INHERITANCE OF RESISTANCE TO BROWN SPOT DISEASE IN UPLAND RICE IN UGANDA

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A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND SEED SYSTEMS OF MAKERERE UNIVERSITY

JUNE 2015

ABSTRACT

Brown spot disease, caused by *Bipolaris oryzae*, is among the biotic threats to rice production in Uganda. The disease is responsible for significant economic losses as it results in loss of both grain quality and yield. Cultural and chemical control measures have been used to reduce the severity of the disease and increase the growth and yield of rice, but breeding resistant varieties to brown spot is the most cost effective measure for disease management.

In this study, field trials and screen house experiments were conducted at the National Crops Resources Research Institute (NaCRRI) – Namulonge, Uganda during 2013/2014 with the objective of identifying new sources of resistance to brown spot. A hundred germplasm were screened for brown spot disease resistance under rain-fed conditions in the field using an alpha lattice design and replicated twice. Plants were artificially inoculated with the disease pathogen and disease scored at 15, 30, 45 and 60 days after inoculation. The inheritance of resistance to brown spot disease was investigated through the nine-parent full diallel mating design. The F_1 progenies were advanced to F_2, and then F_2's together with parents were evaluated against brown spot disease when the panicle had fully emerged. Second filial (F_2) generation progenies of specific crosses were also characterized for their segregation patterns.

There was significant variation for brown spot resistance among genotypes tested. Among the hundred tested rice lines, eighteen lines were rated as highly resistant, fifty one resistant, twenty seven as moderately resistant and four lines were susceptible. The effects of both general combining ability (GCA) and specific combining ability (SCA) were significant, indicating that both additive and non-additive effects respectively, were important in determining resistance to brown spot. The preponderance of GCA effects ($2\sigma^2gca$ / ($2\sigma^2gca + \sigma^2sca$) = 0.29) suggested that non-additive genetic effects were predominant compared to

additive genetic effects. Broad sense coefficient of genetic determination (BS-CGD) was 0.83, indicating that most of the variability was genetically controlled. Narrow sense coefficient of genetic determination (NS-CGD) was low (0.24), indicating that non-additive effects were more influential than additive effects. Reciprocal effects were significant, suggesting that cytoplasmic genes effects played a role in modifying brown spot resistance. Care should, thus be taken when selecting the female parents during hybridization, as it has been observed the maternal effects plays a role in conditioning the resistance. The segregation patterns within specific crosses suggested that brown spot resistance could be explained by the presence of one or two dominant genes.

The resistant lines thus identified may be brought forward and involved in a rice breeding program for the development of brown spot resistant lines. These results further suggest that a breeding program based on planned crossing would be effective for improving the resistance to brown spot in rice varieties adapted in Uganda.

DECLARATION

I **Marco Martin Mwendo** hereby declear that this thesis is my original work and has not been submitted for a degree to any University.

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DEDICATION

This work is dedicated to my lovely wife Jenipher and our beloved daughters, Sharon and Abigail; for your love and support and my parents Mr. and Mrs. Martin Mwendo; you have nurtured me into both a respectful and responsible man. May Almighty God offer you a long life!!

ACKNOWLEDGEMENTS

I thank God for granting me life and good health to accomplish this training. This training was sponsored by Innovative Agricultural Research Initiative (*i*AGRI) through the Regional Universities for Capacity Building in Agriculture (RUFORUM). I am very grateful for their support.

I would like also to express my sincere appreciation to my supervisors Dr. Mildred Ochwo-Ssemakula and Dr. Jimmy Lamo for their patience, encouragement, support and insight throughout the course of my study. Their commendable guidance, advice and positive criticism made this thesis.

I sincerely thank the Ministry of Agriculture, Food Security and Cooperative in Tanzania for permitting me to pursue the study at Makerere University. Special acknowledgments also go to Mr. Geoffrey Kirenga, the Chief Executive Officer at Southern Agricultural Growth Corridor of Tanzania (SAGCOT), Dr. Firmin Mizambwa, the Chief Executive Officer at the Agricultural Seed Agency (ASA) and Mr. Philemon Kawamala, the Marketing and Distribution Manager at ASA, for their insightful advice.

I would like to extend my thanks to Professor Paul Gibson and Dr. Richard Edema, the Coordinator of the Regional Master's Program in Plant Breeding and Seed Systems at Makerere University for their advice, constructive suggestions, moral support during the whole period of my study at the University. I will also always be indebted to Ms Pauline Gibson for her kindness and efforts towards improving my communication skills.

Finally, I extend special thanks to my family for their understanding, endurance and encouragement during the time I was away from home undertaking this study. Their support greatly helped me to accomplish my studies successfully.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OFAPPENDICES

CHAPTER ONE: INTRODUCTION

1.1 Background

Sub-Saharan Africa (SSA) faces challenges in its efforts to improve the lives of the 30% of its population that suffers from extreme poverty and food insecurity. As more than 70% of the populations depend on farming and related activities, agricultural development needs to play a major role in improving this situation. Fortunately, Africa has an abundant supply of natural resources that can support a huge expansion in food, specifically in rice production (Balasubramanian *et al*., 2007).

Rice (*Oryza sativa* L.) is a major food crop for the world's majority. It is mainly produced in Asia contributing over 90% of the world's total production. The total global land area covered by rice is 162 Mha with 10 Mha in Africa (FAOSTAT, 2012). The average annual world rice production increased gradually between 2002 and 2012 from 569 to 738 Metric tons (FAOSTAT, 2012). Because of the strong demand for rice, expansion of the area under rice production in SSA is greater than for any other crop (Balasubramanian *et al.*, 2007). In SSA, from 2007 to 2012, rice yield increased by 30% and this increase is at a faster rate than the world average from 3.3 to 8.4% due to increased use of technological innovation, such as improved varieties and improved crop management in general (Africa Rice, 2013).

Rice is becoming an important food in Eastern, Central, and Southern Africa. In recent years, the relative growth in demand for rice is faster in SSA than any-where else in the world. Demand for rice has been rising due to population growth and a shift in consumer preference for rice, especially in urban areas (Balasubramanian *et al*., 2007). Rice's importance as a food and commercial crop

in East Africa is based on several attributes. The crop can feed the region's growing urban population, while providing a source of income to resource-poor farmers in rural areas. The crop has also been identified for its potential to eradicate poverty (ECARRN, 2005). In Tanzania it is the second most important cereal food crop (FAOSTAT, 2002), while in Uganda it ranks first as a commercial cereal crop (NARO, 2005), and has the highest output-to-input ratio among cereals grown in Uganda (NAADS, 2003; Kijima *et al.,* 2006). Rice has a higher return on investment with an output–to-input ratio of 1.83 when compared with common cereals such as maize hybrids (1.2) and sorghum (1.6) (Kijima et al., 2006).

The popularity of upland rice in Uganda is fairly recent, and is attributed to a high rate of return on investment at 1.8, compared to other cereal crops that attained only 1.2 *(Kijima et al.,* 2006). Its popularity is also due to increased promotion by stakeholders, availability of improved rice varieties, and increased demand and consumption, particularly, among the urban population and neighboring countries. However, the average yields are around 1.5 t/ha as opposed to 3.5 t/ha under irrigated conditions (Odogola, 2006). Despite a continuous increase in the area under cultivation, rice yields remain very low. By 2005 the actual on-farm yields stood at 1.5 t/ ha for both wetland and upland rice production systems. The potential yields for these systems are, however, 4.5 and 5.5 t/ha, respectively, leaving a yield gap of at least 3.0 t/ha (FAOSTAT, 2005). NERICA's potential yield in sub-Saharan Africa is 5 metric tons per hectare with the use of fertilizers, but on farmers' fields in Uganda only 2.2 metric tons are realized (Hyuha *et al*., 2007). The yield gap is attributed by both biotic and abiotic factors (Talwana *et al*., 2008).

1.2 Rice production constraints

Rice production in Africa is affected by a wide range of constraints in the production system especially environmental, biotic, management, and socioeconomic (Ngala, 2013). The abiotic constraints limiting rice production in both rain fed and irrigated areas in sub-Saharan Africa include biophysical constraints and high reliance on low-input and rain fed agriculture (Defoer *et al.*,2002). Most of these stresses are associated with water availability (drought and excess water), soil problems (salinity, nutrient deficiencies and toxicities) and extreme temperatures (heat and cold). These stresses fluctuate seasonally and vary spatially. They adversely affect rice growth and productivity, resulting in reduced yields (Defoer *et al.*, 2002). Among the biotic factors weeds are the principal constraint, followed by insect pests such as stem borers, African rice gall midge- AfRGM (*Orseolia oryzivora*) and rice bugs (*Leptocorisa oratorius*) as well as diseases, which include *Rice yellow mottle virus* (RYMV, genus Sobemovirus), blast (*Magnaporthe grisea*) and brown spot (*Bipolars oryzae*) (Balasubramanian *et al*., 2007). In all ecosystems, rats and birds are also serious problems for rice. Climate change adds more uncertainties, particularly for smallholder subsistence farmers (Dramé *et al*., 2013). Stabilizing crop yields and limiting yield losses will undoubtedly reduce resource-poor African farmers' vulnerability.

1.3 Problem statement

Brown spot is one of the most important diseases of rice worldwide. The disease is caused by the fungus *Bipolaris oryzae* (Breda de Haan) Shoem, common in both rain-fed and upland rice production systems (Singh & Singh, 2000). The loss caused by the disease affects both yield and grain quality (Savary e*t al*., 2005). Heavily infected grains are not suitable for human consumption, which may partly explain the impact of the disease in the Great Bengal Famine of 1942 (Barnwal *et al*., 2013). The pathogen infects the coleoptiles (causing blighting),

leaves (forming oval, dark brown to purplish-brown spots) and damages the photosynthetic activities, ultimately killing the leaf. The reduction in yield can be as high as 45% in severe infection and 12% in moderate infection (IRRI, 1983). Brown spot disease in rice cultivation under aerobic conditions has resulted in 27.5% lower yields than for flooded rice (Patel *et al.,* (2010) cited in (Yaqoob *et al*., 2011). Blighting of seedlings can result in 10-58% seedling mortality.

In East Africa, particularly Tanzania, the disease is widespread in upland , rain-fed lowland and irrigated wetland rice growing ecologies, with losses ranging from 12 - 43%, although up to 90% yield losses have been reported during epidemics (Mwalyego *et al*., 2011), and 50 – 80% may occur, depending on the crop susceptibility, disease severity and agro-ecology (Raymundo, 1980). In Uganda, the disease was first reported in 2003 (Biruma *et al*., 2003), and later in 2005 in association with seed transmission (Kawube *et al*., 2005). Farmers in Uganda subsequently confirmed the disease as the second most important disease of upland rice after rice blast (Odogola, 2006). In 2011 the disease was ranked as the third most important disease after *Rice yellow mottle virus* (RYMV) disease and leaf blast (Adur *et al*., 2011).

The main varieties grown by farmers in Uganda are local ones which are susceptible to many diseases including brown spot (Kawube *et al*., 2005; Odogola, 2006). Rice is further predisposed to brown spot disease infection when grown in soils that are nutrient deficient and poorly drained (Zadoks, 2002). The disease is more predominant under low input agriculture, and is thus considered the "poor rice farmer's disease" which has caused severe and chronic losses in South and Southeast Asia (Savary *et al*., 2000). It is also common in poorly drained soils that are rich in organic matter (Savary *et al*., 2005) and has been associated with the occurrence of drought (Savary *et al*., 2005), macro-nutrient deficiency or both factors (Ou , 1985). Various measures have been employed worldwide to control the disease. These include application of various agronomic practices, pesticides,

biological control and use of resistant varieties. However, very few resistant cultivars are available for practical use (Biswas *et al*., 2011).

1.4 Justification

In Uganda, control of brown spot disease is done by use of cultural practices and spraying chemicals (fungicides) like mancozeb on rice in the field, since the practice of intensive rice cultivation creates favourable conditions for disease development (Biswas *et al*., 2011). With the evident increase in rice cultivation in Uganda (FAOSTAT, 2008), challenges are already being faced in disease management using cultural practices, either alone or in combination with chemicals because such practices are not feasible for disease management in large scale production. In addition, chemical control is known to be environmentally unfriendly, expensive and requires expertise that is generally lacking among most resource-poor farmers in Uganda (Kawube *et al*., 2005). The use of resistant varieties is appropriate for resource poor farmers because it does not require additional costs and is environment-friendly (Khoury & Makkouk, 2010). Varieties with resistance to brown spot disease have been identified (Malavolta *et al*., 2002). Satija *et al*.,(2005) identified 15 *Oryza sativa* entries out of 124 that were classified as resistant to the disease (with less than 5% severity), while Mosharraf *et al*. (2004) identified one resistant variety out of 29 entries. Varieties with partial resistance and three quantitative trait loci (QTL) for brown spot disease resistance have been identified (Sato *et al*., 2008). However, no major genes with resistance to brown spot have been identified to date (Sato *et al*., 2008). The use of resistant cultivars is the most economical and environmentally friendly method for the management of rice brown spot (Dela Paz *et al*., 2006; Savary *et al*., 2011).

In Asia the sources of resistance to brown spot were reported to be available within the rice germplasm and can be used for the development of disease-resistant lines (Yaqoob *et al.*, 2011). There are, however, varietal differences in

susceptibility to the disease (Datnoff & Lentini, 2003). These variations are due in part to the considerable level of diversity that has been reported to occur within the *Bipolaris oryzae* species, with significant differences in aggressiveness being recorded even among isolates of the same fingerprint type (Kamal & Mia, 2009). The resistance developed in most cultivars, lasts for only a few years due to breakdown of resistance in the face of high variability within pathogen populations. As a result, there is need to develop strategies for developing durable resistance so that plants will be protected over a longer period of time in a wide geographical area (Fengming & Robert, 2001).

The use of rice genotypes developed elsewhere may not be very effective in Uganda because of the variability of the brown spot pathogen that has been studied and reported by Kamal & Mia (2009) to occur within the *Bipolaris oryzae* species. To overcome this problem, locally available plant materials and pathogen isolates need to be used in breeding for resistance. The mode of inheritance of resistance within germplasm also needs to be understood. This study identified lines that are both adapted to Uganda and resistant to brown spot. The mode of gene action governing resistance, was also elucidated, thus facilitating the introgression of brown spot resistance genes into preferred rice genotypes.

1.5 Objectives

1.5.1 Overall objective

To contribute towards increased rice yields by providing information on potential sources of resistance to brown spot in genotypes adapted to Uganda.

1.5.2 Specific objectives

1. To determine the reaction of local and improved rice germplasm to brown spot disease.

2. To determine the relative importance of GCA & SCA for resistance to brown spot disease in crosses of resistant x susceptible genotypes.
3. To characterize the segregation pattern of reaction to brown spot in F_2 progeny of selected resistant and susceptible crosses.

1.6 Hypotheses

1. There are genotypes resistant to brown spot disease among local and improved germplasm in Uganda.
2. The general combining ability and specific combining ability between crosses are equally important in brown spot resistance.
3. The segregation pattern in F_2 progeny will indicate quantitative inheritance of brown spot resistance that is primarily additive in nature.

CHAPTER TWO: LITERATURE REVIEW

2.1 Botany, genetics and distribution of rice

Rice is an annual grass of the Gramineae family, and belongs to the genus *Oryza*, which contains approximately 22 species, of which 20 are wild and two are cultivated, *O. sativa* (Asian rice) and *O. 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). Vaughan *et al.*, (2003) have proposed a new nomenclature for cultivated and wild rice in Asia: *O. sativa* sensu lacto subspp. *indica* and *japonica*, and *O. rufipogon* sensu lacto subspp., respectively, with *nivara* referring to annual and *rufipogon* to perennial. In addition, two new wild species have been recognised in the genus *O. glumaepatula* and *O. malapuzhaensis* (Vaughan *et al*., 2003). The wild relatives in the genus *Oryza*, together with weedy rice and various rice varieties function as an exceptionally valuable gene pool that can be used to enlarge the genetic background of cultivated rice in breeding programs (Kush & Brar, 1998).

Oryza glaberrima (genome AA, 2*n* = 24) is a cultivated rice species endemic to Africa. *O. sativa* (genome AA, 2*n* = 24) is spread through large parts of the world and is more diverse than *O. glaberrima*. *Oryza sativa* is broadly divided into *indica* and *japonica* subspecies. The genus *Oryza* is believed to have originated in Gondwanaland, the ancient land mass from which India, Africa, South America and Australia drifted apart. The two cultivated species, namely *O. sativa* and *O. glaberrima*, are considered to have evolved later by independent and parallel evolutionary processes in the Asian and African continents. *O. glaberrima* originated around 1500 BC in swampy basins of the upper river delta of Niger in West Africa, which is the primary centre of origin of this species (Sarla & Mallikarjuna, 2005).

Oryza sativa is the most widely grown of the two cultivated species. It is grown worldwide, including Asian, North and South American, European Union, Middle Eastern and African countries. *O. glaberrima* however, is grown solely in West African countries. *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) has developed inter-specific varieties known as New Rice for Africa (NERICA), from crosses between *O. sativa* and *O. glaberrima* species. These varieties are widely released in Africa (WARDA/FAO/SAA, 2008).

2.2 Rice introduction, varieties grown and importance of the crop in Uganda

Rice was reported to have been introduced to Uganda by Indian traders as early as 1904 (Bigirwa *et al*., 2005) cited in (Odogola, 2006) but it did not gain popularity until the late 1940's. Since then, rice production has progressively increased in Uganda. After the 1940s, rice cultivation was increasingly taken up by few subsistence farmers who grew varieties such as Cakala, Matama, Kawemba, Kigaire and Seena which were introduced into Uganda from Tanzania (Odogola, 2006). During the 1950s, Uganda developed more interest in rice, apparently to feed its growing population that included returnees from the Second World War as well as institutions such as schools, prisons and hospitals. By 1966 to 1976 large scale production of irrigated swamp rice was initiated in Uganda through a partnership between the Uganda government and the Peoples Republic of China (Odogola, 2006). In 1981 - 83 average annual rice production in Uganda was 14,667 hectare, rising to a mean of 78,667 hectares in 2001-2003 and to 93,000 equivalents to 140,000 metric tons of milled rice (UBOS, 2004; FAOSTAT, 2012).

In 2002, superior upland and lowland NERICA (New Rice for Africa) varieties were released to farmers by the National Agricultural Research Organization (NARO) and included four upland rice varieties NARIC 1, NARIC 2 and New Rice of Africa (NERICA) 1 and 4 (Lamo *et al*., 2010). However, farmers also grow various landraces that include Sindano, Supa and Bungala which are under group of *Oryza sativa* (Lamo, 2010). By 2004 – 2012 the area under rice production had gradually increased from 121,000 to 212,000 hectares (FAOSTAT, 2012). Production in Uganda is growing fast and there is an accelerated shift in production from other cereals such as maize, millet, and sorghum to rice (Lamo *et al*., 2010).

2.3 Nature, symptoms and management of brown spot disease

The brown spot symptoms innitially appear as small circular oval spots on the first seedling leaves if the planted seeds are diseased (seed borne). The fungus can also survive on infected rice staw and stable. Soil and some weed hosts also have been reported as inoculum reservoirs (Biswas *et al*. 2008). It spreads from plant to plant in the field by spores (air-borne) (Sato *et al*., 2008). The fungus *Bipolaris oryzae* causes brown spot disease and is responsible for failure to germinate, rotting of seeds, roots and coleoptiles, and poor seedling vigour (Malavolta *et al*., 2002). The disease causes severe yield loss where rice is grown under stress conditions. The pathogen infects the coleoptiles and causes blighting, and turns the leaves oval in shape with dark brown to purplish-brown spots that rigorously damage photosynthetic activity ultimately killing the leaf (Figure 1) (IRRI, 1983).

Figure 1: Symptoms of brown spot disease (Source: Rice Knowledge Bank http://www.knowledgebank.irri.org)

Brown spot disease can be managed by improving soil fertility through regular monitoring of soil macro-nutrients and the application of required fertilizers. These fertilizers are unfortunately often costly and require many cropping seasons before becoming effective. The use of fungicides, such as iprodione, propiconazole, azoxystrobin, trifloxystrobin, and carbendazimis are effective in disease management (Mandal & Jha, 2008). Benzoic acid sprays have also been reported to significantly reduce the disease and increase grain yield and its components (Shabana *et al*., 2008).

Several studies have been made on genotypic variability in rice for resistance to the disease (Ohata & Kubo, 1974; Deren *et al.*, 1994). In these studies, rice varieties such as Tadukan and Tetep offered sufficient quantitative resistance to brown spot and were agriculturally useful (Ohata & Kubo, 1974). Screening of upland rice germplasm to Eastern India has revealed partial and complete resistance to the brown spot pathogen is expressed by several genotypes under field conditions (Shukla *et al*., 1995). The sources of resistance amongst *Oryza sativa* entries it seems to be few (Barnwal *et al*., 2013) and researchers have been exploring other pools, especially *O. nivara*.

2.4 Inheritance of resistance to brown spot disease

Host resistance has been deployed in brown spot management. Goel *et al*. (2006) analysed the inheritance of resistance to brown spot from crosses involving *Oryza nivara* germplasm and hypothesized that additive, dominant and epistatic gene interactions were involved. Sato *et al*. (2008) identified three quantitative trait loci (QTL) in the cultivar Tadukan (qBS2, qBS9, qBS11) on chromosomes 2, 9 and 11, respectively (qBS11 being considered as having a major effect. However, Katara *et al*. (2010) identified 10 QTLs, some of which may be common to the results noted by Sato *et al*., (2008).

Modes of inheritance of resistance have also been studied. Nagai & Hara (1930) reported resistance to be dominant. In contrast, Adair (1941) recorded it as recessive, involving several genes. Balal *et al.* (1979) found two dominant genes that were associated with resistance, while one gene was associated with susceptibility. Harahap (1979) suggested as few as two major genes and some minor modifying genes may control the resistance to infection. In contrast, Hau & Rush (1981) indicated the resistance mechanisms were operative during the pre-penetration period, but that these were not the major disease resistant mechanisms as observed the pathogen have extracellular sheath which adheres to wax crystals and might enable the fungus to attach to the leaf surface and facilitate infection.

2.5 Methods in evaluation of inheritance of resistance to brown spot disease

In a breeding programme, the genetic composition and nature of gene action for traits of interest within breeding stock needs to be understood in order for improvement to be realized. Genotypes are then crossed in order to create variability for a character to be improved (Fehir, 1987). During hybridization, various combinations of genetic material should be expected in order to provide opportunity for genes of each parent to recombine with those of every other

parent. One of the techniques which are widely used to obtain information about the genetic systems governing the inheritance of attributes to be improved and assessing the potential of different crosses and predict their performance in subsequent generation is through use of diallel analysis.

2.5.1 Diallel mating

A diallel cross is a set of all possible mating combinations between genotypes (Hayman, 1958). According to Griffing (1956) a diallel cross may be a full diallel, in which all parents are crossed to make hybrids in all possible combinations, or a half diallel (without reciprocals). The diallel crossing method is best used when parental values of their response towards traits of interest are not well known, and if the breeder is interested in all the crosses. Diallel crosses are traditionally employed to evaluate the general and specific combining abilities of parents (Griffing, 1956). The information on combining ability and type of gene action responsible for expression of different traits helps in defining the potential contribution of the parents and in estimating the proportion of genetic components controlling the resistance (additive, non-additive and maternal effects).

2.5.2 Estimates of reciprocal effects

Maternal effects and sex-linkage give rise to differences between reciprocal crosses. In diallel cross analyses, the presence of these effects will cause biases in estimates of the genetic component of the variation (Crusio, 1987). The variation in an individual's phenotype may be determined not only by the genotype and environment but also by the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Weiner *et al.*, 1997). Unfavorable interactions between the nuclear and cytoplasmic factors in the zygotic cell may prevent normal embryo development. Therefore, making reciprocal crosses between a numbers of genotypes of each species increases the opportunity for the favorable nuclear-cytoplasmic interaction

required for hybrid seed formation (Fehr, 1987). Reciprocal crosses provide a means of overcoming undesirable interactions among the embryo, endosperm and maternal tissue since it provides the best opportunity to obtain a favorable relationship between the tissues of developing seed (Fehr, 1987).

2.5.3 Estimates of combining ability

Combining ability is defined as the ability of the parents to combine among each other in the hybridization process so that favorable genes or characters are transmitted to their progenies (Fehr, 1987). The terms general and specific combining ability were originally defined by Sprague & Tatum, (1942). General combining ability is used to designate the average performance of a line in hybrid combination while specific combining ability is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved (Kronstad & Foote, 1964). Panhwar *et al*. (2008) defined general combining ability as the average performance of a line in a series of crosses, whereas specific combining ability is defined as the deviation in performance of the hybrid from the expected productivity, based upon the average performance of the lines involved in hybrid combination (Panhwar *et al*., 2008). The combining ability turned out to be more important as testing progressed and better lines were selected (Kronstad & Foote, 1964).

2.5.4 Heritability

The effectiveness of selection for a trait depends on the relative importance of genetic and non-genetic factors in the expression of phenotypic differences among genotypes in a population, a concept referred to as “heritability”. The heritability of a character to be adopted has a major effect on the method to be selected for population improvement, inbreeding and other aspects of selection. For a

character of high heritability, single plant selection may be effective and relatively ineffective for one with low heritability (Fehr, 1987).

Heritability can be defined as the ratio of genotypic variance (σ^2g) to the phenotypic variance (σ^2_{ph}). Genotypic variance is a result of variation caused by genetic differences among individuals. It is sum of additive, dominance and epistatic variances (Fehr, 1987). The additive effects is the portion of genotypic value that determines the mean performance of the progeny while dominance is the difference between genotypic value and the breeding value (additive effects) of an individual, and epistatic variance is the genotypic value of an individual for a quantitative character that can be influenced by the interaction of alleles and genotypes at different loci (Falconer, 1981). Heritability can be expressed in either broad or narrow sense. Broad sense heritability (H) is a ratio of genotypic variance to the phenotypic variance, $\sigma^2 g/ \sigma^2_{ph} = (\sigma^2_A + \sigma^2_D + \sigma^2_I)/ \sigma^2_{ph.}$ Narrow-sense heritability (h^2) is the ratio of the additive genetic variance to the phenotypic variance, $\sigma^2_{A} / \sigma^2_{ph}$ (Fehr, 1987). Poehlman and Sleper (1995) define narrow sense heritability as the proportion of resistance that is controlled by additive gene effects. It gives a more accurate estimate of the gain from selection. This knowledge would help in planning appropriate breeding strategies.

In Uganda, the mode of inheritance has not been studied since the disease was first reported in 2003 (Biruma *et al.*, 2003). This is the first study that aims to identify new sources of resistance to brown spot disease relevant to Ugandan growing conditions, and to clarify the mode of inheritance to the disease.

CHAPTER THREE: REACTION OF LOCAL AND IMPROVED RICE GERMPLASM TO BROWN SPOT DISEASE

3.1 Introduction

Rice production in Uganda is increasing rapidly in areas suitable for production. Despite the increment in area under cultivation, however Uganda does not produce sufficient amounts for domestic consumption. For example in the period 1990-2010 local production was at 42-58 percent and imports 58-42 percent of the national rice consumption (PMA, 2009). These levels translate into imports of 77,600 and 33,000 tonnes of rice that was imported in the years 2000 and 201l, respectively. The low productivity is attributed to a number of factors of which pests and diseases are the most serious production constraints for both upland and lowland rice in Uganda (Musiime *et al*., 2005). *Bipolaris oryzae*, among other seed-borne disease is reported as the most prevalent pathogen with an incidence range of 3-94% (Biruma *et al*., 2003). In order to overcome low productivity, the National Crops Resources Research Institute (NaCRRI) in Uganda acquired improved rice lines from Centro Internacional de Agricultura Tropical (CIAT), Madagascar, AfricaRice and Tanzania in order to widen the genetic pool of germplasm in the country. However, the reactions of these materials against biotic and abiotic constraints had to be understood before introducing them into breeding programs.

Savary *et al.* (2000) conducted field surveys and reported that among the many diseases occurring in rice fields in Asia, sheath blight and brown spot accounted for the highest yield losses. In similar studies, the degree of yield loss was, however, reportedly dependent on the host plant resistance, pathogenic variability and environmental conditions. Aerobic rice also suffered from 27.5% more reduction in yield than flooded rice, due to the high incidence of disease (Patel *et al*., 2010). The severity of brown spot disease can be managed through

development of resistant lines (Mew, 1991; Patel *et al*., 2010). Economic analyses suggest that modern varieties with disease resistance contribute 7 to 10% yield gain in rice production (Evenson, 1998). Therefore, in order to identify rice germplasm resistant to brown spot, materials must be screened under low water conditions since the disease is more severe under aerobic conditions (Yaqoob *et al*., 2011). In the present study, 100 rice genotypes were evaluated against brown spot disease under rain-fed conditions with the purpose of identifying parents for future breeding programmes for resistance to the disease.

3.2 Study area

The field trials were conducted at the National Crops Resources Research Institute (NaCRRI) – Namulonge in Uganda, located at 0^0 32" N of the Equator and 32^0 37" E. The institute lies in an area that receives a bimodal cycle of rainfall typical of the central region of Uganda. It is 27 km north of Kampala, at an elevation of 1150 meters above sea level with an average rainfall of 1200 mm per year.

3.3 Materials and methods

3.3.1 Germplasm used and experimental design

One hundred germplasm accessions comprising of interspecific (NERICA lines) and intraspecific lines from CIAT, Madagascar, Africa Rice, Tanzania and locally bred lines. There was little information on reaction to brown spot and had to be evaluated for such resistance (Appendix 1**)**. These accessions were sown in the field nursery by dribbling at 5 x 20 cm spacing (three seeds per hill) in 5-row plots measuring 1 m in length. Each entry was replicated twice in an alpha lattice experimental design.

3.3.2 Inoculum used

The fungus can spread from plant to plant and, in the field, by air borne spores. To ensure maximum disease pressure, inoculum was prepared and the plants artificially infected. In this procedure, leaves and panicles with symptoms of brown spot were sampled from different locations in an infected rice field, approximately 35 m apart, at National Crops Resources Research Institute (NaCRRI) – Namulonge in Uganda. These disease samples were transferred to the laboratory, where by the 100 g of infected samples collected were crushed in 10 ml of double distilled water, using sterile mortars and pestles until 80% of the leaves and panicles were crushed. The inoculum was applied at two weeks after planting onto the rice leaves using the finger-rubbing technique. Inoculation was repeated two days later to ensure the inoculation was successfully done (Mogga *et al*., 2012).

3.3.3 Data collection and analyses

Disease severity was scored at intervals of 15, 30, 45 and 60 days after inoculation (Campbell & Madden, 1990) following the standard evaluation system (SES) for rice (IRRI, 2002), as given in Table 1. The severity rating scale ranged from 1 (highly resistant) to 9 (highly susceptible). Symptoms scored were typical of brown spots and included leaf spots that were small, oval or circular and dark brown. Larger lesions usually had the same color on the edges, with a pale grayish center. Most spots had a light yellow halo around the outer edge (IRRI, 1983).

Table 1: The standard procedure adopted for recording brown spot disease severity in rice

Scale	Affected Leaf Area	Host response
1	No incidence	Highly resistant
2	Less than 1%	Highly resistant
3	1-3%	Resistant
4	4-5%	Resistant
5	11-15%	Moderately resistant
6	16-25%	Moderately susceptible
7	26-50%	Susceptible
8	51-75%	Susceptible
9	76-100%	Highly susceptible

Source: IRRI, 2002

The statistical linear model (Little & Hills, 1977) was used to analyse the test entries that were evaluated for resistance to brown spot in the screened germplasm and is outlined below:

$$Y_{ijk} = Y...+ R_j + G_i + B/R_{jk}+ e_{ijk}$$

Where;

$Y\ldots$= overall mean,

R_j = replication effect of the j^{th} genotype,

G_i = effect of the i^{th} genotype,

B/R_{jk} = block/reps effect of the jk^{th}, and

e_{ijk} = the environmental effect of the ijk^{th} observation.

Data collected on the disease score was subjected to analysis of variance (ANOVA) using Genstat software, 14^{th} edition (GenStat, 2012) in order to obtain the mean squares and differences in the mean for disease severity. The relative Area Under Disease Progress Curve (rAUDPC) was calculated in order to evaluate the germplasm reaction to the disease using method of (Shaner &

Finney, 1977). The mean severity scores across eight weeks and rAUDPC were used to evaluate the reaction of the materials to brown spot disease.

The formula for computing rAUDPC is:

$$rAUDPC = \frac{\sum(T_{i-1} - T_i)*(D_{i+1} + D_i)/2}{T_{Total}*100}$$

Where:

T_i: is the i^{th} day after emergence when the estimation of disease was made

D_i: is the estimate of the percentage of leaf area covered with lesions at T_i and T_{Total} are the number of days after emergence at which the final disease assessment was recorded.

3.4 Results

3.4.1 Response of rice genotypes to brown spot disease

Disease symptoms observed on rice genotypes were typical of brown spot disease, as observed in the field (Fig. 2). The responses of the rice genotypes to brown spot disease in two replications were significantly different for 30 and 45 days screening period (Table 2, Appendix 3). The blocking effect had significant effect in the variation of disease response for the 15 and 60 days (at $P \leq 0.1$, 0.05 respectively). There was high variation in response of germplasm to disease from 30 days of sreening onwards (at $P \leq 0.001$) from highly resistant to susceptible (Table 2, Appendix 3) indicating a range of host resistance available from the tested germplasm.

Figure 2: Symptoms of brown spot observed on susceptible rice lines at Namulonge

Table 2: Summary ANOVA table for brown spot severity on rice at 15, 30, 45 and 60 days of disease scoring

Source of variation	d.f	Var. 15 days	Var. 30 days	Var. 45 days	Var. 60 days
Total	199				
Rep	1	0.25^{ns}	1.13^{+}	2.0^{+}	0.01^{ns}
Rep. Block	8	0.160^{+}	-	-	2.12^{*}
Entries	99	0.09^{ns}	1.97^{***}	1.55^{***}	1.537^{***}
RCB error	99	-	0.458	0.636	-
LEE	72	0.09^{ns}	-	-	0.915

Var = Variance; d.f = degree of freedom; + = significant at $\alpha = 0.1$; * = statistically significant at $\alpha = 0.05$; *** = very highly significant at $\alpha = 0.001$; ^{ns} = statistically not significant.

3.4.2 Resistance rating of rice germplasm

Of the 100 accessions screened, 18 lines scored 2 (indicating less than 1% disease symptom), and are as such considered highly resistant. Fifty two (52) lines scored 3, indicating resistance against the disease. The results further revealed 27 lines to be moderately resistant (Figure 3) and four lines (including the check) were susceptible against the brown spot disease (Appendix 2). Most of the materials from Africa Rice were resistant to brown spot disease and few of them were moderately resistant to susceptible. Uganda land races as well as materials from Madagascar and few from CIAT were moderately resistant. Materials from Tanzania, Pakistan and NaCRRI – Namulonge were susceptible.

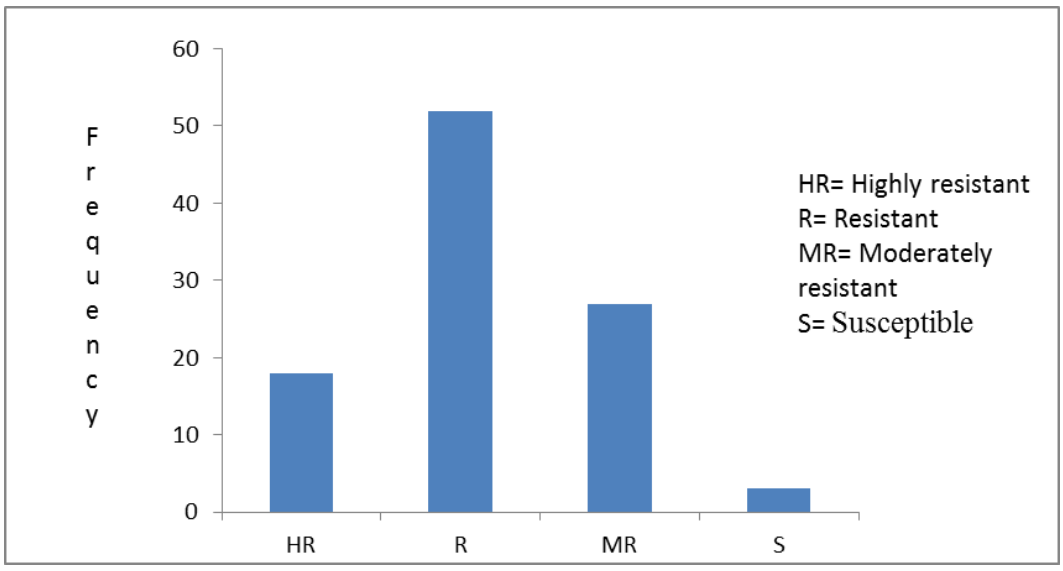


Figure 3: Summary for 100 screened germplasm for resistance to brown spot

3.4.3 Response of selected parental lines

The parental lines selected for crossing were nine which comprised of highly resistant to susceptible lines. The selections of parental lines were based on the level of brown spot resistance observed during screening. In genotypes tested, some are already released varieties adapted to Ugandan growing condition and

have been grown for some years including NERICA 1 which has aroma but susceptible to the disease, NERICA 4 and 10 have good yield potential and they have shown to be resistant to the disease furthermore NERICA's are nutrients responsive and early maturing varieties. K5 and P4R1 are moderately resistant and suseptible respectively but they locally adapted materials. There was high variation in response of germplasm to disease from 30 days of sreening onwards (at $P \leq 0.001$) from highly resistant to susceptible (Table 2, 3 & 4, Appendix 3) indicating a range of host resistance available from the tested germplasm. The means score for the susceptible genotypes were higher as compared to resistant genotypes which had lower mean scores (Figure 4).

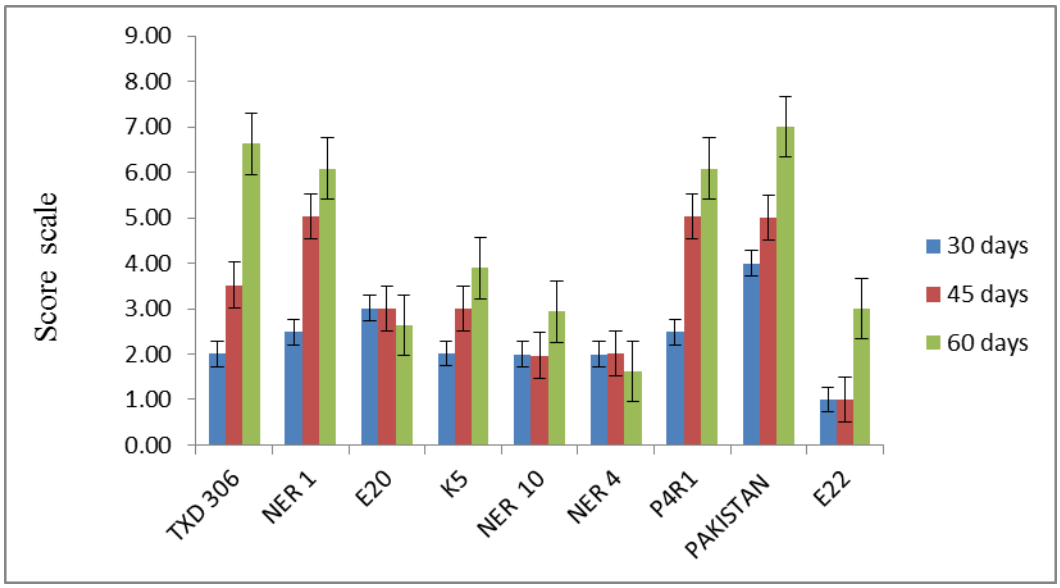


Figure 4: Means for resistance to brown spot in selected parental materials

The data on disease scores was collected sequentially throughout the growing season. The rAUPDC means are calculated and plotted on the bar graph to compare the response of selected accessions to brown spot disease. The selection of parental lines was based on the fact that some are already released varieties grown by farmers and have desirable characteristics although they lack some attributes that need to be improved. These materials also displayed different levels

of disease severity as shown by rAUDPC (Figure 5) with the susceptible ones having higher rAUPDC mean.

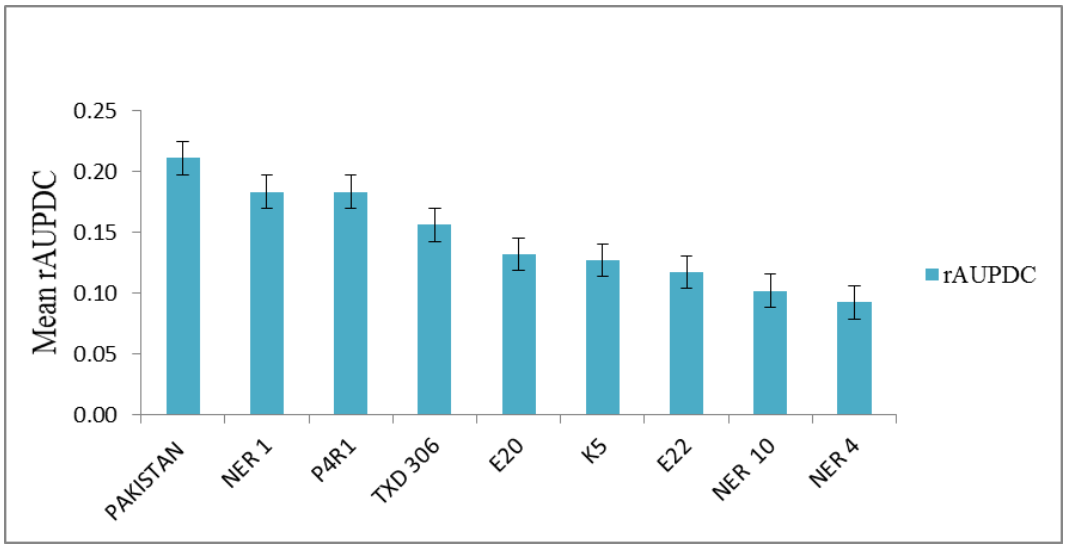


Figure 5: rAUDPC means for resistance to brown spot in selected parental materials

3.5 Discussion and conclusions

Disease screening using lines from International Rice Research Institute (IRRI), reported by various researchers has indicated that rice genotypes differ markedly in their resistance to brown spot disease, from highly resistant to highly susceptible (Tariq *et al*., 2012; Yaqoob *et al*., 2011). Mosharraf *et al*. (2004) reported that reactions of different genotypes to brown spot ranged from resistant to moderately susceptible, while Hossain & Kulkarni (2001) and Castano *et al*. (1990), reported variability for response to various diseases, and categorized the germplasm into groups for highly susceptible to highly resistant responses to various rice diseases. The materials used in this study reacted in a similar manner. At 60 days post-inoculation, most germplasm had attained maximum disease scores and they displayed different leves of incidence and severity that was highly resistant, resistant, moderately resistant and susceptible genotypes.

The results were based on the visual observations of the diseased rice plants and suggested a wide variation in the germplasm against brown spot adapted to Ugandan growing conditions and could be very useful in the breeding of resistant varieties. On the basis of these findings, it could be suggested that, sources of resistance identified from resistant rice germplasm might be exploited in breeding programs for the development of disease resistant varieties after determining their genetics. If these tested accessions had the required trait, then these could be directly utilized as sources of resistant genes in breeding programs. For inheritance study, nine parents out of the 100 screened lines were selected.

CHAPTER FOUR: INHERITANCE OF RESISTANCE TO BROWN SPOT DISEASE

4.1 Introduction

Host plant resistance to disease is an effective and economical way to manage brown spot disease (Savary *et al.*, 2011). The search for sources of resistance to brown spot has been a long-standing effort. Nagai & Hara (1930) reported resistance to be dominant. In contrast, Adair (1941) recorded it as recessive and involving several genes. Sato *et al.* (2008) identified partial resistance in some varieties and three quantitative trait loci (QTL) for disease resistance.

The population screened for resistance to brown spot disease has revealed most materials from Africa Rice have resistance to disease; however there are farmer's preferred varieties that have been in use with attributes like aroma and high yielding e.g. NERICA 1 and K5 but are susceptible to disease also were screened. Uganda rice breeding program has materials to be released but are susceptible to brown spot disease. With the importance of disease in Uganda (Biruma *et al*., 2003; Kawube *et al*., 2005; Adur *et al*., 2011), there is a need to transfer the genes of resistance to these materials which could be of high value to farmers. The need to know how these genes could be transferred it is of paramount important. In additional to the previous studies where findings were based on different parental lines, this study aimed at ascertaining heritability of resistance to brown spot in generations of selected crosses to Uganda growing conditions.

4.2 Materials and methods

4.2.1 Genetic materials used

There were nine parents used in this study that were selected from screening of one hundred germplasm accessions. The materials also were from different origin like Africa Rice (Benin), NaCRRI- Namulonge (Uganda), Pakistan, Tanzania and local Ugandan grown variety. The selection was based on the level of brown spot resistance observed during screening, as shown in Table 3. Most of these materials have been grown except the new lines E22, E20, TXD 306 and Pakistan which are still under trials at National Crop Resource Research Institute (NaCRRI) – Namulonge.

Table 3: Parents and their origin used in full diallel crosses for brown spot resistance.

No.	Entry code	Origin	Resistance designation
1	NERICA 4	Africa Rice	Highly resistant
2	NERICA 10	Africa Rice	Resistant
3	E 20	Africa Rice	Resistant
4	E 22	Africa Rice	Resistant
5	K5	Local - Uganda	Moderately resistant
6	P4R1	NaCRRI- Namulonge	Susceptible
7	NERICA 1	Africa Rice	Susceptible
8	TXD 306	Tanzania	Susceptible
9	PAKISTAN (UP)	Pakistan (Jica)	Susceptible

4.2.2 Population development

The nine selected parents were crossed in a full diallel mating design in the screen house at the National Crop Resource Research Institute (NaCRRI) – Namulonge in Uganda. The Diallel model 1, method 1 of Griffing (1956) was used to determine the effects of general (GCA) and specific (SCA) combining abilities for different parents and crosses. This method was expected to provide unbiased estimates of population parameters (Griffing, 1956; Dabholkar, 1992; Singh & Chaudhary, 2004). A fixed model was used due to the fact that the parents were selected based on the level of resistance portrayed during screening.

The parents were raised in 10 litre buckets in six phases (3 sets), planted at one to two-week intervals in order to synchronise their flowering. After 14 days from sowing, seedlings were transplanted (3 seedlings/pot) in 4 replicates, and were supplied with 2g/pot of Diamonnium phosphate fertilizer when transplanted and Urea (46% N) two weeks after transplanting. Before crossing, female parents were emasculated using a vacuum emasculator (Fig. 6) as previously described (Coffman & Herrera, 1980; Lamo, 2010) and covered to avoid cross pollination. Emasculated flowers were hand pollinated to generate F_1's, including reciprocals. The pollinating panicles were collected from male plants for crossing. After crossing they were covered with paper bags to avoid pollination from unwanted parents for at least two weeks. During crossing one can expect to get from few to several seeds per plant depending on its crossability as shown in Figure 7. The crossability of a given parental group and grain sterility traits in a given crossing environment would be important for successful crosses to be achieved (Lamo, 2010).

Emasculation process

Vacuum emasculator

Figure 6: Emasculation of female rice parents using a vacuum emasculator

Figure 7: Successful rice parental crosses

4.3 Generation of F_2 population

Seeds from F_1's were harvested when the panicle had lost its green color, usually about 25 days after pollination (Navarro & Virman, 1987). The seeds from each panicle were kept in separate envelopes and labeled with the cross codes for easy identification. Before planting, F_1 seeds were surface sterilized using a solution

containing 70% ethanol and 1% sodium hypochlorite and then rinsed with sterilized and distilled water (Oyebanji *et al*., 2009). Sterilized seeds were pre-germinated in petri dishes containing moist tissue paper and incubated at 30^{o}C to accelerate germination (Fig. 8a). After seed germination they were transferred to cups (40 cc) filled with sterilized soil and reared for one week before transplanting them in pots (Fig. 8b). After two weeks, the seedlings were transplanted to 10 litre buckets filled with sterilized soil in the screen house (Fig. 8c).

a: Pre-germination of F_1's seeds

b: Seedlings in cups

c: Transplanted F_1's in buckets

d: F_1's planted along with parents

Figure 8: Summary procedure for raising F_2 populations in the screen house for study of their reaction to brown spot disease

To ensure the harvested plants was a real cross between the two parents, the F_1 plants were planted adjacent to their parents (Fig. 8d) and inspected for self-fertilization before and after flowering. Plants that were not true crosses were

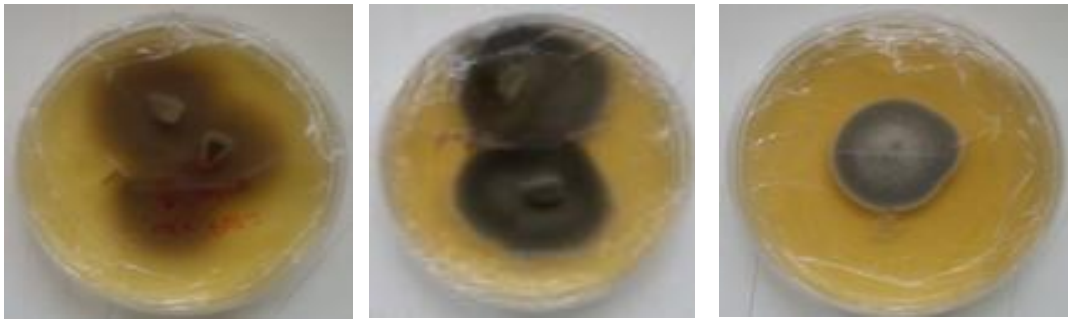
identified by expression of a simply inherited morphological character of the parents. These characters included presence of awns, culm color, plant height and stigma color that are known to be inherited through dominant genes. Mature seeds were harvested and sun-dried in their paper bags for two weeks.

4.4 Diallel F_2's for evaluation of brown spot

Heritability of resistance to brown spot disease was estimated in the F_2 generation of selected crosses and their parents. The F_2 plants and their parents were planted in the field at NaCRRI- Namulonge using an alpha- lattice design with two replications at spacing of 5 x 10 cm (one plant per hill). The plants were supplied with 25 kg N ha^{-1} at two weeks after transplanting. Standard cultural practices like watering and hand weeding were carried out regularly.

4.5 Isolation, identification and inoculation of pathogen

Infected leaves and panicles with symptoms of brown spot were sampled from different location in the rice fields at NaCRRI-Namulonge. Leaves and panicles were transferred to the laboratory and gradually isolated the causal agents from disease samples following the method of Xia *et al*. (1993). Samples were trimmed, washed by sterile distilled water and placed on potato dextrose agar in petri dishes at 25–30°C for 2-3 days. Petri dishes were then incubated at 20- 26°C in the dark or artificial light supplied by fluorescent light on a 12 h light/dark photoperiod for 10-20 days (Sivanesan, 1987). To avoid bacterial contamination antibiotic ampicilin at 50mg/L was incorporated in the media (Motlagh *et al*., 2006).



***Colony of isolate* 1** ***Colony of isolate* 2** ***Colony of isolate* 3**

Figure 9: Pure isolates maintained on PDA amended with ampicilin at 50mg/L at 24°C in the dark

Three isolates were obtained from infected rice samples. The characteristics exhibited by Isolate 1 included colonies that were effuse, dark blackish brown and velvety (Fig. 9) as that of *Bipolaris bicolar* (Motlagh et al., 2006). Aerial mycelium was fluffy, pale brown to dark brown. The characteristics exhibited by Isolate 2 included conidial colonies that grew, spread, and were grey to dark grey (Fig. 9). Aerial mycelium was fluffy, cottony and pale to mid yellowish. These features resembled *Bipolaris victoriae* as reported by Motlagh *et al*. (2006). The characteristic exhibited by Isolate 3, were grey to dark grey conidial colonies grew and spread rapidly. Aerial mycelium was fluffy and cottony (Fig. 9). Conidiophores were single or in small groups, straight to flexuous, pale towards the apex and septate. Conidia were usually curved, fusoid or obclavate, occasionally almost cylindrical, pale to mid golden brown and smooth (Figure 10). The typical characteristics such as shape and color of colony, morphology of conidium and conidiophores were similar to *B. oryzae* (formerly known as *Helminthosporium oryzae)* (Figure 11) (Ellis, 1971; Sivanesan, 1987; Motlagh *et al*., 2006) was the reason for its selection to be used to inoculate the plants.

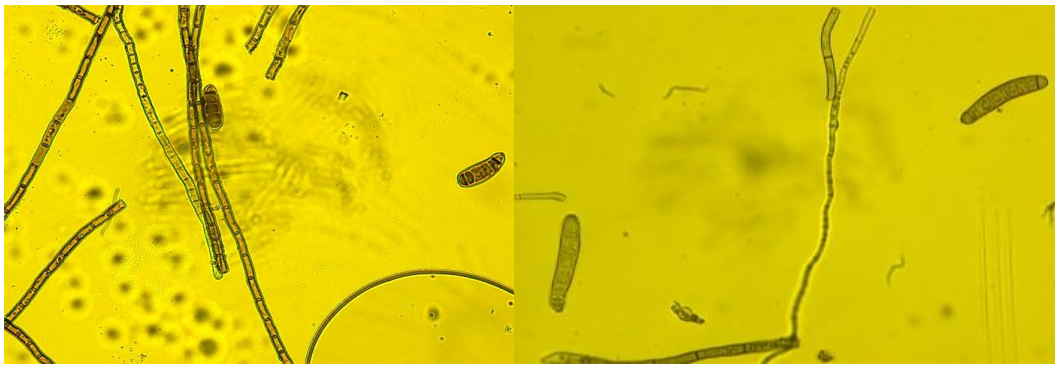


Figure 10: Conidiophore with mycelium of isolate 3 (x400)

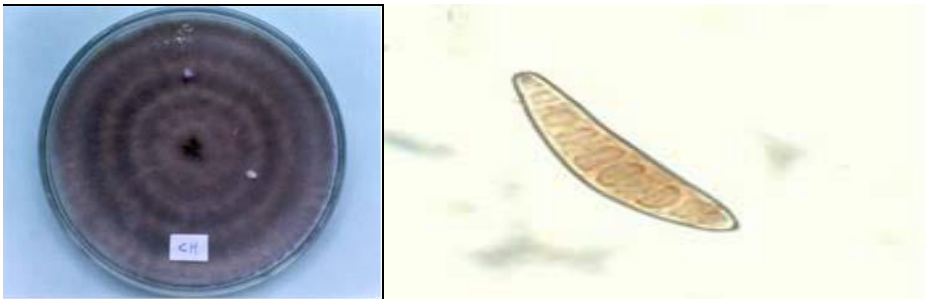


Figure 11: Colony and Conidium (×460) of *Bipolaris oryzae*. Source (Motlagh *et al*., 2006

The F_2 plants and their parents were inoculated mechanically with Isolate 3 which had typical characteristics of *Bipolaris oryzae* for evaluation two weeks after transplanting by conidia suspension (1 x 10^5 conidia mL^{-1}) spraying (Sato *et al*., 2008). To increase surface absorption, 1% Tween-20 was incorporated into conidia suspension (Motlagh *et al*., 2006).

4.6 Data collection and analysis

Disease severity data were scored at full panicle stage following the Standard evaluation system (SES) for rice (Table 1) (IRRI, 2002). The rating scale varies from 1 (highly resistant) to 9 (highly susceptible). Other data recorded were plant height, number of tillers, number of productive tillers, days to heading, days to flowering and grain weight.

The F_2 plants and their parents were evaluated to determine whether the resistance is controlled by major or minor genes using quantitative statistical techniques such as heritability, general and specific combining ability. The combining ability and reciprocals effects were estimated through diallel analysis adopted from Singh & Chaudhary (2004), following Griffing (1956) method 1 model 1, as described in Table 4.

Table 4: Skeleton analysis of variance for combining ability using Griffing's method 1 model 1

Source	Df	Ms	F-calc	Expected Ms	Var. components
Crosses	80				
GCA	8	Mg	Mg/Me	$\sigma^2e + 2p / (p-1) [\sum g_i^2]$	$\sigma^2g = (Mg – Me)/2p$
SCA	36	Ms	Ms/Me	$\sigma^2e + 2p / (p-1) [\sum\sum s_{ij}^2]$	$\sigma^2s = (Ms – Me)/2$
Recip	36	Mr	Mr/Me	$\sigma^2e + 2p / (p-1) [\sum\sum r_{ij}^2]$	$\sigma^2r = (Mr – Me)/2$
Error	160	Me		σ^2e	$\sigma^2e = Me$

Baker's ratio; $X = 2\sigma^2g / (2\sigma^2g + \sigma^2s)$

NS- CGD = $2\sigma^2g / (2\sigma^2g + \sigma^2s + \sigma^2e) \approx h$

BS- CGD = $(2\sigma^2g + \sigma^2s) / (2\sigma^2g + \sigma^2s + \sigma^2e) \approx H$

P = parents, Mg, Ms, Mr and Me = mean squares for GCA, SCA and reciprocals, respectively. g_i, s_{ij}, and r_{ij} = effects of GCA, SCA and reciprocals, respectively. NS- CGD = narrow-sense coefficient of genetic determination, BS-CGD = broad-sense coefficient of genetic determination.

The statistical linear model for this analysis was:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + rij + e_{ijk}$$

Where, μ = overall mean, gi = GCA effect of the ith parent, gj = GCA effect of the jth parent, sij = SCA effect of the ijth genotype, rij = reciprocal effect of the ij^{th} genotype, and e_{ijk} = the environmental effect of the ijk^{th} observation.

The ratio of GCA variance to SCA variance was also estimated according to Baker, (1978) as;

$X = 2\sigma^2 g / (2\sigma^2 g + \sigma^2 s)$. This ratio suggests the relative significance of additive versus no-additive effects (Baker, 1978).

Where,

$\sigma^2 g$ = GCA varience,

$\sigma^2 s$ = SCA varience.

The standard errors (S.E) of estimated GCA and SCA and reciprocal effects were calculated using the formula provided by Dabholkar, (1992) as modified by Gibson (2012, personal communication) for missing crosses:

$$\text{S.E. } gi = \text{sqrt } ((p/ni)*(p - 1/(r*2p^2))\ \sigma^2 e;$$

$$\text{S.E. } s_{ij} = \text{sqrt } ((p^2 - 2p + 2 / (r*2p^2)))\ \sigma^2 e$$

$$\text{S.E.} r_{ij} = \text{sqrt } 1/2\ \sigma^2 e$$

Where; S.E.gi is the standard error for GCA effect of the i^{th} parent, S.E.s_{ij} is the standard error for SCA effect of the ij^{th} genotype, S.E.r_{ij} is the standard error reciprocal effect of the ij^{th} genotype, r is the proportion of reciprocals that are present and $\sigma^2 e$ is the error mean square.

4.7 Results

The present diallel study comprised of forty (40) crosses, twenty eight (28) parental combinations and twelve (12) reciprocal crosses. Although some crosses were missing, each parent was included in enough combinations to provide reliable estimates of general and specific combining abilities effects.

4.7.1 Response of F_2 populations to brown spot disease

The crosses TXD 306 x NER 1 (mean = 5.7); NER 4 x NER 10 (mean = 3.0); TXD 306 x NER 4 (mean=3.7); NER 1 x NER 4 (mean = 3.7); P4R1 x PAKISTAN (mean = 5.7) and P4R1 x NER 4 (mean = 3.0) had mean disease severity scores lower than their respective parents, indicating greater resistance. The rest of crosses had mean score higher than their respective parents (Table 5). Furthermore, several of the reciprocal crosses were numerically different in resistance to brown spot as shown underlined in Table 5. In general, the average mean of crosses (mean = 5.0) was equal to the average parental mean (mean = 5.1).

Table 5: Mean severity scores for F_2 populations in a 9 x 9 diallel

				Male					
Parents	K5	PAK	TXD306	E20	E22	N1	N4	N10	P4R1
Female									
K5	**5.7**		5.7						
PAK		**7**			5	7			6.3
TXD306			**7**	5		5.7	3.7		
E20	6.3	3.7		**3**		7	3.7		6.3
E22	5.7	5	5	3	**3**	5	5	5	
N1	5	5	6.3	5		**5.7**	3.7		
N4	5	7	4.3	5		4.3	**4.3**	3	4.3
N10	5.7			5	3	4.3	5	**3.7**	
P4R1		5.7		5.7		6.3	3		**7**
SEM =	0.88								
LSD =	1.26								
CV =	13%								
Average mean parents = 5.1									
Average mean crosses = 5.0									

PAKS = Pakistan upland; N = Nerica; the scores are based on 1 – 9 scale: (1-2) = highly resistant; (3 - 4) = resistant; (5 - 6) = moderately resistant; (7 - 8) = susceptible; (9) = highly susceptible; F_2 crosses (above diagonals) and their reciprocals (below diagonals).

4.7.2 Analysis of varience in F_2 progenies for resistance to brown spot

The analyses of variances showed highly significant differences among crosses ($P \leq 0.001$; Table 6). The differences among genotypes progenies were contributed by both general combining ability (GCA) and specific combining ability (SCA). The GCA and SCA mean squares were highly significant ($P \leq 0.001$). The reciprocal effects were significant at $P \leq 0.05$ (Table 6). The Baker's ratio was

moderate, with a value of 0.29. The broad sense coefficient of genetic determination was high (0.83) unlike the narrow sense coefficient of genetic determination (0.24) (Table 6).

Table 6: Analysis of variance for combining ability using Griffing's method 1 model 1 for brown spot disease scores in F_2 populations and their parents

Source	df	MS	Fcalc	VarComp	ExpMeanSq
Crosses	39	0.94^{***}	4.94		
GCA	8	1.37^{***}	7.21	0.137	σ^2 e' + 8.61*σ^2GCA
SCA	19	1.11^{***}	5.84	0.664	σ^2 e' + 1.38*σ^2SCA
Recipr	12	0.39^{*}	2.05	0.294	σ^2 e' + 2σ^2Recip
Error	39	0.19			σ^2e'
Baker's Ratio = $(2\sigma^2 \text{ gca})/ ((2\sigma^2 \text{ gca}) + (\sigma^2 \text{ sca}))$					= 0.29
NS-CGD = $(2\sigma^2 \text{ gca})/ (2\sigma^2 \text{ gca} + \sigma^2 \text{ sca} + \sigma^2 e) \approx h^2$					= 0.24
BS-CGD = $(2\sigma^2 \text{ gca} + \sigma^2 \text{ sca})/ (2\sigma^2 \text{ gca} + \sigma^2 \text{ sca} + \sigma^2 e) \approx H$					= 0.83

*, *** = statistically significant at $\alpha = 0.05, 0.001$ respectively; the calculation for coefficient of genetic determination are based on entry means.

4.7.3 Estimates of general combining ability effect

The moderately resistant parent K5, PAKISTAN and P4R1 had significant positive GCA effect, indicating it contributes to susceptibility (Table 7). Lines E22, NER 4 and NER 10 had significant negative GCA effects ($P \leq 0.01, 0.001, 0.001$ respectively) indicating they contributed towards resistance. Line E20 had negatively non-significant GCA effects. Line TXD 306 had non-significant positive GCA effect (Table 7).

4.7.4 Estimates of specific combining ability effects

The estimates of specific combining ability effects are presented in Table 8. The crosses K5 x NER 1; TXD 306 x NER 4; NER 4 x P4R1; NER 1 x NER 4; PAKISTAN x E20 and NER 1 x NER 10 had significant negative SCA effects ($P \leq 0.05, 0.01, 0.01, 0.001$ respectively). Line TXD306 x NER 1 had a significant positive SCA effect ($P \leq 0.05$), indicating the crosses were resistant to brown spot disease.

Table 7: Estimation of general combining ability effects for brown spot resistance for parents

Parents	Parental mean	GCA effects	S.Egca
K5	5.7	0.53^{***}	0.066
PAKISTAN	7.0	0.35^{***}	0.044
TXD306	7.0	0.16^{ns}	0.056
E20	3.0	-0.09^{ns}	0.036
E22	3.0	-0.23^{**}	0.044
NER 1	5.7	0.38^{***}	0.033
NER 4	4.3	-0.63^{***}	0.030
NER 10	3.7	-0.42^{***}	0.056
P4R1	7.0	0.31^{**}	0.056

, * = highly significant at $\alpha = 0.01, 0.001$ respectively; ^{ns} = not significant at $\alpha = 0.05$

Table 8: Estimation of specific combining ability effects for brown spot resistance in F_2 population

Male

Parents	K5	PAKS	306	E20	E22	NER 1	NER 4	NER10	P4R1
Female									
K5			-0.19^{ns}	0.73*	- 0.13^{ns}	- 0.74*	0.27^{ns}		
PAKS				- 0.76***	- 0.12^{ns}	- 0.06^{ns}	2.11^{ns}		
306				- 0.23^{ns}	0.23^{ns}	0.63*	- 0.03**		
E20						0.38^{ns}	0.22^{ns}	0.68*	0.45^{ns}
E22						0.01^{ns}	1.35***	0.15^{ns}	
NER 1							-0.59***	- 1.13***	1.14***
NER 4								0.05^{ns}	- 1.02**
NER10									
P4R1									

*, **, *** Significant at $\alpha = 0.05, 0.01, 0.001$ respectively; ns = not significant at $\alpha = 0.05$; PAKS = Pakistan upland; TXD 306; NER = Nerica

4.7.5 Reciprocal effects

The reciprocal effects are presented in Table 9. In F_2 generation, the cross NER 10 x E22 showed significant negative reciprocal effects at $p<0.05$ (Table 9). The differences between reciprocal crosses indicate maternal contribution towards moderating the resistance (Crusio, 1987). The cross NER 4 x E20 and NER 4 x NER 1 showed significant positive reciprocal effects at $p<0.05$ (Table 9).

Table 9: Reciprocal effects for brown spot resistance in F_2 populations

Parents	K5	PAKS	306	E20	E22	NER1	NER 4	NER10	P4R1
K5									
PAKS									
306									
E20									
E22		0.17^{ns}							
NER 1		-0.50^{ns}	-0.33^{ns}	-0.50^{ns}					
NER 4			0.50^{ns}	0.67^{*}		0.67^{*}			
NER10					-0.67^{*}		0.17^{ns}		
P4R1		-0.17^{ns}		-0.17^{ns}			-0.17^{ns}		

* = significant at α = 0.05; ^{ns} = not significant at α = 0.05; PAKS = Pakistan upland; 306 = TXD 306; NER = Nerica

4.8 Discussion and conclusions

Selection of parents to be involved in population development is very important to plant breeders. The decision process involves identifying the character to be improved, understanding how the characters are inherited and identifying the sources of parental germplasm (Fehr, 1987).

The analysis of variance for resistance to brown spot revealed highly significant differences among parents and F_2 progenies tested. This shows genetic diversity among the crossed parents and their respective crosses. In this study, the overall mean of parents and of their respective crosses were more similar, with the inheritance being attributed by both additive and non-additive factors (dominance and epistasis). Goel *et al*. (2006) got similar results when studying the inheritance of resistance to brown spot; they reported the inheritance to be determined by additive, dominant and involved epistatic gene interactions. The performance for cross between TXD 306 x NER 1 (mean = 5.7); NER 4 x NER 10 (mean = 3.0);

TXD 306 x NER 4 (mean=3.7); NER 1 x NER 4 (mean = 3.7); P4R1 x PAKISTAN (mean = 5.7) and P4R1 x NER 4 (mean = 3.0) performed better than their respective parents, possibly due to complementary alleles from their parents (Fehr, 1987).

The general combining ability effects were significant ($P \leq 0.001$), indicating that the parents of different genotypes contributed differently to the resistance to brown spot. From the low Baker's ratio (0.29), the performance of progenies cannot easily be predicted from the parents due to dominance of non-additive gene effect. With a low gca/sca ratio, one needs to choose several parents and include more crosses in order to be more certain to obtain resistant progenies (Prof. Gibson 2012, personal communication). The resistant parents E22, NER 4 and NER 10 had significant negative GCA effects indicating they contributed to resistance. The parent K5 which is moderately resistance had significant positive GCA effect indicating it contributes towards susceptibility to brown spot. The susceptible parent TXD 306 had a positive non-significant GCA effect indicating that it contributed less susceptibility than expected. The susceptible parents PAKISTAN and P4R1 had significant positive GCA effect indicating their expected contribution towards susceptibility. The parent NERICA 1 had non-significant positive GCA effects indicating it did not contribute to resistance. The parent E20 had non-significant negative GCA effects indicating it did not contribute to resistance. The desirable parents are those with significant GCA effects in the right direction for the trait of interest (Dabholkar, 1992; Singh & Chaudhary, 2004). Therefore, NER 4, E22, and NERICA 10, were best combiners for resistance to brown spot. These parents can be used in the breeding programme to introduce resistance genes to locally adapted rice germplasm.

The study has shown highly significant SCA effects ($P \leq 0.001$), suggesting that brown spot resistance was also controlled by non-additive (dominance/or epistasis) gene effects. Significant SCA effects indicate that the progeny resistance levels of certain parental combinations were significantly higher or

lower than the predictions based on parents' GCA. Hence improvement of resistance to brown spot could be accomplished by selection of crosses having high SCA effects and advancing progenies to later filial generations. The parents NER 1 x NER 4; TXD 306 x NER 4; NER 1 x K5; E20 x PAKISTAN; NER 10 x NER 1 and NER 4 x P4R1 had significant negative SCA effects indicating they contributed to resistance. The crossed parents between TXD 306 x NER 1; E20 x K5; NER 10 x E20; NER 1 x P4R1 and E22 x NER 4 displayed significantly positive SCA effects indicating they have little value as they will contribute to high frequencies of susceptible progenies (Dabholkar, 1992).

The study revealed significant reciprocal effects for NER 10 ($P \leq 0.05$) and NER 4, suggesting the presence of cytoplasmic or maternal effects contributing to brown spot resistance. Thus care should be taken to use the more resistant parent as female, if possible, when making crosses for resistance to brown spot as it has been observed that the maternal effects plays a role in conditioning the resistance. For selected crosses, determination of segregation patterns would provide more insight on the number of genes controlling resistance to brown spot disease in the materials evaluated.

CHAPTER FIVE: SEGREGATION PATTERN OF BROWN SPOT REACTION IN F_2 PROGENIES OF SELECTED RESISTANT AND SUSCEPTIBLE RICE CROSSES

5.1 Introduction

Sources of resistance to brown spot disease have been identified among improved rice populations evaluated in Uganda. The resistance to brown spot is mainly due to both additive and non-additive (dominant or epistatic) genes. However maternal effects were observed in some materials. These results agree with the findings reported by other researchers on inheritance of resistance to the disease (Goel *et al*., 2006) except in the case of maternal effects occurring. Determination of segregation patterns was the next logical step towards understanding the interactions between the genes determining resistance at different loci within the segregating populations. Two alleles at a single locus always affect the same character and characters that are under the control of one or two loci usually show distinct qualitative traits, with discontinuous variation (Burnet, 1986). Some phenotypic variations range from one extreme to another and the individuals cannot be easily classified into distinct groups. Such variation is generally under the control of many genetic loci, each one with a small effect on the phenotype, thus, showing polygenic inheritance. When the variation in a quantitative character is plotted as a frequency distribution, with each character falling somewhere in a continuum ranging from the lowest to the highest measurements, it is described as continuous variation (Burnet, 1986).

Epistasis within the segregating rice populations under evaluation caused deviation from the common phenotypic ratio in F_2 of 9:3:3:1 that is associated with segregation of two independent genes, each with complete dominance, which do not interact (Fehr, 1987). This study was therefore designed to ascertain the

level of epistatic interaction between crosses of resistant and susceptible genotypes.

5.2 Material and methods

5.2.1 Study site

The study was conducted in the field at National Crop Resources Research Institute (NaCRRI) – Namulonge in Uganda, located at 0^0 32" N of the Equator and 32^0 37" E. The institute is at an elevation of 1150 meters above sea level. It receives bimodal rainfall pattern averaging about 1200 mm per year, and has a climate ranging from tropical wet to mild and dry with marginally humid conditions.

5.2.2 Genetic material

Out of forty crosses obtained from diallel crossing, 20 to 60 F_2 plants from crosses between resistant and susceptible families were selected to be used in this study, as shown in Table 10. Only crosses with enough F_2 progenies were selected for studying the segregation pattern of brown spot reaction.

Table 10: F_2 rice populations used in the study

Crossed parents	Status of their parents
TXD 306 x NER 4	S x R
NER 1 x NER 4	S x R
E22 x PAKS	R x S
E20 X NER 1	R x S
NER 4 X TXD 306	R x S
NER 4 X NER 1	R x S
E20 X PAKISTAN	R x S

R = Resistant; S = Susceptible

5.2.3 Population development

The F_2 populations were planted in the field using an alpha-lattice design with two replications. The plants were supplied with 25 kg N ha^{-1} at two weeks after transplanting. Normal management, like watering and weeding was done regularly. The plants were inoculated mechanically by conidia spraying as described previously.

5.2.4 Data collection and analysis

Disease symptoms were scored at full panicle emergence stage following the Standard Evaluation System (SES) for rice (Table 1) (IRRI, 2002). Each plant was scored for disease severity.

The frequency distribution of trait measurements (histogram) was used to study the segregating F_2 populations for the nature of inheritance and number of genes influencing brown spot resistance (Fehr, 1987). To determine distinct phenotypic classes and segregation ratios, this was compared with theoretical ratios using the Chi-square goodness-of-fit test. Lines with disease scores of 1 - 2 were considered highly resistant, 3 - 4 were considered resistant, 5 - 6 were considered moderately resistant, 7 - 8 were considered susceptible and 9 were considered highly susceptible (IRRI, 2002). For analyses, the phenotypic ratios were modified to highly resistant, resistant and moderately resistant were grouped as resistant (R), and all higher ratings (7 – 9) were grouped as susceptible (S) (Ongom, 2010) to best fit the reduced phenotypic classes due to epistasis effects exhibited, and enable determination of the departure of observed frequencies from hypothesized frequencies.

5.3 Results

5.3.1 Frequency distribution for brown spot severity in F_2 populations

The F_2 distribution histograms of brown spot resistance for crosses between resistant x susceptible are presented in Figures 12 - 16. The frequency distribution of F_2 progeny for E20 x NER 1; NER 4 x NER 1; NER 4 x TXD 306 and TXD 306 x NER 4 displayed a discrepancy between the mid-parents (MP) and the mean for each cross (Figure 14, 15, 16 respectively) except E20 x PAKISTAN; E22 x PAKISTAN and NER 1 x NER 4 (Figure 12, 13, 15 respectively).

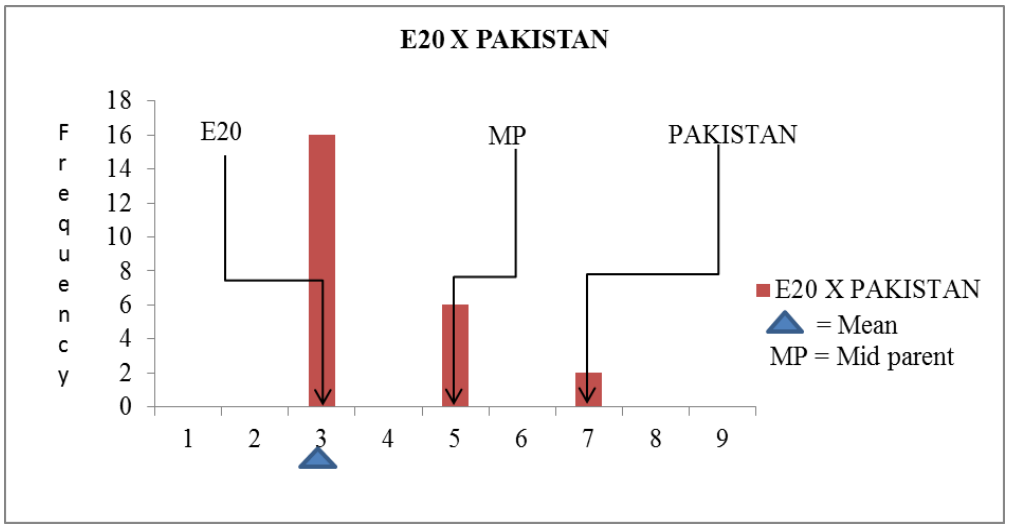


Figure 12: F_2 populations of a cross between resistant E20 and susceptible Pakistan genotype. Most of the progenies displayed between the mean and the mid-parent.

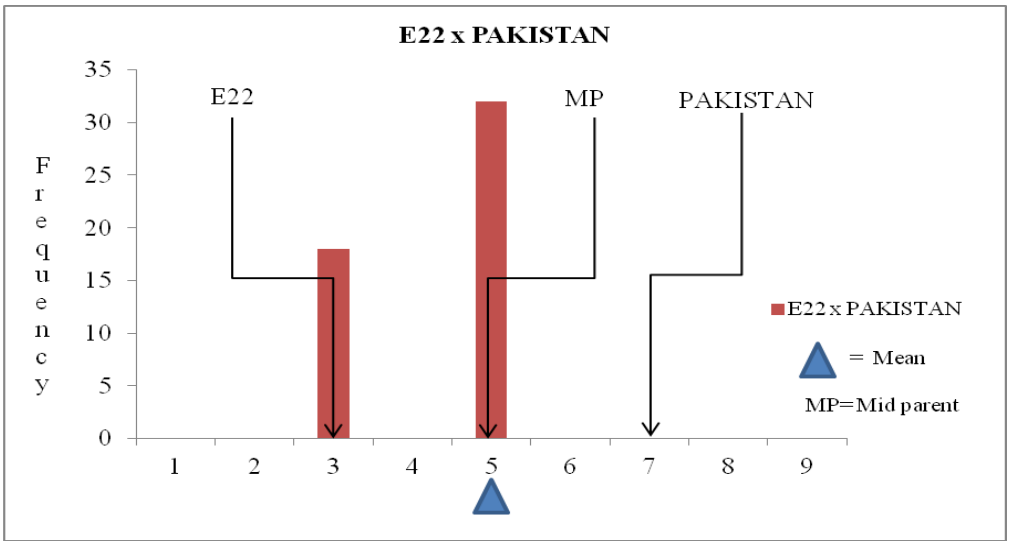


Figure 13: F_2 populations of a cross between resistant E22 and susceptible Pakistan. The progenies are displayed within the range of mean and mid-

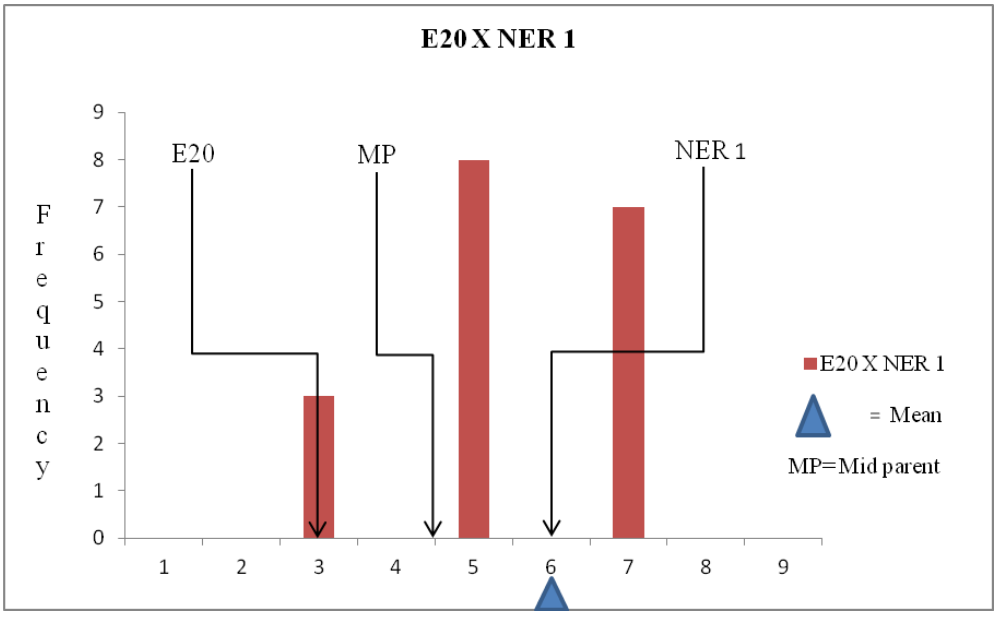


Figure 14: F_2 populations of a cross between resistant E20 and susceptible NERICA 1. The progenies displayed a discrepancy between the mean and mid-parent.

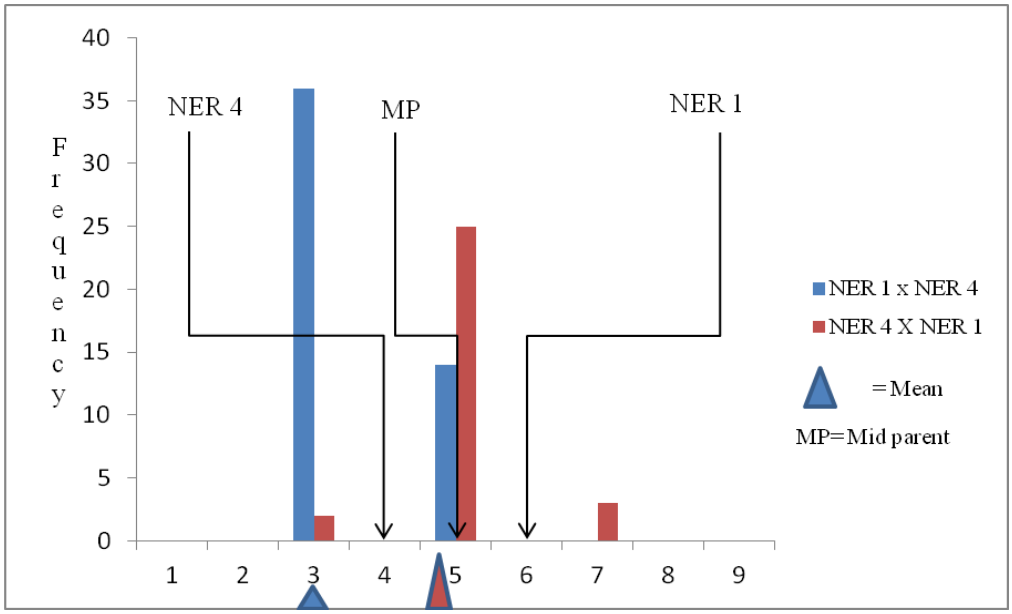


Figure 15: F_2 populations of a cross between resistant NERICA 1 and susceptible NERICA 4 and its reciprocal (NERICA 4 x NERICA 1). The progenies displayed a discrepancy between the mean and mid-parent except for the cross NERICA 1 x NERICA 4.

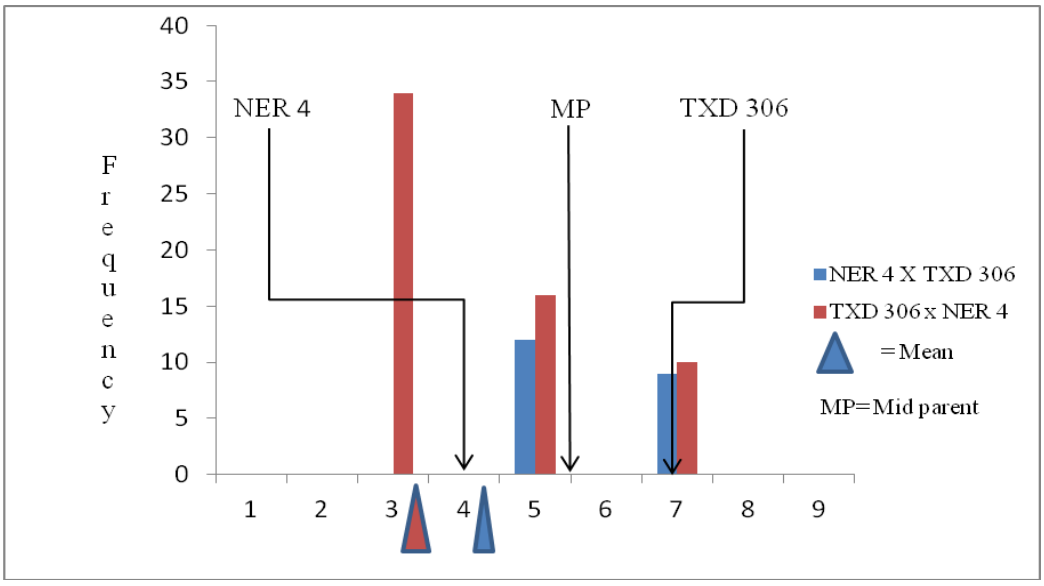


Figure 16: F_2 populations of a cross between susceptible TXD 306 and resistant NERICA 4 genotype and its reciprocal (NER 4 x TXD 306). The progenies are displayed a discrepancy between the mean and the mid-parent.

5.3.2 Chi-square goodness of fit for brown spot resistance

The F_2 progenies from the crosses made have shown distinct phenotypic classes for brown spot scores. The observed segregation with the hypothesized ratios is shown in Table 11.

The crosses TXD 306 x NER4; NER 1 x NER 4; NER 4 x NER 1 and E22 x PAK best fit a 3:1 ratio, suggesting the presence of at least one gene showing dominance. The cross E20 x NER 1 and NER 4 x 306 best fit a 9:7 ratio, suggesting the presence of two complementary dominant genes (duplicate recessive epistasis) (Fehr, 1987; Poehlman & Sleper, 1995). The cross E20 x PAK fit a 15:1 ratio, suggesting the presence of duplicate dominance genes. The cross NER 4 x TXD 306 fit both ratios of 9:7 and 3:1.

Table 11: Phenotypic segregation ratios for resistance / susceptibility to brown spot in F_2 population

F_2 populations			Observed		Expected		$\sum(Oi - Ei)^2 / Ei$	χ^2 prob.
Cross	NoP	Type	R	S	R	S		
Best fit rato 3:1								
TXD 306 x NER 4	60	S x R	50	10	45	15	2.222^{ns}	0.136
NER 1 x NER 4	60	S x R	50	10	45	15	2.222^{ns}	0.136
NER 4 x NER 1	30	R x S	27	3	28	2	3.60^{ns}	0.058
E22 x PAK	60	R x S	50	10	45	15	2.222^{ns}	0.136
E20 x NER 1	18	R x S	11	7	14	4	1.852^{ns}	0.174
NER 4 x TXD306	21	R x S	12	9	16	5	3.571^{ns}	0.058
E20 x PAK	18	R x S	16	3	18	6	3.555^{ns}	0.136
Best fit ratio 9:7								
E20 x NER 1	18	R x S	11	7	10	8	0.172^{ns}	0.678
NER 4 x TXD306	21	R x S	12	9	12	9	0.006^{ns}	0.934
Best fit ratio 15:1								
E20 x PAK	18	R x S	16	2	17	1	0.725^{ns}	0.394

NoP = No of plants: χ^2 = Chi- square test; O = number of plants observed; E = number of plants expected; R, S resistant and susceptible parents respectively; PAK = Pakistan; NER = NERICA; ^{ns} = non-significant at $p \leq 0.05$ probability level.

5.4 Discussion and conclusions

The separation of allelic pairs and their distribution to different cells during meiosis results into phenotypic expression of and individual. In this study, the F_2 progeny for the selected crosses between resistant and susceptible rice genotypes displayed phenotypically-distinct classes of brown spot scores indicating qualitative inheritance that is primarily controlled by one or few genes. The crosses TXD 306 x NER4, NER 1 x NER 4, NER 4 x NER 1 and E22 x PAK

conformed to a 3:1 ratio, suggesting the presence of at least one gene showing dominance (Allard, 1999). Similary, Nagai & Hara (1930) reported the inheritance of resistance to brown spot disease to be dominant.

The crosses E20 x NER 1 and NER 4 x 306 agree to a 9:7 ratio, indicating presence of complementary dominant alleles (duplicate recessive epistasis) (Fehir, 1987). The recessive allele at either of the two loci has masked the expression of the dominant allele at the two loci (Fehir, 1987). The cross E20 x PAK conformed to a 15:1 ratio indicating presence of a dominant allele at either of two loci which masked the expression of recessive alleles at the two loci (duplicate dominant epistasis) (Fehr, 1987). Balal *et al*. (1979) also found that two dominant genes were associated with resistance, while one gene was associated with susceptibility. Similary, Harahap (1979) suggested that as few as two major genes and some minor modifier genes may control the resistance to infection. Goel *et al*. (2006) reported the inheritance of resistance to involve additive, dominant effects as well as interaction between loci for the inheritance of resistance to brown spot from crosses involving *O. nivara* germplasm.

Based on comparison of the mean with the mid-parent, most crosses showed dominance of resistance, indicating resistance is controlled by one or few genes. This suggests the individual alleles of a major gene can be predicted and readily identified on the basis of the genotype (Fehr, 1987). The genes for resistance could be transferred from one genotype to another through family-based breeding programs like pedigree selection, single seed descent and back crossing. Backcrossing method can result into capturing of additive gene effects from resistant genotypes which will improve resistance in the susceptible genotype.

CHAPTER SIX: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The present study was conducted in order to understand the nature of brown spot resistance in upland rice in Uganda. The major objectives were: (1) to determine the reaction of local and improved rice germplasm to brown spot disease, (2) to determine the relative importance of GCA & SCA for resistance to brown spot disease in crosses of resistant and susceptible genotypes, and (3) to characterize the pattern of segregation for reaction to brown spot in F_2 progeny of selected crosses of resistant by susceptible lines.

Brown spot is a chronic disease that affects millions of hectares of rice every growing season, grown by some of the most resource-poor farmers. Despite its widespread occurrence and impact, much still needs to be understood about brown spot (Barnwal *et al*., 2013). Brown spot is by far one the strongest yield reducers amongst rice diseases today. Further, there is indication that brown spot is becoming more frequent and severe as drought is becoming more frequent (Savary *et al*. 2005), possibly due to increased variability in rainfall. Reported yield losses in relative terms vary widely from 4 to 52 % (Barnwal *et al*., 2013) up to 90% during epidemics in Tanzania (Mwalyego *et al*., 2011). In Uganda the brown spot rank as the third most important disease of rice (Adur *et al*., 2011). Mechanism of resistance to brown spot still remain to be defined and linked to results generated by breeders (Barnwal *et al*., 2013).

From this study, the key findings were:

- Fifty two (52) rice genotypes were identified that are resistant to brown spot disease (Appendix 2), which could be used in rice breeding program for developing brown spot resistant lines.

- Three (3) isolates obtained from one location, National Crop Resources Research Institute (NaCRRI) – Namulonge in Uganda were found to have

morphological characteristics similar to three species of *Bipolaris* namely *B. oryzae, B. victoriae* and *B. bicolar*. The occurance of multiple species of *Bipolaris* has been reported by Kamal & Mia, (2009).

- Assessment of inheritance revealed that both GCA and SCA were significant for brown spot scores, indicating that both additive and non-additive genes effects are involved in resistance to brown spot disease in the nine parental lines used in the diallel cross. The resistance could therefore, be improved through single seed descent and pedigree selection, although backcross breeding could also be used, although it would capture only the additive effects.

- Both additive and non-additive gene effects were major factors for brown spot resistance in the rice progenies evaluated; selection for brown spot resistance is expected to be most effective in advanced generations.

- The level of resistance in progenies could be partially predicted from the GCA of their parents.

- Cytoplasmic gene effect has a role in modifying resistance to brown spot. The resistant genotype should be used as the female parent.

In order to contribute to the design of a breeding program suitable for developing resistance to brown spot disease, the following recommendation can be made:

1. Though resistant lines to brown spot disease were identified, it is likely that other sources of resistance exist that were not included in this study. It is therefore recommended to continue screening more germplasm so as to enrich the genetic base for resistance to brown spot.

2. Little is known about the diversity and pathogenicity of *Bipolaris* strains in Uganda. There is need to characterize the fungal strains, determining their biological variation, diversity, and variation in their pathogenicity.

3. The F_2 progenies were evaluated in a single environment. The results may be overestimated since the effects of genotype x environment interactions on the results were not studied. There is need to evaluate in multiple environments for good selection of genotypes with resistant to brown spot disease.

4. Further studies are recommended to understand better, the mode of gene action for resistance to brown spot using better techniques such as use of marker assisted selection, more isolates from different locations and ecologies, and diverse sources of resistance.

5. The estimate of narrow-sense heritability showed a low value (0.24). Therefore selection for brown spot resistance is expected to be most effective in advanced generations such as 5-6.

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APPENDICES

Appendix1: List of germplasm screened for brown spot disease reaction

Entry	Source/origin
E1CV	Africa Rice
E11CV	Africa Rice
E10	Africa Rice
P27H4	NaCRRI- Namulonge
E186	Africa Rice
E51	Africa Rice
P8H13	NaCRRI- Namulonge
E123	Africa Rice
E-3	NaCRRI- Namulonge
E104	Africa Rice
E99	Africa Rice
E16	Africa Rice
E135	Africa Rice
E8CV	NaCRRI- Namulonge
P26H6	NaCRRI- Namulonge
P27H3	NaCRRI- Namulonge
P3R1	NaCRRI- Namulonge
P55H7	NaCRRI- Namulonge
E134	Africa Rice
E78	Africa Rice
P5H6	NaCRRI- Namulonge
P62H17	NaCRRI- Namulonge
E3	Africa Rice
E164	Africa Rice
E21	Africa Rice
E20	Africa Rice
P5H12	NaCRRI- Namulonge

E25	Africa Rice
E9CV	NaCRRI- Namulonge
P48H13	NaCRRI- Namulonge
P25H10	NaCRRI- Namulonge
E10CV	NaCRRI- Namulonge
E34	Africa Rice
E22	Africa Rice
P59H13	NaCRRI- Namulonge
E45	Africa Rice
P58H17	NaCRRI- Namulonge
E168	Africa Rice
E7CV	NaCRRI- Namulonge
E122	Africa Rice
E59	Africa Rice
E156	Africa Rice
E18	Africa Rice
E130	Africa Rice
E6	Africa Rice
E182	CIAT
E4CV	NaCRRI- Namulonge
E2CV	NaCRRI- Namulonge
P24H1	NaCRRI- Namulonge
E5CV	NaCRRI- Namulonge
E44	Africa Rice
P24H10	NaCRRI- Namulonge
E56	Africa Rice
E-5	Africa Rice
GRS OO57	Africa Rice
E121	Africa Rice
E199	Africa Rice
E124	Africa Rice

E6CV	NaCRRI- Namulonge
E109	Africa Rice
E15	Africa Rice
E187	Africa Rice
E191	Madagascar
E54	Africa Rice
P29H1	Africa Rice
E178	Africa Rice
P26H2	NaCRRI- Namulonge
P35H3	NaCRRI- Namulonge
P19H15	NaCRRI- Namulonge
E-4	NaCRRI- Namulonge
E12CV	Africa Rice
E179	CIAT
E9	Africa Rice
P26H11	NaCRRI- Namulonge
E148	Africa Rice
P23H1	NaCRRI- Namulonge
E140	Africa Rice
P6H15	NaCRRI- Namulonge
P23H10	NaCRRI- Namulonge
E77	Africa Rice
E118	Africa Rice
E165	Africa Rice
E65	Africa Rice
E145	Africa Rice
E32	Africa Rice
K5	Uganda-Local
P5H14	Africa Rice
E64	Africa Rice
P24H11	NaCRRI- Namulonge

E136	Africa Rice
E84	Africa Rice
E3CV	NaCRRI- Namulonge
E194	Madagascar
E2	Africa Rice
E95	Africa Rice
TXD 307	Tanzania
NER 1	Africa Rice
P4R1	NaCRRI- Namulonge
PAKISTAN (UP)	Pakistan
TXD 306	Tanzania

Appendix 2: A list of germplasm screened for brown spot disease and their resistance designation

Entry	Mean scores	Source/origin	Designation
E1CV	1.62	Africa Rice	HR
E11CV	1.89	Africa Rice	HR
E10	1.95	Africa Rice	HR
P27H4	2.04	NaCRRI- Namulonge	HR
E186	2.06	Africa Rice	HR
E51	2.08	Africa Rice	HR
P8H13	2.08	NaCRRI- Namulonge	HR
E123	2.12	Africa Rice	HR
E-3	2.13	NaCRRI- Namulonge	HR
E104	2.13	Africa Rice	HR
E99	2.15	Africa Rice	HR
E16	2.45	Africa Rice	HR
E135	2.47	Africa Rice	HR
E8CV	2.47	NaCRRI- Namulonge	HR
P26H6	2.47	NaCRRI- Namulonge	HR

P27H3	2.47	NaCRRI- Namulonge	HR
P3R1	2.47	NaCRRI- Namulonge	HR
P55H7	2.49	NaCRRI- Namulonge	HR
E134	2.52	Africa Rice	R
E78	2.52	Africa Rice	R
P5H6	2.52	NaCRRI- Namulonge	R
P62H17	2.52	NaCRRI- Namulonge	R
E3	2.58	Africa Rice	R
E164	2.58	Africa Rice	R
E21	2.58	Africa Rice	R
E20	2.64	Africa Rice	R
P5H12	2.64	NaCRRI- Namulonge	R
E25	2.65	Africa Rice	R
E9CV	2.65	NaCRRI- Namulonge	R
P48H13	2.65	NaCRRI- Namulonge	R
P25H10	2.68	NaCRRI- Namulonge	R
E10CV	2.72	NaCRRI- Namulonge	R
E34	2.74	Africa Rice	R
E22	2.74	Africa Rice	R
P59H13	2.75	NaCRRI- Namulonge	R
E45	2.79	Africa Rice	R
P58H17	2.79	NaCRRI- Namulonge	R
E168	2.91	Africa Rice	R
E7CV	2.91	NaCRRI- Namulonge	R
E122	2.93	Africa Rice	R
E59	2.93	Africa Rice	R
E156	2.95	Africa Rice	R
E18	2.97	Africa Rice	R
E130	2.99	Africa Rice	R
E6	2.99	Africa Rice	R
E182	3.04	CIAT	R

E4CV	3.04	NaCRRI- Namulonge	R
E2CV	3.04	NaCRRI- Namulonge	R
P24H1	3.04	NaCRRI- Namulonge	R
E5CV	3.06	NaCRRI- Namulonge	R
E44	3.10	Africa Rice	R
P24H10	3.10	NaCRRI- Namulonge	R
E56	3.12	Africa Rice	R
E-5	3.13	Africa Rice	R
GRS OO57	3.14	Africa Rice	R
E121	3.22	Africa Rice	R
E199	3.22	Africa Rice	R
E124	3.23	Africa Rice	R
E6CV	3.23	NaCRRI- Namulonge	R
E109	3.24	Africa Rice	R
E15	3.24	Africa Rice	R
E187	3.25	Africa Rice	R
E191	3.29	Madagascar	R
E54	3.41	Africa Rice	R
P29H1	3.41	Africa Rice	R
E178	3.43	Africa Rice	R
P26H2	3.43	NaCRRI- Namulonge	R
P35H3	3.45	NaCRRI- Namulonge	R
P19H15	3.47	NaCRRI- Namulonge	R
E-4	3.52	NaCRRI- Namulonge	MR
E12CV	3.52	Africa Rice	MR
E179	3.54	CIAT	MR
E9	3.54	Africa Rice	MR
P26H11	3.56	NaCRRI- Namulonge	MR
E148	3.58	Africa Rice	MR
P23H1	3.58	NaCRRI- Namulonge	MR
E140	3.60	Africa Rice	MR

P6H15	3.62	NaCRRI- Namulonge	MR
P23H10	3.63	NaCRRI- Namulonge	MR
E77	3.64	Africa Rice	MR
E118	3.68	Africa Rice	MR
E165	3.68	Africa Rice	MR
E65	3.75	Africa Rice	MR
E145	3.79	Africa Rice	MR
E32	3.89	Africa Rice	MR
K5	3.89	Uganda-Local	MR
P5H14	3.89	Africa Rice	MR
E64	4.02	Africa Rice	MR
P24H11	4.08	NaCRRI- Namulonge	MR
E136	4.14	Africa Rice	MR
E84	4.18	Africa Rice	MR
E3CV	4.25	NaCRRI- Namulonge	MR
E194	4.39	Madagascar	MR
E2	4.49	Africa Rice	MR
E95	5.02	Africa Rice	MR
TXD 307	5.08	Tanzania	MR
NER 1	6.00	Africa Rice	S
P4R1	6.08	NaCRRI- Namulonge	S
PAKISTAN (UP)	6.56	Pakistan	S
TXD 306	6.63	Tanzania	S
Mean	**3.00**		
CV%	**32%**		
LSD	**1.90**		

HR= highly resistant; R= resistant; MR= moderately resistant; S= susceptible

Appendix 3: ANOVA tables for screening germplasm at 15, 30, 45 and 60 days

Table 1: ANOVA table for brown spot severity at 15 days of disease scoring

Source of variation	df	variance	F cal	F prob.
Total	199			
Rep	1	0.25^{ns}	1.56	0.25
Rep.Block	8	0.16^{ns}	1.78	0.09
Entries	99	0.09^{ns}	0.97	0.55
Residual	91	0.09		
LEE	72	0.09		

^{ns} = statistically not significant at $\alpha = 0.05$

Tabl 2: ANOVA table for brown spot severity at 30 days of disease scoring

Source of variation	df	SS	MS	Fcal	F prob.
Total	199				
Rep	1	1.13	1.13^{+}	0.45	0.12
Entries	99	107.66	1.97^{***}	2.37	< 0.001
Residual	91	45.38	0.46		

*** = very highly significant at $\alpha = 0.001$; $^{+}$ = statistically significant at $\alpha = 0.1$

Table 3: ANOVA table for brown spot severity at 45 days of disease scoring

Source of variation	df	SS	MS	F cal	F prob.
Total	199				
Rep	1	2	2 $^{+}$	3.14	0.08
Entries	99	153.78	1.55***	2.44	<0.001
Residual	99	63	0.64		

$^{+}$ = significant at α = 0.1; *** = very highly significant at α = 0.001

Table 4: ANOVA table for brown spot severity at 60 days of disease scoring

Source of varia-tion	df	variance	F cal	F prob.
Total	199			
Rep	1	0.01^{ns}	0.004	0.946
Rep.Block	8	2.12**	2.436	0.019
Entries	99	1.537***	1.68	<0.001
Residual	91	0.870		
LEE	81	0.915		

** = statistically significant at α = 0.01; *** = very highly significant at α = 0.001;
^{ns} = statistically not significant at α = 0.05