Genetic Studies on Drought Tolerance and Grain Shattering in Rice

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General Abstract

Grain yield of rice can be enhanced by breeding for drought tolerance and optimum shattering. New generations of rice, the interspecific fixed lines known to carry more drought tolerance traits, are a potential source for improvement of the Ugandan landraces but they frequently carry genes for grain shattering. Information gathered through surveys to understand rice production constraints and farmersqpreferred rice traits are limited and they are generally unranked. The objectives of this study were seven-fold. They were to 1) determine farmersqperceptions on the key production constraints and preferences for rice in Uganda, 2) develop and appraise cheap and efficient rice breeding equipment, 3) determine anthesis and the grain shattering pattern of rice, 4) characterize for drought tolerance and grain-shattering ability in rice populations in Uganda, 5) investigate crossability between *O. sativa* and the new interspecific fixed lines, and 6 and 7) determine the inheritance and gene action controlling drought tolerance and grain shattering traits. The experiments were conducted at Namulonge, Uganda, from 2006 to 2009.

Data on farmersq perceptions of the key production constraints and preferences for rice in Uganda were obtained through participatory rural appraisal (PRA) methods and a formal household survey of 380 farmers from three regions in Uganda during March 2007 to June 2008. The results showed that the variety NERICA 4 was the most preferred by farmers. Rice yellow mottle virus disease, rice blast and African rice gall midge were major constraints under lowland conditions, while drought was the major constraint under upland conditions. The preferred traits in traditional varieties included aroma characteristics, grain size and the texture of cooked grains. Cultivars that combine high yield potential, early maturity, drought tolerance, aroma characteristics and special market attributes should be included in the breeding objectives.

A new anther suction device that uses an ordinary household vacuum cleaner was fabricated. Its overall units include a pipette tip, silicone tubing, hose tubes, a metallic stand, a rubber stopper and a household vacuum cleaner of 1600 w capacity. It is efficient, cheaper, less bulky and has lower replacement costs than the commercial one. In addition, a hand-held single grain tester, which is efficient, cheaper and more suitable for field studies than the laboratory tester, was developed and appraised.

Studies to determine the grain-shattering pattern in rice were conducted by making assessments at 10, 20 and 30 days after anthesis. The results revealed that the highest level of discrimination among the varieties was between 10 days and 20 days after anthesis. Among the genotypes tested, NERICA rice attained anthesis earlier than CT rice and *O. sativa* attained anthesis later than both rice groups. Appropriate methods of planting the parental lines were proposed for pollination to be synchronised.

Interspecific rice and *O. sativa* were characterized in the field for drought tolerance and grain-shattering ability. There was high variability wihin species in grain shattering and drought tolerance traits, suggesting that selection could be effective. The distribution of grain shattering scores was not normal, suggesting that some selection had taken place for the trait, unlike the drought tolerance traits and leaf roll scores where normal distributions were observed.

Results of the crossability test between *O. sativa* and interspecific fixed lines revealed that some genotypes had high crossability when used as males, or as females, or as both females and males. The best male genotype combiners were CT 16334(2)-CA-2-M, WAB 365-B-1H1-HB and IRAT 257, while the best females combiners were CT 16334(2)-CA-11-M, CT 16317-CA-4-M and IRAT 325. In addition, additive effects were more important that non-additive effects for pollen viability and seed set in the crosses. Genotypes with acceptable crossability and varying levels of drought tolerance and grain-shattering ability were selected for further studies.

The selected genotypes were crossed in a North Carolina II mating scheme, and as pairs of crosses, and used to understand the genetic mechanisms of drought tolerance at the reproductive and vegetative stages. Evaluation of the populations revealed that additive effects for filled grains were more important under drought stress and non-drought stress conditions than the non-additive effects. There were high heritability estimates for leaf roll under drought stress, implying that additive effects were more important.

There was a higher contribution of additive effects for grain-shattering ability than non-additive effects in the populations. Allelic tests for the shattering trait revealed that a single gene was in 46% of the crosses; two genes in another 46% of the crosses had duplicate dominant effects as well as complimentary dominant/recessive effects; and there were

three genes in four percent of the crosses. Segregation ratios of 3:1 and 1:3 were also found in two crosses. This could be due to a reversal of the function of the genes. Another surprising finding was that the segregation ratio of 3:1 was found in a cross with loose grain attachment to the pedicel in an allelism test. This segregation was probably due to unfixed loci in some of the interspecific lines.

This study identified rice breeding priorities for Uganda, prepared breeding tools and provided relevant information for generating lines that have improved drought tolerance and optimum grain-shattering ability by determining the modes of gene action for these two traits.

Declaration

I   **JIMMY LAMO**   declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain any other personsqdata, pictures, graphs or information unless specifically acknowledged as being sourced from other persons.

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Signed

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Jimmy Lamo (Candidate)

As the candidateqs supervisors, we agree to the submission of this thesis.
Signed

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Professor Pangirayi Tongoona (Supervisor)

Signed

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Dr John Derera (Co-supervisor)

Dedication

To God my Saviour through whom

all things are possible!

Table of Contents

List of figures

List of Tables

List of plates

List of appendices

Acknowledgement

Introduction to the thesis

0.1   Significance of rice

Rice provides nutrition for more people in the world than any other crop, especially in developing countries (Phillips et al., 2005). It is, however, the least traded cereal commodity worldwide, with less than 7% of the worlds annual production entering international markets (FAOSTAT, 2008). Initiatives aimed at promoting local production where conditions are favorable are considered viable strategies to avoid competition for this commodity. Rice is also recognized as a crop that can feed large populations based on several of its attributes, including good storability and that it requires less energy to prepare. In Africa, it was identified as a source of food to support the population growth, based on similar successes in Asia in the United Nations International Year of Rice concept of 2003 and its subsequent adoption by the New Partnership for African Development (NEPAD)(FAO, 2003).

Currently, Africa produces about 9% of the global rice production (FAOSTAT, 2008). Of the rice produced in Africa, up to 60% comes from West Africa and 15% is from East Africa (FAOSTAT, 2008). Among African countries, it is only Egypt that is self-sufficient in rice. Rice production has, however, been increasing in Sub-Saharan Africa (SSA) (Table 0.1). For instance, in the 2001-2005 period rice production grew by 3.2% per year and by 3.5% a year from 2006-2008 in this region (ECARRN, 2005; FAOSTAT, 2008). Despite these increases in production during 2001-2008, the population growth rate was higher at 2.9% and 3.8% per year in 2001-2005 and 2006-2008, respectively. The demand gap is, therefore, growing. For instance, the rice imported in 2007 alone was valued at $3.6 billion in SSA (FAOSTAT, 2008). There is therefore a deficit, which can be satisfied only through the use of scarce foreign currency.

Table 0.1 Average paddy production during 2001-2008 (million tonnes)

Sub-region	2001-2005	2006	2007	2008
West Africa	7.55	9.32	10.6	15.0
Central Africa	0.45	0.48	05.3	07.2
Eastern Africa	3.89	0.46	05.1	06.1
Southern Africa	0.18	0.20	05.2	06.2
SSA	**12.07**	**14.2**	**26.2**	**34.5**

Source: ECARRN, 2005; FAOSTAT, 2008.

In Uganda, rice ranks first as a commercial cereal crop (NARO, 2005) and has the highest output-to-input ratio among cereals grown in the country (NAADS, 2003; Kijima et al.,

2006). Indeed, 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). Recently, there has been a steady increase in Uganda in the area planted with rice. From 2003 to 2007, the area under rice increased from 86,000 ha to 119,000 ha (FAOSTAT, 2008). However, the yield average declined from $1.5\,t\,ha^{-1}$ in 2003 to $1.4\,t\,ha^{-1}$ in 2007 (FAOSTAT, 2008). Despite low levels of rice production in Uganda, the per capita consumption has been growing steadily from 5 kg in 2003 (FAOSTAT, 2003) to 8.3 kg in 2008 (FAOSTAT, 2008). It is projected that the per capita rice consumption will increase to 15.6 kg in 2017. The total consumption is expected to be high, considering the current population growth rate of 3.2% (UNFPA, 2008). It is a strategic crop in the government's plan to modernize agriculture because traditional cash crops, namely cotton, coffee and tobacco, are experiencing lower and unpredictable market prices when compared with rice (PMA, 2007).

Rice is cultivated under three production systems in Uganda: upland (60% of the cultivated rice area), rain-fed lowland (36%) and irrigated wetland (4%), with each system contributing 40%, 42% and 18%, respectively, of the total rice produced in the country (JICA, 2007). These production systems have had changes in emphasis over time. Rain-fed lowland production was the main system in use from 1904 to 1970, followed by irrigated rice production (1971 to 1995) and upland rice (1995 to current date) (Workman and Elide, 1999; Odogoola, 2006; JICA, 2007). A common denominator in the three production systems is that there is high potential for rice production. For instance, about 70% of the country's arable land is suitable for rain-fed upland rice production (Ogwang, 2002; JICA, 2007).

0.2 Drought constraint

Despite the high potential of Uganda for rice production, major constraints to production were drought, pests, diseases, access to quality seed and poor cultural practices, according to a survey conducted in 2002 (NARO, 2005). All of these constraints were later mentioned in a survey conducted in 2005 but the lack of equipment for harvesting and processing, and lack of appropriate tools for cultivation were indicated as well (Odogoola, 2006). Considering these constraints, drought is reported as the most important constraint to upland rice production in the country (NARO, 2005; NARO, 2007). It consistently remained a major rice production constraint despite the release of five new varieties in the past eight

years (NARO, 2005; NARO, 2007). Drought is indeed the most important abiotic stress factor in rice production, both in terms of degree and coverage worldwide (Balasubramanian et al., 2007). It has a global dimension in that about 18 M t of rice worth $3.6 billion is lost each year due to drought worldwide (Conway, 2004). A yield loss of up to 15-20% due to drought has been reported in most parts of Africa (Lafitte et al., 2003), especially in rain-fed production environments (Sahrawat, 2004). It has emerged as a critical rice production constraint in East Africa (ECARRN, 2005) and in Uganda in particular (Odogoola, 2006).

In response to the drought problem, rice breeding has been undertaken worldwide in phases using different base populations. In the 1970s and 1980s, progenies from *O. sativa* japonica x *O. sativa* indica crosses, involving upland and irrigated rice types, were core progenitors used repeatedly in the development of rice types with drought tolerance (Chang et al., 1974; Chang et al., 1982; Chang et al., 1985). Evidence of the transmission of the drought tolerance trait was realized and new lines were developed (Lafitte et al., 2006). Genetic barriers to making these crosses were identified and were used to select appropriate crossing techniques (Hung and Chang, 1976). After 1990, it was demonstrated that *O. sativa* was reaching a ceiling as a source for drought tolerance (Nanda, 2000; Yang, et al., 2004). The traits of a wild relative of one cultivated species, *Oryza glaberrima,* showed exceptional but unexplained transmission of the drought tolerance trait (Brar and Khush, 1996; Moncada et al., 2001). Contrary to the earlier notion that being a complex trait it is impossible to be improved on through breeding (Bernier et al., 2005), breeding for improved drought tolerance should be explored considering that it is now very possible. Unlike previous breeding initiatives that used *O. sativa* parents, the current generations of interspecific rice developed using the drought-tolerant parent *Oryza glaberrima* are potential sources of drought tolerance that should equally be explored.

0.3   Grain-shattering ability

Other than drought stress, reduced grain recovery due to loose grain attachment (shattering) or tight grain attachment (non-shattering) at maturity affects rice yield in Uganda. Problems associated with loose grain attachment are a serious constraint for irrigated rice systems (Abong, 1999), while low grain recovery due to tight grain attachment has been reported in upland rice varieties in the country (NARO, 2007). Loose grain attachment leads to direct yield loss and seed emergence in the field as volunteer plants,

3

which leads to the contamination of subsequent rice types. Tight grain attachment leads to incomplete grain recovery and frequent grain breakage during threshing. In order to get the maximum grain recovery and minimum losses during harvesting, an optimum level of grain shattering is preferred. Among the upland rice genotypes released in Uganda, some genotypes developed from *O. sativa* x *O. glaberrima* (interspecific lines) crosses have reduced acceptability due to too tight grain attachment to the pedicel, yet they have several other important traits (Lamo et al., 2007). However, they seem to be more tolerant to drought stress than most upland landraces (NARO, 2005; Lamo et al., 2007; NARO, 2007). The interspecific lines are indeed an important source of drought tolerance traits (Majerus et al., 2007).

Loose grain attachment is important worldwide due to three main reasons. Firstly, relatives of *Oryza sativa* form an important part of the diets of several communities worldwide, but they are known to be susceptible to shattering. For instance, wild rice is a delicacy and an African indigenous rice type. *O. glaberrima* has preferred cooking traits in the USA and West Africa, respectively (Vaughan et al., 2004). Secondly, the natural out-crossing of cultivated rice, the Asian rice *O. sativa* and African rice *O. glaberrima,* with wild rice types leads to weedy rice types that are high in shattering (Langevin et al., 1990). It is a common problem in many rice farming areas worldwide. Thirdly, and perhaps most importantly, breeders perform wide crossing and generate new genotypes with improved biotic and abiotic stress responses, but grain-shattering ability is often transmitted to the new generations (Brar and Khush, 1996). Currently, no parallel breeding programme to improve grain-shattering ability is taking place elsewhere in the world to Uganda's benefit. Besides, Uganda is unique in the distribution of relatives of *Oryza sativa*, known to be precursors of grain shattering (Kiambi et al., 2005). This situation makes the improvement of materials for acceptable levels of shattering a worthwhile research objective.

Cultivated rice is a mutant progenitor of wild rice that was domesticated for reduced shattering ability from the shattering source (Doebley, 2006; Konishi et al., 2006). Over time, the selection of rice for acceptable threshing ability has been conducted progressively with respect to methods used for threshing. For instance, when threshing is conducted by hand more loose attachment is preferred than when threshing is done using machines. Current information shows that the shattering trait varies within cultivated rice types. For instance, the Asian cultivated rice *Oryza sativa* (japonica) is less shattering than *Oryza*

sativa (indica), while the African cultivated rice *Oryza glaberrima* is prone to shattering (Konishi et al., 2006). Similarly, information on new interspecific fixed lines developed through *Oryza sativa* x *Oryza glaberrima* crossing is still limited. The *O. glaberrima* is a more recently domesticated species than *O. sativa*. While *O. glaberrima* was domesticated over 3,500 years ago, O. sativa was domesticated over 10,000 years ago. Using *O. glaberrima* for generation of segregating populations for studies in order to understand the nature of transmission of the grain shattering trait could be very informative. Understanding the nature of transmission of the grain shattering trait using *O. glaberrima* could guide rice breeding because genes for resistance to several useful traits have been extracted from relatives of *O. sativa* but grain shattering, frequently dragged into the new generations, needs to be optimized. A study output that explains the genetics of drought tolerance as well as the grain shattering trait guides breeding work aimed at improving drought sensitive landraces using the *O. glaberrima*.

Despite the considerable contribution of breeding to rice production, Uganda has until recently not had a rice breeding programme. However, three interspecific lines generated from *O. sativa* x *O. glaberrima* in other breeding centres and widely deployed in various countries were released in Uganda based on preferred traits, including tolerance to drought (NARO, 2005; Lamo et al., 2007; NARO, 2007). Accelerated acceptability of this variety is being undermined by poor grain threshing ability (Lamo et al., 2007). Besides, a large collection of *O. sativa* and interspecific rice is available from major rice breeding centres. It is important that this germplasm is characterized for the two traits, before its use in studies of the transmission of drought tolerance and grain shattering in rice germplasm in Uganda. In order to transfer valuable drought resistance sources from this new generation of the fixed interspecific lines into landraces, it is important to understand the mode of transmission of both drought tolerance and the grain shattering trait in these populations. Thus, there is a need to undertake genetic studies between landraces and different interspecific fixed lines of *O. sativa* x *O. glaberrima* crossing background. Therefore, studies were initiated in 2006 to address farmersqpreferences and the modes of transmission of drought tolerance and grain shattering traits from 2006 to 2009.

0.4   Famer preferences

Although drought stress and grain shattering traits are important in the country, an overall ranking of rice production constraints that could be improved on through breeding is lacking.

There are scanty reports of rice production constraints based on reported incidences. For instance, 13 different seed-borne diseases (Biruma et al., 2003), drought (Lamo et al., 2007), poor grain threshing ability (Lamo et al., 2007) and rice yellow mottle virus disease (RYMV)(Pinel and Fargette, 2006) were reported but not ranked. More so, farmers seem to require very defined variety characteristics because recently released rice varieties are still grown alongside traditional landraces (NARO, 2005; NARO, 2007). In order to guide breeders to develop preferred rice types, a clear understanding is required of what varieties farmers have access to at a particular time, constraints associated with them, their characteristics and preferred varietal traits. A survey to gather this information was one of the objectives of this study.

0.5 Development of breeding tools

In order to develop appropriate rice breeding tools, considering that formal rice breeding had not been initiated in Uganda, some critical techniques and procedures needed to be pre-tested and standardized before use. One such tool is flower emasculating equipment required to prevent self-fertilization and facilitate crossing. The flower emasculator is a critical tool because rice florets are small and anthers emerge from the base of stigmas, making it difficult to remove anthers without damaging the stigmas using simple breeding hand tools. The commercial equipment is manufactured in a few advanced laboratories and their prices are prohibitive for breeders in SSA. There are two types of flower emasculators and one is from the International Rice Research Institute (IRRI) in the Philippines while the other is from Cornell University in the USA (Jennings et al., 1979; Coffman and Herrera, 1980). Both pieces of equipment work on the principle of suction, which was used to fabricate a new one using locally available materials, as an objective of this research.

To date, the methods of estimation of grain shattering are subjective in nature. The methods that are less influenced by environmental conditions are laboratory based and are not portable, and therefore not suitable for field evaluation. The available field equipment is a digital breaking strength equipment device described by Li et al. (2006). It is an improved version of equipment developed by Ichakawa et al. (1990) but is not yet available for commercial use. Considering the need for an accurate estimation of grain shattering, for use in the overall objective of this study and other similar work on the genetic study of grain shattering, a simple hand-held grain-shattering tester was fabricated.

0.6 Flower anthesis pattern and other agronomic traits

Successful rice breeding requires knowledge of the rice anthesis pattern, especially its duration and intensity. The anthesis pattern is a crop adaptation trait that is influenced by latitude and other environmental factors. For instance, varieties known to flower early in the morning in tropical countries flowered late in temperate countries (Tao et al., 2003). In addition, it is a trait that is associated with grain shattering according to Fukuta et al. (1994). It is, therefore, important that the anthesis pattern is understood for a given location for practical breeding. This information is still lacking for the Ugandan germplasm where rice breeding is at the beginning and thus, an anthesis pattern study forms part of the current study. Other agronomic traits that determine success in rice breeding according to Tao et al. (2003), are pollen viability, seed set, panicle exertion and tillering capacity. The work further indicated that rice varieties that have very low pollen viability are not appropriate for use as pollen parents instead; they are suitable as parents in hybrid rice production. Seed set directly influences success of making crosses because it is the progeny from making crosses. According to (Jennings et al 1979), panicle exertion is varietal characteristics that determine whether a given rice variety can be used in making crossing . Varieties that are not fully exerted do not have properly developed florets. These makes it difficult as parents during hybridization. Similarly, optimum tillering capacity determines how appropriate a given plant is as a parental line. Information on these agronomic traits for any population is important for any rice breeding work.

0.7 Rice crossability and other reproductive barriers

Although wide crossing (especially between *O. sativa* and *O. glaberrima*) has opened a new gene pool for rice improvement, the incorporation of it in routine, conventional breeding is still limited by difficulty in crossing the two species due to reproductive barriers. These barriers are expressed in the form of poor seed set, poor germination, low seedling survival and filled grains (Sano, 1986; Jones et al., 1997; Tao et al., 1997; Tao et al., 2003). Some of these barriers to making crosses have been overcome by first understanding their nature and extent. For instance, reproductive barriers to making crosses between *O. sativa* indica and *O. sativa* japonica were identified and successfully used to select appropriate crossing techniques for making crosses (Hung and Chang, 1976). Currently, a few difficult techniques are used to overcome crossability barriers in *O. glaberrima* and *O. sativa* crosses. Findings of work so far conducted on crossing *O. sativa* with fixed generations of

O. sativa and *O. glaberrima* show that it is promising (Efisue et al., 2008). It is considered that interspecific fixed lines generated from *O. glaberrima* x *O. sativa* crosses could be further explored for use in improvement of currently grown *O. sativa* landraces. Another preliminary finding that hybrids generated from wild rice and *O. sativa* crosses have higher crossability with *O. sativa* strengthens the need to undertake this study. For example, in a study on natural pollen dispersion between cultivated rice and some wild rice, low crossability was realized (1.2-2.19%)(Chen et al., 2004), while hybridization between cultivated rice and the F1 lines yielded crossability in the range of 1-52% (Langevin et al., 1990). This study sought to test whether the interspecific lines could be used for improving *O. sativa.* This information was crucial for effective improvement of landraces using *O. glaberrima*

0.8   Rice grain-shattering pattern

The precise determination of the shattering trait could guide breeding for an acceptable shattering ability in rice, but the trait is associated with crop development and therefore its determination requires knowledge of the grain-shattering pattern. Information on the pattern of expression of the traits that are associated with crop development has been used successfully in improving those traits through breeding. For instance, information on the pattern of kernel shattering in wheat (Clarke and DePauw, 1983), and chalkiness and translucency in rice (Shi et al., 2002), was used in breeding for improved threshing ability in wheat and improved grain quality in rice, respectively. Information on the pattern of grain shattering in rice is lacking. This information is important because gene expression and the genetic effects at different growth stages for rice may vary.

0.9   Research objectives

The overall goal of the research was to improve rice productivity through the development of new varieties with drought tolerance and optimum grain-shattering ability.

The specific objectives of the research were to:
    i)   determine farmers' perceptions of the key production constraints and their preferences for rice in Uganda;
    ii)   develop and appraise a cheap and efficient rice flower emasculator for breeding and a grain tester for shattering ability;
    iii)   determine the grain-shattering pattern and the anthesis trend in rice;

iv)   characterize rice genotypes for drought tolerance and grain-shattering ability;

v)   determine the crossability and inheritance of crossability parameters, namely seed set, seed germination ability and seedling vigour in crosses between *O. sativa* and interspecific fixed lines;

vi)   determine the inheritance and gene action controlling drought tolerance in rice; and

vii)   determine the inheritance and gene action controlling rice grain-shattering trait.

0.10   Research hypotheses

The hypotheses tested in the study were:

i.   farmers can rank drought and shattering in terms of importance in relationship with other constraints;

ii.   drought tolerance is controlled mostly by additive gene effects,

iii.   grain shattering is controlled mostly by additive gene effects,

iv.   interspecific fixed lines are cross compatible with *O. sativa* genotypes, and

v.   Genes responsible for shattering from different genetic backgrounds are allelic.

0.11   Research hypotheses

This thesis is made up of ten sections, that include nine chapters as shown below:

Introduction to thesis

Chapter One: Literature review

Chapter Two: Farmersq preferred rice varietal traits, their weaknesses and implications for breeding in Uganda

Chapter Three: Development of flower emasculator and grain shattering tester for rice

Chapter Four: Grain shattering pattern and diurnal anthesis trend

Chapter Five: Characterization of rice germplasm for grain shattering and drought tolerance

Chapter Six: Reproductive barriers and gene action for seed set and pollen viability in crosses between Oryza sativa L. and interspecific rice

Chapter Seven: Gene action and inheritance of drought tolerance traits in populations generated from crossing interspecific with intraspecific rice genotypes

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CHAPTER ONE
Literature Review

1.1   Introduction

This chapter provides a context for the research by reviewing theory relevant to: a) rice taxonomy, b) interspecific rice, c) critical advances in knowledge on genetics of drought tolerance (DT), d) genetics function of grain shattering (GS) in rice, and e) cross compatibility. Challenges and limitations of improving drought tolerance and grain threshing ability through breeding are highlighted as well. Farmersqpreferences are reviewed as well. Knowledge gaps addressed in this reported research were also identified.

1.2   Rice taxonomy

The rice tribe, Oryzeae, is distributed in the tropical and temperate regions worldwide (Vaughan et al., 2004). Two species of cultivated rice, namely Asian rice *Oryza sativa* L. and African rice *Oryza glaberrima* Steud belong to the genus *Oryza*, family Poacae and tribe Oryzeae. The genus *Oryza* has 22 wild species (2n=24,48) representing 10 genomic types (Vaughan et al., 2004). The two cultivated rice species belong to genomic group AA (2n=24). Within *O. sativa* there are three subspecies namely the *indica* Kato, *japonica* Kato and *javanica* (Roschevicz, 1931). The subspecies *japonica* has two strains, namely *tropical* and *temperate*. *Oryza sativa* is grown worldwide and *O. glaberrima* is grown in West Africa (Jones et al., 1997a). Considerable rice research has been conducted on *O. sativa,* while research on *O. glaberrima* has been limited and focused on only collecting, characterizing and undertaking introgression breeding.

Rice cultivars can be divided into three ecological varieties: *Indica* which are of tropical and sub- tropical distribution, *Javanica* which grow in Indonesia and *Japonica* of temperate distribution (Vaughan et al., 2004). The *Indica* varieties are long-grained rice, *Javanica* are broad-grained and *Japonica* also short-grained. The cultivars can be distinguished based on many characteristics; these include adaptation to different water regimes, growth habit and height, shapes, size and color of the culm, leaf blade, panicle, hull and grain, and degree of pubescence. *Japonica* is notable for becoming sticky when cooked because it is high in amylopectin.

1.3 Interspecific rice

Interspecific lines are genotypes that are developed through crossing plants from different species. In rice, genotypes developed through crossing the two cultivated species, *O. glaberrima* and *O. sativa* L or crossing either of the two with any of their relatives generates interspecific genotypes. The interspecific lines may be generated as a bridge to extract valuable genes from the relatives of the cultivated rice or simply to generate new rice genotypes. For instance, among the relatives of cultivated rice, four species that are found in Africa are used for extraction of valuable genes through generation of interspecific lines. They include *O. barthi* A. Chev. and Roehr for resistance to green leafhopper, bacterial blight and drought avoidance (Khush, 1997), *O. longistaminata* Chev. and Rohr for drought tolerance (Vaughan, 1994; Khush, 1997), *O. punctata* Ktoschy ex Steud for resistance to brown plant leafhopper and leaf hopper and *O. brachyantha* Chev. and Roehr have resistance to rice yellow mottle virus (RYMV), stem borers, leafhopper, whorl maggot and tolerance to lateritic soil (Vaughan, 1994; Khush, 1997).

Interspecific lines generated through crossing the cultivated rice *O. sativa* and *O. glaberrima* are important rice introductions in the country. The proportion of parental genomic contribution of *O. sativa* to the new genotypes is higher than *O. glaberrima*. For instance in a cross between IR64 (an *Oryza sativa* subsp. *indica* variety) a lowland variety and Tog5681 (an *Oryza glaberrima* variety) the estimated average *O. sativa* genome coverage was found to be 78.2% (Ndjiondjop et al., 2008a). In another generation involving a cross between *O. sativa* tropical japonica and *O. glaberrima* called CG 14, the mean *O. sativa* genome coverage was about 87.4% with a range of 79.0% to 94.4% (Ndjiondjop et al., 2008b). These are the rice lines called NERICA an acronym for New Rice for Africa. Other interspecific lines developed through crossing another *O. sativa* tropical japonica line, CAIAPO and *O. glaberrima* are called the CT lines. The acronym CT is an abbreviation for **C**I**A**T line which refers the International Centre for Tropical Agriculture (CIAT) (Guimaraes et al., 1996). Currently, several CT lines and NERICA lines have been characterized under Ugandan conditions (Lamo et al., 2007) and the possibility of using them for improving landraces is an urgent research need.

The *O. glaberrima* and *O. sativa* belongs to the same genome complex called the AA genome that has 24 chromosomes. They contain a high level of diversity that is sexually compatible with *O. sativa* and can be accessed via crossing and selection. Interspecific

crosses between O. sativa and two African species, *O. glaberrima* and *O. barthii,* have demonstrated the utility of targeted introgression as the basis for gene identification and plant improvement. These relatives are known to be good sources of genes for tolerance to biotic and abiotic stresses and the transgressive behavior of progeny derived from interspecific crosses has been demonstrated in several studies.

1.4. Drought tolerance in rice

1.4.1 Physiological implications of drought

Normal plant growth is generally affected whenever drought occurs. In rice, the function of root growth and distribution, biomass production, peduncle elongation, anther dehiscence, and the extent of xylem cavitation are directly affected when drought occurs (Lafitte et al., 2003). However, drought is most damaging just before and during the anthesis stages of rice development (Ekanayake et al., 1989). Takeoka et al. (1992) showed that when water stress is imposed, multiple pistils and the fusion of ovaries to form gigantic structures occur. They further found that other effects of drought stress were changes in the number of stamens, poor development of stamens, poor development of cuticle structure on the anther epidermis and poor development of pollen. Rice varieties that can maintain growth in relatively dry soils are rated as drought-tolerant varieties (O'Toole and Chang, 1978). Rice varieties that lack the capacity to combine the ability to maintain biomass production in water-limiting soils, especially at anthesis stage, as well as high yield potential in favorable conditions both in terms of time (seasons) and space (locations), are considered susceptible and are not selected for production (O'Toole and Chang, 1978) and are considered susceptible to drought stress and not selected for production. The process of selecting genotypes based on phenotypic values can be improved on through breeding if the genotypic aspects of drought tolerance are used in the selection.

1.4.2 Nature and mechanisms of rice response to drought stress

The way in which rice responds to drought depends on the type of rice variety, the extent of the environmental stress, and the interaction between the two factors (Atlin, 2003). These three-way factors may cause biomass reduction when their effects are moderate, or complete crop failure when severe. Because the severity of drought stress varies with rice variety, varietal response to drought is a major factor exploited during breeding for improved drought stress (Fukai and Cooper, 1995). Rice responds differently to drought during the seedling development, vegetative and reproductive stages. Of the three growth stages, the

reproductive stage is the most sensitive to drought, especially during the reduction division of pollen mother cells (Sarkarung et al., 1995).

Other than the growth stages of rice, the severity of drought depend on the timing of the stress. This aspect is discussed extensively elsewhere (Lafitte et al., 2003). Drought may be early, intermittent or late in the growth cycle of rice. Early drought characteristically occurs during the vegetative growth stages and the crop may not recover if the stress is severe. Intermittent drought occurs at any of the growth stages of rice and it is difficult to predict repeatability of this drought type. Late drought occurs during the reproductive growth stages and affects anthesis, grain number and hence grain yield (Mackill et al., 1996). Delay in anthesis and reduced panicle elongation are the most easily assessed effects of late drought stress (Mackill et al., 1996).

Mechanisms of resistance to drought are adaptive traits that are triggered by drought itself. There are three broad resistance mechanisms, namely drought escape, drought avoidance and drought tolerance (Lafitte et al., 2003). Of the three mechanisms, drought escape does not function in the presence of drought but rather the crop completes its life cycle before serious tissue water deficits occur (O₫oole and Chang, 1978). There are diverging views on its being a drought resistance mechanism. While other workers on drought tolerance view it as the most effective adaptive mechanism for drought resistance by evading drought stress, some scientists consider that it lacks capacity to withstand drought stress. Recovery from drought stress is another drought resistance mechanism included by other scientists working on drought (Lafitte et al., 2003). Recovery from drought occurs through the maximization of the available water rather than maximizing water use efficiency (Lafitte et al., 2003). It functions as long as the wilting point is not reached. The leaf is the most easily observed phenotypic response to wilting.

Drought avoidance takes place when a crop has enhanced water uptake and reduced water loss in the presence of drought stress (Yue et al., 2006). Mechanisms of drought avoidance include increased water uptake, reduced water loss from the crop, deep root structure, and early stomatal closure at the beginning of the period of water deficit (O₫oole and Chang, 1978). The crop responds to this type of drought by maintaining turgor, which involves osmotic adjustment and antioxidant capacity (Hanson et al., 1995).

Drought tolerance functions by reducing dehydration in rice. It is considered to be the most important mechanism for rice breeding. Selection for drought tolerance can be conducted through direct yield selection or the use of secondary traits. Selection for direct yield is effective when conducted under a target environment (Atlin, 2003). The selection of rice under drought tolerance can also be conducted by monitoring secondary traits for yield potential (Lafitte et al., 2003). The effectiveness of selection for secondary traits to improve yield under drought stress has been demonstrated in *O. sativa* and a few interspecific rice genotypes (Efisue, et al 2009). An understanding of the genetic transmission of secondary traits in different populations of interspecific lines is therefore important.

1.4.3 Drought tolerance traits and breeding sources

Several crop improvement methods have been employed to address the problem of drought stress, but improving root related traits, reducing growth duration, and developing stability in anthesis has had more focus. In the case of root related traits, deep root (Mambani and Lal, 1983; Fukai and Cooper, 1995; Kondo et al., 2000) and root volume are important in avoiding drought (O'Toole and Chang, 1978). A few secondary traits, namely, anthesis delay, spikelet sterility (Venuprasad et al., 2002), and leaf water potential (LWP)(O'Toole and Chang, 1978; Lafitte et al., 2003) have been used for selection for drought tolerance. Reduced stomatal closure, leaf rolling, and the development of thick cuticle have also been demonstrated to be associated with drought tolerance (O'Toole and Chang, 1978; Sarkarung et al., 1995).

Several germplasm breeding sources have been identified for use in breeding for drought tolerance. These include wild rice, *O. barthi, O. autraliensis, O. perennis, O. longistaminata, O. rufipogon* (Brar and Khush, 1986), and cultivated African rice, *O. glaberrima* (Sano et al., 1984; Sitch et al., 1989; Jones et al., 1997a; Maji et al., 2001; Sarla and Mallikarjuna Swamy, 2005). Seventeen such new lines, developed from *O. glaberrima* and *O. sativa* crosses, with better drought tolerance than the *O. sativa* species, are under production in several countries in Africa (WARDA, 2005).

Methods so far used to successfully develop drought tolerant rice include conventional crossing and techniques that include the embryo rescue technique (Jones et al., 1997b). There is also evidence that marker assisted selection (MAS) based approaches for drought tolerance that involve introgression of alleles with large additive effects on yield under

stress could be possible (Li et al., 2006c). Studies taken to understand the transmission of drought tolerance traits in interspecific lines may have a high chance of identifying valuable drought tolerance traits because the genome comprises up to 10% of the *O. glaberrima* component.

1.4.4 Inheritance of drought tolerance traits

Genetic variation exists in rice germplasm for drought escape, avoidance and tolerance (O'Toole, 2005). Of the three mechanisms, drought tolerance and drought avoidance are direct responses of plants under drought stress conditions. In order to effectively use traits that have been identified for drought tolerance in breeding, several studies have been conducted to assess their transmission. A summary of heritability scores for five traits is shown in Table 1.1. The highest narrow sense heritability was found in maturity date. Delay to anthesis, percent of fertile spikelets, and leaf death score also showed high heritability. Delay to flower and percent leaf sterility are most effective when the timing of the stress coincides with the late reproductive growth stage of the crop (Lafitte et al., 2003). A recent study found that the heritability of the number of filled grains under drought at anthesis is specific and gives clear information on the genotypic response to stress (Lafitte et al., 2003). It is not only a highly informative indicator of the severity of drought stress, but it is considered to be the most important determinant of yield under drought stress conditions (Ekanayake et al., 1989; Yue et al., 2006). The proportion of filled grains under drought stress could, therefore, be used for genetic studies on drought tolerance. The least heritability score was reported on canopy temperature, partly because it is easily influenced by environmental conditions. Estimation of heritability using leaf death score gives high heritability, but it is a destructive method that is not appropriate for early generations of materials.

Table 1.1 Selected secondary traits recommended for drought tolerance breeding in rice

Trait	Relationship to stress yield	Growth stage for selection	Earliest generation for selection	Heritability approximately
Flowering/ maturity date	Reliability for stress timing: effective for terminal stress	Flowering	Single plants at F_2	0.9
Flowering delay	High for stress at flowering	Flowering	Small plot	0.6
Percent fertile spikelets	High for stress at flowering	Near or at maturity	Single plants at F_2	0.6
Leaf death score	Negative and moderate	All stages	Single plants at F_2	0.7
Canopy Temperature	Negative and fairly high	Pre-flowering	Small plot	0.2

Source: Adapted from Lafitte et al., (2003)

Other than secondary traits, heritability of yield under drought stress has been variously studied. Studies conducted by Atlin et al. (2005) indicated that there is weak correlation between heritability of yield under stress when compared with that under non-stress conditions. A similar study by Bernier et al. (2005), on the genetic control of yield under reproductive stress in an F_3 population of a cross between Vandana, a drought-tolerant Indian upland cultivar, and Way Rarem, a drought-sensitive Indonesian upland cultivar also showed that the heritability of yield was 0.69 and 0.54 under stress and non-stress conditions, respectively. The genetic correlation between the two was 0.24. This observation was further supported by work by Bernier et al. (2008) using a QTL on chromosome 12. Indeed, QTLs with large effects for drought tolerance have been reported in other rice populations (Gao et al., 2005).

In another study involving yield response under stress, up to 50% of yield variation was found under severe drought in upland conditions for an F2 segregating population generated from crossing two upland tropical japonica varieties, Vandana and Way Rarem (Yue et al., 2006). This finding confirms an earlier finding that broad-sense heritability for yield in stress environments was similar to that in non-stress conditions, indicating that selection for yield under stress conditions can also be effective for selection under favorable conditions (Atlin, 2003). In the case of populations derived from *O. glaberrima* x *O. sativa* crosses, similar information on the transmission of the drought tolerance trait is lacking, yet improved drought tolerance was reported in progenies developed from O. *glaberrima* x *O. sativa* crosses (Sano et al., 1984; Jones et al., 1997a; Maji et al., 2001).

1.4.5 Mode of gene action for drought tolerance

Current information on the mode of gene action for drought is that different populations show different mechanisms for drought tolerance. For instance, both additive and epistatic effects for drought tolerance are reported in some populations (Bernier et al., 2005). In another study, both additive and dominance effects were found important in the inheritance of characters associated with drought tolerance at the vegetative stage (Ekanayake et al., 1985). On the other hand, seedling stage leaf length, leaf rolling, leaf drying, and root length showed highly significant additive effects when several rice populations were studied (Ekanayake et al., 1985; Ekanayake et al., 1989). In yet another study, a major dominant gene that controlled leaf rolling under drought stress was found (Singh and Mackill, 1990).

In the case of the inheritance of maximum root length, number of thick roots, and root volume, both additive and dominance effects contributed equally to the transmission of the trait when drought was imposed at the tillering stage (Ekanayake et al., 1985). The inheritance of root dry weight was due to both additive and dominance effects in F2 populations, but additive effects contributed more at the F3 stage. Severe drought, when applied skillfully, amplifies the genetic differences between lines (Lafitte et al., 2003). In another population, a yield-increasing allele was derived from the susceptible parent under stress, indicating that epistatic effects were important in explaining drought resistance (Alagu et al., 2005). Later, pleiotropism for leaf rolling and leaf drying was found in the expression of drought tolerance (Rajendra Prasad et al., 2005). Overall, there is evidence that secondary traits, of *O. sativa* genotypes could be used in improvement for drought tolerance. Studies by Fujii et al. (2005), using interspecific rice showed that vegetative characters were associated with drought tolerance. Further information on the inheritance of these traits in *O. sativa* x interspecific crosses is not available in the literature.

1.4.6 Breeding for drought tolerance

Effective breeding for drought tolerance has been conducted by targeting secondary traits. These include seedling vigour (Choi et al., 2003), stomatal closure (Price et al., 2002), filled grains and anthesis delay (Pantuwan et al., 2002; Lafitte et al., 2003). Breeding for drought tolerance using seedling vigour and tillering capacity in rice could also be efficient and cost effective. It is already being used in wheat breeding (Munns and Richards, 2007) and the contribution of plant vigour to increased drought tolerance in rice has been demonstrated to be possible (Namuco et al., 2009). Breeding for improved stomatal closure is important

because it is associated with yield reduction, however targeting stomatal closure is profitable only during early stress; when the drought is short and mild, it is not desirable (Price et al., 2002). Another trait used for drought tolerance (DT) is the leaf water potential measure (LWP). It is strongly correlated with spikelet sterility under drought stress (Lafitte et al., 2003). A major problem with the LWP trait is that it is laborious to measure and therefore suitable only for a limited test population. Delayed anthesis and panicle elongation are also targeted for breeding but are effective only if the period of water deficit is short and panicle elongation resumes following a brief period of stress. Slow panicle exertion implies that the panicle reaches anthesis when part of the panicle is still within the leaf sheath (Liu and Burke, 2006) and the majority of seeds in the covered sheath remain unfilled (Ji et al., 2006). Based on these advantages, workers on drought tolerance have recently been using this trait for drought tolerance breeding (Yue et al., 2006).

Other than secondary traits, putative traits for drought tolerance have been reported by various researchers (Table 1.2). Of eight traits currently considered for breeding, leaf roll is the easiest to score using minimal facilities, while root depth, root pulling resistance, osmotic adjustment, and relative water content and water use efficiency require sophisticated referencing of the environment before they are used effectively in the improvement of drought tolerance. Leaf roll is currently used in selection for drought tolerance during the vegetative growth stage (Pham et al., 2006).

Table 1.2 Putative traits for drought tolerance identified but under further testing

Trait	Proposed function	Reference
Deeper and thicker roots	Explore greater soil volume	Yadav et al. (1997).
Root pulling-resistance	Root penetration into soil layers	Pantuwan et al. (2002)
Root penetration ability	Explore greater soil volume	Clark et al. (2000)
Osmotic adjustment	Turgor maintenance	Lilley et al. (1996)
Membrane stability	Leaves functioning at high temperatures	Tripathy et al. (2000)
Leaf rolling score	Reduce transpiration	Courtois et al. (2000)
Leaf relative water content	Maintenance of plant water status	Courtois et al. (2000)
Water use efficiency	High dry weight gain with transpiration.	Specht et al. (2001)

Source: Adapted from Lafitte et al. (2003)

1.4.7 Efficiency of breeding for drought tolerance

Previous studies have shown that the relationship between yield loss and drought stress level, the drought tolerance index, was often negatively correlated with potential yield and was also dependent on the actual developmental stage of the plants when the stress treatment was applied (Price and Courtois, 1999; Venuprasad et al., 2002). This observation was based on the estimation and exclusion of drought escape and avoidance mechanisms from tolerance. Recently, a plant-wise drought treatment technique was successfully used to separate drought avoidance (DA) from drought tolerance (DT) (Yue et al., 2006). This finding makes breeding for drought tolerance attainable because precise estimation of phenotypic expressions makes selection of promising lines easy and accurate.

1.5. Breeding for reduced losses due to grain shattering

1.5.1 Origin and causes of grain shattering

Grain shattering (GS) is a primitive trait common in wild ancestors of rice. It is characterized by the shedding of seeds as soon as the spikelets mature. It is postulated that the trait was positively selected for during rice domestication and a natural mutation that involved an amino acid substitution occurred (Li et al., 2006a). According to Vaughan et al. (2008), a single domestication event for *Oryza sativa,* based on the functional allele for non shattering as well as five other single nucleotide polymorphisms, occurred in the region of the Yangtze River valley. Studies to determine the origin of grain shattering in a wide range of rice populations found that the non-shattering allele was present in all the *O. sativa* varieties surveyed, including members of indica, tropical and temperate japonica subpopulations. The allele for non shattering, and not the non-shattering plants themselves, was dispersed in the generated population. It is possible that one subspecies was domesticated, was subsequently crossed to local wild rice, and carried to new locations. At the moment, it is considered that artificial selection favored mutations that reduced shattering without losing it completely. This view is supported by the fact that continuous variation in shattering is observed in cultivated rice (Oka, 1988).

Grain shattering is controlled by genetic, morphological and physiological factors (Fukuta, 1995; Jin et al., 1995). Morphological and physiological processes that lead to grain shattering begin with the development of an abscission layer between the spikelet and pedicel (Fukuta, 1995). The degree of grain shattering is inversely associated with the rate

of degeneration of cells at the base of the sterile glume (Fukuta, 1995). The morphology of the abscission layer varies with rice varieties (Jin and Inouye, 1982; Jin, 1986; Jin et al., 1995). Some varieties do not have an abscission layer and are non shattering, while other varieties have a non-degrading abscission layer (Jin and Inouye, 1982; Li et al., 2006a). Other varieties have a partially developed abscission layer on the palea side of the pedicel, while some varieties have a complete abscission layer on the palea side and an irregular abscission layer on the lemma side (Fukuta et al., 1994). Detailed studies on developmental gene function have revealed that two shattering genes, *sh4* and *sh2*, are important for abscission layer formation (Fukuta and Yagi, 1998).

1.5.2   Inheritance and gene action for grain shattering trait

Transmission of the shattering trait in *Oryza sativa* has been widely studied using conventional genetic approaches. Studies by Kadam (1936) showed that the inheritance of the shattering trait was controlled by a few genes. Work by Chandraratna (1964) confirmed the earlier report that shattering was controlled by a few single genes and revealed further that the trait was controlled by several genes in some rice populations. More studies confirmed that a few genes controlled the GS habit (Eiguchi and Sano, 1990; Oba et al., 1990; Fukuta and Yagi, 1998). Hu et al. (1964), however, used *O. sativa* indica x *O. sativa* indica cross generations to study the segregation pattern of GS and concluded that the trait was inherited as a monogenic and recessive trait. In studying the inheritance of GS, Tang and Morishima (1988) found various segregation patterns, ranging from monogenic to continuous, depending on the populations generated. According to studies by Konishi et al. (2006), indica rices have higher shattering levels than japonicas and crosses between indica and japonica display transgressive segregation for the trait. In another study conducted by Fukuta and Yagi (1998), using highly susceptible *O. sativa* indica rice crossed with resistant mutants, a high broad sense heritability of 0.86 was realized. A detailed analysis revealed that the source of resistance was the gene *sh4*. These findings indicate that there is a diverse nature of transmission for the shattering trait within the intraspecific populations. In the case of interspecific fixed lines crossed with *O. sativa*, there is no information on the transmission of the trait, despite increased initiatives to use wide crossing in breeding.

Several mechanisms that influence the expression of the GS trait have been reported. The modes of action of genes for grain shattering vary greatly in diverse rice populations, with

some exhibiting recessivity and others dominance. Studies involving *O. sativa* and different relatives of rice revealed that the mode of gene action varies within populations. Fukuta and Yagi (1998) reported a recessive gene response in genes *sh1, sh2,* and *sh4*, while *sh3* and *sh5* were dominant. This is in contrast with earlier reports that solely attributed the nature of inheritance to the control of dominant genes (Chandraratna, 1964). In studies involving *O. sativa* and *O. glaberrima,* both recessivity and dominance were responsible for transmission of the trait (Li et al., 2006a). Subsequent studies involving relatives of *O. nivara* showed that both dominant and additive gene action were responsible for the transmission of the shattering trait, with *sh4* and *sh3* being dominant and *sh8* additive (Li et al., 2006a). In another study, *sh4* was a recessive locus in several wild rices, namely *O. rufipogon*, *O. glumaepetula*, *O. nivara*, and *O. meridionalis*, but showed varying degrees of dominance once crossed with cultivated rice (Li et al., 2006a).

1.5.3 Association of grain shattering trait with other quantitative traits

Other than the selection of rice mutants that have optimum shattering ability, there is no information on breeding work with the major objective of improving grain recovery. However, high linkage frequencies have been identified between genes that confer shattering with genes that modulate other functions. These include shattering inhibitor genes (Nagai et al., 2002), blast resistance (Li et al., 2006a), seed dormancy (Oka, 1988; Cai and Morishima, 2002), panicle spreading (Nagao and Takahashi, 1963; Eiguchi and Sano, 1990), red pericarp and dwarfism (Oba et al., 1990).

Other traits that show a linkage with grain shattering are lack of legule (Sobrizal et al., 1999), dwarfism (Kobayashi, 1973; Yokoo and Saito, 1986) and phenol staining (Sobrizal et al., 1999). Any breeding work that aims at eliminating these traits will indirectly affect the shattering capacity of the crop. For instance, if breeding is done to eliminate the red pericarp in rice because it contains anthocyanin pigments that are known to reduce the bioavailability of iron and protein, the shattering capacity is compromised. In the case of the agronomic traits in *O. sativa* and *O. sativa* crosses, Agarwal et al. (1989) found high heritability and genetic gain for both spikelet numbers per panicle and shattering percentage, but there was no association between the two traits. However, Mohanty and Sahu (1968) found a high association between grain shattering and the number of spikelets per panicle in another population. A breeding objective that targets grain shattering and

traits associated with it at the start of the breeding process could lead to optimum grain-shattering ability.

1.6. Genetic function of grain shattering

Detailed quantitative trait locus analyses (QTL) using *Oryza sativa* have found that the GS trait is quantitatively inherited. In a study involving F2 populations derived from a cross between Nipponbare (japonica) and Kasalath (indica), five putative QTLs on chromosomes 1, 2, 5, 11 and 12 were identified (Fukuta et al., 1995). Detailed studies of their functions indicated that chromosome 1 expressed the trait very strongly, but the other four loci expressed it more weakly. The QTL at the putative locus on chromosome 1 explained the value of the LOD score and the variance by 45.5% and 68.6%, respectively. This high LOD for a few loci probably explains why the effect of a few genes is reported in several classical genetic analyses. A similar study conducted using a different rice population developed from *O. sativa* and other relatives of rice showed that loci *sh4* contributed more than 65% of the phenotypic variation (Xiong et al., 1999; Cai and Morishima, 2000; Cai and Morishima, 2002; Li et al., 2006a). In addition, Li et al. (2006b) analyzed three gene loci, *sh4*, *sh3* and *sh8,* and showed that they contributed 69%, 3.1% and 3.6% of the phenotypic variation, respectively, confirming the importance of *sh4*. Recently, s*h1* and *sh4* have been confirmed as playing the more important role in seed shattering than others identified (Onishi et al., 2007a). In practical breeding, they are the loci targeted for rice improvement.

Overall, QTLs for shattering have been reported on all the 12 chromosomes of rice except for number 10 (Xiong et al., 1999; Zhong et al., 1999; Bres-Patry et al., 2001; Cai and Morishima, 2002; Thomson et al., 2003; Gu et al., 2005; Lee et al., 2005; Ji et al., 2006; Konishi et al., 2006; Li et al., 2006a; Onishi et al., 2007a). However, this information was obtained using wild and weedy rice forms. Within the *O. sativa* populations, five putative QTLs have been identified (Fukuta., 1995; Onishi et al., 2007b). It now remains to be explained which of the known QTLs are present in the population derivatives from *O. sativa* and *O. glaberrima* crosses, which are target populations for rice improvement. Considering that gene *sh4* and *sh1* contribute the highest levels of phenotypic variation, and also that *sh4* was detected in wild x cultivated rice crosses while *sh1* was detected in cultivated rice (*Oryza sativa*), detailed studies on populations derived from *Oryza glaberrima* and *Oryza sativa* crosses using cloned *sh4* and *sh1* primers could guide the identification of these genes in *O. glaberrima.* These two genes form part of five genes already mapped in the rice

genome. The five genes are *sh1* on chromosome 11 (Nagao and Takahashi, 1963), *sh2* chromosome 1 (Oba et al., 1990), *Sh3* on chromosome 4 (Nagai et al., 2002), *Sh4* on chromosome 3 (Fukuta, 1995; Fukuta and Yagi, 1998) and *sh-h* on chromosome 7 (Ji et al., 2006). This parallel information on the shattering gene in wild rice could be useful in quickly understanding the function of shattering genes in *O. glaberrima*.

1.7.   Interspecific rice breeding

Currently, relatives of cultivated rice have been demonstrated as providing useful genes for various stress tolerances. Some of the important genes include resistance to brown plant hopper (BPH), bacterial blight (BB), blast and tungro disease (Brar and Khush, 1996). In addition, they include drought tolerance and tolerance to acid sulfate but, unfortunately, poor crossability is a major limitation to more efficient exploitation of these traits (Sitch et al., 1989). Efforts to overcome these barriers are important research options for rice improvement. Another benefit of such wide crosses includes cytoplasm male sterility (CMS) development, which is used in hybrid rice production. Among the relatives of *Oryza sativa, O. glaberrima*, *O. barthi,* and *O. longistaminata* have been used in the generation of new rice types. Moreover, some varieties with wide compatibility (WCV) can be used to solve the problem of sterility (Ikehashi and Araki, 1984). With the great potential for using WCVs for the elimination of sterility, extensive screening of WCVs is necessary.

1.7.1   Nature of cross compatibility

Crops have natural isolation mechanisms for maintaining individual species, the major one being the limitation of out-crossing. Isolation promotes genetic differentiation and permits different populations to coexist without losing their identity, but the degree of isolation is inversely correlated to out-crossing. In cultivated rice and its relatives (weedy rice, wild rice and other cultivated species), different degrees of out-crossing have been reported. For example, Asian rice (*Oryza sativa* L.) has a 0-1% out-crossing rate and African rice (*Oryza glaberrima)* has a 7-55% out-crossing rate (Heuer and Meizan, 2003). This natural adaptation makes it difficult to cross any two rice species, depending on the degree of reproductive barriers that exist. Common genetic reproductive barriers include lack of seed set, pollen infertility, spikelet infertility and reduced plant vigour (Kitampura, 1962; Bougerol and Pham, 1989). Other forms of barriers are morphological and associated with domestication. For instance, wild rice types often have longer, exerted (protruding) stigmas that are more exposed to pollen from nearby plants than those of cultivars, which tend to

remain at least partially within the hull and are more protected from non-self pollen (Oka, 1988). However, in cultivated rice, anthers are shorter than the stigma and pollen is released shortly after the florets open. In the relatives of *O. sativa*, pollen is released long after the florets have opened and the anthers are longer, thus increasing the chance of out-crossing (Oka, 1988).

To date, work on cross compatibility between *O. sativa* and its relatives (wild rice, weedy rice and other cultivated rice types) has been reported. For example, Sunisa et al. (2009) found that *O. rufipogon* and *O. sativa* are interfertile but produce different rates of seed set. In crosses involving *O. sativa* and *O. glaberrima,* seed set varying between 30-56% was found (Jones et al., 1997a; Heuer and Meizan, 2003).

Generally, lower seed set has been recorded in crosses where *O. sativa* is female than where *O. glaberrima* is female, indicating that unidirectional effects were important and probably maternal effects were the cause in some sets of crosses (Sitch et al., 1989; Heuer and Meizan, 2003). Although both *O. glaberrima* and *O. sativa* are in the same genome group, the AA group that would make homologous paring complete and the progenies fertile, deviations are frequently observed and genic slips were suspected (Brar and Khush, 1996). However, an earlier study indicated that negative reproductive traits followed a Mendelian pattern in some *O. glaberrima* and *O. sativa* crosses (Chang, 1976), while other workers found that meiotic irregularities caused deviation from the Mendelian pattern in crosses involving other *O. glaberrima* and *O. sativa* populations (Heuer and Meizan, 2003), thus supporting the view of Brar and Khush (1996) that crossability between *O. glaberrima* and *O. sativa* is influenced by variation at genic level. In the case of hybrids developed through crossing interspecific fixed lines (*O. glaberrima* x *O. sativa* derived lines) with *O. sativa,* there is only speculation that reproductive barriers in the crosses between them is minimal. To understand the nature of gene transfer between species, there is a need to know what types of genetic mechanisms are involved in reproductive barriers between species.

1.8. Farmers' preferences

Flower emasculation refers to the process of making the male organs of a plant's floret non functional. This can be done through removal of anthers or by killing the anthers using hot

water (Chandraratna, 1964; Coffman and Herrera, 1980). The method that involves removal of anthers was described by Coffman and Herrera (1980) and Jennings et al. (1979). When the inflorescence has reached anthesis, a few florets (spikelets) that are ready for anthesis the following day are selected a day before. The tip of each of the selected spikelets are cut about one-third off its longitudinal length. The anthers are then carefully removed with forceps or through use of suction equipment. All the six anthers are removed through the cut end while avoiding damaging the stigmas. All other flowers (spikelets) are removed from the selected panicle. Each panicle is then covered with a bag that is spore impermeable bag until the next morning to avoid natural contamination of other pollen. The next morning, at the time anthesis starts, the emasculated florets are opened and pollen from selected parents deposited onto their stigmas. It is recommended that the materials are kept in dark room and taken out just before crossing operation.

The use of forceps to remove anthers is difficult and prone to errors. Other anther suction devices are preferred because they are more efficient than the technique that involves the use of forceps. Two types of flower emasculation devices are currently used. These devices operate on the principal of air suction force, where individual anthers are forcefully pulled off the florets (Chandraratna, 1964; Coffman and Herrera, 1980; Navarro and Virmani, 1987). The first emasculator type is a prototype developed by the International Rice Research Institute (IRRI) and the second type is a modification of the IRRI prototype (Coffman and Herrera, 1980). The second type of emasculator was developed with a view of reducing weight and for use in remote areas where there is no electricity. It uses a simple portable and cheap 12 V power unit. Both emasculators are manufactured in advanced fabrication centres and their prices are prohibitive for breeders in Sub-Saharan Africa.

The hot water technique of flower emasculation was described by Jennings et al. (1979). It involves immersing the rice panicles in to the hot water of 43^0 C for 5 to 7 min. Then the anthers in a flower ready to anthesis on the following day are killed and at the same time the flower becomes open by the stimulation of the hot water. All flower except the opened ones are removed.

1.9.   Grain shattering assessment and evolution grain shattering testers

Several methods have been employed in assessing grain shattering trait. To date seven methods have been used to assess grain shattering. In the first method, panicles are

29

shaken and grains that fall off are recovered, counted and grouped (Ramiah and Rao, 1953). However, owing to variation in the density of the panicles, the impact of shaking is liable to be variable. The second method involves cutting panicles and placing them on a smooth wooden board with an inclination to a horizontal board. The tip of the board is fixed facing downwards to allow grain recovery (Rao, 1935). A glass roller is released and allowed to roll over the panicles. Shattering is computed from the ratio of the number of grains detached to total number of grains on the panicle. The third method, involves counting grains that fall freely at 30 days after anthesis. In this method, the number of scar marks and non-grain bearing pedicels are assumed to have shattered. It is a common method used in screening grain shattering in cereals. The fourth method involves dropping panicles from some height on to a hard surface and the grains that get dislodged from inflorescence are counted (Ramiah and Rao, 1953; Sahu and Sahu, 1981). This method was later improved by placing panicles on a wooden frame and allowing it to be released from a spring so that it strikes an inclined board (Jacobi et al., 1974). The effect of varying panicle densities is reduced. However, it is still suitable for laboratory work only.

The fifth and sixth method were developed by Ichakawa et al. (1990). In the fifth method, a conventional rice thresher was modified. Panicle stalks are cut to a length of 500mm from the remaining top. The stalks are divided into standard size units, which are then manually fed to the testing device. It is suitable when large sample sizes can be used for assessment but cumbersome for field experiments. The sixth device measures the force required to separate grains from panicles directly. In the measurement, static test of shattering ability is estimated. However, it is still too cumbersome for field experiments. The seventh equipment is a digital breaking strength equipment device described by Li et al. (2006b) . It uses a digital force gauge (FGC-1B, Shimpo, Japan) and operated by attaching it upside down to a stand (FGC-50L) (Li et al., 2006b). This is the most appropriate in terms of accuracy but not practical for field screening of large numbers of genotypes. It is also not under commercial production at the moment.

1.10. Farmers' preferences

Information on farmersqpreferences for rice varieties has been documented in many rice-growing regions. In terms of field performance, farmers prefer varieties that provide a high and stable yield, but their choices are determined not only by their own personal

preferences but also by their perceptions of the interests of consumers and processors. In the case of rice, consumers have high culinary preferences, especially regarding cooking and eating qualities. A popular and preferred rice variety is expected to have preferred grain, cooking and eating qualities (Tan et al., 1999). However, quality is often difficult to identify as it is defined by end users and their preferences are highly variable (Sall et al., 2000; Traore, 2005). Generally, however, west African markets and domestic consumers prefer non-waxy rice that is medium-grain in size (Buddenhagen, 1986; Traore, 2005). In East Africa, both non-aromatic and aromatic types with medium and long grain size have been reported (ECARRN, 2005). Farmersq preferences can guide breeders in the development of new rice varieties (Sall et al., 2000). This was found to be the case in the breeding of cassava in Ghana (Manu-Aduening et al., 2006) and maize in Zimbabwe (Derera et al., 2006). In Uganda, there is limited information on farmer variety preferences for guiding breeders in rice improvement.

1.11. Summary of literature

The review demonstrated that the exploitation of genes for resistance to several biotic and abiotic constraints has been through intraspecific breeding and lately, interspecific breeding. It also underscored the need to use wide crossing in order to understand the function of grain shattering and drought tolerance traits.

Problems associated with crossing rice with its relatives were highlighted and the need to understand the problem in the new generations of the interspecific rice was reviewed. In particular, justification for using interspecific fixed lines as one set of parents was provided. This review also provided referenced information on farmersqrice variety preferences, the state of rice breeding in terms of tools for breeding, adaptation of rice types to different eco-zones and current procedures for assessing grain-shattering ability. The review also pointed out that breeding for tolerance to drought and optimum grain-shattering ability could be enhanced.

This literature review established that progress has been made in improving rice for drought tolerance and grain shattering using biotechnology approaches. However, most of the biotechnology approaches have limited use at the moment in terms of usable products. Therefore, much work for the improvement of rice for drought tolerance and grain-shattering ability lies in its genetic improvement using conventional breeding. In order to exploit the

31

genetic potential of the crops, studies to understand the genetic function of genes for drought tolerance and grain shattering in landraces and interspecific fixed lined are required.

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CHAPTER TWO

Farmers' Preferred Rice Varieties and their Implications for Breeding in Uganda

Abstract

Uganda has initiated rice breeding but little information is available on production constraints and farmers' preferred varietal characteristics. A study was carried out to identify farmers' perceptions of rice cultivars grown, production constraints, preferred characteristics and their implications for breeding in the country. Data were obtained through participatory rural appraisal (PRA) methods and a formal household survey of 380 farmers from 5 districts representing major rice production systems in Uganda from March 2007 to June 2008. Data obtained through multiple listing was subjected to chi-square tests to determine independence between different districts and the different variables using the SPSS 15.0 statistical package. Simple tallying, ranking, pair-wise ranking and Kendall's Coefficient of Concordance (W) were appropriately used to analyze data using Genstat package 12.0. Seventeen different crops were grown by the interviewed farmers. Rice was ranked fourth in importance after maize, beans and sweet potatoes. Thirteen rice cultivars were being grown by farmers, of which NERICA-4 was the most preferred (78%). Majority of farmers preferred high yield, high market price and aroma with 60.9%, 58.3% and 49.4% of farmers, respectively mentioning them. Low yield was the most important varietal weaknesses making 64.6% of the responses. Susceptibility to drought stress and unacceptable grain quality were important varietal weaknesses in all districts surveyed. Twenty-two cultivars previously grown had already been dropped, of which varieties, SUPA, IRAT112 and TOX 4 had the highest frequency of 14.6%, 12.0% and 6.9%, respectively. The major reasons for dropping rice varieties were rice blast disease, long growth duration, rice yellow mottle virus (RYMV) disease and low market price with frequency of 16.8%, 6.0%, 4.6% and 4.4%, respectively. African rice gall midge (AfrRGM) and RYMV varied across districts as a reason for dropping rice varieties at $P=0.05$ level of significance, while low yield, drought and rice blast, varied at $P=0.01$. The study confirmed that rice is an important crop in Uganda. Farmers preferred cultivars that combine high yield potential, early maturity, drought tolerance, aroma characteristics and special market attributes targeting specific ecologies should be the breeding strategy. The implications of these outcomes for similar niches elsewhere are also discussed.

2.1   INTRODUCTION

Rice is becoming a major commercial food crop in East and Central Africa (ECARRN, 2005). In Uganda, it is the fourth most important cereal food crop after maize, sorghum and finger millet (FAOSTAT, 2007). It has higher returns on investment than most other staple cereal crops in the country, with an input to output ratio of 1.83 compared with maize-hybrid (1.2) or sorghum (1.6)(Kijima et al., 2006). The total annual rice production grew from 153,000 MT in 2005 to 162,000 MT in 2007, making it twelfth in ranking in Africa (FAOSTAT, 2007).

Despite this increasing trend in production, an average of $60 million is spent annually on its importation. This reliance on the world market for rice is becoming a more risky and expensive strategy. It is therefore important that local rice production is promoted, but this requires breeding programmes that cater for specific agro-ecological constraints and farmersq preferences. The comparative commercial advantage of rice over other cereal crops in Uganda, its increasing consumption by urban dwellers, coupled with the low and unpredictable market price of traditional cash crops (Odogoola, 2006), has made rice production a priority of the Ugandan government in an action plan dubbed Prosperity for All (PMA, 2007) for eradicating poverty and creating wealth. A strategic option to increase rice production is to increase yield through breeding. This is plausible considering that there is an expansion in the production area but the yield average is only modest (Candia et al., 2008; FAOSTAT, 2008).

The participation of farmers in plant breeding is critical in ensuring that technologies developed are accepted and their adoption increased (Lightfoot et al., 1988; Sperling et al., 1993). Generally, farmersqneeds are broad ranging, including soil moisture, soil conditions related to labour, food availability, intercropping and weed competition (Sperling et al., 1993; Sall et al., 1998). In the case of rice, culinary and cultural preferences regarding taste, colour, consistency, size of grain, cooking time, processing quality and suitability for preparation into different food forms are also of paramount importance (Ashby et al., 1989; Sall et al., 1998). Farmersq local varieties are an important germplasm in plant breeding programmes because they have several traits preferred by farmers (Danial, et al., 2007; Singh et al 2004).

In Uganda, the critical information required for rice breeding is limited. A clear understanding is needed of the varieties farmers have access to at a particular time, the constraints associated with them, their characteristics, uses and farmer preferences. Farmersqperceived rice production constraints are listed in a few survey reports but they are not ranked. These constraints need to be ranked in order to allocate limited resources to critical breeding goals. For example, up to 13 different seed-borne diseases have been reported in rice in Uganda but are not ranked according to importance (Biruma et al., 2003). However, preliminary findings show that rice blast (*Pyricularia Oryzeae* Matsu & Tomoy) has the highest incidence (Ogenga-Latigo, 1995). Recent reports indicated that constraints on rice production include termite damage, bird damage and losses due to drought effects and storm damage (NARO, 2005; Odogoola, 2006). Isolates with symptoms of RYMV disease, collected from eastern Uganda, confirmed the presence of rice yellow mottle virus (Pinel and Fargette, 2006). Imanywoha et al. (2002) mentioned that most upland rice varieties are susceptible to rice drought stress. Lamo et al. (2007) indicated that drought was precarious and could undermine upland rice production in the country.

Farmer participation in variety selection is a key component of plant breeding, where farmer involvement is pivotal. In Uganda, three forms of farmer participation in rice variety selection have been reported. Firstly, in an approach that involved testing new varieties with farmers and recommending their production without official release (Abong, 1999). Secondly, rice introductions were tested on-station and farmers were involved only during on-farm testing. A variety that became very popular, IRAT 112, was tested and deployed using this approach (Abong, 1999). Thirdly, introductions were tested using participatory variety selection (PVS) in collaboration with the African Rice Centre (formerly known as the West African Rice Development Association-WARDA). Five upland varieties, Namulonge Rice (NARIC) 1 and 2 and New Rice for Africa (NERICA) 1, 4 and 10 were released using a modified form of the PVS (NARO, 2007). However, loose grain attachment to the pedicel was observed as a major reason for discarding several potential lines. A common denominator in these variety selections is that the materials were developed based on regionally identified breeding priorities. The development and deployment of farmer preferred rice varieties that address local situations in Uganda would be more easily achieved if farmers were involved early on in the selection of new varieties. Engaging farmers in the varietal development process would help breeders understand their needs

and preferences, which in turn would help in the selection of appropriate genetic materials from them (Sthapit et al., 1996; Witcombe, 1996; Thelma et al., 2003; Sharma et al, 2006;).

2.2   Objectives of the study

The overall goal of the study was to identify farmersqneeds and preferences in rice varieties using participatory rural appraisal approaches (PRA).

The specific objectives were to:

i.   identify rice production constraints;

ii.   understand farmersqpreferred rice traits and characteristics; and

iii.   assess the status of drought and grain shattering in rice in the country.

2.3   MATERIALS AND METHODS

2.3.1   Study area and sampling procedure

The study was conducted in Northern, Eastern and Western regions that form the traditional (Northern and Eastern) and new (Western) rice farming zones of Uganda (Abong, 1999; Kijima et al., 2006). Within the Northern region, there is an irrigated rice scheme covering about 750 ha. The region also has an extensive rain-fed lowland area covering 30,000 ha. The Eastern region has long experience in rice production, with two major irrigated rice schemes covering more than 3000 ha developed and under cultivation since 1945 (Abong, 1999). Several small-scale rain-fed lowland rice production projects, under out-growersq schemes, are being conducted under the Japan International Cooperation Agencies (JICA) arrangement in the Northern and Eastern regions (Odogoola, 2006). Western region has a relatively short experience in rice production but accelerated production of upland rice has been witnessed since 2002 when new upland rice varieties were released in the country (Kijima et al., 2006).

Districts and sub-counties were selected using a stratified sampling approach in two stages. Selection of the districts and sub-counties was based on preliminary information on contrasting experiences in rice production and production levels. For example, farming communities that had been in rice production for less than five years were considered new while those that had been in rice production for more than 5 years were considered as experienced rice farmers. Two districts, Lira and Dokolo, from the Northern zone, Iganga and Bugiri (Eastern) and Masindi (Western) were selected, and two to three sub-counties

were selected per district. Individual farmers were selected by randomly targeting those who grew rice, using a list from the Department of Agriculture. 350 individual farmers from the five selected districts were interviewed. The individual farmers were randomly selected from a list of rice farmers provided by the extension staff who were part of the survey team. Another 30 key informants comprising farmers with more than 10 yearsqexperience in rice production (10), rice millers (10), and rice traders (10) were selected for group discussions. The key farmers were Of the 30 key informants, comprising 14 males and 16 females, 12 were from the Eastern region, 8 from the Northern region and 10 from the Western region.

2.3.2 Data collection and analysis

Quantitative and qualitative approaches were used to gather data. In the quantitative approaches, information was gathered mainly through survey and group discussions, while qualitative approaches involved making observations in the study area. A workshop was conducted in May 2007 at Mukono Zonal Agricultural Research and Development Institute (MUZARDI) to identify key rice production zones, general constraints and sampling procedures.

Individual interviews were conducted using pre-tested, semi-structured questionnaires to capture information on the farmersqdemographic characteristics, their preferred varieties and the susceptibility of these varieties to various biotic and abiotic stresses; problem listing, observations and cross-checking tools were used. The survey was conducted from March 2007 to June 2008 by a team comprised of the principal investigator, three enumerators and local extension staff. Group discussions focused on salient issues that accrued from the survey of individuals. These areas were the criteria of selecting high-yielding varieties, the period when different varieties were introduced, and key variety strengths and weaknesses. Responses to some questions asked during the interview had a more than one possible answer. It is referred to as multiple listing.

Data obtained through multiple listing were subjected to chi-square testing to determine independence between different districts and the different variables using SPSS (version 15.0) computer package. Chi-square tests (X^2) for fixed ratio hypothesis for the variables and districts were then used. Information from group discussions was tallied, ranked and compared using pair-wise ranking procedures. This method was used to determine preferred traits, major weaknesses of rice varieties grown and the rice varieties grown.

Kendall's Coefficient of Concordance (*W*) was used to compare responses among males and females using the Genstat package 12.0.

2.4 RESULTS

2.4.1 Demographic characteristics and cropping systems

Eighty seven percent of the farmers interviewed were male. Their average family size was six persons. They grew mainly upland rice, although 20% grew both upland and lowland rice or irrigated rice. Information on the average age of the farmers, education level, proportion of rice sold and preferred methods of cooking rice is summarized in Table 2.1.

Table 2.1 Demographic characteristics and rice consumption among farmers interviewed

Category	Groups			
Average age	Age in years 35			
Formal education level	Primary education (48%)	Informal education (25%)	Secondary education (16%)	Institutions (11%)
Proportion of rice sold	Rice marketed (86%)		Rice consumed (14%)	
Preferred method of cooking	Boiled (60%)		Cooked with meat (30%)	

A total of 17 different crops were grown by the farmers interviewed. Chi-square analysis revealed that crops grown by the farmers differed significantly among the districts (Table 2.2). Chi-tests for fixed ratio hypothesis for the row variables (different crops) were then applied separately for each row. The choice of crops varied significantly across districts, except for maize and beans at 1% and sunflower at 5% level of significance. Maize, beans, sweet potatoes, rice and groundnuts were the most important crops grown. Of the five districts, Lira had the highest proportion of farmers who chose rice as a major crop (85.7%), followed by Bugiri (35%).

Table 2.2 Farmers' preferences (%) for different crops in five districts in Uganda

Crop	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	X^{2a}
Maize	98.6	80.0	97.1	87.1	77.1	88.0	4.31
Beans	87.1	81.4	64.3	94.3	85.7	82.6	6.35
S.flowerr	1.4	0.0	4.3	0.0	4.3	2.0	12.17*
Tobacco	4.3	2.9	0.0	1.4	8.6	3.4	14.17**
Cassava	11.4	5.7	0.0	2.9	14.3	6.9	25.48**
Sugarcane	0.0	0.0	14.3	4.3	4.3	4.6	31.48**
Sorghum	7.1	12.9	0.0	0.0	0.0	4.0	38.36**
Bananas	7.1	0.0	28.6	8.6	14.3	11.7	44.28**
S.potatoes§	80.0	21.4	52.9	24.3	38.6	43.4	50.89**
Vegetables	2.9	0.0	30.0	2.9	4.3	8.0	62.00**
G.nuts«	21.4	11.4	75.7	20.0	22.9	30.3	72.21**
Millet	35.7	0.0	1.4	0.0	10.0	9.4	90.98**
Sesame	32.9	0.0	0.0	0.0	5.7	7.7	91.93**
Cowpea	34.3	0.0	0.0	0.0	5.7	8.0	96.00**
Rice	85.7	14.3	40.0	35.0	14.3	37.9	105.66**
Cotton	11.0	30.0	17.1	0.0	14.3	14.5	160.20**
Others [b]	0.0	1.4	4.3	0.0	5.7	2.3	14.54**
	285.8	238.7	233.3	100.6	62.6		921.00

Calculated chi-square value=**1823.2** and tabulated values at 0.05 and 0.01 probability level are 83.68 and 93.22 respectively, at df= (17-1)*(5-1)=64. NR, data not recorded

Totals more than 100 because of multiple response

[a]= Calculated chi-square for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01 probability level is 9.49 and 13.28 respectively.

[b]=Others included (green gram, yams and pigeon peas)

(«G. nuts=Ground nuts; r S. flower=Sunflower, r S. potatoes=Sweet potatoes)

2.4.2 Rice cultivars grown and their preferred traits and weaknesses

Thirteen rice cultivars were being grown by farmers in the selected districts, of which NERICA 4 (New Rice for Africa) was the most frequently grown (78%) (Table 2.2). Chi-square analysis revealed that the chance of choosing to grow all the rice varieties varied significantly at the 1% level of significance, except for the varieties Congo, TOX 4, TOX 5 and IRAT 112. The choice to grow NERICA 1 varied significantly at the 5% level of significance, while all other varieties were significant at 1% level (Table 2.3).

Table 2.3 Farmers' preferences (%) for different rice varieties in five districts in Uganda

Variety name	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	X^{2a}
Congo	000.0	00.0	00.0	00.0	00.8	00.2	003.20**
TOX 4	000.0	00.0	00.0	00.0	01.4	00.3	005.60**
TOX 5	000.0	00.0	00.0	00.0	01.4	00.3	005.60**
IRAT 112	0.0	2.9	0.0	0.0	1.4	0.9	7.76
NERICA 1	7.1	10.0	0.0	11.4	10.0	7.7	10.90*
NERICA10	5.7	0.0	0.0	0.0	1.4	1.4	17.16**
NERICA 4	100.0	81.4	94.3	57.1	58.6	78.3	20.11**
TOX 9	0.0	0.0	0.0	5.7	11.4	3.4	30.40**
NARIC 1	15.7	1.4	1.4	4.3	18.6	8.3	32.86**
SUPA	10.0	17.1	0.0	0.0	1.4	5.7	40.69**
SUPARICA	0.0	10.0	25.7	5.7	27.1	13.7	42.99**
SIENA	0.0	0.0	0.0	14.3	25.7	8.0	68.12**
NARIC 2	0.0	0.0	0.0	58.6	12.9	14.3	180.27**
	69.9	**63.0**	**61.6**	**166.5**	**104.6**		**465.66**

Calculated chi-square value=465.66 and tabulated values at 0.05 and 0.01 probability level is 65.17 and 73.68 respectively, at df= (13-1)*(5-1) =48. NR, data not recorded
Totals more than 100 because of multiple response
[a]=Chi-square calculated for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01, probability level is 9.49 and 13.28 respectively.

Generally, high-yielding varieties were the most preferred by the farmers, followed by varieties with a high and stable market price. The calculated chi-squared value for farmersq preferred rice traits suggested that the traits differed significantly among the districts (Table 2.4). Further analysis of each trait across the districts revealed that preferences for high head recovery, medium grain size, medium plant height and resistance to pests, drought and disease did not differ significantly across all districts. Preference for high-yielding rice, white grain rice and early maturity rice varied significantly at the 5% level of significance across all districts. Other traits, including aroma, high market value, good taste and soft texture when cooked, differed significantly across the districts at the 1% level of significance. Overall, high yield potential, high market value and aroma were the most important. In Lira, aroma was the highest ranked trait (86%) followed by high market value (81%) and high yields (80%), respectively. Good taste was considered very important in Lira (60%) and Iganga (54%) districts (Table 2.3).

Table 2.4 Farmers' preferences (%) for rice traits in five districts in Uganda

Preferred traits	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	χ^{2a}
High head recovery	4.3	4.3	2.9	2.9	5.7	4.0	1.37
Medium grain size	7.1	7.1	4.3	4.3	5.7	5.7	1.38
Medium plant height	4.3	5.7	1.4	2.9	4.3	3.7	2.86
Resistant to pests	5.7	15.7	8.6	12.9	7.1	10.0	6.98
Drought tolerant	1.4	1.4	2.9	0.0	5.7	2.3	8.26
Disease resistant	10.0	21.4	14.3	8.6	10.0	12.9	8.52
White grains	1.4	2.9	0.0	2.9	7.1	2.9	9.89*
Early maturity	0.0	10.0	10.0	4.3	7.1	6.3	11.42*
High yield	80.0	48.6	45.7	61.4	68.6	60.9	13.25*
Good taste	60.0	34.3	54.3	31.4	25.7	41.1	22.09**
Resistant to Lodging	28.6	5.7	12.9	7.1	14.3	13.7	24.09**
Easy to thresh	5.7	0.0	7.1	0.0	14.3	5.4	25.92**
Soft when cooked	20.0	4.3	27.1	17.1	40.0	21.7	31.84**
High market price	81.4	17.1	54.3	68.6	70.0	58.3	42.73**
Aromatic	85.7	75.7	44.3	15.7	25.7	49.4	75.53**
Others[1]	4.3	2.9	0.0	4.3	5.7	3.4	5.44
	77.3	**83.7**	**21.8**	**42.9**	**65.8**		**291.56**

Calculated chi-square value=291.56 and tabulated values at 0.05 and 0.01 probability level is 79.08 and 88.38 respectively, at df= (16-1)x(5-1)=60. NR, data not recorded
Totals more than 100 because of multiple response
[a]=Chi-square calculated for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01 probability level is 9.49 and 13.28 respectively.
[1] Other traits that included grain husk color, color of grain tip and presence awns

Major varietal weaknesses were obtained through multiple listing approaches by farmers. The responses were compared across the districts using chi-square analysis and the results suggested that the varietal weaknesses did differ significantly among the districts, at the 5% and 1% level of significance. These implied that the occurrence of the varietal weaknesses was dependent on the districts (Table 2.5). Further analysis to compare individual weaknesses across the districts showed that drought and low grain quality were equally important, but low yield, termite damage and the grasshopper effect differed at the 5% level. The remaining weaknesses, including bird damage, loose grain-shattering ability, damage by African rice gall midge (AfRGM) and RYMV, varied significantly among the districts at the 1% level of significance, based on farmersqresponses (Table 2.5).

Table 2.5 Farmers responses (%) on weakness of varieties grown in five districts in Uganda

Weakness	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	χ^{2a}
Drought	4.3	2.9	1.4	1.4	2.9	2.6	2.31
Low grain quality	1.4	2.9	0.0	2.9	4.3	2.3	4.70
Low yield	51.4	60.0	87.1	57.1	67.1	64.6	11.84*
Termites	0.0	4.3	0.0	0.0	1.4	1.1	12.24*
Grasshopper	5.7	5.7	0.0	4.3	11.4	5.4	12.28*
RYMV	0.0	5.7	0.0	0.0	0.0	1.1	22.80**
AfRGM	8.6	1.4	0.0	0.0	0.0	2.0	27.96**
Poor threshability	14.3	4.3	2.9	1.4	0.0	4.6	28.05**
Birds damage	4.3	5.7	1.4	2.9	18.6	6.6	29.00**
Stem borers	2.9	2.9	2.9	35.7	18.6	12.6	67.61**
	57.1	**35.3**	**32.6**	**52.7**	**41.0**		**218.79**

Calculated chi-square value=218.79and tabulated values at 0.05 and 0.01 probability level is 51.00 and 58.62 respectively, at df= (10-1)*(5-1)=36. NR, data not recorded
Totals more than 100 because of multiple response
[a]=Chi-square calculated for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01 probability level is 9.49 and 13.28 respectively.

2.4.3 Previously grown rice varieties and reasons for dropping by farmers

Twenty-two cultivars previously grown had been dropped by farmers (Table 2.6). The chi-square value of the 22 rice varieties dropped revealed that the varieties differed among the districts. Further chi-square tests for fixed ratio hypothesis for the row variables (different varieties dropped) revealed that the varieties including IRAT 112, SUPA, Kenya, Kaiso, WITA 7, K85, SIENNA, TOX 4 and NP 3 differed in choice to be dropped by farmers among the districts. There was no significant difference in choosing to drop the remaining 10 varieties. In the group discussions, the varieties WAB 189, Basmati and Sindano, not mentioned by the individual farmers as rejected, were indicated as having been dropped (Table 2.7). The time when varieties were grown shows that few varieties were being grown in the 1970s and 1980s (Table 2.7).

Table 2.6 Percentage of interviewed farmers who dropped different rice varieties in five districts in Uganda

Variety	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	χ^{2a}
K98	2.9	2.9	0.0	2.9	4.3	2.6	3.82
TOX 5	4.3	2.9	0.0	2.9	4.3	2.9	4.28
Kilombero	4.3	2.9	0.0	2.9	4.3	2.9	4.28
K23	4.3	2.9	0.0	2.9	4.3	2.9	4.28
WITA 9	1.4	2.9	0.0	2.9	4.3	2.3	4.70
K264	5.7	2.9	0.0	2.9	4.3	3.1	5.66
Jaggery	1.4	2.9	0.0	2.9	5.7	2.6	6.97
Others	1.4	2.9	0.0	2.9	5.7	2.6	6.97
NARIC 1	1.4	5.7	0.0	2.9	5.7	3.1	8.30
WITA 6	7.1	12.9	8.6	2.9	4.3	7.1	8.57
SUPARICA	1.4	4.3	0.0	2.9	8.6	3.4	12.69*
SINDANO	10.0	10.0	0.0	2.9	8.6	6.3	13.32**
NARIC 2	12.9	8.6	0.0	4.3	4.3	6.0	15.97**
TOX 4	11.4	5.7	0.0	2.9	14.3	6.9	20.42**
NP3	12.9	2.9	0.0	2.9	4.3	4.6	20.85**
SIENNA	1.4	5.7	20.0	8.6	4.3	8.0	25.86**
K85	15.7	2.9	0.0	2.9	4.3	5.1	28.81**
WITA 7	7.1	4.3	0.0	18.6	4.3	6.9	28.87**
Kaiso	18.6	21.4	4.3	2.9	4.3	10.3	30.96**
Kenya	1.4	1.4	8.6	21.4	22.9	11.1	39.48**
Supa	27.1	32.9	4.3	2.9	5.7	14.6	55.78**
IRAT 112	17.1	34.3	0.0	4.3	4.3	12.0	65.49**
	90.9	**98.8**	**105.9**	**58.4**	**62.4**		**416.33**

Calculated chi-square value=47850.2 and tabulated values at 0.05 and 0.01 probability level is 106.4 and 117.06 respectively, at df= (22-1)*(5-1)=84. NR, data not recorded
Totals more than 100 because of multiple response
[a]=Chi-square calculated for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01 probability level is 9.49 and 13.28 respectively.

Table 2.7 Time-line of popular rice types grown in Uganda and farmers' ways of grouping in terms of growth duration, production zones, their preferred characteristics and key weaknesses

Rice variety	Growth duration months	Rice types	Time started growing	Preferred traits	Weaknesses
[1]K types (K5, K264, K85, K98, K23)	Three and half	[9]Swamp	1972	- high yield - medium growth duration -not pasty when cooked	- red rice - susceptible to rice blast - susceptible to ARGM
[2]Sindano	Five and half	[10]Upland	1970	- high yield -not pasty when cooked	- susceptible to rice blast - long growth duration
Supa	Five and half	Swamp	1970	- high yield - medium growth duration - aromatic	- pasty when cooked - susceptible to rice blast
Bungala	Four	Swamp	1960	- medium growth duration - -not pasty when cooked	- susceptible to rice blast
Basmati	Four	Swamp	1970	- medium growth duration - aromatic	- low yielding - susceptible to rice blast
[3]WAB 189	Four	Swamp	2002	- high yield - medium growth duration	- susceptible to rice blast - susceptible to ARGM
[4]TOX 4	Four	Swamp	2001	- high yield - medium growth duration	- susceptible to rice blast - susceptible to ARGM
[5]WITA 7	Four	Swamp	2002	- high yield - medium growth duration	- susceptible to rice blast - weak culm
WAB 450	Three and half	Upland	2002	- high yield - medium growth duration	- susceptible to rice blast
TOX 5	Four	Swamp	2002	- high yield - medium growth duration - -not pasty when cooked	- susceptible to rice blast - susceptible to ARGM
[6]IRAT 112 (NARIC-1)	Three and half	Upland	1985	- high yield - medium growth duration	- low tolerance to drought - pasty when cooked
[7]NERICA-4	Three and half	Swamp and upland	2003	- high yield - medium growth duration -not pasty when cooked	- difficult to thresh (tight grain attachment)
[8]SUPARICA-1	Three and half	Upland	2003	- high yield - medium growth duration -not pasty when cooked	- red rice - susceptible to rice blast - susceptible to ARGM
NARIC-2	Three and half	Upland	2003	- medium growth duration	- low tolerance to drought
NERICA-1	Three and half	Upland	2007	- medium growth duration	- aromatic
NARIC-10	Three	Upland	2007	- short growth duration	- early maturing

Foot-note:
1. K-Types: Rice varieties tested at Kibimba rice scheme; 2. Supa: Supa V-88 rice variety; 3. WAB: **W**ARDA (West African Rice Development Association)-**A**DRAO **B**ouaké. **B**ouaké is located in Côte d'voire; 4. TOX : (**T**ropical **O**ryza **C**rosses); 5. WITA: **W**ARDA at I**ITA** rice ; 6. IRAT: (Institut de recherches agronomiques tropicales et des cultures vivrières) ; 7. NERICA: **N**ew **R**ice for **A**frica; 8. SUPARICA: **Su**pa **Ric**e; 9. Swamp: Rice grown under lowland and irrigated environments; 10. Upland: Rice grown rainfed upland environments
Source: 2007 PRA survey information by the authors

A chi-square analysis to test if the reasons for dropping rice varieties revealed that the reasons differed significantly across the districts (Table 2.8). The chi-tests for fixed ratio hypothesis for the row variables (reasons for dropping varieties) indicated that low yield, RYMV and AfRGM differed significantly at the 1% level, while drought, rice blast disease and low market value differed significantly at the 5% level of significance. Other constraints were equally important across the districts. Overall, the main reason for dropping the varieties was rice blast disease (Table 2.8).

Table 2.8 Farmers' responses (%) on reasons for dropping previously varieties in five districts in Uganda

Item	Lira	Dokolo	Iganga	Bugiri	Masindi	Mean	χ^{2a}
Grain discoloration	2.0	1.0	0.0	2.0	3.0	1.6	3.25
Soil fertility	1.0	2.0	0.0	2.0	3.0	1.6	3.25
Termites	2.0	1.0	0.0	2.0	3.0	1.6	3.25
Aroma	6.0	2.0	3.0	2.0	3.0	3.2	3.38
Birds	1.0	2.0	0.0	0.0	2.0	1.0	4.00
Others	1.0	2.0	0.0	2.0	4.0	1.8	4.89
Long duration	10.0	6.0	8.0	4.0	2.0	6.0	6.67
Lack of seeds	6.0	2.0	0.0	2.0	4.0	2.8	7.43
Low market price	9.0	6.0	0.0	3.0	4.0	4.4	10.27
Blast	24.0	21.0	18.0	15.0	6.0	16.8	11.36*
Drought	9.0	4.0	0.0	2.0	4.0	3.8	11.79*
Low yield	9.0	2.0	0.0	1.0	3.0	3.0	16.67*
RYMV	12.0	6.0	0.0	2.0	3.0	4.6	18.96**
AfRGM	1.0	12.0	0.0	2.0	3.0	3.6	25.89**
	50.3	24.3	30.6	7.7	18.2		131.04

Calculated chi-square value=131.041 and tabulated values at 0.05 and 0.01 probability level is 68.08 and 78.38 respectively, at df= (14-1)*(5-1)=52. NR, data not recorded
Totals more than 100 because of multiple response
[a]=Chi-square calculated for each row, at df=(5-1)=4, *=significance at 5%, **=significance at 1%, tabulated values at 0.05 and 0.01 probability level is 9.49 and 13.28 respectively.

2.5 DISCUSSIONS

The finding that up to 86% of rice produced was sold confirms an earlier report that most rice produced in Uganda is not for domestic consumption but for the market (NARO, 2005). Rice production is indeed a major form of employment not only during cultivation but also during processing and marketing (Odogoola, 2006). This is contrary to the situation in other regions where rice is consumed largely by the producers (FAOSTAT, 2007). For example, in Senegal about 30% of domestic production is consumed by the producing households themselves and only about 7% of rice produced enters international markets (FAOSTAT,

2007). The results of farmersqpreferred traits showed that market traits were paramount in the selection of rice varieties, thus confirming the need to meet the market demand in addition to individual farmersqdemands. It is, therefore, important that the Ugandan farmers produce rice that meets market demands.

Results showed that there were no differences across rice farmers for choice of crops grown, except for beans and maize; this suggested that rice cultivation did not influence the selection of these key food crops by farmers. Maize and beans are important food security crops in Uganda and only the excess of domestic demand is sold to the market (Odogoola, 2006). However, where there was significant variation in the selection of crops, this could be because most of them were for the market, especially cotton, sunflower and tobacco. It is likely that farmersqselection of these crops for production depended on the suitability of the area for production.

Variety NERICA 4 was the most widely grown rice variety (Table 2.2). Although it is resistant to pests and diseases, is higher yielding than local cultivars, has white grains when hulled and is aromatic, which is preferred by traders, it is difficult to thresh (NARO, 2007). Aroma and high yield are important traits for the selection of varieties across all districts (Table 3). Rice blast disease was not mentioned by farmers as a major constraint on NERICA 4 yet rice blast was reported earlier as a major production constraint (Ogenga-Latigo, 1995). It is therefore likely that NERICA 4 has durable resistance for this disease. Hence, the variety could be used as a parent for introgressive breeding. Resistance to blast was one of the positive attributes of NERICA 4 at the time of release for production (NARO, 2007).

Low yield is a major weakness of the rice types grown in the country. The average national rice yield is 1.5 t ha^{-1} (FAOSTAT, 2007) while the yield potential is 4.5 t ha^{-1} (FAOSTAT, 2007; NARO, 2007). High-yielding varieties are a motivation for farmers. Farmersqstrong preference for high-yielding varieties in Uganda was therefore not surprising because there are few high-yielding varieties. For instance, a summary of rice varieties grown in the country shows that between 1970 and 2002, only three upland rice varieties were under production and high yield was the major reason for their retention. However, a section of farmers grew low yielding but aromatic rice varieties. For instance, the variety Basmati has been under production since 1970 (Table 2.7). Similarly, farmers in the irrigated ecologies

of West Africa generally considered high yield as the most important trait in the selection of rice varieties (Efisue et al., 2008).

All of the farmers interviewed grew upland rice. They indicated that upland rice production was relatively new but they were strongly motivated by the relatively lower labor demand for its production as well as the higher competitive advantage over other crops as a commercial commodity. However, upland production zones are known to be diverse and their production constraints vary greatly across environments. In a study conducted in Asia, the conventional system of rice breeding under lowland and irrigated production systems was found to be very effective due to the homogeneity of these production environments, but the upland rice environment is very different (Atlin, 2003). The current study has demonstrated that the agro-ecological, environmental and socio-economic situations are diverse. For example, in Lira drought was the most important reason for dropping some varieties. Again, in Lira and Dokolo susceptibility to drought was ranked as the most important weakness of the varieties being grown. Drought effect is a widespread problem worldwide and its effect is most critical during the anthesis stage (Atlin, 2003). In Sikasso, Mali, for example, farmers preferred short duration rice as a way of avoiding drought (Efisue et al., 2008). In Uganda, farmers selected an early maturing variety, NERICA 10, during Participatory Variety Selection (PVS) as a strategy to avoid drought effects (NARO, 2007). Farmers in Lira and Dokolo also dropped the long-term variety SUPA due to susceptibility to drought. These responses support earlier views that drought could be a disincentive to farmers to invest and intensify their commercial rice production (Lightfoot et al., 1988; Satheesh, 1996).

Another characteristic preferred by farmers in the northern districts (Lira and Dokolo) was aroma. This could be because most rice farmers were exposed only to rice varieties that were aromatic. The varieties, Supa, Bungala and Basmati, introduced to these regions were indeed aromatic (Abong, 1999). In the same region, the insect pest AfRGM was mentioned as a major pest in Lira and Dokolo. This work confirms an earlier report by Abong (1999) that the insect pest was prevalent in the region.

Farmers in Bugiri and Iganga districts preferred the rice varieties NERICA 4 and NARIC 2 for their bold and white milled grain characteristics. In these two districts, rice blast and low grain quality were the main reasons for dropping several varieties. They also mentioned

high market prices as the first reason for selecting certain rice varieties. Grain quality traits, aroma and "good taste" (soft when cooked) were the second and third reasons respectively for selecting varieties. Eastern Uganda is a traditional rice farming area and they are particular about culinary characteristics.

In western Uganda (Masindi district), high market price was mentioned as the main reason for selecting certain rice varieties, followed respectively by high yield trait and softness when cooked. However, they mentioned the problems of rice blast, drought, low market value and lack of seeds as major reasons for dropping some rice varieties, while a major problem with the varieties being grown was stem borers. This region is the leading area in maize production and there is a natural pressure of stem borer on rice as an alternative host to the pest (Obaa et al., 2005). This could explain why there was a high incidence of stem borer damage in Masindi. It is proposed that breeding that focuses on tolerance to stem borer damage, high yield and possibly tolerance to bird damage should be initiated. Concurrently, Masindi farmers lacked the seed of suitable varieties and rice blast was a problem. This observation is consistent with the fact that recently released rice varieties were resistant to blast but the seed was not yet available in adequate quantities. Farmers who did have access to improved seed still had the rice blast problem.

The current study produced evidence that in the 1980s and 1990s, there was a gap in making new rice varieties available to farmers, as reflected in the time line of rice production. The gap in the release of rice varieties could exist because during this period rice was not ranked as a high priority crop in the country's crop research areas (Abong, 1999). Considering that rice production areas are diverse, there is therefore an urgent need to embark on rice breeding with a focus on specific agro-ecologies now that the current study has demonstrated that rice varietal preferences are structured according to the region.

Farmers indicated that panicle weight and density, known components of yield in rice (Samonte et al., 1998), were important in their selection. It was therefore likely that farmers' participation in variety selection could end in high yields. Other traits not directly linked to yield components were considered to have a direct impact on yield: grain size, thickness of husk and shattering ability. These traits are linked to grain quality attributes, milling quality and grain recovery, respectively. Farmers seem to consider yield not only in the field but

also in the store and in seed form. A similar observation was made in maize evaluation in Uganda (Obaa et al., 2005).

2.6   CONCLUSION AND IMPLICATIONS FOR BREEDING

This work demonstrated the importance of rice in Uganda. The results indicated that farmers had a clear understanding of the major constraints involved in rice production; these include drought, poor threshing ability, low yield and diseases. Farmers expressed the need for better varieties that can enhance the commercial production of rice. The varieties should be drought tolerant, of short duration, high yielding and adaptable to farmersqspecific needs. This study showed that some varietal preferences varied among the districts. Therefore, breeding needs to allow different selection indices to be factored in to cater for different preferences and stress conditions across regions. For example, in the North, aromatic rice was preferred, while in the East farmers rated culinary characteristics and in the West, resistance to stem borer. These should be factored in. For successful adoption, rice breeders should pay attention to these traits along with their compatibility in cropping systems, in developing new varieties for Uganda and similar niches in sub-Saharan Africa. Non-varietal constraints like a lack of input and nutrient management packages need to be tested and recommended as part of production packages. This will enhance variety adoption and productivity. Overall, rice breeding needs to be client oriented and should include participatory methods for identifying farmersqpreferences.

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CHAPTER THREE

Development of Flower Emasculator and Grain Shattering Tester for Rice

Abstract

The rice flower emasculator is a critical tool for making crosses because rice florets are small. The equipment is, however, expensive for breeders in Sub-Saharan Africa. A new anther suction device that uses an ordinary household vacuum cleaner was fabricated. Its overall units include pipette tips, silicon tubing; hose tubes, metallic stand, rubber stoppers and the household vacuum cleaner of 1600 w capacity. It was validated with a prototype commercial equipment and found equally efficient and yet cheaper. Grain shattering, also called grain shedding, is the relative ease with which rice seed is dislodged from the pedicel at maturity. Several methods used to assess grain shattering are qualitative and they tend to underestimate yield loss due to the constraint. The available equipment for the accurate, quantitative assessment of grain shattering is mostly laboratory based. However, a prototype developed recently in Japan for fieldwork is available but the cost is prohibitive. An improved grain-shattering tester that uses a hand-held device has been fabricated. It is an improvement on the fixed laboratory device. It has the following modifications: a crocodile clip instead of a bench clump, and a helical spring for testing tensile material instead of a tension gauge. The new device is affordable, handy for fieldwork and effective for large, experimental trials. Both pieces of equipment were designed and developed at the University of KwaZulu-Natal's mechanical workshop. Their efficiency was tested at the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda.

A.   A simple and Efficient Flower Emasculating Equipment

A.1   Introduction

In order to develop an efficient rice breeding program the, it is necessary that effective tools are available. One such tool is a flower emasculating equipment. Flower emasculation refers to the process of removal of the male organs of a plant's floret in order to prevent selfing and facilitate crossing. The flower emasculating equipment is used to remove the anthers through suction to facilitate hybridization. Two types of flower emasculation devices are currently used. These devices operate on the principal of air suction force, where individual anthers are forcefully pulled off the florets and are used by plant breeders (Chandraratna, 1964; Coffman and Herrera, 1980; Navarro and Virmani, 1987). The first emasculator type is a prototype developed by the International Rice Research Institute

(IRRI) and the second type is a modification of the IRRI prototype (Coffman and Herrera, 1980). The IRRI prototype has the following specifications: Model-1022-V103-G272X, Horse Power (HP) 0.75, Voltage (V) 115-220, and revolution per minute (RPM 1725). The Gast Manufacturing Corporation manufactures the model. The second type of emasculator was developed with a view of reducing weight and for use in remote areas where there is no electricity. It uses a simple, portable and cheap 12 V power unit. The overall weight is 6.35 kg while the former weighs 21 kg. It uses diaphragm oil-less pumps manufactured by Gast Manufacturing Corporation. The assembling of the power unit was conducted by Cornell University.

The rice flower emasculators currently in use are manufactured in advanced fabrication centres and their prices are prohibitive for breeders in Sub-Saharan Africa. In addition, this equipment cannot be serviced in workshops in most African countries. Instead of using manufactured pumps, a common household vacuum cleaner pump is used. In this paper, a new vacuum-assisted anther remover designed at the University of KwaZulu-Natal (UKZN) in South Africa is presented. It uses simple household and laboratory facilities with modifications using modest workshop facilities.

A.2   Construction and operation of flower emasculator

The schematic design of the new emasculator is shown in Fig. 3.1. It comprises the following materials: pipette tips of 1000µl capacity, silicon tubing, PVC manifold tube, braided hose tube, metallic stand made of aluminium tubing, rubber stopper, epoxy putty and household vacuum cleaner of 1600 w capacity.

The construction of the equipment involves the following steps:

a.   Cut a 1.5-2.0m piece of silicon tubing of internal diameter (ID) 8mm and outside diameter (OD)16mm then push the larger end of a 1000µl capacity pipette to one end of the tube to fit in an air-tight position as shown in Plate 3.1A.

b.   Cut the tip of the pipette at a slant of 30% at about 5cm from the tip. Separately, fabricate a PVC block to fit manifold tubes (6 attachments) (Plate 3.1B). Each opening on the PVC tube is fixed into the open end of the silicon tubing. The six silicon tubes with the pipette end are attached to the end of the braided hose tubes. The PVC block is 16.5cm long and has an external diameter of 1.5 cm. One end of the PVC block is also machined and fitted to one end of a 1.5-2.0 m braided hose of 13 mm ID x 17 mm OD (Plate 3.1B). The point of attachment between the braided

hose and the PVC block has a thin layer of epoxy putty in order to make the attachment firm and air tight so that a constant sucking force is maintained.

c.   Attach the open end of the braided hose to a household vacuum cleaner hose sleeve, cut at 20cm from the tip via a ball valve (Plate 3.1C).
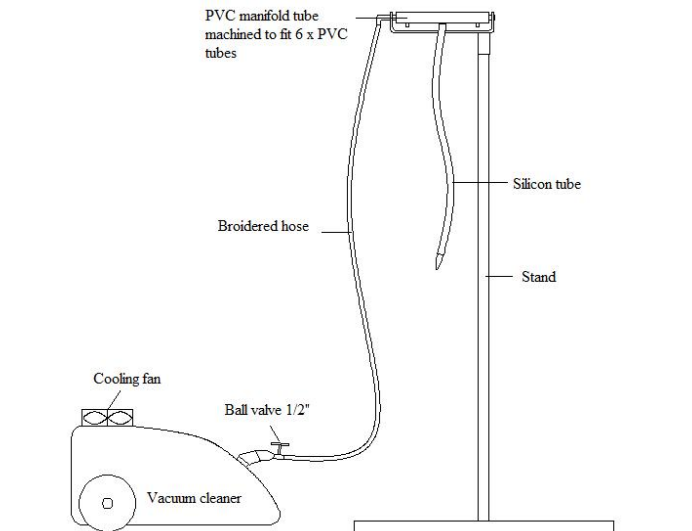
IFigure 3.1   Schematic diagram of the suction equipment

A household vacuum cleaner, 1600W Electrolux type, model Ultima (H5005), was used. It is permanently fixed onto a suction tube using an epoxy putty layer. The equipment is then supported by an adjustable stand (Plate 3.1D). The stand is adjustable to suit different plant heights and has a hook that holds the PVC block for facilitating emasculation. The complete, assembled equipment is shown in Plate 3.1D.

Air flow rate or suction pressure is adjusted to about 500 mm Hg air-flow meter at the end of the silicon tubes, which are attached to the 1000μl-capacity pipette. The air flow rate is tested by adjusting the flow and testing until a point when proper extraction is achieved. The air flow at the pipette throttle is adjusted to 0.3 m/s and 0.5 m/s at the open end of the braided hose.

The equipment is operated by switching on the power and adjusting the suction to 500 mm Hg suction pressure. The pipette tip is then placed at a position approximately 5 mm from tip of the floret. It serves as a pollen extraction tip. When the anthers are

60

sucked, a characteristic snap sound is heard. The pipette is dipped in 1.25% w/w sodium hypochlorite for 5 minutes before the start of the work and after each different set of rice genotypes.
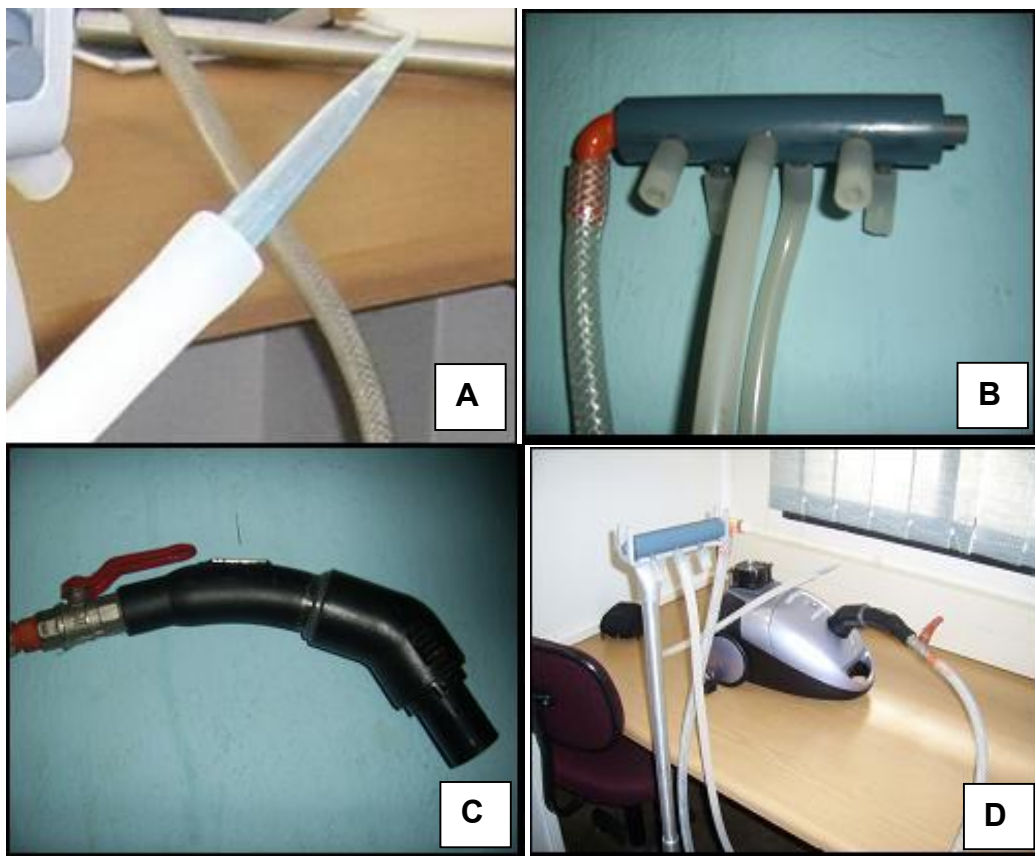
Plate 3.1A Pipette tip

Plate 3.1B Air tight connections

Plate 3.1C Attachment to vacuum cleaner

Plate 3.1D Assembled equipment

A.3 Technical details of the flower emasculator

The technical details are shown in Fig. 3.2. It comprises the front, top and end elevations. A detailed longitudinal section of the PVC block is shown in Fig. 3.2A. A detailed plan of the pressure control nobe is shown in 3.2B. Furthermore, a detailed section of the air cooling unit is shown in Fig. 3.2C. In addition, a detailed top view of the PVC block is shown in 3.2D.

FRONT ELEVATION

END ELEVATION

DETAIL A

TOP ELEVATION
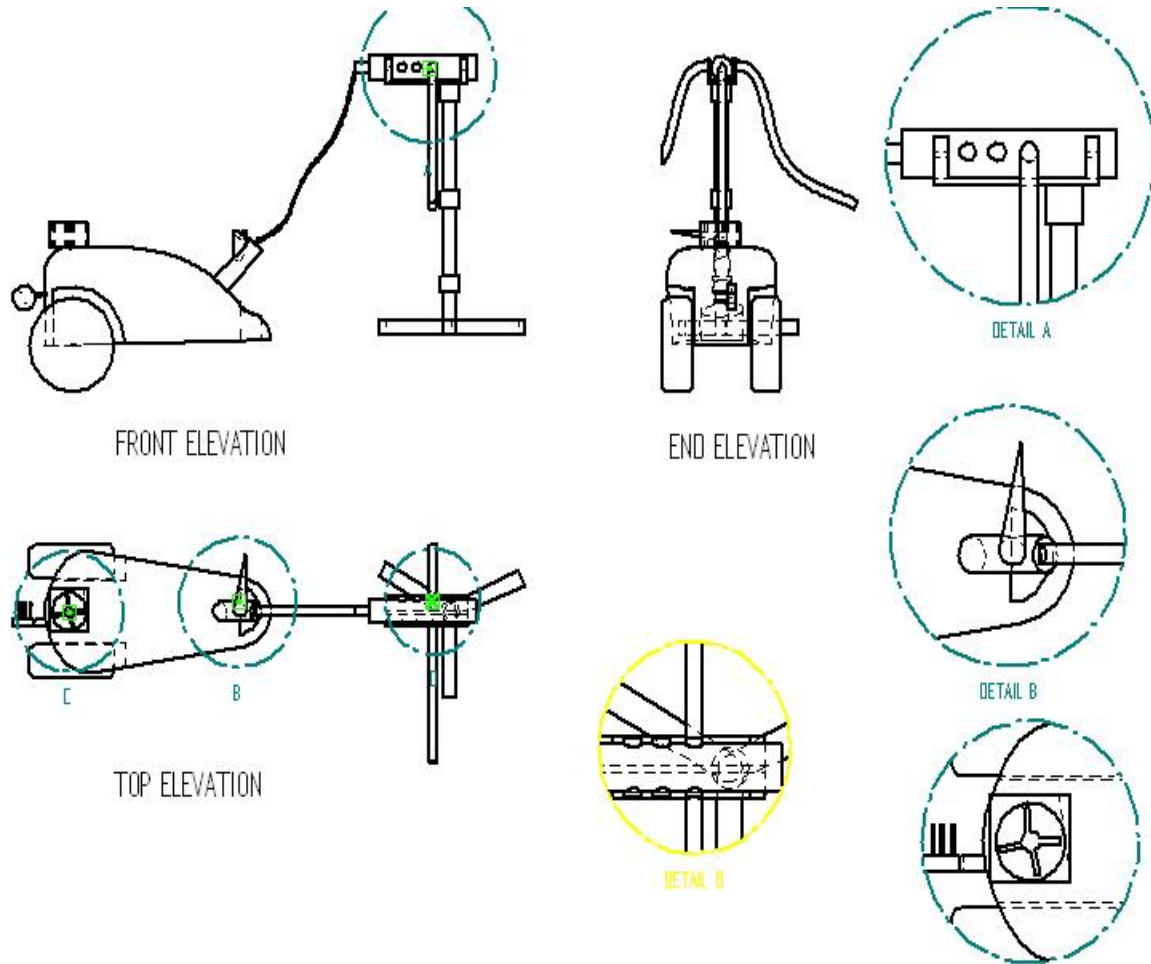
DETAIL B

DETAIL D

Figure 3.2   Detailed plan of flower emasculator

Cooling unit c

A.4 Appraisal of the equipment

In order to test the performance of the UKZN-designed equipment, four rice varieties were planted for use in this study. The experimental design was RCDB with the treatments being two emasculating machines, namely the UKZN and IRRI designs. The procedures for flower

emasculation used by various authors have been described (Jennings et al., 1979; Coffman and Herrera, 1980). The plants selected as female were observed and assessed for readiness for emasculation after emergence from the boot and prior to anthesis. This was when 50-60% of the panicles had emerged. The upper and lower florets on the panicle were cut off with scissors and the tip of each spikelet was clipped off at about one-third or one-half of the longitudinal length of the florets. Emasculation was conducted in the late afternoon (16:00-17:00 hrs). The emasculated panicles were tagged and enveloped in a glassine bag.

Pollination was conducted using the standard procedures (Coffman and Herrera, 1980). According to the procedure, fresh pollen was collected by carefully cutting the pollen panicles before anthesis and placing it at the base of the panicle in beakers filled with water. The panicles were watched for anther extrusion. At maximum pollen production, the male panicles were gently tapped over the female panicles and they were immediately covered with pollination bags. The plants were watered regularly until the 28th-30th day. It was when the seed had just turned from green to straw brown. The panicles were cut, dried and threshed. The glume remnants were removed and the F1 seeds from each panicle cross placed in envelopes with cross identification details. An additional set of plants was similarly emasculated and immediately covered with pollination bags as a control to test the effectiveness of the emasculation. The failure to set seed showed that the emasculation was effective. A preliminary study was conducted to compare seed set when new equipment and the IRRI equipment was used to make crosses and there were no significant differences in seed set at (P=0.05). Further studies have been recommended to compare the efficiency of seed set.

The basic structure of the flower emasculator involves three sections . namely, the suction unit, a suction hose and a tip for anther suction. The most expensive component is the power suction unit. In the new equipment made at UKZN the suction force is provided by the vacuum cleaner that costs $400 while the motor for the IRRI equipment costs $800, according to 2008 quotations. Overall, the UKZN model costs $850 while the IRRI prototype costs $2,100 excluding freight costs (Table 3.1). Overall, the new equipment is 2.2 times cheaper than the commercial one. The UKZN design has less total weight; it weighs 6 kg as opposed to the 21 kg of the IRRI model. The replacement cost of the suction unit is $600 lower for the UKZN unit than for the IRRI unit. The new equipment and the commercial one

63

were equally efficient in flower emasculation. However, how long it would take before a major service or replacement of the whole equipment was required was not tested.

Table 3.1 Comparison of prices of two flower emasculating machines

Units/ parts	Cost on USD	
	UKZN design	**IRRI design**
Suction unit	400	1200
Hose and clips	250	600
Suction tip	200	300
Total cost	**850**	**2100**

From the appraisal study, it can be concluded that this new equipment has four strengths. It is equally efficient in flower emasculation, cheaper, less bulky and has lower replacement costs than the commercial flower emasculators. However, its robustness is not known. Rice breeders in Tanzania and Kenya are currently using this equipment and are achieving the expected results.

B.   Development and Appraisal of a New Cheap and Efficient Single Grain Shatter Tester for Rice

B.1   Introduction

Grain shattering is a serious problem that occurs at the end of the growth cycle of rice, but comparatively little attention has been accorded to it, when compared with other constraints that emerge early in the crop's growth cycle. Part of the reason for little appreciation of its importance is probably due to lack of quality data on yield loss. Yield losses in the range of 0% to 38% has been reported in when susceptible varieties are grown (Jacobi et al., 1974; Sahu and Sahu, 1981; Agarwal et al., 1989). Current advances in rice breeding that involve crossing of *O. sativa* with its relatives*,* known to have high shattering ability, has made the grain shattering problem to re-emerge (Fukuta et al., 1995a).

Precise estimation of grain shattering in cultivated rice is important in three ways. In the first case, it helps in ranking the importance of grain shattering among other constraints. Secondly, it guides estimation of genetic parameters for the trait. The trait is controlled by genetic, morphological and physiological factors. The genetic component is controlled quantitatively (Fukuta, 1995a,b). Better understanding of its transmission would require quantitative assessment of shattering ability in a population segregating for the trait. The

last reason for importance is that it guides selection of varieties with acceptable threshing abilities.

To date, methods of estimation of grain shattering are subjective in nature. The methods that are less influenced by environmental conditions are laboratory based and are not portable therefore not suitable for field evaluation. Seven methods have been developed and used to assess grain shattering ability. In the first method, panicles are shaken and grains that fall are recovered, counted and grouped (Ramiah and Rao, 1953). However, owing to variation in the density of the panicles, the impact of shaking is liable to be variable. The second method involves cutting panicles and placing them on a smooth wooden board with an inclination to a horizontal board. The tip of the board is fixed facing downwards to allow grain recovery (Rao, 1935). A glass roller is released and allowed to roll over the panicles. Shattering is computed from the ratio of the number of grains detached to total number of grains on the panicle. The third method, involves counting grains that fall freely at 30 days after anthesis. In this method, the number of scar marks and non-grain bearing pedicels assumed to have shattered. The second and third methods are suitable for use only under laboratory conditions. The fourth method involves dropping panicles from some height on to a hard surface and the grains that get dislodged from inflorescence are counted (Ramiah and Rao, 1953; Sahu and Sahu, 1981). This method was later improved by placing panicles on a wooden frame and allowing it to be released from a spring so that it strikes an inclined board (Jacobi et al., 1974). The effect of varying panicle densities is reduced. However, it is still suitable for laboratory work only.

The fifth and sixth equipment were developed by Ichikawa et al. (1990). In the fifth device, a conventional rice thresher was modified. Panicle stalks are cut to a length of 500mm from the remaining top. The stalks are divided into standard sized units, which are then fed manually to the testing device. It is suitable when large sample sizes can be used for assessment but cumbersome for field experiments. The sixth device measures the force required to separate the grains from panicles directly. In the measurement, static test of shattering ability is estimated. However, it is still too cumbersome for field experiments. The seventh method involves a digital breaking strength equipment device described by Li et al. (2006b). It uses a digital force gauge (FGC-1B, Shimpo, Japan), which is operated by attaching it upside down to a stand (FGC-50L) (Li et al., 2006). It is an improved version of the equipment developed by Ichakawa et al. (1990). Rice panicles from the main stem or

65

primary tiller are harvested and fixed upside down to the force gauge using a flat chuck tensile grip and each grain is pulled down by a forcep, and the maximum tensile strength measured at the moment of pedicel breakage. This is the most appropriate in terms of accuracy but it is not practical for the field screening of large numbers of genotypes. It is also not under commercial production at the moment.

The new grain shattering equipment is a modification of a device developed by Ichikawa et al. (1990). It was designed, constructed and tested at the University of KwaZulu-Natal. In the device, the modifications were the following: 1) a single holding clip for holding the grain instead of two holding clips for the grain and the detached panicle; 2) the panicle is not detached from the plant; 3) a helical spring for testing tensile material instead of a tension gauge.

B.2   Construction and operation of the grain shattering tester

The assembled equipment is in Plate 3.2. Its construction takes the following steps:

a.   Fix a 10 newton force (10N) spring constant helical spring on to a PVC block machined in the groove of a spring jacket. The other end of the spring is fixed onto a crocodile clip type K260. One Newton is equal to 9.8gf.

b.   Attach a cork onto one end of the spring to allow estimation of force required to pull the spring.

c.   Make a helical spring from 0.5mm diameter spring wire and estimate the pulling force for minimum and maximum tensile force it can estimate using a standard laboratory force gauge. The spring pulling force is adjusted by altering spring diameter to measure force ranging from 0 to 400 gram force (gf). Different weights in units of 50gf are attached to the spring and the spring is allowed to stretch until it stabilizes. The calibration is conducted under minimum air resistance. The spring end is marked at the edge of the aluminum cover plate and engraved with the weight label.

In operating this device, the adjustable cork is set to the zero mark and the clip is allowed to clamp a single grain. The pedicel of the clamped grain is held firmly with one hand and the other hand pulls the grain off the pedicel. The whole equipment is held parallel to the longest axis of the seed, which is kept at its natural position in relation to the axis of the panicle. The grain is pulled gently until it is dislodged from the pedicel. In the process of

pulling, the spring extends while pushing a cork upwards and it remains in a fixed position when the grain is dislodged. The position of the cork corresponding to the graduated marks on the aluminum label is then read. This reading corresponds to the minimum force required to dislodge the grains. The general posture for grain testing is in Plate 3.3.
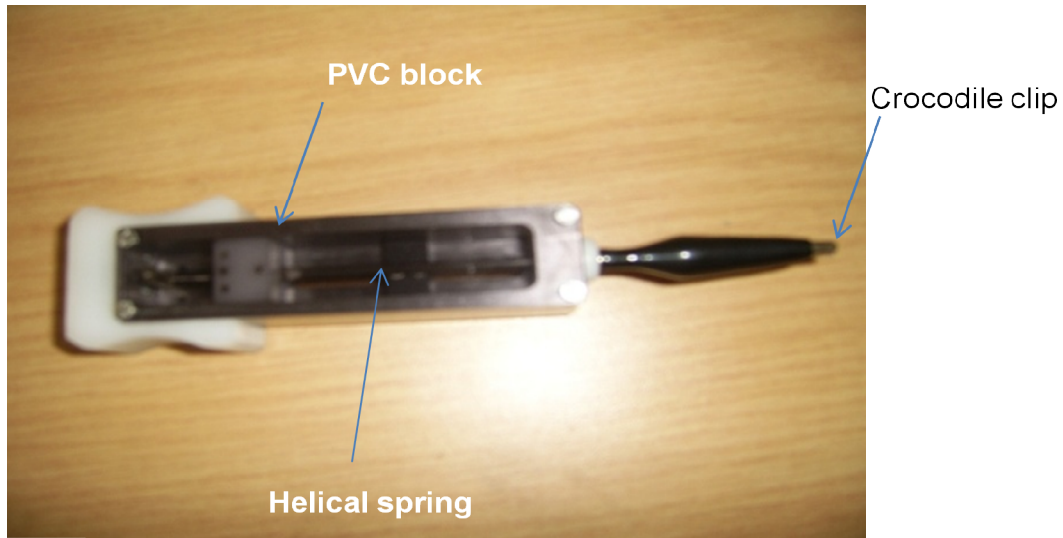
Plate 3.2 Photo of assembled grain shattering equipment

Plate 3.3   Demonstrating use of grain shattering tester in the field

B.3   Technical details of the grain shatter tester

This single grain rice shattering device was designed, constructed and tested at University of Kwa-Zulu Natal. The front and end view of the device is shown in Fig. 3.3. The first

detailed is tensile adjustment gauge, front view (Detail A) and side view (Detail C). It is made up of a PVC engineering block. The cork slides on a smooth Aluminum surface with graded marks. A crocodile clip, type K260, is attached at the end of the pulling rod (Detail B). In the aluminum box, a 1mm diameter helical stainless steel spring calibrated to 400gf tension was used.

B.4　　Appraisal of the equipment

In order to test the performance of the UKZN designed equipment, 10 rice varieties with known shattering ability based on the hand squeeze and shaking method were tested. The experimental design was split-plot with the main plot being hand estimation of shattering and the second was the UKZN new equipment. The sub-plot was the 10 varieties. Procedures for testing the grain shattering involved modifying method described by Ramiah and Rao (1953). In this method, 10 primary panicles that were ripened at 30 days after flowering were carefully harvested and placed on a clean sheet. Each panicle was gently squeezed and the panicle pulled out slowly. The grains that were dislodged are counted. All the remaining grains are also harvested and separately counted. The grain shattering degree was the ratio of grain recovered to total number of grains in each panicle. The percentage grain shattering ability for each variety was calculated.

Figure 3.3   Detailed sketch of the single grain shattering tester

In order to take records on shattering using the new grain shattering tester, five plants from each of the 10 varieties planted in 1x1-m sub-plots were sampled and the panicles of the primary plant were tagged on the date when 50-60% of the inflorescences had reached anthesis. The tagging was done between 10:00 am and 3:00 pm, the time when most inflorescences flowered at Namulonge. All the florets that flowered earlier along with those that had not flowered on the date tagged were carefully excised off the peduncle immediately after tagging. The flowering date was considered to be the day when the middle portion of the panicle length had flowered. Record of shattering was taken for 10 seed per panicle on the 30th day after flowering. Overall, 50 records were taken per plot.

Results of the minimum force required for each variety, the corresponding mean yield loss and the standard grouping of the varieties according to African Rice Center (ARC) seed import notes is shown in Table 3.3. In the Table 3.3, three lines considered to be high shattering according to ARC notes required less than 30gf for dislodging using the single

grain tester and yield loss of over 50% was recorded. Varieties that were non-shattering required more than 100gf for dislodging them and less than 20% yield loss was realized when the method was used. When the percent yield loss was regressed on minimum force required to dislodge the grains (R^2=86.3), it was found that there was high correspondence in the way the two methods estimated the importance of grain shattering (Fig 3.4).

Generally, higher gram force was required to dislodge grains that are difficult to thresh. According to Ichikawa et al. (1990), the minimum force required to dislodge the grains was 180gf while high shattering populations needed less than 150gf to dislodge. Different grouping of rice populations based on grain threshability has been reported. When threshing is conducted by machine in Japan, a higher level for threshability was reported (Ichikawa et al., 1990). Indeed rice varieties that were machine threshed required higher minimum force to dislodge the grains. For instance, in Japan, a mean force required to dislodge the grain in a collection of rice from other places were 200gf and 150gf for lowland and upland rice types, respectively (Fukuta, 1995b). In India and China, where threshing is conducted by hand, however, the mean force required to dislodge the grains for a collection of rice were 50gf and 70gf (Fukuta, 1995b). This new equipment was therefore able to discriminate the populations for grain threshing ability and strongly corresponds with the standard grouping conducted by African Rice Center. Considering that the use of hands to assess grain shattering is cumbersome and prone to error, this new equipment is presented for use under field conditions.

Table 3.2 Mean shattering degree (gf) and yield loss (%) for 10 reference rice types

	NERICA 6	FKR 14	WITA 3	WITA 7	WITA 12	WAB 56-104	WAB 365-B-1H1-HB	IDSA 10	WAB 56-39	FKR 41
Shatter degree										
Mean	23.5	26.0	27.5	29.0	37.0	65.0	106.0	108.0	117.0	128.0
Standard Error	1.7	0.7	1.1	1.3	2.8	7.1	6.6	6.0	5.6	6.7
Standard De	11.7	4.9	7.6	9.3	19.7	50.0	47.0	42.4	39.9	47.3
Range	50.0	25.0	25.0	25.0	75.0	1175.0	175.0	175.0	175.0	175.0
Minimum	0.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	50.0	50.0
Maximum	50.0	50.0	50.0	50.0	100.0	200.0	200.0	200.0	225.0	225.0
CV (95.0%)	3.3	1.4	2.2	2.6	5.6	8.9	13.4	12.0	11.3	13.4
Yield loss										
Mean	65.3	63.5	64.9	57.4	32.9	29.0	19.8	16.7	13.7	12.4
Standard Error	3.5	3.1	4.3	5.2	2.3	2.5	2.5	1.9	1.1	0.6
Standard De	11.1	9.8	13.8	16.5	7.2	7.9	8.0	5.9	3.5	2.0
Range	31.5	28.9	50.7	53.0	25.7	26.4	29.1	15.5	9.7	6.4
Minimum	52.4	50.6	38.1	40.2	21.2	20.7	1.0	9.5	7.8	9.5
Maximum	83.9	79.5	88.8	93.2	47.0	47.1	30.1	25.0	17.5	15.9
CV (95.0%)	7.9	7.0	9.8	11.8	5.2	5.7	5.7	4.2	2.5	1.4
Africa Rice rating[1]	Shatter	Shatter	Shatter	Shatter	Normal	Normal	Not-shatter	Not shatter	Not-shatter	Not-shatter
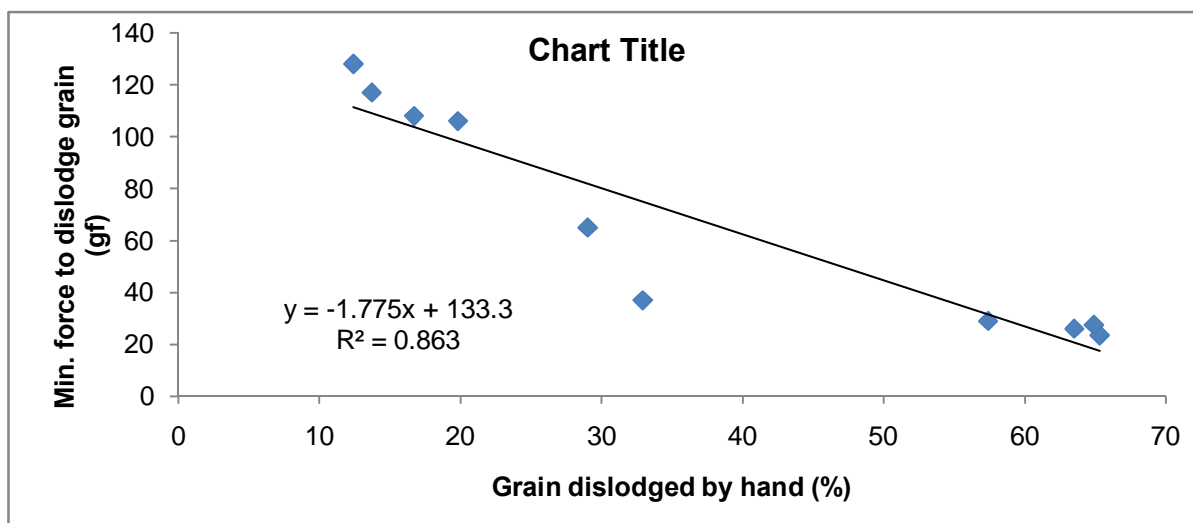
‡Figure 3.4   Regression of grain dislodged by hand to minimum force required to dislodge the grains

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CHAPTER FOUR
Grain Shattering Pattern and Diurnal Anthesis Trend of Rice

Abstract

Precise determination of the pattern of grain shattering, a major problem during harvest and post harvest crop stages, could guide rice breeding to minimize yield losses due to grain shattering. The significance of testing grain shattering tendency at the growth stage when the greatest discrimination for the trait could be measured was examined. To increase the chance of success in making crosses in rice, information on rice anthesis pattern, a trait known to vary with locations and genotypes, was assessed. A field trial comprising of 191 genotypes were planted in a 25 x 8 alpha lattice design with two replications. Among the genotypes, there were three rice groups namely CT genotypes, NERICA genotypes and the *O. sativa*. Records were was taken on grain shattering at 10 days, 20 days and 30 days after flowering. The number of plants that had anthesis were also counted at two hour intervals starting from the first day of anthesis till the time there was no anthesis for two consecutive days were also recorded. Daily temperature and humidity was also recorded. The data was analyzed using mixed model GLM in GENSTAT. There was highly significant (P=0.001) variability in the grain shattering score at 20-days and 30-days after flowering and significant (P=0.01) at 10 days after flowering. The mean tensile gram force (gf) required to dislodge the grains decreased from 10-days to 30-days after flowering. Results demonstrated that the shattering trend in the three rice groups CT, NERICA and *Oryza glaberrima* genotypes decreased in a similar trend. The highest level of discrimination among a segregating population could be achieved at 20 days after flowering. Results also showed that NERICA genotypes had flowered by 10:00 hrs and that a few CT line had flowered by 10:00 hrs as well. This could be due to the *O. glaberrima* in genes in their genome. The *O. glaberrima* rice types flower early as a way of avoiding heat stress. Findings of this study than on cold and wet days, the *Oryza sativa* flowered over a longer period than on hot and dry days implied that the populations used had special adaptation for avoidance of cold stress. This study found that the period of spikelet flowering varied over a lower range than when compared with studies in other locations. This could have been due to the fact that temperatures, humidity and length of days which are key determinants of the degree of flowering is more uniform in Uganda and are optimum for rice production. Since the country is equal days and night length and the temperature and humidity optimum for rice anthesis and grain filling breeding could be conducted throughout the year.

**4.1	INTRODUCTION**

Grain shattering (GS) is a trait whose expression varies with rice development stages (Li et al., 2006b). At present, grain shattering is a major drawback to a widely preferred breeding method called wide hybridization because it is frequently carried in new genotypes generated. Shattering ability is influenced by three factors. First is the crop morphological structure especially abscission layer development. The trend of development of the abscission layer with crop development has been extensively studied (Konishi et al., 2009). Another factor is environmental conditions especially humidity, temperature and the impact of storm. The third factor that influences grain shattering ability is genetic constitution of the crop. The effect of both abscission layer and environmental factors are high during the later stage of crop development. This is the stage when most studies to assess grain shattering have been done (Fukuta, 1995a). In order to gain more insight into the effect of genes responsible for shattering, earlier stage of grain development could be used. Preliminary works by Ji et al. (2006) suggested that there is less influence of environment in the earlier stages of grain development. A comprehensive understanding of grain shattering pattern is important because it would guide identification of the critical stage when there is maximum discrimination among segregating populations during breeding. In the process, the best parental lines will be identified for breeding for optimum grain shattering ability.

Information on pattern of expression of traits that are associated with crops development has been successfully used in improving different traits in crops. For instance, information on pattern of kernel shattering in wheat (Clarke and DePauw, 1983) and chalkiness and translucency in rice (Shi et al., 2002) were used in breeding for improved threshing ability in wheat and improved grain quality in rice, respectively. In the case of GS ability in rice, development of morphological structures called abscission layer contribute to grain shattering (Jin et al., 1995). However, other workers have confirmed that abscission layer development is not the sole factor that control grain shattering because some shattering rice that lack abscission layers have been identified (Li et al., 2006b). Other than the role of abscission layer on grain shattering, genetic control of grain shattering is very important (Jin et al., 1995). Variation might exist for gene expression and genetic effects at different growth stages, but studies to verify require precise determination of shattering in real time. In this study, the shattering pattern of several rice cultivars that vary from landraces of *O.*

sativa and improved *O. sativa* to derived fixed lines of *O. sativa* and *O. glaberrima* were examined to identify the most appropriate stage for assessing grain shattering.

Flowering time often referred to as heading date in cereal crops, is an important trait for rice production as well as the development of new varieties. Developing early-flowering or photoperiod-insensitive cultivars has been a major objective of rice breeding for several decades. Flowering time is controlled by many genes, which are expressed or suppressed in close interaction with environmental factors such as day length and temperature (Lee, 2007). However, for every flowering time in terms of duration of crop growth, the duration a rice genotype continues producing pollen and time of the day when it flowers (anthesis) are equally important. These traits are also influenced by rice genotype, environmental condition and the interaction of the two species (Tao et al., 2003). One of the important environmental stresses is water stress. Diurnal flowering trend is associated with drought tolerance and it varies among crop varieties. For instance, *O. glaberrima* are known to flower early in the morning and *O. sativa* flowers latter in the day. However, the flowering pattern of genotypes developed through crossing *O. glaberrima* and *O. sativa* is not available yet it is very important for using these new generations of rice in breeding. It is important that flowering pattern, that is the duration a rice genotype continues producing pollen and time of the day when it flowers, is understood for specific environmental conditions. In this study, duration a rice genotype continues producing pollen and time of the day when it flowers was explored.

4.2   Objectives of the study
The specific objectives of the study were to:
i.   determine the pattern of shattering of rice grain, and
ii.   determine the daily anthesis pattern of different rice genotypes, and
iii.   test the effect of daily variation in temperature and humidity on anthesis pattern, in Uganda.

4.3   MATERIALS AND METHODS
4.3.1 Study location and plant materials
A total of 191 rice introductions from major rice breeding centers were used in the study. Of the 191 materials 77 were *O. sativa* comprising of 45 from the the African rice centre (ARC); 15 lines from International Rice Research Institute (IRRI), (13) Mali, three from Uganda and

one from China. Among the introductions, there were three *O. glaberrima* accessions. The remaining 111 lines were interspecific ones developed from *Oryza sativa* x *Oryza glaberrima* crosses, comprising 18 lines from ARC and 93 lines from the International Centre for Tropical Agriculture (CIAT), Colombia. The genotypes from CIAT were code named the CT series. The 93 interspecific lines from CIAT were BC_4F_1s developed from crossing CAIAPO, a tropical *Oryza sativa* Japonica from Colombia, with RAM 24 (*Oryza glaberrima*) (Guimaraes et al., 1996). The *O. glaberrima* accessions named %Riz African du Mali+ (RAM) were taken from the germplasm collections of the Institut dqEconomie Rurale (IER) at Sikasso in Mali. The introductions were planted on the 12th December 2006 at Namulonge in central Uganda at 0° 32' N latitude and 32° 53'qE longitudes. The soils of the location were clay loam. The period December to March is characteristically the long, dry season. The period December to March is a long dry season in Uganda.

4.3.2   Experimental design

Each of the 191 genotypes were planted as a single entry except WAB 880-1-27-9-2-P1-HB, WAB 450-24-2-3-P-38-1-HB, NERICA 6, WAB 365-B-2-H1-HB, NERICA 3, NERICA 5 and NERICA 10 were planted twice, while genotype CG 56-104 was planted three times to increase the degrees of freedom for them and thus improve on their estimated mean values. The experiment was laid out as an alpha-lattice design in an 8 x 25 arrangement in two replicates. A total of 36 hills were planted in each 1x1-m experimental plots spacing of 20cm x 20cm. About five seeds were planted per hill using the line dibbling method before thinning to a single plant three weeks after germination. Supplementary irrigation was applied at a rate of 20mm per week. The first level of fertilizer was applied at the rate of 30kgN ha^{-1}, 15kgP ha^{-1} and 15kgK ha^{-1}. At booting 24kgN ha^{-1} was also applied as topdressing.

4.3.3   Data collection

Record of shattering trait was conducted by determining the degree of shattering of rice using a hand held grain-shattering tester fabricated at the University of Kwa Zulu Natal. It is a modification of the laboratory based grain shatter tester used by Ichikawa et al. (1990). The modifications were; 1) a single holding clip for holding the grain instead of two holding clips for the grain and the detached panicle; 2) panicle is not detached from the plant; 3) testing was conducted in the field. Grain shattering ability was measured by determining the minimum force required to dislodge the individual grains by pulling the grains until it is

dislodged. A hand held breaking tensile strength (BTS) equipment was used. The range of the reading of the equipment was between 0gf and 400gf. The gauge was rigidly attached to a haemostat clip, which closed on the seed. The gauge and the clip were held parallel to the longest axis of the seed, which was kept at its natural position in relation to the axis of the panicle.

Five plants from each of the 1m x1m sub-plots were sampled and the panicles of the main primary plant were tagged on the date when 50-60% of the inflorescences had flowered. The tagging was done between 10:00 am and 3:00 pm, the time when most inflorescences flowered at Namulonge. All the florets that reached anthesis earlier along with those that had not reached anthesis on the date tagged were carefully excised off the peduncle immediately after tagging. The date for tagging the plants was based on visual observation of panicle length when about 50% of the panicle length had reached anthesis. This date varies with variety type and weather during the reproductive stage and it varied between 5 and 8 days after initiation of anthesis. Anthesis date was the day when the first day when inflorescence was observed. Records of shattering were then conducted on the flowering at 0, 10, 20 and 30 days after flowering. The method involved sampling ten florets of grains per main culm. Overall, 50 records were taken per plot.

Record on the number of spikelets that had reached anthesis was taken every two hours. Initially the main culm of four plants, randomly selected per plot, was tagged before the flowering stage. The four plants were monitored every day and the first day any of them reached anthesis was taken as the day anthesis began for that genotype. On the following days after flowering started, record of number of spikelets that flowered were recorded at two hour intervals starting at 08:00hrs and ending at 18:000hrs. A spikelet that is flowering has anthers that are extended out of its floret. The flowering is considered to have ended when no record is taken for three consecutive days. Record on the daily temperature and humidity was assessed from the weather station at Namulonge (Table 4.1).

Table 4.1 Mean daily humidity and temperature on days when record on anthesis was taken

	High humidity and low temperature		Low humidity and High temperature	
	Temperature		Humidity	
Time of the day	9:00 am	2:00pm	9:00 am	2:00pm
Mean at different times (cold day, humid)	16.2	27.7	86.2	70.6
Mean at different times (hot, dry day)	16.0	30.0	74.4	60.8

4.3.4 Statistical analysis

The data was analyzed using Genstat version 12 (Payne et al., 2007). First, the variates were subjected to Analysis of Variance (ANOVA) using the varieties as factors. The auto-regression was to estimate and exclude field spatial variation (Gilmour et al., 1997). Finally, a generalized linear model was used to analyses the data, with variety as main factor and different times of data collection nested within variety. The mean anthesis pattern for selected genotypes, when the humidity was high and the temperature low, was summarized and the days when the temperature was high and humidity was low, was compared with the days when humidity was high and the temperature was low.

The ratio of range to LSD was calculated used by Iqbal and Clarke (2003) to select variables that best decide variation within populations. It has more power in discrimination among variables when comparing with other variables that have different scales of measurements.

4.4   RESULTS

4.4.1 Grain shattering pattern

Significant differences (P<0.01) were observed among genotypes for grain shattering at 10 days, after the first record of flowering in each panicle, but it was highly significant at 20 days and 30 days after flowering (P<0.01) (Table 4.2).

Table 4.2 Mean square for shattering ability for three record dates

Source of variation	df	duration in days after flowering		
		10 days	20 days	30 days
Rice genotypes	190	14901**	5052.7***	3262.2***
Rice groups	2	6	93.2	10.8
Residual	206	10902	856.6	478.0

** = P < 0.01, *** = P < 0.001

The mean tensile shattering force for 10 high shattering *O. sativa* varieties, 5 moderate shattering varieties and 10 least shattering varieties are presented but the mean, Least significant difference (LSD), coefficient of variation in percentage (CV%), Range/LSD and variance is for all the 191 lines tested (Table 4.3). They were the best 10, middle 5 and least 10 in terms of shattering degree to give a representation of all the varieties studied. The mean tensile gram force (gf) required to dislodge the grains for the 191 genotypes decreased with time from 10 days to 30 days after anthesis. The shattering strength at 10 days after anthesis was of 1.4 times higher than that at 20 days. At 20 days, however, the mean shattering strength was only 1.3 times more than that at 30 days after flowering. The LSD at 0.05 were similar between 0.2gf and 0.5gf. The ratio of range to $LSD_{0.05}$ for 10 days after anthesis was more than three times the value at 20 days to 30 days after anthesis. The variance levels were similar ranging between 6 to 9.

4.4.2 Diurnal variation in anthesis in rice

Results of the time of the day when flowering commenced (TDF) in the main stems of four NERICA genotypes, nine CT genotypes and twelve *Oryza sativa (*12) are presented in Table 4.4. At 10:00 hrs, all the NERICA had started anthesis and a few rice CT genotypes. The peak flowering was generally between 12:00 and 14:00 with CT 16324-CA-9-M, CT 16344-CA-9-M, CT 16350-CA-5-M and NERICA 9 showing over 50% of the spikelets had reached anthesis stage. On the other hand, CT 16344-CA-9-M, CT 16346-CA-20-M and CT 16350- CA-5-M had produced over 50% total pollen production at 14:00 hrs. All the genotypes stopped flowering before 18:00hr except CT 16344-CA-9-M and IRAT 13 that stopped before 16:00hrs.

Table 4.3 Pedigrees, origin and grain shattering mean scores at 10-d, 20-d and 30-d after flowering for selected *O. sativa* genotypes

Genotype (name/no)	Pedigrees	Origin	Grain shattering (gram force)		
			10-d	20-d	30-d
High shattering					
UPL RI 5	-	IRRI	73.8	19.5	29.5
CT 16337-CA-7-M	CT 16337-CA-7-M	CIAT	74.5	25.8	19.2
IR 77298-5-6	IR 77298-5-6	IRRI	82.3	55.1	37.8
CT 16324-CA-10-M	CT 16324-CA-10-M	CIAT	85.5	34.8	15.0
WAB 638-1-B-10A5-1	WAB 638-1-B-10A5-1	WARDA-ARC	87.5	64.3	18.2
CT 16310(2)-CA-7-M	CT 16310(2)-CA-7-M	CIAT	92.1	56.9	29.8
WITA 1	-	WARDA-ARC	94.3	65.1	42.0
IR 74371-54-1-1	IR 74371-54-1-1	WITA 1	94.6	84.1	43.2
IR 71524-44-1-1	IR 71524-44-1-1	IRRI	98.4	60.4	22.0
IR 64		IRRI	101.0	49.0	33.0
Moderate shattering					
CT 16337-CA-9-M	CT 16337-CA-9-M	CIAT	155.8	114.0	89.5
CT 16311(2)-CA-1-M	CT 16311(2)-CA-1-M	CIAT	156.5	59.2	51.0
CT 16342-CA-19-M	CT 16342-CA-19-M	CIAT	157.0	25.8	23.5
CT 16345-CA-13-M	CT 16345-CA-13-M	CIAT	157.3	81.0	57.0
CT 16307-CA-15-M	CT 16307-CA-15-M	CIAT	157.3	66.9	43.8
Low shattering					
WBK 25 F4	-	WARDA-ARC	222.9	162.3	90.2
Sikamu	-	Uganda	225.0	166.5	125.8
WAB 56-39	-	WARDA-ARC	226.2	164.2	117.0
WBK 39 (F3)	-	WARDA-ARC	226.5	155.5	135.5
CO 39	-	WARDA-ARC	227.0	170.5	148.8
NERICA 13	-	WARDA-ARC	233.1	182.5	141.4
NERICA 15	-	WARDA-ARC	240.8	163.7	112.5
WAB 96-3	-	WARDA-ARC	243.6	161.0	143.5
WAB 880-1-27-9-2-P1-HB	-	WARDA-ARC	246.3	106.3	71.0
IRAT 104	-	WARDA-ARC	182.0	164.8	152.0
Transformed values1					
Mean			**12.60**	**9.08**	**7.64**
LSD $_{0.05}$			**0.26**	**0.29**	**0.26**
CV %			**20.70**	**32.0**	**35.00**
Range/ LSD $_{0.05}$			**160.60**	**49.6**	**49.20**
Variance			**6.76**	**8.26**	**7.17**

[1]The mean, LSD$_{0.05}$, CV%, Range/LSD$_{0.05}$ and variance of square root transformation of data is presented.

The spikelets attained anthesis stage in the later time of days on days that were cold and wet when compared with the days when it was hot and dry (Fig 4.1). On cold and wet days, the CT lines had maximum flowering at 14:00hrs (Fig 4.1a). However, the same genotypes had peaked flowering at 12:00 hrs on hot and dry days, except genotype CT 16346-CA-20-M (Fig 4.1b). In the case of NERICAs, the maximum flowering on cold and wet days and hot dry days were all 12:00hrs (Fig 4.1c and Fig 4.1d) except NERICA 9 and NERICA 15 that flowered most at 14:00hrs on hot and dry days.

Table 4.4 Flowering duration of selected 25 selected rice genotypes

| No | Variety/Line | Percent number of florets per panicle that flowered | | | | | | Total flowered |
		0800 hrs	1000 hrs	1200 hrs	1400 hrs	1600 hrs	1800 hrs	
1	880-19	0.0	0.0	25.0	34.2	1.1	0.0	456
2	Bonanca	0.0	0.9	44.8	30.5	2.2	0.0	599
3	Caiapo	0.0	1.0	44.0	45.2	2.9	0.0	714
4	CK 73	1.0	5.6	35.6	29.8	4.5	0.0	585
5	CT 16313-CA-4-M	0.0	2	29.0	32.7	3.4	0.0	510
6	CT 16317-CA-4-M	0.0	1.8	36.6	25.3	2.1	0.0	503
7	CT 16324-CA-9-M	0.0	3.0	56.0	46.7	2.9	0.0	832
8	CT 16334(2)-CA-2-M	0.0	5.2	37.6	52.6	7.5	0.0	1572
9	CT 16342-CA-25-M	0.0	1.0	35.0	33.6	1.2	0.0	536
10	CT 16344-CA-9-M	0.0	0.0	54.5	51.0	0.0	0.0	806
11	CT 16346-CA-20-M	0.0	2.0	41.0	56.3	1.4	0.0	770
12	CT 16350- CA-5-M	0.0	1.6	48.6	51.0	4.8	0.0	810
13	CT 16355-CA-15-M	0.0	1.4	41.1	29.3	2.9	0.0	571
14	Golden	0.0	1.8	24.5	14.7	0.1	0.0	312
15	IRAT 104	0.0	3.0	25.0	28.1	0.8	0.0	438
16	IRAT 13	0.0	0.0	22.4	36.1	0.0	0.0	447
17	IRAT 257	0.0	0.0	37.0	16.6	2.1	0.0	428
18	IRAT 325	0.0	4.3	22.3	24.9	0.4	0.0	396
19	NERICA 15	0.0	3.0	25.0	20.3	2.9	0.0	388
20	NERICA 9	0.0	3.0	59.6	25.1	3.1	0.0	694
21	VANDANA	0.0	0.0	39.0	39.7	1.3	0.0	617
22	WAB 365-B-1H1-HB	0.0	5.6	32.9	45.3	3.5	0.0	667
23	WAB 450-I-B-38-HB (NERICA1)	0.0	18.0	30.0	25.1	3.4	0.0	585
24	WAB 56-104	0.0	1.8	41.0	32.5	3.7	0.0	1208
25	WITA 1	0.0	1.0	43.0	25.3	3.9	0.0	562
	Mean value	0.0	2.7	37.2	34.1	2.5	0.0	
	LSD$_{0.05}$	0.14	9.70	18.68	27.17	2.35	*	
	Var	18.70	1.15	2.7	1.7	6.3	*	
	F value	<.001	0.36	0.0	0.1	<.001	*	

In the case of NERICA lines, maximum flowering was at 14:00hrs cold and wet days (Fig 4.1e) but peaked at 12:00 hrs on hot and dry days (Fig 4.1f). On the hot dry days most CT genotypes had more than 70% spikelet flowered at 12:00 hrs, while the NERICA and *O. sativa* genotypes had less than 60%. On the cold and wet days, however, both CT genotypes and *O. sativa* had more than 60% spikelets flowered.
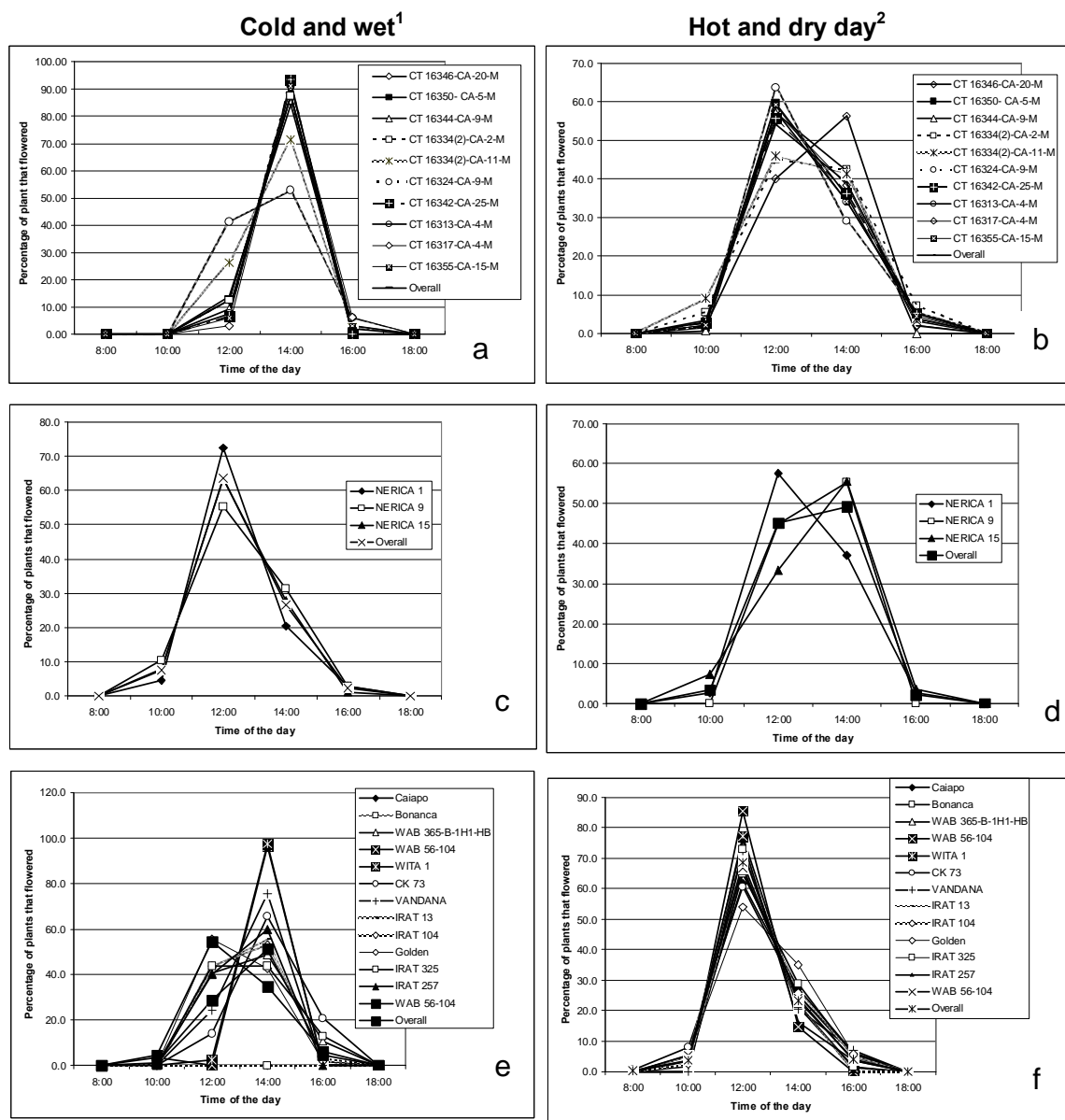
Figure 4.1 Pattern of anthesis of different rice types on hot and dry days compared with cold and wet days

Key:

1. [1]cold and wet day = max temp 27.7±17, min temp 16.2±17, RH 86.2±4
2. [2]hot and dry day = max temp 30.0±11, min temp 16.0±10, RH 74.4±5
a) Flowering pattern of CT genotypes during hot and dry days, b) Flowering pattern of NERICA genotypes during hot and dry days, c) Flowering pattern of *Oryza sativa* genotypes during hot and dry days, d) Flowering pattern of CT genotypes during cold and wet days, e) Flowering pattern of NERICA genotypes during cold and wet days, and f) Flowering pattern of *Oryza sativa* genotypes during cold and wet days.
3. [3]rice types = rice types groups were: *Oryza sativa*, *O. glaberrima*, interspecific rice derived from CG 56- 104 (*Oryza sativa*) and *Oryza glaberrima crosses* and CAIAPO (*Oryza sativa*) and *Oryza glaberrima*

4.4.3 Duration of pollen production in rice

Mean anthesis duration calculated based on total number of spikelets that flowered on each day is in Table 4.4. The mean number of days from start to end of anthesis was 7 to 10 days. Peak anthesis was on the 4th and 5th day after start of anthesis and it represented about 45% of all the plants that had reached anthesis. In the range of the 3rd to 6th day after anthesis, about 77% of the plants had reached anthesis. Genotypes CT 16334(2)-CA-2-M, CT 16334(2)-CA-2-M and CT 16324-CA-9-M, had the longest anthesis period of 10 days.

Table 4.5 Flower duration for selected 25 genotypes

No	Variety/Line	Percent number of panicles that had reached anthesis for the first 10 days after start of anthesis										Total flowered
		1	2	3	4	5	6	7	8	9	10	
1	880-19	0.0	12.4	20.7	30.4	15.1	12.3	6.7	2.3	0.0	0.0	456
2	Bonanca	3.4	7.1	9.8	13.4	16.1	19.9	16.4	9.7	4.2	0.0	599
3	Caiapo	5.0	9.6	10.6	18.5	21.7	18.4	13.7	2.5	0.0	0.0	714
4	CK 73	1.6	6.7	23.1	29.2	22.6	13.3	3.2	0.3	0.0	0.0	585
5	CT 16313-CA-4-M	3.9	7.6	8.0	18.2	22.1	20.3	12.9	7.1	0.0	0.0	510
6	CT 16317-CA-4-M	2.8	9.8	17.5	25.1	21.1	14.9	8.8	0.0	0.0	0.0	503
7	CT 16324-CA-9-M	2.2	3.1	8.9	12.3	19.6	18.4	16.4	11.9	5.7	1.7	832
8	CT 16334(2)-CA-2-M	3.9	6.4	10.3	14.3	18.5	17.5	13.5	9.6	5.1	0.9	1572
9	CT 16342-CA-25-M	6.9	5.0	8.2	16.0	23.5	17.7	09.1	11.0	2.8	0.0	536
10	CT 16344-CA-9-M	1.3	2.6	13.0	23.4	22.8	17.8	14.2	4.7	0.2	0.0	806
11	CT 16346-CA-20-M	0.5	2.2	9.2	21.9	32.5	27.2	3.0	2.2	1.3	0.0	770
12	CT 16350- CA-5-M	0.8	2.5	8.8	16.4	24.4	23.9	11.0	5.5	4.8	1.9	810
13	CT 16355-CA-15-M	2.4	6.6	11.3	20.5	24.6	18.9	11.1	4.6	0.0	0.0	571
14	Golden	0.0	5.1	23.7	28.7	19.7	13.0	7.3	01.3	1.3	0.0	312
15	IRAT 104	0.0	7.6	18.8	27.8	26.0	10.4	8.3	1.2	0.0	0.0	438
16	IRAT 13	0.0	6.8	15.3	25.4	19.1	14.7	7.2	6.1	5.5	0.0	447
17	IRAT 257	0.0	2.6	14.3	25.3	21.9	15.5	12.5	4.3	3.8	0.0	428
18	IRAT 325	0.0	7.1	25.3	26.5	20.8	13.3	3.1	3.8	0.0	0.0	396
19	NERICA 15	0.0	7.0	19.7	20.9	24.4	13.8	11.5	2.6	0.0	0.0	388
20	NERICA 9	0.4	3.5	13.4	19.8	23.8	21.1	16.0	1.9	0.0	0.0	694
21	VANDANA	0.5	4.1	17.4	29.8	24.7	18.3	5.3	0.0	0.0	0.0	617
22	WAB 365-B-1H1-HB	4.1	9.8	20.2	20.4	23.7	12.8	9.1	0.0	0.0	0.0	667
23	WAB 450-I-B-38-HB (NERICA 1)	2.8	9.6	20.8	25.7	24.1	11.2	5.9	0.0	0.0	0.0	585
24	WAB 56-104	1.0	7.6	12.7	24.0	21.0	17.5	10.9	4.6	0.6	0.0	1208
25	WITA 1	0.5	5.5	16.1	26.4	29.1	19.2	3.2	0.0	0.0	0.0	562
	Mean	1.8	6.3	15.1	22.4	22.5	16.9	9.8	4.1	1.51	0.2	
	LSD$_{0.05}$	3.9	7.9	12.5	13.8	12.3	12.3	8.6	7.4	4.6	1.9	
	Var	2.01	0.95	1.5	1.30	0.8	0.84	2.02	2.14	1.90	0.79	
	F.value	0.04	0.55	0.1	0.20	0.8	0.66	0.05	0.0	0.05	0.7	

4.5   DISCISSIONS

4.5.1   Shattering pattern

The high levels of significance for grain shattering scores at 10 days, 20 days and 30 days after anthesis shows that the scores have adequate variability to warrant selection. The similar ratio of range to LSD and variance suggests that the record has similar precision and reliability for explaining variability in the record on the dates. According to Gomez and Gomez (1984), the ratio of range to LSD and variance are indicators of precision of a record. The finding that ratio of range to $LSD_{0.05}$ for 10 days after anthesis was more than three times the value at 20 days to 30 days after anthesis implies that more reliable information on variability in shattering trait could be got at 10 days after anthesis. This implies that the highest level of discrimination among a segregating population could be got 20 days after anthesis. This work confirms findings of Jin and Inouye (1982) that the tensile strength to dislodge rice grains drops drastically between the seven (7) and 21 days after heading based on record taken at weekly intervals after heading. In the current study, the greatest drop in tensile strength to dislodge the grains was between 10 and 20 days after heading. Similarly, Ji et al. (2006) found the highest reduction in mean tensile force for grain shattering between 15 and 20 days after heading. The period 20 days after heading is equivalent to about 15 days after heading, depending on variety and environmental conditions (Jennings et al., 1979). This work therefore tallies with the preliminary finding by Ji et al. (2006) which was conducted using *O. sativa*. In this study however, both *O. sativa* and interspecific lines were tested. It could that the fact that interspecific rice has up to 87% genome coverage comprising *O. sativa* component (Ndjiondjop et al., 2008a; Ndjiondjop et al., 2008b), most of the genetic block found in O. sativa are present in the interspecific lines. This finding is suggest that accurate estimation of shattering level should be conducted at between 10 days and 20 days after anthesis. Traditionally, shattering degree is used 30 days after heading in studies. This stage is greatly influence by environment according to Mohanty and Sahu (1968).

The findings that the greatest drop in shattering trend was within 10 and 20 days after anthesis could be due to the trend development of abscission layer. Recent findings by Konishi et al. (2009) revealed that one layer of cells division occurs at the base of rice grains around 2 weeks before heading and then lignin disposition starts at the base expect the separation layer cells around 1 week before heading. The lignin layer is responsible for separation of the grains from the floret (Fukuta et al., 1994). Our record taking at 10 days

after anthesis may have occurred after the separation of the walls to make the grains shatter had already occurred. At 20 days after anthesis when we took the second record, shattering varieties were already distinctly different thus similar to the third record taking at 30 days after anthesis. This study demonstrated that the shattering trend decreased with time. The highest level of discrimination among a segregating population could be achieved at 10 days after flowering contrary to the traditional date when shattering level was always recorded.

4.5.2 Diurnal variation in flowering in rice

Results of this study that NERICA genotypes had reached anthesis by 10:00 hrs and that a few CTs line also had reached anthesis by 10:00 hrs could be due to transfer of traits of stress avoidance from *O. glaberrima* to these two rice groups. Both CT lines and NERICAs are genotypes developed through crossing *O. glaberrima* with *O. sativa* japonica. *O. glaberrima* genotypes do under go anthesis early as a way of avoiding heat stress (Prasad et al., 2006; Jagadish et al., 2008). Heat stress leads to pollen sterility and poor seed development (Prasad et al., 2006; Jagadish et al., 2008). This early anthesis of *O. glaberrima* has been widely reported. Jagadish et al. (2008) for instance found that *O. glaberrima* attained anthesis one and half hours after dawn in the United Kingdom. Other studies similarly showed that *O. glaberrima* reached anthesis at around 9:30 hrs within the tropics (Nishimaya and Blanco, 1980; Prasad et al., 2006).

The pattern of anthesis of the spikelets on cold and wet days was similar to that on hot and dry days but that peak times varied gave clues to the level of adaptation of the three rice groups to weather conditions. The early anthesis of both NERICAs and CT genotypes was expected since they are interspecific genotypes generated from crossing *O. sativa* with *O. glaberrima*. However, the wide range of time over which NERICA attained anthesis when compared with CT lines could be due to special adaptation of the *O. sativa* parents used in the generation of NERICAs since the *O. glaberrima* parents were all accessions adapted to a common agro ecology in West Africa (Jones et al., 2007b). The *O. sativa* parent for NERICAs were CG 56-104, a widely adapted *Oryza sativa japonica* in West Africa (Jones et al., 2007b). In order for these varieties to produce seed in the areas with high diurnal temperatures in West Africa, anthesis was spread over some time. The *O. sativa* parent for CT lines was, however, CAIAPO, a widely adapted rice variety in South America, where temperature peaks are lower than in West Africa. The findings of this study that the cold

and wet days, the *Oryza sativa* flowered over a longer period than on hot and dry days implied that the populations used had special adaptation for avoidance of cold stress. On cold and wet days, the *Oryza sativa* reached anthesis over a longer period than on hot and dry days. The *Oryza sativa* that are used as upland rice are mostly the japonica types (Jones et al., 1997c). The japonicas are more adapted to lower temperatures than the indicas (Nishiyama, 1984; Matsui et al., 2000). It is likely that the adaptation of the japonicas is by spreading anthesis time over a long period. In most tropical countries, the rice shed pollen from 9:00 hrs to 12:00 hrs.

The implications of these findings to rice breeding are that for genotypes that have anthesis during the later time of the day than target genotypes designed to cross with, methods that induce early anthesis should be involved in the management of the former genotypes. One method commonly used to induce early anthesis is by keeping the plant for longer hours in darkness during reproductive growth stages. Rice is a short day plant and keeping it in dark places for longer triggers stress responses that initiates early anthesis (Izawa, 2007). Most rice varieties are short-day plants and keeping such varieties in dark places for extended periods trigger stress responses that initiate early flowering (Izawa, 2007). In addition, information on anthesis duration helps in the hybridization planning and staggering the planting of genotypes.

4.5.3   Duration of pollen production in rice

Results of this study show that the period of spikelet anthesis varied from 7 to 10 days. In a study involving red rice, however, an anthesis range of 8 to 17 days was recorded (Shivrain et al., 2006). The current study found that the period of spikelet anthesis varied over a lower range than when compared with studies in other locations. This could have been because temperatures, humidity and length of days, which are key determinants of the degree of anthesis, are uniform in Uganda and are optimum for rice production. Since the country's days and nights are of equal length, and the temperature and humidity levels are optimum for rice anthesis and filled grains, breeding could be conducted throughout the year. The temperature, humidity and length of days, which are key determinants of the degree of anthesis are conducive for rice production in Uganda. For instance, the temperature range of 16^0C to 30^0C, which was reported in the growth period of rice in this experiment, is very suitable for rice development. Temperatures of more than 35^0C require special adaptation

by the crop to mechanisms that include delayed anthesis or early anthesis (Nishiyama, 1984; Matsui et al., 2000).

4.6   CONCLUSION

This study identified the time of peak anthesis in rice in Uganda and the influence of environmental conditions on rice anthesis patterns. The most appropriate growth stage to assess the grain shattering trait of 10 days after anthesis was also determined. In addition, the optimum time to make crosses in Uganda is between 10:00 am and 2:00 pm. However, if the genotypes are of interspecific rice types they will reach anthesis earlier. Generally, rice reached peak anthesis between 1:00 pm and 2:00 pm on cold and wet days. The study also found that *O. sativa* rice shed pollen over a longer period on cold and wet days. The peak anthesis time of rice in Uganda was three to six days after the first day of anthesis. Based on these findings, grain shattering can be assessed with more precision and rice breeding should be carefully planned to get maximum pollen shedding for making crosses in rice.

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CHAPTER FIVE

Characterization of Rice Germplasm for Grain Shattering and Drought Tolerance

Abstract

Drought stress and grain shattering are major constraints of rice production in Uganda. It is important that the germplasm is characterized for these traits in order to select genotypes with preferred performance for production and select parents for use in breeding for optimum grain shattering and drought tolerance. The specific objective of this study was to identify lines/varieties with diverse performance in terms of tolerance to drought at vegetative stage, and reproductive stage and grain shattering ability for use in the breeding program. An alpha lattice design with 25x8-arrangement was used to evaluate 191 genotypes during 2006/7dry season. Drought stress was managed, by applying supplementary irrigation of 20mm/5days whenever it did not rain, until peak tillering stage when irrigation was terminated. After 12 days of water stress, irrigation was resumed, when 30% of the available soil water was used up. Irrigation was again terminated when the penultimate leaf was fully expanded. Spikelet fertility score varied from 26.49 to 92.12%, mean leaf roll varied from 1.17 to 6.33 and mean grain breaking tensile strength from 6 to188gf). Nine out of the 189 genotypes were more drought tolerant than all the standard drought tolerant checks. Of three rice groups that were used in the study, only two were significantly distinguishable using canonical discriminate analysis. About 32% and 48% of the population had high tolerance to drought at reproductive and vegetative stages, respectively. Results also showed that, 53% and 41% of the population had loose and tight grain attachment to the pedicel, respectively. Among the genotypes, 18 had optimum grain shattering ability for maximum grain recovery during harvest and minimal loss due to grain shattering. The 18 genotypes also had high tolerance to drought stress under both reproductive and vegetative stages. There was adequate variability in the population to warrant selection of parents for use in the breeding programs with similar conditions[1].

5.1 INTRODUCTION

Rice is a major food-cash crop in Uganda but average yield on-farm is still very low at 1.4t - 1.5t ha^{-1} (FAOSTAT, 2008), yet varieties with yield potential of about 3t ha^{-1} under normal cropping conditions are already available (NARO, 2007). Yield losses due to drought stress and grain shattering are among the major constraints of rice production in the country (NARO, 2007). Drought stress is important partly because the available rice varieties are not tolerant to drought stress and that rice production is expanding to parts of the country that have less dependable rainfall. Frequent drought still limits full realization of the potential of released rice varieties (Imanywoha et al., 2002). At the moment, upland rice cultivars that are characteristically produced under rain-fed environments are being promoted by the Ugandan government as an alternative to the irrigated rice due to increased cultural, health and environmental reservations on continued use of the wetlands for rice production (Odogoola, 2006; PMA, 2007).

As a consequence of promotion of upland rice, three varieties were released in 2002 and two in 2007 (NARO, 2007). Of the five varieties, the most popular one showed stronger attachment of the grain to the pedicel than the rest and was difficult to dislodge during threshing leading to poor grain recovery at harvest (Lamo et al., 2007). In addition, several potential lines could not be presented for release despite meeting all preferred qualities except loose shattering or tight grain attachment on to the pedicel. The majority of these lines that were discarded were introductions developed through *O. sativa* x *O. glaberrima* crossing and had improved tolerance to common biotic and abiotic stresses (Lamo et al., 2007). It is hypothesized that there is potential to improve rice for grain shattering once the mode of gene action for grain shattering is adequately understood for a given population (Li et al., 2006b). Informative output on the mode of gene action can be generated when relatively few but representative genotypes are used in generating segregating populations. Credible information on mode of gene action guides genetic resource enhancement in any breeding program. However, it can only be successful when appropriate methods of selection of parents are employed. Choice of parents that are genetically divergent is important in crop hybridization especially when exploitation of transgressive segregants is the breeding objective. Besides, genetic uniformity, or lack of genetic diversity, is considered to increase the potential vulnerability of the crop to biotic and abiotic constraints (Chatel et al., 1996). Appropriate choice of parents would provide representative genotypes

for transmission of drought tolerance and grain shattering in rice germplasm in Uganda. This is plausible considering that heritable variability in rice populations for drought tolerance exists (Atlin, 2003; Lafitte et al., 2003).

Quantitative methods allow for more precise classification than qualitative methods, especially when appropriate statistical methods are used to analyze the data. Among multivariate analysis methods, canonical models provide information on quantitative differences among genotypes. Studies conducted elsewhere have demonstrated that drought effect could be assessed at vegetative and reproductive stages using leaf roll scores and filled grains (Satyanarayana et al., 2000). Also, quantitative assessment of grain shattering degree is a preferred methods of determining variability in rice populations (Ichikawa et al., 1990). Selection of parents for use in the genetic study for grain shattering ability and drought tolerance was the focus of this work. The genetic base of rice was broaden prior to the study by introducing rice from other germplasm centres with diverse shattering and drought tolerance traits. So far, no study to characterize rice germplasm in Uganda in terms of grain shattering and tolerance to drought stress has been conducted simultaneously.

5.2   Objectives of the study

The overall aim of this study was to select parents with diverse performance in terms of drought tolerance and grain shattering for use in genetic studies.

The specific objectives were to:

i.   assess the performance of different rice groups under imposed drought stress, and

ii.   characterize rice lines based on their differential tolerance to drought and grain shattering.

5.3   MATERIALS AND METHODS

5.3.1 Study environments

This experiment was conducted at National Crop Resources Institute (NaCRRI), at Namulonge in central Uganda at 00^0 32qN latitude and 32^0 53qE longitudes with altitude of 1,150m above sea level. The soils of the place are clay loam. The period December to March is characteristically the long dry season but mean long term annual rainfall is 1,270mm. The monthly means of maximum and minimum air temperature, rainfall and relative air humidity during the period the experiment was conducted is shown in Table 5.1.

Table 5.1 Monthly mean maximum and minimum temperature, rainfall and rainy days

	Max Temp (^{0}C)	Mini Temp (^{0}C)	Mean monthly Rainfall (mm)	Rainy days days
December	28.0	15.9	4.8	15
January	28.9	16.6	1.2	24
February	28.7	16.5	3.7	17
March	29.2	16.6	1.6	24
April	28.7	16.6	3.0	16

5.3.2 Plant materials

A total of 191 rice introductions from major rice breeding centers were evaluated. Of the 191 materials, 77 were *O. sativa* comprising 45 from African Rice Centre, 15 lines from International Rice Research Institute (IRRI), 13 from Mali, three from Uganda and one from China. Among the introductions, there were three *O. glaberrima* accessions. The remaining 111 were interspecific lines developed from *Oryza sativa* x *Oryza glaberrima* crosses comprising 18 from ARC and 93 the International Center for Tropical Agriculture (CIAT), Colombia coded as the CT series. However, among the interspecific samples, two genotypes namely WAB 880-1-27-9-2-P1-HB and WAB 450-24-2-3-P-38-1-HB were duplicates from different repeated introductions from IRRI and WARDA-Africa Rice Centre. The 93 interspecific lines from CIAT were BC_4F_1s developed from crossing CAIAPO, a tropical *Oryza sativa* japonica from Colombia with RAM 24 (*Oryza glaberrima*).

5.3.3 Experimental design

The experiment was planted on the 12th December 2006. All the 191 genotypes planted but WAB 880-1-27-9-2-P1-HB, WAB 450-24-2-3-P-38-1-HB, NERICA 6, WAB 365-B-2-H1-HB, NERICA 3, NERICA 5 and NERICA 10 were planted twice, while genotype CG 104 was entered three times. The experiment was laid out as an alpha-lattice design in a 8 x 25 arrangement in two replicates. A total of 36 hills were planted in each 1m x1m experimental plots spacing of 20cm x 20cm. About five seeds were planted per hill using the line dibbling method before thinning to a single plant three weeks after germination. The field management included irrigation, fertilizer application and hand weeding. The irrigation was applied at the rate of 20mm per week. The first level of fertilizer was applied at the rate of 30kgN ha^{-1}, 15kgP ha^{-1} and 15kgK ha^{-1}. The fertilizers used were urea and NPK. At booting 24kgN ha^{-1} was also applied as a topdressing.

5.3.4 Data collection

Records were taken on grain shattering and drought tolerance score. Grain shattering degree was measured at 30 days after booting for each selected individual plant sampled. Shattering degree was determined using the method described by Elliot and Perlinger (1977) but with modifications detailed in (Lamo et al., 2007). Instead of cutting the panicles before assessing the shattering score, a hand held breaking tensile strength (BTS) equipment fabricated at the University of Kwa-Zulu Natal was used in the field with the plants not harvested. The equipment measured the minimum force required to dislodge individual grains by pulling according to Porter et al. (1994). The unit was calibrated in gram force (gf) and the BTS was designed to measure from 0gf to 400gf. The record involved randomly sampled five plants from each of the 1m x1m sub-plots. A detailed description of its operation is in Chapter 3 of this thesis. The main primary panicle was tagged for each plant on the date when 50-60% of the inflorescences had florets open (flowered). Tagging was done between 10:00 am and 3:00 pm, the time when most inflorescences flowered at NaCRRI site. All the florets that flowered earlier along with those that had not flowered on the date tagged were carefully excised off the peduncle. On the 30th day after tagging, record on shattering was taken. A total of ten grains per panicle were sampled.

The reaction of the different materials to drought was assessed at the vegetative and reproductive growth stages. In order to assess the reaction of the varieties at vegetative stage, irrigation was terminated when about 50% of the plants in the whole experiment had attained maximum tillering. The assessment at tillering stage was considered for a whole experimental area because all the varieties used were of similar duration to maturity period, 110-130days. The assessment of tillering capacity, however, was taken plot by plot every four days from the start of tillering. Data was taken on leaf rolling at the time when 30% of the available moisture had been lost from the soil at 20cm depth. The available soil moisture was taken using the ECHO soil moisture tester (Decagon Devices, Inc Pullman, Washington USA). The leaf roll score was taken using 0-9 scale: 0, healthy; 1, shallow V-shaped leaves; 3, deep V-shaped leaves; 5, fully capped leaves; 7, leaf margin tightly held in U-shape; 9, tightly rolled leaves (Gregorio and Cabuslay, 2005). This is a modification of the standard SES scoring system for rice developed by IRRI (IRRI, 2002).

In order to assess drought stress during reproductive growth stage, drought stress was imposed by terminating irrigation when about 50% of the populations had reached a point

when interauricular distance between the flag leaf and penultimate leaf was zero (Ji et al., 2007). It is the period when it is about 10 days before flowering. It is the time when the penultimate leaves were fully expanded. Rainfall during the trial period was recorded. Irrigation was resumed 14 days later when 30% of the available water had been lost from the soil at 20-cm depth. The available soil moisture was taken using the ECHO soil moisture tester (Decagon Devices, Inc Pullman, Washington USA). All the grains from each panicle were hand threshed and dried. The filled and unfilled grains are then separated using floatation methods. The numbers of filled and unfilled grains are then counted.

Overall three treatments of water stress were applied, first was from 20th January to 3rd February, 2007. It was imposed to evaluate early maturing varieties. The second treatment was applied between 10th February and 24th February, 2007. It was imposed to evaluate early maturing varieties for reproductive stage stress tolerance and late maturing varieties for late vegetative stage stress tolerance (Fig. 5.1). The last treatment was from 3rd march to 17th March, 2007. It was imposed to evaluate late maturing varieties for reproductive stage stress tolerance.
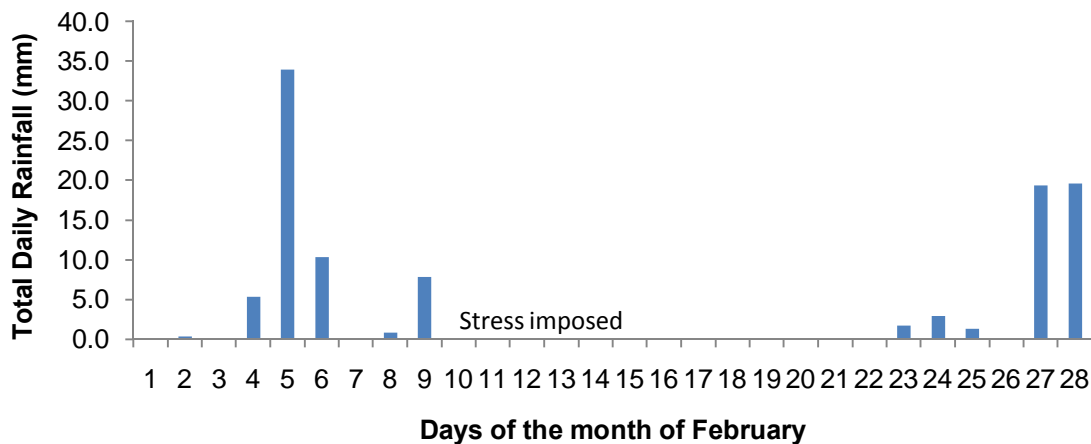
Figure 5.1   Second water stress event for late vegetative and early reproductive stage varieties

5.3.5   Statistical analysis

The data was analyzed using Genstat version 12. First, the data was subjected to unbalanced Analysis of Variance (ANOVA) using the varieties and rice groups as factors. Second, the variates were subjected to ANOVA by mixed model with auto-regression with only rice groups *O. sativa*, *O. glaberrima* and interspecific lines as factors. The auto-

regression was to estimate and exclude field spatial variation (Gilmour et al., 1997). The shattering and drought sensitivity mean values were tested for correlation before subjecting to canonical discriminate analysis according to Hair et al. (1987). In the analysis, the mean of the canonical discriminant function was the centroid. The Mahalanodis squared distance was used for difference between the group centroids. The mean values for the three traits was finally subjected to hierarchical clustering using m̄70gf, >71<100gf, ¯100gf grouping for grain shattering ability and m̄85, >85 and for spikelet fertility. The ratio of range to LSD was calculated used by Iqbal and Clarke (2003) to select variables that best decide variation within a population. It has more power in discrimination among variables when comparing with other variables that have different scales of measurements.

5.4   RESULTS

5.4.1   Variation within the rice populations

The reaction of the 1191 genotypes to early drought tolerance at reproductive stage and the shattering ability score revealed that spikelet fertility score varied from 26.49 to 92.12%, mean leaf roll varied from 1.17 to 6.33 and mean grain breaking tensile strength (6-188gf). The distribution of the mean shattering score was skewed to the left, while the leaf roll score values almost normal and spikelet sterility skewed to the right side of the distributed (Fig 5.2, Fig 5.3 and Fig 5.4).
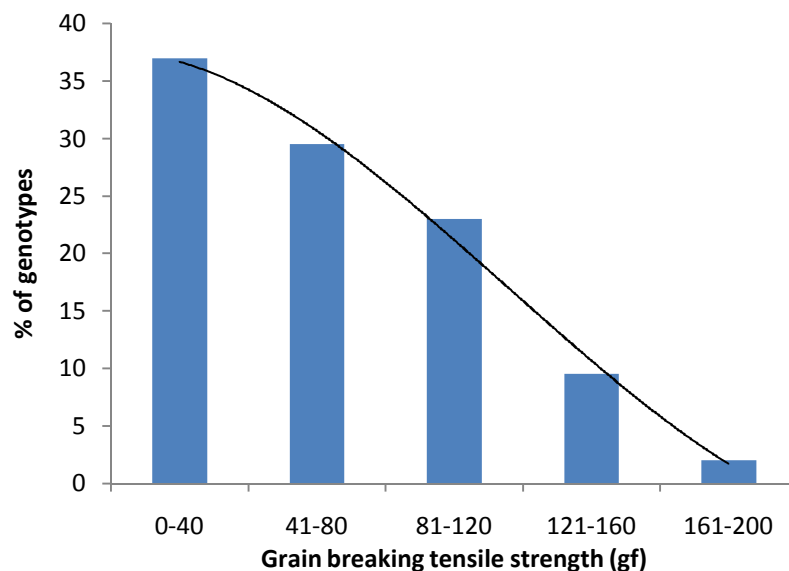
Figure 5.2   Distribution of genotypes according to mean shattering ability
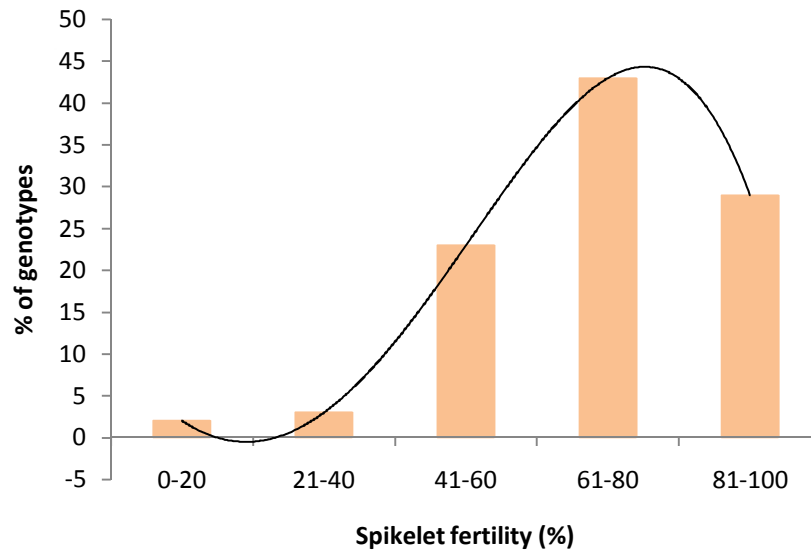
Figure 5.3   Distribution of genotypes according to percent spikelet fertility
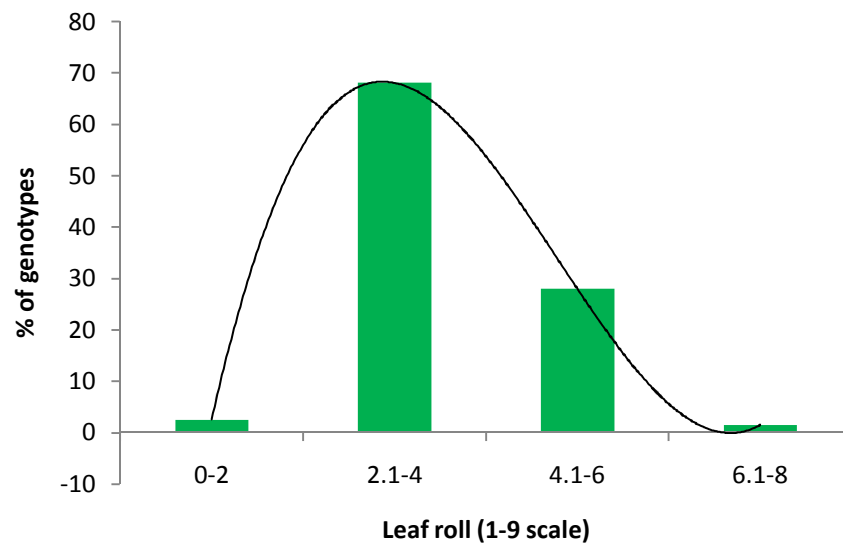
Figure 5.4   Distribution of genotypes according to mean leaf-roll score

5.4.2   Variance components among rice genotypes and correlation among variates

Results showed that there was significant variation for spikelet fertility, leaf roll and grain shattering score for the genotypes but not for the genotype groups (Table 5.2). The discriminate canonical variates, which is the linear combination of variates, namely grain shattering score, leaf roll score and spikelet fertility, identified two major groups based differences between the variates. In the first group, spikelet fertility had the highest contribution to variation while in the group leaf roll had the highest contribution. The first

canonical variate accounted for 65% of the variation among the three variates. The second variate accounted for 32% and both the variates are significant (P=0.001) (Table 5.4).

When the data was subjected to canonical analysis for grain shattering ability, grain fertility score and leaf roll using the separate groups, *O. sativa*, interspecific lines and *O. glaberrima* a scatter of the centroids was constructed (Fig. 5.5). At five percent level of significance, there was an overlap in the population *O. sativa* and interspecific lines. The visual variation in *Oryza glaberrima* is that it is distant from both *O. sativa* and interspecific and the circle is the largest.

Table 5.2 Mean square for spikelet fertility, leaf rolls and shattering for all genotypes and different genotype groups

Change	d.f.	Spikelet fertility	Leaf rolls	Shattering
Group	1	0.39	2.51	0.18
Genotype	190	10.81***	1.30***	5.98***
Group.Var	10	1.22	0.75	0.78
Residual	178	26.70	1.50	432.

Table 5.3 Linear correlation of different variates on the canonical variate

Trait	Canonical discriminate variates	
	1	2
Variance of shattering ability compared with all variates	0.02	-0.04
Variance of leaf roll score compared with all variates	0.02	0.17
Variance of spikelet fertility compared with all variates	0.18	0.07
P level of significance	0.01	0.01
Variance accounted for	65.0	31.9
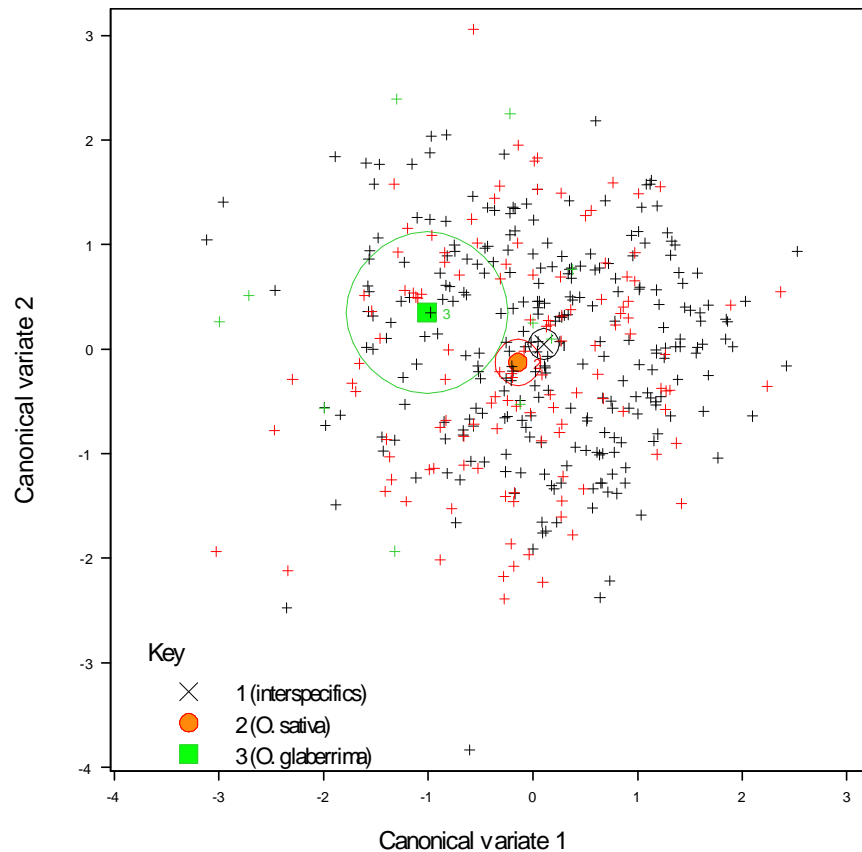
Figure 5.5 the canonical leadings of independent variables on the two canonical discriminant variates of genotypes

5.4.3   Major groups in the rice genotypes

Among the test materials, 10 reference genotypes for grain shattering ability were included. A summary of their grain shattering score is shown in Table 5.5. Generally, high shattering genotypes had shattering scores of less than 30gf while non-shattering genotypes had shattering scores of more than 100gf. The acceptable grain shattering degree for maximum recovery of grain during harvesting and threshing varies with methods of harvesting and threshing. When harvesting is done by hand, a shattering degree of 70gf to 100gf is preferred but when threshing is by machine, shattering degree of 50gf to 80gf is acceptable (Fukuta, 1995). In Uganda where grain threshing is done by hand, 12 genotypes with shattering degree in the range of 50gf to 80gf were identified (Appendix 1). Four reference genotypes for drought tolerance at vegetative stage were also included. Their performance in terms of susceptibility to drought at vegetative stage is shown in Table 5.6. All the genotypes had leaf roll score in the range of 4.0 to 7.0. Similarly, 15 genotypes rated as tolerant to drought had spikelet fertility of 70% and above except ITA 212. However, varieties rated as less tolerant to drought had spikelet fertility values of less than 70%.

Table 5.4 Performance of reference genotypes for grain shattering ability using a grain shatter tester

Genotype	Standard (WARDA)	Evaluation (gf)
NERICA 6	Shattering	23.5
FKR 14	Shattering	26.0
WITA 3	Shattering	27.5
WITA 7	Shattering	29.0
WITA 12	Normal	37.0
WAB 56-104	Normal	65.0
WAB 365-B-1H1-HB	Non- shattering	106.0
IDSA 10	Non- shattering	108.0
WAB 56-39	Non- shattering	117.0
FKR 41	Non- shattering	128.0

Table 5.5 Performance of genotypes for drought tolerance at Namulonge site

Vegetative stage drought stress			Reproductive stage drought stress		
genotype	standard WARDA	leaf roll	genotype	standard WARDA	spikelet fertility (%)
NERICA 1	Poor tolerance	3.2	MOROBEREKAN	Tolerant	76
WAB 56-104	Poor tolerance	4.3	NERICA 5	Tolerant	70
CG 14	Poor tolerance	5.0	ITA 212	Tolerant	63
LAC 13	Poor tolerance	3.1	NERICA 7	Tolerant	88
			NERICA 3	Tolerant	74
			IDSA 110	Tolerant	74
			IRAT 13	Tolerant	90
			VANDANA	Tolerant	88
			CG 14	Tolerant	84
			SALUMPIKIT	Tolerant	80
			OS 6	Tolerant	71
			CO 39	Tolerant	88
			NERICA 1	Tolerant	79
			WAB 96-3	Tolerant	72
			IRAT 216	Tolerant	73
			NERICA 5	Less Tolerant	67
			NERICA 10	Less Tolerant	67
			IRAT 104	Less Tolerant	66

Genotypes that have grain shattering score in the range of ‾50 m80gf, spikelet fertility more
that 70% and mean leaf roll score of less than 3.5 are shown in Table 5.7. A total of 18 out
of 191 genotypes were in this group, 12 of which were CT lines. Among the 18 genotypes,
two namely WAB 56-104 and IRAT 216 are known to have resistance to drought during
vegetative and reproductive stages, respectively.

Table 5.6 List of genotypes with optimum shattering score, high spikelet fertility and minimal leaf roll score under drought stress

No	Genotypes	Spikelet fertility (%)	Shattering score (gf)	Leaf roll score (0-9)
11	CT 16341-CA-8-M	72.6	61.0	2.3
19	CT 16334(2)-CA-11-M	78.3	55.1	2.6
24	CT 16338-CA-6-M	84.5	75.5	2.5
26	CT 16324-CA-9-M	74.3	78.0	2.2
38	CT 16315(1)-CA-16-M	74.4	69.0	2.3
63	CT 16333(2)-CA-6-M	80.2	61.1	3.2
66	CT 16333(1)-CA-22-M	96.7	58.4	2.3
67	CT 16313-CA-4-M	83.8	69.7	2.9
73	CT 16329-CA-5-M	78.0	76.8	3.2
75	CT 16313-CA-3-M	86.7	53.5	2.3
79	CT 16311(1)-CA-6-M	71.1	58.2	2.3
94	Caiapo	78.7	67.2	2.7
114	WAB 56-104	77.7	71.3	3.4
115	WAB 450-1-B-P-62-HB	88.8	72.1	2.2
145	IRAT 216	73.3	70.5	3.3
174	WBK 40 (F4)	79.3	63.4	1.8
194	Golden	83.1	56.5	3.3
3	CT 16346-CA-10-M	90.0	56.8	3.5
Overall	**Mean**	**67.0**	**57.0**	**3.40**
	LSD $_{0.05}$	**1.88**	**6.30**	**0.16**
	CV%	**13.2**	**53.0**	**23.0**
	Range/LSD	**17.6**	**15.3**	**156.0**
	Variance	**18.6**	**11.5**	**20.1**

A list of only 30 genotypes including top 20 and bottom 10 least performing genotypes in terms of spikelet fertility are presented in Table 5.8. However, up to 60 genotypes have spikelet fertility score of more than 80% (Appendix 5.1). Among the top 20 genotypes, three namely NERICA 7, CO 39 and VANDANA were reference materials for high drought the tolerance at reproductive growth stage.

Results of the top twenty and least performing genotypes performance in terms of shattering score are shown in Table 5.9. Up to 53% of the population had shattering ability score of 30gf or less and 41% had shattering ability score of 100gf and above (Appendix 5.1). In addition, a list of only 30 genotypes including top 20 and bottom 10 performing genotypes in terms of leaf roll score are presented in Table 5.10. However, a total of 91 genotypes have leaf roll scores of less than 2.5 (Appendix 5.1). Among the top 20

genotypes, three namely IDSA 110 and CO 39 were reference materials for high drought tolerance at the vegetative stage.

Table 5.7. The top 20 and bottom 10 genotypes in terms of percent spikelet fertility

No	Genotypes	Spikelet fertility (%)	Shattering score (gf)	Leaf roll score (0-9)
Top 20 genotypes				
112	WAB 56-50	96.3	143.4	3.2
53	CT 16333(1)-CA-18-M	91.1	24.1	5.0
34	CT 16326-CA-3-M	89.1	71.5	4.7
101	NERICA 14	88.7	121.4	4.8
108	WAB 56-39	88.6	117.9	3.3
137	NERICA 7	88.1	86.5	4.3
132	CO 39	87.8	138.4	3.2
142	VANDANA	87.6	81.7	4.5
124	NERICA 6	87.4	47.8	2.9
83	CT 16340-CA-9-M	86.7	39.3	3.8
190	NERICA 17	86.6	83.4	3.8
45	CT 16329-CA-10-M	85.5	27.9	2.7
177	WBK 35 (F3)	84.9	121.2	3.3
92	CT 16315(1)-CA-1-M	84.7	101.0	2.8
1	CT 16330(1)-CA-2-M	84.1	65.6	3.8
165	IR 64	83.8	33.5	1.7
188	NERICA 15	83.5	113.8	4.5
90	CT 16307-CA-5-M	83.5	49.2	3.8
10	CT 16353-CA-17-M	83.4	23.8	4.2
30	CT 16324-CA-10-M	83.1	17.4	2.6
Bottom10 genotypes				
169	IR 57514-PMI 5-B-1-2	49.4	18.2	2.5
80	CT 16316-CA-2-M	49.4	13.9	3.0
106	IDSA 6	49.1	57.4	2.2
104	ITA 123 (FKR 28)	47.9	47.9	3.2
175	RAM 118	47.8	34.8	6.3
49	CT 16346-CA-11-M	47.8	14.5	3.3
32	CT 16312(1)-CA-1-M	47.3	55.1	2.7
166	IR 77298-14-1-2	45.7	25.3	3.4
155	LAC 23	43.8	54.1	3.0
65	CT 16307(1)-CA-2-M	27.9	29.0	1.8
Overall	Mean	67.0	57.0	3.40
	LSD$_{0.05}$	1.88	6.30	0.16
	CV%	13.2	53.0	23.0
	Range/LSD	17.6	15.3	156.0
	Variance	18.6	11.5	20.1

Table 5.8. The top 20 and bottom 10 genotypes in terms of grain shattering score

No	Genotypes	Spikelet fertility (%)	Shattering Score (gf)	Leaf roll Score (0-9)
Top 20 genotypes				
6	CT 16330(1)-CA-3-M	80.0	188.2	1.8
43	CT 16330(1)-CA-12-M	73.4	176.0	2.7
4	CT 16323-CA-30-M	65.3	175.9	4.8
193	NERICA 13	69.5	152.6	4.2
18	CT 16334(2)-CA-2-M	74.6	149.5	3.8
196	IRAT 325	69.5	144.0	3.8
112	WAB 56-50	96.3	143.4	3.2
147	IRAT 104	71.6	142.7	3.2
132	CO 39	87.8	138.4	3.2
197	IRAT 257	61.8	137.7	3.3
176	WBK 39 (F3)	65.5	137.4	2.8
149	WAB 96-3	58.3	134.7	4.5
118	WAB 450-I-B-38-HB (NERICA 1)	79.3	134.6	4.3
186	NERICA 11	70.9	133.2	4.3
109	FKR 41	72.1	128.8	3.0
134	NERICA 9	65.0	128.0	3.5
195	Sikamu	79.5	122.1	4.0
101	NERICA 14	88.7	121.4	4.8
177	WBK 35 (F3)	84.9	121.2	3.3
139	NERICA 3	75.1	119.2	3.9
Bottom 10 genotypes				
54	CT 16333(1)-CA-7-M	72.7	17.7	2.7
30	CT 16324-CA-10-M	83.1	17.4	2.6
183	RAM 24	73.6	16.6	5.7
198	Jaggery	60.9	14.8	5.3
59	CT 16345-CA-12-M	68.4	14.7	2.2
161	WAY RAREM	56.8	14.5	2.9
49	CT 16346-CA-11-M	47.8	14.5	3.3
80	CT 16316-CA-2-M	49.4	13.9	3.0
9	CT 16350- CA-5-M	67.8	10.6	3.4
85	CT 16342-CA-4-M	50.2	7.2	3.7
Overall	**Mean**	**67.0**	**57.0**	**3.40**
	LSD$_{0.05}$	**1.88**	**6.30**	**0.16**
	CV%	**13.2**	**53.0**	**23.0**
	Range/LSD	**17.6**	**15.3**	**156.0**
	Variance	**18.6**	**11.5**	**20.1**

Table 5.9 The top 20 and bottom 10 genotypes in terms of leaf roll score

No	Genotypes	Spikelet fertility (%)	Shattering score (gf)	Leaf roll score (0-9)
Top 20 genotypes				
153	IRAT 16	64.2	23.5	1.2
150	NERICA 12	66.9	116.8	1.3
62	CT 16345-CA-3-M	67.7	24.4	1.4
20	CT 16338-CA-12-M	75.2	108.7	1.5
165	IR 64	83.8	33.5	1.7
6	CT 16330(1)-CA-3-M	80.0	188.2	1.8
13	CT 16338-CA-10-M	59.7	62.7	1.8
126	ITA 321	57.9	51.3	1.8
65	CT 16307(1)-CA-2-M	27.9	29.0	1.8
50	CT 16310(2)-CA-5-M	78.9	49.0	2.0
82	CT 16342-CA-18-M	60.7	34.2	2.0
39	CT 16307(1)-CA-4-M	64.5	64.7	2.1
119	BG 90-2	51.0	41.4	2.1
7	CT 16353-CA-10-M	69.0	35.4	2.1
159	UPL RI 5	78.2	30.5	2.1
106	IDSA 6	49.1	57.4	2.2
131	OS 6	71.2	42.5	2.2
140	IDSA 110	52.9	26.0	2.2
128	FKR 14	63.4	25.6	2.2
69	CT 16313-CA-11-M	67.8	21.6	2.2
Bottom 10 genotypes				
56	CT 16354-CA-6-M	63.1	21.5	4.8
29	CT 16344-CA-12-M	56.3	71.9	5.0
46	CT 16353-CA-11-M	81.3	33.8	5.0
53	CT 16333(1)-CA-18-M	91.1	24.1	5.0
158	IR 74371-54-1-1	51.0	39.4	5.1
198	Jaggery	60.9	14.8	5.3
183	RAM 24	73.6	16.6	5.7
12	CT 16344-CA-9-M	76.9	39.0	5.8
72	CT 16317-CA-4-M	57.8	30.3	5.8
175	RAM 118	47.8	34.8	6.3
Overall	**Mean**	**67.0**	**57.0**	**03.40**
	LSD$_{0.05}$	**1.88**	**6.30**	**00.16**
	CV%	**13.2**	**53.0**	**23.0**
	Range/LSD	**17.6**	**15.3**	**156.0**
	Variance	**18.6**	**11.5**	**020.1**

5.5 DISCUSSION

5.5.1 Variation for grain shattering, leaf roll and spikelet fertility

In the population under study, the distribution of genotypes in terms of minimum force required to dislodge grains was skewed to the lower scores of the distribution curve. The skewness indicated that most of the materials assessed had low scores for force required to dislodge the grains indicating that they have high tendency to shatter. This observation is contrary to the findings in studies conducted in *O. sativa* populations in India, Japan, Indonesia and the USA where all germplasm collected had normal distribution (Fukuta, 1995a). A common denominator to all the genotypes tested by Fukuta (1995a) was that they were earlier selected for preferred traits, including acceptable grain shattering ability. Genotypes used in the current study included 93 lines of a group called CT lines. The CT lines used were a third backcross generation of *O. glaberrima* x *O. sativa* crosses. The majority of these materials were segregating and selection for normal shattering ability had not been conducted on them (Guimaraes et al., 1996). Besides, they comprised of lines developed through crossing CAIAPO, a popular South America upland variety, preferred for acceptable threshability with line RAM 24 a characteristically shattering *Oryza glaberrima* accession according to Jones et al. (1997c). In addition, the collection included NERICA rice types that were developed through crossing *O. sativa* and *O. glaberrima.* The fact that CT lines along with NERICAs had *O. glaberrima* as one of the parents in their crosses, also that *O. glaberrima* is generally high shattering, the presence of 107 these genotypes in a total of 191 genotypes could explain why the grain shattering of the population tended to cluster on the lower side.

The normal distribution of the population for the leaf roll score could be explained by the fact that selection for improved vegetative stage drought tolerances has not been conducted in most breeding populations. It is widely reported that vegetative stage drought tolerance is not as important as the reproductive stage drought tolerance (Lafitte et al., 2003) and less attention had been vested to it in terms of population improvement. In the case of spikelet fertility, the skewness of the population to the right side of the population distribution indicating that they had high spikelet fertility could be because grain filling is one of the aspects breeders use in the selection for lines. Spikelet fertility has been one of the criteria used in screening new populations (IRRI, 2002; Lafitte et al., 2003). The current materials were fixed lines already selected at the respective breeding centres.

There were significant differences among the genotypes in terms of leaf rolling, spikelet fertility and grain shattering ability. This implies that the collection had adequate variability in terms of the three traits to warrant selection. In addition, significant differences for grain shattering between the three groups of rice, *O. sativa*, *O. glaberrima* and interspecific lines suggested that there were real differences in the genotype groups under study for the three traits. This was expected in the case of *O. sativa* and *O. glaberrima* because they are not only different species but also of populations that were domesticated during different periods. The *O. sativa* was domesticated from either one or both of closely related species; O. *nivara* and O. *rufipogon* (Oka, 1988). The domestication of rice is associated with selection for non-shattering alleles and the frequency for shattering alleles decreased (Li et al., 2006a). Since *O. sativa* had an earlier domestication, it is likely that it had more of the non-shattering genes.

The discriminate canonical variates, which is the linear combination of variates (Hair et al., 1987), in this case the grain shattering score, the leaf roll score and the spikelet fertility, identified two groups based differences between variates. It further revealed that the first group, the first canonical variate accounted for 65% of the variation among the three variates while the second accounted for 32%. First, the fact that this two groups account for 97 percent of the variation implies that they are adequate to used to study variability in the population, based contribution of the variates (Sharma, 1995). In addition, the highest value in group 1 with 65% implies that it can reliably explain variation in 65% of the population. However, the fact spikelet fertility had the highest contribution to variation in group 1, implies that it explains much of the variation in the population. According to Vayyey and van Santen (2002), the variate with high contribution of variation to the also has high genetic variability. In this study, genetic variability could indeed be high in this population due to the fact that they are materials generated for drought tolerance by crossing the landraces that are susceptible to drought stress and *O. glaberrima* that are known to have high tolerance to drought. Spikelet fertility is an indicator of drought tolerance and these traits have had genetic variation as a result of crossing parents with high and low spikelet fertility. According to Falconer and Mackay (1996) high genetic variability is realized when parents with high and low performance in terms of some traits are crosses.

5.5.2 Rice groups

There was more similarity between *O. sativa* and O. *glaberrima*. This observation could be due to the fact that much of the variation in the averages genome coverage of hybrids developed through crossing *O. sativa* and *O. glaberrima* are in the range of 78.2% to 94.4% depending on the parental line used (Ndjiondjop et al., 2008a). This finding implies that much of the traits of *O. sativa* could be retained in the new generation and they were thus similar to the *O. sativa*. It could be possible to identify new lines that have special traits but having much of the phenotypes of *O. sativa.*

5.5.3 Characterization for grain shattering ability, leaf roll and spikelet fertility

In order to confirm that the method of assessing grain shattering degree was appropriate, a total of 10 genotypes that have known shattering were included among the 191 test materials. Four shattering genotypes according to WARDA (2006) had minimum force required to dislodge them in the range of 0gf to 30gf (Table 5.4). Similarly, all reference genotypes that were rated as non-shattering had shattering degree of more than 70gf. Two varieties that were rated as normal in terms of shattering degree had minimum score required to dislodge the grain of more than 30gf and less than 70gf. This finding showed that the method used to test grain shattering ability was suitable for this population.

Reference genotypes with known performances in terms of tolerance to drought at the reproductive stage and grain shattering ability were included among the test genotypes in this study. There was coherence in the grouping of reference materials and the grouping according to our work. A total of 14 out of 15 genotypes rated as tolerant to drought according to WARDA (2006) had spikelet fertility of more than 70%. A total of three reference genotypes rated as poor in tolerance to drought according to WARDA (2006) had spikelet fertility of less than 70%. The three genotypes rated as low in response to drought tolerance in this study are NERICA 5, NERICA 10 and IRAT 104. In another study, NERICA 5 gave higher yield than average yield under drought, while NERICA 10 provided lower yield under drought stress (Manneh and Ndjindjop, 2008). This apparent difference could be due to the mechanisms for drought tolerance in the variety. While the Africa rice centre work used basically root and other shoot traits as a drought tolerance trait, in our study leaf roll and spikelet fertility were used. Rice is known to avoid drought stress through stomatal closure and development of thick cuticle (Lafitte et al., 2003). Generally, however,

these findings on the 14 genotypes confirm that the extent to which stress was imposed was adequate to discriminate between populations varying in tolerance to drought.

Genotypes that have grain shattering score in the range of ‾50ɳ80gf, spikelet fertility more that 70% and mean leaf roll score of less than 3.5 were 18. These ranges are approximately for the optimum levels for acceptable threshability (Ji et al., 2006), optimum spikelet fertility for drought tolerance (Lafitte et al., 2003) and acceptable leaf roll score for drought tolerance at vegetative stage (Singh and Mackill, 1990a). The population had about 9% of lines with already prefer trait. Most breeding work employ selection pressure of 10% (Falconer and Mackay, 1996), there for the was adequate variability in the population worth use for selection. On addition the that there was high variation between the top performers and the least performers in terms of grain shattering ability, leaf roll and spikelet fertility, it implies that these populations can be used for improvement of the trait through breeding if the gene responsible for their transmission are known. Among the 18 out of 191 suitable genotypes, 12 of which were CT lines. They also form the majority of genotypes listed as high performers in terms of the three traits. The CT lines for instance were developed through crossing CAIAPO, a tropical *O. sativa spp. japonica* an *O. glaberrima.* These differences between the CT lines and CAIAPO are not conclusive. The CAIAPO is adapted to upland acid soil conditions in Brazil (EMBRAPA, 1997). The CT lines are a third backcross generation of a cross between, the parent CAIAPO and *O. glaberrima* (RAM 24) (Jones et al., 1997a). The *O. glaberrima* has several escape mechanisms for tolerance to drought. It withstands drought and recovers fast from short drought periods through presence of thin leaves and roots. The thin leaves roll quickly to retain water and thin roots penetrate the soil effectively, thus accessing soil moisture. The evidence for the importance of these traits in breeding for drought tolerance has been reported (Dingkuhn et al., 1999; Maji et al., 2001).

The values of LSD, CV, ratio for range over LSD and variance shows that for the traits spikelet fertility, shattering score and leaf roll presented in Tables 5.7-5.10 and Appendix 1 are for the whole sample set. Spikelet fertility and leaf roll had low coefficient of variation indicating the observation could be very reliable. The shattering ability score was high, more than 53% and transformation did not improve on the value. This trait was measured at a time when environmental influence was very high yet the equipment used was precise to units if gram force therefore the error value became big. When less accurate methods that

108

use large samples were used, the variation was less (Ichikawa et al., 1990). It is advisable that the equipment used in the current study be used at the stage when environmental effects like controlled laboratory conditions. A ratio of range to LSD of more than three is considered appropriate for using a given trait in variation analysis (Iqbal and Clarke, 2003). This is based on its reliability for measuring spread and it dummies any extreme value that range and normally include. In the current study, all the variates could be informative in assessing variability in the population. In the current work, however, leaf roll was more reliable. More so the variances were similar indicating the traits could be used equally to assess variation in the populations.

5.6   CONCLUSION

The study has provided critical information on variability in rice germplasm in terms of tolerance to drought and grain shattering ability. It showed that:

There was adequate variability in the population for the traits grain shattering ability, leaf roll and filled grains. However, among the three, filled grains could be the most informative.

Of the three rice groups *O. sativa*, interspecific lines and *O. glaberrima* there was high similarity between *O. sativa* and interspecific lines.

Nine percent of the germplasm had optimum performance in terms of grain shattering ability, leaf roll and filled grains.

Rice lines that could be used for improvement of rice for grain shattering ability, leaf roll and filled grains were identified.

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Appendix 5.1 List of genotypes that fit in the major groups based on low grain shattering, spikelet fertility score and leaf roll scores[1]

Genotypes	Sp.fert (%)	Shat. (gf)	Leaf roll 0-9		Genotypes	Sp.fert (%)	Shat. (gf)	Leaf roll 0-9
IRRI 132 (IR55423-01)	56.7	19.2	4.3		CT 16333(20-CA-18-M	77.1	47.6	2.8
CT 16337-CA-6-M	69.6	20	3.3		CT 16341-CA-9-M	79.4	48.2	2.7
IR 80508-B-57-3-B	71	20	3		CT 16328-CA-10-M	54	49.1	4.7
CT 16313-CA-19-M	81.1	20.4	2.3		CT 16311(2)-CA-1-M	70.6	52.6	4.2
CT 16342-CA-19-M	71.1	20.9	2.7		CG 14	72.4	53	3.75
SALUMPIKIT	79.5	21.2	3.3		CT 16345-CA-4-M	67.4	61.4	2.4
WAB 638-1-B-10A5-1	73.4	22.3	3.7		CT 16341-CA-4-M	66.1	62	2.3
WAB 189	79.2	22.4	3.8		CT 16333(2)-CA-20-M	80.5	63.3	3.7
CT 16315(1)-CA-5-M	72.2	22.7	3.2		CT 16308-CA-5-M	64.2	68.8	3
CT 16319-CA-4-M	82	23.2	3		Cirad 409	63	69.8	3.3
CT 16331-CA-8-M	58.5	24	3		CT 16334(1)-CA-2-M	77.4	72.4	3.8
CT 16311(1)-CA-1-M	52.2	24.2	3.9		IR 71525-19-1-1	66.1	72.6	4.5
CT 16337-CA-7-M	55.7	24.5	3		NERICA 2	71.3	78.8	4.3
CT 16345-CA-13-M	57.3	24.9	4.2		CT 16338-CA-19-M	64.8	79.5	2.8
BOUKE 189	66.9	25.2	2.8		CT 16312(2)-CA-6-M	54	80.1	2.8
CT 16324-CA-15-M	73.9	27	2.3		CAN 90-25	77.9	80.8	4.7
WAB 368-1-B-10A5-1	49.9	27.3	4.7		MOROBEREKAN	75.8	82.9	3
WITA 3	75.7	28.3	3.3		CT 16329-CA-7-M	75.2	84.3	3.8
WABIR 12979	61.3	29.2	4.7		CT 16344-CA-3-M	51.9	86.6	3.8
WITA 7	57.1	29.6	4.3		NERICA 4	75.4	86.6	3.3
IR 71524-44-1-1	79.1	30.3	4.7		WBK 42 (F4)	78	88.5	3.8
CT 16333(1)-CA-3-M	76.3	31.3	3		IRAT 144	67.3	89.3	2.8
WBK 190 (F5)	68	31.7	3.7		WAB 450-24-2-3-P-38-1-HB	60	89.3	4.4
CT 16328-CA-12-M	64.6	31.8	2.3		CT 16338-CA-8-M	60.7	89.5	3.8
CT 16345-CA-8-M	79.2	31.9	2.4		CT 16337-CA-9-M	82.3	93	4
CT 16333(1)-CA-15-M	56.9	32.8	3.1		Curinga	80	93.2	2.8
CT 16307(1)-CA-8-M	74.9	33.1	3		NERICA 18	60.9	95.5	4
WITA 2	54.3	33.3	2.3		WBK 25 F4	61.7	96.1	4.8
CT 16345-CA-14-M	57.4	33.6	2.3		NERICA 10	67.3	96.3	3.1
IRAT 13	80.2	33.7	3.3		NERICA 16	51.1	96.6	4.3
CT 16356(1)-CA-8-M	70.2	35.2	4.3		WBK 41 (F4)	75.2	98.1	4.3
CT 16310(2)-CA-7-M	69.15	35.72	3.6		CT 16342-CA-25-M	82.4	98.5	4.7
CT 16334(1)-CA-12-M	79.4	36.1	2.5		CT 16338-CA-29-M	59.9	100	3.1
WITA 12	74.7	37.1	4.7		WBK 150 (BC1F2)	72.3	101.2	3.3
ITA 212	62.8	37.9	4.2		WAB 365-B-2-H1-HB	64.2	103.6	4
WITA 1	72.2	38	4.8		WAB 365-B-1-H1-HB	71.7	106.3	3.2
Lideranza	76.4	38.9	3.8		IDSA 10	74.2	108.4	2.3
CT 16356(2)-CA-9-M	68.1	39.2	2.5		WBK 03 (F4)	60.1	110.7	2.6
IR 77298-5-6	52.9	41	2.8		NERICA 5	66.7	110.8	2.3
CT 16344-CA-8-M	68.5	41.4	2.3		WAB 365-B-1H1-HB	64.8	111.2	3.2
IR 2071-625-1-252 (IR 36)	52.5	43.6	2.5		WAB 450-1-B-P-33-HB	73.9	111.7	2.8
CT 16346-CA-20-M	63.5	44.9	4.7		NERICA 8	57.2	116.6	3.8
CT 16307-CA-15-M	64.4	45.4	2.4		WAB 450-I-B-38-HB (NERICA 1)	79.3	134.6	4.3
Bonanca	68	45.6	3.8					
CT 16315(1)-CA-12-M	53.7	45.7	4.4		Mean	67.0	57.0	3.40
CK 73	68.9	46.7	3.3		LSD$_{0.05}$	1.88	6.30	0.16
CT 163313-CA-8-M	81.5	46.9	2.5		CV%	13.2	53.0	23.0
					Range/LSD	17.6	15.3	156.0
					Variance	18.6	11.5	20.1

[1] The genotypes did not fall in any of the groups discussed in Tables 5.6 to 5.9
Blue is for genotypes that have shattering degree of >50 and <80 gf.
Blue= genotypes that had acceptable grain threshability

CHAPTER SIX
Crossability and Gene Action for Seed Set and Pollen Fertility in Crosses between *Oryza sativa* L. and Interspecifc Rice

Abstract

Crossability and pollen fertility between *O. glaberrima* x *O. sativa* crosses is low yet the *O. glaberrima* is an important source of resistance to various stresses. The interspecific genotypes could be useful in improving *O. sativa* if information on crossability and sterility are available. This study had the objectives of determining crossability in crosses and the mode of gene action for pollen fertility in F1 crosses generated from interspecific genotypes and *O. sativa*. Twenty seven genotypes comprising 12 *O. sativa* and 17 interspecific genotypes were used in this study. Each *O. sativa* was crossed with randomly selected interspecific genotypes. Reciprocal crosses were also performed. The crosses were conducted using females planted in pots but males planted in the field. Crossability was assessed on the seed set and hybrid performance in terms of seed germination percentage and seedling survival was assessed as well. Pollen staining technique was used to assess pollen viability. The seed set in crosses involving interspecific genotypes as females were similar to the seed set in crosses involving *O. sativa* as females. Parents that had high crossability were identified for use as female and male parents. The best male genotype combiners were CT 16334(2)-CA-2-M, WAB 365-B-1H1-HB and IRAT 257, while the best female combiners were CT 16334(2)-CA-11-M, CT 16317-CA-4-M and IRAT 325. There were high germination rates with a mean of 88% and range 75% to 96% while seedling survival had a mean of 68% and a range of 57% to 90%. The mean percentage pollen viability was 77.8% with the highest levels recorded in crosses involving genotype code 105 (WAB 365-B-1H1-HB) as the female. There was higher additive effects for seed set than non-additive effect. However, there was higher additive effects for parents that have at least one parent with tight grain attachment to the pedicel. Similarly, additive effects were more important that non-additive effects for pollen fertility. In conclusion, interspecific fixed lines could be used for improving *O. sativa* landraces based on seed set, seedling survival and genetic transmission of seed set and pollen fertility.

6.1   INTRODUCTION

Rice is a major source of dietary energy for more than 60% of the world's population. There are two cultivated species, namely *Oryza glaberrima* and *Oryza sativa* L., and both species are cultivated in Africa. Currently, a new rice generation has been developed through *Oryza glaberrima* x *Oryza sativa* crosses. These new rice genotypes have been released for production in many countries in sub-Saharan Africa. They carry genes for resistance to biotic and abiotic stresses, including iron toxicity (Majerus et al., 2007), bacterial blight (Yogesh et al., 2007), rice yellow mottle virus (Attere and Fatokun, 1983), African rice gall midge (Singh et al., 2004), cyst nematodes (Lorieux et al., 2003), drought (Majerus et al., 2007) and weed competitiveness (Haefele et al., 2004). Continued unlocking of these potential of *O. glaberrima* is undermined by difficulty in making crosses (Brar and Khush, 1996). The difficulty is a result of varying degree of sexual reproductive barriers between the two species (Sano, 1986).

Hybridization between *O. glaberrima* and *O. sativa* is expected to be easy based on the similarity in chromosome number (Nayar, 1973), but low success in crossing has been reported in the form of spikelet fertility (Chang, 1976; Sano et al., 1979; Jones et al., 1997b; Tao et al., 1997) and seed set (Jones et al., 1997b). There is little consensus on the patterns of crossability between the two species. For instance, Chu et al. (1969) indicated that there was no crossing barriers after getting 39-42% crossability between the two species. However, Jones et al. (1997b) found about 15% success in crosses, while Sano (1986) found differences between different crossing sets of the two species. It is now convincingly clear that interspecific crossability operates as a partial barrier effected by environment, cytoplasm, genotype of *O. glaberrima* and genotypes of *O. sativa* (Sano, 1986). Grain sterility is controlled genetically by nuclear genes and nuclear x cytoplasmic gene interactions. This aspect is useful in hybrid rice production. However, the objective is to improve seed set, the pollen fertility is an important and information on its genetic control could be useful in breeding. It is known that environmental conditions especially dehydration and heat stress affect pollen fertility (Nepi et al., 2001). Nonetheless, the trait is also controlled genetically. Therefore, understanding 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.

It is considered that interspecific fixed lines generated from *O. glaberrima* x *O. sativa* crosses, could be easier to hybridize with *O. sativa* landraces than the case when the *O. glaberrima* is used. This is based on preliminary findings that hybrids generated from wild rice and *O. sativa* crosses have higher crossability with *O. sativa.* For example, in a study on natural pollen dispersion between cultivated rice and some wild rice, low crossability was realized (1.2-2.19%) (Chen et al., 2004) while hybridization between cultivated rice and the F1 lines had crossability in the range of 1-52% (Langevin et al., 1990). These findings make understanding of the crossability between fixed lines generated from *O. glaberrima* with *O. sativa* crosses an attractive option for three reasons. First, it could guide selection of appropriate parents and choice of procedures for making crosses between the interspecific lines and *O. sativa*. Second, information on crossability could give a prediction of natural gene flow between *O. sativa* and *O. glaberrima.* This could translate into a practical guide for minimum distance between different interspecific genotypes and *O. sativa,* for seed production and cultivation processes. Thirdly, the current trend of losing landraces due to a few negative traits may be reversed and the overall rice gene pool instead broadened using the interspecific rice generations.

The interspecific rice has a high potential for use in improving rice landraces. In Uganda, a few new interspecific lines have been released in the country, namely NERICA 1, NERICA 10 and NERICA 4. They were released based on advantages that include resistance to rice yellow mottle virus disease (RYMV), African rice gall midge (AfRGM), rice blast and drought tolerance. Moreso, other interspecific lines superior to the released NERICAs in terms of drought tolerance have been identified (Lamo et al., 2007). The new rice types could therefore be used in the improvement of landraces that are susceptible to these traits if the crossability is understood. This study was therefore undertaken in order to explore the possibility of using interspecific fixed lines developed from *O. sativa* x *O. glaberrima* crosses for improving to *O. sativa* landraces in Uganda and elsewhere in the world.

6.2   Objectives
The overall aim of this study was to assess seed set and spikelet fertility of F1 progenies generated from crosses between *Oryza sativa* and interspecific fixed lines
The specific objectives were to:
i.   evaluate seed set pattern in crosses between *O. sativa* and interspecific rice;

ii.   determine germination percentageof F1 progenies generated from crosses between *O.sativa* and interspecific rice; and

ii.   investigate combining abilities for seed set and pollen fertility in F1 progenies, generated from *O. sativa* x interspecific genotypes crosses.

6.3   MATERIALS AND METHODS

6.3.1   Germplasm

The pedigrees of 12 *O. sativa* and 17 interspecific rice lines used in this study are presented in Table 6.1. All the materials were collected as seed at Namulonge, in Uganda. They included popular rice varieties and interspecific fixed lines developed through *O. sativa* x *O.glaberrima* crosses. The interspecific lines represent two rice groups. First are lines developed using, *O. sativa* japonica, CG56-104, with *O. glaberrima*. The second group is lines developed using, a popular upland *O. sativa* japonica in South America called CAIAPO as one of the parents. The other parent was one of the *Oryza glaberrima* accessions.

6.3.2   Crosses

In order to generate F1 populations from the *Oryza sativa* and interspecific parental lines crosses were attempted using all the 27 between *Oryza sativa* and the interspecific genotypes. The crosses were conducted using the North Carolina II mating design in sets with reciprocals. However, the sets for some crosses could not be used in crosses due to low pollen and lack of synchronization of flowering time of the day. Therefore, each female parent, *O. sativa* or interspecific was crossed with one to six males of the interspecific or *O. sativa* lines, respectively. All the 27 parents were crossed with three different males except genotypes 2, 193 and 129 that were each crossed with six different parental genotypes. Genotype 192 was crossed with two other genotypes and genotype 177 was crossed with a single genotype.

The female parents were pre-germinated in petri dishes for 5 days and then seedlings transferred to 8L (diameter 24cm) plastic buckets containing clay loam soil. The pots were kept flooded with water to a depth of 5 cm of water but drained every fortnight to proper soil aeration. Twenty plants of each genotype were planted in the plastic pots, two plants per pot on four planting dates staggered at 10-day intervals. Fertilizers, NPK and Urea were

applied at recommended rates. Ten to fifteen panicles of each variety and 15 to 20 spikelets per panicle were emasculated and used in the crossability test.

Pollination was conducted following the procedures described by Coffman and Herera (1980) using a new flower emasculating equipment fabricated at University of Kwa-Zulu Natal, South Africa. Panicles of female plants were prepared for pollination by selecting spikelets with normal shape of development and emasculation conducted a day before pollination between 4:00 p.m and 6:00 p.m. The emasculated panicles were covered with waxy pollination bags. The bags were opened just before facilitating pollination. The emasculated panicles were pollinated by tapping the pollen grains from a pollen donor at the time of the day when the male parents were flowering. The pollen parent panicles were prepared for pollination by cutting at a length 15-20cm below the base of the panicle on the day they were to be used and dipped in a container water water to avoid withering. The pollen panicles used per cross were 1 to 3. In some cases, the panicles were collected from different plants of the target genotype. Similarly, reciprocal crosses were attempted. The pollinated flowers were covered with waxed paper bags. Immediately after making crosses, panicles were bagged until the 28th-30th day when the grains turned straw green in color. Seeds from each cross were harvested and stored at 4^0C.

In order to test whether the method of emasculation did not affect the outcome of the crossability a control set of 26 parental genotypes, 10 panicles each, were included in the experiment. All the 10 panicles were emasculated from a set of five plants which were randomly selected; covered by bagging and pollen from the same variety used to pollinate the plants the following day. The remaining five plants were taken to the field were the parents had reached flowering stage to allow natural pollination to take place. After two days in the field, all the sets of plants were brought to the rain-out shelter where crosses were being conducted.

6.3.3   Evaluation of F1 hybrids
The F1 seeds recovered from the crosses were sterilized by dipping in 1.25% Sodium hypochlorite for 15mins to control any fungal infections. The seeds were pre-germinated by placing in sterilized petri-dishes, layered with moistened filter papers (Plate 6.1). On the fifth day, record of germination was taken and all newly germinated plants were immediately transferred to 300ml sterilized plastic containers filled with sterile soil. On the 20th day,

successful seedlings were established in an alpha lattice design in a 35 x 2 arrangement with two replicates in a rain-out shelter. Each treatment was established in single-row plots each measuring 1.2m. The plants received fertilizer application at recommended 50 kg N/ha applied in two splits. First application was done at 40 days after planting and the second at 50 days after planting.

Table 6.1 Pedigree of parental lines used in the crossability studies

Var. No.	Source	Designation	Pedigree	Type
2	CIAT	CT 16346-CA-20-M	BC4 Caiapo and O.glaberrima	Interspecific
9	CIAT	CT 16350- CA-5-M	BC4 Caiapo and O.glaberrima	Interspecific
12	CIAT	CT 16344-CA-9-M	BC4 Caiapo and O.glaberrima	Interspecific
18	CIAT	CT 16334(2)-CA-2-M	BC4 Caiapo and O.glaberrima	Interspecific
19	CIAT	CT 16334(2)-CA-11-M	BC4 Caiapo and O.glaberrima	Interspecific
26	CIAT	CT 16324-CA-9-M	BC4 Caiapo and O.glaberrima	Interspecific
27	CIAT	CT 16342-CA-25-M	BC4 Caiapo and O.glaberrima	Interspecific
67	CIAT	CT 16313-CA-4-M	BC4 Caiapo and O.glaberrima	Interspecific
72	CIAT	CT 16317-CA-4-M	BC4 Caiapo and O.glaberrima	Interspecific
88	CIAT	CT 16355-CA-15-M	BC4 Caiapo and O.glaberrima	Interspecific
94	CIAT	Caiapo	CNAX 104 B-18 PY-2B/PEROLA	*O. sativa*
96	IRRI	Bonanca		*O. sativa*
105	WARDA	WAB 365-B-1H1-HB	*O .sativa*	*O. sativa*
114	WARDA	WAB 56-104	IDSA 6/IAC 164	*O. sativa*
118	WARDA	WAB 450-I-B-38-HB (NERICA 1)	WAB 56-104/CG 14//WAB56-104	Interspecific
121	WITA 1	-	-	*O. sativa*
129	IRRI	CK 73	unknown	*O. sativa*
134	WARDA	WAB 450-B-136-HB (NERICA 9)	WAB 56-104/CG 14//WAB56-104	Interspecific
138	WARDA	WAB 450-B-136-HB (NERICA 8)	WAB 56-104/CG 14//WAB56-104	Interspecific
141	IITA/WARDA	IRAT 13	560/Variety from Zaire	*O. sativa*
147	IITA/WARDA	IRAT 104	IRAT 13/MOROBERAKAN	*O. sativa*
188	WARDA	WAB 881-10-37-18-3-P1-HB (NERICA 15)	CG 14/WAB 181-18//WAB181-18	Interspecific
191	WARDA	WAB 881-10-37-18-12-P3-HB (NERICA 18)	CG 14/WAB 181-18//WAB181-18	Interspecific
193	WARDA	NERICA 13	-	Interspecific
194	China	Golden 1	*O sativa japonica*	*O. sativa*
196	IITA/WARDA	IRAT 325	IRAT 112/TOX 490-1	*O. sativa*
197	IITA/WARDA	IRAT 257	IRAT 112/TOX 490-1	*O. sativa*
192	WARDA	WAB 189	WAB 189 BBB8 HB	*O. sativa*

IRAT 112=IRAT 13/Dourado Precose 689;

'Plate 6.1 Grains in petri-dishes

6.3.4 Data collection and analysis

Data was collected on seed set, seed germination rate, seedling survival and pollen grain fertility. At maturity, 28-30 days after pollination, seed set count was conducted by harvesting seed from the pollinated panicles. The record included number of spikelets emasculated and pollinated and number of grains formed. The data on seed set was taken for the first 250 spikelets that were emasculated. The germination percentage was scored following the procedures described by Miura et al. (2004). According to the method any seed with radical length more than half the length of the grain and the shoot equal or more than the length of the grain is scored as seed that has already germinated. Seedling survival score was taken by counting the shriveled seeds and dead plants and the total number of plants. The record was taken at one week after germination. Data on seedling vigour was collected by taking heights of all the plants per plot. This involved measuring the whole plant from the base to the top of the upper most leaf at 10 days after germination.

The pollen viability study involved collecting five spikelets at the time of anthesis from a single plant in each plot. Essentially, one plant per treatment was sampled. The spikelets were taken to the laboratory, anthers removed, squashed and mounted under the microscope in 1% acetocarmine. Direct observation of the pollen grains was done. Observations for each microscope field were made for more than 60 spores and counting made on five microscope fields. The pollen grains that appeared spherical and darkly stained by the acetocarmine were recorded as viable while the unstained and spherical or unstained and irregular in shape were recorded as non-viable. Seeds were harvested and kept at 4^0C for use in subsequent studies. Some plants did not have filled grains and the tillers were instead split and maintained.

119

Genetic analysis for seed set and pollen viability were performed in Genstat Computer Package. Crosses were fixed while replications were considered random. The North Carolina design II (NCII) analysis was performed using the following model.

Where

GCA effects of males and females were estimated using the formula (Dabholkar, 1999):

$$\hat{g} \qquad \overline{} \qquad \overline{}$$

$$\hat{g} \qquad \overline{} \qquad \overline{}$$

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6.4   RESULTS

6.4.1   Seed set

Results of the crosses are shown in Table 6.2 to 6.6. Out of 96 crosses made 13 (8.6%) did not yield any seed and five out of the 13 crosses had interspecific genotypes as females (Table 6.2). Among the crosses that yielded seeds, the average seed set was 6% at standard deviation of 6.2% and the range was 0.4% to 32%. The ratio of range to LSD was 25.4. A summary of the best ten and the least ten in terms of seed set are in Table 6.3. The highest seed set was for cross IRAT 325 x CT 16334(2)-CA-2-M and the least seed set was for cross WAB189 x CT 16317-CA-4-M. Results of the ranking for mean seed set for each female when crossed with different male parents are in Table 6.4. Out of the 27 parental lines used as females, 11 (42%) gave over 5% seed set when crossed with different males. The best female parental genotypes were 196, 72 and 67, while the least in performance were 188, 129 and 122.

Table 6.2 List of crosses that aborted

Female parents		Male parents
WITA 1	x	CT 16350- CA-5-M
WITA 1	x	CT 16346-CA-20-M
CK 73	x	CT 16344-CA-9-M
CT 16350- CA-5-M	x	Bonanca
IRAT 13	x	WAB 450-I-B-38-HB (NERICA 1)
CK 73	x	NERICA 15
WAB 450-I-B-38-HB (NERICA 1)	x	Bonanca
WAB 450-I-B-38-HB (NERICA 1)	x	CK 73
NERICA 15	x	CK 73
WAB 189	x	CT 16346-CA-20-M
WAB 189	x	CT 16355-CA-15-M
IRAT 104	x	CT 16346-CA-20-M
CT 16346-CA-20-M	x	IRAT 104

Table 6.3 The best ten and least ten mean seed set for different crosses

Female		Male	% seed set (no of seeds)[1]
Ten least seed set			
Bonanca	x	CT 16346-CA-20-M	0.4 (1)
CK 73	x	CT 16342-CA-25-M	0.4 (1)
WAB 881-10-37-18-3-P1-HB (NERICA 15)	x	IRAT 13	0.4 (1)
NERICA 13	x	CT 16317-CA-4-M	0.4 (1)
CT 16344-CA-9-M	x	CK 73	0.8 (2)
CK 73	x	WAB 450-I-B-38-HB (NERICA 1)	0.8 (2)
WITA 1	x	CT 16344-CA-9-M	1.2 (3)
WAB 881-10-37-18-3-P1-HB (NERICA 15)	x	Bonanca	1.2 (3)
IRAT 257	x	CT 16346-CA-20-M	1.2 (3)
IRAT 104	x	CT 16317-CA-4-M	1.2 (3)
Ten best seed set			
Caiapo	x	CT 16313-CA-4-M	12.8 (32)
CT 16342-CA-25-M	x	IRAT 13	13.6 (34)
WAB 450-B-136-HB (NERICA 8)	x	WAB 365-B-1H1-HB	14.4 (36)
CT 16334(2)	x	Golden 1	14.4 (36)
CT 16334(2)	x	WAB 56-104	15.6 (39)
CT 16313-CA-4-M	x	Golden 1	17.2 (43)
WAB 365-B-1H1-HB	x	WAB 450-B-136-HB (NERICA 8)	17.6 (44)
CT 16317-CA-4-M	x	IRAT 257	25.2 (65)
IRAT 325	x	WAB 365-B-1H1-HB	26.0 (67)
IRAT 325	x	CT 16334(2)-CA-2-M	32.0 (80)
	Overall	**Mean**	**6.2**
		SE	**0.7**
		Range/LSD	**25.4**
		Variance	**35.8**

[1]The mean seed set was calculated based on a sample of 10 plants a total of and 250 spikelets per cross

Table 6.4 Mean seed set for each female when crossed with different males

Female lines		Set of males lines	Percentage seed set[1]	Type
196	x	18,105,134	20.4	I
72	x	197,193,147	13.1	II
67	x	114,194,94	11.7	II
19	x	114,194,94	11.6	II
18	x	193,138,196	9.1	II
134	x	193,138,196	8.9	II
105	x	193,138,196, 194	8.7	II
94	x	19,67,26	8.3	I
27	x	141,96,129	8.3	II
138	x	18,105,134	6.5	II
114	x	19,67,26	5.9	I
26	x	114,194,94	4.8	II
141	x	27,118,188	4.7	I
9	x	122,96,129	4.4	II
197	x	2,72,88	4.3	I
88	x	197,193,147	3.7	II
194	x	19,67,26	3.2	I
177	x	105	3.2	I
193	x	2,72,88,18,105,134	3.1	II
2	x	122,96,129, 197, 193, 147	2.7	II
96	x	9, 2, 12, 27, 118,188	2.5	I
12	x	122, 96,129	1.6	II
147	x	2, 72, 88	1.3	I
118	x	141, 96,129	1.2	II
188	x	141, 96,129, 198	1.1	II
129	x	9, 2, 12, 27, 118,188	1.0	I
122	x	9,2,12	0.4	I
	Overall Mean		**6.2**	
	SE		**0.7**	
	Range/LSD		**25.4**	
	Variance		**35.8**	

I = *O. sativa*

II = interspecific rice

[1]The mean seed set was calculated based on a sample of 10 plants a total of and 250 spikelets per cross

Results of the male sets performance are in Table 6.5. Among the males, however, the highest seed set were genotypes 18, 105 and 197 while the least in performance was for genotypes 96, 9 and 118. The mean rank of seed set when genotypes were used both as females and as males is shown in Table 6.6. Genotypes 197, 18 and 105 consistently had the highest seed set while genotypes 96, 129 and 118 had the least seed set levels.

Table 6.5 Mean seed set for each male when crossed with different females

Female lines		Set of male lines	Percentage seed set	Type
193,138,196	x	18	13.4	II
193,138,196, 177	x	105	13.3	II
2,72,88	x	197	11.6	I
19,67,26, 105	x	194	10.3	I
19,67,26	x	114	10.1	I
18,105,134	x	138	10.1	I
18,105,134	x	196	9.1	I
7,72,88,18,105,134	x	193	7.7	II
114,194,94	x	67	6.5	II
114,194,94	x	26	6.4	II
27,118,188	x	141	5.9	I
122,96,129, 197,193,147	x	2	5.3	II
19,67,26	x	94	4.9	I
141,96,129	x	27	4.7	II
114,194,94	x	19	4.0	II
2,72,88	x	147	3.3	I
9,2,12	x	122	3.3	I
197,193,147	x	88	3.3	II
193,138,196	x	134	2.9	II
188	x	198	2.8	II
9,2,12 27,118,188	x	129	2.5	I
122,96,129	x	12	2.3	II
141,96,129	x	188	2.1	II
197,193,147	x	72	2.0	II
9,2,12,27,118,188	x	96	1.7	I
122,96,129	x	9	1.6	II
141,96,129	x	118	0.8	II
	Overall Mean		6.2	
	SE		0.7	
	Range/LSD		25.4	
	Variance		35.8	

I = *O. sativa,* II = interspecific rice
[1]The mean seed set was calculated based on a sample of 10 plants a total of and 250 spikelets per cross

Table 6.6 Overall mean seed set for each parents when used as male and as female

Female			Male			Overall	
Var. no	Mean seed set	Rank	Var. no	Mean seed set	Rank	Mean	Rank
197	4.3	1	197	11.6	3	2.0	1
18	9.1	5	18	13.4	1	3.0	2
105	8.7	7	105	13.3	2	4.5	3
67	11.7	3	67	6.5	9	6.0	4
114	5.9	11	114	10.1	5	8.0	5
138	6.5	10	138	10.1	6	8.0	6
19	11.6	4	19	4.0	15	9.5	7
94	8.3	8	94	4.9	13	10.5	8
26	4.8	12	26	6.4	10	11.0	9
27	8.3	9	27	4.7	14	11.5	10
194	3.2	19	194	10.3	4	11.5	11
141	4.7	13	141	5.9	11	12.0	12
196	20.4	17	196	9.1	7	12.0	13
134	8.9	6	134	2.9	19	12.5	14
72	13.1	2	72	2.0	24	13.0	15
2	2.7	20	2	5.3	12	16.0	16
193	3.1	25	193	7.7	8	16.5	17
88	3.7	16	88	3.3	18	17.0	18
147	1.3	23	147	3.3	16	19.5	19
9	4.4	14	9	1.6	26	20.0	20
188	1.1	18	188	2.1	23	20.5	21
12	1.6	22	12	2.3	22	22.0	22
122	0.4	27	122	3.3	17	22.0	23
96	2.5	21	96	1.7	25	23.0	24
129	1.0	26	129	2.5	21	23.5	25
118	1.2	24	118	0.8	27	25.5	26

6.4.2   Seed germination, seedling survival and pollen viability

The germination percentage of 88% was realized for 17 parents when crossed with different male parents. Genotypes number 2 and 94 had the highest germination rate and genotype 27 had the least (Table 6.7). Results of the seed survival rate are shown in Table 6.8. Female parent 72 had the highest level of seed survival while 26 had the least. Results of pollen viability is presented in Table 6.9. The mean percentage pollen viability was 77.8% with the highest levels recorded in crosses involving genotype code 105 (WAB 365-B-1H1-HB) as the female..

Table 6.7 Germination score of F1 hybrids

Common Females[1]	Different Males	Percent germination	Different Males	Percent germination	Different Males	Percent germination	Overall Mean	Rank
2	129	88.0	122	100.0	96	100.0	96.0	1
18	138	91.7	19	88.0	196	90.3	90.0	6
19	94	83.3	194	88.9	114	87.2	86.5	12
26	114	71.4	94	90.0	194	84.2	81.9	15
27	96	76.9	129	66.7	141	82.2	75.3	17
67	94	80.0	114	93.3	194	93.0	88.8	8
72	193	88.2	147	83.3	197	78.5	83.3	9
88	147	71.4	197	77.9	193	91.7	80.3	16
94	19	100.0	26	90.5	67	93.8	94.8	2
96	9	100.0	27	100.0	118	75.0	91.7	5
105	194	100.0	196	83.3	193	76.9	86.7	11
114	67	90.9	26	83.3	19	84.2	86.1	13
134	138	90.0	193	90.9	196	84.0	88.3	9
138	134	100.0	18	77.8	105	88.9	88.9	7
193	134	90.0	8	83.0	23	87.0	86.7	11
194	19	100.0	26	88.9	67	90.9	93.3	3
196	134	100.0	105	91.0	18	87.5	92.8	4
					Mean		87.7	
					LSD$_{0.05}$		2.6	
					Range/ LSD		7.9	
					Variance		29.3	

[1]Each female in the set was crossed with three different males.

Table 6.8 Seedling survival of F1 score

Common Females[1]	Different Males	Percent germination	Different Males	Percent germination	Different Males	Percent germination	Overall Mean	Rank
2	129	90.9	122	100.0	96	4.2	65.0	14
18	138	72.7	19	95.5	196	35.7	68.0	13
19	94	80.0	194	90.6	114	38.4	69.7	11
26	114	60.0	94	88.9	194	24.7	57.9	17
27	96	80.0	129	90.0	141	48.5	72.8	9
67	94	100.0	114	96.5	194	48.4	81.6	4
72	193	6.7	147	86.7	197	88.7	90.7	1
88	147	80.0	197	99.8	193	12.2	64.0	16
94	19	100.0	26	100.0	67	31.5	77.2	5
96	9	75.0	27	100.0	118	4.4	89.8	2
105	194	60.0	196	100.0	193	33.0	64.3	11
114	67	100.0	26	86.7	19	22.1	69.6	12
134	138	94.4	193	5.0	196	29.7	73.0	8
138	134	100.0	18	85.7	105	40.5	75.4	7
193	134	100.0	8	100.4	23	26.5	75.6	6
194	19	100.0	26	100.0	67	10.6	70.2	10
196	134	87.5	105	85.3	18	78.8	83.9	3
					Mean		68.2	
					LSD$_{0.05}$		4.8	
					Standard error		2.4	
					Range/ LSD		8.5	
					Variance		97.0	

127

Table 6.9 Percentage pollen viability of F1 generation for six females genotypes

Female	Male	Percentage pollen viability[1]
18	138,193,196	78.4
105	138,193,196	81.6
134	138,193,196	80.9
141	2,118,188	76.1
96	2,118,188	71.4
129	2,118,188	78.8
	Mean	77.8
	$LSD_{0.05}$	2.1
	Range/LSD	7.3
	Variance	20.0

[1]Pollen viability was determined using pollen staining technique

6.4.3 Gene action and relative importance of GCA and SCA effects for seed set and pollen viability

The mean square values for seed set for male, female and the male x female parents for five sets of populations A, B, C, D and E are shown in Table 6.10. A detailed list of parental lines are presented in Appendix 6.1. The mean squares for male, female and male x female were significant for the seed set in sets A, D and E. There was, however, high level of significance for male and female interactions but not female mean squares. In set C the mean squares for males and females were highly significant. Results of the GCA effects for female and male parents are shown in Table 6.10. The total GCA effects for both male and female parents were more than 60% for all the sets except set B. The set B had nearly equal total GCA effects with the SCA effects. The total GCA effects for set A was 51% and the SCA effects was 49%. The ratio of the mean square value for female compared with males is shown in Table 6.9. The ratios varied from 1.1 to 3.1.

Table 6.10 Mean square value for seed set for male and female sets of parents

Source of variation	SET A[1]		SET B[2]		SET C[3]		SET D[4]		SET E[5]	
	d.f	ms	d.f.	ms	d.f.	ms	d.f.	ms	d.f.	ms
Female (GCA)	5	9.41***	5	3.69**	5	6.51***	5	7.18***	5	7.28***
Male (GCA)	4	8.90***	4	2.38	4	5.38***	4	2.32***	4	3.28***
Female x Male (SCA)	8	5.40***	7	3.77***	8	1.66***	6	1.22***	8	1.82***
Relative contribution of sum of squares to GCA[6]										
GCA Female		37		34		48		68		54
GCA Male		28		18		32		18		21
SCA		34		49		20		14		23
Ratio of Female MS to Male MS		1.1		1.6		1.3		3.1		2.2

[1] Both parental sets used in the set A have tight grain attachment to the pedicel

[2] Both parental sets used in the set B have loose grain attachment to the pedicel

[3] Both parental sets used in the set C have moderately tight rain attachment to the pedicel

[4] Parent with tight grain attachment crossed with parents with loose grain attachment to the pedicel

[5] Parent with tight grain attachment crossed with parents with loose grain attachment to the pedicel

[5] Relative contribution of sum of squares to GCA is calculated by calculating the ratio of each component sum of squares to the total of the sum of squares of GCA female, GCA male and SCA

Results of the analysis of variance for pollen viability is presented in Table 6.11. A summary of the mean pollen viability is presented in Appendix 6.2. The male x female interaction mean square was significant ($P < 0.1$) for the pollen viability. There was significant ($P < 0.05$) mean square for both female effects and male effects. The GCA effects for female and male parents are shown in Table 6.11 as well. The total GCA effect for both male and female parents were more the 67%, while the SCA effects contributed to the remainder 33%.

Table 6.11 Mean square of pollen viability

Source of variation	df	Mean square value
Reciprocal	1	215.1
Female (GCA)	5	598.9**
Male (GCA)	5	575.2**
Female x Male (SCA)	7	411.4*
Residual	16	
Relative contribution of sum of squares to GCA		
GCA female	34	
GCA male	33	
SCA	33	
Ratio of female mean square value to male mean square value		1.04

[5] Relative contribution of sum of squares to GCA is calculated by calculating the ratio of each component sum of squares to the total of the sum of squares of GCA female, GCA male and SCA

6.5   DISCUSSION

6.5.1   Seed set, germination percentage, seedling survival and pollen viability

Results of this work that seed set ranged from 0.4% to 32% agree with findings by Selvanathan and Khanna (1991) that seed set in the range of 2.6 to 35.7% was found in crosses involving *O. sativa* parents. However, in the current study crosses were between *O. sativa* and interspecific lines. The similarity in the seed set between our parental sets and that reported by Selvanathan and Khanna (1991) indicates that the interspecific lines could be used for rice improvement using conventional methods of hybridization. In the work by Selvanathan and Khanna (1991) the method of crossing used by Coffman and Herrera (1980) was used. The methods used in the current study were similar to this method. A high variability in seed set was, however, found in crosses involving *Oryza sativa* ssp. indica and. Japonica ssp with seed set of 1 to 97% (Katayama, 1974). It is likely that the new interspecific genotypes used in the current study are quite similar to the O. sativa used in the studies by Katayama (1974). Overall, this finding suggests that closeness in genome composition of interspecific rice lines with their *O. sativa* parents reported by Ndjiondjop et al. (2008b) could have had the genes responsible for seed set.

The seed germination rate and seedling survival reported in this work was high. Interspecific hybrids generated from crossing *O. sativa* and *O. glabberima* however, have lower seedling survival (Jones et al 2007). This could be because crossing the interspecific

rice with the *O. sativa*, as the case in this study resulted in the genome being much similar to the *O. sativa* than the 90% contribution reported in the F1 generated from *O. sativa* x *O. glaberrima* crosses (Ndjiondjop et al 2008). It is well established that higher protein levels are in interspecific rice (Mahmoud, et al., 2028). Protein is essential for seedling vigour. It is likely that these proteins in interspecific rice combined positively with the *O. sativa* protein leading to the high germination rate and seedling survival observed.

In this study, *Oryza sativa* parents that may not be used for crossing using conventional method were identified based on repeated trial test for crossing under the experimental conditions in Uganda. The genotypes include WITA 1, WAB 189, ITA 104 and IRAT 13. Interestingly, all these materials have a common parent called CO 9 (IRRI, 2008). In a situation where these genotypes are required for crossing with interspecific rice types, then other breeding methods should be employed. This finding also suggests that gene flow involving these genotypes could be minimal under field conditions. This aspect needs to be further tested. If it is found to be working, then they could be used as border rows in evaluation of breeding genotypes.

In this study, there was variation in the crossability parameter, seed set, among the female parents. The best female parents were identified as IRAT 325, CT 16317-CA-4-M and CT 16313-CA-4-M. Of the three genotypes, CT 16317-CA-4-M and CT 16313-CA-4-M are interspecific genotypes. This implies that they could be used in breeding and for genetic studies. In the same vein the best male parents were, IRAT 257, CT 16334(2)-CA-2-M and WAB 365-B-1H1-HB. Among the best females, some interspecific genotypes were also identified. In breeding and genetic studies where reciprocal crosses are not required, judicious selection of the parents from among the best female parents and best male parents could be undertaken. In addition, parents those had high seed set both as females and as males were identified. The best three male as well as female parents were again the best female parents namely, IRAT 257, CT 16334(2)-CA-2-M and WAB 365-B-1H1-HB.

The germination percentage of 88% recorded in this study was high when compared with germination of hybrids generated from crosses involving *Oryza sativa* with the relatives that were in the same genome group, the AA group. For instance, *Oryza sativa* was crossed with *Oryza meridionalis,* germination score of 52% was realized (Pham et al., 2006). In the case of the cross between *Oryza sativa* with *Oryza meridionalis*, the parents instead of the

interspecific genotypes were used. It is likely that interspecific genotypes generate F1 lines that have higher vigour traits.

6.5.2 Combining ability for seed set

The findings of this study that there was highly significant mean square values for seed set for male, female and the male x female parents for sets A, D and E suggests that both additive and non-additive effects were important in the inheritance of seed set. Further, comparison of the sets using GCA effects showed that the total GCA effects for both male and female parents for the three sets A, D and E were more than 60%. These observations indicated that additive effects were more important in the transmission of this trait. These three populations have one thing in common, that they have at least one parent with tight grain attachment to the pedicel (Lamo et al., 2007). Other workers also reported that varieties that shatter are not suitable for making crosses because they frequently abort before grain formation (Kennard et al., 2002). The set C, however, had genotypes with moderate grain shattering ability and they had high additive effects with no non-additive effects for seed set. It is likely that selection of rice genotypes with acceptable moderate shattering ability would indirectly lead to selection for lines with more additive influence. This aspect needs to be explored further so that breeding for moderate grain shattering ability is conducted along with improvement for high seed set.

The current study found that the ratio of female to male mean square value was more than 3.1 in set D. This value suggests that maternal influence was probably significant in the population under study. According to Kearsey and Pooni (1996), the ratio of female to male mean squares estimate the role of maternal effects. As a rule of thumb, a ratio of female mean square of 3:1 implies that maternal effects are significant (Kang, 1994). Further the F test was significant at (P=0.05). These parents need to be selected in a way that takes consideration of which parents to use as female in the improvement of filled grains.

6.5.3 Combining abilities for pollen grain viability

Results that female, male and male x female interaction mean squares were significant for the pollen viability imply that both additive effects and non-additive effects were important. In addition, the finding that the GCA for both male and female parents were more than 67%, while the SCA contributed to the remainder 33% indicates that additive influence was more important than the non-additive effect. However, the fact that the female mean square to

male mean square was 1.04 implied that maternal influence was not significant. Similar studies on the combining abilities for pollen viability are not available, however the existing work shows that there is wide variability for this trait in segregating populations. For instance, a study conducted by crossing interspecific genotypes with *O. sativa* found wide variation in filled grains but did not report on pollen grain fertility like the case in the current work (Ikeda et al., 2009). Instead, pollen viability ranging from 79% to 84% was also reported in crosses involving various *O. sativa* rice genotypes with IR 36, and viability in the range of 2% to 99% was found in crosses involving *O. sativa* ssp. indica and ssp japonica variation (Selvanathan and Khanna, 1991).This work therefore contributes information on an aspect that is less studied, the cross between interspecific rice and *O. sativa*. For instance, available information on crosses involving relatives of O. sativa is limited to crosses between O. sativa and its relatives. For instance work involving crossing *O. sativa* and *O. rufipogon*, a relative of cultivated rice in the same genome group as *O.glaberrima*, showed pollen viability in the range of 1% (Song, 2004). Similarly, crosses between *O. sativa* and *O. nivara and O. punctata, O. longistaminata* yielded low pollen viability of less than 10% (Song, 2004). In the current study, pollen viability had mean of 53%, an indication that the use of interspecific fixed lines to cross with *O. sativa* improved pollen viability with that of F1 generations.

The cytoplasm of *O. glaberrima* are known to have suppressant effects on hybrid sterility in japonicas (Yabuno, 1977) and indicas (Yabuno, 1977). The evidence of these different findings was based on crossing the *O. sativa* genotypes with *O. glaberrima* and analyzing the F1 generations, except the work by Ikeda (2009) that involved crossing the interspecific lines with *O. sativa*. The current finding takes a different aspect by studying pollen grain fertility instead of filled grains using interspecific lines.

6.6   CONCLUSION

This study provided information that could guide rice breeders as they use the new generations of rice, interspecific rice in improving *O. sativa* rice. The following findings were realized in the current study:

> The seed set in crosses involving interspecific genotypes were similar to the seed set in crosses involving *O. sativa* parents. Parents that had high crossability were identified for female, male as well as female and male parents. The best male

genotypes combiners were CT 16334(2)-CA-2-M, WAB 365-B-1H1-HB and IRAT 257, while the best females combiners were CT 16334(2)-CA-11-M, CT 16317-CA-4-M and IRAT 325.

The mean percentage pollen viability was 77.8% with the highest levels recorded in crosses involving genotype code 105 (WAB 365-B-1H1-HB) as the female.

There were a high germination rate with a mean 88% and a range 75% to 96% while seedling survival had a mean of 68% and range of 57% to 90%.

There were higher additive effects for seed set than non-additive effect. However, there were higher additive effects when least one parent had tight grain attachment to the pedicel. Similarly, additive effects were more important than the non-additive effects for pollen viability.

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Appendix 6.1 List of parents used in different sets of crosses

Genotype no	Parent type[1]	Genotype no	Breeding line	Type
SET A				
	F and M	18	CT 16334(2)-CA-2-M	Interspecific
	F and M	105	WAB 365-B-1H1-HB	*O. sativa*
	F and M	134	NERICA 9	Interspecific
	F and M	138	NERICA 8	Interspecific
	F and M	193	NERICA 13	Interspecific
	F and M	196	IRAT 325	*O. sativa*
SET B				
	F and M	2	CT 16346-CA-20-M	Interspecific
	F and M	9	CT 16350- CA-5-M	Interspecific
	F and M	12	CT 16344-CA-9-M	Interspecific
	F and M	96	Bonanca	*O. sativa*
	F and M	121	WITA 2	*O. sativa*
	F and M	129	CK 73	*O. sativa*
SET C				
	F and M	114	IDSA 6/IAC 164	*O. sativa*
	F and M	194	Golden 1	*O sativa japonica*
	F and M	94	CNAX 104 B-18 PY-2B/PEROLA	*O. sativa*
	F and M	19	CT 16334(2)-CA-11-M	Interspecific
	F and M	67	CT 16313-CA-4-M	Interspecific
	F and M	26	CT 16324-CA-9-M	Interspecific
SET D				
	F and M	141	560/Variety from Zaire	Interspecific
	F and M	96	Bonanca	Interspecific
	F and M	129	CK 73	*O. sativa*
	F and M	27	WITA	*O. sativa*
	F and M	118	WAB 450-I-B-38-HB (NERICA 1)	Interspecific
	F and M	188	WAB 881-10-37-18-3-P1-HB (NERICA 15)	Interspecific
SET E				
	F and M	197	IRAT 257	*O. sativa*
	F and M	193	WAB 880-19	Interspecific
	F and M	147	IRAT 13/MOROBERAKAN	*O. sativa*
	F and M	2	CT 16346-CA-20-M	Interspecific
	F and M	72	CT 16317-CA-4-M	Interspecific
	F and M	88	CT 16355-CA-15-M	Interspecific

[1]Parent type F and M implies that the genotypes was used as female and male

CHAPTER SEVEN
Gene Action and Inheritance of Drought Tolerance in Populations Generated from Crossing Interspecific with Intraspecific Rice

Abstract

Drought is the major production constraint of upland rice. Accordingly, the development of drought-tolerant varieties is the single most important attribute for upland rice. This study investigated the nature of inheritance of drought tolerance in crosses between interspecific and intraspecific rice genotypes using secondary traits. Three separate experiments were conducted, using *O. sativa* and fixed interspecific lines derived from *O. glaberrima* and *O. sativa* crosses. The aim of the first experiment was to investigate the inheritance of drought tolerance at the reproductive growth stage. Eighteen crosses were generated from two sets of 3 x 3 parents using the North Carolina mating design II (NCD II). All the 18 F2 and the 12 parents were evaluated in a 2 x 15 alpha lattice design, with two replicates under a rain-out shelter and non-stress conditions in the field. In the second experiment, the magnitude and direction of gene action for drought tolerance at the reproductive stage was determined in five populations P1, P2, F1, F2 and F3 generated from a drought tolerant x susceptible cross using generation mean analysis (GMA). The materials were planted in the dry season and drought was imposed by terminating irrigation at the stage of panicle initiation. The third experiment aimed at examining the inheritance of drought tolerance at the vegetative stage. Populations were generated from seven crosses involving seven parents. All the seven parents, F1 and F2 populations were planted in a 3 x 7 alpha lattice. Drought stress was imposed during the vegetative growth stage. Results from the NCD II analysis showed that both GCA and SCA effects within sets were significant, indicating that both additive and non-additive effects were important for filled grains, grains per panicle, leaf area, plant height, tiller number and panicle number. The SCA effects were not significant for tiller number. Results of the GMA were that only additive effects were significant for filled grains in the additive-dominance model. Additive genes were important for the transmission of leaf roll under stress at the vegetative stage in the third experiment.

7.1 INTRODUCTION

Drought is a major stress factor in rain-fed lowland and upland rice environments worldwide. The upland rice ecosystem, in particular, makes up to 40% and 45% of rice growing areas in Africa and South America, respectively (Atlin, 2003). In sub-Saharan Africa (SSA), there is increasing expansion of rice production from traditional irrigated production areas to rain-fed environments, where the drought problem is an inherent challenge. It has emerged as a critical rice production constraint in East Africa (ECARRN, 2005), particularly in Uganda (Odogoola, 2006).

A cost-effective means of managing the drought in rice is to develop varieties for different water stress environments (Fukai and Cooper, 1995). In the development of rice that is adapted to drought-prone environments, resistance to drought and escape from drought are the preferred options. Drought escape functions by way of having a short developmental cycle. Drought resistance is attained through drought avoidance or tolerance (Levitt, 1980). Improvement of traits has been achieved by crossing *O. sativa* japonica with *O. sativa* indica (Moncada et al., 2001). Upland rice landraces in Uganda are in this category with some preferred varieties being susceptible to drought. For instance, yield is about 1.0 t ha^{-1} (Odogoola, 2006), yet similar varieties yield up to 3.5 t ha^{-1} elsewhere in the world (Fischer and Fukai, 2003). It is therefore important that the yield potential of the landraces is improved.

Currently, a new generation of rice, the interspecific rice genotypes, offers potential for use in breeding for improved drought tolerance. It was generated by crossing cultivated African rice, *Oryza glaberrima,* with cultivated Asian rice, *Oryza sativa* vs. japonica. The *O. glaberrima* also provides preferred traits that include resistance to rice yellow mottle virus (RYMV) (Paul et al., 2003), resistance to African rice gall midge (AfRGM) (WARDA, 2000) and drought (Majerus et al., 2007). RYMV and AfRGM are, however, major constraints under irrigated production zones in Uganda, based on findings by Odogoola (2006), making drought stress a major constraint for upland production areas.

A few new interspecific lines have been released in the country, namely NERICA 1, NERICA 10 and NERICA 4, albeit they are susceptible to drought (Kijima et al., 2006). Considering that the interspecific rice genotypes have high osmotic potential (Majerus et al.,

2007), high tillering and leaf rolling capacity, and high plant vigour (Jones et al., 1997b), it is likely that the few released NERICA rice lines carry these preferred traits. Interspecific genotypes that are superior to the released NERICAs in terms of drought tolerance have been identified (Lamo et al., 2007). These introductions could be used to improve existing rice genotypes. However, there is no information on their combining ability with popular landraces. Moreover, a vast majority of Uganda's rice landraces are intolerant to drought (Imanywoha et al., 2002). There has been difficulty in phenotypic evaluation of traits under drought stress that frequently led to low estimation of heritability values (Lafitte et al., 2003). This has been improved through the use of hydroponics, rain-out shelters and the referencing of evaluation sites (Yue et al., 2006). In Uganda, a rain-out shelter and drought screening site has been referenced for drought tolerance assessment (Lamo et al., 2007). Referencing is the process of determining the duration of drought stress that is adequate to discriminate within the rice population for tolerance to drought stress (Lafitte et al., 2003). Genotypes that show varying levels of tolerance to drought have been selected (Lamo et al., 2007), but information on the transmission of those traits is lacking.

Knowledge of the genetics of drought resistance or its component traits is important for effective breeding. In rice, most of the knowledge about drought tolerance has been based on root related traits. For instance, Chang et al. (1985) and Ekanayake et al. (1985) separately studied the inheritance of root traits related to drought tolerance. Recently, however, secondary traits with high heritability for drought tolerance . that is, leaf drying, leaf rolling, filled grains, root traits and water use efficiency . have been reported (Singh and Mackill, 1990; Garrity and O'Toole, 1994; Fukai and Cooper, 1995; Lafitte et al., 2003). Among the secondary traits used in evaluation under drought, filled grains is arguably the most preferred (Atlin, 2003; Lafitte et al., 2003; Jongdee et al., 2006). It is an indirect index for drought tolerance, easy to score and is strongly associated with yield (Lafitte et al., 2003). Information on the inheritance of filled grains is limited. However, anther and spikelet dehiscence, known to be affected by drought and correlated with filled grains, have been widely studied for genetic variation under drought stress (Liu et al., 2006). Based on inheritance studies, anther dehiscence was effectively used in the selection of drought-tolerant upland rice cultivars (Ekanayake et al., 1989). Variety evaluation using anther dehiscence is difficult, time consuming or expensive. An understanding of the transmission of filled grains would guide rice breeding for improved drought tolerance.

Leaf roll is a drought resistance trait that is exhibited through drought avoidance mechanisms (Singh and Mackill, 1990). Leaf roll reduces transpiration and absorption of radiant heat and light, in effect reducing leaf water potential (Lafitte et al., 2003). Singh and Mackill (1990) found that a single gene was responsible for leaf roll in rice in *O. sativa*. However, up to 11 QTLs have been mapped for leaf rolling (Hsiao et al., 1984), suggesting that more than one gene could be responsible for the leaf roll trait. Studies on the genetics of leaf roll under drought stress were conducted using *O. sativa* rice, which does not have direct application in the new generation of the interspecific derived rice types. The present study was, therefore, conducted to examine the nature of gene action governing drought tolerance at the reproductive stage and associated secondary traits in newly generated fixed lines developed through *O. sativa* x *O. glaberrima* crossing and popular *O. sativa* landraces.

7.2 Objectives of the study

The specific objectives of the study were to:

i.   investigate the mode of gene action for tolerance to drought stress during the reproductive growth stage in rice;

ii.   determine the correlation between filled grains and other traits under stress and non-stress conditions; and

ii.   determine the heritability estimates for tolerance to drought during the vegetative growth stage in rice.

7.3 MATERIALS AND METHODS

7.3.1 Germplasm and generation of segregating populations

Three groups of populations were generated. In the first population, a total of 12 genotypes consisting of five *O. sativa* and seven fixed interspecific lines were selected from collections previously characterized for tolerance to drought in Uganda (Lamo et al., 2007). The interspecific fixed lines comprised three New Rice for Africa (NERICA) rice and four CT rice lines (Table 7.1). One group of six parents was established in a 3 x 3 crossing scheme consisting of SET A and the other group formed SET B. The two sets were for studies on drought tolerance focusing on using the filled grains trait.

A second group of populations was generated in a separate crossing involving genotypes CT 16334 (2)-CA-2-M and WAB 450-1-BL1-136-HB (Table 7.1). Five generations involving

the two parents, F1, F2 and F3 were used in the second experiment. The third experiment involved crossing the genotypes in Table 7.1 as 12 x 121, 12 x 129, 2 x 121, 2 x 129, 2 x 147, 72 x 147 and 27 x 197. The individual F1s were selfed to generate F2 generations. These five generations were used for studies on drought tolerance focusing on using the filled grains trait. The third group comprised seven genotypes in SET D. They were used for studies on drought tolerance at the vegetative growth stage.

Table 7.1 Rice genotypes used in the three experiments

Experiment	Genotype no	Parent type	Genotype no	Breeding line	Type
	SET A				
		Female	18	CT 16334(2)-CA-2-M	Interspecific
		Female	105	WAB 365-B-1H1-HB	O. sativa
		Female	134	NERICA 9	Interspecific
		Male	138	NERICA 8	Interspecific
		Male	193	NERICA 13	Interspecific
1		Male	196	IRAT 325	O. sativa
	SET B				
		Female	2	CT 16346-CA-20-M	Interspecific
		Female	9	CT 16350- CA-5-M	Interspecific
		Female	12	CT 16344-CA-9-M	Interspecific
		Male	96	Bonanca	O. sativa
		Male	121	WITA 2	O. sativa
		Male	129	CK 73	O. sativa
	SET C				
2		Female	18	CT 16334 (2)-CA-2-M	Interspecific
		Male	138	WAB 450-1-BL1-136-HB	Interspecific
	SET D	Female			
		Female	2	CT 16346-CA-20-M	Interspecific
		Female	12	CT 16344-CA-9-M	Interspecific
3		Female	72	CT 16317-CA-4-M	Interspecific
		Female	121	WITA	O. sativa
		Male	147	IRAT 104	O. sativa
		Male	197	IRAT 257	O. sativa
		Male	129	CK 73	O. sativa

7.3.2 Experimental design

Experiment 1: Inheritance of drought tolerance

Thirty genotypes comprising 18 F2 progenies from sets, A and B, along with the 12 parents (18, 105, 134, 138, 193, 196, 96, 121, 129, 2, 9 and 12) were used in this experiment. The 30 entries were established in a rain-out shelter at the National Crops Resources Research

Institute (NaCRRI) in Namulonge (Fig 7.1). Namulonge is located at $0°$ 31' N, $32°$ 35' E, with a mean altitude of 1,150 m above sea level. The rain-out shelter was constructed using translucent sheets for the roof and wire mesh on the sides of the structure to prevent rain water and to allow free air circulation, respectively. In the rain-out shelter, standard troughs that are 1 m wide, 8 m long and 1.5 m deep were filled with soil from a fallow field in Namulonge. Four troughs were made and filled with the soil, referred to as strips. The seeds were planted in a 2 x 15 alpha lattice design. Two strips represented a replicate. The 12 parental genotypes were planted in 3 rows planted across the 1 m wide strips, while the F2 populations were planted in 6 rows. The plant-to-plant spacing was 15 cm, making the plant population 36 parental lines and 72 F2 lines.

A second set of the 30 entries was planted in the field under optimal conditions. These conditions involved irrigating the field at 20 mm per week during the period when there was no rain. In both trials, a 2 x 15 alpha lattice design planted in two replicates was used. Two seeds from each generation were drilled at a depth of 3 cm at a spacing of 20 cm x 20 cm in each plot. In order to reduce border effects, 20 cm spaces were left between plots. The 12 parental genotypes were planted in 5-row and 3-column plots while the F2 populations were planted in 5-row and 6-column plots. Overall, there were 15 plants per replicate of the parents and 30 plants per replicate of each F2 genotype, thus the total number of plants was 30 and 60 for the parents and F2, respectively. Similar studies by Ali et al. (2003), Govinda, and Virmani (1988) in rice were conducted using a minimum of 30 plants at F2 generation. The sample size in this study was therefore considered adequate. The plants were thinned to one plant per hill. Supplementary irrigation water was applied at 20 mm per week. Standard cultural practices, including hand planting and hand weeding, were followed. The crops were fertilized with 25 kg N ha^{-1} at 20-25 days after transplanting (DAT) and the same rate at 40-45 DAT to enhance plant vigour.

Drought stress was imposed by terminating irrigation when about 50% of the populations had attained an interauricular distance between the flag leaf and penultimate leaf of zero, that is the period about 10 days before anthesis (Ji et al., 2007; Itoh et al., 2008). This method of identifying the stage of imposing drought was applied both in the field and in the rain-out shelter. In general, this is the time when the penultimate leaves were fully expanded. Rainfall during the trial period was recorded.

In the field experiment, irrigation was applied using sprinkler irrigation. The field was irrigated every three days before imposing drought stress. On the day the irrigation was terminated, the field was irrigated to field capacity in the evening between 5:00 pm and 6:00 pm and was resumed 14 days after termination using sprinkler irrigation. The duration of drought stress was determined by testing the level of soil moisture daily using the ECHO soil moisture tester (Decagon Devices, Inc Pullman, Washington, USA). On the day when 30% of the available water had been lost from the soil at a 20 cm depth, irrigation was resumed. In the rain-out shelter, water was applied using hand irrigation cans but water was calculated for each strip at 140 L per week, which is equivalent to 20 mm per week.

The number of filled grains was counted per panicle at grain maturity. Two panicles from each plant were randomly collected and the number of filled grains was determined using the floatation method (IRRI, 2002). The F1 grains were carefully hand threshed and dried. The grains were subsequently poured onto a 500-100 ml beaker containing water. The grains were then stirred for 1 minute to expel any pockets of air within the bulk of grain and left to stand for three minutes to allow filled grains to sink to the bottom. The seeds that floated were carefully skimmed off using strainers. The filled and floating grains were dried separately and the total number of filled and unfilled grains was counted. Grain weight was not measured because the results would have been misleading. The unfilled and filled grains when compared on the basis of weight requires standardizing weight of filled grains and extrapolating but some entries had all plants having unfilled grains, thus there was no filled grains weight for comparison.

Experiment 2: Gene action for drought tolerance

In this experiment, all five populations generated from crossing; lines P1 and P2, and their F1, F2 and F3 genotypes were planted following a randomized complete block design (RCBD) with two replicates. Two seeds from each generation were drilled at a depth of 3 cm at a spacing of 20 cm x 20 cm in each experimental unit (plot) in the field at NaCRRI. The generations P1, P2 and F1 were planted in 5-row and 3-column plots while F2 and F3 were planted in 5-row and 6-column plots. Overall, there were 15 plants per replicate of the parents and 30 plants per replicate of each F2 genotype, thus the total numbers of plants were 30 and 60 for the parents and F2, respectively. The cultural practice in experiment 1 was followed, and drought stress was imposed following the procedures in experiment 1.

Experiment 3 Heritability studies for leaf roll trait

The third experiment was conducted using the seven parents, seven F1 and seven F2 populations. Each of the overall 21 entries was planted in a 3 x 7 alpha lattice design with two replications. The parental generations and F1s were planted in 5-row and 3-column plots, while F2 progenies were planted in 5-row and 6-column plots. The plants were thinned to one plant per hill. Supplementary irrigation was applied at 20 mm per week. Standard cultural practices were followed.

The reaction of the different materials to drought stress was assessed at the vegetative growth stage. In order to assess the reaction of the varieties at the vegetative stage, irrigation was terminated when about 50% of the plants had attained maximum tillering. Maximum tiller numbers were attained when the daily total tiller number ceases to increase following the mean tiller number recorded every three days. The leaf roll score was taken using a 0-9 scale: 0, healthy; 1, shallow V-shaped leaves; 3, deep V-shaped leaves; 5, fully capped leaves; 7, leaf margin tightly held in U-shape; 9, tightly rolled leaves (Gregorio and Cabuslay, 2005). It is a modification of the standard SES scoring system for rice developed by IRRI (IRRI, 2002).

Figure 7.1 Rain- out shelter used to evaluate drought in segregating populations

7.3.3   Statistical analyses

Data was analyzed in three parts, namely analyses of variance, Residual Maximum Likelihood (REML), regression and generation means. The analysis of variance was performed for different traits associated with drought tolerance in the two sets of populations, A and B, pooling for both stress and non-stress environments. The set A and set B populations comprised seven parents each (Table 7.1). Using REML, the separate

sets were analyzed for each trait. The analyses of the variance components of genotypes were further partitioned into variations due to parents and crosses.

General analyses of variance were performed for filled grains, grains per panicle, leaf area, plant height, tiller number and panicle number of all hybrids including checks. Genetic analyses for the six parameters of experimental hybrids were then performed in Genstat (Payne et al., 2007) as a fixed effects model across two locations:

Where $1, 2, 3;$ $1, 2, 3;$ $1, 2;$ $1, 2, 3, 4, 5, 6, 7, 8;$ $1, 2;$

denotes the mean variate score of the line of a mating of the th female line,

the th male line, in the th block, within set and in the th location

The terms of the model were de ined as follows:

Grand mean;

the average effect of the th set

GCA effect of the th female line nested within th set

GCA effect of the th female line nested within th set

SCA effect speci ic to cross of the th female and th male nested within th set;

average effect of th location

effect of the th replication nested within the th set and th location

interaction between set effects and the location

and   interaction between location and GCA nested within sets

interaction between location and SCA nested within sets

pooled error or random experimental error

Genetic correlations between any two traits taken in the same environment was calculated using Genstat statistical package, 12 (Payne et al., 2007).

Generation mean analysis of the genotypes CT 16334 (2)-CA-2-M crossed with WAB 450-1-BL1-136-HB was used to determine additive, dominant and epistatic effects following Hayman (1958) model. The various generations did not have equal variances therefore weighted inverse of the variances were used in subsequent analysis according to Nigam (2001). Regression analysis procedures were used to find the best fit model. It is a

graphical method used to compare the additive model with additive-dominance models. Any effect that was not significant at 5% level was excluded from the model. The parameters were fitted using weighted mean squares as described by Rowe and Alexandria (1980).

A scaling test was conducted using linear combinations of various means according to Hayman (1958) and Jinks and Jones (1958) to detect the presence of non-allelic interactions that are known to bias estimates of additive and dominance components in the populations when present. However, in this case where F3 populations are used instead of backcross populations, the additive effects estimate is for both additive effects and additive x additive interaction effects. Similarly, the dominance effect combined both dominance effects and dominance x dominance interaction effects as a single estimate (Sharma, 1995). This is not a major drawback considering that most breeding work exploits additive effects and dominance effects. Besides, generation of F3 populations is much easier than making backcrosses in rice where self pollination and small floret size make crossing difficult. Standard errors of generation means were computed by performing nested analysis of variance following methods used by Nunir et al. (2007).

In order to verify the number of genes involved in the transmission of traits associated with drought tolerance Castle-Wrights formulae described by Sharma (1995) was used.

The formula is as follows:

$$ \quad 1 \qquad 2\ {}^{2}/\,8 \qquad 2 \qquad 1 $$

Where, is the estimate number of genes, 1 is the mean of parent 1, 2is the mean of parent 2, 2 is variance of 2 population and 1 is variance of 1 population.

An estimate of degree of dominance or Potency Ratio (PR) was conducted using the formula described by Peter and Frey (1966).
The formula for F1 generation is as follows:

$$ 1 \qquad 1 \qquad / \qquad 1 \qquad . $$

Where,

 the average of the two parents that is , 1 2 /2, the 1 being the the superior parent and 2 the inferior parent involved in the 1cross.

In F_2 generation,

$$2 \quad 2 \quad 2 \quad / \quad 1 \quad .$$

Where,

is the average of the two parents that is, $1 \quad 2 /2$, the 1 being the superior parent and 2 the inferior parent involved in the 2 cross.

The scales used for both 1 and 2 are $= 0$, no dominance; 1.0, overdominance; 1.0, partial dominance; 1.0, complete dominance.

In order to determine heritability, a set of 7 crosses were subjected to regression analyses using offspring regression involving regressing 2 on 1 progenies (Vogel et al., 1980; Caster, 1982), and 1 progenies on mid-parents (Sharma, 1995; Falconer and Mackay, 1996). The regression model was ; where Y is the relationship between 1 and mid-parents and 2 with 1; X is the intercept and C the constant.

Data generated on agronomic traits associated with drought stress were used to calculate susceptibility indices. Data on yield could not be used because the sample size did not provide the minimum weight required to use the method described by Fischer and Mauer, (1978).

$$\frac{\quad}{\quad} / \quad .$$

Where

= mean of the parameter measured in stress environment

= mean of the parameter measured in non-stress environment

= stress intensity

$$1 \quad \rule{3cm}{0.4pt}$$

7.4 RESULTS

7.4.1 Gene action

Generalized linear analysis for different traits pooled across sets and sites are presented in Table 7.2. Results showed that both GCA and SCA effects within sets for filled grains, grains per panicle, leaf area tiller number and number of panicles per plant were significant

(P = 0.001), while only the GCA effects within sets for tiller number were significant (P = 0.001) but not the SCA effects.

. Table 7.2 Pooled mean square for spikelet fertility and other secondary traits under drought stress and non drought stress environments

	df	Spikelet fertility	Grains per panicle	Leaf area	Plant height	Tiller number	Panicle number
				Mean square value			
Env[1]	1	146.5***	1441.2***	1335.9***	109.3***	1534.8***	1708.8***
Set	1	41.5***	13.6***	35.0***	10.1***	0.7	14.1***
Set / GCA$_f$[2]	4	10.1***	10.5***	17.3***	7.3***	3.2**	5.1***
Set / GCA$_m$[3]	4	5.8***	11.4***	12.8***	26.5***	8.3***	3.8**
Set / SCA	8	3.6***	8.5***	8.3***	26.5***	1.2	14.6***
Env x Set	1	14.9***	15.4***	35.4***	1.9	6.3**	13.2***
Env x Set / GCA$_f$	4	14.8***	8.4***	14.7***	1.4	4.5***	0.3
Env x Set / GCA$_m$	4	5.6***	7.9***	11.9***	3.7**	2.5**	0.1
Env x Set / SCA[4]	8	2.8**	7.2***	8.3***	1.8	1.6	0.5

*, **, *** - P < 0.05, P < 0.01, P < 0.001
[1]Environment; [2]GCA$_f$ = General combining ability for female parents , [3] GCA$_m$= General combining ability for male parents; SCA= Specific combining ability.

The male and female mean squares were all significant (P = 0.05) for the filled grains under drought stress (DS) and non-drought stress (NDS) conditions for the A set population (Table 7.3). There was significant (P<0.05) mean square for male x female interaction for the filled grains under NDS for set A. In the case of the B crossing set, the male x female interaction mean squares were significant under DS and NDS conditions. In addition, the mean square of male and female were significant under DS but not under NDS. The male, female and male x female interaction mean squares were all highly significant (P = 0.001) for the total number of grains per panicle under NDS conditions for the A set and significant (P = 0.05) under DS conditions. In the case of B crossing set, male, female and the male x female interaction, mean squares were significant under NDS conditions but not the case under DS conditions. The set A had highly significant (p < 0.001) male, female and male x female mean squares for leaf area under NDS conditions. Mean squares for male and male x female interactions were significant for leaf area under DS conditions but not the case of female mean square. The results of the B crossing set revealed that, male, female and the male x female interaction mean squares were all highly significant (P < 0.001) under the NDS and DS conditions.

The male x female interaction mean squares were all highly significant (P = 0.001) for the plant height under DS and NDS conditions for the A and B populations. There was significant mean square for female effects under NDS but not under DS conditions. In the case of B crossing set, male, female and the male x female interaction mean squares were all highly significant under both NDS and DS conditions. Mean squares for male, female and male x female interaction mean squares were not significant for the tiller number under NDS conditions for the A populations. There was, however, significant mean square for male and the male x female interactions but not female mean squares under DS conditions. On the other hand the B crossing set had male, female and the male x female interaction mean squares all highly significant under both DS and only the male under NDS conditions.

Table 7.3 Mean squares for spikelet fertility, total grains per panicle, leaf area, plant height and tiller number under drought and non-drought stress

| | | Mean Square values | | | |
| | | Set A | | Set B | |
Source	d.f	Drought stress	Non-drought stress	Drought stress	Non-drought stress
Fertility[1]					
Male	2	3.21*	12.90*	0.51*	1.86
Female	2	4.07*	9.01*	2.58*	5.40
Male x Female	4	1.56	6.84*	3.29*	0.80**
Total grains panicle[-1]					
Male	2	4.58*	8.60***	2.39	12.28***
Female	2	4.14*	9.86***	1.38	9.51***
Male x Female	4	2.64*	9.40***	0.62	5.83***
Leaf Area					
Male	2	3.09*	11.00***	6.96***	15.51***
Female	2	1.40	16.21***	10.01***	16.01***
Male x Female	4	4.76***	9.74**	17.74***	5.34***
Plant height					
Male	2	4.46*	4.45	26.70***	26.44***
Female	2	1.26	1.20**	7.66***	7.27***
Male x Female	4	8.14***	8.14***	15.70***	14.70***
Tiller no					
Male	2	23.13*	42.99	26.70***	4.61**
Female	2	3.36	49.08	7.66***	0.75
Male x Female	4	2.17***	13.97	15.70***	1.67

[1]Spikelet fertility in percentage

7.4.2   Relative contribution of GCA and SCA

General Combining ability (GCA) for female and male (GCA_f and GCA_m) in A populations under DS and NDS conditions are presented in Fig 7.2. The total GCA for both male and female parents (GCA_t) under DS were more than 55% for all five traits except leaf area that had 42%. All the SCA values were less than 50%. The SCA effects of tiller number under DS was 14% and the male x female interaction was not significant (Table 7.3). Similarly, the SCA effects for filled grains were not significant under DS conditions (Table 7.3). This finding, therefore implies that the additive effects were more important than non-additive effects for filled grains, total number of grains per panicle, plant height and tiller number.

Under NDS, however, filled grains, leaf area and tiller number had GCA_t more than 55%. The total number of grains panicle^{-1} and plant height had nearly equal GCA_t when compared with SCA. This finding implies that the additive effects are more important than non-additive effects for filled grains, leaf area and tiller number, while additive and non-additive effects had nearly equal effects for total number of grains panicle^{-1}. However, the lack of significance in the male x female interactions for tiller numbers (Table 7.1) makes the importance of SCA not valid.
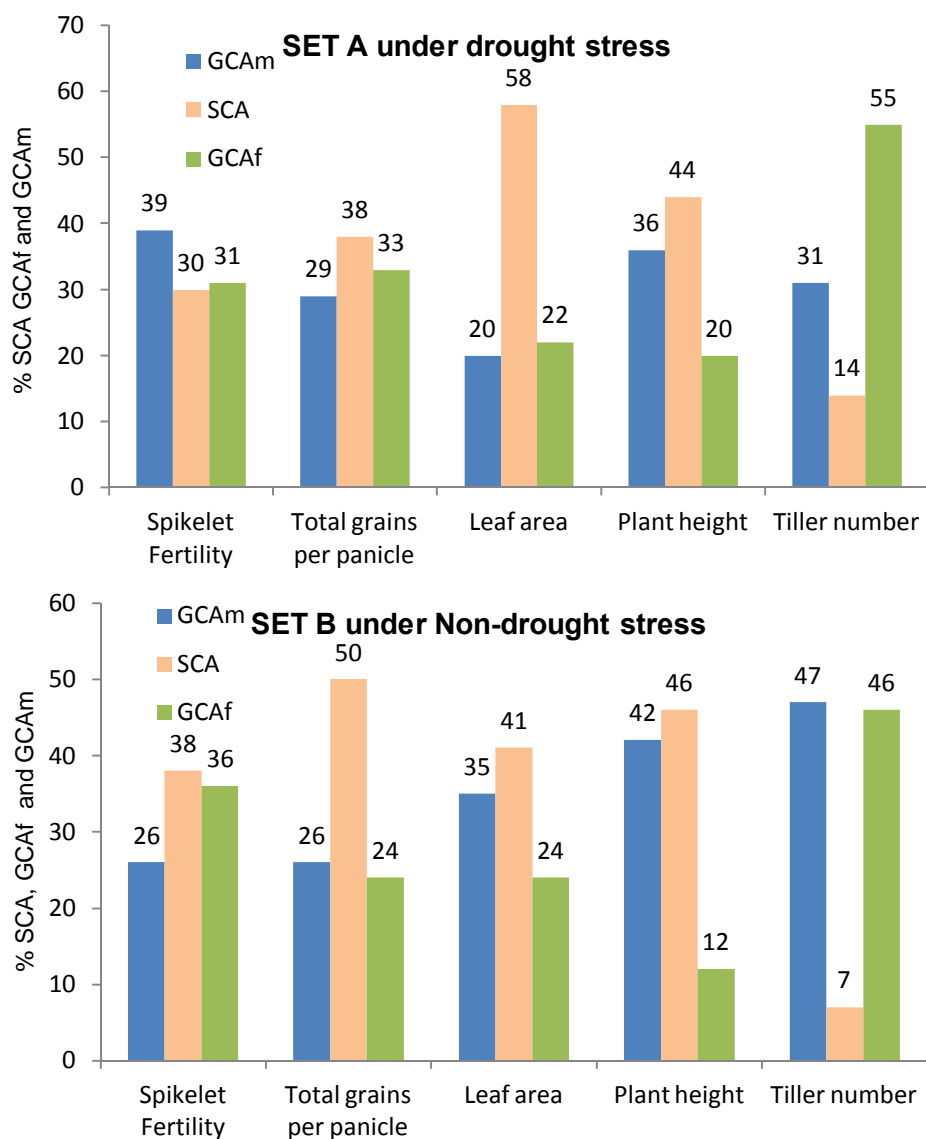
Figure 7.2. Relative (%) contribution of sum of squares to GCA and SCA for A set under drought stress and non drought stress

Results of the GCA effects for female and male (GCA_f and GCA_m) for B populations under DS and NDS conditions are shown in Fig 7.3. The GCA total (GCA_t) under drought was more than 55% for filled grains, total number of grains panicle^{-1} and tiller number under DS. The GCA_t and SCA for plant height were nearly equal, while a very high SCA value of 68% was found for leaf area. All the SCA values were less than 50%, moreover the SCA effects for tiller number were not significant (Table 7.3) and that of total number of grains per panicle were also not significant (Table 7.3). This finding implies that the additive effects were more important than non-additive effects for filled grains, total number of grains per panicle, and tiller number, while additive and non-additive effects had nearly equal effects

for plant height. Under NDS conditions, however, filled grains, total number of grains panicle^{-1}, leaf area and tiller number had GCA$_t$ more than 55%. The plant height had nearly equal GCA$_t$ when compared with the SCA. This finding implies that the additive effects were more important than non-additive effects for filled grains, total number of grains panicle^{-1} ,leaf area and tiller number, while additive and non-additive effects had nearly equal effects for plant height.
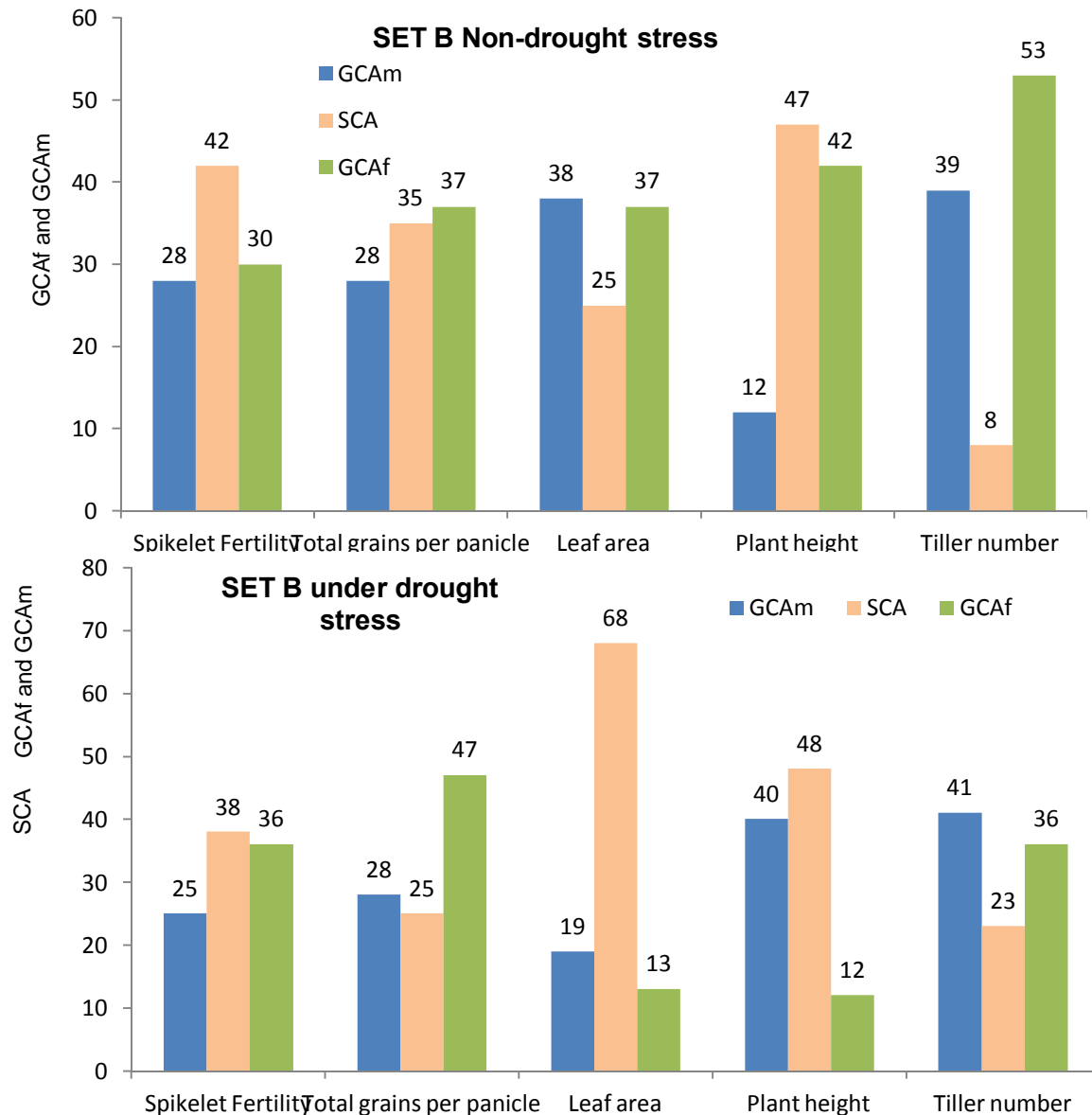
Figure 7.3. Relative (%) contribution of sum of squares to GCA and SCA for B set under drought stress and non drought stress

Results of the ratios of female mean square to male mean square for the A populations under DS and NDS conditions are in Fig 7.4. Under drought stress, the ratio of female to

male mean square were all less than 1 except filled grains. In the NDS condition, only plant height and filled grains had ratios of female mean square to male mean square less than 1. There was similarity in the plant height and total number of grains per panicle under drought and non-drought stress conditions. Overall, the mean square value for female was higher for most traits under NDS conditions than under drought stress conditions.

SET A under drought stress

SET A under drought stress
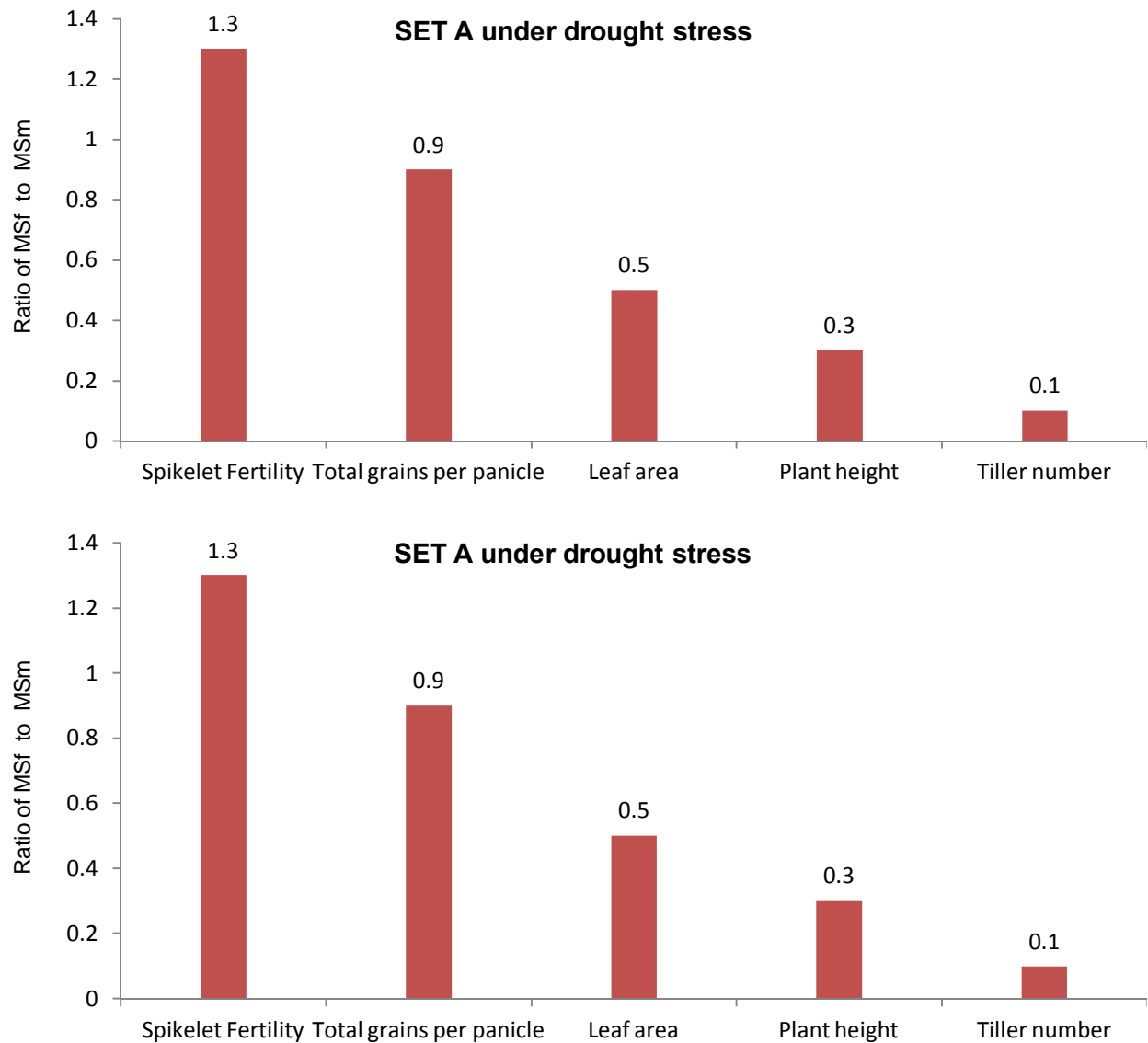
Figure 7.4. Ratio of mean square for female to mean square for male in A set under drought stress and non drought stress

Ratios of female mean square to male mean square in the B populations under DS and NDS conditions are shown in Fig 7.5. Under drought and non-drought stress conditions, the ratio of female to male mean square were all less than 1.5 except filled grains. The traits plant height and tiller number had equal values of mean square ratios. Only filled grains under drought stress conditions was significant at P = 0.01. There was similarity in the plant height and total number of grains per panicle under DS and NDS conditions. Overall, the least ratio was 0.3 for tiller number tested under NDS while the highest ratio was 5.2 for filled grains number under DS, respectively.
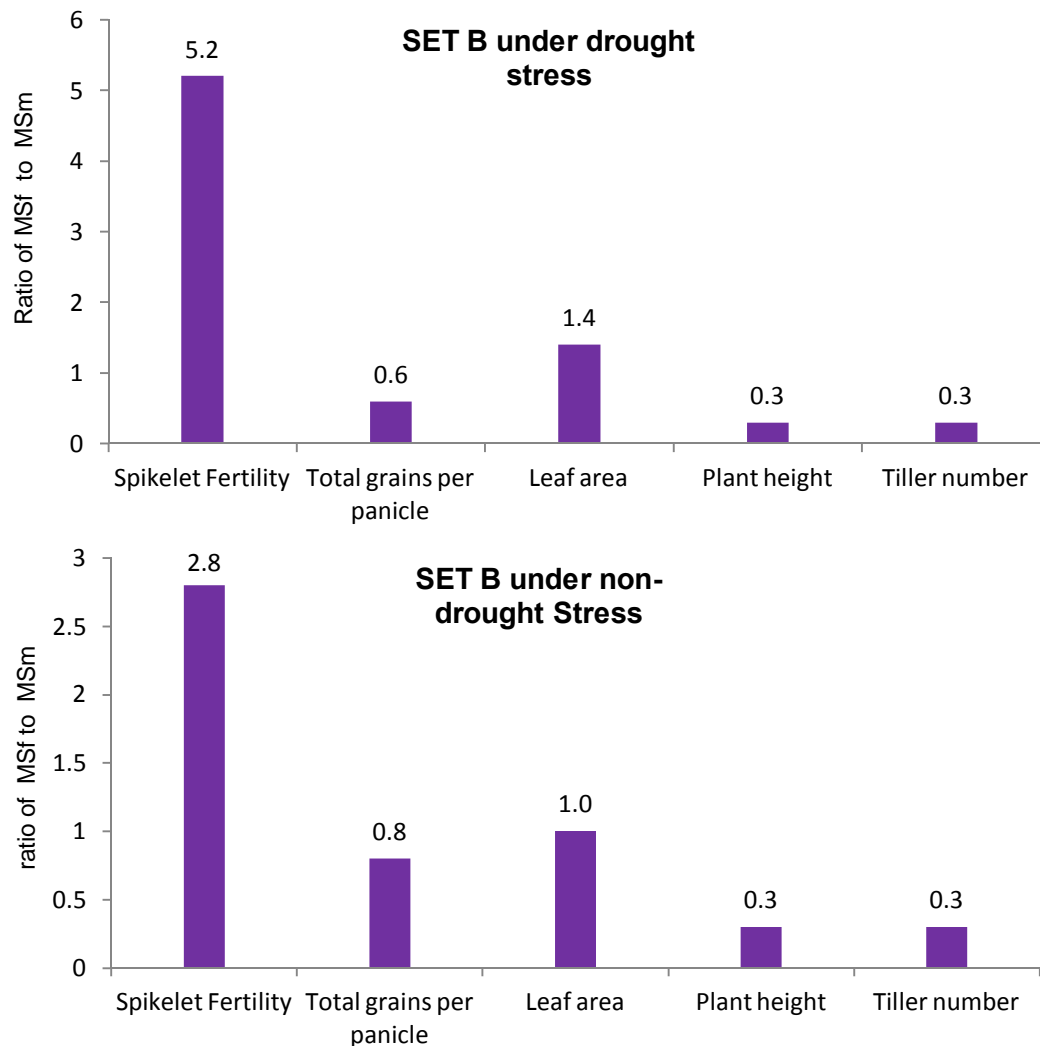
Figure 7.5. Ratio of mean square for female to mean square for male in B set under drought stress and non-drought stress

7.4.3 General combining ability effects

Table 7.4 shows the GCA effects for filled grains for interspecific and intraspecific rice. The GCA values for filled grains were the only one presented because other secondary traits had weak correlation with the filled grains which is a trait associated with drought tolerance. Positive GCA effect is desirable in breeding for improved drought tolerance. Strong negative values of GCA effects of parents show contribution of GCA towards low filled grains while high positive values show high filled grains. Since both GCA effects and SCA effects were significant for filled grains, the individual values for both GCA and SCA effects are presented (Table 7.4 and 7.5). Parents CT 16350- CA-5-M, IRAT 325 and NERICA 9 had positive and significant scores of filled grains under NDS conditions (Table 7.4). In the DS environment, CK 73 was highly positive and significant at P=0.001 while CT 16344-CA-9-M, NERICA 9 and CT 16346-CA-20-M had positive and significant filled grain scores at P = 0.01.

Table 7.4 Estimates of general combining ability (GCA) effects for filled grains under drought and non drought stress conditions

	Filled grains	
	Non- drought stress	Drought stress
Female		
NERICA 8	-0.46	-3.42**
NERICA 13	-1.69	3.45**
IRAT 325	2.15*	-0.03
Bonanca	-2.80**	1.04
WITA 1	-4.98***	-6.23***
CK 73	-2.18*	5.19***
Male		
CT 16334(2)-CA-2-M	-1.29	-1.18
WAB 365-B-1H1-HB	-0.71	-2.12*
NERICA 9	2.00*	3.30**
CT 16346-CA-20-M	-2.54**	-2.26*
CT 16350- CA-5-M	5.26***	-1.16
CT 16344-CA-9-M	-2.72**	3.42**
SE	**±0.83**	**±0.97**

*** = significant at P=0.001 (4.14); ** = significant at P=0.01 (2.98); * = significant at 0.05 (2.15)

7.4.4   Specific combining ability effects

Superior crosses were observed, with positive SCA effects (Table 7.5). Under non-drought stress conditions crosses WITA 1 x CT 16350- CA-5-M, Bonanca x CT 16346-CA-20-M and CK 73 x CT 16344-CA-9-M had significant filled grain score of 0.01%, 0.01% and 0.001%, respectively. The cross WITA 1 x CT 16344-CA-9-M had significant filled grain score at 0.05 level of significance. In the drought stress conditions, the cross NERICA 8 x WAB 365-B-1H1-HB were highly significant P=0.001, Bonanca x CT 16344-CA-9-M and IRAT 325 x CT 16334(2)-CA-2-M positive and significant at 0.01.

Table 7.5 Estimates of specific combining ability (SCA) effects for filled grains under drought and non drought stress conditions

| | | | Filled grains | |
			Non drought stress	Drought stress
NERICA 8	x	CT 16334(2)-CA-2-M	-3.46	-12.86***
NERICA 13	x	CT 16334(2)-CA-2-M	1.47	4.48*
IRAT 325	x	CT 16334(2)-CA-2-M	0.73	7.21**
NERICA 8	x	WAB 365-B-1H1-HB	-0.59	8.53***
NERICA 13	x	WAB 365-B-1H1-HB	-2.01	-4.93*
IRAT 325	x	WAB 365-B-1H1-HB	1.90	-5.71*
NERICA 8	x	NERICA 9	3.60	0.92
NERICA 13	x	NERICA 9	-1.12	3.91
IRAT 325	x	NERICA 9	-0.46	-1.52
Bonanca	x	CT 16346-CA-20-M	6.15**	-3.19
WITA 1	x	CT 16346-CA-20-M	-6.39**	-0.88
CK 73	x	CT 16346-CA-20-M	-2.32	1.86
Bonanca	x	CT 16350- CA-5-M	1.30	-1.74
WITA 2	x	CT 16350- CA-5-M	7.12**	-1.63
CK 73	x	CT 16350- CA-5-M	-3.17	2.26
Bonanca	x	CT 16344-CA-9-M	-10.27***	6.02**
WITA 2	x	CT 16344-CA-9-M	4.24*	-3.67
CK 73	x	CT 16344-CA-9-M	3.30***	1.12

*** = significant at P=0.001 (4.14); ** = significant at P=0.01 (2.98); * = significant at 0.05 (2.15)

7.4.5   Summary of analysis of generation of means

The mean, variance, mean variance of the records of filled grains for P1, P2, F1, F2, and F3 are shown in Table 7.6. The F2 populations had the highest variance followed by F3 and F1. Scaling tests for dominance x dominance and additive x additive interactions were non-significant for both levels. Dominance main effects were not significant, but additive main

157

effects were significant at P = 0.01. When the mean scores were fitted to an additive model it fitted with r^2=0.77 (Fig 7.6). The estimate of the number of genes that control filled grains trait based on Castle-Wrights method was 0.9^1 1 gene. Estimate of the degree of dominance in the F1 and F2 generation based on the Peter and Frey (1966) method was -3^1 0 and 0.9^1 1 level of dominance, respectively.

The narrow sense heritability in the generations from the cross between CT 16334 (2)-CA-2-M and WAB 450-1-BL1-136-HB using regression of F1 on mid-parents and F2 to F1 based on single seed decent are shown in Fig 7.7 and 7.8, respectively. In the F1 to mid-parents regression, heritability of 60% was realized but when F2 was regressed onto F1 means, the heritability estimate was 74%.

Table 7.8 Summary of generations in variety 18 x 138 cross, scaling test, and components of means for spikelet fertility score

Descriptive summary of generations

Generations	d.f	Mean	Variance	Mean Variance
P1	29	77.80	42.92	2.59
P2	29	56.93	16.89	1.90
F1	29	74.73	80.47	2.49
F2	59	72.07	141.08	1.20
F3	59	64.60	81.87	1.08

Scaling test for spikelet fertility

Interactions	Scale	SE	d.f	t(Scale/SE)
dominance x dominance	-15	3.559	146	-1.184[NS]
additive x additive	3	3.361	176	0.893[NS]

Components of means (three parameters)

Gene effects	Expectation estimates	SE	t=(component/SE)	df
Mean	57.0	1.176	48.46**	59
Additive effects	10.5	0.707	14.85**	58
Dominance effects	0.8	36.842	0.22	147
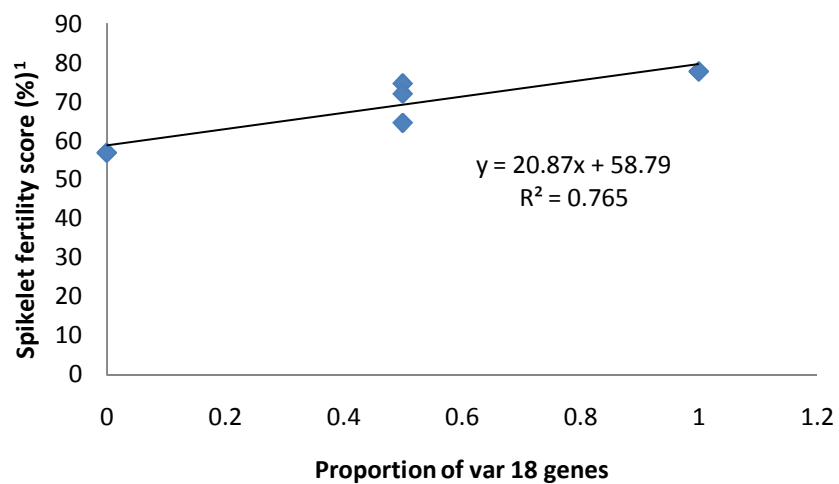
Figure 7.6. Proportions of genes contributing to spikelet fertility score
Mean spikelet fertility score was Best Linear Unbiased Estimator (BLUE) of the traits
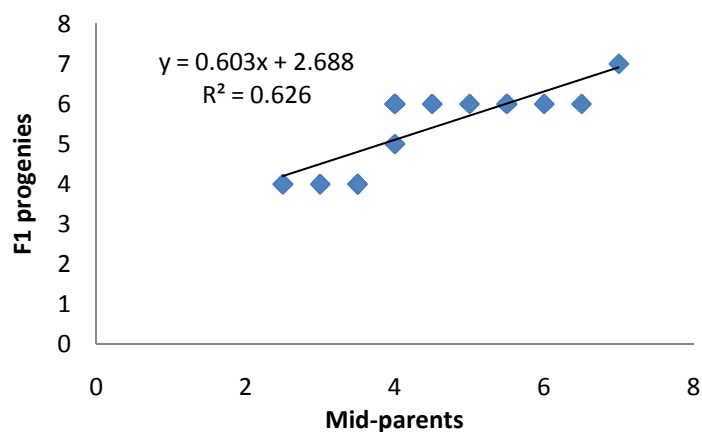
Figure 7.7 Regression of F1 progenies on mid-parents for 12 x 138 cross using spikelet fertility
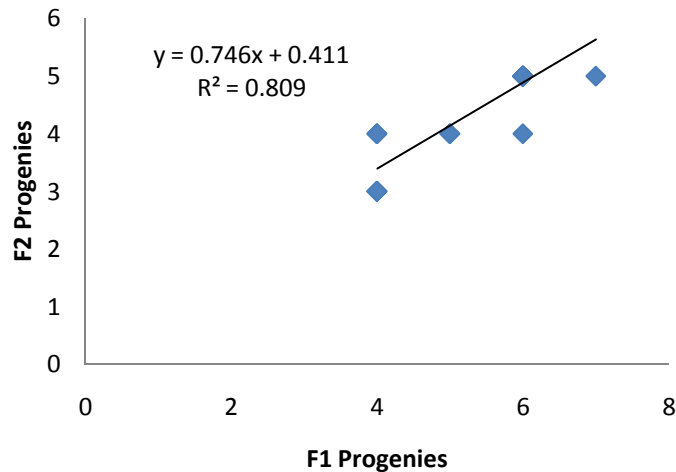
Figure 7.8 **Regression of F2 progenies on F1 parental means for 12 x 138 cross using spikelet fertility**

7.4.6 Correlation between spikelet fertility and other traits

Phenotypic correlations between filled grains and other traits for set A under DS and NDS are shown in Table 7.7. The highest correlation coefficient was observed between leaf area and total number of grains per panicle under NDS condition (r = 0.94) followed by filled grains with leaf area (r=0.5) under NDS conditions. There were highly significant correlations (P=0.001) for filled grains and total grain weight under DS and NDS conditions, leaf area and filled grains under NDS conditions, total number of grains per panicle and tiller number under DS conditions and total number of grains per panicle and tiller number with leaf area. There were significant correlations (P=0.05) between leaf area and plant height as well as number of panicles per plant with plant height. There was significant correlation for number of panicles per plant and plant height under NDS and DS, respectively. All the significant correlations were positive except filled grains with total number of grains per plant under DS.

160

Table 7.7 Correlation coefficients among spikelet fertility and other traits for drought in A populations under drought stress and control

	Spikelet fertility		Total grains		Leaf area		Panicle no		Tiller no	
	Drought Stress	Non Stress	Drought Stress	Non Stress	Drought Stress	Non Stress	Drought Stress	Non Stress	Drought Stress	Non Stress
Total grains	-0.12***	0.28***								
Leaf Area	-0.02	0.50***	0.06	0.95***						
Panicle No	0.02	0.04	0.06	0.05	0.06	0.06				
Tiller no	-0.03	0.03	0.13***	0.04	0.05	0.06	-0.03	0.03		
Plant height	-0.04	-0.00	0.11	-0.00	0.11**	0.02	0.08*	0.12**	0.04	0.00

a* and ** = P < 0.05 and P < 0.01, respectively.

Phenotypic correlations among filled grains and other traits for population B under DS and NDS are presented in Table 7.8. The highest correlation coefficient was observed between leaf area and total number of grains per panicle under NDS condition ($r^2 = 0.90$) followed by filled grains with leaf area ($r^2=0.54$). There were significant correlations (P=0.05) for filled grains and total grain weight, leaf area as well as number of panicles per unit area. Other pairs were total number of grains per panicle and leaf area under NDS conditions and number of panicles per plant with tiller number under DS and NDS conditions. The correlation between plant height with filled grains and total number of grains both under NDS conditions were significant (P=0.05). Other traits that were significantly different were leaf area with plant height, leaf area and panicle number. Although all the pairs that were significant were positive, the majority were weak relationships.

Table 7.8 Correlation coefficients among spikelet fertility and other traits for drought in B populations under drought stress and control

	Spikelet fertility Drought Stress	Spikelet fertility Non Stress	Total grains Drought Stress	Total grains Non Stress	Leaf area Drought Stress	Leaf area Non Stress	Panicle no Drought Stress	Panicle no Non Stress	Tiller no Drought Stress	Tiller no Non Stress
Total grains	-0.20	0.20***								
Leaf Area	-0.05	0.54***	-0.02	0.90***						
Panicle No	0.04	0.13	0.08	0.08	-0.03	0.11**				
Tiller no	0.00	0.05	-0.02	0.07	-0.03	0.07	0.12**	0.37**		
Plant height	-0.07	0.11**	0.07	0.07**	0.11**	0.14**	0.07	0.06	0.03	-0.04

a* and ** = $P<0.05$ and $P<0.01$, respectively.

7.4.5 Heritability estimates for leaf roll under drought stress in rice populations

The narrow sense heritability estimates for leaf-roll scores under drought stress for seven different crosses is presented in Table 7.9. Consistently, there was higher heritability estimates for regression of F1 on mid-parents than the estimates when F2 was regressed on F1 progenies except for the cross between parent 12 and parent 121. Consistent heritability estimates for both methods were r crosses 12 x 129 and 12 x 121. The heritability estimates for regression of F2 on F1 progenies were approximately half of the estimates for regression of F1 on mid-parents for crosses 2 x 147 and 2 x 121.

Table 7.9 Summary of parental, F1 and F2 means and heritability estimates using F1 on mid-parents and F2 on F1 progenies for leaf-roll scores under drought stress

	Parent 1 Var	Parent 1 Mean	Parent 2 Var	Parent 2 Mean	F1 Mean	F2 Mean	F1 on mid-parents h^2	F2 on F1 h^2
1	12	5.9±1.5	121	3.2±1.9	5.4±0.9	4.5±0.8	0.57	0.68
2	12	5.9±1.5	129	4.0±1.4	5.3±1.0	4.7±0.6	0.65	0.65
3	02	4.8±1.3	121	3.2±1.9	4.6±1.2	4.6±0.5	0.86	0.38
4	02	5.9±1.1	129	4.0±1.4	4.1±1.1	4.4±0.5	0.61	0.37
5	72	5.6±1.1	147	3.9±1.5	5.2±1.2	4.6±0.5	0.94	0.47
6	02	4.8±1.3	197	3.6±1.4	5.1±0.8	4.6±0.5	0.66	0.49
7	02	4.8±1.3	147	3.9±1.5	5.2±1.1	4.8±0.5	0.86	0.42

7.4.6 Susceptibility index for grains per plant, leaf area and tiller number

Table 1 shows the total number of grains per plant, leaf area, and indices of susceptibility for 18 rice lines in two sets of F2 populations. The results indicate that population A had higher phenotypic correlation for leaf area and tiller number than population B. The cross IRAT 325 x CK 73 was most susceptible to drought stress when parameters total number of grains and leaf area were assessed in population A. The highest susceptibility to drought stress based on tiller number was observed for the cross NERICA 12 x Bonanca in the population A. Results of susceptibility index for population B showed that the cross CT 16334(2)-CA-2-M x CT 16344-CA-9-M was susceptible to drought stress when the parameter total number of grains was assessed. The cross NERICA 9 x CT 16350- CA-5-M was the most susceptible when leaf area was considered while the cross NERICA 13 x Bonanca was most susceptible when tiller number was assessed.

Table 7.10. The total number of grains, leaf area and tiller number and indices of susceptibility for 18 populations

		Total no. of grains		Leaf area		Tiller number	
Set A[1]		SI[2]	DS[3]	SI	DS	SI	DS
Female	Male						
NERICA 8	Bonanca	0.85	274.6	0.54	15495	1.24	5.5
NERICA 13	Bonanca	0.81	248.2	0.88	19940	1.58	6.2
IRAT 325	Bonanca	1.16	311.6	1.21	24795	1.16	5.3
NERICA 8	WITA 1	1.09	349.3	1.23	28085	0.99	5.3
NERICA 13	WITA 1	1.04	399.6	1.25	29567	0.77	5.1
IRAT 325	WITA 1	0.42	168.6	0.31	10907	0.84	5.4
NERICA 8	CK 73	1.37	304.0	1.17	22423	0.72	4.0
NERICA 13	CK 73	0.98	333.3	1.15	28620	0.80	4.8
IRAT 325	CK 73	1.36	386.3	1.37	28665	1.02	5.1
	Phenotypic correlation	**0.78**		**0.95**		**0.82**	
Set B[4]							
CT 16334(2)-CA-2-M	CT 16346-CA-20-M	0.84	210.8	0.77	14140	0.79	5.3
WAB 365-B-1H1-HB	CT 16346-CA-20-M	0.76	221.4	0.54	12593	0.99	5.0
NERICA 9	CT 16346-CA-20-M	1.02	232.7	0.84	17217	0.58	5.2
CT 16334(2)-CA-2-M	CT 16350- CA-5-M	1.23	336.6	0.98	21973	0.70	5.0
WAB 365-B-1H1-HB	CT 16350- CA-5-M	0.75	215.2	1.18	14787	1.26	5.9
NERICA 9	CT 16350- CA-5-M	1.61	357.2	1.67	27657	1.24	5.7
CT 16334(2)-CA-2-M	CT 16344-CA-9-M	1.25	323.8	1.33	25922	0.83	4.9
WAB 365-B-1H1-HB	CT 16344-CA-9-M	0.72	231.7	0.77	16341	0.97	5.1
NERICA 9	CT 16344-CA-9-M	0.85	241.2	0.99	19482	0.95	4.8
	Phenotypic correlation	**0.93**		**0.84**		**0.65**	

[1]Set A = Parents used were more tolerance to drought stress than parents used in set B.
[2]SI = Susceptibility Index, [3]DS = Response under drought stress condition
[4]Set B = Parents used were less tolerant to drought stress than parents used in Set A.

7.5   DISCUSSIONS

7.5.1   Genetic analysis for spikelet fertility and other agronomic traits

The analysis of F2 crosses revealed the various components of gene action controlling various drought tolerance traits in rice. Both male GCA and female GCA effects were significant for filled grains under both DS and NDS for the A populations and the B populations under DS. Generally, the population B was generated from parents that had

lower filled grains level (detailed in Chapter 5). The finding that the additive effects were more important than non-additive effects for total number of grains filled grains in set A under NDS and B under NDS and DS implies that additive effects control the traits in different populations and the non-additive effects varied with populations under study. This result is contrary to the finding by Mohaptra and Mohanty (1985) that filled grains was predominantly controlled by non-additive gene effects under drought stress. However, in the study by Mohaptra and Mohanty (1985), the populations were generated by crossing *O. sativa* with *O. sativa.* High additive effects were, however, found in rice genotypes when stress imposed was soil salinity instead of drought stress (Akbar et al., 1985). The mechanism of drought tolerance in *O. glaberrima*, a parent of the interspecific rice used in the current study was reported to be different from that of *O. sativa* (Fujii et al., 2005). This could explain the apparent differences in findings of the current study when compared with that of Mohaptra and Mohanty (1985). Based on the current study, breeding methods that involve selection in the early generations are recommended. The methods include single seed decent, pedigree selection and modified bulk methods. Studies using more populations generated from *O. sativa* and interspecific rice could confirm our finding that the importance of SCA varies with population under study.

Findings of this study that non-additive effects for total number of grains per panicle was important in both set A and set B under NDS conditions, implies that breeding methods that involve late selection could improve drought tolerance under NDS using number of grains per panicle trait. The use of yield components including grains per panicle has been demonstrated to be effective in improving yield under drought stress by selecting under NDS conditions (Atlin, 2003). The differences between the responses under DS and NDS conditions for total number of grains per panicle could be due to fewer loci within the set B that could segregate for the trait than in A. Set B comprised of lines with more susceptibility to drought stress than those in set A.

Additive effects for number of grains per panicle were important in all the population in set A and set B under DS and NDS. This implies that breeding methods that involve selection in the early generations especially, single seed decent, pedigree selection and modified bulk methods could improve drought tolerance through selection of number of grains per panicle. In another study involving *O. sativa* parents that included susceptible, moderately susceptible, moderately resistant and resistant lines, number of grains per panicle was

reported to be controlled by additive effects under NDS conditions (Ahmad et al., 1985). Genes with additive effects were predominant in the inheritance of number of grains per panicle (Mohapatra and Mohanty, 1985). Both additive and non-additive effects were nearly equal in populations in set A under NDS. These set of populations could be used to improve drought stress using methods that involve selection in the early and late generations of the populations. These methods include modified bulk methods and repeated crossing at the segregation stage. Similarly, additive and non-additive gene effects were significant for number of spikelets per panicle under both normal and saline conditions, and repeated crossing has successfully been used to improve salinity tolerance (Narayanan and Sree Rangasamy, 1990).

Results of this study that non-additive effects for leaf area were more important than additive effects in both set A and set B under DS conditions, implied that breeding methods that involve late selection could improve drought tolerance under both conditions. In addition, the findings that additive effects were more important than non-additive effects for the populations in sets A and B under NDS implied that selection methods that involve early selection could be employed under NDS. In the populations in sets A and B, interspecific rice genotypes generated from *O. glaberrima* crosses were the majority of the parents (Table 7.1). *O. glaberrima* is known to have high vegetative growth as a drought stress adaptation mechanisms (Jones et al., 1997c; Fujii et al., 2005). It is likely that these traits were transmitted to the populations under study and it is expressed more under DS than under NDS conditions.

Results of this study that additive and non-additive effects for plant height were nearly equal with contribution for total GCA varying between 45% and 55% for both set A and set B under DS and NDS conditions, implied that that breeding methods that involve both early and late selection could be employed in the improvement of drought tolerance using this trait. Modified bulk method of selection method could be appropriate. In another study involving *O. sativa* parents that included susceptible, moderately susceptible, moderately resistant and resistant lines, plant height was controlled by additive effects under NDS conditions (Ahmad et al., 1985). In the current study, both additive and non-additive effects were important when the B generations were tested under DS and NDS conditions. Drought traits were controlled quantitatively.

The current study found that additive effects were the more important in the transmission of drought tolerance using tiller number as evidenced by the lack of significance for male x female interaction effects for tiller number. This finding is contrary to the work reported by other scientists that non-additive effects were more important under drought stress conditions (Sarker et al., 2002; Efisue et al., 2009). In another study, however, expression of tiller number, under both NDS and DS situations was found to involve non-allelic gene interaction (Narayanan and Sree Rangasamy, 1990).

Overall, in situations where non-additive effects are more important, selection should be delayed until later generations. In these type of populations, repeated crossing in the segregating generations may be useful to pool all the desirable genes in one genotypes according to Manonmani and Fazlullah Kahn (2003). The modified bulk method is another useful method of improvement. However, when additive affects are more important, then a modified pedigree method currently employed in Africa Rice Centre, that involves bulking before evaluation is appropriate. However, when both additive and non-additive effects are important, two options can be taken depending on the objective of the breeding and the relative importance of the additive or non-additive effects. In case the objective is to develop hybrid rice, as it is planned in Uganda, then pure line selection should be employed. In this approach, additive effects will be extracted because rice is autogamous (Chandraratna, 1964). In a situation where both additive and non-additive gene action are to be exploited, a modified bulk breeding method would hasten the rate of genetic improvement. Similar exploitation of both additive and non-additive gene action has been conducted in the improvement of cold tolerance (Acharya, 1987) and sodicity tolerance in rice (Geetha et al., 2006).

7.5.2   Combining abilities for filled grains under drought stress

Generally, there was no clear distinction in combining ability between *O. sativa* and interspecific rice lines under non-drought stress conditions but the interspecific lines were better combiners under drought stress conditions. Among the *O. sativa* line, IRAT 325 was a good general combiners while CT 16350-CA-5-M and WAB 450-B-136-HB (NERICA 9) were good combiners under non-drought stress conditions. In the drought stress condition, however, CK 73 an *O. sativa* genotype was the best combiner for improved filled grains. Other parents with lower levels of significance were CT 16344-CA-9-M, WAB 450-B-136-HB (NERICA 9) and CT 16346-CA-20-M.

Specific combining ability analysis revealed that crosses WITA 1 x CT 16350- CA-5-M, Bonanca x CT 16346-CA-20-M, CK 73 x CT 16344-CA-9-M were best under NDS condition. The cross CK 73 x CT 16344-CA-9-M had both parents good combiners indicating additive x additive type of gene action. It is expected that these crosses could provide transgressive segregants that could be selected using pedigree methods (Chakraborty et al., 1994). The others crosses had mixed combiners, therefore additive and non-additive gene action could be the major contributors. In such crosses bulk breeding methods could exploit both gene actions.

7.5.3 Relative importance of maternal effects for spikelet fertility

Information on male and female effects is scanty for most agronomic traits especially under drought stress. The higher GCA effects for females than that of males under NDS conditions, in the A populations for filled grains but not in the DS conditions suggested that maternal effects may have influenced the trait expression under NDS. In the B population, the GCA effects for females were higher than the effects for males under DS conditions. Similarly, findings of this study that there were higher GCA effects for females than for males in the B populations for total number of grains per panicle under both DS and NDS indicated that maternal influences were probably important. The finding that GCA effects for females were nearly double that of males under DS and NDS for plant height in the B populations suggested that maternal effect probably influenced the expression of the trait. Observation that GCA effects for females was higher than the males under DS showed that maternal effect probably influenced tiller number. Similarly, the results that GCA effects for females were higher than those for males under NDS for tiller number in the case of B population implying that maternal effects influenced this trait.

According to Kang (1994) the magnitude of the ratio of sum of squares of female to male sets can guide selection of crosses that can be used in reciprocal crosses to confirm the presence of maternal effects. In a situation where the number of males and females are balanced, Kearsey and Pooni (1996), found that the ratio of female to male mean squares estimated the role of maternal effects. As a rule of thumb a ratio of female mean square of 3:1 implies that maternal effects are significant (Kang, 1994). In our estimates, ratios of 2.8 and 5.2 for filled grains number under NDS and DS, respectively was found in B populations and were considered to be controlled by maternal effects. This is probably the

first report of maternal effects for filled grains. However, maternal influences can only be confirmed with analysis of reciprocal crosses. A number of reproductive linked traits such as seed dormancy are influenced by maternal effects (Gu et al., 2003). In rice maternal effects have been detected in milled rice recovery and brown rice recovery according to Shi and Zhu (1998), brown rice width (Zhang et al., 2006), amylose content (Gravois and McNew, 1993) and seed shape (Qi et al., 1983). In the case of traits that are influenced mainly by maternal effects, the F2 populations are expected not to have high segregation (Shi and Shu, 1998). Stress is known to influence filled grains. For instance, Ratho and Pradhan (1992), found that cold temperatures induced genetic male sterility that translated into differential levels of filled grains. Such information in the case of drought is not known.

7.5.4 Generation means for filled grains under drought stress

There were significant differences among generations for filled grains indicating the presence of sufficient genetic variability. Variability for various traits of rice has been reported (Singh and Mackill, 1990; Garrity and OʻToole, 1994; Fukai and Cooper, 1995; Lafitte et al., 2003). The scaling test showed that additive genetic effects but not dominance and epistatic genetic effects were important in the inheritance of filled grains. Fitting means of filled grains on the additive model showed that additive effects accounted for 77% of the genetic variation. In addition, the finding that dominance level was 0 in the F1 population showed that there were no dominance effects.

The generation means analysis confirmed that additive effects were significant in the transmission of filled grains in the populations generated. This study had no inconsistencies in detecting that additive effects were the most important genetic factor in the population under study. In addition, results that narrow sense heritability was high indicated that a high proportion of genetic components of variance can be fixed in segregating generations. Since the selection was conducted under drought stress, it is appropriate that selection for improved drought stress is conducted as early as F2 in the study location. According to Lafitte (2003), it is appropriate that selection for improved drought stress is conducted using heritability estimates for target traits. There is limited information on the inheritance of filled grains trait under drought stress. However, various reports indicated that additive effects were the main components that controlled the transmission of this trait under high temperature (Sun et al., 1989; Ali et al., 1995). A single gene pair was estimated to control

filled grains under drought stress. A single gene was found to be responsible for the transmission of filled grains under high temperatures (Sun et al., 1989; Ali et al., 1995).

7.5.5   Correlation between spikelet fertility and other traits

The inter-character associations among the six characters in the A and B populations under DS and NDS suggest that these responses were controlled differently except leaf area and total number of grains under NDS and to a moderate extent filled grains and leaf area under NDS. They had high moderate to high positive correlations. This implied that improvement of the drought tolerance by improving filled grains could as well improve total number of grains per panicle as well as leaf area of the flag leaf. Similarly, spikelet sterility was found to be strongly correlated with total number of grains under heat stress in another study (Heuer and Meizan, 2003). In addition, the correlations between filled grains and leaf area suggested that leaf area, which is easy to score, could be a moderate predictor for filled grains under NDS. These findings indicate that it should be possible to combine leaf area of the flag leaf and total number of grains in the breeding for high filled grains by judicious planning of NDS conditions.

7.5.6   Heritability estimates for leaf roll under drought stress

The narrow sense heritability estimates were generally high with estimates for regression of F2 on F1 progenies approximately half of the estimates for regression of F1 on mid-parents. The high narrow sense heritability point to the importance of additive gene action in the genotypes studied. Knowledge about narrow sense heritability is important in determining selection efficiency in breeding. According to Gravois and McNew (1993) when additive effects are high in a self-pollinated crop like rice, then it is appropriate to select at various levels of inbreeding, because the affects are transmissible from one generation to another. In this population pedigree, mass selection or single descent seed method of breeding may be adopted for the exploitation of additive genes in the early generations. This finding is consistent with work by Efisue et al. (2009) who detected high and significant heritability estimates of leaf scores in inter-specific rice populations under drought stress. Sirualt (2007) also found that additive effects were the most important components in the control of drought stress in wheat. The putative trait, leaf rolling, as reported by Lafitte et al. (2003) could be useful in breeding for improved drought tolerance. This is important especially in the new generation of the interspecific lines that are reported to have higher leaf roll responses as a mechanism to avert drought (Fujii et al., 2005).

7.5.7 Susceptibility index for grains per plant, leaf area and tiller number

The results that population A had higher phenotypic correlation for leaf area and tiller number than population B implies that there is a high chance of indentifying lines that are tolerant to drought stress when population B is used. Zhu et al., (2005) similarly identified rice lines that were tolerant to heat stress. Results that the cross IRAT 325 x CK 73 was most susceptible to drought stress when parameters total number of grains and leaf area were assessed in population A implies that the cross progenies may not provide lines with tolerance to drought stress. Similarly, NERICA 12 x Bonanca in the population A and CT 16334(2)-CA-2-M x CT 16344-CA-9-M for set B should not be advanced for selection. Based on results of the phenotypic correlations and susceptibility indices, genotypes that could be used for making crosses are WAB 365-B-1H1-HB x CT 16344-CA-9-M and IRAT 325 x WITA 1.

7.6   CONCLUSION

This study provided information on the gene action for drought tolerance at reproductive and vegetative stages of crosses between interspecific and O. sativa genotypes. Evidence of additive, non-additive, additive x additive, and dominance effects were tested for drought stress at reproductive stage, while heritability was estimated for drought tolerance at vegetative stage.

Additive effects were the most important components that controlled filled grains in most of the populations. Breeding methods that involve selection in the early generation could be helpful in improving rice for filled grains. These methods include pedigree breeding, pure line selection, mass selection, single seed decent and progeny selection. In a few crosses however, non-additive effects controlled proportion of filled grains. Methods that involve a delay in selection of genotypes would be appropriate for improving filled grains in these populations. Modified bulk methods of selection is proposed. Test for magnitude of the gene action for filled grains using additive-dominance model confirmed that additive gene effects were the most important and additive x additive as well as additive x dominance effects were not important. Genotypes O. sativa, namely WITA 1, IRAT 325, CT 16350-CA-5-M and WAB 450-B-136-HB (NERICA 9) were good combiners under non-drought stress condition for filled grains. In the drought stress condition, however, CK 73 an O. sativa was

the best combiner for improved filled grains. Specific combining ability analysis revealed that crosses WITA 2 x CT 16350- CA-5-M, Bonanca x CT 16346-CA-20-M, WITA 2 and CT 16344-CA-9-M were best under NDS condition. Further analysis of susceptibility indices indicated that crosses WAB 365-B-1H1-HB x CT 16344-CA-9-M and IRAT 325 x WITA 1 had populations with the highest tolerance to drought.

There was weak correlation between filled grains and other agronomic traits implying that the traits could not be effectively used in selection for improved filled grains under drought and non-drought stress conditions. This study also found high heritability for leaf roll under drought stress conditions populations implying that additive effects were important. Pedigree selection methods, single seed descent mass selection and progeny selection is proposed for its improvement. Overall, there was evidence that a few major gene and several minor genes controlled filled grains in rice. Breeding methods that encourage early selection should be employed. Methods that include pedigree selection, purelines selection, single seed descent should be employed in the breeding for optimum grain shattering ability.

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CHAPTER EIGHT

Inheritance of Grain Shattering Trait in Rice Generated from Interspecific and Intraspecific Crosses

Abstract

Rice genotypes generated from wide crosses can provide important traits for stress tolerance. However, these genotypes frequently shatter and information on the genetic effects that condition grain shattering in rice is limited. This study was conducted to: (a) investigate the mode of gene action for the shattering trait; (b) determine the segregation pattern and allelic relationships for the grain shattering trait, and (c) test whether maternal effects play a role in determining grain shattering. Twenty-six lines were mated according to a North Carolina Design II scheme in sets. The F1 progenies were evaluated under a rain out shelter, while the F2 generations were evaluated in the field. The 58 populations and 11 parental inbred lines were evaluated in a 3 x 23 alpha lattice design with two replicates, at Namulonge. Both   GCA effects and SCA effects within sets were significant indicating that both additive and non-additive effects were important. The relative contribution of GCA and SCA effects were female GCA 28%, male GCA 30% and SCA 42% further conforming that breeding that targets additive effects or non-additive effects or both could be possible. Segregation pattern of 50 crosses were tested for allelism. Results showed that the shattering trait was controlled by the same genes in 23 crosses; two genes in 23 crosses with duplicate dominant effects as well as complimentary dominant/recessive effects; and three genes in one of the crosses. In one cross involving shattering parents a 3:1 ratio of nonshattering : shattering was observed. This was unexpected as the lines were supposed to be fixed. Probably there were still some unfixed loci for shattering in the parents involved and the classification used in this study was arbitrary such that the parents used could have been carrying different alleles for shattering. In crosses involving shattering and nonshattering parents a ratio of 3:1 and the reverse ratio of 1:3 were detected implying reversal of gene effects in different genetic backgrounds. Maternal effects were detected in seven out of 10 crosses tested. The significance of additive and non-additive effects implies that breeding methods that involve selection in the early generation like pedigree selection and seed descent methods or modified bulk methods, respectively could be used to improve on this trait. This study also demonstrated that two genes were generally responsible for transmission of this trait. In conclusion, a few major genes and some minor genes are responsible for the control of grain shattering in the rice populations under study.

8.1 INTRODUCTION

Grain shattering is the tendency of grains to fall off easily when they become mature. Loose attachment of grains to the pedicel, also called high shattering ability (HSA), is a major constraint to rice production. Yield losses of up to 100% occur when susceptible varieties are grown and harvesting is delayed which is a common practice under small scale farming. However, losses in the range of 0% to 38% have been reported in experimental trials (Jacobi et al., 1974; Sahu and Sahu, 1981; Agarwal et al., 1989). A combination of factors, which include storms and cool periods at maturity, aggravates this problem (Vennum, 1988; Porter et al., 1994). Grain shattering is a widespread problem in most rice growing areas, especially the USA, where wild rice is a delicacy, West Africa where a high shattering rice, African rice (*Oryza glaberrima*), is cultivated and all parts of the world where *Oryza sativa* varieties are grown. The importance of grain shattering is increasing due to two factors. First, frequent genetic degeneration of cultivated rice varieties by way of out-crossing with wild relatives of the crop increases the problem of grain shattering. Second, wide crossing as a method to improve rice for various constraints is becoming a major breeding method. However, the grain shattering trait frequently found in wild rice is also dragged into the new materials generated. Currently, any new generation of rice with high shattering ability is simply discarded during early generations of the populations. This practice may undermine the current quest by breeders to broaden the genetic base of cultivated rice. In Uganda, GS is an important yield limiting factor among other constraints of rice according to recent surveys (Odogoola, 2006). Rice farmers prefer varieties with optimum grain shattering ability in order to maximize grain recovery.

Mohanty and Sahu (1968) considered grain shattering to be a problem that occurred whenever *O. sativa* japonica was crossed with *O. sativa* indica because *O. sativa* indica is a less shattering variety than *O. sativa* japonica. In the last two decades, however, few studies aimed at understanding the genetic nature of grain shattering showed that it is linked to several traits and constraints. For instance, Sahu and Sahu (1981) found that shattering blocked accumulation of starch prematurely and led to poor grain quality and quantity. Other studies found that GS was linked to several important negative traits including, blast resistance (Li et al., 2006a), seed dormancy (Cai and Morishima, 2000b), panicle spreading (Eiguchi and Sano, 1990), phenol staining (Sobrizal et al., 1999) and red pericarp (Oba et al., 1990). It is also associated with several important quantitative traits

namely panicle length, tiller numbers, panicle weight and plant height, pericarp color (Oba et al., 1990) as well as dwarfism (Oba et al., 1990) and ligule length (Sobrizal et al., 1999). Therefore, grain shattering is a trait that deserves more consideration to understand than it was earlier viewed.

Understanding the inheritance and expression of grain shattering trait would guide breeding for acceptable shattering ability. To date conventional genetic analysis conducted on GS found that a few genes controlled GS habit (Eiguchi and Sano, 1990; Oba et al., 1990; Fukuta and Yagi, 1998). Hu et al. (1964) used *O. sativa* indica and *O. sativa* indica cross generations to study the segregation pattern of GS and concluded that the trait was inherited as a monogenic recessive trait. In studying the inheritance of GS Tang and Morishima (1997) found various segregation patterns ranging from monogenic to continuous variation, depending on the crosses. In another study on gene function, gene *sh4* was identified as a recessive locus in several wild rices, namely *O. rufipogon*, *O. glumaepetula*, *O. nivara*, *O. meridionalis* but showed varying degrees of dominance in crosses with cultivated rice. On the contrary, gene *sh4* along with *sh1* and *sh2* were found to be recessive while the other two *sh3* and *sh5* were dominant in other populations (Fukuta and Yagi, 1998). When *O. nivara* was crossed with *O. sativa*, however, the modes of gene action of the alleles were both dominant and additive (Burke and Arnold, 2001). In studies involving *O. sativa* and *O. glaberrima* both recessivity and dominance were responsible for transmission of the trait (Li et al., 2006a). It is important to note that the mode of gene action and transmission of the traits vary with populations under study.

Relatives of cultivated rice serve as reservoirs of the grain shattering gene. In populations generated through crossing relatives of rice with *O. sativa*, the shattering gene was identified in 11 out of the 12 chromosomes (Xiong et al., 1999; Cai and Morishima, 2000a; Bres-Patry et al., 2001; Thomson et al., 200s3; Lee et al., 2007; Onishi et al., 2007b). Of these genes, five have been mapped in the rice genome namely *sh1* on chromosome 11 (Nagao and Takahashi, 1963), *sh2* on chromosome 1 (Oba et al., 1990), and *Sh3* on chromosome 4 (Eiguchi and Sano, 1990; Fukuta and Yagi, 1998; Nagai et al., 2002). Other genes already mapped are *Sh4* on chromosome 3 (Fukuta and Yagi, 1998) and *sh-h* on chromosome 7 (Ji et al., 2006). Relatives of cultivated rice are therefore attractive germplasm to use for better understanding of the genetics of grain shattering in rice.

Some of the mapped genes responsible for grain shattering have been cloned based on their importance as factors for transmission of this trait. For instance, the loci *sh4* contributes the highest phenotypic variation, more than 65%, for grain shattering in *O. sativa* and other relatives (Xiong et al., 1999; Cai and Morishima, 2000b; Li et al., 2006a). A second grain shattering QTL (*qSH1*) contributed 68% of total phenotypic variation in *indica* and *japonica* rice crosses (Konishi et al., 2006). This information implies that a small number of genes are probably responsible for large variation in the grain shattering trait. Populations having these genes could be useful for conventional breeding once identified because they are likely to be transmitted easily to new generations.

Successful development of new populations of rice through crossing *O. sativa* with *O. glaberrima* has been conducted. They are likely to provide more insight into the nature of transmission of GS trait because they have *O. glaberrima* parents that are a more recent domestication population than *O. sativa* and have more conserved shattering traits (Vaughan et al., 2004; Li et al., 2006a). Besides testing segregation pattern of grain shattering, it is important that any allelic relationships that may exist with the rice germplasm is determined. There is no published report about the allelic relationship between grain shattering genes in rice in general. The current development of the new generation of rice developed through wide crossing involving *O. glaberrima* has made it necessary to investigate the allelic relationships of these indica varieties with interspecific fixed lines developed through crossing *O. sativa* japonica with *O. sativa*.

In Uganda, it is almost a decade since new rice types developed from crossing *O. sativa* and *O. glaberrima* were introduced, and during this period several lines tested using Participatory Variety Selection were retained for production and by 2009 three varieties were released. However, a major reason for rejection of otherwise promising lines was loose grain attachment to the pedicel and in some cases too tight grain attachment. It is very appealing to integrate these new rice lines into landraces in the country. It is for this reason that this study was initiated with the aim of determining the inheritance and expression of grain shattering in landraces in the country and all *O. sativa* x *O. glaberrima* derived introductions. This study also would allow for a better understanding of genetic control of shattering for use in rice breeding elsewhere in the world.

8.2   Objectives of the study

The specific objectives of the study were to:

i.   investigate the mode of gene action for grain shattering;

ii.   determine the segregation pattern and allelic relationships for grain shattering in F2 populations; and

iii.   test whether maternal effects play a role in determining grain shattering.

8.3   MATERIALS AND METHODS

8.3.1 Study location

Progenies were evaluated in the first crop growing season of the year 2009 at the National Crops Resources Research Institute (NaCRRI), Namulonge Uganda for their grain shattering ability. NaCRRI is located 27 kilometers north of Kampala (0^0 31qN, 32^0 35qE), with a mean altitude of 1,150m above sea level. The long term annual rainfall is 1,270mm and the distribution is bimodal. Relative humidity varies between 76% and 88%. The study was conducted during January to May period of 2009.

8.3.2 Germplasm used in the study

Twenty six lines obtained from International Rice Research Institute (IRRI), Africa Rice Centre-West African Rice Development Association, (ARC-WARDA), International Center for Tropical Agriculture (CIAT), Mali, and Ugandan landraces were used in the study (Table 8.1). They were previously screened and grouped based on shattering level in Uganda (Lamo et al., 2007). The 26 materials comprised of 17 interspecific derived lines and 8 *O. sativa*. These materials were used to develop segregating populations for the shattering trait. Crosses were made in sets of three parents, where three intraspecific lines in one set were used as females and crossed with three interspecific derived lines from another set used as males, according to a North Carolina Design II Mating Scheme (Hallauer and Miranda, 1988). The main sets were the intraspecific and interspecific derived lines. Five sub-sets comprising Tight shattering x Tight shattering, Loose x Loose, Normal x Normal Tight x Loose and Loose x Tight were realized. Reciprocal crosses were made for each crossing set. Overall, 90 crosses were expected but only 62 were advanced to F2 due to low seed set in some crosses (see Chapter 6). The F1 seed from the 62 crosses was planted under a rain-out shelter and selfed by bagging at anthesis to generate F2 seed. Of

the 62 F1 crosses only 58 F2 new generations were realized. A summary of the parental lines of the crosses is presented in Table 8.1.

8.3.3   Experimental design

All 58 F2 progenies along with 11 parents were planted under irrigated conditions in the field in an alpha lattice design (23 x 3-arrangement) in two replicates. Each treatment was established in 15-row plots each measuring 5m x 6m at 20cm x 20cm plant spacing. The plants were thinned to one plant per hill. Irrigation water was applied at 20mm per week. Standard cultural practices including hand planting and hand weeding were followed. The crops were fertilized with 25 kgN ha^{-1} at 20-25 days after transplanting (DAT) and the same rate at 40-45 DAT to enhance plant vigour.

Table 8.1 Origin, pedigree and parental lines of different parental test lines

Pedigree	Source	Pedigree	Cross type	Shattering ability group
CT 16346-CA-20-M	CIAT	-	Interspecific	Loose
CT 16350- CA-5-M	CIAT	-	Interspecific	Loose
CT 16344-CA-9-M	CIAT	-	Interspecific	Loose
CT 16334(2)-CA-2-M	CIAT	-	Interspecific	Tight
CT 16334(2)-CA-11-M	CIAT	-	Interspecific	Normal
CT 16324-CA-9-M	CIAT	-	Interspecific	Normal
CT 16342-CA-25-M	CIAT	-	Interspecific	Loose
CT 16313-CA-4-M	CIAT	-	Interspecific	Normal
CT 16317-CA-4-M	CIAT	-	Interspecific	Tight
CT 16355-CA-15-M	CIAT	-	Interspecific	Tight
Caiapo	CIAT	-	Interspecific	Normal
Bonanca	IRRI	-	Interspecific	Loose
WAB 365-B-1H1-HB	WARDA	-	Interspecific	Tight
WAB 56-104	WARDA	-	Intraspecific	Normal
WAB 450-I-B-38-HB	WARDA	NERICA 1	Interspecific	Loose
-	WARDA	WITA 2	Intraspecific	Loose
-	WARDA	CK 73	Intraspecific	Normal
WAB 450-B-136-HB	WARDA	NERICA 9	Interspecific	Tight
WAB 450-1-BL1-136-HB	WARDA	NERICA 8	Interspecific	Tight
-	IITA/WARDA	IRAT 13	Intraspecific	Tight
-	IITA/WARDA	IRAT 104	Intraspecific	Loose
WAB 881-10-37-18-3-P1-HB	WARDA	NERICA 15	Interspecific	Loose
-	WARDA	NERICA 13	Interspecific	Tight
		Golden 1	Intraspecific	Tight
-	IITA/WARDA	IRAT 325	Intraspecific	Tight
-	IITA/WARDA	IRAT 257	Intraspecific	Loose

Key: Loose ≤70; Normal 70-100gf; Tight ⁻ 100gf

8.3.4 Data collection

The grain shattering trait was tested taking the following procedures. According to the method, flower development was monitored daily by inspecting each plot during the reproductive stage to select and group plants according to date of flower exertion. This was done in order to synchronize date of recording grain shattering to be 30 days after anthesis. Since the process of anthesis in rice progresses from the top of the inflorescence, only the middle portion of the panicle was assessed for uniformity. On the day when 50-60% of inflorescences of the main primary panicle had been exerted from the sheath, all the florets that flowered earlier along with those that had not reached anthesis were carefully excised off the peduncle, and the panicle immediately tagged. Records on shattering ability were taken by individually pulling off the grains and recording the minimum force it took to dislodge them. The gauge and the clip were held parallel to the longest axis of the seed, which was kept at its natural position in relation to the axis of the panicle when pulling the individual grains. The equipment was designed at the University of Kwa-Zulu Natal as described in chapter three of this thesis. A minimum of 40 plants were sampled. Similar studies by Ali et al. (2003) and Govinda and Virmani, (1988) in rice evaluated a minimum of 30 plants at F2 generation and accurate information was generated.

8.3.5 Statistical analysis

The data for grain shattering ability of the F2 populations was analyzed. Overall data for sets were subjected to Generalized Linear Model with female and males nested in sets. In the next analysis, each set was analyzed separately. In a second set of analysis, the observed distribution of shattering of the individual plants in each cross was tested for goodness of fit for different segregation patterns. Observed and expected phenotypic segregation ratios of F2 generations were tested by using chi-square (X^2) for goodness of fit, assuming one, two or three gene models for grain shattering and non-grain shattering generations. In the third set of analysis, 10 crosses were tested in pairs   for difference between the means using t-test. Each cross and its reciprocal was compared. All the analyses were conducted using Genstat version 12 (Payne et al., 2007). The fixed model for the NCD II design in according to Sharma (1995) is presented.

Where   1, 2, 3;   1, 2, 3;   1, 2;   1, 2, 3, 4, 5, 6, 7, 8;

183

denotes the mean force required to dislodge the line of a cross of the th female line, the th male line in the kth block, withinset p and in the qth location

The terms of the model were de ined as follows:

    Grand mean;

    the average effect of the th set

    GCA effect the th female line nested within th set

    GCA effect the th male line nested within th set

    SCA effect speci ic to crossof the th female and th male nested within th set;

    effect of the th replication nested within the th set

    pooled error or random experimental error

The combining ability estimates were calculated based on the methods described by Singh and Chaudhary (1985), and Huff and Wu (1992) as follows:

Independent GCA effects were calculated for male and female parents using the same formula.

GCA was regarded as significantly different from zero using a t-test, where

    0/   at degree of freedom of error.

SCA was calculated using the formula

8.4   RESULTS

8.4.1 Genetic analysis

Results of analysis for grain shattering pooled across sets in Table 8.3. Both the GCA and SCA effects within sets for grain shattering were significant (P = 0.001). In addition, the relatives contribution of GCA and SCA effects to the cross sum of squares varied between 28% and 42%. The total GCA effects for males and females were higher than the SCA effects.

Table 8.3 Mean square value for spikelet fertility

Source	d.f.	Mean square value
Set	3	28.55***
Set / Entry	27	31.57***
Set / Female	8	39.12***
Set / Male	7	32.36***
Set x Female x Male	12	28.55***
Relative (%) contribution of sum of squares to GCA and SCA		
GCA female		28
GCA male		30
SCA		42

Three different sets were analyzed separately and the results are shown in Table 8.4. A detailed parental list is in Appendix 8.1. The male, female and male x female mean squares were all highly significant for the grain shattering trait in the Tight x Tight set of crosses. The GCA effects for the females were larger than those of males in this set of crosses. In the case of Loose x Loose set, female and male GCA effects were highly significant but not the male x female interaction effects. The GCA effects for males were higher than the effects for females in this case. In the Tight x Loose crosses, the GCA effects due to females and males were highly significant whilst the SCA effects were non-significant.

Table 8.4 Mean square for shattering for different sets

Fixed term	Tight x Tight		Loose x Loose		Tight x Loose	
	d.f.	Mean Square Value	d.f.	Mean square Value	d.f.	Mean Square Value
Female	2	86.69***	2	36.91***	2	29.83***
Male	2	12.22***	2	69.91***	1	34.63***
Female x Male	4	21.49***	4	2.39	1	1.22
Relative (%) contribution of sum of squares to GCA and SCA						
GCA female	61		GCA female	33	GCA female	62
GCA male	8		GCA male	63	GCA male	36
SCA	30		SCA	3	SCA	1

8.4.2 Test for allelism

The distribution of the grain shattering score of 23 (46%) F_2 progenies out of 50 were generally unimodal and the skewness and kurtosis of the distributions are in Table 8.5. The skewness varied between 0.02 and 0.10 while the kurtosis varied from 2.5 to 2.9. The skewness indicated the normality of the distribution while kurtosis indicated the peak of the distribution. The number of observed plants in each peak for the 27 remaining crosses are

summarized in Table 8.6. All the 27 crosses segregated in a bimodal pattern and the number of individual plants that were in each peak is in Table 8.5. These crosses were tested for goodness of fit to different segregation ratios for resistance versus susceptibility to grain shattering. A total of 12 crosses (24%) did not differ significantly from 15:1 ratio and (19%) 9:7, (4%) 7:9, (2%) 63:1, (4%) 3:1 and (2%) 1:3 were observed. The ratios show that a few genes, one to three, confer resistance to grain shattering.

Table 8.5 Skewness and kurtosis of F2 populations in crosses with unimodal distribution

Cross combinations	Group	No of plants	Skewness	Kurtosis
WAB 365-B-1H1-HB x IRAT 325	Tight x Tight	189	0.03	2.61
IRAT 104 x CT 16355-CA-15-M	Loose x Loose	69	0.10	2.50
NERICA 13 x WAB 365-B-1H1-HB	Tight x Tight	94	0.05	2.68
NERICA 13 x NERICA 9	Tight x Tight	80	0.08	2.50
NERICA 13 x CT 16334(2)-CA-2-M	Tight x Tight	71	0.06	2.58
Golden 1 x CT 16334(2)-CA-2-M	Tight x Tight	75	0.05	2.68
Golden 1 x CT 16313-CA-4-M	Loose x Loose	60	0.04	2.50
IRAT 325 x WAB 365-B-1H1-HB	Tight x Tight	67	0.04	2.68
IRAT 325 x NERICA 9	Tight x Tight	158	0.08	2.70
IRAT 257 x CT 16355-CA-15-M	Loose x Loose	80	0.03	2.58
CT 16324-CA-9-M x WAB 56-104	Normal x Normal	79	0.09	2.50
CT 16346-CA-20-M x Bonanco	Loose x Loose	80	0.03	2.58
CT 16355-CA-15-M x NERICA 13	Loose x Loose	60	0.05	2.90
Bonanca x WAB 450-I-B-38-HB	Loose x Loose	102	0.05	2.68
Bonanca x CT 16344-CA-9-M	Loose x Loose	79	0.07	2.70
Bonanca x CT 16342-CA-25-M	Loose x Loose	78	0.10	2.58
CT 16350- CA-5-M x CK 73	Loose x Loose	80	0.10	2.58
CT 16334(2)-CA-2-M x NERICA 8	Tight x Tight	40	0.08	2.68
CT 16346-CA-20-M x WITA 2	Loose x Loose	40	0.08	2.50
CT 16313-CA-4-M x WAB 56-104	Normal x Normal	40	0.03	2.68
Caiapo x CT 16324-CA-9-M	Normal x Normal	40	0.02	2.58
CT 16342-CA-25-M x CT 16342-CA-25-M	Loose x Loose	55	0.06	2.60
NERICA 9 x IRAT 325	Tight x Tight	78	0.06	2.90

8.4.4 Maternal effect

Means square value, LSD, and mean shattering score normal and reciprocals of 10 crosses is presented (Table 8.7). Of the 10 crosses, three had no significant difference between the crosses. These were crosses number 1, 2 and 3. The crosses that did not show maternal effect comprised five *Oryza sativa* and one interspecific line. All the crosses that showed maternal effects were those involving interspecific lines. It is clear from the results that maternal effect for grain shattering was more common in crosses involving *O. sativa* and

interspecific lines. Of the crosses that demonstrated no significant effect for grain shattering one each was of Tight grain shattering x Tight grain shattering, Loose x Loose and Normal x Normal.

Table 8.6 Phenotypic segregation ratios for resistant to shattering (Non Shatter:Shatter) in F2 populations that fitted on 15:1, 9:7, 7:9, 63:1, 3:1 and 1:3 genetic model

Cross	Phenotype level[1]	No of plants	Observed		Expected		X^2
			NS[2]	S[3]	NS	S	
Ratio 15:1							
WAB 365-B-1H1-HB x NERICA 8	m100	90	78	3	84.4	5.6	1.71
NERICA 9 x WAB 365-B-1H1-HB	m160	80	78	2	75	5	1.92
NERICA 9 x NERICA 13	m150	48	47	1	45	3	1.42
NERICA 8 x NERICA 9	m125	40	38	2	37.5	2.5	0.11
NERICA 8 x CT 16334(2)-CA-2-M	m125	80	78	2	75	5	1.92
CK 73 x CT 16346-CA-20-M	m140	40	39	1	37.5	2.5	0.96
CT 16344-CA-9-M x CK 73	m150	79	78	1	74.1	4.9	3.35
Bonanco x CT 16346-CA-20-M	m110	80	78	2	75	5.0	1.92
CT 16350- CA-5-M x CK 73	m160	40	38	2	37.5	2.5	0.11
WAB 56-104 x CT 16324-CA-9-M	m130	80	78	2	75	5	1.92
CT 16313-CA-4-M x Caiapo	m100	76	72	4	71.3	4.8	0.13
CT 16342-CA-25-M x IRAT 13	m110	28	26	2	26.3	1.8	0.04
Ratio 9:7							
WAB 56-104 x CT 16313-CA-4-M	m150	80	45	35	43.3	33.7	0.12
WBK 35 (F3) x NERICA 8	m100	80	42	38	45.0	35.0	0.46
CT 16334(2)-CA-2-M x IRAT 325	m110	79	41	38	44.4	34.6	0.61
CT 16334(2)-CA-11-M x WAB 56-104	m75	80	43	37	45.0	35.0	0.20
Bonanco x NERICA 15	m110	124	68	56	69.8	54.3	0.10
Bonanco x CT 16350- CA-5-M	m50	78	46	32	43.9	34.1	0.24
WAB 56-104 x CT 16334(2)-CA-11-M	m140	88	62	26	66	22	0.97
CK 73 x CT 16350- CA-5-M	m150	42	24	18	23.6	13.5	1.51
IRAT 13 x CT 16342-CA-25-M	m120	80	50	30	45.0	28.1	0.68
Ratio 7:9							
NERICA 9 x NERICA 8	m150	80	34	46	35	35	0.05
CT 16342-CA-25-M x IRAT 257	m180	43	21	22	18.8	24.2	0.45
Ratio 63:1							
CT 16317-CA-4-M x IRAT 104	m150	119	116	3	117	2	0.44
Ratio 3:1							
CT 16344-CA-9-M x WITA 2	m150	69	50	19	51.7	17.3	0.41
NERICA 1 x Jaggery	m125	52	42	10	39	13	0.92
Ratio 1:3							
WAB 365-B-1H1-HB x Golden	m125	39	12	27	9.8	32.2	0.63

[1]Phenotype Level = The point along graphical representation of all the shattering value where two peaks of the bimodal separates
[2] NS = Non shattering plants
[3] S = Shattering plants

Table 8.7 Means square value, LSD, and mean shattering score for crosses and their reverse

	Cross (F and F')	Cross type	Error df	Mean square value	LSD	Mean Normal (gf)	Mean Reciprocal (gf)
1	WAB 365-B-1H1-HB x IRAT 325	T x T	302	1.2	5.5	188.5	185.1
2	Bonanca x CT 16344-CA-9-M	L x L	64	2.2	12.5	193.0	182.8
3	CT 16313-CA-4-M x WAB 56-104	N x N	78	1.6	10.8	189.1	196.0
4	WAB 450-B-136-HB x NERICA 8	T x T	106	76.7***	14.4	175.8	112.4
5	WAB 450-B-136-HB x IRAT 122	T x T	100	6.3***	9.9	181.0	168.5
6	CT 16346-CA-20-M x Bonanca	L x L	100	13.0***	19.9	117.7	153.6
7	CK 73 x CT 16350- CA-5-M	L x L	78	47.7***	20.3	115.5	186.1
8	WAB 56-104 x CT 16334(2)-CA-11-M	N x N	58	17.3***	11.8	203.8	179.2
9	WAB 56-104 x CT 16324-CA-9-M	N x N	77	113.9***	4.3	189.1	144.8
10	CT 16334(2)-CA-2-M x NERICA 8	T x T	148	14.7***	18.3	152.9	117.1

Key; T x T= Tight shattering xTight shattering; Lx L= Loose shattering x Loose shattering; Normal Shattering x Normal Shattering

8.5 DISCUSSIONS

8.5.1 Combining ability

The significant mean squares due to the female and male GCA and the significant female x male interactions (SCA) indicated that both additive types of gene action due to males and females as well as non-additive types of gene action, respectively, were important for determining grain shattering ability. The male and female effects are the components associated with general combining ability (GCA), while the female x male interactions are associated with specific combining ability. The components associated with SCA effects were lower than those, which are, associated the total GCA effects, for all sets pooled indicated the relative importance of GCA effects for grain shattering in rice and suggested that the additive type of gene action was more important than the non-additive effect. This type of gene action has also not been verified for grain shattering in rice, although simple inheritance has also been found (Hu et al., 1964).

In the population generated from crosses involving parents with loose grain attachment to the pedicel, the Loose x Loose populations, female and male effects were highly significant but not the male and female interaction effects. The high GCA effects with male contributing to most and SCA being insignificant suggests that selection of parents and selection during the segregating generation could improve for optimum grain shattering which is desirable. Similarly, the cross involving Normal x Normal rice had high GCA and male effect is more

important. These findings demonstrate that additive genes worth selection are present in the crosses involving loose-loose, tight-tight and normal-normal.

8.5.2 Allelism

There was no apparent segregation in 46% of the crosses as evidenced in the unimodal distribution of the mean shattering scores of F2 populations. This finding suggests that genes responsible for resistance to grain shattering in these populations were the same in each cross. However, this study could not confirm which genes are present in the different populations. Grain shattering is a domestication linked trait. Some domestication linked traits, for example seed dormancy, exhibit distorted segregation from Mendelian pattern (Brar and Khush, 1996). Allelic tests coupled with gene identification could guide breeding for improved grain shattering if such distortion of segregation occurs. Allelism has not been reported before in grain shattering. However, allelism has been used in identifying sources of semi-dwarfism in rice populations. For instance, through allelic tests, two popular short statured rice varieties, Dee-geo-woo-gen (DGWG) and Jikkoku were confirmed to carry the same gene for dwarfism (Kikuchi, 1986). The study by Kikuchi (1986) further tested the F3 population of the crosses and confirmed that the genes for shattering were recessive. These two lines formed the back-bone parents of Green Revolution in rice. Similarly, results of the current segregations need to be confirmed at F3.

The two groups of plants, the shattering and non shattering, suggested that one or two major genes were responsible for transmission of grain shattering in these populations. The segregation ratios 15:1, 9:7 and 7:9 formed 85% of the segregating populations. These ratios imply that two genes were segregating at two independent loci. The data that segregation ratio of 15:1 indicated a duplicate dominant epitasis involving two genes was responsible for transmission of shattering trait. Previous genetic studies also suggested that at least two genes were responsible for the transmission of shattering (Tang and Morishima, 1988).

The observed segregation ratios of 9:7 and 7:9 indicated that gene action controlling grain shattering ability in those populations were due to two pairs of complementary dominant genes that function for tight to loose grain attachment. In the 9:7 ratio, it could be that the two dominant genes in this cross are responsible for loose grain attachment rather than tight grain attachment trait. This observation is not new. Recently, Li et al (2006a) found that

dominance controlled the transmission of grain shattering. Similarly, Elliot and Pellinger (1977) crossed five plants with loose attachment and five plants that have tight grain attachment and evaluated S_1, F_1, and F_2 progenies and proposed that two complementary dominant genes were responsible for their observed segregation ratios. Alternatively, the genes responsible for tight grain attachment could be expressed but one of the two may be a modifier. Gene modifiers have been reported for grain shattering in rice (Fukuta et al., 1995b).

In the 7:9 ratio, there is a possibility that the two complementary genes are recessive rather than dominant. Partial recessivity has been reported in grain shattering for gene sh2 (Oba et al., 1990). It could be that one of the two genes reported is sh2. Another work by Tang and Morishima (1988) found a ratio of 1:6:9 indicating partial resistance involving two genes in crosses involving *Oryza sativa* japonica (T65) and a Nepalese weedy rice (C9489). The two gene three loci model that satisfies the 7:9 ratio has been reported for grain shattering in crosses involving *Fagopyrum homotropicum* and *F. esculentum* (Wang et al., 2005).

When the source of resistance for any trait involves three independent genes, then 63:1 ratio is expected (Suzuki et al., 1989). In this study crosses involving CT 16317-CA-4-M x IRAT 104 that were non shattering and shattering, respectively had this segregation pattern. Recessive and dominant genes could be designated as S_aS_a S_bS_b S_cS_c for the non shattering parents and s_as_a s_bs_b s_cs_c for the shattering parents. All the three recessive genes s_as_a, s_bs_b and s_cs_c are required for grain shattering trait. Wide genetic variation in the degree of shattering has been found in cultivated rice (Uchiyamada et al., 1985). Three genes that control shattering *sh1* (Nagao and Takahashi, 1963), *sh2* (Oba et al., 1990; Ogi et al., 1993), and sh3 (Eiguchi and Sano, 1990) have been located on rice chromosomes 11, 1, and 4, respectively. It could be possible that these genes are responsible for grain shattering.

One cross had segregation pattern fitting the 3:1 ratio implying that a sinle gene was responsible. This cross had parents CT 16244-CA-9-M and WITA 2 which were both classified as loose shattering with mean shattering scores of 39gf anf 33gf, respectively (detailed in Chapter 5). This observation was surprising because the parents involved in the cross were supposed to be fixed. The reason for this outcome was not clear but the lines involved may not have been fixed for shattering and also the arbitrary scale used to group

genotypes for shattering may classify genotypes with different alleles for the trait. Two other crosses NERICA x Jaggery and WAB 365-B-1H1-HB had segregation ratios of 3:1 and 1:3, respectively. The parents had loose grain attachment and normal grain attachment. This is a reversal of the gene effects, which implies that the same genes switched their function from being dominant to recessive in different genetic backgrounds. Segregation ratio of 1:3 found in this study is has been reported before. A segregation ratio of 1:3 was found in a cross involving an *Oryza sativa* japonica (T65) and a Nepalese weedy rice (C9520) implying that seed shattering is controlled by a single gene in which shattering is dominant to nonshattering based on F2 populations (Tang and Morishima, 1988). In addition Ohnishi 1999 found the 1:3 ratio in a cross involving cultivated buck wheat, *Fagopyrum esculentum* and its shattering ancestor *F. esculentum* ssp. ancestrale Ohnishi (Onhishi, 1999). In cross between *Fagopyrum homotropicum* and *F. esculentum* however, Wang et al. (2005) found a 1:3 ratio using a two gene three loci model one parent was dominant at all the three alleles Sh1_Sh2_Sh3 and the other homozygous at one locus, sh1sh1 SH2SH2SH3SH3. In the current study it is not known if the same genes that are showing reversal in different populations.

8.5.3 Maternal effect

The observed results that 70% of the crosses tested had significant reciprocal differences suggests that there was both cytoplasmic and nuclear influence in the transmission of the grain shattering ability. There is no report of the influence of cytoplasm in the control of grain shattering. However, in studies involving *O. glaberrima* and *O. sativa*, there was poor crossability when *O. glaberrima* was a female, indicating presence of maternal effect (Jones et al., 1997). All the crosses that showed maternal effect are crosses involving interspecific lines. It is clear from the results that maternal effect for grain shattering was more common in crosses involving *O. sativa* and interspecific lines. Maternal effect is a rare and little studied genetic parameter. Maternal effects along with cytoplasmic effects have been demonstrated to be useful in the development of hybrid rice. The maternal effects involve any influence of a female parent exerted to progeny otherwise than by direct transmission of nuclear genes (Mc Laren, 1981).

When parents that had high resistance to grain shattering (tight grain attachment) were crossed to each other, female GCA was over 10 times higher than the male GCA. This observation suggests that maternal effects could be important in the transmission of the

shattering trait in this population. Out of four sets of crosses tested in this group, three had high maternal effects (Table 8.6). Overall total GCA for male and female was higher than SCA. The importance of maternal effect in the Tight x Tight set is that appropriate females could be selected when breeding for reduction of tightness of the grains onto panicles. This is important in the improvement of relatives of *O. sativa* that are known to have loose attachment of grains onto panicles. Similar effects of maternal parent, found in intercalary elongation of rice is used to select appropriate females for improvement of rice that is grown under varying water levels including floating rice to avoid submergence (Chen and Aes, 1990).

8.6   CONCLUSION

This study found information on combining abilities, segregation patterns and maternal effects for grain shattering ability in populations generated through crossing *O. sativa* and interspecific lines. The key findings are as follows:

> Generally, there was higher contribution of additive effects than non-additive effects to grain shattering in the populations. This implied that selections of segregating populations during the early generations could be effective.

> The majority of crosses generated populations that fitted a 2-gene model.

> Maternal effects were detected in some crosses.

> A 3:1 ratio from a shattering X shattering cross was probably due to unfixed loci in interspecific lines and the arbitrary grouping of genotypes for shattering.

> A 3:1 ratio and a 1:3 ratio in shattering X normal crosses suggested a single gene controlling the shattering trait but showing reversed function in different genetic backgrounds. This findings need to be confirmed using F3 populations.

Overall, there was evidence that a few major genes and several minor genes controlled grain shattering in rice. Breeding methods that encourage early selection should be employed could be exploit the effects of the major genes while selections in the later generations could exploit the effects of the minor genes. The early selection methods could include pedigree selection, pure lines selection, mass selection single seed descent while modified bulk methods could exploit the minor genes effects.

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Appendix 8.1 List of genotypes used in different sets of crosses for grain shattering trait

Group	Genotype no	Parent type	Genotype no	Breeding line	Type
Tight x Tight	Tight	Female	18	CT 16334(2)-CA-2-M	Interspecific
	Tight	Female	105	WAB 365-B-1H1-HB	*O. sativa*
	Tight	Female	134	NERICA 9	Interspecific
	Tight	Male	138	NERICA 8	Interspecific
	Tight	Male	193	NERICA 13	Interspecific
	Tight	Male	196	IRAT 325	*O. sativa*
Loose x Loose	Loose	Female	141	IRAT 13	Interspecific
	Loose	Female	96	Bonanca	*O. sativa*
	Loose	Female	129	CK 73	*O. sativa*
	Loose	Male	188	NERICA 15	Interspecific
	Loose	Male	27	CT 16342-CA-25-M	Interspecific
	Loose	Male	118	WAB 450-I-B-38-HB (NERICA 1)	Interspecific
Loose x Tight	Loose	Female	2	CT 16346-CA-20-M	Interspecific
	Tight	Female	72	CT 16317-CA-4-M	Interspecific
	Loose	Female	88	CT 16355-CA-15-M	Interspecific
	Tight	Male	147	IRAT 104	*O. sativa*
	Tight	Male	193	NERICA 13	Interspecific
	Loose	Male	197	IRAT 257	*O. sativa*

Research Overview

Introduction

The study was conducted with the aim of exploring possibilities of using new generations of rice, the interspecific fixed lines, which are drought tolerant, but frequently carry genes for grain shattering, as a source for improvement of the Ugandan landraces. The first part were preliminary studies that included determining farmersᵍ perception on the key production constraints and preferences for rice in Uganda, developing rice-breeding tools, determining anthesis and grain shattering pattern of rice, and characterizing rice populations in Uganda. In the second part, studies were conducted to determine crossability and the inheritance and gene action for drought tolerance and grain shattering in generations from crosses between *O. sativa* and new interspecific fixed lines. A summary of the research findings and their implications are presented.

1.1   Summary and implications of research findings

Chapter 2. Farmers' perceptions on the key production constraints and their preferences for rice in Uganda

Major findings of this study were:

Unreliable rainfall was the predominant rice production constraints in upland environment.

Rice yellow mottle virus, rice blast disease and African rice gall midge were major constraints in lowland rice production.

Positive attributes of traditional varieties included aroma characteristics, earliness, large white grains and softness when cooked.

Negative attributes of some varieties being grown were late maturity and yield loss due to grain shattering.

Up to 78% of the area under upland production was under a variety released in 2002, NERICA 4.

An early maturing variety NERICA 10 and an aromatic variety NERICA 1 was selected by farmers for production.

The unreliable rainfall in the upland conditions implied that drought is the most important constraint in farmersqfields. Farmers have no appropriate control strategy for this constraint, which means that yield will continue to be compromised if drought tolerant varieties are not developed. Similarly, the finding that rice yellow mottle virus, stem borers, rice blast disease and African rice gall midge were more important under lowland rice production systems implies that varieties with resistance to these pests should be developed to enhance yield in this environments.

The fact that farmers described aroma characteristics, earliness, large white grains and softness when cooked as preferred traits in landraces and went ahead to adopt a semi-aromatic variety released in 2002, implies that the farmers are not only willing to adopt new varieties that have these important traits but also able to judge before taking new rice types. A further observation that farmers selected an early maturing variety NERICA 10 and an aromatic variety during variety selection confirms that farmers are in need of varieties with these traits. In addition, the evidence that in the period in 1980s to 1990s, there was a gap in making new rice varieties available to farmers as reflected in the time line of rice production implies that there is an urgent need to embark on rice breeding with a focus on specific agro-ecologies. This work provides critical information required to begin rice breeding in Uganda.

Breeding for resistance to rice yellow mottle virus, stem borers, rice blast disease and gall midge are major breeding priorities in the International Rice Research Institute (IRRI) and Africa Rice Centre (ARC). In addition, early maturity drought tolerance and grain quality trait are major breeding priorities IRRI, ARC and CIAT. Rice breeding in Uganda could benefit from these centres through germplasm exchange and use of laboratory services.

Overall, breeding for resistance to key biotic stresses, especially drought and biotic stress is recommended. Preferably, the widely grown variety, NERICA 4, could be improved on using an introgression scheme. Farmers need to be involved in the variety selection process so that they get a sense of recognition, responsibility and ownership of the new varieties developed.

Chapter 3. Development of flower emasculator and grain shattering tester

Key outputs from this chapter were:

A new flower emasculating equipment that is as efficient as commercial one but cheaper, less bulky and had lower replacement costs has been developed.

A new single grain shatter tester that is portable and equally efficient when compared with laboratory grain testers has been developed.

The new flower emasculating equipment developed and found to be equally efficient, cheaper, less bulky and has lower replacement costs could be promoted for rice breeders in sub-Saharan Africa where facilities are in most cases limited. The new flower emasculator is made using a common household vacuum cleaner, which is cheap and readily available. Overall, it is 2.2 times cheaper than the commercial one. The new rice flower emasculator is a valuable tool for rice breeding, especially the ones in Africa who frequently lack capital to purchase the traditional on manufactured emasculators that are expensive. Another equipment, the hand held single grain shatter tester also developed in the current study is cheap but more accurate in estimating grain shattering than the common method of shaking the panicles. It is valuable equipment for rice breeding. It is important because at the moment rice breeding using relatives of the cultivated species, is very important yet the relatives of rice are naturally shattering types.

Chapter 4. Rice shattering pattern and anthesis trend

Major findings of this chapter were:

The greatest drop in the force required to dislodge rice grains is between 10 days after anthesis and 20 days after anthesis.

Among the rice types tested, NERICA rice genotypes had anthesis earlier than CIAT rice lines and the *O. sativa* flowered later than both rice groups.

The finding that the greatest drop in the force required to dislodge rice grains was between 10 days after anthesis and 20 days after anthesis implies that the highest level of discrimination among the varieties could be achieved when grain shattering is measured at this stage. This finding tallies with findings of Jin and Inouye (1982), that the tensile strength to dislodge rice grains drops drastically between seven and 21 days after heading based on record taken at weekly intervals after heading. Similarly, Ji et al. (2006) found the highest

reduction in mean tensile force for grain shattering between 15 and 20 days after heading. It is recommended that grain shattering be assessed at 10 days after anthesis.

Another result of this study that NERICA rice genotypes flowered earlier than CT rice genotypes and the *O. sativa* flowered later than both rice groups implies that appropriate methods of making *O. sativa* flower early should be employed so that pollination is synchronized.

Chapter 5. Characterization of rice germplasm for grain shattering and drought tolerance

This chapter provided information that:

> High genetic variability for grain shattering and drought tolerance was observed was in the germplasm collected. The mean shattering scores were also skewed to the lower values of shattering while the leaf score values and spikelet sterility were normally distributed

High genetic variability for grain shattering and drought tolerance was observed. These imply that suitable parents for improvement for the two traits could be identified. The mean shattering scores were skewed while the leaf score values and spikelet sterility were normally distributed. The skewness of shattering score to the lower values was that most of the materials assessed had low scores for force required to dislodge the grains indicating that they have high tendency to shatter. This observation is contrary to the findings in studies conducted in *O. sativa* populations in India, Japan, Indonesia and the USA where all germplasm collected had normal distribution (Fukuta, 1995). A common denominator to all the genotypes tested by Fukuta (1995) was that they were earlier selected for preferred traits, including acceptable grain shattering ability. However, the normal distributions of the leaf score values and spikelet sterility was probably because majority of these materials had not been selected.

It is proposed that use be made of the identified lines with tolerance to drought and low grain loss due to shattering in breeding program in Uganda. It is also important that more characterization studies be conducted on more landraces and more traits that includes aroma, grain size and growth duration before using them in the breeding program.

Chapter 6. Reproductive Barriers and Gene Action for Seed Set and Pollen viability in Crosses between *Oryza sativa* L and Interspecific Rice Genotypes
The outcomes from this chapter were:

Parents that had consistently high seed set when used as males, or as females or as both males and females were identified.

Additive effects were more important than non-additive effects for pollen viability and seed set in crosses between *Oryza sativa* and interspecific lines.

The fact that genotypes that had high crossability when used as male parents, female parents or as male and female parents were identified implied that these interspecific genotypes could successfully be used in rice breeding. A further finding that additive effects were more important than non-additive effects for pollen viability implies that parents could be selected for breeding new genotypes with high pollen viability. The study identified genotypes IRAT 325, CT 16317-CA-4-M and CT 16313-CA-4-M as the best female parents in terms of crossability, and genotypes IRAT 257, CT 16334(2)-CA-2-M and WAB 365-B-1H1-HB as the best females. Consistently, genotypes IRAT 257, CT 16334(2)-CA-2-M and WAB 365-B-1H1-HB were ranked as the best female and male parents. These parents are recommended for used in breeding that involves crossing with interspecific rice x landrace crosses. The best male genotypes combiners were CT 16334(2)-CA-2-M, WAB 365-B-1H1-HB and IRAT 257, while the best females combiners were CT 16334(2)-CA-11-M, CT 16317-CA-4-M and IRAT 325 are recommended for breeding.

 Another aspect of this study that additive effects were more important that non-additive effects for pollen viability needs further investigation because other scientists found that nuclear cytoplasmic interactions are major contributors in the transmission of pollen viability. Models for genetic function of pollen viability based on sporo-genotypic interactions have been proposed. Several gene loci   have been reported (Sano, 1990; Doi et al., 1998; Doi et al., 1999). In addition, three gamete eliminator loci and two pollen killer loci have been found in *O. glaberrima* (Dayun et al., 2002). The current observation requires more investigations into the mode of transmission of this pollen viability.

Chapter 7. Gene action and inheritance of drought tolerance traits in populations generated from interspecific with intraspecific rice

The following information were generated in this chapter:

There was generally low and insignificant correlation between filled grains and other agronomic parameters.

Additive effects for filled grains under drought stress and non-drought stress were more important than the non-additive effects and there was high heritability for leaf roll score under drought stress.

The fact that additive effects for filled grains under drought stress were more important than the non-additive effects implied that selection of parental lines and hybridization using selection methods that involve selection in the early generation would be effective. However, a lack of significant correlation between filled grains and other agronomic parameters under drought stress implied that those traits could not be used in the breeding for improved filled grains under drought stress. In sets of crosses where both non-additive influences and maternal effects were detected under drought stress and non-drought stress conditions breeding for improved drought tolerance should involve selection during later generations of the crop.

The genotypes with high positive GCA WITA 1, IRAT 325, CK 73, CT 16350-CA-5-M and WAB 450-B-136-HB (NERICA 9) could be used in pedigree breeding, pure line selection for improved tolerance to drought. in condition for filled grains. Crosses WITA 1 x CT 16350-CA-5-M, Bonanca x CT 16346-CA-20-M, WITA 1 and CT 16344-CA-9-M with high positive SCA could be selected using methods that involve delayed selection like modified bulk method.

Chapter 8. Inheritance of grain shattering trait in rice generated from crossing interspecific with intraspecific rice.

This chapter generated the following information:

The chi-square analyses of segregation pattern showed that there was no segregation in 46% of the crosses but 46% gave 15:1, (33%) 9:7, (7%) 7:9, (4%) 63:1, (7%) 3:1 and (4%) 1:3 for resistant: susceptible ratio.

Generally, there was higher contribution of additive effects for grain shattering ability than non-additive effects in the populations. However, a few involving non shattering parents showed evidence of maternal effects.

Results of the test for allelism revealed that shattering was controlled by the same genes in the crosses that had no segregation; two genes in another 46% of the crosses with duplicate dominant effects as well as complimentary dominant/recessive effects; and three genes in four percent of the crosses.

A surprising result was segregation ratios of 3:1 when both parents had loose grain attachment to the pedicel. The observed segregation could be due to unfixed loci for shattering in the interspecific and *O. sativa* parents used.

Two crosses, NERICA x Jaggery and WAB 365-B-1H1-HB had segregation ratios 3:1 and 1:3, respectively. The parents had loose grain attachment and normal grain attachment. This is a reversal of genetic effects, which implies that genes that switched their function from being dominant to recessive were present. Other studies involving different rices found 1:3 or 3:1 ratios, implying that a single gene was involved but affected by other factors like genetic backgrounds. This observation is very important because knowledge of each parent contribution to segregation guides breeding. Further studies are required to understand this apparent reversal of gene effects. At the moment, it is difficult to explain.

Overall, there was evidence that a few major genes and several minor genes controlled grain shattering in rice. Breeding methods that encourage early selection should be employed. Methods that include pedigree selection, pure lines selection, mass selection, single seed descent should be employed in the breeding for optimum grain shattering ability.

Conclusion:
In this work, rice production constraints were identified and ranked. Farmers and consumersꞌ rice preferences were also identified. The rice breeding priorities for upland, irrigated and different regions were also determined. Furthermore, new breeding tools, namely for flower emasculation and grain quality testing were developed and appraised. In this study the appropriate growth stage to screen for grain shattering and the appropriate

trend of anthesis of different rice varieties were determined. Parental lines with high variability for grain shattering and drought tolerance were identified as well. The parental genotypes were used to gain more understanding on grain crossability between *O. sativa* and interspecific fixed lines. The genotypes with high seed set, high pollen viability and desirable transmission of pollen viability traits were identified. The preceding preliminary work set a condition suitable for genetic studies of inheritance and gene action controlling drought tolerance and grain shattering traits. Good tolerance to drought stress has been identified in the F2 generations and this program needs to continue with selection and making crosses using identified parents. It will be necessary to involve farmers in the selecting the best genotypes. Based on the finding in this study, breeding for optimum grain shattering ability and improved proportion of filled grains under drought would require breeding methods that involve selection in the early stages of segregation and the late stages of segregation due to the presence of both major gene and several minor genes for the two traits, respectively.

In this study, complete failure of grains to fill was realized in some F1 plants and these plants were maintained through technique of tiller splitting. It is likely that male sterility controlled the observed unfilled grains. Another aspect of this work that some important agronomic traits were controlled by non-additive gene effects opens an opportunity to explore the development of this suspected male sterility genes for hybrid rice production in Uganda.

References

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