, these superior genotypes for the different traits can be used if a breeder needs to improve specific traits or to combine traits in a single rice variety.

5.3 Recommendations and future directions

Resistant genotypes are most preferable by the farmers and the breeders rather than using bactericides, which sometimes cause environmental pollution and health problems. The existence of significant genotypic variation for reaction to BLB disease and other desirable traits including grain yield suggests that it is possible to develop high yielding and BLB tolerant or resistant rice cultivars for production in Tanzania. However, for acceptance of the new rice cultivars, it would be important to combine yield and disease resistance with grain quality traits.

However, there were three local varieties, Mwangaza, Kalamata and Kalundi that were resistant to BLB, and other improved varieties, especially the NERICAs were the best for disease resistance. These genotypes are therefore recommended to be used in breeding programmes aimed at developing resistant varieties for the all the three ecologies in Tanzania.

Traits that were conditioned by high heritability were days to early flowering, days to maturity, grain length and grain yield per plot. Thus, they can quickly be improved under a condition with no disease pressure through recurrent selection procedures aimed at accumulating the desirable additive genes. On the other hand, the traits lodging and dead heart were highly influenced by the environment resulting in low broad sense heritability estimates. Therefore, selection based on these traits would wait until later generations. Hybridization can be a choice for developing cultivars with high yield, disease resistance and other desirable yield components. In genotype x environment interactions, high resistant and stable genotypes were identified across the test environments using the GGE biplot models. The GGE biplot showed that NERICA 2 and LOWLAND NERICA 6were the most stable and resistant genotypes. However, since the results of this study were based on a single year data, more temporal and spatial environments will be needed to give meaningful recommendations.