

**STUDIES OF DROUGHT TOLERANCE IN INTERSPECIFIC PROGENIES OF
ORYZA GLABERRIMA (STEUD) AND *O. SATIVA* (L) AND AN APPRAISAL OF
THE USE OF MALE GAMETOCIDES IN RICE HYBRIDISATION**

BY

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Thesis Abstract

Rice production in Sub-Sahara Africa is hampered by drought stress, low farmer adoption of improved varieties, un-adapted germplasm, problems of crossability between species and problems of efficient emasculation. This study was initiated to: (1) determine farmer preferences for rice varieties and production practices, (2) identify drought tolerant parental lines for use in interspecific crosses, (3) determine crossability between different rice species, (4) identify drought tolerant interspecific segregants from crosses, and (5) study the efficacy of ethrel and gibberilic acid (GA₃) as chemical hybridising agents.

Participatory rural appraisal (PRA) was conducted in Sikasso region in the month of September 2005 to determine farmer preferences for varieties and production constraints. A total of 125 rice farmers from 10 villages participated. This study found that farmer preferences for rice traits did not coincide with the breeders' objectives and trait preferences varied across ecologies. In upland and lowland rice ecologies, the most preferred characteristic was short duration. In the lowland tall plants was most preferred and short duration trait ranked second. Long duration ranked as the second most preferred trait after yield in the irrigated rice ecology. The estimated grain yield reduction was 60% due to drought stress from all ecologies.

Sixteen genotypes including seven *Oryza glaberrima* Steud, six interspecific (*O. sativa* x *O. glaberrima*) inbred genotypes and two improved *O. sativa* L. subsp (japonica) genotypes, with OS 6, as drought tolerant check were screened for drought tolerance at the vegetative stage during the dry seasons of 2004 and 2005 at Samanko research station, in Mali. Most of the genotypes could be regarded as exhibiting drought tolerance and avoidance mechanisms for most of the morpho-physiological characters under study. High selection index was observed for WAB 450-I-B-P-103-HB in the two season experiments. Drought stress tolerance (little leaf drying) was significantly correlated with leaf rolling, leaf area index, and plant height. Fast drought recovery was significantly correlated with drought tolerance, tiller number and leaf area index. Effects of genotypes, environment and their interactions were significant for tiller number, root dry weight and relative leaf water content. The most stable genotype was RAM 3 for tiller number and relative leaf water content. Thirteen genotypes were selected on the basis of these traits for rice hybridisation.

A factorial arrangement involving three levels of gametocide concentrations and five application stages of the female rice parent was undertaken for each gametocide ethrel and GA₃ for two seasons. Per cent empty spikelets and male sterility increased with concentration of ethrel. Male sterility induction of 41.5% and 42.8% at 2000 ppm was achieved in 2004 and 2005, respectively, while at 4000 ppm male sterility induction was 40.5 % and 46.1% in 2004 and 2005, respectively. The best application stage of ethrel that induced highest male sterility was at P4 (panicle primordium differentiation + spikelet primordium differentiation) of 46.6% in 2004 and 49.2% at P1 (panicle primordium differentiation) in 2005. There was no measurement on GA₃ for sterility due to lodging. Total male sterility was not achieved therefore, these gametocides could not be recommended for use as hybridising agents.

Crossability was studied in four groups: group A (*O. glaberrima* x *O. sativa*), group B (*O. glaberrima* x Interspecific), group C (Interspecific x *O. sativa*) and group D (Interspecifics x Interspecific). High per cent seed set was obtained in group D of 19.08% and group A had 9.29%, in group B the seed set was 11.26% and group C had 6.62 %. The three best combiners as female parents based on per centage seed set were CG 14 (*O.*

glaberrima), WAB 450-IBP-105-HB and WAB450-I-B-P-103-HB (interspecifics), and for male parents were NERICA 3, NERICA 2 (interspecifics) and WAB 375-B-9-H3-2 (*O. sativa*).

Genetic components of variance and heritability estimates were studied in F3 and F4 generation progenies from North Carolina II design mating scheme. General combining ability (GCA), mainly due to female and specific combining ability (SCA) were significant for tiller number, plant height and specific leaf dry weight indicating that additive and non-additive genetic effects controlled these traits. Heritability estimates for drought tolerance and leaf rolling were highly significant for all the populations examined, ranging from 0.32 to 0.72 and 0.22 to 0.56, respectively. Drought tolerance expressed as little leaf drying was significantly associated with less leaf rolling, larger leaf area index, and taller plants. Some genotypes and progenies such as WBK 39-B-B-B, WBK 30-B-B and WAB 450-I-B-P-103-HB were identified as being drought tolerant with the ability to recover quickly from drought stress.

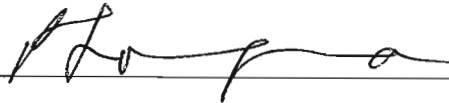
There was a strong desire for tall plants and short duration rice in upland and lowland ecologies, while high yield was preferred in irrigated ecology. Therefore, the best breeding strategy would be ecosystem breeding targeting each of the ecologies. Screening for secondary traits that have been identified to be significantly associated with rice grain yield could be used for direct selection for grain yield under drought stressed conditions. Progenies identified, as drought tolerant would be considered for use as source germplasm in breeding that target Sikasso region and any other similar ecologies. This study therefore suggested that participatory research approaches that involve all stakeholders in rice production would be valuable in identifying and generating drought tolerant and adoptable rice cultivars in the tropics of Africa with considerable impact on food security.

Declaration

Research presented in this thesis represents original work by the author and has not been otherwise submitted in any form for degree or diploma to any University. Where use has been made of the work of others it is duly acknowledge in the text.

A handwritten signature in black ink, appearing to read 'Andrew A. Efisue', written over a horizontal line.

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Dedication

This thesis is dedicated to my mother, even after the early death of my father; she worked tirelessly to get me educated.

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Introduction to thesis

Rice production in Africa

Rice is the most agro-ecologically diverse of the world's major crops. It is grown under deep and shallow flooding, in the dryland (upland), and in coastal mangrove swamps. It is also, grown in the temperate, sub-tropical and tropical climates, and at altitudes exceeding 2000m. Rice forms the nutritional basis of the world's population and it is estimated that 40% use rice as a major source of calories (FAO, 2001). Globally, rice provides 20% of energy and 15% of per capita protein and by the year 2025, it is estimated that more than 8.3 billion people will live on earth and 50% of them will be rice eaters. Therefore, the current world rice production of approximately 584.9 million tons (FAO, 1999) must be increased by 70% to meet this demand.

Africa is the least producer of rice globally. Her production on the average was 14.6 million t per year (1989-1996) and rose to 17.00 million t in 1999 (FAO, 1999), which could be translated to about 1.5% increase per annum. This increase was attributed mainly to the increase in the land area under cultivation. The population of Africa is increasing at an average of 3.0% per annum (ThinkQuest, 1998), thus leaving a wide gap between production and consumption (Table 1). According to FAO rice statistics reports, no African countries are self sufficient in rice except Egypt; thus, supplement to fill this gap comes from importation using scarce foreign currency.

In 2004, average paddy rice production in West Africa was 8.06 million t while the gap (between production and consumption) was 3.73 million t in 2003. As of 2002, the total consumption was 7.87 million t of milled rice and per capita consumption in the same year was 30.07 kg (FAOSTAT data, 2005).

Rapid changes in the dietary preferences of West African consumers have resulted in higher demand for rice, thereby creating an imbalance between production and consumption in the region. The sudden shift in consumer preferences is attributed to rural-urban migration coupled with women moving into the work force. Cooking rice saves time, as it is very easy to prepare.

Table 1: The production, importation and consumption of rice in West Africa

<i>Average totals per year</i>	<i>1961-1974</i>	<i>1975-1984</i>	<i>1985-1994</i>	<i>1995-1998</i>
Production (1000t)	1335	2081	3456	4567
Consumption (1000t)	1336	2852	4688	6234
Gap (between production and consumption) (1000t)	424	1340	1982	2423
Per capita consumption (kg)	26	33	39	43
<i>Average growth rate (%)</i>	<i>1961-1974</i>	<i>1975-1984</i>	<i>1985-1994</i>	<i>1995-1998</i>
Production	3.9	3.8	3.8	3.7
Consumption	6.3	6.2	6.2	6.2
Gap (between production and consumption)	9.0	9.2	9.4	9.3
Per capital consumption	2.0	2.1	2.0	2.0

Source: Adapted from FAO, 2001 Internet database

Participatory Rural Appraisal for farmers' perceptions, and preferences

In Sikasso region of Mali, many upland rice varieties have been introduced but with low adoption by the farmers. The reasons could stem from lack of farmers' preferred traits and perceptions in the development of rice varieties. Some varieties that were adopted are poor yielding due to the effects of drought stress. Methods of rice cultivation, harvesting and use vary from community to community and as well as farming systems. The adoption of rice varieties would therefore vary from community to community. This implies the need for farmers' involvement in the breeding programme and their traits preferences considered in the development of new varieties of rice.

Participatory Rural Appraisal (PRA) is being used as a tool for involving communities in the development processes. It is a participatory approach, which emphasis local knowledge and assistance to local people to make their own appraisal, analysis and plans. Joshi and Witcombe (1995) reported that participatory approach was a rapid and cost effective method of identifying farm-preferred rice cultivars. They reported that this approach in India revealed a number of important characters that would have not been identified in breeders'

experiments. Lightfoot and Ocado (1998) also reported success in using participatory approach methods in jointly developing new ways to control a crucial weed problem in the Philippines.

Sikasso region is semiarid transition zone with abundant annual rainfall of 1100mm thus favouring upland rice cultivation. The erratic single rainfall pattern in the region results in recurrent drought at any stage of the rice development. Thus, late season and intermittent droughts characterize the drought pattern of the region. This region accounted for 10.8% of the national harvested areas (FAOSTAT, 2001) and also over 80% of upland rice production in Mali. Therefore, with these favourable conditions for upland rice ecology, PRA was conducted in this region to determine farmers' perceptions on drought stress management practises and varietal trait preferences.

Rice production constraints with emphasis on drought stress

The major rice production constraints in most ecologies of Africa where rice is grown are biotic and abiotic factors. Biotic factors include weeds, rice yellow mottle virus and blast, while drought stress, nitrogen and iron deficiency are the most devastating of the abiotic factors. These cause major damage in Sub-Saharan Africa, where the main stakeholders are small scale and poor resource farmers. Other important constraints are: poor water management practices, low yielding cultivars, poor accessibility to credit for the purchase of inputs, and regrettably, bad government policies on rice production by encouraging rice importation and less budgetary allocation to agriculture. Due to the effects of drought and the aforementioned constraints, the average yield in farmers' fields is about 1.0 t ha⁻¹ and the potential is between 2.5 to 4.5 t ha⁻¹ in the upland rice ecologies (WARDA, 1997).

The economic effects of drought are enormous, at whichever stage it occurs and is a serious limitation to yield. Rice plants are most susceptible to water stress at the reproductive stage of plant development. Drastic reduction of grain yield occurs when stress coincides with critical phenological events at reproductive stage such as panicle initiation and anthesis (Price and Courtois, 1999). Widawsky and O'Toole (1990) reported that yield losses due to drought stress at anthesis and seedling stages combined was higher than the yield loss due to

weeds. In upland rice ecology, drought occurs at various stages of plant development. Drought stress occurring at the vegetative stage causes delay in time to flower, affects leaf expansion and leaf rolling, which subsequently reduces total grain yield (Pantuwan *et al.*, 2004).

No single selection criterion or screening methodology for drought tolerance in rice has been found adequate because drought is highly unpredictable over time and space. However, under drought stressed conditions, some morphological and physiological traits have been identified as putative and respond to drought stress (O'Toole, 1982; Lilley and Fukai, 1994). These putative traits include leaf drying, leaf rolling, leaf area reduction and osmotic adjustment. These relationships among morpho-physiological traits could be used for breeding strategies that target secondary traits for indirect selection for grain yield under drought stress conditions, as they indirectly affect the pathways of high yield in rice genotypes.

This study therefore, focused on drought stress as one of the major production constraints of rice in the region. Different rice species screened for drought stress tolerance and associated morphological and physiological traits at the vegetative stage of rice growth in upland rice ecologies. The different species were used as parents in the interspecific breeding for drought tolerance.

The use of chemical gametocides in hybrid rice production

Currently, rice hybridisation is done manually. The morphology of rice flower allows one seed per floret to be produced. The small flower size and manual pollen emasculation are labour intensive. The vacuum emasculator that sucks the anthers is an expensive mechanical device for flower emasculation. In West Africa, most national agricultural research institutes (NARS) may not be able to afford this vacuum emasculator and the costs of the maintenance.

Chemical gametocides can be useful in inducing male sterility in rice, thus facilitating hybridisation for possible F1 production and general crossing for breeding purposes. The

efficacy of gametocides in population breeding in inducing male sterility has been reported (Beek, 1986). Review of literature reveals that an efficient gametocide must produce almost 100% male sterility without affecting female fertility. Apart from the lethal effects on female organs, information of dosage rate, spraying schedule and stage of spraying the plant, which confound the efficacy of this technology has not been adequately addressed. This is because the efficacy of gametocides depends on genotypes, season and location. This study would therefore appraise the use of gametocides as chemical hybridising agents in an attempt to develop an appropriate methodology for rice hybridisation for the region.

Crossability of *Oryza glaberrima* x *O. sativa*

Asian rice, *Oryza sativa* L. is high yielding and is gradually displacing *O. glaberrima* (Africa rice) in terms of cultivation. *Oryza glaberrima* is still widely cultivated in many West African countries however. This is because it is well adapted to local production systems and has some resistance to important biotic and abiotic production stresses (Jones *et al.*, 1997a) such as weeds, blast and rice yellow mottle virus for biotic stresses and drought for abiotic stress. The *O. glaberrima*, unlike the Asian rice, possesses some undesirable agronomic traits such as lodging and seed shattering that result in low grain yield. Although, Asian rice possesses good agronomic traits coupled with high grain yield potential, there is limited resistance to many of the biotic and abiotic stresses of the region (WARDA, 1997).

The use of *O. glaberrima* genes to improve Asian rice for adaptation to West African production systems is therefore necessary and important for rice production in the region. Interspecific hybridization is a cross between two, sometimes, distinctly related species. Plant breeders undertake interspecific crosses because of the need to resolve some breeding problems. Incompatibility barriers limit the success of these crosses. However, this is genotype dependent. The success of interspecific crosses varies with the rice species used as maternal parent and interaction between the parental genotypes and the cytoplasm. Successive backcrosses, embryo rescue through anther culture and double haploids have been used to overcome these barriers (Jones *et al.*, 1997b).

This study used successive backcrosses technique in generating fertile seeds for drought screening. The current study exploited these possibilities by developing interspecific progenies between different rice species for drought tolerance in upland rice ecologies.

Genetics of rice traits

The effectiveness of a drought screening procedure is best measured by the genetic heritability estimates achieved for target traits, whether the focus is conventional or marker-assisted plant breeding. Selection of parents is one of the most important steps towards developing drought tolerant lines as genetic variance and heritability estimates for grain yield decline under stress (Blum, 1988). Secondary traits under lowland rice ecologies that are associated with drought stress at the vegetative and reproductive stages have been identified. These traits are leaf rolling, leaf drying and relative leaf water content at the vegetative stage and delayed flowering time at the reproductive stage (O'Toole, 1982; Pantuwan *et al.*, 2004). Information on genetic variances and their effects have contributed to rice improvement and to the understanding of the gene action involved in the expression of heterosis and economically important quantitative traits. However, there is no sufficient information of secondary traits that are associated with drought stress under upland rice ecologies with interspecific progenies in the region.

Genotype by environment interaction and variety stability

The development of rice varieties that can adapt to a wide range of environments is of utmost importance to a rice breeder in population improvement. Rice genotypes respond differently to drought stress, which is influenced by their interaction with environmental factors such as soil nutrients and climate. Adaptability of a genotype to diverse environments is usually tested by its level of interaction with the target environments. A genotype is considered stable if it has a higher mean performance for the measured traits such as yield with less fluctuation across environments (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966). The stability of a genotype for a particular trait is genetically controlled, hence the effects of genotype x environment interaction, which confound the selection of desirable genotypes will be examined in this study. Information on the effects of genotypes, environments and their interactions with rice traits under different drought

stressed water regimes in this region are yet to be established. Therefore, this study evaluated rice genotypes under stressed and non-stressed conditions across seasons. The effects of genotypes, environments and their interactions on rice traits were examined.

Objectives of the study

The goal of this study was to develop drought tolerant rice that is adaptable, high yielding and adoptable by farmers in the tropics of Africa. The specific objectives of this study were:

- (1) to determine farmers' varietal trait preferences and perceptions on drought and management practices in Sikasso region in Mali,
- (2) to identify genotypes with good crossability as parents for use in interspecific hybrids production,
- (3) to study the efficacy of gametocides as chemical hybridising agents for use in rapid hybridisation of rice genotypes, and
- (4) to identify drought tolerant interspecific genotypes at the vegetative stage of rice development and traits and genetic components that are associated with drought tolerance under stress and non-stressed conditions.

The hypotheses of this study were:

- (1) Farmers' trait preferences coincide with breeders' objectives,
- (2) Morpho-physiological traits associated with drought tolerance can be identified at the vegetative stage of rice plant development,
- (3) Drought tolerant genotypes, which are crossable, can be identified,
- (4) Gametocides are useful for rapid hybridisation in rice breeding, and
- (5) There is genetic variation for morph-physiological and phenological traits associated with drought tolerance in rice genotypes.

Structure of the thesis

The following experiments were designed to achieve these objectives in different chapters. The review of literatures revealed the works that have been done and where information is lacking most particularly on drought stress (Chapter 1). Participatory rural appraisal was conducted to appraise rice production practices and general constraints in the Sikasso region in Mali (Chapter 2). Based on the PRA results, parental lines were screened for drought

tolerance at the vegetative stage of plant development as more farmers in the upland rice ecologies experienced drought at this stage (Chapter 3). Chemical hybridising agents (gametocides) were tested for their efficacy in rice hybridisation and production of large quantities of seeds (Chapter 4). Results from the parental lines screening and the past performance of these lines were used in the selection of parents for hybridisation (Chapter 5). The derived progenies were subjected to drought stress at vegetative stage in the dry season and compared with their performance under stressed and non-stressed experiments (6). The genetic components of variation were studied both under stressed and non-stressed conditions (Chapter 7). The overview for the study is presented in Chapter 8.

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CHAPTER 1

Review of the literature

1.1 Introduction

In this chapter, literature is reviewed on, taxonomy and origin of rice, domestication and distribution of *Oryza glaberrima* (Steud) and *O. sativa*, climatic environments of rice production, major rice ecologies in West Africa, and participatory rural appraisal. The literature review also covers use of gametocides in hybrid rice production, interspecific hybridisation in rice. The effects of drought stress at different growth stages in rice, mechanisms of drought tolerance and screening methodology for drought tolerance in rice were also examined.

1.2 Taxonomy and origin of rice

Rice belongs to the family *Gramineae* (*Poaceae*) of the tribe *Oryzaceae* and genus *Oryza*. Most *Oryza* species are diploid ($2n = 24$), although some are tetraploids ($4n = 48$) (Table 1). It consists of about 26 species; the exact number is subjective and varies from one author to the other. Vaughan (1994) identified 23 *Oryza* species and grouped them into four complexes. Among the *Oryza* species, only two, *O. sativa* and *O. glaberrima* are cultivated the others are wild species. The taxonomy of the genus *Oryza* is well defined (Vaughan, 1994) and the relationships between species based on molecular and cytogenetic studies have been established (Katayama, 1997; Wang *et al.*, 1992; Aggarwal *et al.*, 1997).

The genus *Oryza* is believed to have originated in Gondwanaland (Khush, 1997), the ancient landmass, which was believed to be from where India, Africa, South America and Australia drifted apart (Chang, 1985). The *O. sativa* is widely grown in tropical and temperate zones, but *O. glaberrima* is endemic to West Africa (Linares, 2002). It is generally believed that *Oryza sativa* and *Oryza glaberrima* evolved from different wild relatives. The progenitor of *O. sativa* is believed to be the wild annual genus *O. nivara*, derived from wild perennial rice *O. rufipogon* (synonymous to *O. perennis*). In a parallel evolutionary pathway, the progenitor of *Oryza glaberrima* is the wild annual genus *O. breviligulata* (synonymous to *O. barthii*) derived from the wild perennial *O. longistaminata* (Chang, 1985; Khush, 1997). The origin and genomic classification of *Oryza* species in Africa is summarised (Table 1).

Table 1: The origin and genomic classification of *Orzya* species in Africa

Species	2n	Genome	Origin
<i>O. sativa</i> (cultivated)	24	AA	Asia
<i>O. glaberrima</i> (cultivated)	24	A ^g A ^g	West Africa
<i>O. stapfii</i> (weed species)	24	A ^g A ^g	West Africa
<i>O. barthii</i>	24	A ^g A ^g	West Africa
<i>O. longistaminata</i>	24	A ¹ A ¹	Tropical Africa
<i>O. brachyantha</i>	48	FF	West & Central Africa
<i>O. eichingeri</i>	24	CC	West & Central Africa
<i>O. punctata</i>	48	BBCC	East & Central Africa
<i>O. punctata</i>	24	BB	East & Central Africa
<i>O. schwein furthiana</i>	48	BBCC	Tropical Africa

Source: Adapted from Vaughan and Morishima, 2003; Ng et al., 1983.

1.3 Domestication and distribution of *O. glaberrima* and *O. sativa*

Oryza glaberrima was domesticated along Niger River some 2,000 to 3,000 years ago (Porteres, 1976), although Carpenter (1978) claimed that it was under cultivation in West Africa around 3,500 years ago. Recently, Murray (2005) reported that *O. glaberrima* has been under cultivation since 1000 BC. The exact date and year of its domestication varies among authors but it is generally believed to have been more than 35 centuries ago in West Africa.

This species spread to two secondary centres of diversification: one on the coast of Gambia, Casamance and Guinea Bissau and the other in the Guinea forest along the arc of Sierra Leone through Western Ivory Coast down to Nigeria (Porteres, 1976). The early colonial history of *O. glaberrima* begins when the early Portuguese explorers to the West African coast discovered the cultivation of rice in the floodplains and marshes of the upper Guinea coast. In their account, dated between 15th to 16th centuries, the vast fields of rice under cultivation were reported. Richards (1996) confirmed the previous observation by mentioning the many varieties of rice that were grown in the Gambia area. The Mende people of Sierra Leone used African rice soaked in palm oil as a component of their ritual

sacrifices to their ancestors (Richards, 1996). Casamance, one of the two centres of diversity of *O. glaberrima*, was inhabited by the Felupos (the Jola) in the west corner of Senegal and they used traditional African rice to propitiate the spirits for the link between crops and the ancestors. The Jolas' practised a labour intensive form of wet rice cultivation including tilling the soil, diking and bunding the paddy field using a local shovel called kajandu (Linares, 2002). This system has undergone transformation with the use of modern methods of rice production such as good rainwater control, puddling and fertilizer application. Linares (2002) reported that drought experienced by these centres of diversification in the last two decades has caused a notable loss of diversity in the rice species of *O. glaberrima*, more particularly in the lower Casamance region. This long history of domestication of *O. glaberrima* species, make them adaptable to the farming systems of the region. These could be the reason why some West African rice farmers, especially in Mali, Guinea and Casamance region still grow *O. glaberrima* species, despite presence of the high yielding Asian rice. The surviving species are believed to be drought tolerant and have spread to many countries in West Africa. This study used *O. glaberrima* species as parents to introgress some of their good agronomic traits in the development of drought tolerant interpecific progenies.

Oryza sativa was domesticated over 8, 000 to 15, 000 years ago (Normile, 2004). It is grown world wide under a wide range of agro-climatic conditions. *O. sativa* can be distinguished into three ecological varieties (ecotypes), *indica* (tropical and sub-tropical), *japonica* (temperate) and *javanica* (grown in Indonesia). The most widely grown and researched of these ecotypes are *indica* and *japonica*. The common distinguishing features are that *japonica* grains are shorter and wider than the *indica* grains. The *indica* has profuse tillering and soft plant tissues, while *japonicas* are medium to low tillering with hard plant tissue (OECD, 1999). The argument still continues amongst the Asian countries as to who started the domestication of rice. However, there are records of rice in ancient Hindu scriptures and literatures dated back to 1300 BC. At Lothal in Gujarat, southwards extension of the Harappa and Mohenjodero cultures of the Indus Valley, there was evidence of rice cultivation dated to 2300 BC (Deshaprabhu, 1966). That domestication of rice started in India or Indochina cannot be in doubt due to the abundance of wild rice species and varietal

diversity of cultivated rice there (Watabe *et al.*, 1976; Kumar, 1988). The importance of rice in religious and custom rituals may suggest that India is the heartland of rice. However, some schools of thought believe that domestication process started in China due to the divergence of the two species (*japonica* and *indica*). Apart from this, the cultural practices such as puddling and transplanting, which were first developed in North and Central China, also laid credence to China as the center of origin (De Datta, 1981). There is no argument however that the Asia arc is the centre of origin of rice and that it later spread to other parts of the world. This study used some high yielding Asian rice as donor parents to improve the indigenous African rice *O. glaberrima*. A summary of some major distinguishing features between the two cultivated rice species is shown (Table 2).

Table 2: Summary of some distinguishing characters between *Oryza glaberrima* and *O.sativa*

Character	<i>Oryza gaberrima</i>	<i>O. sativa</i>
Distribution	Endemic to West Africa	Cosmopolitan
Habit	Annual	Essentially perennial
Varietal differences	Limited variation	High variation
Lodging	Frequent	Rare
Drought tolerance	High	Poor
Ligules	Short (6mm), oblong, thick	Long (40-45mm), pointed thin
Panicle branching	None or few	Many
Grain number	75-100 grains/panicle	250 grains/panicle
Grain (milling property)	Difficult to mill	Easy to mill
Seed dormancy	High	Low
Seed shattering	High	Low
Protein content	High	Low

Source: Adapted from Sarla and Swamy, 2005.

1.4 Climatic environments of rice production

Most of the rice growing areas in the world are located in the tropics (Yoshida, 1981). These areas are between the tropics of Cancer (23.5⁰N) and the tropic of Capricorn (23.5⁰S) and extend to as far as 49⁰N and 35⁰S of the latitudes. Rice also grows from sea level to an

altitude of 2,500m or more (Khush, 1997). Although, rice is primarily a tropical and subtropical crop, the best grain yields have been obtained in the temperate region. This is attributed to lower temperature during ripening, which gives more time for grain filling, long day length and high level of solar energy during the ripening period (Yoshida, 1981). These phenomena are also conducive to lower grain yield obtained during the wet season versus the dry season. However, due to the prevalence of biotic and abiotic factors during dry seasons, lower grain yield is obtained in most cases as compared to the wet season. During the vegetative stage of a crop, poor solar radiation slightly affects yield and yield components, because the plant would recover during the reproductive stage. However, it has a pronounced effect on spikelet number during the reproductive stage and ripening of the grains (Yoshida, 1981).

Temperature regime greatly influences growth duration and growth pattern. High temperatures are destructive to plant growth. This is because high temperature enhances depletion of internal water status of the plant through high transpiration. Extreme temperature may also cause drought stress by enhancing evapotranspiration of the soil. Mackill *et al.* (1982) reported that extreme heat stress might also cause poor production and shedding of pollen. When the environmental temperature is below a critical threshold, transpiration rate is reduced as well as photosynthesis. This reduction causes glutting of soil nutrients, which could result in toxicity stress. Temperature below a critical threshold causes reduction in pollen mother cell division, which usually induces a high percentage of spikelet sterility (Satake, 1976). When this occurs especially at the reproductive stage, the spikelet number per plant also decreases. This study will select for high temperature tolerant rice since mean monthly temperature in Mali ranges from 35 °C to 43 °C.

1.5 Major rice ecologies

Classification of ecological regions where rice is grown is yet to be resolved due to overlap of several contiguous ecosystems favourable for rice growth. Rice ecologies are classified according to source of water supply into two broad terms as rainfed and irrigated. In South and Southeast Asia, Barker and Herdt (1979) classified rice lands into irrigated, shallow rainfed, deepwater and upland. In West Africa, Buddenhagen (1978) classified rice

ecologies into four main types and eight sub-types based on climate, soil conditions, water regime and management practices. WARDA (1997) defined six ecological areas favourable for rice production in West Africa (Table 3). This study will focus on the rainfed upland rice ecology. This is because it is an important rice production ecology, particularly in West Africa. This ecology represents 57% of total rice area and 40% of regional production (Table 3). In Mali, rainfed upland rice ecology accounts for 10.8% of the national rice production.

Table 3: Six ecologies for rice production in West Africa

Ecology	%Rice grown area	Yield potential t ha ⁻¹	Farmer' yield t ha ⁻¹	Major constraints
Rainfed upland	57	2.5-4.5	1.0	Weed, drought, Blast, soil acidity, nitrogen and iron deficiency
Rainfed lowland	20	3.0-5.5	1.4	Weed, drought, Rice Yellow Mottle Virus (RYMV), water control, and nitrogen deficiency.
Lowland irrigated	5	5.0-7.0	2.8	RYMV, gall midge, iron toxicity and nitrogen deficiency,
Sahelian irrigated	7	5.0-8.5	3.5	Salinity, cold, RYMV, alkalinity weeds and nitrogen deficiency
Mangroove swamp	5	2.5-6.0	2.0	Sulphate acidity, salinity and crabs
Deep water/floating	6	3.0-4.5	0.7-0.9	Drought, weed at seedling stage, lodging susceptibility and crabs.

Source: De Datta, 1981; WARDA, 1997, 2003.

1.6 Participatory rural appraisal

Participatory rural appraisal (PRA) is being used as tool for involving communities in the varietal development processes. It is a participatory approach, which emphasises local knowledge and assistance to local people to make their own appraisal, analysis and plans. Joshi and Witcombe (1995) reported that the participatory approach is a rapid and cost effective method of identifying farmers-preferred rice cultivars in India and it also revealed

a number of important characters that would have not been identified in breeders' experiments. Lightfoot and Ocado (1998) also reported success in using participatory approach methods in jointly developing new ways to control a crucial weed problems in the Philippines. In this study, a PRA was conducted to evaluate farmers' traits preferences, perception in relation to drought and crop management practises in Sikasso region of Mali. Participatory Rural Appraisal was conducted in this region of Mali to determine farmers' perceptions and preferences as well as management practises under drought conditions that often occur. This enabled this study to determined ways of ameliorating effects of drought and enhances upland rice production in the region.

1.7 The use of gametocides in hybrid rice production

It is difficult and labour intensive to obtain large quantities of crossed seeds from autogamous (self-fertilizing) plants like rice. The application of male-sterility inducing substances would permit the production of hybrid seeds on a large scale for commercial exploitation. Chemical hybridising agents (gametocides), which have no detrimental effects on the female organs but induce male sterility, could be used to facilitate level hybridisation programme. Gametocides have been studied in rice as shown in (Table 4).

Table 4: Gametocides used to induce male sterility in crop hybridisation

Gametocide	Crops / stage	Efficacy	Side effects	References
Ethrel	1 Rice at booting stage	High level of pollen sterility at 8000ppm	Reduced plant height, panicle length,	Aswathanarayana and Mahadevappa, 1991; 1992.
	2 Rice at spikelet differentiation and pollen mother cell formation	Reduced number of spikelets and hastened flowering at 8000ppm	Plant height, panicle length and exsertion were not significant	Manjula and Ibrahim, 1999a & b.
	3 Rice at panicle initiation	High level of pollen sterility at 10,000 and 12,000 ppm	Reduction of panicle, spikelet size	Shamsi <i>et al.</i> , 1996
	4 Flag leaf Emergence	Induced sterility at 1,000 and 3,000ppm		Chan and Cheah, 1983
Gibberellic acid	Rice at booting	High level of pollen sterility	Increased plant height, and	Aswathanarayanaand Mahadevappa, 1991;

Gametocide (GA ₃)	Crops / stage stage	Efficacy at 800ppm	Side effects panicle length	References 1992
Natrium arsenate	1 Rice at panicle initiation	Less effective in inducing pollen sterility at 800 and 1,000ppm		Shamsi <i>et al.</i> , 1996
	2 Rice at panicle initiation	Induced sterility at 3 spraying methods with 600ppm		Rao <i>et al.</i> , 1996.
	3 Rice at panicle initiation	Less effective in inducing pollen sterility at 800 and 1,000ppm	No significant effects on plant height and panicle length	Satyanarayana <i>et al.</i> , 1996.
Sodium methyl Arsenate (SMA)	1 Rice at panicle initiation	High level of pollen sterility at 500 and 600ppm	Significant reduction in plant height and panicle length	Satyanarayana <i>et al.</i> , 1996
	2 Rice at panicle initiation	High level of pollen sterility High level of pollen sterility at 500 and 600ppm	Significant reduction in plant height, panicle length and spikelet size	Shamsi <i>et al.</i> , 1996
Streptomycin	Rice at spikelet differentiation and pollen Mother cell formation	Less effective in inducing pollen sterility at 8000ppm	Reduction in plant height, panicle length and exsertion and delayed flowering	Manjula and Ibrahim, 1999a & b; Pradhan, <i>et al.</i> , 1991
Ethyl 4'fluorooxanilate and Ethyl 4'bromooxanilate	Rice, between Stamen-pistil primordial	High pollen and spikelet sterility at 1500ppm	Least phytotoxicity and high seed set	Ali <i>et al.</i> , 1999
Nirma synthetic Detergent	Rice at tillering and booting	High level of male sterility at 3% Concentration at tillering and 6% concentration at booting		Sing, 1999

Gametocide	Crops / stage	Efficacy	Side effects	References
Maleic hydrazide	1 Rice at spikelet differentiation and pollen cell mother formation.	High pollen and spikelet sterility at 6000ppm	Reduced plant height, panicle exertion delayed flowering and increased spikelet number panicle	Manjula and Ibrahim, 1999a, b.
	2 Rice at booting stage	High level of pollen sterility at 0.2%	Reduction in panicle length and poor exertion	Aswathanarayana and Mahadevappa, 1991; 1992
I1, I2, I3 and TO3 (Pyridazone derivative)	Rice at 15 days before heading	Complete male sterility	I2 and I3 decreased nucleic acids, RNA in anthers, protein and proline content, while TO3 decreased proline only	Zhang <i>et al.</i> , 1996.
FW-450	1 Beets, clovers and turnips at 0.9-8.1 mg per dm ² .	Moderate to very high degree of male sterility.	Degeneration of pollen kernels	Wit, 1960
	2 Soybean at 0-1000 ppm	Delayed flowering and sterility	Reduced meristem activity, killed terminal buds, and reduced plant height.	Starnes and Hadley, 1962

The use of gametocides such as Ethrel (2-chloro ethyl phosphoric acid) in developing hybrid varieties in cereals has also been reported (Manjula and Ibrahim, 1999a, b). They reported reduction in number of spikelets and hastening flowering for ethrel when applied at spikelet differentiation and pollen mother cell formation stages. Aswathanarayana and Mahadevappa (1991; 1992) reported high pollen sterility when ethrel and Gibberellic acid (GA₃) were applied at rice booting stage. Effective gametocides need to produce almost 100% male sterility in the female parent without affecting female fertility. The efficiency of gametocides depends on the dose, genotype and stage of application (Manjula and Ibrahim, 1999).

The major problems attributed to these gametocides are methods of application, optimum stage of application and the dose rate of the gametocides. There are environmental and seasonal, as well as phytotoxic effects, on the efficacy of these gametocides. Therefore, it becomes imperative to appraise some of these gametocides and develop appropriate

methodology for this technology in the region. Ethrel and Gibberellic acid were investigated in the current study to address some of the aforementioned problems, because they are readily available in the local markets and affordable.

1.8 Interspecific hybridisation in rice

Interspecific hybridization is a cross between two, sometimes, distinctly related species. Plant breeders undertake interspecific crosses because of need to resolve some breeding problems. Some of these reasons include the need to transfer one or more genes from one species to another, developing new character expression, producing a new level of ploidy species and determining the relationships between species (Hadley and Openshaw, 1980). In rice, *O. glaberrima* and *O. sativa* are the most frequently used species in interspecific hybridisation. This is because of their broad genetic diversification stemming from their long history of domestication and cultivation in West Africa (Carpenter, 1978; Linares, 2002). Cytological studies and a physical map of the rDNA loci on the chromosomes of *O. sativa* and *O. glaberrima* are almost identical (Ohmido and Fukui, 1995). African rice, *Oryza glaberrima*, is a reservoir of many important agronomic traits such as weed competitiveness, drought tolerance and ability to respond to low agricultural input conditions (Jones *et al.*, 1997a; Sarla and Swamy, 2005). Other wild species of *Oryza* have also been identified as rich sources of agronomically important genes such as wild *Oryza* species of *O. meyeriana* complex belonging to the GG genome, which contain genes for drought tolerance (Elloran *et al.*, 1992).

Introgression of good agronomic traits from *O. glaberrima* and wild relatives into elite lines of *O. sativa* has been reported (Jones *et al.*, 1997a; Martinez *et al.*, 1997). Jones *et al.* (1997a) reported introgression of early maturity, rapid seedling growth and high tillering from *O. glaberrima* into segregating BC₂F₃ populations in *O. sativa* crosses. Interspecific hybrids derived from the crosses between *O. sativa* and *O. glaberrima*, developed by WARDA and called NERICA (New Rice for Africa), combined some useful traits from both parents. The progenies combined traits such as high yield potential from *O. sativa*, high spikelet number from *O. glaberrima*, rapid vegetative growth and high nitrogen responsiveness (Jones *et al.*, 1997b). Dingkuhn *et al.* (1998) observed that the progenies

between *O. sativa* x *O. glaberrima* had similar grain yield, tiller number, resistance to lodging and grain shattering to the *O. sativa* parent. Transgressive segregation between 5 and 15% for yield were obtained over the recurrent parent when *O. rufipogon*, *O. barthii* and *O. glaberrima* were hybridised with improved rice cultivars (Martinez *et al.*, 1997). Alien genes such as grassy stunt virus resistance from *O. nivara* and bacterial leaf blight resistance from *O. longistaminata* have been introgressed into *O. sativa* (Jena and Khush, 2001) as have genes for resistance to blast from *O. brachyantha* into *O. sativa*.

Genes from *O. glaberrima* and other wild relative species have several advantages over other methods of gene transfer for rice improvement, as they are not hazardous and are environmentally friendly (Jena and Khush, 2001). However, the use of these species is limited due to several barriers, such as genome incompatibility and chromosome non-homology, which may limit successful gene transfer (Khush and Brar, 1992). Another barrier is the almost inviable pollen of the hybrids (F₁s), which hinders heterogenetic recombination (Brar and Khush, 1986; Jena and Khush, 1990). Studies on sterility of *O. glaberrima* x *O. sativa* interspecific hybrids conducted by Sano (1990) and a mapping analysis by Lorieux *et al.* (2000) suggested that genomic loci from the short arm of chromosome 5 (S5) might be the underlying causes for sterility of hybrids.

Some of the methods used to overcome these barriers are cytogenetic studies, which showed that fertility depends on the cytoplasm of the parent involved in the cross (Yabuno, 1977; Bouharmont *et al.*, 1985), stimulation of pollen to germinate (Sitch and Romero, 1990), use of bridging species and embryo culture (Hadley and Openshaw, 1980). Other methods include the “Contig line concept” proposed by Ghesquiere *et al.* (1997). This concept bypasses the sporogametic interaction by monitoring sterility loci using molecular markers. Also, the uses of successive backcrosses, embryo rescue through anther culture and doubled haploids (Jones *et al.*, 1997b) have been used.

Significant variations in the number of seeds set were observed between the crosses of *O. glaberrima* accessions and *O. sativa* ssp *indica* parents (Heuer *et al.*, 2003), thus suggesting importance of parents’ selection in the interspecific hybridisation. Dayun *et al.* (1997)

reported differences in crossability in crosses involving thirteen *O. glaberrima* and *O. sativa* accessions. Li *et al.* (1997) reported hybrid breakdown in interspecific F₄ populations of rice, which was largely due to additive epistatic loci, and additive-by-additive interactions for grain yield and yield components. Hybrid breakdown in interspecific crosses could be seen when a vigorous and fertile interspecific hybrid fails to perform under normal conditions and or when they give rise to very weak or sterile progenies (Hadley and Openshaw, 1980). Similarly, Yu *et al.* (1997) observed that additive epistasis was largely responsible for the grain yield and its components in F₃ populations of rice. They concluded that epistasis played a major role in the inheritance of quantitative traits as well as the genetic basis of heterosis.

Hybrid breakdown is one of the major problems of interspecific hybrids of which this study will address. This study involved a series of crosses, between some of the elite NERICAs from WARDA with other *Oryza* species to broaden their genetic base. Also, crosses involved *O. glaberrima* and improved *O.sativa* with the intention of broadening their genetic bases to overcome the problem of hybrid breakdown.

1.9 Effects of drought stress at different growth stages in rice

Vegetative stage: The vegetative growth stage has been divided into two major components; (i) the basic vegetative and (ii) the photoperiod sensitive phases. The vegetative stage starts at the emergence of rice seedling and ends with panicle initiation of the rice plant (IRRI, 1996). Seedling establishment is highly sensitive to water stress (Cruz and O'Toole, 1984). Delay in seedling emergence has physiological effects on its establishment in the field. The seedlings usually show less vigour and poor plant stand under drought stress. Singh *et al.* (1996) reported that one-week delay due to mild stress resulted in about 64 % seedling emergence in rice and grain yield was 30% lower than the non-stressed condition.

There are different opinions on the relevance of vegetative stage drought screening. Lilley and Fukai (1994) reported that effect of water deficit during vegetative stage growth may result in an insignificant reduction in grain yield. This is because the rice plant may recover

totally when favourable conditions resume. However, Mackill *et al.* (1996) argued that susceptible lines at the vegetative stage are unlikely to have good drought tolerance at the reproductive stage. Thus, susceptible lines can be discarded at the vegetative stage. Other reports have shown some relevance for drought tolerance screening at vegetative stage of rice development. Boonjung and Fukai (1996) observed that drought stress occurring close to panicle initiation reduced grain yield of rice. This is similar to the report of Wopereis *et al.* (1996) that the number of panicles was affected at maturity when drought stress occurred at mid to late tillering of the vegetative stage. Generally, grain yield is significantly associated with yield components such as panicle number per m², number of spikelets per panicle and panicle weight. Effects of drought stress on traits that are linearly associated with yield components such as leaf area index and tiller number at the vegetative stage may lead to reduction in grain yield.

Rice varieties express differential responses to drought at the vegetative stage. Some varieties may fail to produce normal panicles even when stress is relieved far ahead of the flowering stage (Murty and Ramakrishnayya, 1982). Effects of drought on morpho-physiological traits of rice at the vegetative stage have been extensively reviewed (O'Toole and Cruz, 1980; O'Toole, 1982; Lilley and Fukai, 1994). They reported that drought stress occurring at vegetative stage caused leaf drying, leaf rolling, and reduction in leaf area index, plant height, tiller number and dry matter production. These may have direct effects on grain yield. Cruz *et al.* (1986) reported that under mild water stress at the vegetative stage, rice grain yield was reduced by about 15 to 27% depending on the drought stress intensity. Drought stress occurring at the vegetative stage of rice was reported to cause delay in time to flowering. This delay depends on intensity, time, and period of drought stress (Novero *et al.*, 1985). Wopereis *et al.* (1996) corroborated this finding that delay in time to flower was longer when drought stress occurred during early tillering than mid-tillering stage.

Therefore in this study, rice genotypes will be screened for drought tolerance at the vegetative stage with the objective of identifying genotypes with potential drought tolerance for the region. This is because about 90% of the upland rice farmers that participated in participatory rural assessment experienced drought at the vegetative stage of the crop

development (Chapter 2). This is partly due to the erratic rainfall pattern during the cropping period as well as early cessation and uneven distributed of rainfall in Sikasso region of Mali.

Reproductive stage: The reproductive stage in rice is considered to be between panicle initiation and flowering (anthesis). This involves many phenological stages such as panicle initiation, panicle growth and exsertion. Other noticeable stages are booting, flowering, embryogenesis, and fertilization. Drought stress during the reproductive stage can significantly result in reduction of grain yield. When it occurs during panicle developmental stages, panicle emergence from the flag leaf sheath may be affected. Failure to emerge could lead to complete sterility of those spikelets left inside the leaf sheath (Namuco and O'Toole, 1980). Ekanayake *et al.* (1989) reported that when drought occurs during reproductive stage, panicle exsertion and anthesis are inhibited, so the spikelets are not fertilized resulting in drastic yield reduction. Moisture stress at booting and flowering also reduces dry matter production, delayed panicle exsertion, and resulted in uneven flowering (Murty and Ramakrishnaya, 1982).

Some drought response traits have been identified at the reproductive stage. Rice genotypes respond differently to drought with respect to panicle water potential (PWP) especially if drought occurred just before flowering. Pantuwan *et al.* (2002) reported that genotypes that maintain high PWP during the drought stress just before flowering are associated with high grain yield, harvest index, and drought responsive index (DRI). Bidinger *et al.* (1987) developed this relationship as $DRI = (\text{actual yield} - \text{predicted yield}) / \text{standard error of the predicted yield}$. They used DRI to identify lines with tolerance or susceptibility to drought stress.

When drought occurs from the heading stage up to anthesis period, it causes spikelet sterility, which results in poor pollen shedding and subsequent failure of pollination (Ekanayake *et al.*, 1989, 1990). High-temperature stress has also been shown to prevent dehiscence of anthers. The ability to maintain anther dehiscence under exposure to high temperature is under the control of a few genes with additive effects (Mackill and Coffman, 1983). The mechanism that controls these traits appears also to confer tolerance to drought

induced spikelet sterility (Mackill and Ekanayake, 1986). The phenology in terms of time to flowering or time to maturity has been identified as a constitutive trait. Constitutive traits are traits that do not require drought responsive genes and / or stress conditions for expression (Blum, 2002). These traits could be manipulated in the development of rice genotypes for intermittent or late season drought environments.

Maturation stage: The maturation stage of rice is regarded as the period between anthesis and harvest. Significant variation exists among genotypes in response to drought stress during this period. Tolerance to drought stress is often expressed in terms of per centage reduction in yield between stressed and non-stressed conditions. Dikshit *et al.* (1987) reported that a long dry period during the maturation period reduced the grain yield by 10-91%. They found a significant correlation between maturity prolongations and yield reduction due to drought stress. Kobata and Takami (1983) observed that when drought stress occurred at maturity, the effect on total grain yield was less severe as compared to the vegetative and reproductive stages. This may be attributed to assimilate redistribution to the essential parts of the plant during this period. At the ripening phase, drought stress induces spikelet sterility, while at the grain filling stage; grain weight is also reduced (O'Toole, 1982). Maintenance of high leaf water potential and delayed leaf senescence has been identified as desirable traits during this stage (Murty and Ramakrishnayya, 1982).

1.10 Mechanisms of drought tolerance

The mechanisms of drought tolerance in the field have been described as escape, avoidance and tolerance (Levitt, 1972; Turner, 1986). Drought recovery is another mechanism.

Drought escape mechanism: Drought escape in this context could be related to the ability of a rice genotype to escape harsh environmental conditions by early completion of its life cycle. Turner (1986) defined drought escape as a phenological development of the plant matching the available soil water conditions. According to O'Toole and Chang (1978), drought escape is the most effective adaptive mechanism as far as rice productivity is concerned. Rice grain yield has been increased and stabilised in drought prone areas by growing early maturing varieties to match the rainy period (Mackill *et al.*, 1996). Turner

(1979) presented three drought escape mechanisms that enable crop plants to minimise the effects of drought. They are early maturity, developmental plasticity such as asynchronisation of tillers with plant development and remobilisation of stem reserves stored before anthesis into the grain. Another method suggested by O'Toole and Chang (1978) is to use photoperiod sensitive cultivars whose reproductive stages are photo-periodically controlled to coincide with favourable conditions, therefore, allowing the crop to complete all the phenological events under adequate water regime. This current study selected genotypes that are early maturing and evaluate them for drought tolerance.

Drought avoidance mechanism: Drought avoidance represents the plant's ability to maintain a high level of water status or turgor under conditions of increasing soil moisture deficit. According to O'Toole (1982), an avoidance strategy involves genotypes gaining access to additional resources. These resources are reserves of soil water from soil layers already explored, or from deeper soil layers, which have not been previously explored. Fukai and Cooper (1995) reported that improving root traits for gaining access to additional reserves was more important than improving shoot traits for reducing loss of reserves.

However, there is no identifiable single plant trait conferring stress avoidance. A combination of traits is involved (Turner, 1986; Acevedo, 1987). Genotypic variation in the root system of rice and other crops has been reported (O'Toole and Bland, 1987). Some root characteristics such as root length, depth and density have been associated with the maintenance of a high level of internal plant water status under upland conditions (O'Toole and Chang, 1979; Ekanayake *et al.*, 1985a). Root length and density has also been associated with cultivars with high soil water extraction ability under moisture stressed conditions (Puckridge and O'Toole, 1981; Lilley and Fukai, 1994). Cultivars with dense roots maintained high levels of internal plant water during the stress period. The root density largely determines the extent to which roots can extract water from the adjacent soil. Root pulling resistance has been used as a rapid method to screen for drought avoidance. This is because high root pulling resistance was associated with high leaf water potential (O'Toole and Bland, 1987). The use of root pulling resistance in the study of root depth variation under lowland conditions showed that the force required to pull the plant is positively

correlated with the portion of the root system that remains in the soil (Ekanayake *et al.*, 1986).

Other traits that have been implicated in the avoidance mechanism are deep roots, root thickness (larger diameter) and low hydraulic resistance in the root system (Ekanayake *et al.*, 1986). Deep rooting is significantly associated with drought avoidance (McWilliam, 1989). Deep roots enable the plant to extract water beyond rooting zones into the subsoil. Drought avoidance under field conditions is significantly correlated with high root-to-shoot ratio of rice varieties. This ratio is a measure of a plant's ability to absorb water from deep soil layers (Yoshida and Hasegawa, 1982). The root axial resistance can be improved upon by breeding for larger xylem vessels in the main seminal root (Passioura, 1982). Fukai and Cooper (1995) reported that root axial resistance could be important in determining the rate of water flow from the root to the shoot. Therefore, a high level of resistance may restrict water extraction from deep soil layers. Haque *et al.* (1989) observed that root diameter was positively correlated with cross sectional area of the xylem vessels and negatively correlated with drought score (leaf drying).

As the root system is an undisputably important characteristic in relation to drought avoidance, its exploitation in developing drought resistant rice cultivars will enhance rice production in the region. Therefore, in this study root biomass in relation to drought will be examined during stress and after stress relief.

The first noticeable morphological change with the onset of drought is leaf area reduction. Shedding leaves or death of leaves and reduction in the leaf size could cause leaf area reduction. Water stress decreases the leaf area, which reduces the intercepted solar radiation thus affecting the rate of photosynthesis (Singh and Mackill, 1991).

Leaf rolling score under stressed conditions could be used reliably as an estimator for rice dehydration avoidance (O'Toole and Cruz, 1980). However, leaf rolling is often regarded as a drought avoidance mechanism (O'Toole and Chang, 1978) and /or a symptom of the stress level of the plant (Henderson *et al.*, 1995). In rice, leaf drying and leaf rolling are criteria

often used in assessing levels of drought tolerance. O'Toole and Moya (1978) reported that the use of visual scoring techniques, based on either leaf rolling or leaf drying, were highly correlated with leaf water potential. A limitation on the use of visual scoring as an index for drought tolerance is that it does not distinguish between drought tolerance and avoidance mechanisms (Ingram *et al.*, 1990).

Stomatal closure is regarded as a drought avoidance mechanism in rice for reducing water loss under water stressed condition (O'Toole and Cruz, 1980). Genetic variation exists within rice genotypes in the sensitivity of stomatal conductance to leaf water status (Dingkuhn *et al.*, 1989; Price *et al.*, 1997). The relationship between stomatal conductance and crop performance in the field under drought condition is yet to be established. However, rapid stomatal closure is believed to slow down transpiration and carbon dioxide movement and thus reduced photosynthesis and water use efficiency.

Cuticular resistance is believed to be associated with epicuticular wax deposit in rice. This association may be responsible for cultivar's drought avoidance in dryland conditions (O'Toole *et al.*, 1979). Leaf orientation to the incoming radiation under water stress conditions has been used as an indicator for drought avoidance in rice. This is based on the principle that leaf orientation would reduce the radiation load on the leaves. Therefore, it allows the plant to dissipate less energy as latent heat (Turner, 1982). The importance of leaf orientation to drought avoidance is yet to be known.

Leaf canopy temperature in rice can be measured by infrared thermometer. The canopy temperature has been used as a drought avoidance indicator (Maurya and O'Toole, 1986). This is based on the principle of the cooling effect of transpiration as high transpiration rate occurs in the canopy under lower temperatures. Genotypic variation exists within rice for canopy temperature and this variation is related to leaf water potential in rice (O'Toole *et al.*, 1984). Canopy temperature of rice genotypes under drought stress conditions is negatively correlated with midday leaf water potential and turgor pressure (Turner *et al.*, 1986). Canopy temperature at anthesis is linearly correlated to spikelet sterility (Maurya and

O'Toole, 1986). An increase in canopy temperature had as much as 0.2 per cent increases in spikelet sterility (Chang and Loresto, 1986).

Under drought condition, abscisic acid (ABA) concentration in the leaves increases rapidly; often several fold (Austin *et al.*, 1982). Varietal differences in the accumulation of ABA exist in rice, but its relationship with drought avoidance is yet to be established (Austin *et al.*, 1982). Innes *et al.* (1984) observed that varieties of wheat respond to drought by producing high concentration of leaf ABA. These varieties do use less water and produce higher yields under drought stress. Abscisic acid in the leaf is involved in the regulation of stomatal conductance. It is believed that ABA in the leaf enhances osmotic potential of the guard cells resulting in stomatal closure. This may lead to reduction in transpiration and rate of photosynthesis (Boyer and Mcpherson, 1976). Metabolites such as proline and protein-nitrogen have also been used as drought avoidance indicators in rice. Accumulation of proline in plant parts of rice during water stress has been observed (O'Toole, 1982), while total-nitrogen and protein-nitrogen decrease as the amino-nitrogen increases in the plant shoot under drought stress in barley (Tully *et al.*, 1979). The roles of these metabolites under drought stress are still unknown.

Water stress significantly affects plant biomass production. Biomass production decreases linearly with increasing stress intensity (Novero *et al.*, 1985; Turner *et al.*, 1986). It is almost an irreversible trait when affected by drought stress. As biomass production is a function of water use efficiency, its reduction may ultimately limit potential grain yield of rice even when favourable conditions return (Begg, 1980; Puckridge and O'Toole, 1981).

The shoot is very important in plant productivity. Some morpho-physiological traits of the shoot have been identified as drought responsive. Incorporating these traits into the breeding programmes in developing drought tolerant varieties will enhance rice production. Therefore in this study, secondary traits such as leaf drying, leaf rolling, leaf area index and some other shoot parameters were examined under drought stress and non-stress conditions.

Drought tolerance mechanism: Drought stress tolerance represents the plant's relative ability to sustain the least injury to life functions at decreasing levels of tissue water status or turgor. Drought tolerance usually involves the development of low osmotic potentials. It is one of the major characteristics of plant species found in arid environments (Morgan, 1984). Osmotic adjustment is considered to be the net increase in intracellular solutes that usually occurs in response to various environmental stresses (Steponkus, 1980). The rate of osmotic adjustment is a function of stress intensity. Osmotic adjustment, or accumulation of solutes by cells, is a process by which water potential can be decreased without an accompanying decrease in turgor (Taiz and Zeiger, 1991). This has been considered to be a drought tolerance mechanism and genotypic variation for osmotic adjustment has been found in many crops including rice (Lilley and Ludlow, 1996).

Changes in dehydration tolerance occur with different rates of tissue drying. Slow rates of tissue drying enhance dehydration tolerance, possibly due to the accumulation of solutes such as sugars and proline that protect proteins and aid in recovery. Another advantage gained when tissues experience osmotic adjustment are leaf rolling and drying at lower leaf water potential than when tissues do not adjust. Thus, osmotic adjustment allows photosynthesis to be maintained longer under moisture stress (Hsiao *et al.*, 1984).

Leaf elongation is one of the major activities during plant development that increases the interception of solar radiation and may lead to high grain yield. Leaf elongation is very sensitive to drought stress and could stop in a severe drought condition. This phenomenon is regarded as a drought avoidance mechanism in rice (McWilliam, 1989). This causes reduction in the leaf area index (Dingkuhn *et al.*, 1989; Singh *et al.*, 1995). In rice, there is a significant linear relationship between leaf area and specific leaf dry weight. High specific leaf dry weight (SLDW) is associated with thicker leaves and a significant negative relationship was also found between SLDW and transpiration relative to the control (Cabuslay *et al.*, 1999). Larger SLDW is correlated with higher net assimilation rate (Ohno, 1976). As transpiration is primarily related to leaf area, increasing photosynthetic rate per unit leaf area could be another way of enhancing water use efficiency. Therefore, selection for higher SLDW under water stressed conditions may lead to higher grain yield in rice.

Genotypes that could maintain high leaf area under drought stressed condition would be desirable as an indicator of drought tolerance (Mc William, 1989).

Dehydration tolerance is reported to be stable from generation to generation and is related to field performance under drought. It is strongly developed in species and cultivars that do not have dehydration avoidance mechanisms (O'Toole *et al.*, 1978). Thus, morpho-physiological characters of both root and shoot systems could be exploited to develop multiple drought tolerant cultivars that would combine both drought avoidance and tolerance mechanisms.

Drought recovery mechanism: Drought recovery is often defined as the ability of a plant to resume growth and yield after drought stress, with a minimum of yield loss. This is observed to be heritable (O'Toole and Chang, 1978). Fast recovery rates from drought stress have been reported to be significantly associated with high tillering ability, less leaf rolling and unrolling, and low drought score (Chang *et al.*, 1982). A fast recovery rate is also associated with delayed heading, decrease in grain size and weight. Thus, drought recovery ability is an important determinant of grain yield (De Datta and Seshu, 1982) and could be more important than drought tolerance under mild stress. The positive relationship between drought tolerance and drought recovery ability could be used to assist rice farmers to predict plant population when rain or irrigation resumes (Malabuyoc *et al.*, 1985). Genotypes with high drought recovery ability would minimize hazards of total crop failure. The genotypes that can recover from drought stress will produce more yield as compared to genotypes that do not recover. This study screened genotypes for fast recovery rate and associated traits.

1.11 Screening methodology for drought tolerance in rice

Selection of screening environment depends partly on the way in which drought tolerance is to be assessed. If the screening is targeting a yield-based drought tolerance, then, the environmental conditions in the screening environment must be a duplicate and / or a replica of the target environments that affect yield (Bidinger, 2002). If drought stress screening is targeting stress responses or tolerance mechanisms, then the use of dry season or more arid locations would be ideal. This is because natural occurrence and severity of stress in these locations can be controlled through management of irrigation or the sowing date. Drought

stressed environments can be created artificially by using rainout shelters but this is feasible only for small-scale screening. Planting during the off-season or in dry locations (hotspots) could also be used to simulate drought. These environments have advantages of screening large numbers of materials, as they are reliable and cheap. However, genotype-by-season interaction effects may confound drought effects. Thus, on whichever basis drought stress screening was conducted; the screening environment must be a representative of the target environments.

Drought screening methodology adaptable at the vegetative stage of rice: Protocols have been developed for drought tolerance screening at the vegetative stage. Some protocols are glasshouse and field based. Maurya and O'Toole (1986) reported root box and hydroponic culture techniques in the screening for root characteristics in the glasshouse. In the root box technique, seedlings are established either in a wooden or metal box. The seedlings then grow into a desirable stage for evaluation. Root-shoot ratio and vertical distribution of roots of different rice varieties have been studied using these techniques. Traditional upland rice varieties have been identified with high root-shoot ratios, while significant positive correlation was found between lower root-shoot ratios with rice varieties that are drought susceptible. High root-shoot ratios correlate with rice varieties that are field tolerant to drought stress (Yoshida and Hasegawa, 1982). Ekanayake *et al.* (1985b) used hydroponic culture for root inheritance studies. They found that root characters such as length, mass, density and thickness were associated with high level of plant water status.

Chang *et al.* (1974) proposed mass screening techniques for drought tolerance evaluation at the vegetative stage under upland field conditions. This technique involves irrigating the test materials for 40 days and then withholding water for 20 days to simulate drought. Water management during the vegetative stage is very important, especially in the timing of stress imposition. Pantuwan *et al.* (2004) withheld water for 46 days after sowing to simulate drought in a lowland rice ecology, while water was withdrawn at 30 days after seedlings emergence, in upland and irrigated rice ecologies (Malabuyoc *et al.*, 1985; Singh and Mackill, 1991). However, imposition of drought stress at the maximum tillering stage gives effective drought tolerance screening for any rice-growing environment (Yoshida, 1981).

During the stressed period, morpho-physiological characters of the entries are evaluated. Such characters are leaf rolling, leaf drying, leaf area, plant height, tissue death, and recovery ability after stress relief. This information is then used for the selection of drought tolerance. As imposition of drought stress at maximum tillering is considered to be more effective, this study imposed drought stress at maximum tillering at the vegetative stage growth of rice.

Drought screening methodology adaptable at the reproductive stage of rice: Screening for drought stress at the reproductive stage of rice involves techniques that are different from the vegetative stage. Garrity and O'Toole (1994) suggested a mass screening method by staggering the planting date of the genotypes during the dry season. This is to synchronize flowering dates of the genotypes. This is because when water is withdrawn, the early maturing genotypes may escape the severity of the drought stress as compared to late maturing ones. Rice is very sensitive to stress at flowering. Therefore, flower synchronisation among genotypes is very important but difficult to achieve. It requires accurate information on flowering time of the genotypes to be tested (Lafitte, 2003), which is a major drawback of this technique. Garrity and O'Toole (1994) advised that the identified genotypes for drought tolerance in the dry season should be screened for yield performance in the target environment during the wet season.

Fukai *et al.* (1999) proposed a wet season field screening technique for the reproductive stage of rice. The technique involves two separate experiments; stressed and non-stressed during flowering. The stressed experiment evaluates the genotypes performance under stressed conditions using rainout shelters, while non-stressed experiments provide information on yield potential of the genotypes. These concurrent evaluations for drought stress and yield performance could offer an advantage over the dry season screening technique. This technique however requires larger experimental plots, seed lots and labour.

In conclusion, whichever screening protocols are to be adapted for drought tolerance screening, the protocols should be effective and reliable. Their effectiveness would depend

on the ability to identify high heritability and genetic variations for the screening in the target environments.

1.12 Genotype by environment interaction and stability of rice across environments

Breeders are interested in the development of rice genotypes, which can adapt to a wide range of environments. These rice genotypes should perform well across environments in which they are grown. That is, they should show less genotype by environment (G x E) interaction over these environments, so that their performance could be considered to be stable. Genotypes by environment interactions are present when there is a change in the relative performance of a genotype across environments. This interaction could influence the nature and selection of a desirable genotype across environments. Genotype by environment interaction often confounds the effective selection for desirable traits. Environments used by breeders are usually better than the farmers' environments and selection under these environments may not produce stable genotypes. Lawrence and Senadhira (2001) suggested that selection for stable genotypes should be done initially in poor environments, then, further stability testing could be carried out over a wide range of intended target environments. This could be feasible, however, most research institutes has a wide range of ecologies whereby the target environments could be simulated, thus reducing the cost of screening. Multi-location trials can also be used to enhance selection of stable genotypes. Fuakai and Cooper (2002) reported large G x E interaction for grain yield of rice under rainfed lowland conditions.

Undoubtedly, G x E interactions confound the selection achievable for desirable and stable genotypes in a breeding programme. Many methods have been used for analyses and interpretation of patterns of G x E interactions (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966). As stability of traits like yield across environments is of utmost importance to breeders, Finlay and Wilkinson (1963) proposed a model to test the stability of genotypes over a wide range of environments by regression. The observed value of the genotypes (yield) is regressed on the grand mean of the environment (environmental index); the regression coefficient is thus regarded as a stability parameter. Eberhart and Russel (1966) proposed a deviation from the regression line as a stability parameter. Based on these

models, they defined a stable genotype as having unit regression coefficient over environments ($b = 1.0$) and minimum deviation from the regression line ($S^2_{di} = 0.0$). Genotypes with regression coefficient greater than one ($b > 1.0$) are regarded as sensitive to changes in the environments and have below average stability. These genotypes are said to be better adapted to favourable environments. While genotypes with regression coefficient less than one ($b < 1.0$) are said to be less sensitive to changes in the environment and have above average stability. These genotypes are said to be specifically adapted to poor environments. The mean value of the genotypes across environments is another important factor to be considered in the determination of genotype stability. For instance, high mean yield value across environment is preferable to low mean yield in stability determination.

Drought environments cause a reduction in genetic variance and heritability estimates for yield (Fukai and Cooper, 1995). Effective management of stress environments could assist in reducing the effects of G x E interaction and improved heritability estimates of traits. This is because the magnitudes of the G x E interaction are linear function of the environmental effects. Stress environmental errors could be managed by increasing the number of replications within and across environments, increasing the number of years and environments for drought stress screening. Significant variation existed within rice genotypes in response to drought stress. Therefore, good physiological knowledge is required to differentiate patterns of response of genotypes to environmental stresses to enhance selection efficiency of desirable genotypes. This study evaluated genotypes in two different stress regimes: stressed and fully irrigated water regimes for the selection of stable genotypes across the different stress regimes.

2.13 Selection and target environments

Due to some difficulties such as inadequate drought screening facilities in the target environments, genotypes are screened and selected for drought stress tolerance outside the target environments. Bidinger (2002) emphasised that the selection environments (S) should be designed to be representative of the target environments (T). Selection environments could only be useful if they could predict reliability of performance of a genotype in the target environment (Atlin, 2003). This could be difficult to achieve in relation to drought stress as drought stress severity varies with locations and season (Cooper, 1999). However,

Atlin (2003) indicated that it could be possible to predict genotype performance if selection for drought tolerance was based on genetic correlation between selected and target environments. Atlin (2003) suggested various methods of maximizing genetic correlation such as selecting genotypes that perform well both in drought stressed and non-stressed conditions. The genetic correlation (R_g) is calculated as described by Atlin (2003) as:

$$R_g = \text{Cov}(S, T) / \text{square root of } \delta^2 S \times \delta^2 T; \text{ where Cov = covariance and } \delta^2 = \text{variance.}$$

Heritable secondary traits under drought stressed conditions are considered reasonable to predict genotype performance in the target environment (Banzinger *et al.*, 2000). Atlin (2003) and Banzinger *et al.* (2000) suggested secondary traits that could help to identify drought tolerant genotypes under drought stressed conditions. They proposed traits that are highly heritable during grain filling, traits that are associated with high grain yield and increased grain yield.

This study evaluated genotypes using two types of moisture regimes, drought stressed and non-stressed (fully irrigated). The drought stressed was used to predict performance under drought stressed conditions while the non-stressed predicted performance under favourable conditions.

Conclusion

The *O. glaberrima* is a reservoir of genes reported to be tolerant to many biotic and abiotic stresses of the region, they were used to improve the cultivated rice cultivars in the region. This current study engaged farmers through a PRA in investigating their farming systems and production ecologies (target environments) in Sikasso. The application of male-sterility inducing substances would permit the production of hybrid seeds on a large scale for commercial exploitation. Effective gametocides need to produce almost 100% male sterility in the female parent without affecting female fertility. The efficiency of gametocides depends on the dose, genotype and stage of application. This study appraised ethrel and gibberellic acid as gametocides or hybridising agents in hybrid rice production. Drought stress could occur at any stage of rice development and its effects depended on genotypes,

severity and seasons. Morpho-physiological traits that have been identified to be associated with drought stress were explored in this study to screen rice genotypes at the vegetative stage for drought tolerance. Genotypes and traits were identified that exhibited different mechanisms of drought stress tolerance.

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CHAPTER 2

Farmers' perceptions on rice varieties in Sikasso region of Mali and their implications for rice breeding

Abstract

Rice has become a staple food as well as a source of income to many farmers in Mali and the rest of West Africa, but for various factors production does not match the demand. Rice varieties developed to date for the region have not taken into account farmers' preferences and perceptions on varieties and management practices. As a result, farmers have adopted few of the improved rice varieties that have been released, suggesting that farmer's perceptions and preferences might not coincide with breeding objectives. This study was therefore conducted to determine farmers' preferences for rice cultivars, and perceptions on drought stress and management practices. Participatory rural appraisal (PRA) was conducted in Sikasso region in September 2005. A total of 125 farmers, comprising 68 males and 57 females, were randomly selected from 10 villages in the upland, lowland and irrigated ecologies. The PRA tools included individual interviews, group discussions and transect walks across the fields. Results indicated that farmers' preferences, crop management and ranking of production constraints differed significantly across ecologies. The most preferred trait in upland ecology was short duration rice and tall plants. In terms of grain quality, long red grains were preferred in the upland ecology. In the lowland ecology, tall varieties were most preferred followed by short duration and long white grains. In irrigated rice ecology, high grain yield was the most preferred trait followed by long duration, but were indifferent about grain quality. Seemingly, farmers in the lowland and upland ecologies were prepared to trade off yield for grain quality and plant height, which might be different from breeders' selection criteria. Farmers' high preference for tall varieties in the upland and lowland ecologies was also in sharp contrast with the model of dwarf rice varieties responsible for the green revolution in Asia. These findings suggest that a different plant idiootype would be required. Generally, drought was perceived to be a major problem causing an estimated yield reduction of about 60% in all ecologies, indicating the need to breed for drought stress tolerance. Common drought management strategies that were employed by farmers included planting of early maturing cultivars that escape late season drought and use of drought tolerant varieties. Sole cropping was practiced in all the ecologies. There were differences in the number of farmers who applied fertilizer, which increased from 4% in the irrigated, 26% in lowland to 73% in the upland ecologies. Female farmers were more predominant in the rainfed lowland and irrigated ecologies than the upland ecology. Our results suggested that the best breeding strategy would be ecosystem breeding for each of the ecologies.

2.1 Introduction

Mali is a landlocked country located in the interior of West Africa between 12° W and 4° E longitude and 10 and 25° N latitude. Mali is bounded on the north by Mauritania and Algeria, to the east by Niger, to the south by Burkina Faso, Côte d'Ivoire, and Guinea, and to the west by Senegal. A decade ago, there was a noticeable increase in rice production in Mali. This increase was attributed to an increase in cultivated land area and better floods along the Niger River (FAOSTAT 2005). In 2005, rice production estimate in Mali was 718,086t on a cultivated area of 451, 000ha. Although cultivated areas have expanded, there was a marginal increase in average yield from 1.5 to 1.6 t ha⁻¹. The effects of biotic and abiotic stresses were attributed to limiting the increase in average yield. Literature showed that biotic stresses such as rice yellow mottle virus, blast and rice gall midge and weeds and abiotic stress including drought are the most devastating stresses reducing yield (WARDA, 1997). Rice consumption rate has increased in West Africa and Mali in particular. Rural-urban migration and women moving into the work force were attributed to the sudden shift in consumer preference for rice. In Mali, the per capita consumption rate of rice (kg yr⁻¹) has increased from 34.6 in 1990 to 44 in 1995. Therefore, there is a production gap that is being supplemented by importation of about 75 000t (FAOSTAT, 2001).

Increasing average production potential would be an important step in closing this production gap. This would involve a series of activities such as knowing the causes of low adoption rate of the varieties of rice in the region, farming systems and production constraints. In Sikasso region rice culture methodology varies from one community to another. These variations include methods and time of sowing, cropping systems and ways of preparing rice for eating. The low adoption rate of rice varieties by the farmers in this region could be attributed to the lack of understanding of these variations of rice culture in the region. In some communities, rice is intercropped with maize or sorghum and rice panicles are harvested with knives, tall variety rice would be easily be adopted as compared to dwarf rice variety. Some communities prepared rice into paste for eating. Rice varieties with high amylose content would be the most preferred in these communities. Therefore a good knowledge of the rice production systems in this region would enhance its production.

This necessitated this study to conduct a participatory rural appraisal in Sikasso region of Mali.

The main rice ecologies in Mali are rainfed upland, rainfed lowland, irrigated lowland, and flood prone. These ecologies depend on the amount of rainfall and the flooding along the Niger River. There are five major zones of rice production of which four are located along the floodplain of river Niger (Figure 1). It is not known if farmer preferences differ in these contrasting rice ecologies.

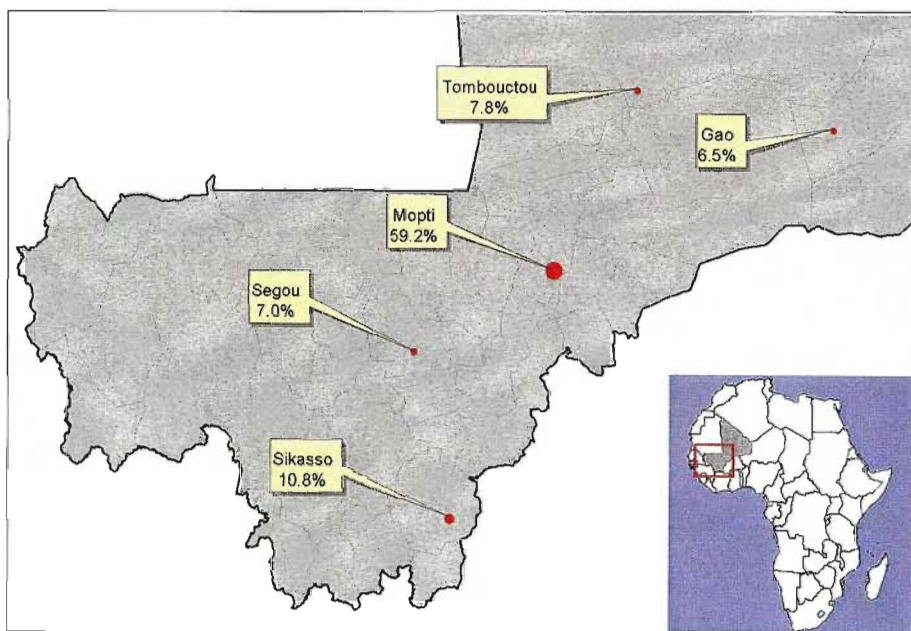


Figure1. Major rice production zones and their per cent of the national area under rice cultivation of Mali

2.2 The objectives of this study were:

To determine farmers' variety traits preferences, perceptions in relation to drought and crop management practices in Sikasso region of Mali.

2.2.1 Hypothesis

Farmers in Sikasso region of Mali are aware of their variety traits preferences and production practices. These variety preferences coincide with breeders' objectives.

2.3 Methodology

2.3.1 Description of the study area

This study was conducted in Sikasso region of Mali (Figure 1). This region is a semi-arid transition zone with annual rainfall of 1100mm. Rice is produced in rainfed lowlands in the form of seasonally flooded valley bottoms, rainfed upland and irrigated. This region accounted for 10.8% of the national harvested area (FAOSTAT, 2001) and also over 80% of upland rice production. The popular upland rice varieties grown in the area are Dourada precoce, IRAT 10, IRAT 13 and IRAT 112. These varieties are commonly grown in combination with maize, millet, sorghum and cotton.

The activities of NGOs (Non Governmental Organization) compliment the state extension services in the distribution of improved rice seeds in this region. Although, there is an appreciable increase in rice production and area cultivated in the region (IER, personal communication, 2004), the maximum production potential has yet to be attained. In the region, less than 10% of the cultivable land for upland rice is put into use. These drawbacks in rice production are attributed to production constraints such as drought, weeds, lack of good quality seeds and absence of farmers' trait preferences in the varieties. This makes this region favourable for the conduction of a Participatory rural appraisal (PRA).

The PRA was conducted in September 2005. This is the peak period of upland rice production activities. This enabled this study to extract maximum information about rice production in the region. Sikasso town is centrally placed among the participating villages. The farthest village from Sikasso town is about 53 km, while the nearest is about 15 km (Figure 2). In terms of production ecology, the study covered upland, lowland and irrigated rice ecologies.

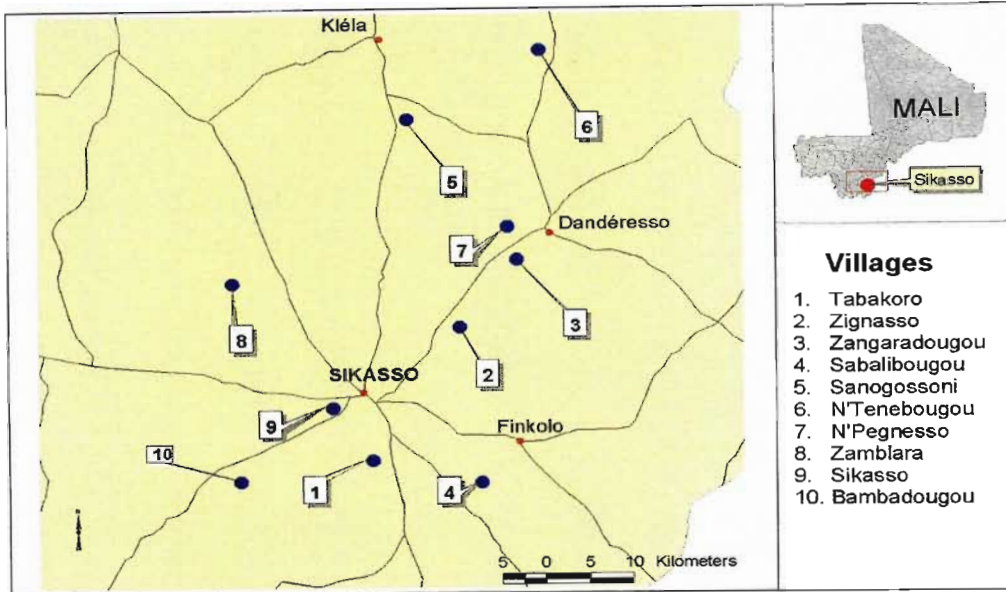


Figure 2: Villages under appraisal and location within the region

2.3.2 Sampling procedures

This study was conducted in collaboration with Mali agricultural institute (Institut de Economie Rurale (IER) du Mali) in Sikasso region. Institut de Economie Rurale agricultural extension unit at Sikasso presented a list containing more than 30 villages of potential rice growers in the region. Ten villages were randomly selected from the list for the appraisal (Figure 2). A visit was made to these villages for the selection of participating farmers and fixing dates for the appraisals. The visiting team was comprised of the principal investigator, a social officer and two extension officers from IER. In each village, the team met with the village heads and briefed them of the mission. The village heads then summoned meetings of the clan leaders and encouraged them to cooperate and participate in the appraisal. In most of the villages, all the rice farmers were eligible to participate, because of their small number (Table 1). The only exception was where a village had more than twenty rice farmers. In this case, a number of twenty farmers were randomly selected for the appraisal. The total sample size was 125, which comprised 68 male farmers and 57 female farmers (Table 1). This represented 54.4% and 45.6% of male and female farmers, respectively.

Table 1: Total number of farmers and gender number in each village surveyed

Village	Male	Female	Total number of farmers per village
Tabakoro	14	2	16
Zignasso	7	11	18
Zangaradougou	7	7	14
Sabalibougou	3	3	6
Sanogosso	9	2	11
N'Tenebougou	9	11	20
N'Pegnesso	7	5	12
Zamblara	2	9	11
Sikasso	1	0	1
Bambadougou	9	7	16
Total number of farmers	68	57	125

2.4 Data collection

2.4.1 Rapport building and interview techniques

Team members: The team comprised five members, the project coordinator, agronomist and agricultural extension agent from Institut de Economie Rurale (IER) du Mali in Sikasso, Social officer/gender expert and field technician from WARDA upland rice programme. The team members met for a brainstorming session to determine what information was necessary and how it could be best obtained. Interviewing techniques, topics and sub topics and of areas to be investigated were developed to guide the discussion (Table 2). We also sensitized ourselves on rapport building as suggested by Pokharel et al. (1997). This was achieved by allowing sufficient time with the participants, listening to them, learning more about issues under consideration and showing genuine interest in the local issues. Most importantly farmers were treated and respected as per their local custom. For example, farmers were greeted first in their local language. Rapport building created an enabling environment for the team to collect reliable information from Sikasso region.

Table 2: Topics of information obtained and techniques used during the interview

(a) Information on rice production	Techniques used during interview*
Sowing date	Iteration and probing
Methods of sowing	Iteration, probing and observation
Information on fertilizer application	Iteration and probing
Weed control	Iteration, probing and observation
Access to credit facility	Iteration and probing
Rice ecology	Iteration and probing
Land preparation	Iteration and probing
Source of agricultural information	Iteration and probing
Farmers association	Iteration and probing
(b) Information on managing drought stress	Techniques used during interview*
Cropping system	Iteration, probing and observation
Varieties cultivated and number	Iteration, probing and observation
Preferred traits and why?	Preference and pairwise ranking
Area cultivated	Iteration, probing and observation
What stage of plant growth drought stress set in?	Iteration and probing
Drought coping mechanisms	Iteration and probing
Grain yield in absence of drought	Iteration and probing
Grain yield when there is drought	Iteration and probing

2.4.2*Techniques used during interview

The techniques used during interview as described by Grandstaff and Grandstaff (1989) were:

Iteration: Iteration is a technique whereby the same question is repeatedly asked in different situations to confirm the given information. The ability to reframe and formulate a new

question has better advantages, especially within the interview context. This method was very helpful as most of our participants were illiterate. Questions were often reframed more than two times before arriving at the farmer's real opinion.

Probing: The main function of a probe is to encourage the respondent to answer more fully and accurately. Furthermore, it helps to structure the respondent's answer and makes sure that all topics of interest are covered. The principles of the "six helpers" (who, what, where, why, when and how) to establish the basic situation were employed. For example, to determine the area cultivated, farmers were asked "what is the land area cultivated", and "how much grain was harvested". The grain yield was therefore related to the land area cultivated.

Observation: This involved transect walks along farmers' fields and direct observations of rice storage facilities. Random measurements of area cultivated were taken in some farmers' fields. This technique was used to verify the collected information generated from the individual interview and group discussions.

Preference ranking: This allows the PRA team to determine the preferences of individual farmers. Farmers were asked to list some of the important traits of the rice varieties they were sowing or had grown before. By iteration and probing, farmers were able to put these traits into preference ranking.

Pairwise ranking: Pairwise comparison of some rice varieties was conducted to determine why one variety was preferred over another. The results enabled the PRA team to understand the traits farmers used to differentiate varieties

2.4.3 Methods employed for data collection

Three methods were employed in collecting data: focus group discussions, focus group questionnaires and field visits. A semi-structured interviewing method was employed to guide some specific questions during the interview (Table 2).

Group discussion: Group discussion was held to obtain community level information in an informal way at each village (Figure 3). The discussion was guided by a semi-structured questionnaire to avoid distraction and ensured that the main objectives were addressed during the discussion. However, farmers were allowed to raise their own issues for discussion. All the selected rice farmers in each village participated in the group discussions.



Figure 3: Group discussion at Tabakoro village in Sikasso rice growing zone

Individual interviews: Individual farmers were interviewed separately. This enabled individual farmers to express their own opinions without any influence from the community (Figure 4). During the interview, practical demonstrations and drawings were frequently used. For instance, the team used 50 kg of jute bags to demonstrate the quantity of harvest. This enabled the estimation of the actual grain yield at harvest.



(a)



(b)

Figure 4: Individual discussion at Zangardougou (a) and Zamblara villag (b)

Field visit: The field visits were conducted to verify some of the information given during group discussions and individual interviews. This involved transect walks and direct observations. Two farmers and one farmer's association fields were randomly selected per village for the field visits. The research team and the farmers had a transect walk across the field. Random measurements of the area cultivated were taken in some farmers' fields. The team visited some of the rice storage facilities and observed the quantity and quality of rice grain. One of the field visits was to a rice farmers association (Figure 5).



Figure 5: Field visit to women association farm of Zamblara village

2.5 Data analysis

The data generated from the group discussions, questionnaires and field visit were used for the analysis. Mean values and frequency distribution expressed in per centages were calculated using excel computer software (Microsoft, 2000).

2.6 Results

The appraisal exercise was conducted for five days and two villages were appraised per day. During the appraisal, we observed that farmers from three rice ecologies: rainfed upland (UPL), rainfed lowland (LWL) and irrigated (IR) were represented (Table 3). There were also farmers who cultivated more than one of the three rice ecologies. In general, 58%, 23%, and 18% of the participating farmers were cultivating in lowland, upland and irrigated rice ecologies, respectively (Table 3).

Table 3: Number of participating farmers per village and ecology

Village	UPL*	LWL*	IR*
Tabakoro	16	0	0
Zignasso	3	15	0
Zangaradougou	1	13	3
Sabalibougou	2	4	0
Sanogossoni	1	10	0
N'Tenebougou	1	16	8
N'Pegnesso	3	10	0
Zamblara	5	7	1
Sikasso	1	0	0
Bambadougou	0	7	14
Total	33	82	26

* UPL = upland , LWL = lowland and IR = irrigated ecologies

The number of males and female farmers per ecology were also noted and expressed as a percentage. Gender participation in rice production varied across ecologies. In the upland rice ecology, the number of male farmers was more than female farmers, while more female farmers participated in lowland and irrigated rice ecologies (Figure 6).

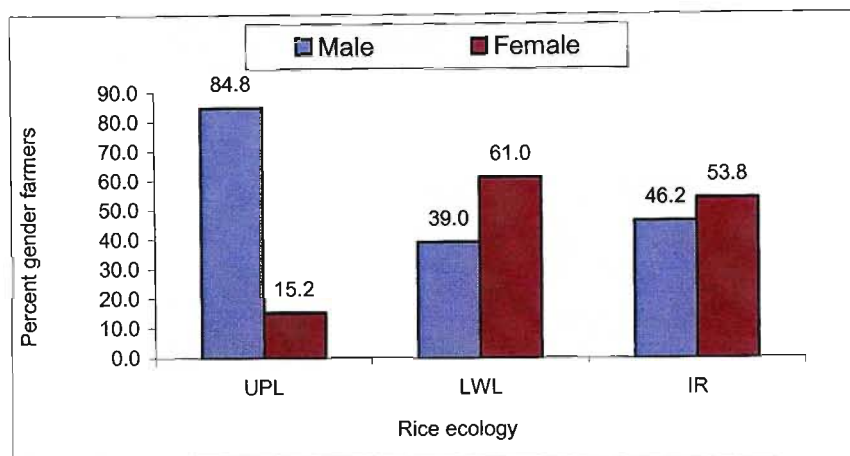


Figure 6: Per cent gender participants per ecology

* UPL = upland , LWL = lowland and IR = irrigated ecologies

2.6.1 Preferred rice characteristics

Farmers' preferences for rice varietal traits differed significantly across ecologies (Table 4). Tall plant trait was the most preferred characteristic across ecologies. This trait ranked highest in the lowland ecology, while it ranked second in upland ecology. Short duration rice was the most preferred trait in the upland ecology as compared to other ecologies. However, it ranked second across ecologies. Long and medium duration rice was the most preferred traits by farmers in the irrigated rice ecologies followed by lowland and upland ecologies, respectively. In the irrigated ecology, all farmers preferred high yielding rice. In other ecologies, less than 30% of the respondents preferred high yielding rice. Farmers' preference for grain quality traits such as size and colour varied across ecologies. In upland rice ecology, more than 61% of the respondents preferred long red grains as compared to respondents in other ecologies. Long white grain was most preferred in the lowland ecology. However, irrigated rice farmers were indifferent about grain quality. Rice with high tillering ability was most preferred in the irrigated ecology. The least preferred traits were short plant, long panicle and taste (Table 4).

Table 4: Per centage farmers preferring traits in different rice ecologies in Sikasso region of Mali.

Preferred rice traits	Upland ecology	Lowland ecology	Irrigated ecology	Across ecologies
Tall plant height	67.0	67.0	23.0	56.0
Short duration	89.0	48.0	33.0	52.0
High yield potential	27.0	29.0	100.0	32.0
Long duration	5.0	34.0	41.0	31.0
Long red grain	61.0	29.0	8.0	31.0
Long white grain	3.0	47.0	8.0	28.0
High tillers number	6.0	9.0	23.0	28.0
Medium duration	5.0	18.0	26.0	18.0
Short plant height	3.0	8.0	4.0	6.0
Short white grain	3.0	12.0	NR	3.0
Short red grain	3.0	4.0	NR	1.0
Long panicle	NR	NR	8.0	1.0
Taste	NR	NR	4.0	1.0

NR = data not recorded

2.6.2 Effects of drought on rice

Drought stress was important both at the vegetative and reproductive stage of rice growth depending on the ecology. Most rice farmers in the upland and lowland rice ecologies experienced drought at the vegetative stage, while a higher per centage of farmers experienced drought at the reproductive stage in the irrigated rice ecology (Figure 7). Also, genotypic variation existed within rice in response to drought stress. A minority of farmers experiencing drought did not sow rice during the normal sowing period of the ecology.

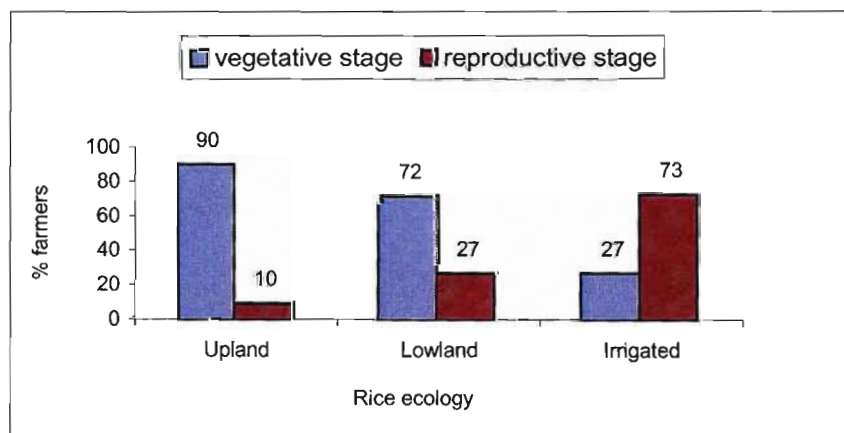


Figure 7: Per centage of farmers who experienced drought at vegetative and reproductive growth stage of rice in upland , lowland and irrigated ecologies.

There was grain yield reduction due to drought stress in the three rice ecologies (Figure 8). By ecology, estimated yield ranged from 806 kg ha⁻¹ to 1250 kg ha⁻¹ under non-stress, and from 314 kg ha⁻¹ to 482 kg ha⁻¹, under stress. Under both stress and non-stress, yield was highest under irrigation, followed by lowland, and least in upland ecologies (Figure 8). Rice grain yield reduction in all ecologies was estimated to be more than 50% due to drought stress.

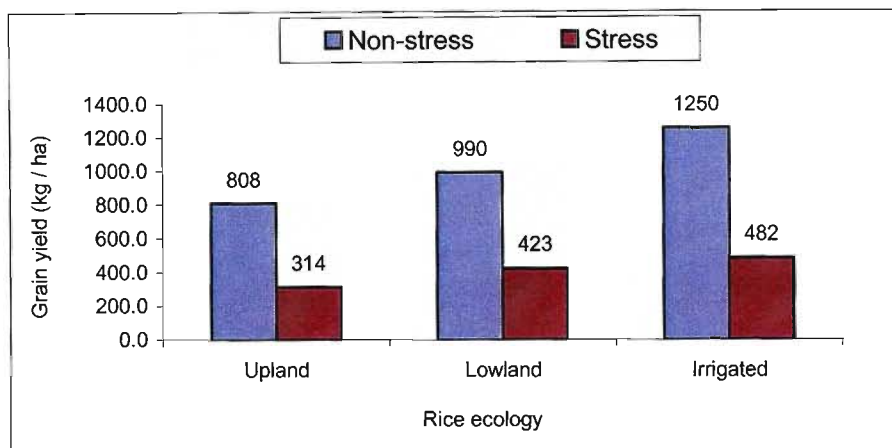


Figure 8: Effects of drought stress on estimated grain yield in different rice ecologies

Rice farmers adopted some drought management strategies. Some farmers practised one or more mechanisms depending on their financial status. Irrespective of the ecology or stage of drought development, about 45.6 % of the total farmers did not know what to do during drought. The common avoiding management strategies against drought were the use of early maturing varieties, tolerant varieties and change of varieties. Surrounding each plot with soil mounds to catch water and early planting were the least preferred management strategies against drought stress (Figure 9).

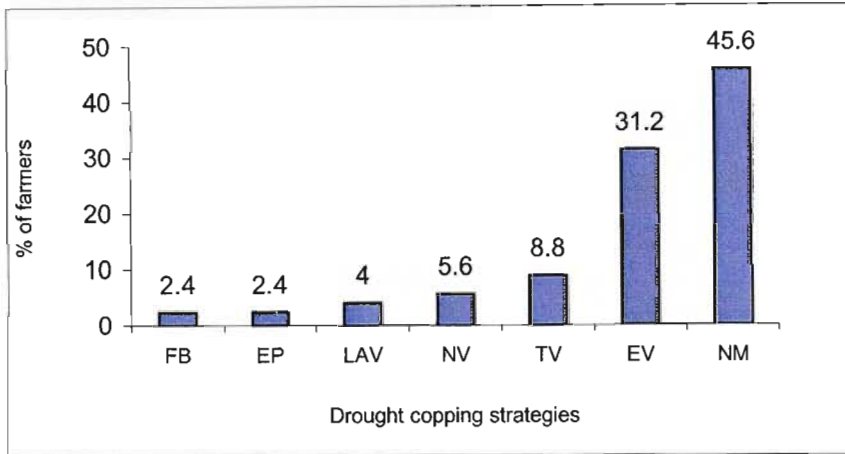


Figure 9: Per centage of farmers adopting different drought management strategies

Use of tolerant variety (TV); Use of early maturing variety (EV); No mechanism (NM); Adaptive/ local variety (LAV); Field bounding (FB); Early planting (EP) and Change to new variety (NV)

2.6.3 Crop management practices

The rainfall in this region starts in April and peaks in August and decreases speedily till October. In the upland rice ecology, farmers reported sowing in May, June and July, but the majority sown in July. Farmers reported sowing rice in April, May, June and July, with the majority sown in May in lowland ecology. In the irrigated rice ecology, sowing took place mainly in May, followed by June and only a few sows in July (Figure 10). Most farmers in the region also engaged in other farming activities such cotton, sorghum and millet cultivation, and as well, cultivated in the three rice ecologies. They therefore prioritized the sowing dates, which may fall outside the main sowing date for the ecology.

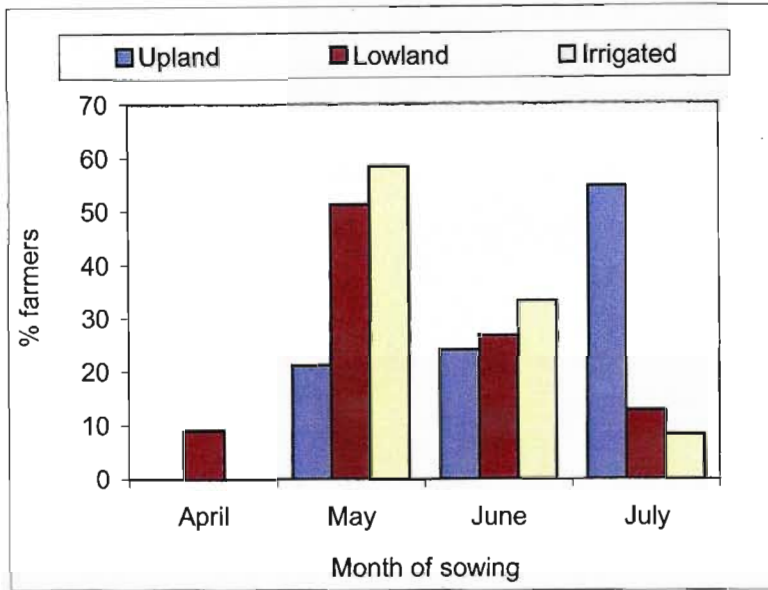


Figure 10. Times of sowing in different rice ecologies

The methods of sowing varied from one village to another (Figure 11). This variation was often related to traditional practices as well as availability of labour and land. The team observed that land area cultivated by farmers varied between 0.5 and 1.5 ha. Drilling and dibbling methods of sowing required more labour and maximized the use of land as compared to broadcasting. Broadcasting was the most popular sowing method in lowland and irrigated rice ecologies. Reasons attributed to these were that apart from labour shortage, the moisture condition of these ecologies ensured maximum germination of the seeds. Across ecologies 66%, 26% and 8% of the rice farmers practiced broadcasting, dibbling and drilling methods of sowing, respectively, from irrigated, lowland and upland ecologies.

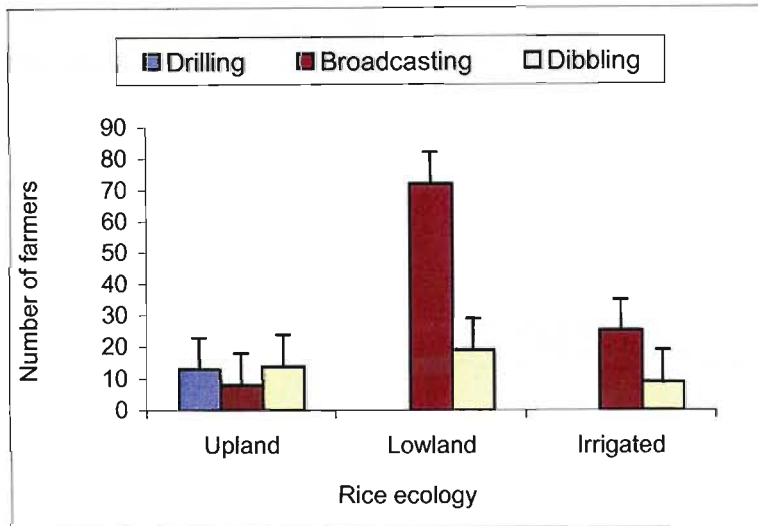


Figure 11: Number of farmers using different methods of crop establishment

The use fertilizer in rice production varied across ecologies (Figure 12). A higher percentage of the upland rice farmers applied fertilizer than those in the lowland and irrigated rice ecologies.

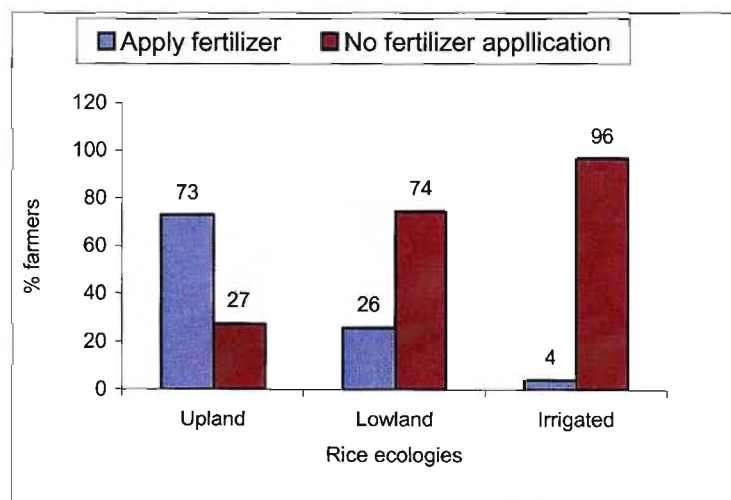


Figure 12: Fertilizer use in different rice ecologies

High numbers of farmers weeded their crop twice, at maximum tillering and before flowering stages of the rice growth (Figure 13).

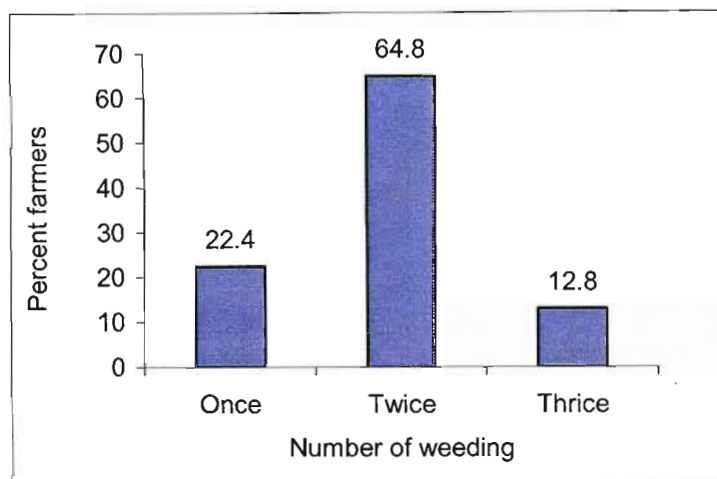


Figure 13: Weeding practices averaged over participating farmers across ecologies.

Farmers in the irrigated rice ecology grew many rice varieties as compared to farmers in the other rice ecologies. The range of rice varieties grown per farmer varied across the ecologies (Table 5). The mean number of rice varieties grown per farmer was three in the irrigated ecology, two and one in the lowland and upland rice ecologies, respectively. A maximum of three to six varieties were grown in different ecologies (Table 5). The research team could not classify the rice varieties according to their species. This was because most of the farmers could not give full description of the varieties. However, most of the red pericarp rice varieties were thought to be *glaberrima* species.

Table 5: Mean number of rice varieties grown per farmer in the different ecologies

Rice ecology	Mean number of varieties grown	Minimum	Maximum
Rainfed upland	1	1	3
Rainfed lowland	2	1	4
Irrigated	3	2	6

Land preparation was done mainly by animal traction. More than 90% of the farmers used animal traction, while others used manual labour. Sole rice was the most common rice based cropping system in the region. More than 75% of the rice farmers practiced sole cropping. Other farmers intercropped rice with cotton, maize, sorghum and millets.

2.6.4 Other information on rice production in the region

The team established that the main sources of obtaining new seeds were through exchange with neighbours, non-governmental organizations (NGO) and agricultural extension agents of the region. Radio also served as a source of information to most of the farmers. Information from the participants was that NGOs were assisting the community to form associations, which may not necessarily be farmers' associations. Through the associations, most of the farmers could afford to buy or obtain agricultural inputs like fertilizers.

2.7 Discussion

In Sikasso region, more women than men were involved in the cultivation of rice in the lowland and irrigated rice ecologies. These ecologies were also used for vegetable production, which attracted more women than men, since vegetables were generally perceived as the women's crop. Vegetables were commonly grown after rice had been harvested from the land. The women used the proceeds from these to assist in family needs. Apart from satisfying the home needs, the excess was sold at the market for cash income. In case of upland rice cultivation, there were more men than women farmers involved. This was because crops like maize, sorghum, millets or cotton performed better than vegetable crops after rice had been harvested from the land. Crops like maize, sorghum, millets or cotton were usually referred to as men's crops in the region.

2.7.1 Preferred traits

In both upland and lowland rice ecologies, the most preferred rice characteristics were short duration, and tall plant varieties. Farmers in these ecologies believed that short duration rice could escape drought stress and gave them enough time to grow more crops in a year than long duration rice. The reasons for high preference for tall plants were that the majority of the farmers harvested rice panicles using knives while standing. Tall plant varieties may also be resistant to lodging. Tall plants lessened the burden of bending. Apart from this, tall plants had long rice straws. Farmers used these straws mixed with clay in building tents. The green revolution in Asia was based on dwarfing genes for developing rice varieties, this finding does not coincide with current breeding objective for dwarf rice. Therefore, very different genes for plant height are required for this region. Other preferred rice

characteristics were long red grains in the upland and long white grains in the lowland. Farmers believed that red rice was more appealing and could be eaten without seasoning, thus reducing the cost of preparation. It was observed that all the improved rice varieties introduced into the region were of white pericarp, which does not coincide with farmers' preference. Therefore, breeding for red pericarp rice is important for these categories of farmers. In the irrigated rice ecology, all farmers preferred high yielding varieties. The current grain yield in farmers' field was estimated to be about 800, 1000 and 1250 kg ha⁻¹ for upland, lowland and irrigated rice ecologies, respectively in Sikasso region (Figure 8). This trend was similar to the potential yield of about 3500, 5000 and 6000 kg ha⁻¹ for upland, lowland and irrigated rice ecologies (WARDA, 1997). The significant yield gap could be attributed to some of these factors such as drought stress, poor yielding varieties and lack of agricultural inputs like fertilizers. This emphasized the importance of developing new varieties that are drought tolerant, high yielding and adaptable to farmers' needs. Preference for long, short or medium duration varieties creates flexible time for other farming activities. For instance an early maturing rice variety will be harvested early before the commencement of other crops like sorghum and millet. Across ecologies, most farmers preferred rice that is high yielding, tall and short duration. Therefore, rice breeders should pay attention to these traits in developing new varieties for Sikasso region of Mali. Currently, a few of such traits of rice varieties are available in the region.

Overall, significant differences between farmers for rice trait preferences in different production ecologies indicated that an ecosystem breeding approach would be the best strategy. Thus use of different selection indices in each of the ecologies is implied. In developing such selection indices, greater weight should be given to early maturity followed by tall plant types in the upland ecology and tall plant types and early maturity, respectively for lowland ecology. In the irrigated ecology, based on Table 4 results, greater weight should be given to yield, late and short duration rice.

2.7.2 Drought

Drought is one of the major natural hazards that has been associated with food shortage and in a severe situation it may lead to famines. The erratic rainfall and uneven distribution

during the season significantly affects rice production. In the upland rice ecology, few farmers started sowing rice seeds in May and the number increased gradually until it reached a peak in July. Results indicated that this erratic rainfall and uneven distribution caused intermittent drought. Lilley and Fukai (1994) and Boonjung and Fukai (1996) reported that drought at vegetative stage delays time to flowering, causes a reduction in dry matter production and tiller numbers. The most preferred trait in upland rice ecology was short duration suggesting that new early maturing rice varieties would be adopted. Thus, early sowing and short duration rice could serve as drought escape mechanisms in these rice ecologies, thus corroborating similar reports by Cooper *et al.* (1999). Similar trends were also observed in the lowland rice ecology. In addition, tall plant type was a highly preferred trait both in upland and lowland rice ecologies. Tall plants have been associated with deep root systems (Ekanayake *et al.*, 1985). Deep rooting is significantly associated with drought tolerance (McWilliam, 1989) as these varieties would be able to exploit water from the deep soil reserves. Tallness of a plant is often used as an indicator of plant vigour and high nutrient use efficiency. Breeding for tall plants will be advantageous in drought prone ecologies of Sikasso region and the varieties would be adoptable in the areas where they are most preferred. In this region, rice is normally harvested by women who are normally carrying children on their backs and bending would be a major problem.

In the irrigated rice ecology, however, more farmers experienced drought at the reproductive stage of rice growth. Most irrigated rice varieties were photoperiod sensitive and flowered when day length was short in October to November. Due to an erratic rainfall pattern in the region, their flowering often coincided with low water level in the reservoirs. This resulted in the rationing of irrigation water, these contributed to drought experienced at the reproductive stage in this ecology.

Some farmers adopted different management strategies against drought, which lessened its effect on the crop. Almost half of the participating farmers had no knowledge of drought management practices. This is partly due to lack of information and their lack of financial capabilities to install physical structures like wells to store irrigation water for the farm during this period. The activities of the national extension services and NGOs need to be strengthened in the delivering of quality and reliable information to the farmers. This PRA

has shown the importance of breeding early maturing and drought tolerant rice varieties in particular for rainfed upland and lowland ecologies.

2.7.3 Crop management practices

The broadcast method of sowing was commonly practised in this region as compared to other sowing methods examined. Farmers believed that it was very fast, less labour intensive and cheap. Perhaps, this is why farmers in the rainfed lowland and irrigated ecologies cultivated two to three rice varieties at a time while the upland rice farmers could only handle one variety because of the sowing methods being practiced there. However, it is expensive in terms of seeds. The broadcast method of sowing requires a large quantities of seed to sow a hectare of land. It requires a seed rate of 60 to 80 kg ha⁻¹ as compared to drilling or dibbling methods, which require 40 to 50 kg ha⁻¹ (De Datta, 1981). Seed supply may likely be a major constraint in rice production in this region, especially in the rainfed lowland and irrigated rice ecologies. Therefore, farmers would be encouraged to adopt efficient planting methods, and perhaps use the surplus seed as grain or cultivate more land.

The majority of the upland rice farmers applied fertilizer in contrast to other rice ecologies. Traditionally, upland rice farmers relied on extended periods of bush fallow to restore soil fertility. Population growth and land shortage has forced many farmers to reduce the fallow period and that is now being supplemented by the use of fertilizer (WARDA, 1997). Soils in the lowland and irrigated ecologies are believed to be richer in terms of soil nutrients than the upland soil. This is because erosion carries nutrients and debris from the upland into the valleys and flood plains of these ecologies. Hence the farmers applied less fertilizer in lowland and irrigated ecologies. In irrigated ecology, high sunlight would enable farmers to obtain an appreciable yield with less fertilizer as compared to other ecologies. Rice breeders should therefore develop rice varieties that respond to nitrogen and other essential plant nutrients.

Sole cropping of rice was the most common cropping system practised in the region, as they believed it was easier to maintain than mixed cropping. The majority of the farmers that weeded three times were lowland and irrigated rice farmers. As weeds are naturally more

endemic in the lowland and irrigated ecologies than in the upland rice ecology. Majority of the farmers weeded twice mostly at maximum tillering and booting stage of rice growth. Land preparation is commonly done by animal traction because the animals are available and cheap.

Conclusion

This study has identified early maturing and tall varieties among the most important traits by farmers in the rainfed production ecologies in Sikasso region. High yielding varieties, which are early maturing and tall will be highly desirable in this region. Farmers in the rainfed lowland and upland ecologies were also particularly keen on colour traits of rice grain with moderate emphasis on yield whilst farmers in the irrigated lowland ecology rated yield and late maturity as the most important. This finding deviated from current objective of most plant breeders for dwarf rice and well as white pericaps rice introduced into the region.

Farmers used more fertilizer in the upland ecology than in the lowland and irrigated ecologies. Female farmers were more prevalent in the lowland and irrigated ecologies than in the upland ecology. Farmers in all ecologies reported drought stress. These results imply the breeding of varieties for specific ecologies.

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CHAPTER 3

Screening of parental genotypes for drought tolerance at vegetative stage of rice development

Abstract

Drought stress is a limiting factor in rice production in Sub-Sahara Africa. It can occur at any stage of rice development. Drought stress during the vegetative stage could cause an irreversible damage to the plant. Morpho-physiological traits have been used as indirect measures of drought stress tolerance in rice but these are genotypes and environmental dependent. This study was conducted to determine morphological and physiological traits associated with drought stress tolerance, and examine genotype-environment interactions under water-stress conditions at the vegetative stage of rice growth in upland rice ecologies. Sixteen genotypes including seven *Oryza glaberrima* Steud, six interspecific (*O. sativa* x *O. glaberrima*) inbred genotypes and two improved *O. sativa* L. subsp (*japonica*) genotypes, with OS 6, as drought tolerant check were used. They were screened for drought tolerance at the vegetative stage during the dry seasons of 2004 and 2005, at Samanko research station, in Mali. In 2004, one stressed experiment was established while in 2005, two experiments were established: stressed and fully irrigated, which served as a control. Overhead sprinkler irrigation was applied for 35 days after sowing three times weekly. Irrigation was withdrawn in the stressed experiments for 21 and 43 days in 2004 and 2005, respectively to induce stress. Genotypes reacted differently in response to drought stress for the characters examined. Most of the genotypes examined under drought stress could be regarded as exhibiting drought tolerance and avoidance mechanisms for most of the morpho-physiological characters under study. High selection index was observed for WAB 450-I-B-P-103-HB in the two seasons experiments. Drought stress tolerance was significantly associated with less leaf rolling, larger leaf area index (LAI), and taller plants. Faster drought recovery was significantly associated with drought tolerance, fewer tillers and larger leaf area index. Effects of genotypes, environment and their interactions were significant for tiller number, root dry weight and relative leaf water content (RLWC). The most stable genotype was RAM 3 for tiller number and relative leaf water content. Drought tolerance was associated with many traits at the vegetative stage. Therefore, screening for drought responsive traits such as leaf drying and rolling, large LAI and RLWC at the vegetative stage of rice growth is very important for rice production.

3.1 Introduction

In most ecologies of Africa where rice is grown, drought is a serious limitation to yield. This is because a larger proportion of rice fields in Sub-Sahara Africa depend solely on rainfall (De Datta, 1981). In view of this, breeding for drought tolerance is very important. Drought tolerance has been identified as a complex trait, which involves complex interactions of morpho-physiological characteristics (Blum, 2002). In rice, the effect of drought varies with rice genotypes, intensity, timing and duration of stress (Pantuwan *et al.*, 2002).

Drought stress during vegetative stage can cause irreversible damage to the plant. The damage may lead to reduction in the leaf area, tiller number and reduction in grain yield (Cruz *et al.*, 1986). When drought stress occurs at the tillering stage of rice, its effect leads to significantly reduced plant height and induces leaf rolling. The vegetative stage of the plant could be prolonged and delay in time to flowering can occur even after drought stress has been relieved (Murty and Ramakrishayya, 1982; Boonjung and Fukai, 1996).

Screening of genotypes at the vegetative stage for drought stress tolerance is therefore important. Early screening has been used to identify cultivars and breeding genotypes that are drought tolerant (IRRI, 1996a). The screening methodology allows breeders to screen large numbers of genotypes at an early generation in the breeding programme and leads to rejection of susceptible materials at an early stage. The performance of these materials at the reproductive stage may vary, but Mackill *et al.*(1996) reported that susceptible genotypes at the vegetative stage are unlikely to perform better at the reproductive stage.

Some morpho-physiological traits of rice have been identified that respond to drought stressed conditions. These drought stress responsive traits are indicators in the screening of genotypes for drought tolerance. They have been identified both at the vegetative and reproductive stages of rice development (O'Toole, 1982; Pantuwan *et al.*, 2004). At the vegetative stage, leaf rolling, relative leaf water content, osmotic adjustment and deep root system have been identified as putative traits for drought tolerance (Singh and Mackill, 1991; Blum, 2002; Pantuwan *et al.*, 2002). Ability of rice to recover fast after stress relief is an important trait under drought stress conditions. The ability of rice to recover from drought stress is often based on the greenness of old leaves and fast emergence of young leaves after drought stress relief. This ability could avoid total crop failure and is an important determinant of grain yield (Chang *et al.*, 1974; De Datta *et al.*, 1975).

Indigenous African rice, mostly *O. glaberrima*, is more adapted to harsh environmental conditions than *O. sativa*. This adaptation stemmed from its long cultivation in the continent (WARDA, 2000). Although *O. glaberrima* is associated with poor and low grain yield, it has good agronomic potentials (Jones *et al.*,1997a). Reports revealed that *O. glaberrima*

genotypes are drought tolerant and recover fast from drought stress when conditions improve (Jones *et al.*, 1997a; Dingkuhm *et al.*, 1999). Interspecific genotypes derived from crosses between *O. sativa* and *O. glaberrima* have been identified with good tolerance to drought stress. This tolerance has been associated with rapid vegetative growth, droopy lower leaves, high tillering ability, early maturity and good grain quality (WARDA, 1995; Jones *et al.*, 1997a).

Genotype-environment interaction is an important factor in the selection of good materials and is of utmost importance to the plant breeders (Eberhart and Russell, 1966). Wider adaptability and stability of genotypes are considered as the most important traits in breeding programmes for drought tolerance. This is because there are significant variations in phenology, morphology and physiology of rice genotypes across environments (Wade *et al.*, 1999; Cooper *et al.*, 1999). Identification of putative traits in rice genotypes that are stable in diverse environments will be indicative of good donors for drought tolerance.

3.2 Objectives of the study

The objectives of this experiment were:

1. to determine morphological and physiological traits associated with drought tolerance during water-stress at the vegetative stage of rice growth in upland rice ecologies,
2. to determine genotype-environment interactions during water-stress, and
3. to identify the best performing genotypes as parents for a drought tolerance-breeding project.

3.3 Hypothesis of this study

Morpho-physiological traits of rice respond similarly to drought stress.

3.4 Materials and methods

Experimental site and climate: Two dry season experiments were conducted in 2004 and 2005 at ICRISAT Research Station, Samanko, Bamako in Mali (latitude 12.23^o N, longitude 7.57^o W, altitude 380m above the sea level).

3.4.1 Soil properties and environmental parameters

The soil properties are summarised (Table 1). A graduated soil auger was used for sampling the soil. Six core soil samples per replication at 0-20 cm depth were taken and bulked to form a composite and two sub-samples were taken per composite, thus, a total of 8 samples were submitted for analysis at Soil science laboratory in Sotuba at Institut d' Economie Rurale (IER) Bamako, Mali. The soils are acidic and deficient in organic matter and total nitrogen (Table 1). The soil texture is a silty clay loam with very low cation exchange capacity. It was of low fertility. This resulted from inadequate levels of essential nutrients especially nitrogen (N) content, which was 0.071 and 0.058 % for 2004 and 2005 experiments, respectively. The soil organic matter was low, 1.156 and 0.480 % for 2004 and 2005 experiments, respectively (Table 1).

Table 1: Soil properties in 0-20 cm soil depth for the experimental site at Samanko, during 2004 and 2005 dry season

Property	2004	2005
pH (1:1 water)	4.721	4.964
Organic matter (%)	1.156	0.480
Total N (%)	0.071	0.058
P (ppm)	5.929	9.600
K (ppm)	0.156	0.232
Ca (ppm)	2.508	2.776
Mg (ppm)	0.749	0.896
CEC (cmol (+)/kg)	12.142	9.740
Sand (%)	8.500	21.200
Silt (%)	70.357	60.800
Clay (%)	16.214	17.800
Soil texture	Silty clay loam	Silty clay loam

Climatic data was collected from ICRISAT weather station located about 500m from the experimental site. Monthly mean maximum air temperature for the two seasons ranged from 36.0 to 47.5 °C, while the minimum ranged from 14.6 to 32.9 °C. The air temperature

increased gradually from the beginning of the year (Table 2). In 2004, total rainfall received during the experiment was 72.4 mm, and it fell for only 3 days in April and May. Total rainfall for 2005 was 12.2 mm and it fell for 2 and 3 rainy days in March and April, respectively. Percentage air relative humidity was very low and ranged from 45.1 to 59.1 %. This enhanced higher pan evaporation ranging from 6.2 to 10.6 mm day⁻¹ during the experiments (Table 2).

Table 2: Monthly mean maximum and minimum air temperature, pan evaporation, rainfall and air relative humidity during the experiments

Experiments/ month	Maximum Temperature (° C)	Minimum Temperature (° C)	Pan Evaporation (mm day ⁻¹)	Rainfall (mm)	% air relative humidity
<i>2004 experiment</i>					
March	41.8	14.6	10.6	0.0	51.3
April	42.8	19.3	8.9	26.1	54.3
May	43.6	26.0	7.5	46.3	45.1
<i>2005 experiment</i>					
December	39.9	21.1	6.2	0.0	45.6
January	36.0	21.5	7.3	0.0	59.1
February	40.9	24.9	9.3	0.0	57.5
March	45.1	30.0	8.7	8.5	55.0
April	47.5	32.9	9.3	3.7	58.0

3.4.2 Genetic materials

Sixteen genotypes including seven *Oryza glaberrima* Steud, six interspecific (derived from crosses between *O. sativa* and *O. glaberrima*) inbred genotypes and two improved *O. sativa* L. subsp (japonica) genotypes, with OS 6, as drought tolerant check were included in the study. They were screened for drought tolerance at the vegetative stage during the dry season of 2004 and 2005. Six of these *O. glaberrima* genotypes named “Riz African du Mali (RAM)” were selected from the germplasm collections of the Institut d’ Economie Rurale (IER) at Sikasso, in Mali based on their drought recovery ability. RAM 55 was excluded in 2005 because of poor stand establishment and it was replaced with OS 6. Other materials

were selected from the WARDA upland rice-breeding unit based on their previous performance (Table 3).

Table 3: Genetic materials for the experiments

Genotype code	Test entry	Species of test entry
1	RAM 3	<i>Oryza glaberrima</i>
2	RAM 55*	<i>Oryza glaberrim</i>
3	RAM 85	<i>Oryza glaberrima</i>
4	RAM 86	<i>Oryza glaberrima</i>
5	RAM 118	<i>Oryza glaberrima</i>
6	RAM 163	<i>Oryza glaberrima</i>
7	CG 14	<i>Oryza glaberrima</i>
8	TOG 5681	<i>Oryza glaberrima</i>
9	NERICA 2	Interspecific
10	NERICA 3 (tolerant check)	Interspecific
11	WAB 365-B-1-H1-HB	Improved <i>Oryza sativa</i>
12	WAB 375-B-9-H3-2	Improved <i>Oryza sativa</i>
13	WAB 450-I-B-P-103-HB	Interspecific
14	WAB 450-I-B-P-6-1-1	Interspecific
15	WAB 450-I-B-P-105-HB	Interspecific
16	WAB 880-1-38-13-1-P1-HB	Interspecific
17	OS 6 (tolerant check)*	Improved <i>Oryza sativa</i>

* RAM 55 was excluded in 2005 because of poor stand establishment and it was replaced with OS 6.

3.4.3 Crop management and experimental design

The land was ploughed and disc harrowed and levelled before sowing the seeds. Rice seeds were dibbled in shallow holes at the rate of three seeds per hole. The spacing was 20 cm within plants and 25 cm between rows of plot size 1.0 cm x 3.0 cm and plant population was 64 (4 rows and 16 hills per row). The experimental design was a lattice square (4 x 4) with three replications. In the 2004 dry season, only drought stress was established comprising the sixteen genotypes. This is because seeds were not sufficient to conduct a fully irrigated experiment. In the 2005 dry season, this experiment was repeated and an experiment that was fully irrigated throughout the growth of the crop was included as a control. Seedling emergence was observed a week after sowing and the plants were thinned to one plant per hole at 15 d after seedling emergence. The experiments were hand weeded at 15 d and 35 d after sowing prior to fertilizer application. Basal fertilizer was applied at the rate of 200 kg ha⁻¹ of 17-17-17, N-P-K and top-dressed with urea (46% N) at the rate of 100 kg ha⁻¹ at 35 d after sowing. There was no insecticide applied. Overhead sprinkler irrigation was applied for 35 d after sowing, which was at maximum tillering stage, three times weekly. These brought

the soil to field capacity. Simulation of drought at maximum tillering stage is advisable for rice crop (Singh and Mackill, 1991). Irrigation was withdrawn in the stressed experiments for 21 d to avoid rain in 2004 and 43 d in 2005 to induce moisture stress at maximum tillering stage. In 2005, soil water monitoring and crop visual observation approaches were used to determine when to relieved stress.

3.5 Measurements

Measurements of the agronomic characters listed below were taken at weekly intervals in both stressed and control experiments during the stress period. The Standard Evaluation System (SES) for Rice reference manual (IRRI, 1996b) was used for all trait measurements except where stated. The following measurements were taken as observed for the whole plot, otherwise, were stated.

Drought score: Drought score (Dt) was rated weekly based on the leaf drying symptoms observed. Rating of drought score on a scale of 0-9 was done as shown below (De Datta *et al.*, 1988):

Score	0	No symptoms of stress
	1	Slight drying of leaf tips
	2	25% of the length of 25% of all leaves (apart from older leaves that are normally dry)
	3	At least 25% of the length and 26% to 50% of all leaves are dry.
	4	At least 25% of the length of 50% of all leaves are dry; 25% of leaves are fully dried.
	5	50% of all leaves are fully dried
	6	51-69% of all leaves are fully dried
	7	70% of all leaves are fully dried
	8	More than 70% of all leaves are fully dried
	9	All plants are apparently dead

Leaf rolling: A leaf rolling score was adapted from O'Toole and Moya (1978). Drought and leaf rolling score were taken at weekly interval throughout the period of stress between 1300 and 1500 hr to assess the effects of water stress as follows:

Score	0	No leaf rolling
	1	Slight leaves rolling
	2	Leaves rolled into Slight V-shape
	3	Leaves rolled into deep V-shape
	4	Leaves rolled and leaf margins touching
	5	All leaves completely rolled

Plant height and tiller number: Plant height (cm) was measured from soil surface to the tip of the shoot and tiller number recorded within each hill as described by IRRI (1996b).

Plant vigour: Plant vigour (Vg) was taken when 50% of the plants in a population were at 4th leaf stage. A total of five plants per plot were randomly taken for measurement for each of the traits as described by IRRI (1996b):

Score	1	Extra vigorous (very fast growing; plants at 5-6 leaf stage have 2 or more tillers in majority of population)
	3	Vigorous (fast growing; plants at 4-5 stage have 1-2 tillers in majority of population)
	5	Normal (plant at 4-leaf stage)
	7	Weak (plants somewhat stunted; 3-4 leaves; thin population; tiller formation)
	9	Very weak (stunted growth; yellowing of leaves)

Relative leaf water content (RLWC): Relative leaf water content was determined between 1300 and 1500 hr by the method suggested by Barrs and Weatherly (1962). Three young fully expanded leaves from the main stem were randomly selected in each plot and placed in a plastic bag and transported to the laboratory immediately. A rectangular metal cock was used in a sample taken from the middle portion of each leaf and weighed to determine the fresh weight. Turgid weight was determined by weighing the leaf samples left in petri dishes

containing water for four hours. Then they were oven dried at 60^o C for 24 hours and weighed for dry weight determination. Relative leaf water content was calculated as:

$$\text{RLWC} = \{ (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \} \times 100$$

Plant biomass (fresh and dry): Plant biomass was taken at weekly intervals. Two plants were randomly selected per plot and the aboveground part of the plant was cut off and fresh weight recorded. For the root sampling, a hole of 30 cm depth was dug within 10 cm diameter around the plant. Roots were gently removed into a 2 mm mesh screen and washed free of soil with water to ensure minimum root loss for fresh weight determination. The samples were oven dried at 65^o C for 72 hours and weighed for shoot and root dry weights.

Leaf area index and specific leaf dry weight: Two young fully expanded leaves from the main stem were randomly selected in each plot and leaf area (LA) was determined using a leaf area meter (li-3100, Lincoln, NE USA). Leaf area index (LAI) was calculated as described by Yoshida (1981) as follows:

$$\text{LAI} = (\text{sum of the leaf area of all leaves} / \text{unit area where the leaves have been collected}).$$

The specific leaf dry weight (SLDW) was calculated as the area of leaf per leaf dry weight (Jones et al., 1997b; Zaharieva et al., 2001).

Recovery ability: Plant recovery scores were taken at 3 and 10 days after resuming irrigation. Drought stress recovery symptoms were based on healthy plants and leaves as described by IRRRI (1996b) as follows:

Score	1	90-100% recovery
	3	70-89% recovery
	5	40-69% recovery
	7	20-39% recovery
	9	0-19% recovery

Drought tolerance index: Per cent reduction of each character (X) relative to the control was calculated as tolerance index by the method suggested by Reyniers *et al.* (1982) as follows:

$$\text{Drought tolerance index} = (X \text{ control} - X \text{ stress}) / X \text{ control} \times 100$$

Grain yield: Data on grain yield were not obtained due to severe cold harmatan haze that coincided with flowering period of the genotypes. This haze is cold especially at night and caused a severe chilly effect on the plants.

Selection indices

Weights (W_i) for the selection index were assigned based on the relative importance of each measured trait as an indicator of drought stress and grain yield in upland rice ecology (Table 4). The results from the participatory rural appraisal (Chapter 2) on preferred traits by upland rice farmers were also considered in assigning weight on each trait.

Table 4: Sign and weight based on the relative importance of traits in the selection index

Traits	Sign‡	Weight (W _a)	Importance
Drought score	-	5	Little or no leaf drying
Leaf rolling	-	4	Little or no leaf rolling
Plant height	+	3	Tall plant
Plant vigour	+	3	Highly vigorous
Tiller number	-	3	Less tiller number
Leaf area index	+	3	Large leaf area index
Specific leaf dry weight	+	2	Large specific leaf dry weight
Relative leaf water content	+	2	High relative leaf water content
Root dry weight	+	2	High root dry weight
Shoot dry weight	+	1	High shoot dry weight
Root dry weight/ Shoot dry weight ratio	+	2	High Root / Shoot ratio
Recovery score at 3 days after irrigation	-	5	Fast recovery
Recovery score at 10 days after irrigation	-	1	Fast recovery

‡ -ve = not desired; +ve = desired

The phenotypic values were standardized by the method suggested by Banzinger *et al.* (2000) as:

$$P_a = (X - GM) / Sd$$

Where P_a = standardized phenotypic value; GM and Sd are the grand mean and standard deviation of the trait in the experiment, and X = actual value of the trait measured on the genotype. The selection index of (SI) each genotype was calculated as:

$$SI = P_a W_a + P_b W_b + \dots + P_n W_n$$

Where P_a = standardized phenotypic value of the trait observed; W_a = is the assigned weight value to the trait in the selection index.

Genotype by environment interaction

The effect of genotype by environment interaction was tested over three different water stressed conditions for all the traits examined. Stability of genotypes across the environments was examined. The environments were Env-1 for 2004 drought stressed experiment and Env-2 and Env-3 for 2005 drought stressed and fully irrigated experiment, respectively.

3.6 Statistical analysis

Analysis of variance (ANOVA) was performed separately on the individual experiment and combined analysis was used for some traits using Statistical Analysis System (SAS, version 9.1, 2003) to test the significance of differences among genotypes. A combined ANOVA for the three environments was run by PROC GLM in SAS programme. Genotypes by environment interaction and stability of genotypes were determined. The environments and genotypes were considered fixed effect as they were created purposely for these experiments, while replications as random effects.

$$Y_{ijk} = \mu + rk + gi + ej + (ge)_{ij} + e_{ijk}$$

Where y_{ijk} = phenotypic observation on the progenies, rk = replication effect, gi = genotype effects, ej = environmental, $(ge)_{ij}$ = interaction between genotype and environment and e_{ijk} = experimental error due to environmental effects.

Simple linear correlation analysis was computed for all the observations using individual plot means to evaluate their associations using a model described by Gomez and Gomez (1984) as:

$$Y = \alpha + b X$$

Y = phenotypic value for F4 population, X = phenotypic value for F3 population, α = intercept and b = coefficient of linear correlation. Student's t-tests were used for comparisons between treatment means.

3.7 Results

3.7.1 Monitoring the stress condition

Soil moisture content (SMC) of each plot was monitored at 0-20 cm soil depth every five days throughout the stress period using the gravimetric soil analyses method. Graduated soil auger was used for sampling the soil. The weight of fresh soil (Fwt) and soil oven dried for 48 hours at 70⁰ C (Dwt) were taken. Per cent moisture content was calculated as: [(Fwt-Dwt / Fwt)* 100]. There was rapid loss of soil moisture content (SMC) within 15 days from 18.4 to 11.2% in 2004 and 15.2 to 5.6 % in 2005 (Figure 1). Within the same period, 63.4% and 39.3% of water loss was recorded in 2004 and 2005, respectively. Therefore, it could be concluded that drought stress in 2005 was much more severe than in 2004 (Figure 1). The little rise in SMC from 15 to 30 days was due to severe Harmantan haze with high atmospheric humidity resulting in low soil water evaporation.

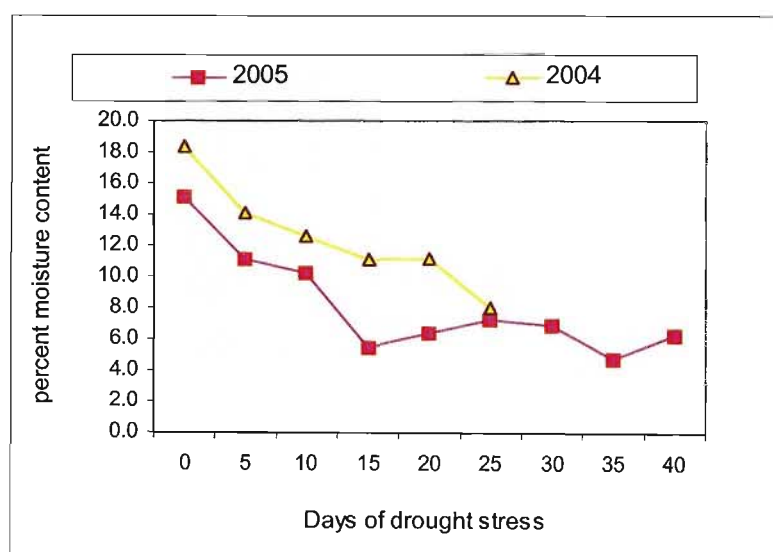


Figure 1: Per cent moisture content for the experiment during 2004 and 2005 dry seasons

3.7.2 Effects of water stress on plant characteristic of rice genotypes

Tests for homogeneity of variance were calculated for each of the traits examined and significant differences were observed among the variances both in 2004 and 2005 dry season experiments. Therefore data from the two seasons were analysed and presented separately (See Appendix I to III). Significant variation was observed in most of the traits evaluated. The mean values and ranges for 12 traits of rice evaluated under drought stressed conditions along with their standard errors are shown in Table 5. Genotypes reacted differently to the effects of drought and significant variation ($P \leq 0.001$) for leaf rolling, plant height and tiller numbers existed among the genotypes (Table 5). The effects of water stress for each genotype during 2004 and 2005 experiments are shown in appendix I to III.

Table 5: Mean values, estimation of standard error (S.e) of the mean for the evaluated traits of 16 rice genotypes under drought stressed condition in 2004 dry season

Rice traits	Mean	Minimum	Maximum	S.e	Probability
Drought score	3	1	7	0.23	***
Leaf rolling score	5	1	7	0.23	***
Plant vigour score	4	1	7	0.29	ns
Plant height (cm)	55.18	34.40	74.60	1.42	***
Tiller number (per plant)	5	2	13	0.40	***
Leaf area index	3.77	2.06	6.45	0.14	ns
Relative leaf water content (%)	59.83	35.59	82.09	2.00	ns
Root dry weight (g)	1.34	0.60	2.51	0.07	*
Shoot dry weight (g)	14.87	7.54	30.83	0.75	ns
Root / Shoot ratio	0.09	0.05	0.25	0.01	***
Recovery score at 3 days after irrigation	4	2	7	0.29	***
Recovery score at 10 days after irrigation	3	1	7	0.20	***

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant.

Overall, selection index values ranged from -27.04 to 26.72 (Figure 2). The best genotype with the highest selection index value was WAB 450-I-B-P-103-HB (genotype 13) and lowest was RAM 118 (genotype 5). The best genotype amongst *O. glaberrima* was CG 14 (genotype 7) and followed by RAM 86 (genotype 4) with selection index value of 6.63 and 1.88 respectively. While amongst the interspecific genotypes, NERICA 3 (genotype 10) had the least selection index value of 4.23 (Figure 2). Amongst the *O. sativa* WAB 365-B-1-H1-HB (genotype 11) had the highest selection index.

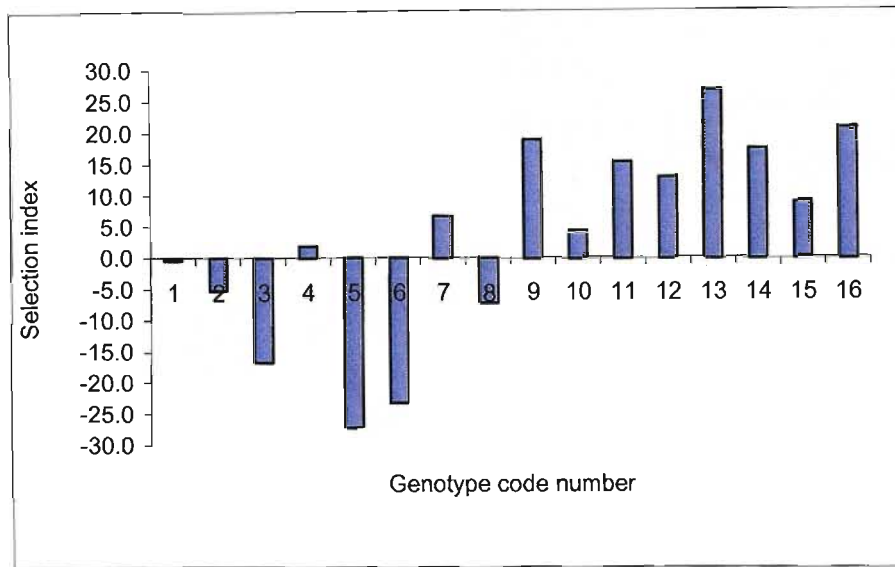


Figure 2: Selection index for 16 rice genotypes under drought stressed during 2004 dry season experiment based on 12 traits. For genotype code, refer to Table 3.

In 2005 dry season experiment, significant genotypic differences were observed in all the traits except relative leaf water content, plant biomass and recovery ability at ten days after stress was relieved (Table 6). Traits values for individual genotypes are shown in appendix II.

Table 6: Mean values, estimation of standard error (S.e) of the mean for the evaluated traits of 16 rice genotypes under drought stressed condition in 2005 dry season

Rice traits	Mean	Minimum	Maximum	S.e	Probability
Drought score	4	2	6	0.15	***
Leaf rolling score	3	2	4	0.10	***
Plant vigour score	5	3	7	0.20	***
Plant height (cm)	47.93	34.06	58.41	0.95	***
Tiller number (per plant)	6	2	16	0.51	***
Leaf area index	2.13	0.77	3.62	0.10	***
Specific leaf dry weight (m ² /kg)	11.27	6.72	14.23	0.24	***
Relative leaf water content (%)	80.11	69.84	87.92	0.56	ns
Root dry weight (g)	0.63	0.30	2.17	0.04	*
Shoot dry weight (g)	7.86	3.12	13.74	0.34	ns
Root / Shoot ratio	0.08	0.04	0.18	0.00	ns
Recovery score at 3 days after irrigation	5	3	7	0.19	*
Recovery score at 10 days after irrigation	3	1	5	0.16	ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant.

In 2005 dry season experiment, genotypes responded differently in contrast to 2004 dry season experiment. Selection index values ranged from -36.79 to 26.43 (Figure 3). The best three genotypes based on selection index were WAB 450-I-B-P-103-HB (genotype 13), WAB 450-I-B-P-105-HB (genotype 15) and TOG 5681 (genotype 8) with a value of 26.43, 21.05 and 19.72, respectively. All the genotypes were better than OS 6 the resistant check except RAM 86, RAM 118 and CG 14 based on selection index value (Figure 3). The best genotypes were WAB 450-I-B-P-103-HB (genotype 13), WAB 365-B-1-H1-HB (genotype 11) and TOG 5681 (genotype 8) for interspecifics, *O. sativa* and *O. glaberrima*, respectively (Figure 3).

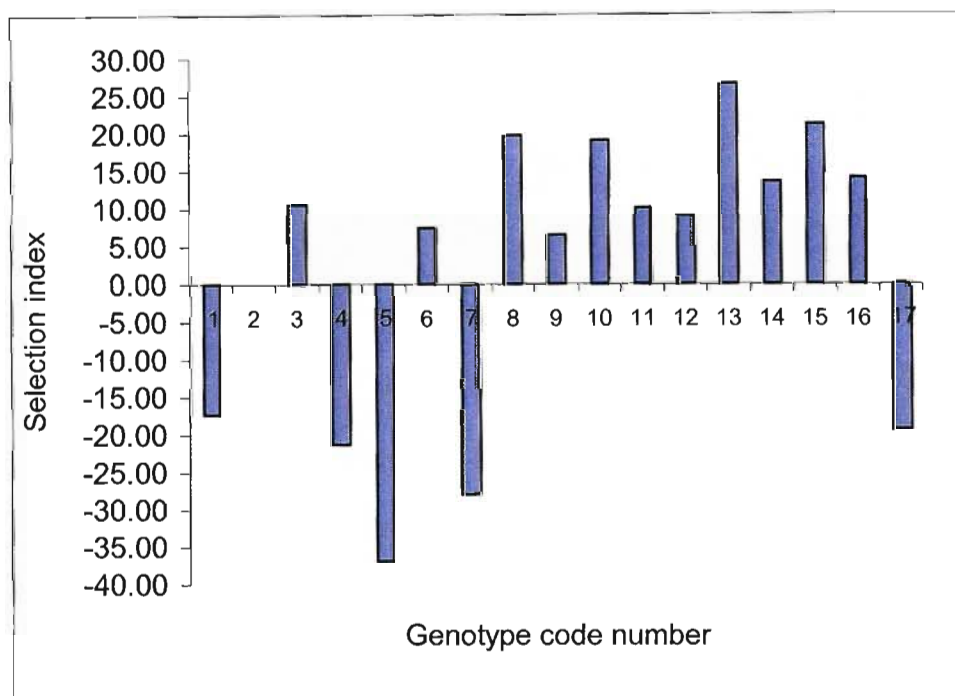


Figure 3: Selection index for 16 rice genotypes under drought stressed during 2005 dry season experiment based on 13 traits. For genotype code, refer to Table 3.

3.7.3 Drought tolerance indices of rice genotypes at the vegetative stage

In Table 7, genotypes with a negative value performed better under drought stress compared to the fully irrigated experiment for that character. While genotypes with a positive value expressed reduction for that character due to drought stress relative to the fully irrigated experiment. In the case of Relative leaf water content (RLWC), genotypes with negative values indicated water loss and positive values indicated water gain by the plant under

drought stress. These values were used as drought tolerance indices to identify genotypes, which are drought tolerant or susceptible (Table 7).

Differential reaction of genotypes to drought stress was observed for all the traits measured. There was a reduction in leaf area index due to drought stress in all the genotypes except TOG 5681, CG 14 and WAB 375-B-9-H3-2 (Table 7).

Table 7: Drought tolerance indices in 2005 dry season trial

Genotypes	Leaf area index	Plant height (cm)	Tiller number	Shoot dry weight (g)	Root dry weight (g)	RLWC (%)	SLDW (m ² kg ⁻¹)
OS 6 (resistant check)	31.2	8.2	35.1	43.8	66.8	-3.0	14.5
RAM 163	21.4	11.6	30.2	47.2	68.0	-0.1	17.9
NERICA 3	19.8	14.0	6.4	31.4	62.0	-10.0	7.8
RAM 3	17.2	0.2	17.3	51.2	70.8	-10.9	13.5
RAM 118	16.1	-1.7	28.2	51.3	71.3	-3.4	8.1
RAM 85	15.1	15.1	16.0	31.1	68.6	-7.2	8.5
WAB 450-I-B-P-105-HB	14.9	20.0	3.1	40.4	67.2	-21.8	1.3
WAB 450-I-B-P-103-HB	13.8	18.6	-8.3	34.8	60.1	-15.4	-9.6
WAB 450-I-B-P-6-1-1	13.6	6.5	-13.3	31.6	73.4	-8.2	0.1
WAB 880-1-38-13-1-P1-HB	11.1	10.7	21.7	50.9	75.4	-3.0	-4.8
RAM 86	4.9	14.6	23.6	42.2	44.1	-12.2	14.6
WAB 365-B-1-H1-HB	4.0	9.1	-16.4	31.3	66.0	-4.3	5.9
NERICA 2	1.3	7.5	18.5	40.5	70.2	-7.7	-12.9
WAB 375-B-9-H3-2	-0.9	7.5	21.7	37.4	69.3	-13.3	-9.1
CG 14	-1.7	22.6	21.1	50.5	64.9	-32.4	0.5
TOG 5681	-19.6	10.1	45.0	-9.1	-2.5	-10.3	0.4

Reduction in tiller number was higher among *O. glaberrima* genotypes than the other rice species and ranged from 16.0% to 45.0%. TOG 5681 was the most affected genotype for tiller number. Plant biomass (shoot and root dry weight) was the most affected of all the characters examined. Reduction in shoot dry weight ranged from 31.1% to 51.3 % and root dry weight ranged from 44.1% to 75.4 %. The *O. glaberrima* genotypes were the most affected among the rice species except for TOG 5681. Reduction in specific leaf dry weight (SLDW) was more pronounced within *O. glaberrima* genotypes. CG 14 and TOG 5681

were the least affected with 0.5% and 0.4% reduction in SLDW, respectively (Table 7). The level of water loss during the drought stress period varied amongst genotypes. No genotypes gained water. However, per cent water loss ranged from 0.1% to 32.4% RLWC. High level of water loss was observed in some genotypes.

3.7.4 Correlation analysis

3.7.4.1 Linear correlation coefficient across genotypes during 2004 dry season

In the 2004 dry season experiment, linear correlation coefficient analyses were carried out across the genotypes as well as among rice species. Genotypes reacted differently to stress intensity, season and environmental conditions. Across genotypes, drought tolerance (Dt) and little leaf drying, were significantly ($P \leq 0.05$) and negatively associated with leaf area index (LAI) and tiller number (TN). It was also significantly ($P \leq 0.01$) and positively associated with leaf rolling (LR) (Table 8). Leaf rolling was significantly positively associated tiller numbers and leaf area index and a significant negative relationship was found between leaf area index and tiller numbers. Higher Relative leaf water content (RLWC), commonly used as measure of water status of plants, was found to be significantly ($P \leq 0.001$) and positively associated with less vigorous plants (Vg) and plant height (PH). Root dry weight (RDm) was significantly positively correlated with shoot dry weight (ShDm) (Table 8).

Table 8 : Linear correlation coefficients among traits studied in a collection of 15 rice genotypes during 2004 dry season

Traits	Dt	LR	LAI	PH	TN	RDm	ShDm	RLWC
LR	0.610**							
LAI	-0.311*	-0.343*						
PH	0.165 ns	0.043 ns	-0.060 ns					
TN	0.293 *	0.500***	-0.438**	0.047 ns				
RDm	-0.129 ns	0.065 ns	-0.047 ns	-0.143 ns	0.202 ns			
ShDm	0.053 ns	0.132 ns	-0.042 ns	0.109 ns	0.120 ns	0.574***		
RLWC	0.066 ns	0.072 ns	0.034 ns	0.906***	0.060 ns	-0.032 ns	0.034 ns	
Vg	-0.103 ns	-0.111 ns	0.136 ns	-0.933***	-0.212 ns	0.003 ns	-0.034 ns	0.928***

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant. Dt = drought tolerant score, LR = leaf rolling score, LAI = leaf area index, PH = plant height, TN = tiller number, RDm = root dry weight, ShDm = shoot dry weight, RLWC = relative leaf water content and Vg = plant vigour.

3.7.4.2 Linear correlation coefficient across genotypes during 2005 dry season

Genotypic variation in response to change in plant characteristics evaluated in 2005 as compared to 2004 exists. This might be due to drought severity. In the 2005 dry season experiment, correlation coefficient analyses across genotypes showed a significant positive relationship ($P \leq 0.001$) between drought tolerance (little leaf drying) and leaf rolling and tiller numbers. While drought tolerance was negatively correlated with leaf area index and tall plants (PH) (Table 9). Leaf rolling was significantly negatively associated ($P \leq 0.001$) with plant height and leaf area index ($P \leq 0.001$) and positively correlated with shoot dry weight (ShDm) ($P \leq 0.05$). Leaf area index had significant positive association with plant height and specific leaf dry weight (SLDW) and negative association with tiller numbers. Tiller number was significantly ($P \leq 0.001$) and negatively associated with plant height and LAI, and also positively associated with root and shoot dry weight. While significant negative association existed between plant height and biomass (shoot and root dry weight) (Table 9).

Table 9: Linear correlation coefficients among traits studied in a collection of 16 rice genotypes during 2005 dry season

Traits	Dt	LR	LAI	PH	TN	RDm	ShDm	RLWC
LR	0.892***							
LAI	-0.485***	-0.568***						
PH	-0.602***	-0.661***	0.590***					
TN	0.758***	0.792***	-0.584***	-0.668***				
RDm	0.299 ns	0.273 ns	-0.150 ns	-0.515***	0.481***			
ShDm	0.259 ns	0.297*	-0.164 ns	-0.325*	0.647***	0.620***		
RLWC	0.062 ns	0.062 ns	0.068 ns	-0.174 ns	0.036 ns	0.172 ns	0.137 ns	
SLDW	-0.070 ns	-0.040 ns	0.529***	0.160 ns	0.001 ns	0.151 ns	0.202 ns	0.225 ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant. Dt = drought tolerant score, LR = leaf rolling score, LAI = leaf area index, PH = plant height, TN = tiller number, RDm = root dry weight, ShDm = shoot dry weight and RLWC = relative leaf water content.

3.7.4.3 Correlation of traits between environments

Simple linear correlation coefficients were analysed to determine the possibility of predicting the performance of genotypes under stressed conditions by their performance in non-stressed (fully irrigated) conditions. Leaf area index, plant height and tiller number under fully irrigated condition were significantly ($P \leq 0.001$) positively correlated with leaf area index, plant height and tiller number, respectively under stressed conditions. Root and shoot dry weight under fully irrigated conditions was significantly correlated with root and shoot dry weight, respectively under stressed conditions (Table 10). Drought tolerance (little leaf drying) was significantly associated with all the characters examined under fully irrigated conditions. Leaf area index and plant height under fully irrigated conditions were significantly ($P \leq 0.001$) and positively correlated with leaf area index, tall plant height and negatively correlated with tiller number and drought tolerance under stressed conditions (Table 10).

Table 10: Correlation coefficients between characters under stressed and non-stressed conditions in 2005 dry season experiments

Observable characters Under stressed conditions	Observable characters under fully irrigated conditions				
	Leaf area Index	Plant height (cm)	Tiller numbers	Root dry weight (g)	Shoot dry weight (g)
Leaf area index	0.81***	0.60***	-0.72***	-0.15 ns	-0.47**
Plant height	0.64***	0.76***	-0.67***	-0.47**	-0.36 ns
Tiller numbers	-0.74***	-0.67***	0.91***	0.36*	0.72***
Root dry weight	-0.31 ns	-0.38*	0.35*	0.45**	0.27 ns
Shoot dry weight	-0.19 ns	-0.08 ns	0.28 ns	0.22 ns	0.38*
Drought tolerance	-0.59***	-0.74***	0.79***	0.48**	0.56***

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant.

Root dry weight in fully irrigated conditions was significantly ($P \leq 0.01$) and positively correlated with drought tolerance under drought stress and negatively correlated with plant height stressed conditions. Shoot dry weight in fully irrigated condition was significantly negatively correlated with leaf area index and positively correlated with tiller number and drought tolerance score under drought stress conditions. Tiller number in fully irrigated condition was significantly negatively correlated with leaf area index and plant height and positively correlated with drought tolerance score under drought stressed conditions (Table 10).

3.7.4. 4 Recovery ability and associated traits

In 2004 dry season, ability to recover from drought stress across genotypes was examined at 3 d and 10 d after stress relief (resumed irrigation). Recovery ability at 3 d and 10 d after stress relief was significantly positively associated with drought tolerance, leaf rolling and negatively associated with leaf area index (Table 11).

Table 11: Correlation coefficient of rice traits with recovery ability at 3 and 10 days after stress relief in 16 rice genotypes in 2004 dry season

Characters	3 days after stress relief	10 days after stress relief
Drought score	0.607***	0.467***
Leaf rolling score	0.615***	0.545***
Leaf area index	-0.384**	-0.291*
Plant height	-0.038 ns	0.586***
Tiller numbers	0.414**	0.248 ns
Relative leaf water content	-0.157 ns	0.502***
Plant vigour	0.048 ns	-0.600***
Shoot dry matter	0.236 ns	0.308*
Root dry matter	-0.033 ns	0.043 ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

In 2005 dry season stressed experiment, significant variation among the genotypes was not observed for most of the characters measured when stress was relieved. However, drought tolerant score and leaf rolling were significantly ($P \leq 0.001$) and positively associated with recovery ability at 3 d and 10 d after stress relief, while tiller number was significantly positively correlated with recovery ability at 3 d and 10 d after stress relief (Table 12).

Table 12: Correlation coefficient of rice characters with recovery ability at 3 and 10 days after stress relief of 16 rice genotypes in 2005 dry season

Traits	3 days after stress relief	10 days after stress relief
Drought score	0.540***	0.462***
Leaf rolling score	0.568***	0.534***
Leaf area index	-0.260 ns	-0.244 ns
Plant height	-0.215 ns	-0.264 ns
Tiller numbers	0.544***	0.430**
Relative leaf water content	0.098 ns	0.107 ns
Specific leaf dry weight	0.016 ns	-0.051 ns
Shoot dry matter	0.260 ns	0.202 ns
Root dry matter	0.062 ns	-0.062 ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

3.7.5 Effects of genotypes-environments interaction on the stability of some rice characters

The nature of genotype by environment (G x E) interactions on rice characters was examined using Finlay and Wilkinson (1963) and Eberhart and Russell's models (1966). The results from only three characters (tiller number, root dry weight and relative leaf water content) that showed significant G x E interaction are presented in Table 13. The G x E interaction was tested against pooled error and it was found to be highly significant ($P \leq 0.001$) for tiller number and relative leaf water content and significant ($P \leq 0.05$) for root dry weight. The mean squares for genotypes and environments for these three characters were highly significant ($P \leq 0.001$) except for relative water content that was significant at $P \leq 0.01$ (Table 13).

Environment was the most dominant source of variation. It contributed the highest percentage of variation (based on the sums of square) for root dry weight and RLWC accounting for 51.2% and 58.3 % of the observed variation, respectively. While genotypes accounted for 69.7 % of the observed variation for tiller number. However, G x E interactions were equally important, accounting for more than 2 times as much of the observed variation due to the effect of genotype for RLWC as well as the effect of environment for tiller number. G x E interactions were low for root dry weight accounting for only 3.2 % of the observed variation (Table 13).

Table 13: Analysis of variance of three rice characters of 16 rice genotypes over three environments

Tiller numbers

Source	df	SS	MS	Probability
Environments (E)	2	139.69	69.84	***
Replications (environment)	5	46.06	9.21	**
Genotypes (G)	15	1357.43	90.50	***
Genotype x Environment (GxE)	30	305.88	10.20	***
Pooled error	75	194.13	2.59	
Total	127	1946.57		

Root dry weight

Source	df	SS	MS	Probability
Environments (E)	2	28.54	14.27	***
Replications (environment)	5	0.59	0.12	ns
Genotypes (G)	15	9.38	0.64	***
Genotype x Environment (GxE)	30	1.78	0.26	*
Pooled error	75	10.54	0.14	
Total	127	55.74		

Relative leaf water content

Source	df	SS	MS	Probability
Environments (E)	2	7920.54	3960.27	***
Replications (environment) +	4	195.50	48.88	ns
Genotypes (G)	15	1178.88	78.59	**
Genotype x Environment (GxE)	30	2698.58	89.95	***
Pooled error	60	1805.28	30.09	
Total	111	13575.13		

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level, respectively; ns = non significant
 + Samples were taken in two replications in environment 1 and 3, and three replications in environment 2.

Stability parameters for tiller number showed that RAM 3 was the most stable with mean tiller number above the trial mean and a regression coefficient approximated to $b_i = 1$. The NERICA 2 and WAB 375-B-H3-2 could be considered as stable also, but they had tiller

numbers below the mean. There were five genotypes with below average stability having regression coefficients greater than one ($b_i > 1$) and eight genotypes above average stability having regression coefficients less than one ($b_i < 1$) (Table 14).

Table 14: Stability parameters for tiller number in 16 rice genotypes grown in three environments.

Genotypes	*Env-1	Env-2	Env-3	Mean	bi ‡ (slope)	S ² di†	Probability
RAM 3	7	9	11	9	0.96	0.03	***
OS 6	12	10	15	13	1.49	2.65	*
RAM 85	4	3	4	4	-0.69	0.99	ns
RAM 86	4	12	15	10	3.60	1.72	***
RAM 163	8	4	6	6	-0.48	2.02	ns
RAM 118	9	11	15	12	2.88	0.05	***
CG 14	8	13	17	13	3.603	1.80	***
TOG 5681	7	3	6	5	-0.11	2.32	ns
NERICA 2	3	4	5	4	1.01	0.15	***
NERICA 3	3	4	5	4	0.51	0.10	***
WAB 365-B-1-H1-HB	2	4	3	3	0.18	0.22	ns
WAB 375-B-9-H3-2	4	5	7	5	1.01	0.01	***
WAB 450-I-B-P-103-HB	3	4	3	3	0.15	0.09	ns
WAB 45-I-B-P-6-1-1	3	5	4	4	0.35	0.41	ns
WAB 450-I-B-P-105-HB	2	3	4	3	0.26	0.03	***
WAB 880-1-38-13-1-P1-HB	3	5	6	5	1.29	0.22	***
Grand mean	5	6	8	6			

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

*Env-1, Env-2 and Env-3 = environments, ‡ = regression coefficients and † = deviation from the regression line

Six genotypes showed no significant variation for the stability parameters for tiller number (Table 14). Only five genotypes had tiller number above the mean of which four were *O. glaberrima* genotypes and the fifth was the check (OS 6). All the genotypic variances deviated either below or above the mean ($S^2di = 0$) (Table 14).

Stability parameters for root dry weight were significant for all the genotypes except TOG 5681. A genotype that could be regarded as most stable was NERICA 3, because it had a root dry weight mean of 1.27 g and stability parameters of “bi” and “S²di” close to one and zero, respectively (Table 15). However, RAM 85, WAB 365-B-1-H1-HB, WAB 450-I-B-

P-103-HB, and WAB 450-I-B-P-105-HB had good stability parameters, but low root dry weight below the mean. The 2005 drought stressed experiments (Env-2) had the lowest mean value (Table 15).

Table 15: Stability parameters for root dry weight in 16 rice genotypes grown in three environments

Genotypes	Env-1	Env-2	Env-3	Mean (g)	bi ‡ (slope)	S ² di †	Probability
RAM 3	1.67	0.46	1.61	1.25	1.09	0.09	***
OS 6	1.12	0.93	2.8	1.61	1.37	0.24	**
RAM 85	1.18	0.51	1.64	1.11	0.95	0.00	***
RAM 86	1.6	1.21	2.17	1.51	0.67	0.11	*
RAM 163	1.41	0.44	1.37	1.07	0.88	0.06	***
RAM 118	0.96	0.46	1.6	1.01	0.92	0.01	***
CG 14	2.39	0.87	4.49	1.92	1.49	0.10	***
TOG 5681	1.12	0.53	0.52	0.72	0.13	0.11	ns
NERICA 2	1.33	0.68	2.28	1.43	1.28	0.02	***
NERICA 3	1.3	0.69	1.81	1.27	0.94	0.00	***
WAB 365-B-1-H1-HB	1.26	0.57	1.69	1.17	0.94	0.00	***
WAB 375-B-9-H3-2	1.52	0.57	1.87	1.32	1.13	0.01	***
WAB 450-I-B-P-103-HB	1.05	0.55	1.38	0.99	0.7	0.00	***
WAB 45-I-B-P-6-1-1	1.51	0.59	2.21	1.44	1.36	0.00	***
WAB 450-I-B-P-105-HB	1.05	0.5	1.52	1.02	0.85	0.00	***
WAB 880-1-38-13-1-P1-HB	1.6	0.54	2.2	1.3	1.32	0.04	***
Grand mean	1.32	0.63	1.82	1.26			

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

*Env-1, Env-2 and Env-3 = environments, ‡ = regression coefficients and † = deviation from the regression line

All the genotypes had significant stability parameters for relative leaf water content. Only RAM 3 could be approximated to be stable amongst the genotypes examined (Table 16). The *O. glaberrima* genotypes could be regarded as the less stable for relative leaf water content, because of their higher deviation from stability parameters as compared to other genotypes (Table 16).

Table 16: Stability parameters for relative leaf water content in 16 rice genotypes grown in three environments.

Genotypes	Env-1	Env-2	Env-3	Mean (g)	bi‡ (slope)	S ² di	‡ Probability
RAM 3	60.17	78.45	70.72	69.78	1.15	0.05	***
OS 6	78.93	80.06	77.76	78.92	1.47	18.12	***
RAM 85	57.87	78.65	73.38	69.97	1.48	7.49	***
RAM 86	53.57	79.64	71.01	68.07	1.25	0.02	***
RAM 163	45.56	78.68	78.61	67.62	1.79	43.68	***
RAM 118	41.52	81.68	79.00	67.40	1.57	19.51	***
CG 14	61.88	79.87	60.31	67.35	0.79	53.11	***
TOG 5681	60.80	78.98	71.58	70.45	0.74	1.26	***
NERICA 2	67.88	80.26	74.51	74.22	0.52	1.12	***
NERICA 3	75.00	76.37	69.40	73.59	0.40	4.62	**
WAB 365-B-1-H1-HB	66.65	76.73	73.57	72.32	0.23	0.66	***
WAB 375-B-9-H3-2	72.32	82.89	73.18	76.13	0.50	10.29	*
WAB 450-I-B-P-103-HB	56.44	82.07	71.14	69.88	1.26	1.09	***
WAB 450-I-B-P-6-1-1	57.07	83.29	76.95	72.43	1.30	2.30	***
WAB 450-I-B-P-105-HB	68.61	82.75	67.92	73.09	1.24	9.85	***
WAB 880-1-38-13-1-P1-HB	63.60	81.36	79.01	74.66	0.31	0.00	***
Grand mean	61.74	80.11	73.00	71.62			

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

*Env-1, Env-2 and Env-3 = environments, ‡ = regression coefficients and † = deviation from the regression line

3.8 Discussion

3.8.1 Effects of drought stress on morpho-physiological characters of genotypes

Drought tolerance is a complex trait and should be defined relative to plant characters. Potential plant yield under drought stress, which is often used as a measure of drought tolerance may not be a reliable selection criterion (Blum, 1982). Most of the genotypes examined under drought stress could be regarded as exhibiting drought tolerance or avoidance mechanisms for most of the morpho-physiological characters under study thus corroborate the previous reports of (O'Toole and Chang, 1979; Cooper, 1995).

The selection indices for genotypes varied both in the 2004 and 2005 dry season experiments. These variations could be attributed to the degree of drought intensity and variation in the environments (Figures 2 and 3). In 2004, genotypes with high selection indices were WAB 450-I-B-P-103-HB, WAB 880-1-38-13-1-P1-HB and NERICA 2. They

are drought tolerant (little leaf drying) and less leaf rolling. They also had high recovery ability three days after stress relief and early flowering. In 2005, the genotype with the highest selection index was WAB 450-I-B-P-103-HB. Other genotypes with high selection index values were WAB 450-I-B-P-105-HB, NERICA 3 and TOG 5681. These genotypes were used as parents in the drought stressed hybridisation programme. Although most of the *O. glaberrima* genotypes such as RAM 118 and RAM 85 had low selection indices, they were used as parents because of their tillering ability and plant biomass. The selection indices showed that WAB 450-I-B-P-103-HB was better as compared to all the genotypes examined. It had consistently low drought scores (little leaf drying) in the two dry season experiments. Therefore it could also be used as part of the gene pool for a drought tolerance breeding programme.

Drought tolerance index for genotypes was computed in 2005 dry season. The drought tolerance index assisted in the determination of the extent of drought stress effect on the genotypes relative to the fully irrigated conditions. The *O. glaberrima* genotypes suffered considerable reduction in leaf area index due to drought compared to interspecifics and *O. sativa* genotypes (Table 7). This reduction in LAI may affect total evapotranspiration and this could be regarded as an avoidance mechanism. The study found that some genotypes such as CG 14, TOG 5681 and WAB 375-B-9-H3-2 maintained reasonable increases in LAI thus exhibiting a drought tolerance mechanism. LAI is an important indirect yield component and its use in the breeding programme could translate into higher yield. Higher reduction in specific leaf dry weight was observed in *O. glaberrima* genotypes. Although this could be regarded as an avoidance mechanism to reduce water loss, it might reduce their yield potential as well (Tables 7). Specific leaf dry weight is an important determinant of potential leaf area production and weed competitiveness (Dingkuhn *et al.*, 1996). Therefore, in a water-limited environment, these interspecific and *O. sativa* genotypes could be evaluated for weed competition as they recorded lower reduction in SLDW.

Relative leaf water content estimates the water status of the leaf tissue relative to its capacity at full turgidity. Therefore, it could be regarded as a measure of water deficit in the plant leaf. Inability of genotypes to express significant differences in RLWC might be due to the fact that the stress level was not severe enough to reveal genetic differences, similar

observations were reported by Liu *et al.* (2004). However, some genotypes showed a higher rate of water loss compared to others during drought stress (Tables 7). This might enhance the photosynthetic apparatus of the plant as evidenced by their higher biomass observed in well-irrigated (control) conditions (Appendix III). Genotypes such as CG 14, WAB 450-I-B-P-105-HB and WAB 450-I-B-P-103-HB had high rate of water loss (Table 7).

The *O. glaberrima* genotypes exhibited higher tillering ability, a character that is significantly correlated with weed competitiveness of upland rice (Fofana *et al.*, 1995). However, the remarkable reduction in tiller number under drought stress relative to the control could affect their yield potential as compared to interspecific or *O. sativa* genotypes (Table 7). This may not be a good trait to use for developing high yielding genotypes. The high tiller number reduction observed in *O. glaberrima* genotypes might be due to a long photoperiod sensitive phase (PSP) in the vegetative stage of the plant development. Lilley and Fukai (1994) reported that root growth of all cultivars had stopped by 54 days after sowing. *O. glaberrima* genotypes may have stopped root growth before the completion of the vegetative stage. General reduction in plant height due to drought stress was expected for all the genotypes (Singh and Mackill, 1991), but it was less pronounced in *O. glaberrima* genotypes compared to interspecific and *O. sativa* genotypes. This could be attributed to a compensatory mechanism involving reducing tiller number to maintain plant height. The *O. glaberrima* genotypes were higher in plant biomass and lower in root to shoot ratio as compared to interspecific and *O. sativa* genotypes. They could be used in developing genotypes with higher plant biomass and a high plant biomass may be indirectly related to efficiency in extracting soil water under favourable conditions.

Rice plants easily roll their leaves under water stress. Leaf rolling is regarded as an indicator of a cultivar's ability to maintain a favourable water status under stress (Lafitte and Courtois, 2002), and it is highly heritable (Price and Courtois, 1999). This study showed that greater leaf rolling was more pronounced within the *O. glaberrima* genotypes. Greater leaf rolling may not be an indication of higher drought stress susceptibility. This could be misleading as some genotypes like RAM 55, TOG 5681 and CG 14 had a lower drought score, but greater leaf rolling, a greater leaf rolling may also be regarded as a symptom of drought stress intensity and adaptive response by the plant thus corroborate the previous

reports (Henderson *et al.* 1995; Maji *et al.*, 2001). Interspecific inbred genotypes generally performed better relative to morpho-physiological characters examined under drought stressed conditions.

3.8.2 Relationships between morpho-physiological characters

Genotypic variation existed in the response to drought amongst the plant characters examined. Drought tolerance (little leaf drying) was significantly associated with less leaf rolling, larger leaf area index, and taller plants. These associations were consistent in the two seasons and between and across rice species. Similar observations were reported (Henderson *et al.*, 1995; Singh and Machill, 1991; Cruz *et al.*, 1986). Thus, these could be regarded as putative-adaptive characters indicative of drought tolerance.

Positive relationship that was observed with lower drought score and lower tiller number shows that genotypes with low tillering ability could be regarded as drought tolerant. This finding is consistent with the report of Chang *et al.* (1982). However, high tiller number was positively associated with greater leaf rolling and larger plant biomass. In water-limited environments, plant biomass production is related to extractable soil water (Puckridge and O'Toole, 1981) as well as assimilates storage in the vegetative organs of the plant (Begg, 1980). Therefore, high tillering genotypes such as the *O. glaberrima* genotypes could perform better in a long dry spell compared with low tillering genotypes. This observation is contrary to the reports (Ichwantoari *et al.*, 1989; Chang *et al.*, 1982) that say higher tillering genotypes were susceptible to drought stress. High tillering was significantly associated with shorter plants, a criterion that could be used in breeding for short plants mostly for rainfed lowland ecologies, where lodging is one of the major constraints in rice production.

The significant correlation observed between characters under irrigated and stressed conditions showed that the performance of genotypes in an off-season experiment could be used to predict their performance under stressed conditions (Table 11). Thus, if planting materials are not limiting, there could be rapid advancement in screening for drought stress using off-season trials. The advantage of this method is that the same environment was used as selection and target environment. The selected genotypes from the dry season could be

evaluated for yield performance in the wet season. This is similar to the screening strategy proposed by Fukai et al. (1999).

In conclusion, drought tolerance was associated with many traits at the vegetative stage, but not all traits appeared to be equally important for different rice species. Developing rice genotypes that are drought tolerant at the vegetative stage is therefore very important for rice production. This is because water stress at the vegetative stage induces a permanent damage in the rice crop that affects rice growth and development.

3.8.3 Recovery ability and associated characters

Drought recovery ability is an important component that could affect grain yield under drought stress conditions (Pantuwan *et al.*, 2002; Wade *et al.*, 1998). It could also assist farmers in predicting whether rice stands in the field could survive when favourable conditions resumed (Malabuyoc *et al.*, 1985). Faster drought recovery was significantly associated with a lower drought score (little leaf drying), fewer tillers and larger leaf area index (Table 10). Lilley and Fukai (1994) observed similar relationships. The *O. glaberrima* genotypes showed poor recovery rate in plant biomass (shoot and root dry weight) at 3 days after stress relief but some recovered fast at 10 days. This phenomenon could be attributed to a long vegetative phase of these genotypes, which may allow sufficient time to recover from stress. This is similar to the findings of Singh and Mackill (1991) for *O. sativa* species.

3.8.4 Genotype-by-Environment interactions on some rice characters

Tiller number, root dry weight and RLWC directly and indirectly influenced plant growth and development in this study. Aside from genotypic variation, there was a differential genotype-by-environment interaction for these characters, confirming the existence of genetic differences among the genotypes, thus indicating that these characters were highly influenced by the changes in environment. Environment was the dominant source of variation for root dry weight and relative leaf water content. This could be exploited for population improvement in the breeding programme. This is particularly important for good root system structure for upland and lowland rice ecologies. Genotype was the main source of variation for relative leaf water content. Genotypes with high relative leaf water content could be used in the breeding of rice cultivars that maintain high internal plant water status

under drought stressed conditions. However, the significant G x E interaction for these characters could be an impediment to selection strategies that aim to improve broad based adaptation. Other characters examined showed no significant G x E interaction, such as plant height, leaf rolling and leaf area index, thus, they might be non-responsive to changes in the environment. Recently, Fuakai and Cooper (2002) reported large G x E interaction for grain yield of rice under rainfed lowland conditions.

Stability parameter (deviation from the regression line) for the three traits examined showed significance for most of the genotypes. This could indicate that the performance of the genotypes across environment could not be predicted, therefore considered unstable for these three traits. A stable genotype is defined as having unit regression coefficient over environments “ $b = 1.0$ ” and minimum deviation from the regression line “ $S^2_{di} = 0.0$ ” (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966). Based on this model, stability parameters for tiller number showed that RAM 3 was the most stable, while NERICA 3, RAM 3, NERICA 2 and WAB 375-B-9-H3-2 were the most stable for root dry weight. The most stable genotypes for relative leaf water content was RAM 3. Genotypes with regression coefficients greater than one “ $b > 1$ ” (Tables 14, 15 and 16) could be regarded as sensitive to changes in the environments, with below average stability. These genotypes could perform better under favourable environmental conditions. While Genotypes that had regression coefficient less than one “ $b < 1$ ” (Tables 14, 15 and 16) were less sensitive to changes in the environment, with above average stability. They could be better adaptable to less favourable environmental conditions (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966). The possible limitations of the regression analysis in measuring stability due to high bias especially with few environments used in the study is acknowledged. Thus, stability of these genotypes should be interpreted with caution.

Conclusion

Most genotypes' performance was affected by drought stress. The best performing genotypes based on selection indices in 2004 season were WAB 450-I-B-P-103-HB, WAB 880-1-38-13-1-P1-HB and NERICA 2. Amongst the *O. glaberrima* genotypes CG 14 and RAM 86 had the highest selection indices in 2004 and TOG 5681, RAM 85 and RAM 163 in 2005 dry season experiment.

Significant correlations were observed amongst morpho-physiological traits examined such as drought tolerance (little leaf drying), which was significantly associated with less leaf rolling, larger leaf area index, and taller plants. This study also observed a significant relationship between morpho-physiological traits in fully irrigated and under drought stressed experiments. Drought tolerance was significantly associated with fast recovery ability. These relationships could be exploited in the prediction of genotypes performance and their development.

The drought tolerance indices showed that the genotypes performed differently in response to drought stress. This drought tolerance index could be used in the population development in a rice breeding programme. Most of the *O. glaberrima* genotypes trade-off their tiller number for plant height under drought stressed conditions.

Effects of genotypes, environment and their interactions were significant for tiller number, root dry weight and relative leaf water content, which should be considered in breeding for these traits. Genotypes identified to be stable, such as RAM 3 for tiller number and relative water content could be used in population improvements.

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APPENDIX I

The effect of water stress on rice characteristics in 2004 dry season experiment

Genotypes	Drought score	Leaf rolling	Leaf area index	Plant height (cm)	Tiller number	Root dry weight (g)	Shoot dry weight (g)	RLWC (%)	Vigour
WAB 450-I-B-P-103-HB	1.00e	1.67d	4.49a	71.3a	3.00b	1.05b	15.85a	56.40a	2.33b
WAB 45-I-B-P-6-1-1	1.67de	3.67c	4.08a	54.00cd	3.67b	1.51b	17.20a	57.10a	5.00ab
CG 14	1.67de	5.00bc	3.15a	36.67f	8.67a	2.39a	15.16a	61.87a	4.33ab
NERICA 2	1.67de	5.00bc	4.04a	52.33cd	3.33b	1.33b	11.83a	67.88a	7.00a
RAM 55	2.00cde	6.00ab	3.05a	44.67ef	10.96a	1.17b	14.57a	48.30a	5.67ab
WAB 375-B-9-H3-2	2.00cde	5.00bc	4.58a	49.67de	3.67b	1.52b	12.93a	72.32a	3.67ab
WAB 880-1-38-13-1-P1-HB	2.00cde	5.00bc	4.23a	52.67cd	3.00b	1.16b	12.39a	63.61a	3.67ab
RAM 3	2.33bcde	6.00ab	4.23a	49.0de	7.33a	1.67b	19.53a	60.17a	5.67ab
TOG 5681	2.33bcde	6.33ab	3.23a	53.33cd	7.00a	1.12b	16.83a	60.80a	4.33ab
WAB 450-I-B-P-105-HB	2.33bcde	5.00bc	4.60a	67.00a	2.33b	1.05b	13.98a	68.61a	2.33b
WAB 365-B-1-H1-HB	2.33bcde	5.33b	4.00a	57.66c	2.00b	1.26b	12.15a	66.65a	5.67ab
RAM 86	2.67bcde	5.00bc	3.08a	59.33bc	4.33b	1.16b	16.78a	53.60a	5.67ab
RAM 118	3.67abcd	6.00ab	3.00a	43.67ef	9.33a	0.96b	11.65a	41.50a	5.00ab
NERICA 3 (check)	4.00abc	5.00bc	3.31a	59.33bc	3.00b	1.3b	12.92a	75.00a	5.00ab
RAM 163	4.33ab	7.00a	2.87a	56.00cd	7.33a	1.41b	16.55a	45.60a	3.00b
RAM 85	5.00a	7.00a	3.75a	65.67ab	4.33b	1.18b	17.84a	57.90a	4.33ab
Grand mean	2.600	5.200	3.772	55.178	4.822	1.339	14.868	59.828	4.467
LSD (0.05)	1.955	1.360	1.569	7.224	2.515	0.633	9.167	19.376	3.027
Probability	**	***	ns	***	***	*	ns	ns	ns

Letter of the same alphabet are not significantly different at: *, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

APPENDIX II

The effect of water stress on rice characteristics in 2005 dry season experiment

Genotypes	Drought score	Leaf rolling	Leaf area index	Plant height (cm)	Tiller number	Root dry weight (g)	Shoot dry weight (g)	RLWC (%)	SLDW (m ² kg ⁻¹)
WAB 450-I-B-P-103-HB	3	2	2.83ab	54.80ab	4	0.57ab	8.17ab	82.07a	13.00a
NERICA 3	3	2	2.37bcd	52.27abc	4	0.70b	9.03ab	76.37a	10.13de
RAM 163	3	2	1.77def	51.03abc	5	0.43b	6.20b	78.67a	9.53ef
TOG 5681	3	2	2.20bcde	51.35abc	3	0.53b	6.57b	79.00a	10.87cde
WAB 450-I-B-P-105-HB	3	3	2.87ab	52.09abc	3	0.50b	6.57b	82.77a	11.17bcde
WAB 45-I-B-P-6-1-1	3	3	2.40abcd	49.45bcd	5	0.57b	7.67ab	83.30a	10.83cde
WAB 365-B-1-H1-HB	3	3	2.70abc	51.01abc	4	0.57b	6.73b	76.73a	11.63abcd
NERICA 2	3	3	2.03cde	47.44cd	4	0.67b	7.07b	80.27a	11.67abcd
WAB 880-1-38-13-1-P1-HB	3	3	3.10a	46.90cd	5	0.53b	6.33b	81.37a	12.77ab
WAB 375-B-9-H3-2	3	3	1.97de	44.51d	5	0.57b	7.30b	82.90a	12.03abc
RAM 85	4	3	2.73abc	56.06a	3	0.50b	6.77b	78.63a	11.30abcd
RAM 3	4	4	1.83def	51.45abc	9	0.50b	8.93ab	78.43a	12.53abc
CG 14	5	4	1.73def	37.18e	13	0.87ab	11.30a	79.90a	12.40abc
RAM 86	5	4	1.50efg	37.77e	12	1.20a	9.60ab	79.67a	12.57abc
OS 6 (resistant check)	5	4	0.93g	36.50e	10	0.90ab	9.03ab	80.03a	8.03f
RAM 118	5	4	1.20fg	47.07cd	11	0.47b	8.37ab	81.70a	9.80ef
Grand mean	4	3	2.135	47.929	6	0.629	7.852	80.113	11.267
LSD (0.05)	0.9	0.6	0.633	5.233	2.2	0.399	3.2579	6.216	1.790
Probability	***	***	***	***	***	*	ns	ns	***

Letter of the same alphabet are not significantly different at: *, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

APPENDIX 111

Mean value of analysis of variance of rice characteristics in fully irrigated experiment in 2005 dry season experiment

Genotypes	Leaf area index	Plant height (cm)	Tiller numbers	Fresh shoot weight (g)	Shoot dry weight (g)	Fresh root weight (g)	Root dry weight (g)	RLWC (%)	SLDW (m ² kg ⁻¹)
WAB 880-1-38-13-1-P1-HB	3.48a	52.49defg	5.94c	44.25bcde	12.96bcd	8.70ab	2.20ab	79.02a	12.20abc
WAB 450-I-B-P-105-HB	3.38a	65.13ab	3.52c	36.74cde	11.03bcd	5.76bc	1.52abc	67.92bc	11.34cd
WAB 450-I-B-P-103-HB	3.31a	67.32a	3.34c	40.01bcde	12.54bcd	5.11bc	1.38bc	71.14ab	11.89bcd
RAM 85	3.27a	66.03a	3.94c	33.91de	9.80cd	6.15ab	1.64abc	73.38ab	12.34abc
NERICA 3	2.95ab	60.79abc	4.57c	43.30bcde	13.16bcd	7.44ab	1.81abc	69.40ab	10.96cd
WAB 45-I-B-P-6-1-1	2.80abc	52.9cdef	4.26c	36.12cde	11.25bcd	6.73ab	2.21ab	76.95ab	10.84cd
WAB 365-B-1-H1-HB	2.80abc	56.09cde	3.28c	34.97cde	9.81cd	6.92ab	1.69abc	73.57ab	12.36abc
RAM 163	2.23bcd	57.77bcd	6.20c	38.46cde	11.80bcd	4.58bc	1.37bc	78.61a	11.63cd
RAM 3	2.21bcd	51.54defg	10.77b	64.39ab	18.30ab	5.15bc	1.61abc	70.72ab	14.49ba
NERICA 2	2.05cd	51.29defg	5.29c	38.31cde	11.87bcd	8.56ab	2.28ab	74.52ab	10.35cd
WAB 375-B-9-H3-2	1.96cd	48.10efg	6.68c	38.58cde	11.64bcd	8.79ab	1.87abc	73.18ab	11.03cd
TOG 5681	1.81d	57.15cd	5.62c	19.67e	6.03d	1.62c	0.52c	71.58ab	10.92cd
CG 14	1.70d	48.05efg	16.77a	69.56a	22.83a	8.46ab	2.49ab	60.31c	12.27abc
RAM 86	1.57d	44.22gh	15.12a	57.29abcd	16.62abc	8.00ab	2.17ab	71.01ab	14.75a
RAM 118	1.42d	46.29fgh	15.31a	60.01abc	17.14ab	5.66bc	1.60abc	79.00a	10.65cd
OS 6 (resistant check)	1.36d	39.77h	15.16a	52.64abcd	16.09abc	10.33a	2.80a	77.76a	9.37d
Grand mean	2.393	54.056	7.859	44.26	13.302	6.744	1.819	73.003	11.709
LSD (0.05)	0.796	7.393	3.144	22.331	6.341	3.906	1.206	8.346	2.437
Probability	***	***	***	*	**	*	ns	*	*

Letter of the same alphabet are not significantly different at: *, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

CHAPTER 4

Appraisal of chemical hybridising agents in rice hybridization

Abstract

Making of planned crosses is difficult in rice because of the morphology and size of the inflorescence. Male gametocides, which can induce male sterility, can play a role in rice hybridisation. This study was undertaken to study the efficacy of two gametocides, ethrel and GA₃ as emasculating agents at ICRISAT research station, Samanko, Mali. A factorial arrangement in a randomised complete block design involving three levels of concentrations of the gametocide and five application stages was undertaken for each gametocide for two seasons. Morpho-physiological trait responses and spikelet sterility data were recorded on the female treated plants. In the case of ethrel, per cent empty spikelets and male sterility increased with concentration peaking between 2000 and 4000 ppm. Male sterility induction of 41.5% and 42.8% at 2000 ppm was achieved in 2004 and 2005, respectively, while at 4000 ppm male sterility induction was 40.5 % and 46.1% in 2004 and 2005, respectively. The best application stage of ethrel that induced highest male sterility was at P4 (panicle primordium differentiation + spikelet primordium differentiation) of 46.6% in 2004 and 49.2% at P1 (panicle primordium differentiation) in 2005. The results were inconsistent between seasons however and ethrel application was phytotoxic to the rice plant causing a reduction in plant height and panicle length. It, however, caused increased tillering, which could be advantageous. In the case of GA₃, it caused an increase in plant height, which resulted in significantly increased lodging. Therefore male sterility data were not obtained. Ethrel and gibberellic acid may not be promising gametocides in hybrid rice production because male sterility did not reach 100% in this study.

4.1 Introduction

Rice has become a staple food in Sub Sahara African countries, which need an urgent increase in rice production. Hybrid rice would increase rice production like in other cereals but production of hybrid seed in commercial quantities is a limiting factor. Rice is self-fertilized crop, which produces a single seed per spikelet. Hand pollination is the only assured way to control crossing in rice. The conventional methods such as hot water and vacuum emasculator used to emasculate the rice flower to effect pollination are tedious, labour intensive and expensive in terms of man-hours per day. Controlled crossing for population improvement is also limited due to insufficient natural sources of male sterile lines. Thus, make it difficult to obtain large quantities of crossed seeds from this autogamous (self-fertilizing) plant.

Chemical hybridisation is one of the alternatives for exploitation for commercial hybrid production. This involves the use of chemicals to create artificial male sterility by spraying

the rice plant with a gametocide. This gametocide induces pollen sterility without hopefully damaging the female organs. Earlier works on this identified Ethrel (Parmer *et al.*, 1979) and Sodium arsenate (Cho *et al.*, 1989) as an effective gamatocide for rice. Gibberellic acid (GA₃) has also been reported to induce male sterility in rice plants (Aswathanarayana and Mahadevappa, 1991). The efficiency of gametocides depends on the dosage rate, genotype and stage of application (Manjula and Ibrahim, 1999). The rate of adoption of gametocides is with varying levels of success (Kaul, 1988). The major problems attributed to these are methods of application, optimum stage of application and the dose rate of the gametocides. There are environmental and seasonal, as well as phytotoxic effects that limit their efficacy.

Review of literature reveals that an efficient gametocide must produce almost 100% male sterility without affecting female fertility. This is because the efficacy of gametocides depends on genotypes, season and location. This study therefore appraised the use of two gametocides (Ethrel and Gibberellic acid) as chemical hybridising agents in an attempt to develop an appropriate methodology for rice hybridisation for this project and region.

4.2 Objective

The objective of this study was to determine the efficacy of ethrel and gibbrellic acid as chemical hybridising agents in rice

4.3 Hypothesis

The use of gametocides is a viable option for the rapid large scale and efficient hybridisation of rice genotypes.

4.4 Materials and methods

There were two experiments established in 2004 and 2005 (wet season) at the ICRISAT research station, Samanko in Bamako, Mali. The experimental materials included an upland rice variety WAB 56-50 (*japonica* spp) as a female parent. It matures in about 110 days. Two male parents used in this experiment were NERICA 2 (interspecific cross of *japonica* background) and early maturing (between 90 to 95 days) and WITA 9 (*indica* spp), which matures in about 120 days. One pollen parent has purple stems while the female parent has

green stems. The other male parent has awns, which the female lacks. These morphological traits of the pollen parents served as genetic markers for true crosses as they are simple, dominant and heritable (WARDA, 1997; Starnes and Hadley, 1962). Ethrel (2-chloro ethyl phosphonic acid) and Gibbrellic acid (GA_3) were the gametocides used in this experiment. Four levels of concentration of the gametocides in aqueous solution were used (Table 1). The two gametocides obtained were in a water-based solution of approximately 480g/l and 32g/l for Ethrel and GA_3 , respectively.

Table 1: Chemical gametocides and concentration levels

Gametocide	Concentration (ppm)
<i>Ethrel</i>	
Control	0
L1	500
L2	2000
L3	4000
<i>GA₃</i>	
Control	0
L1	400
L2	800
L3	1000

The aqueous solutions of the gametocides were applied at five application stages at three growth stages of the plant development. The growth stages were at panicle primordium differentiation, spikelet primordium differentiation and booting of the female parent (WAB 56-50) (Table 2).

Table 2: Number of times of gametocides application on different stages of plant development

Application stage	Plant growth stage	Time of application (DAS)*
P1	Panicle primordium differentiation	45
P2	Spikelet primordium differentiation	55
P3	Booting	70
P4	Panicle primordium differentiation + Spikelet primordium differentiation	45 and 55
P5	Panicle primordium differentiation + Spikelet primordium differentiation + Booting	45, 55 and 70

*DAS: days after sowing

The first experiment was established in the last week of June 2004 (wet season). It was established in a well-tilled upland field, disc ploughed and harrowed, which facilitated hand levelling. A factorial design with 3 x 5 treatment combinations and the control were planted in a randomised complete block design with three replications. The plot size was 2.8 m x 3.0 m with plant spacing of 20 cm within rows and 20 cm between rows. Dried seeds were dibbled on a shallow hole between two to three seeds per hole and thinned to one plant per hole a week after emergence only for the female plant. Five rows of the male parents were stagger-planted on either side of the female parent for flower synchronization at 5 days intervals. The sowing spread from 29th June to 19th July for the males and the female was sown on the 9th July. Basal fertilizer was applied at the rate of 200 kg ha⁻¹ of 17-17-17, N-P-K and top-dressed in two splits with urea (46% N) at the rate of 40 kg ha⁻¹ at 35 d and 60 d after sowing.

Examination of young panicle initiation started 35 d after sowing and 10 tillers per replication were randomly collected cut from the plant base every 3 d. It was observed that the culm was firmly enveloped by the leaf sheaths. Therefore, a sewing needle was used to split and strip the leaf sheaths. The young panicle became more visible at each subsequent sampling dates to the naked eye.

A magnifying glass was used to aid its identification. The young panicle could be described, as been tiny with white hairs and looks translucent. Yoshida (1981) suggested that panicle primordium differentiation was when the young panicle was between 0.5 – 0.9 mm long. He also stated that when it is about 1.0 mm long and above, then the spikelet primordium differentiation stage begins. In this study, the panicle primordium differentiation was when the young panicle was 0.7 mm long and the first application was done, which was at 45 d after sowing. While spikelet differentiation stage was when the panicle was 2.0 mm long, which was at 55 d after sowing. The booting stage in this study was when the young panicle within the flag leaf sheath caused visible bulging of the stem and the flag leaf sheath becomes thickened, which was at 70 d after sowing.

Ten plants from the female parent were randomly selected from each plot (treatment unit). Five of the plants were bagged with red label tags and five plants were un-bagged with blue label tags before the commencement of spraying. Only the female plants tagged were sprayed at each application using a compressed air hand sprayer. The spray mist was directed to the inner most whorl of the leaves at the growing tip, the flag leaf and the next two leaves until run-off occurred. Spraying was done in the early morning hours when the air was still. The male plants were protected during spraying against drift. Wind is the main pollinating agent, although insect activities were visible in the plots.

A second experiment was established on the second week of May 2005 with the same protocol as with the first season except that a new block was used about 500 m away. The relocation was due to termite activities that affected one of the plots at the upper end of the first block. The GA₃ experiment could not be repeated because GA₃ was out of stock and also, it enhanced stem elongation that resulted in severe lodging of the plants during the flowering stage. Therefore, sterility data were not collected. The sowing of the males started from 5th to 26th May 2005, and the female parent was planted on 12th May 2005.

Two experiments were established in the early November 2005. One in the field and the other in the glasshouse from the seeds obtained from the above experiments. The experimental design was randomised complete block in three replications. Similar field preparation and crop managements as described above were used. The principal objectives

of these experiments were to identify the true crosses and classify their segregating ratio in the population using the male genetic markers as described earlier.

4.4.1 Data collection

The following data were collected only from the female plant: plant height, effective tiller number per plant, panicle length, 1000-grain-weight and panicle exertion. These were taken at maturity and the maximum tiller number was taken at panicle initiation (PI) stage. The bagged and un-bagged plants were used to determine the effects of gametocides on spikelet sterility. Full and empty spikelets were counted manually in the laboratory on both bagged and un-bagged labelled plants.

4.4.2 Statistical analysis

Per cent empty spikelet was calculated as the number of empty spikelets divided by the total number of spikelets on the plant. Per cent male and female sterility induced due to gametocide was calculated using these formulae:

$$\% MS = \%P_{ut} - \%P_{bt}$$

$$\% FS = \%P_{uc} - \%P_{ut}$$

Where % MS = per cent male sterility induced; %FS = per cent female sterility induced
%P_{ut} = per cent full spikelets un-bagged but treated; %P_{bt} = per cent full spikelets bagged and treated and %P_{uc} = per cent full spikelets un-bagged control.

Analysis of variance (ANOVA) was performed for all measured traits using Statistical Analysis System (SAS, version 9.1, 2003) to test the significance of differences among treatments.

4.5 Results

4.5.1 Interactive effects of gametocides concentration and plant developmental stages on some characters of rice

Ethrel application stages were significant for panicle length, plant height and per cent empty spikelets, while concentration level was highly significant ($P \leq 0.001$) for plant height and significant ($P \leq 0.05$) for per cent empty spikelets (Table 3). Interaction between application stage, concentration level and year was not significant for all the traits examined except for

plant height, while interaction between application stage and concentration level was significant only for tiller number (Table 3).

Table 3: Mean squares from combined analysis of variance for tiller number, panicle length, plant height and per cent empty spikelets of female plants.

Source of variation	df	Mean squares			
		Tiller number	Panicle length (cm)	Plant height (cm)	% Empty spikelets
Replication	2	4.05*	2.93 ns	60.72 ns	305.69*
Application	4	1.95 ns	34.34***	182.45***	351.69**
Concentration	2	3.48 ns	6.11 ns	375.32***	284.70*
Year	1	61.00***	0.00 ns	2.01 ns	149.30 ns
Concentration x Year	2	9.75***	13.60**	24.30 ns	27.34 ns
Application x Concentration	8	2.89*	1.30 ns	11.23 ns	45.72 ns
Application x Year	4	3.00 ns	10.84**	119.16**	138.52 ns
Application x Concentration x Year	8	2.36 ns	2.29 ns	104.38**	31.91 ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant.

4.5.2 Effects of ethrel concentrations on rice

Empty spikelets increased in order of ethrel concentration level (Table 4). Ethrel induced maximum empty spikelets of 74.4%, which was significantly different from the control (33.1%) applied at 4000 ppm during panicle and spikelet differentiation stages in 2004. However, in 2005, application of ethrel was significant difference between the treatments and the control at ($P \leq 0.05$) (Table 4). Maximum empty spikelet of 78.8% at 4000 ppm during panicle and spikelet differentiation stages was obtained in 2005. In 2004 season, the three levels of ethrel concentration increased tiller number and decreased panicle length and plant height relative to the control in the order of concentration. In 2005 season, the three levels of ethrel concentration decreased plant height relative to the control (Table 4).

4.5.3 Effects of application stages of ethrel on rice

Effects of application stages of ethrel on seed development showed significant variation from year to year. In 2004, ethrel application at P4 (Panicle primordium differentiation + Spikelet primordium differentiation) had the highest empty spikelet of 71.6% on the average

and the lowest was 57.3% at P5 (Panicle primordium differentiation + Spikelet primordium differentiation + Booting). In 2005, ethrel application at P1 (Panicle primordium differentiation) had the highest empty spikelets of 73.5% and lowest was at P3 (Booting) with 60.6%. The mean percentage empty spikelet was 64.4% and 67.0% in 2004 and 2005, respectively (Table 4).

Ethrel application at P5 and P2 (Spikelet primordium differentiation) had the highest tiller number in 2004 and 2005 respectively. Significant variation for panicle length was observed with ethrel application stages and P2 and P1 had the highest panicle length in 2004 and 2005, respectively. Ethrel application significantly decreased plant height and the decrease was more at P5 (Table 4)

4.5.4 Interactive effect of ethrel concentration and application stages on rice

Significant interaction between concentration levels of ethrel and application stages was observed only for tiller number. The interaction increased tiller number and high tiller number was observed between 4000 ppm and P5 in 2004 and between 2000 ppm and P4 in 2005 (Table 4). Interactive effect between concentration, application stages and year was significant only for plant height and caused decreased in plant height. Plant height decreased was higher with the interaction between ethrel at 4000 ppm and P5 in both years.

Table 4: Interactive effects of ethrel concentration and developmental stage of rice plant on traits and seed development of rice

Application	2004				2005			
	Concentration levels			Mean	Concentration levels			Mean
	L1	L2	L3		L1	L2	L3	
<i>% Empty spikelets</i>								
	L1	L2	L3	Mean	L1	L2	L3	Mean
P1	63.6	69.7	64.4	65.9	73.9	71.1	75.7	73.5
P2	61.9	67.4	67.8	65.7	58.9	66.2	67.9	64.3
P3	61.2	61.3	61.6	61.4	59.9	63.3	58.8	60.6
P4	69.2	71.1	74.4	71.6	60.8	69.4	78.8	69.7
P5	49.5	62.2	60.1	57.3	63.7	65.8	71.0	66.8
Mean	61.1	66.3	65.7	64.4	63.4	67.1	70.5	67.0
Control	33.1				66			
LSD (0.05)	12.38***				16.56*			
<i>Tiller number</i>								
P1	6	7	7	7	4	5	5	5
P2	7	7	8	7	4	7	6	6
P3	7	6	7	7	7	5	6	6
P4	6	8	9	8	5	7	4	5
P5	7	7	9	8	5	6	5	5
Mean	7	7	8	7	5	6	5	5
Control	6				6			
LSD (0.05)	1.5*				2.2*			
<i>Panicle length</i>								
	L1	L2	L3	Mean	L1	L2	L3	Mean
P1	25.1	24.2	23.9	24.4	24.1	24	25.3	24.4
P2	26.1	25.2	23.0	24.8	21.3	22.9	22.6	22.3
P3	21.9	20.8	21.1	21.3	23.6	21.9	22.5	22.7
P4	26.1	22.9	22.1	23.7	23.0	23.0	24.7	23.6
P5	21.4	19.9	20.0	20.4	21.7	21.7	21.5	21.6
Mean	24.1	22.6	22.0	22.9	22.7	22.7	23.3	22.9
Control	24.2				23.1			
LSD (0.05)	2.70***				2.29*			
<i>Plant height</i>								
	L1	L2	L3	Mean	L1	L2	L3	Mean
P1	87.6	81.8	75.9	81.8	89.1	91.0	89.3	89.8
P2	87.6	84.6	83.3	85.2	85.8	83.0	77.1	82.0
P3	93.1	86.4	89.8	89.8	86.4	89.9	80.2	85.5
P4	89.5	80.7	74.3	81.5	81.2	77.0	80.2	79.5
P5	78.6	77.6	80.6	79.0	93.4	81.7	70.8	82.0
Mean	87.3	82.2	80.8	83.4	87.2	84.5	79.5	83.7
Control	88.6				87.7			
LSD (0.05)	7.02***				11.18*			

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively.

4.5.5 Per centage sterility induced by ethrel concentration on seeds development in rice

In 2004 season ethrel at 2000 ppm had the highest induction of male sterility of 41.5% followed by 4000 ppm with 40.5%. All the levels of ethrel concentration induced higher male sterility than the control (Figure 1). In 2005 season, the per centage of male sterility induced increased with the strength of Ethrel concentration. All the levels of ethrel concentration were higher than the control except 500 ppm in male sterility induction (Figure 1).

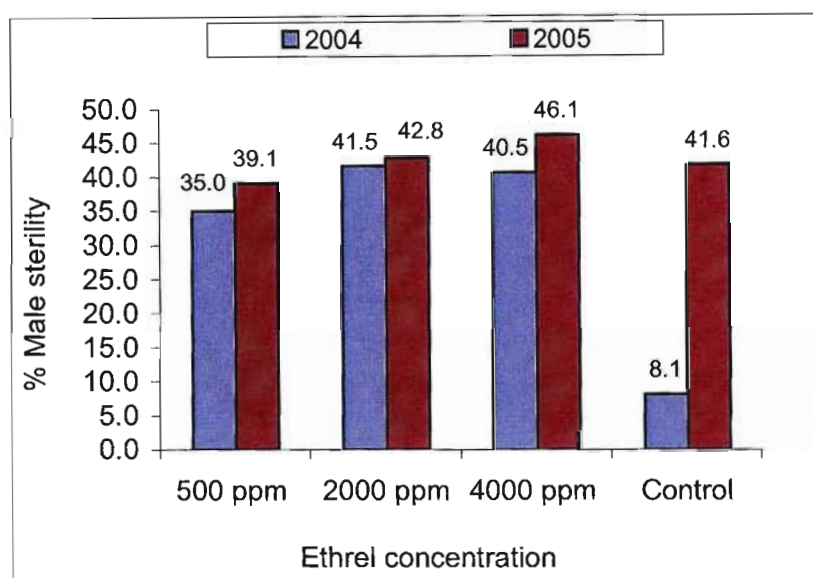


Figure 1: Per centage male sterility induced by ethrel concentration.

Ethrel application had different effects in inducing male sterility at different growth stages of rice. In 2004 season, ethrel application at the P4 stage induced more male sterility by 46.6% followed by P2, which had 41.0% (Figure 2). Ethrel application at P5 had least induction of male sterility with 32.4%. In 2005 season, however, P1 was the highest in inducing male sterility with 49.2% followed by P4 with 45.3% and P3 was the least with 36.3% (Figure 2).

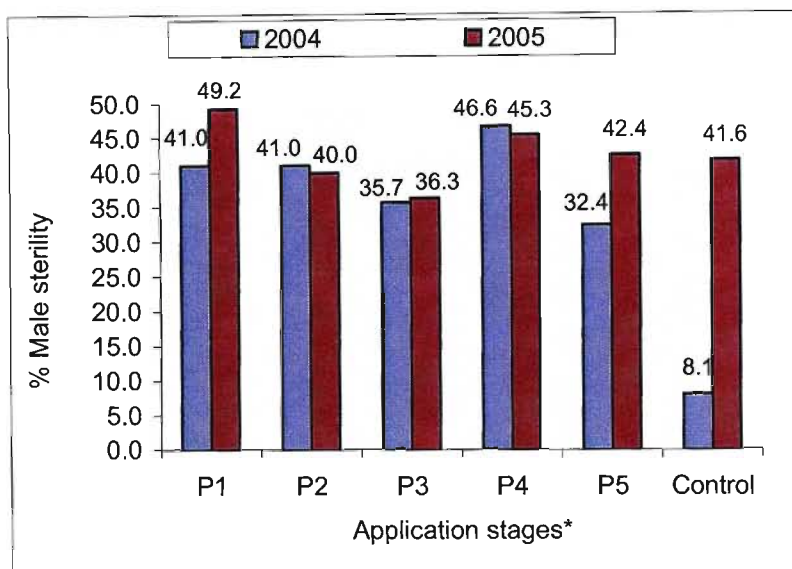


Figure 2: Per centage sterility induced by ethrel application stages.

* Refer to Table 2

4.5.6 Effects of gibberellic acid on rice traits during 2004 season experiment

Gibberellic acid (GA_3) caused an increased in plant height that resulted to severe lodging of the rice plant. Therefore, data on male sterility were not collected. Increased in plant height varied with the strength of the acide. Gibberellic acid decreased tiller number and the decrease also varied with the strength. Interactive effect between concentration levels and application stages of GA_3 was significant for plant height and tiller number. The most significant increases in plant height occurred in L3P4 and P3P5 had the least tiller number (data not shown).

4.5.7 Other information

There was no segregation in the late season experiments sown in the field and in the glasshouse in 2005 season. Therefore no true outcross was observed in any of the treatments.

4.6 Discussion

The results were not consistent during the two seasons. These could be attributed to environmental effects and seasonal differences. The interaction effect between application stages, concentration level and year (application x concentration x year) was not significant

for all the traits examined except plant height. The interaction effect between application stages and concentration level was significant only for tiller number amongst the traits examined in the combined analysis. However, a significant interaction between growth stages application and concentration level was observed in each season. Therefore, season comparisons may not reveal the efficacy of Ethrel treatments in this study.

4.6.1 Effects of ethrel on seed development

The effects of Ethrel in inducing spikelet sterility varied with concentration. Ethrel concentrations at 2000 ppm and 4000 ppm had highest spikelet sterility in the 2004 and 2005 seasons, respectively (Figure 1). This finding was consistent with the work of Asathanarayana and Mahadevappa (1992) in which as the concentrations of Ethrel increased from 500 to 8000 ppm, the per cent seed set decreased in proportion to the concentration. This trial was intended to develop an appropriate methodology for the use of gametocides in rice hybridisation for the region. This finding needs further clarification as to whether the empty grains obtained could have been induced by either male sterility or female sterility or both. Therefore, the outcome of these results could not be generalized but based on the status of this experiment.

The effectiveness of a gametocide at any concentration depends on the growth stage at the time of application and the degree of interaction. Differences in varietal response to gametocides have also been reported (Kaul, 1988; Shamsi *et al.*, 1996). Ethrel application at P4 (Panicle primordium differentiation + Spikelet primordium differentiation) and P1 (Panicle primordium differentiation) induced much higher spikelet sterility in 2004 and 2005 season, respectively than any other application stages. Similar data from Parmer *et al.* (1979) showed that maximum pollen sterility was obtained at panicle initiation stage with Ethrel. Thus, as Ethrel has phytotoxic effect on rice, this may interfere with pollen formation causing abortion after pollen formation or non-dehiscence of anthers (Wit, 1960). This may have resulted in a high number of empty spikelets (spikelets sterility) in all the treatments in this study. The male sterility observed in the control could be partly due to the effects of bagging.

4.6.2 Effects of gametocides on rice

The three levels of Ethrel concentration (500, 2000 and 4000 ppm) significantly increased tiller number in 2004 season and showed a reasonable increase in 2005 season (Table 4). Ethrel is a plant growth regulator. The results showed that it enhanced tillering ability of rice; which could be exploited if the tillers could be effective in grain yield production. The high empty spikelets observed for the three levels of Ethrel concentration implied that the increased tiller numbers did not translate into grain yield production. Dosage rate may have been too high thus causing phytotoxic effects on the rice plant. Panicle length is one of the yield components in rice. The reduction in panicle length by the three levels of Ethrel may affect the total grain yield. Therefore, Ethrel may not be promising as a hybridising agent for commercial hybrid rice production. In gametocides studies (Shamsi *et al.*, 1996) also showed that Ethrel reduced panicle length when applied at 10, 000 and 12,000 ppm but Manjula and Ibrahim (1999) did not observe significant reduction in panicle length. Significant decreases in plant height were observed in the 2004 season in proportion to the Ethrel concentration and appreciable decreases were seen in the 2005 season. Plant height could be reduced through an inhibition of internode elongation (Wit, 1960). This finding was consistent with the work of Aswathanarayana and Mahadevapa (1991), who found that plants treated with Ethrel showed decreases in plant height that were proportional to the concentration and frequency of application.

Gibberellic acid decreased tiller number and the decrease varied with the strength of the acid. Gibberellic acid is a plant growth regulator that increases plant height, which caused a severe lodging of the plants especially at the flowering stage of rice. Lodging is one of the detrimental traits of rice that results in little or no grain in yield. This is because of poor translocation of nutrients and assimilates into the developing seed during the reproductive stage of the crop. The gametocides used in these experiments had effects on morphological and physiological traits of rice at different growth stages of the plant (Tables 4 and 11). Gametocide with high efficacy must induce almost 100% male sterility without affecting female fertility. These results imply that ethrel may not be an ideal gametocide as hybridising agent in rice breeding programme. However, it could be useful with the assistant of genetic marker traits in any of the parents involved in the crosses.

Conclusion

Effectiveness of Ethrel as a gametocide is influenced by environment and development stage of the parents. The inconsistency in the results obtained in 2004 and 2005 seasons demonstrate the effect of the environment. Male sterility increased with increased ethrel concentration, but this also depended on the growth and application stages of the plant. Ethrel concentration at 2000 ppm and 4000 ppm were the best in relation to male sterility induction. Further clarification will be needed to determine treatment effect on male sterility or female sterility or both on the effectiveness of gametocides in sterility induction. Ethrel concentration at 2000 ppm had 41.5% and 42.8% male sterility induction in 2004 and 2005, respectively, while at 4000 ppm male sterility induction was 40.5 % and 46.1% in 2004 and 2005, respectively.

The best application stages of ethrel that induce high male male sterility were when applied at P4 (Panicle primordium differentiation + Spikelet primordium differentiation) and P1 (Panicle primordium differentiation) in 2004 and 2005 respectively. There was no observation of an outcross in all the treatments. Ethrel had phytotoxic effects; it increased tiller number and decreased panicle length and plant height. The GA₃ caused lodging in this study, therefore it may not be a promising gametocide.

Therefore no true outcross was observed in any of the treatments, this methodology would not be used for this project. Future work involving more genotypes and chemical hybridising agents is needed because differential response of genotypes has been reported.

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CHAPTER 5

Crossability of diverse rice genotypes in developing drought tolerant lines for upland rice ecologies in the tropics of Africa

Abstract

Interspecific hybridisation is an important technique used in improving rice populations, by combining desired traits from different species. However, there could be difficulty in making such crosses due to barriers to interspecific hybridization. Therefore, the objective of this study was to determine the compatibility and crossability of rice genotypes from different species. Five *O. glaberrima* genotypes and four interspecific inbred lines were used as female (seed) parents and two improved *O. sativa* and two interspecific inbred lines (NERICA 2 and NERICA 3) as male (pollen) parents to make 36 cross combinations in North Carolina design II mating scheme. Crosses were performed in the glasshouse using vacuum machine to emasculate the flowers. The crosses were performed in four groups: group A (*O. glaberrima* x *O. sativa*), group B (*O. glaberrima* x Interspecific), group C (Interspecific x *O. sativa*) and group D (Interspecifics x Interspecific). In general, all groups recorded poor success rates in crossability. Group A and B had comparable per cent crossability levels of about 10%. Group D had the highest level of crossability with a mean of 19%. The least compatible was group C with 6% crossability rate. Within groups, wide ranges of crossability values were detected as follows: A (1.3-20.5%), B (0.0-28.8%), C (1.1-12.6%) and D (2.2-39.9%). The reasons for these differences were not apparent from this study. The highest per cent seed set were obtained in the cross of CG 14 x WAB 365-B-1-H1-HB (20.5%), CG 14 x NERICA 2 (28.8%), WAB 450-IBP-105-HB x WAB 375-B-9-H3-2 (12.6%) and WAB 450-IBP-105-HB x NERICA 3 (39.9%) for group A, B, C and D, respectively. The best female parent was CG 14 followed by WAB 450-IBP-105-HB, while the best male parent was NERICA 3 followed by NERICA 2. Overall, the study indicated a serious challenge in making interspecific hybrids, because only 11% of 8031 pollinations were successful in setting seeds, compared to 45% within *O. glaberrima* and 70% within *O. sativa* crosses that is obtainable under similar conditions. Higher sterility was observed in backcrosses involving *O. glaberrima* cytoplasm as compared to single crosses. The backcrosses involving *O. glaberrima* cytoplasm were completely sterile with no seed set except with the CG 14 cytoplasm.

5.1 Introduction

Cultivated rice belongs to two species *Oryza sativa* L. and *O. glaberrima* Steud. The former is of Asian origin and is commonly known as Asiatic rice while the latter is known to have been selected and cultivated in parts of West Africa more than 3500 years ago (Carpenter, 1978; Jacquot, 1977). Although high yielding lines from Asia have largely displaced *O. glaberrima*, it is still an important crop in the farming systems of Sub-Saharan Africa. This is because of its adaptation to the local production systems and tolerance to important biotic

and abiotic production stresses. Apart from this, *O. glaberrima* is considered a sacred crop used for traditional rituals and ceremonies (Richards, 1996). The high yielding Asian rice however, easily succumbs to biotic and abiotic stresses and has poor adaptability to the African climate (WARDA, 1997). The improvement of *O. sativa* that combine high yield with tolerance to biotic and abiotics stresses would hence rice production in the region. Therefore *O. sativa* can be improved through interspecific hybridisation. Some useful traits have been found in the gene pool of *O. glaberrima* that could serve as a source of important traits for the development of new varieties tolerant to biotic and abiotic stresses (De Kochko, 1987; Jones *et al.*, 1997a).

Interspecific hybridization (crosses between *O. glaberrima* and *O. sativa*) for plant improvement is complicated by the presence of incompatibility barriers, such as hybrid seed sterility, which hinder heterogenetic recombinations (Brar and Khush, 1986; Jena and Khush, 1990). These barriers also depend on the genotypes used in the crosses. Therefore, there is need to determine the crossability of the genetic materials in this study and viability of their progenies. Thus, developing broad genetic base would enhance the stability of the interspecific progenies in the region.

5.2 Objective

Objective of this study was to determine the compatibility and crossability of rice genotypes included in this study.

5.3 Hypothesis

Rice genotypes included in this study are freely crossable.

5.4 Materials and Methods

5.4.1 Genetic materials used in crosses

The *Oryza glaberrima* lines used in the crosses were selected based on their previous performance during vegetative stage screening for drought tolerance. NERICA 2 and NERICA 3 (*O. glaberrima* x *O. sativa*) are released varieties grown in many West and Central African (WCA) countries. WAB450-I-B-P-103-HB, WAB450-I-B-P-6-1-1, WAB450-I-B-P-105-HB, and WAB880-1-38-13-1-P1-HB were selected from Observational

/ Replicated Yield Trials (OYT/RYT) based on their drought and yield performance, while WAB 365-B-1-H1-HB and WAB 375-B-9-H3-2 were selected from Elite Varietal Trials (EVT) as high yielding varieties. Pedigree and agronomic characteristics of the selected lines are shown in Table 1.

Table 1: Pedigree and agronomic characteristics used in the crosses.

Genotype	Pedigree	Species	Drought score	Tillering ability	Photo-periodicity	Yield potential
RAM 85	Landrace	<i>Oryza glaberrima</i>	Moderately tolerance	Low	Photo-sensitive	Low
RAM 86	Landrace	<i>Oryza glaberrima</i>	Moderately tolerance	High	Photo-sensitive	Low
RAM 118	Landrace	<i>Oryza glaberrima</i>	Moderately tolerance	High	Photo-sensitive	Low
CG 14	Landrace	<i>Oryza glaberrima</i>	Tolerant	High	Photo-sensitive	Low
TOG 5681	Landrace	<i>Oryza glaberrima</i>	Tolerant	High	Photo-sensitive	Low
NERICA 2	WAB 56-104 / CG 14	Interspecific	Highly tolerant	Low	Photo-insensitive	Medium
NERICA 3	WAB 56-104 / CG 14	Interspecific	Tolerant	Low	Photo-insensitive	Medium
WAB 450-I-B-P-103-HB	WAB 56-104 / CG 14	Interspecific	Highly tolerant	Low	Photo-insensitive	Medium
WAB 450-I-B-P-6-I-1	WAB 56-104 / CG 14	Interspecific	Highly tolerant	Low	Photo-insensitive	Medium
WAB 450-I-B-P-105-HB	WAB 56-104 / CG 14	Interspecific	Tolerant	Low	Photo-insensitive	Medium
WAB 880-I-38-13-1-P1-HB	WAB 56-104 / CG 14	Interspecific	Tolerant	Low	Photo-insensitive	Medium
WAB 365-B-1-H1-HB	ITA 257 / WAB 56-57	Improved <i>Oryza sativa</i>	Tolerant	Low	Photo-insensitive	High
WAB 375-B-9-H3-2	TGR 68 / WAB 56-82	Improved <i>Oryza sativa</i>	Tolerant	Medium	Photo-insensitive	High

5.4.2 Cross combinations and pollination method

The crosses were classified into four groups: group A (*O. glaberrima* x *O. sativa*), group B (*O. glaberrima* x Interspecific), group C (interspecific x *O. sativa*) and group D (Interspecifics x Interspecific) (Table 2). Five *Oryza glaberrima* genotypes and four inbred interspecific lines (derived from crosses between *O. sativa* x *O. glaberrima*) were used as female parents and crossed with two improved *O. sativa* L. subsp (*japonica*), and two inbred interspecific lines as pollen parents in a North Carolina design II mating scheme (Comstock and Robinson, 1952). Total of 36 crosses were made (Table 2). A total of 8031 pollinations were made.

Table 2: Parental lines and groupings used in the North Carolina design II mating scheme

Female parent		Male parent
<i>O. glaberrima</i> (group A)	x	<i>O. sativa</i>
RAM 85		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
RAM 86		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
RAM 118		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
CG14		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
TOG 5681		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
Total crosses = 10		
<i>O. glaberrima</i> (group B)	x	Interspecific
RAM 85		NERICA 2
		NERICA 3
RAM 86		NERICA 2
		NERICA 3
RAM 118		NERICA 2
		NERICA 3
CG14		NERICA 2
		NERICA 3
TOG 5681		NERICA 2
		NERICA 3
Total crosses = 10		
Interspecific (group C)	x	<i>O. sativa</i>
WAB450-IBP-103-HB		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
WAB450-IBP-6-1-1		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
WAB450-IBP-105-HB		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
WAB880-1-38-13-1-P1-HB		WAB375-B-9-H3-2
		WAB365-B-1-H1-HB
Total crosses = 8		
Interspecific (group D)	x	Interspecific
WAB450-IBP-103-HB		NERICA 2
		NERICA 3
WAB450-IBP-6-1-1		NERICA 2
		NERICA 3
WAB450-IBP-105-HB		NERICA 2
		NERICA 3
WAB880-1-38-13-1-P1-HB		NERICA 2
		NERICA 3
Total crosses = 8		
Grand total crosses = 36		

Dried seeds were sown in a pot filled with sandy loam soil in the greenhouse in January 2004. There were four sets of plantings staggered at weekly intervals to synchronize flowering. *O. glaberrima* genotypes are photoperiod-sensitive and require short day length to flower. A darkroom was constructed, which reduced the day-length by about eight hours by putting the plants outdoor from 7.0hr to 15.0 hr each day. Emasculation was done in the morning hours or late evening in the glasshouse. This is the period when there is little no air movement.

Emasculation was performed on a plant whose panicle had emerged about half way from the boot. The spikelets that had already undergone anthesis and the immature ones at bottom of the panicle were cut off and only the emasculated spikelets were left in the panicle. The spikelets at the middle portion of the panicle were selected to be the best for emasculation. The spikelet was cut on the slant about half or two-thirds from the base with a sterilized scissors to expose the anthers. Then the anthers were removed by a micropipette attached to vacuum emasculator, which sucks the anthers into a conical flask without damaging the stigma. The emasculated panicle was then covered with a pollinating bag and closed with paper clips or stapler. The optimum time to pollinate was midday to 15.00 hours. This was the period when anthers were open and pollen could be squeezed out or shed when gently touched with a finger. The pollen was removed from the male plant into a Petri dish containing a little water. Bags were gently removed from the seed parent and pollen was transferred onto the stigma by an office pin or forceps. The bag was replaced on the female plant and the date and the parents of the cross were written on the back of the bag. Mature seeds were harvested when they lost their green colour between 25 to 30 days after pollination. The F₁ seed was naked without glums. Seeds from each female panicle were harvested and bagged separately and parents of the cross were recorded.

5.4.3 Data collection and analysis

The following data was collected from the crosses: number of spikelets pollinated and number of matured seeds harvested. Mean values and frequency distribution expressed in per centages were calculated using excel software.

5.5 Results

5.5.1 Success rate of cross pollination between *O. glaberrima* and *O. sativa* genotypes

The group A involved ten crosses made between five *O. glaberrima* genotypes and two improved *O. sativa* lines (Table 2). There were some differences of crossability among the *O. glaberrima* genotypes based on the per centage seeds set (Table 3). The number of pollinated spikelets per cross depended on the synchronization of flowering and it was highest in the cross RAM 85 x WAB 375-B-9-H3-2 and least in RAM 86 x WAB 365-B-1-H1-HB. Significant correlation ($r = 0.68^*$) was observed between the number of spikelets pollinated and number of seeds set (Table 3).

Table 3: Number of spikelets pollinated, number of seeds set and per cent seed set between *O. glaberrima* x *O. sativa*

<i>O. glaberrima</i> x <i>O. sativa</i> (G x S) group A	No.of spikelets pollinated	No. of seeds set	% Seed set
RAM 85 x WAB 375-B-9-H3-2	276	19	6.9
RAM 85 x WAB 365-B-1-H1-HB	204	12	5.9
RAM 86 x WAB 375-B-9-H3-2	196	29	14.8
RAM 86 x WAB 365-B-1-H1-HB	-	-	-
RAM 118 x WAB 375-B-9-H3-2	62	7	11.3
RAM 118 x WAB 365-B-1-H1-HB	170	11	6.5
CG 14 x WAB 375-B-9-H3-2	54	7	13.0
CG 14 x WAB 365-B-1-H1-HB	39	8	20.5
TOG 5681 x WAB 375-B-9-H3-2	77	1	1.3
TOG 5681 x WAB 365-B-1-H1-HB	117	17	14.5
Total	1195	111	9.29
S.e. of mean	27.00	2.76	1.96

Note: Estimates of 70% and 45% seed set were observed in the crosses of *O. sativa* x *O. sativa* and *O. glaberrima* x *O. glaberrima*, respectively under the same condition.

The *O. glaberrima* genotypes were photoperiod sensitive and they flowered almost at the same time in spite of the staggered planting, thus making it difficult to pollinate all the spikelets desired. This also resulted in a shortage of pollen for some particular crosses.

Hence, no crosses were made between RAM 86 x WAB 365-B-1-H1-HB. Therefore, groups A and B had smaller number of spikelets pollinated compared to groups C and D. Five individual crosses had higher seed set than the group mean (Table 3). The three crosses with highest per cent seed set were CG 14 x WAB 365-B-1-H1-HB (20.5%), RAM 86 x WAB 375-B-9-H3-2 (14.8%) and TOG 5681-x WAB 365-B-1-H1-HB (14.5%) (Table 3).

5.5.2 Success rate of cross pollination between *O. glaberrima* and interspecific genotypes

The group B involved crosses between *O. glaberrima* x interspecifics. Five *O. glaberrima* and two interspecific lines were used in ten cross combinations (Table 4). CG 14 was the best female based on the per centage seed set amongst the *O. glaberrima* genotypes followed by TOG 5681. Two interspecific inbred lines (NERICA 2 and NERICA 3) showed high per centage seed set with some female parents (Table 4).

Table 4: Number of spikelets pollinated, number of seeds set and per cent seed set between *O.glaberrima* x interspecific lines

<i>O. glaberrima</i> x Interspecifics (G x IS) group B	No.of spikelets pollinated	No. of seeds set	% Seed set
RAM 85 x NERICA 2	237	8	3.4
RAM 85 x NERICA 3	136	15	11.0
RAM 86 x NERICA 2	85	4	4.7
RAM 86 x NERICA 3	46	0	0.0
RAM 118 x NERICA 2	146	6	4.1
RAM 118 x NERICA 3	50	6	12.0
CG 14 x NERICA 2	160	46	28.8
CG 14 x NERICA 3	100	19	19.0
TOG 5681x NERICA 2	56	11	19.6
TOG 5681 x NERICA 3	103	11	10.7
Total	1119	126	11.26
S.e. of mean	18.80	4.09	2.83

Note: Estimates of 70% and 45% seed set were observed in the crosses of *O. sativa* x *O. sativa* and *O. glaberrima* x *O. glaberrima*, respectively in the same condition.

Five crosses had seed set above the group mean and the three crosses with highest per cent seed set were CG 14 x NERICA 2, TOG 5681 x NERICA 2 and CG 14 x NERICA 3. The worst was RAM 86 x NERICA 3 with no seed set. There was no significant correlation ($r = 0.34ns$) between number of spikelets pollinated and number of seeds set (Table 4).

5.5.3 Success rate of cross pollination between interspecific and *O. sativa* genotypes

The group C comprised photoperiod insensitive genotypes (Table 5), thus synchronizing flowering was not much of a problem. The group C involved cross-pollination of interspecific lines with improved *O. sativa* lines (Table 5). The major problem observed in this group was that at 10-15 days after crossing, some of the embryos began to shrivel, especially when the air temperature was high. The roof of the glasshouse was covered with cardboard paper to reduce the temperature inside the glasshouse. This effort yielded little success, as there was not much difference between inside glasshouse (33 °C to 40 °C) and outdoor temperature.

Table 5: Number of spikelets pollinated, number of seeds set and per cent seed set between interspecific lines x *O. sativa*.

<i>Interspecifics x O. sativa (IS x S) group C</i>	No. of spikelets pollinated	No. of seeds set	% Seed set
WAB 450-IBP-103-HB x WAB 375-B-9-H3-2	180	9	5.0
WAB 450-IBP-103-HB x WAB 365-B-1-H1-HB	513	50	9.7
WAB 450-IBP-6-1-1 x WAB 375-B-9-H3-2	359	4	1.1
WAB 450-IBP-6-1-1 x WAB 365-B-1-H1-HB	693	29	4.2
WAB 450-IBP-105-HB x WAB 375-B-9-H3-2	430	54	12.6
WAB 450-IBP-105-HB x WAB 365-B-1-H1-HB	258	16	6.2
WAB 880-1-38-13-1-P1-HB x WAB 375-B-9-H3-2	659	58	8.8
WAB 880-1-38-13-1-P1-HB x WAB 365-B-1-H1-HB	649	27	4.2
Total	3741	247	6.62
S.e. of mean	68.4	7.41	1.30

Note: Estimates of 70% and 45% seed set were observed in the crosses of *O. sativa x O. sativa* and *O. glaberrima x O. glaberrima*, respectively in the same condition.

The female parent that showed the highest per cent seed set was WAB 450-IBP-105-HB. The best crosses based on per centage seed set were WAB 450-IBP-105-HB x WAB 375-B-9-H3-2, WAB 450-IBP-103-HB x WAB 365-B-1- H1-HB and WAB 880-1-38-13-1-P1-HB x WAB 375-B-9-H3-2 with seed sets of 12.6, 9.7 and 8.8 %, respectively. The worst was WAB 450-IBP-6-1-1 x WAB 375-B-9-H3-2 with 1.1% seed set. There was no significant correlation ($r = 0.58$ ns) between number of spikelets pollinated and number of seeds set (Table 5).

5.5.4 Success rate of cross pollination with interspecifics x interspecifics

The group D was made up of photoperiod insensitive genotypes. The female parents in this group had a higher per centage seed set as compared to other groups. This was because it was an intraspecific cross (Interspecifics x Interspecifics). WAB 450-IBP-105-HB and WAB 450-IBP-103-HB were the best female parents based on per centage seed set. NERICA 3 was the best male parent based on per centage seed set. Four crosses had seed sets less than the mean seed set of the group (Table 6).

Table 6: Number of spikelets pollinated, number of seeds set and per cent seed set between Interspecific lines

Interspecifics x Interspecifics (IS x IS) group D	No.of spikelets pollinated	No. of seeds set	% Seed set
WAB 450-IBP-103-HB x NERICA 2	272	6	2.2
WAB 450-IBP-103-HB x NERICA 3	396	107	27.0
WAB 450-IBP-6-1-1 x NERICA 2	167	25	15.0
WAB 450-IBP-6-1-1 x NERICA 3	112	14	12.5
WAB 450-IBP-105-HB x NERICA 2	104	9	8.7
WAB 450-IBP-105-HB x NERICA 3	321	128	39.9
WAB 880-1-38-13-1-P1-HB x NERICA 2	244	23	9.4
WAB 880-1-38-13-1-P1-HB x NERICA 3	360	65	18.1
Total	1976	377	19.08
S.e. of mean	39.2	16.78	4.21

Note: Estimates of 70% and 45% seed set were observed in the crosses of *O. sativa* x *O. sativa* and *O. glaberrima* x *O. glaberrima*, respectively in the same condition.

The highest synchronization was observed between WAB 450-IBP-105-HB x NERICA 3 followed by WAB 450-IBP-103-HB x NERICA 3. Significant correlation ($r = 0.75^*$) was observed between the number of spikelets pollinated and number of seeds set (Table 6).

5.5.5 Performance of parents as good combiner in the cross combinations

Performance of rice genotypes in the cross combinations showed that CG 14, TOG 5681 and RAM 86 were the three female parents with the highest per cent seed set amongst the *O. glaberrima* genotypes (Figure 1). While WAB105 was the best female parent with the highest per cent seed set amongst the interspecific lines. The worst female parent based on per centage seed set was WAB 611 (Figure 1).

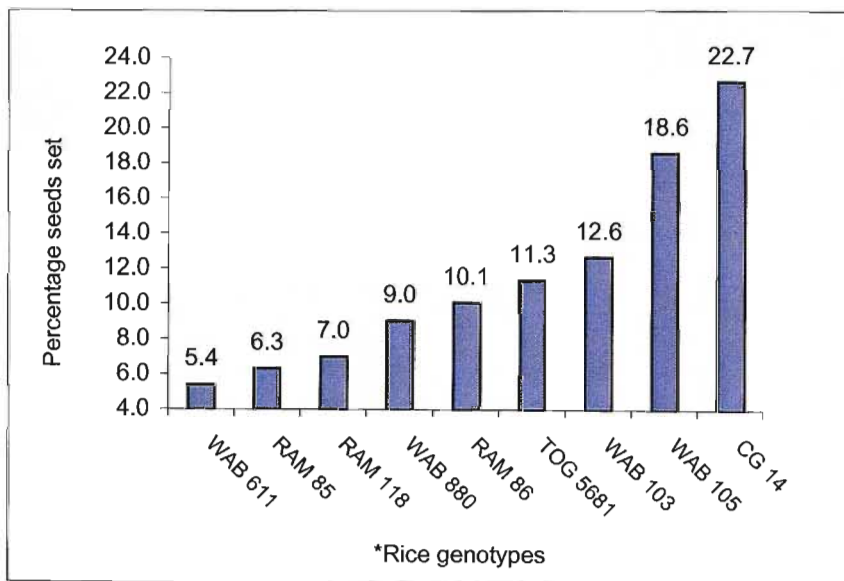


Figure 1: Performance of rice genotypes based on seed set in the cross combinations as female parents; * Rice genotypes were abbreviated.

The best male parent with the highest per cent seed set was NERICA 3, followed by NERICA 2 (Figure 2). The *O. sativa* male parents were lower in per centage seed set than the NERICA lines and WAB 365 was the worst parent with 6.4 % seed set.

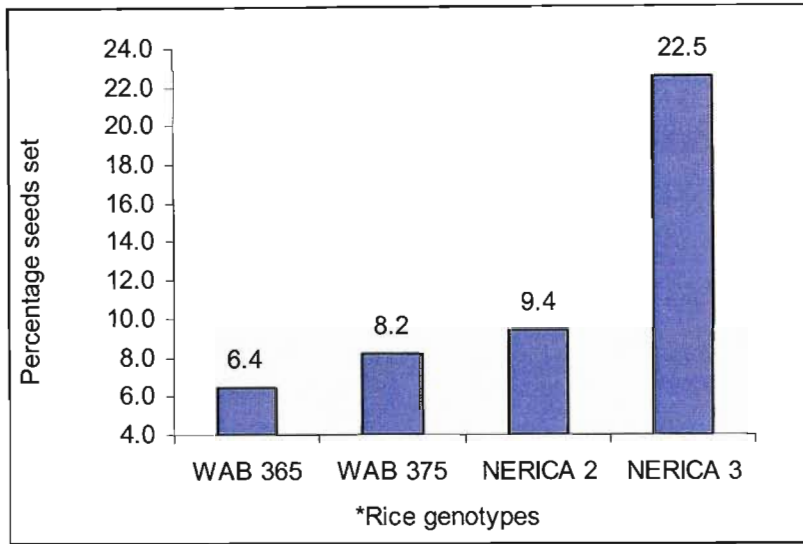


Figure 2: Performance of rice genotypes based on seed set in the cross combinations as male parents; * Rice genotypes were abbreviated.

5.5.6 Comparison of seed set in different types of cross combinations

The average seed sets for each group (A, B, C and D) were presented in Figure 3. The group D involved crosses between Interspecifics x Interspecifics (ISxIS) had the highest success rate of followed by group A involved crosses between *O. glaberrima* and *O. sativa* (GxS) and Group C involved crosses between Interspecifics and *O. sativa* (ISxS) with lowest seed set (Figure 3).

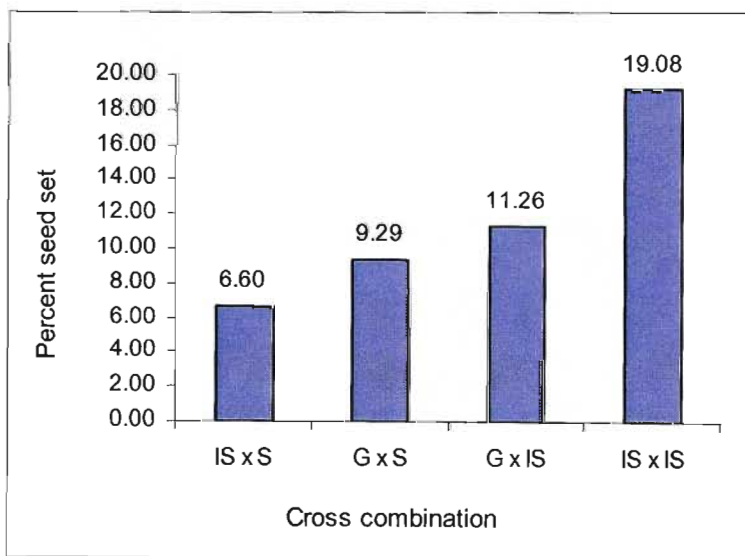


Figure 3: Mean per centage seeds set in crosses between rice species

5.5.7 Success rate in complex cross generations

Complex crosses were carried out using the F₁ plants as female parents crossed to the males parents either as three way or backcrosses. There were no seeds set with the *O. glaberrima* cytoplasm except with interspecifics (Table 7). The (GxS and GxIS) F₁ plants probably contained more of *O. glaberrima* genomes, while S and IS plants contained more of the *O. sativa* genomes. This would widen the genetic distance as compared to (ISxIS) F₁ plants x IS crosses (Table 7). The crosses (ISxIS // IS) had the highest seed set of which half were from backcrosses and the other half from three way crosses. Next best was (ISxS // S) with 4.1% seeds set, of which 84.0% were from backcrosses and the other from three way crosses.

Table 7: Cytoplasmic effects in the backcross and 3-way crosses and their standard deviation

Complex cross	Cytoplasm*	Pollen*	Total No.	No. of seeds	No.cross	% seed set
			Flowers ± S.e.	set ± S.e.		
IS x S // S	IS	S	1822 ± 70.4	75 ± 7.6	16	4.1
IS x S // IS	IS	IS	2232 ± 67.1	44 ± 5.2	16	2.0
IS x IS // S	IS	S	1671 ± 80.0	65 ± 5.2	16	3.9
IS x IS // IS	IS	IS	2243 ± 78.8	180 ± 16.6	16	8.0
G x S // S	G	S	423 ± 29.1	0	10	0.0
G x S // IS	G	IS	1271 ± 39.4	0	20	0.0
G x IS // S	G	S	566 ± 33.0	0	20	0.0
G x IS // IS	G	IS	562 ± 46.4	0	10	0.0

* IS = Interspecific, G = *glaberrima* and S = *sativa*. Cytoplasmic designation was similar to Sano *et al.* (1979).

The complex crosses involving *O. glaberrima* cytoplasm had no seed set (Table 7). However, low seed set was obtained in the crosses involving CG 14 cytoplasm (Table 8). This showed that CG 14 could be a good female parent based on the per cent seed set as compared to *O. glaberrima* genotypes used in this study. High seed set was obtained where WAB 365-B-1-H1-HB was involved as pollen parent (male) (Table 8).

Table 8: Complex cross involving CG 14 in 3-way cross and backcrosses

Cross combination	Type of cross	No. of spikelets pollinated	No. of seeds set	% Seed set
CG 14 x NERICA 2 // WAB 365-B-1-H1-HB	GxIS // S	96	3	3.1
CG 14 x NERICA 3 // WAB 365-B-1-H1-HB	GxIS // S	128	8	6.3
CG 14 x NERICA 2 // WAB 375-B-9-H3-2	GxIS // S	115	1	0.9
CG 14 x NERICA 3 // WAB 375-B-9-H3-2	GxIS // S	104	1	1.0
CG 14 x NERICA 3 // NERICA 2	GxIS // IS	125	2	1.6
CG 14 x WAB 375-B-9-H3-2 // WAB 365-B-1-H1-HB	GxS // S	106	5	4.7
CG 14 x NERICA 2 // NERICA 2	BC	202	4	2.0
CG 14 x NERICA 3 // NERICA 3	BC	233	6	2.6
Total		1109	30	2.71
S.e.of mean		17.86	0.88	0.67

5.6 Discussions

High sterility of hybrid seeds in the crosses between *O. glaberrima* x *O. sativa* and *O. sativa* x *O. glaberrima* have been previously reported (Bouharmont *et al.*, 1985; Jones *et al.*, 1997b; Yabuno, 1977). In this study, 9.29 % seed set in F₁ hybrids in the crosses involving *O. glaberrima* genotypes as female parents was obtained (Figure 3). The per cent seed set was however lower than the crosses between *O. glaberrima* x *O. glaberrima* that had an average of 45% seed set. Thus confirming the existence of incompatibility barriers between the crosses in these groups.

This was higher than Sano (1990), who reported complete sterility while Jena and Khush (1990) observed 1.3% seeds set when trying to introgress genes from *O. officinalis* to *O. sativa*. Jones *et al.* (1997a) reported 5% seed set in the crosses involving *O. sativa* and *O. glaberrima*, and Dayun *et al.* (1997) reported a range from 1 to 15%. The differences in these results may be due to genetic, cytoplasmic and environmental factors. Sano *et al.* (1979) observed that F₁ interspecific hybrids with *O. sativa* background had more seed set when crossed with *O. sativa* pollen than *O. glaberrima* pollen. Environmental temperatures have been implicated to affect meiotic stage of the pollen either at above or below critical

temperature (Yoshida, 1981; Mackill *et al.*, 1982). Similarly, F1 seed parents with *O. glaberrima* background had more seed set with *O. glaberima* pollen than *O. sativa* pollen, thus corroborating this study (Tables 7). This emphasizes the importance of cytoplasm in the crosses involving *O. glaberrima*. The interactions of cytoplasm of *O. glaberrima* with the nucleus of *O. sativa* to induce male sterility have been reported (Carnahan *et al.*, 1972; Lo and Yuan, 1990).

The success rate achieved in the crosses involving *O. glaberrima* x interspecific inbred lines as compared to *O. glaberrima* x *O. sativa* confirms the results of Sano *et al.* (1979) that higher crossability is achieved when *O. glaberrima* is used as pollen parent). The per cent seed set of these crosses however, was about equal (Figure 3). This study observed that the interspecific inbred lines exhibit more of *glaberrima* characters in the cross combinations. To confirm the above information, crosses were made on the cytoplasm of interspecific inbred lines and higher seed set was obtained with crosses with another interspecific line than with crosses to *O. sativa* lines (Figure 3). The per cent seed sets from these crosses were lower than the control crosses between *O. sativa* x *O. sativa* with an average of 70% seed set. These differences could be attributed to incompatibility barriers in these crosses.

Higher sterility was observed in backcrosses involving *O. glaberrima* cytoplasm as compared to single crosses. The backcrosses involving *O. glaberrima* cytoplasm were completely sterile with no seed set except with the CG 14 cytoplasm. These results also agree with similar observations by Bouharmont *et al.* (1985) and Carnahan *et al.* (1972), although fertility improvement after two or more backcrosses has been reported (Heuer *et al.*, 2003; Jones *et al.*, 1997b; Yabuno, 1977) and high per centage seed set was obtained.

Conclusion

Significant variations were observed amongst the genotypes involved in the crosses. The best female parents that combined well based on per centage seed set were CG 14, WAB 450-IBP-105-HB and WAB 450-IBP-103-HB, while the best male parents were NERICA 3 and NERICA 2. These parents could be used as bridging species for interspecific breeding programmes. The best cross combination was between interspecifics x interspecific based on per centage seed set. Higher sterility was observed in backcrosses involving *O. glaberrima*

cytoplasm as compared to single crosses. In fertility improvement, backcrossing that involved *O. glaberrima* cytoplasm may not be promising as compared to *O. sativa* cytoplasm.

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CHAPTER 6

Screening of early generation of rice progenies for drought tolerance

Abstract

Drought is an important constraint of production in major rice ecologies in Mali. However, the traditional upland rice cultivars that possess drought tolerance are low yielding. A breeding approach that will improve the traditional rice by incorporating new genes from other rice species and identifying genotypes combining drought tolerance and high yield at early generation could enhance rice production. This study was conducted to identify drought tolerant progenies in early generations of the breeding cycle and study the relationship between drought tolerance and some morphological and physiological traits. The progenies were generated using the North Carolina II mating design and were advanced by selfing to F3 and F4 generations. Progenies were evaluated in 8 x 8 and 9 x 9 lattice design with two replications, under drought stress and fully irrigated conditions, respectively. Overhead sprinkler irrigation was applied for 40 days after sowing, three times weekly, to maintain field capacity. Irrigation was stopped in the stressed experiment for 50 days to induce stress and then full irrigation was resumed until maturity. A selection index was used to rank the progenies. Selection index values ranged from -42.74 to 20.70. There were 15 progenies with high selection indices. Drought stress caused delay in flowering, which was more pronounced in early flowering progenies as compared to late flowering ones. High tillering progenies had more reduction in tiller number as compared to low tillering progenies under drought stress. Drought tolerance (little leaf drying), taller plants and less leaf rolling were significantly associated with fast recovery ability at 3 and 10 days after stress relief. There was a significant relationship between drought tolerance and less leaf rolling, large leaf area index, tall plants and high plant biomass. Progenies with high selection index performed well under drought stressed conditions and putative traits identified could be used as indicators of drought stress tolerance.

6.1 Introduction

Water stress is a serious production constraint in upland rice ecologies in many countries in Africa. In Mali, rainfall is monomodal and often unevenly distributed. Therefore, upland rice is subjected to varying degrees and duration of drought stress, especially at the vegetative stage. Drought is a limiting factor in rice production due to the poor drought tolerance of rice as compared to other cereals (Lafitte and Bennett, 2003). Developing drought tolerant rice cultivars is thus needed to reduce yield loss and stabilize production in this ecology. As grain yield is the principal objective in most rice breeding programmes, the use of yield measurement to select for drought stress in rice has been considered as a reasonable approach (Atlin, 2003). Grain yield under drought conditions is very difficult to measure, because it is confounded with biotic and abiotic factors. Because of this, there is

considerable interest in identifying more efficient breeding methods based on indirect selection criteria as has been proposed (Falconer and Mackay, 1996; Pantuwan *et al.*, 2004).

There is considerable genotypic variation in response to drought and in adaptation to the upland ecology. Blum (2005) proposed that high yield potential could be combined with drought tolerance provided that relevant dehydration-avoidance factors are not associated with low yield potential. Some morpho-physiological traits have been identified to be associated with grain yield under water-limited environments (Cooper, 1999; Fukai *et al.*, 1999). This may vary according to the severity and time of water deficit (Turner, 1982). In an earlier report by Efisue *et al.* (2005), significant variation was detected within interspecific lines for plant height, leaf rolling and fast recovery ability. Drought stress occurring at the late vegetative stage has led to reduction of yield components that subsequently affect grain yield (Wopereis *et al.*, 1996; Boonjun and Fukai, 1996).

Most of the rice cultivars that have been identified as drought tolerant were screened at the later stages of the breeding cycle when morpho-physiological characters were already fixed. It requires more space, labour and money to evaluate early generations of breeding populations for drought stress. It may be costly for a breeder to subject early generation materials to stress, but the outcome may justify the cost.

Weather conditions in Mali

Mali is located in sub-Saharan vegetation belt in West Africa. It is a landlocked country located in the interior of West Africa between 12° W and 4° E longitude and 10 and 25° N latitude.

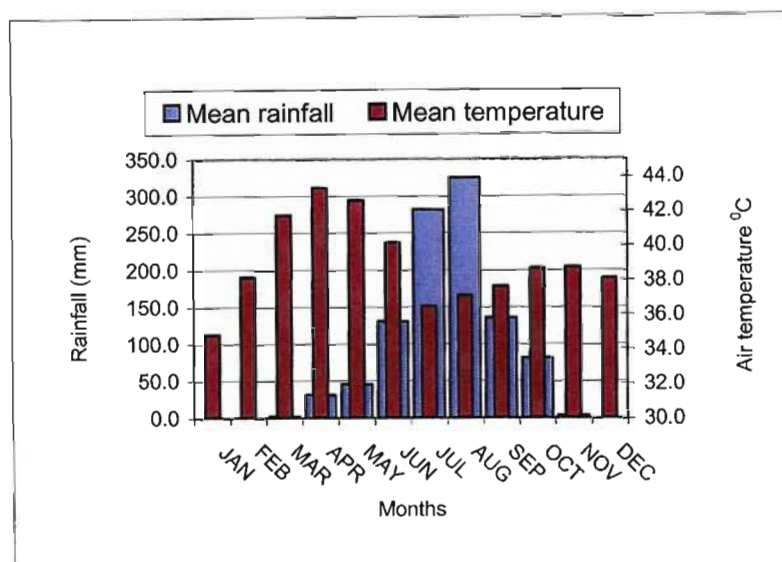


Figure 1: Mean annual rainfall and air temperature at ICRISAT research station, Samako from 1999 to 2005

The annual rainfall regime is monomodal, with distinct wet and dry seasons and air temperature very high during the early months of the year (Figure 1). The rainfall starts mainly in April and increases sharply in August, which is the peak period followed by sharp drop till October. The July and August receive about 60% of the annual rainfall, which shows the uneven distribution of rain in this region. The period between November and March (5 months) is virtually dry and no rain (Figure 1). This period also experiences the harmatan haze that blows from the Sahara desert to the Sahel region of West Africa. The mean monthly rainfall in the period under review was 86.12 mm. Mali has bimodal pattern in monthly air temperature and the air temperature increases from 34.8⁰ C in January to 43.5⁰ C in April, which is the hottest month. The second modal air temperature starts from August and increases gradually to November, and decreases thereafter till January (Figure 1).

6.2 Objectives

The objectives of this study were to:

- (1) to identify drought tolerant progenies in early generations of the breeding cycle, and
- (2) to investigate the relationship between drought tolerance and some morphological and physiological traits in segregating populations of rice at the vegetative stage.

Hypothesis

Morpho-physiological traits associated with drought tolerance can be used to identify drought tolerant progenies in early generations of rice breeding.

6.3 Materials and methods

6.3.1 Soil properties and environmental parameters

The segregating progenies were established at ICRISAT research station, Samanko, Mali in 2005 dry season. The soils were acidic and deficient in organic matter and total nitrogen (Table 1). The soil texture was silty clay loam with very low cation exchange capacity. The soil was of low fertility. This resulted from inadequate levels of essential nutrients, especially Nitrogen (N) content, which was 0.058 %. The soil organic matter was low, 0.480 % (Table 1).

Table 1: Soil properties in 0-20 cm soil depth for the experimental site at Samako, during 2005 dry season.

Property	2005
pH (1:1 water)	4.964
Organic matter (%)	0.480
Total N (%)	0.058
P (ppm)	9.600
K (ppm)	0.232
Ca (ppm)	2.776
Mg (ppm)	0.896
CEC (cmol (+)/kg)	9.740
Sand (%)	21.200
Silt (%)	60.800
Clay (%)	17.800
Soil texture	Silty clay loam

The monthly means of maximum and minimum air temperature, pan evaporation, rainfall and air relative humidity during the experiments were as shown (Table 2). The 2.5 mm rainfall received during the experimental period fell once on the 4th of October 2005. The gradual decrease in temperature and relative humidity was due to severe Harmantan haze with very dry and cool air. Pan evaporation had a monthly mean of 5.18 mm day⁻¹. This season experienced lower temperature and Pan evaporation as well as higher relative humidity as compared to the previous seasons' drought experiment (Table 2).

Table 2: Monthly means maximum and minimum air temperature, pan evaporation, rainfall and air relative humidity during the experiments

Month	Maximum Temperature (° C)	Minimum Temperature (° C)	Pan Evaporation (mm day ⁻¹)	Rainfall (mm)	% air relative humidity
October	38.6	25.5	3.3	2.5	79.0
November	38.9	22.8	4.7	0.0	75.8
December	37.1	23.1	6.2	0.0	69.1
January	37.4	20.2	6.5	0.0	58.7

6.3.2 Genetic materials

The populations that were generated using the North Carolina design II mating scheme (Table 2, Chapter 5) were advanced to F3 and F4 generations for seed increase by single plants selection. A total of four populations were advanced to F3 generation and eight populations to F4 generation only, while twelve populations were advanced to both F3 and F4 generations (Table 3). These populations and their parents accounted for the genetic materials used in this study. Pedigree and agronomic characteristics of the parents used in the crosses shown in (Table 1, Chapter 5). These will enable to identify at which generation of the population may be suitable for drought stress screening and to accelerate their advancement in the breeding programme. Selection of these progenies was based on availability of enough seed to conduct two trials simultaneously as well as for seeds harvest. Therefore the number of progenies in the fully irrigated (non-stressed) experiment was greater than the stressed experiment. All the progenies in stressed experiment were represented in the fully irrigated experiment for comparison.

6.3.3 Cultural details and experimental design

Two experiments comprising drought stressed and fully irrigated (non-stressed) treatments were established simultaneously with F3 and F4 progenies and their parents (Table 3). These enabled the identification of the generation of the population that was be suitable for drought stress screening and to accelerate their advancement in the breeding programme.

The drought stressed experiments contained 64 entries (progenies and their parents) including OS 6 and IR 20 as resistant and susceptible checks, respectively. Entries were sown on the 20th October 2005 in (8 x 8) lattice design with two replications. The fully irrigated experiments, which acted as a control had 81 entries as explained (See 6.3.2) were sown on the 18th October 2005 in (9 x 9) lattice design with two replications, including OS 6 and IR 20 as resistant and susceptible checks, respectively. The dried seed were dibbled on shallow holes at the rate of three seeds per hole with a spacing of 20 cm within rows and 20 cm between rows and thinned to one plant per hole after 15 d of seedling emergence. The plot size was 1.0 m x 2.0 m and a total of 55 plants per plot. The experiments were hand weeded at 15 d and 35 d after sowing prior to fertilizer application. Basal fertilizer was applied at the rate of 200 kg ha⁻¹ of 17-17-17, N-P-K and urea (46% N) was top-dressed at the rate of 100 kg ha⁻¹ at 35 d after sowing. There was no insecticide applied.

Table 3: Genetic materials used in the drought stressed and non-stressed experiments

Germplasm Populations	Pedigree	Generations
WBK 02	RAM 24 x NERICA 2	F4
WBK 03	RAM 24 x NERICA 3	F4
WBK 15	RAM 118 X WAB 375-B-9-H3-2	F4
WBK 28	WAB 450-IBP-103-HB x WAB375-B-9-H3-2	F3, F4
WBK 30	WAB 450-IBP-103-HB x NERICA 3	F3, F4
WBK 32	WAB 450-IBP-105-HB x WAB375-B-9-H3-2	F4
WBK 34	WAB 450-IBP-105-HB x NERICA 3	F3, F4
WBK 35	WAB 450-IBP-6-1-1 x WAB365-B-1-H1-HB	F3, F4
WBK 39	WAB 880-1-38-13-1-P1-HB x WAB365-B-1-H1-HB	F3, F4
WBK 40	WAB 880-1-38-13-1-P1-HB x WAB375-B-9-H3-2	F3, F4
WBK 41	WAB 880-1-38-13-1-P1-HB x NERICA 2	F3, F4
WBK 42	WAB 880-1-38-13-1-P1-HB x NERICA 3	F3, F4
WBK 64	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // WAB365-B-1-H1-HB	F3, F4
WBK 70	WAB 450-IBP-105-HB x NERICA 3 // WAB365-B-1-H1-HB	F3
WBK 75	WAB 880-1-38-13-1-P1-HB x WAB365-B-1-H1-HB // WAB365-B-1-H1-HB	F3
WBK 78	WAB 880-1-38-13-1-P1-HB x NERICA 3 // WAB365-B-1-H1-HB	F3, F4
WBK 87	RAM 118 X WAB365-B-1-H1-HB // WAB375-B-9-H3-2	F4
WBK 100	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // WAB375-B-9-H3-2	F4
WBK 106	WAB 450-IBP-105-HB x NERICA 3 // WAB375-B-9-H3-2	F3
WBK 110	WAB 450-IBP-6-1-1 x NERICA 3 // WAB375-B-9-H3-2	F4
WBK 114	WAB 880-1-38-13-1-P1-HB x NERICA 3 // WAB375-B-9-H3-2	F4
WBK 136	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // NERICA 2	F3, F4
WBK 149	WAB 880-1-38-13-1-P1-HB x NERICA 2 // NERICA 2	F3
WBK 150	WAB 880-1-38-13-1-P1-HB x NERICA 3 // NERICA 2	F3, F4
WBK 184	WAB 880-1-38-13-1-P1-HB x WAB375-B-9-H3-2 // NERICA 3	F3
Parents of populations		
NERICA 2		Parent
NERICA 3		Parent
WAB 365-B-1-H1-HB		Parent
WAB 375-B-9-H3-2		Parent
WAB 450-I-B-P-103-HB		Parent
WAB 450-I-B-P-105-HB		Parent
WAB 450-I-B-P-6-1-1		Parent
WAB 880-1-38-13-1-P1-HB		Parent
RAM 24		Parent
RAM 118		Parent
OS 6		Resistant check
IR 20		Susceptible check

6.3.4 Water management

Stress imposition at the vegetative stage varies with rice ecologies, Pantuwan *et al.* (2004) withheld water at 46 d after sowing to stimulate drought in lowland rice ecology while at 30 d after rice emergence in irrigated rice (Singh and Mackill, 1991; Malabuyoc *et al.*, 1985). However, stimulation of drought at maximum tillering stage is advisable for rice crop (Singh and Mackill, 1991). Overhead sprinkler irrigation was applied till maximum tillering stage (40 d after sowing), three times weekly, to maintain field capacity. Irrigation was stopped in the stressed experiment for 50 d to simulate stress. Soil water monitoring and crop visual observation approaches were used to determine when to relieve stress. Full irrigation resumed again until maturity. The control experiment was irrigated to field capacity throughout the study.

6.4 Data collection

The Standard Evaluation System (SES) for Rice reference manual (IRRI, 1996) was used for all trait measurements except where stated. Measurements were taken at weekly intervals on all traits in both stressed and fully irrigated-control experiments for six weeks during the stress period. The following measurements were taken as observed for the whole plot, unless stated otherwise.

Drought score: Drought score (Dt) was rated weekly based on the leaf drying symptoms observed for the whole plot. Rating of drought score on a scale of 0-9 was done as shown below (De Datta *et al.*, 1988):

Score	0	No symptoms of stress
	1	Slight drying of leaf tips
	2	25% of the length of 25% of all leaves (apart from older leaves that are normally dry)
	3	At least 25% of the length and 26% to 50% of all leaves are dry.
	4	At least 25% of the length of 50% of all leaves are dry; 25% of leaves are fully dried.
	5	50% of all leaves are fully dried
	6	51-69% of all leaves are fully dried
	7	70% of all leaves are fully dried
	8	More than 70% of all leaves are fully dried
	9	All plants are apparently dead

Leaf rolling: A leaf rolling score was adapted from O'Toole and Moya (1978). Drought and leaf rolling scores were taken at weekly intervals throughout the period of stress between 1300 and 1500hr to assess the effects of water stress.

Score	0	No leaf rolling
	1	Leaves slightly rolled
	2	Leaves rolled into Slight V-shape
	3	Leaves rolled into deep V-shape
	4	Leaves rolled and leaf margins touching
	5	All leaves completely rolled

Plant height and tiller number: Plant height (cm) was measured from soil surface to the tip of the shoot and tiller number per plant recorded within each hill as described by IRRRI (1996).

Plant vigour: Plant vigour (Vg) was taken when 50% of the plants in a population were at 4th leaf stage. A total of five plants per plot were randomly taken for measurement for each of the traits as described by IRRRI (1996b):

Score	1	Extra vigorous (very fast growing; plants at 5-6 leaf stage have 2 or more tillers in majority of population)
	3	Vigorous (fast growing; plants at 4-5 stage have 1-2 tillers in majority of population)
	5	Normal (plant at 4-leaf stage)
	7	Weak (plants somewhat stunted; 3-4 leaves; thin population; no tiller formation)
	9	Very weak (stunted growth; yellowing of leaves)

Relative leaf water content (RLWC): Relative leaf water content was determined between 1300 and 1500 hr by the method suggested by Barrs and Weatherly (1962). Three young fully expanded leaves from the main stem were randomly selected in each plot and placed in a plastic bag and transported to the laboratory immediately. A rectangular metal cock was used in sample taken from the middle portion of each leaf and weighed to determine the fresh weight. Turgid weight was determined by weighing the leaf samples put in petri dishes

containing water for four hours. Then they were then oven dried at 60^o C for 24 hours and weighed for dry weight determination. Relative leaf water content was calculated as:

$$RLWC = \{(Fresh\ weight - Dry\ weight) / (Turgid\ weight - Dry\ weight)\} \times 100$$

Plant biomass (fresh and dry): Plant biomass was taken at weekly intervals .Two plants were randomly selected per plot and the aboveground part of the plant was cut off and fresh weight recorded. For the root sampling, a hole of 30 cm depth was dug about 10 cm diameter around the plant. Roots were gently removed into a 2 mm mesh screen and washed free of soil with water to ensure minimum root lost for fresh weight determination. The samples were oven dried at 65^o C for 72 hours and weighed for shoot and root dry weight.

Leaf area index and specific leaf dry weight: Two young fully expanded leaves from the main stem were randomly selected in each plot and leaf area (LA) was determined using a leaf area meter (li-3100, Lincoln, NE USA). Leaf area index (LAI) was calculated as described by Yoshida (1981) as follows:

LAI = (sum of the leaf area of all leaves /unite area where the leaves have been collected).
The specific leaf dry weight (SLDW) was calculated as the area of leaf per leaf dry weight (Jones *et al.*, 1997; Zaharieva *et al.*, 2001).

Recovery ability: Plant recovery scores were taken at 3 and 10 days after resuming irrigation. Drought stress recovery symptom was based on healthy plants and leaves as described by IRRRI (1996):

Score	1	90-100% recovery
	3	70-89% recovery
	5	40-69% recovery
	7	20-39% recovery
	9	0-19% recovery

Drought tolerance index: A tolerance index was calculated as per cent reduction in a character due to drought stress relative to the non-stressed control experiment using the following formula suggested by (Reyniers *et al.*, 1982):

$$\text{Drought tolerance index} = (X \text{ control} - X \text{ stress}) / X \text{ control}) \times 100$$

Selection indices

Weights (W_i) for the selection index were assigned based on the relative importance of each measured trait as an indicator of drought stress and grain yield in upland rice ecology (Table 4). The results from the participatory rural appraisal on preferred traits by upland rice farmers were also used in assigning weight on each trait (see Chapter 2).

Table 4: Sign and weight based on the relative importance of traits in the selection index

Traits	Sign‡	Weight (W_a)	Farmers preference (See Chapter 2)
Drought score	-	5	Little or no leaf drying
Leaf rolling	-	4	Little or no leaf rolling
Plant height	+	3	Tall plant
Tiller number	-	3	Less tiller number
Leaf area index	+	3	Large leaf area index
Specific leaf dry weight	+	2	Large specific leaf dry weight
Relative leaf water content	+	2	High relative leaf water content
Root dry weight	+	2	High root dry weight
Shoot dry weight	+	1	High shoot dry weight
Root dry weight/ Shoot dry weight ratio	+	2	High Root / Shoot ratio
Plant population	+	1	High plant population
Days to 50% flowering	-	4	Early flowering
Recovery score at 3 days after irrigation	-	5	Fast recovery
Recovery score at 10 days after irrigation	-	1	Fast recovery

‡ -ve = not desired; +ve = desired

The phenotypic values were standardized by the method suggested by Banziger *et al.* (2000) as:

$$P_a = (X - GM) / Sd$$

Where P_a = standardized phenotypic value; GM and Sd are the grand mean and standard deviation of the trait in the experiment, and X = actual value of the trait measured on the genotype. The selection index of (SI) each genotype was calculated as:

$$SI = P_a W_a + P_b W_b + \dots + P_n W_n$$

Where P_a = standardized phenotypic value of the trait observed; W_a = is the assigned weight value to the trait in the selection index.

6.5 Statistical analysis

Analysis of variance (ANOVA) was performed separately on the individual experiment and combined analysis was used for some traits using Statistical Analysis System (SAS, version 9.1, 2003) to test the significance of differences among entries. Simple linear correlation analyses were computed for all the observations using individual plot means to evaluate their associations using a model described by Gomez and Gomez (1984) as:

$$Y = \alpha + b X$$

Y = phenotypic value for F4 population, X = phenotypic value for F3 population, α = intercept and b = coefficient of linear correlation.

Tests for homogeneity of variance for the test environments was calculated using Hartley's test as described by Fujino (1976) as:

$$\text{Hartley test } F_{\max} = (\text{Largest } \delta^2 / \text{Smallest } \delta^2)$$

Where δ^2 = variance for the trait under consideration. The critical value (F_c) = $F_{\infty} [k (n-1)]$ if $n_1 = n_2$; but if $n_1 \neq n_2$, $F_c = F_{\infty} [k (n_{\text{maximum}} - 1)]$; n_1 = sample size for group one and n_2 = group two.

If $F_{\max} > F_c$, it indicates that the variances are not equal.

6.6 Results

6.6.1 Monitoring the drought stress condition

Soil moisture content (SMC) of each plot was monitored at 0-20 cm soil depth every five days throughout the stress period using the gravimetric soil analysis method. A graduated soil auger was used for sampling the soil. The weight of fresh soil (Fwt) and soil oven dried for 48 hours at 70⁰ C (Dwt) were taken. Per cent moisture content was calculated as: $\text{Fwt} - \text{Dwt} / \text{Fwt} * 100$. The soil moisture content decreased gradually and by the 21st day of stress, 1.3% of soil moisture was lost (Figure 2), as cracks were observed on the soil surface.

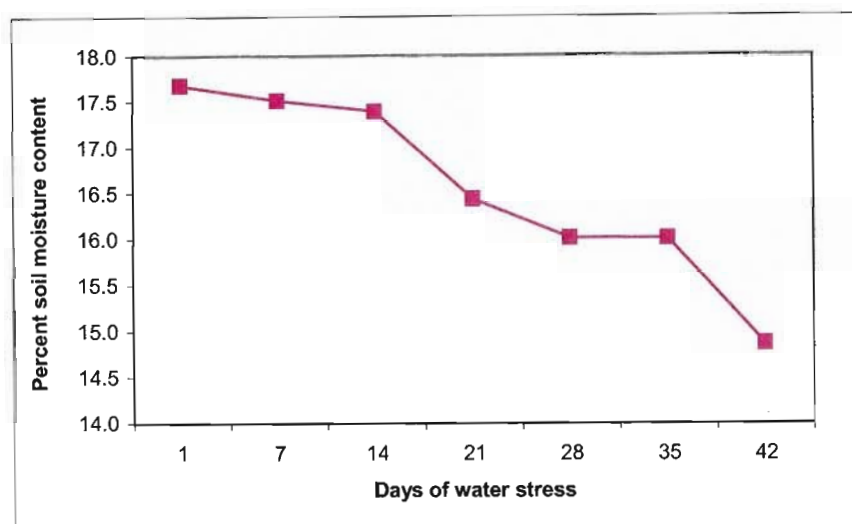


Figure 2: Per cent moisture content monitored at 0.0 to 20 cm in the top 20cm of soil depth.

6.6.2 The performance of progenies and their parents under water stressed condition

The mean values and ranges for 14 traits evaluated under drought stressed conditions along with their standard errors are given in Table 5. Progenies responded differently to drought stress. Most of the progenies were medium flowering as shown by the mean for days to 50% flowering which was 133 days and had high relative leaf water content with a mean of 78.37% (Table 5). Results showed non-significant variation among progenies for relative leaf water content, root dry weight, shoot dry weight and root-to-shoot ratio, and recovery at 3d after irrigation. However, all the other traits were significant (Table 5).

Table 5: Mean values, estimation of standard error (S.e) of the mean for the evaluated traits of segregating progenies of rice and their parents under drought stressed condition

Rice traits	Mean	Minimum	Maximum	S.e	Probability
Drought score	3	2	6	0.10	**
Leaf rolling score	3	2	5	0.08	*
Plant height (cm)	37.56	19.30	50.30	0.60	***
Tiller number (per plant)	4	2	14	0.13	***
Leaf area index	3.88	0.56	7.80	0.14	**
Specific leaf dry weight (m ² kg ⁻¹)	11.33	6.43	23.64	0.22	*
Relative leaf water content (%)	78.37	42.50	92.68	0.91	ns
Root dry weight (g)	0.31	0.10	0.82	0.02	ns
Shoot dry weight (g)	2.89	0.66	6.46	0.12	ns
Root / Shoot ratio	0.13	0.03	1.41	0.01	ns
Plant population (%)	73.06	31	98.18	1.65	***
Days to 50% flowering	133	101	161	1.69	***
Recovery score at 3 days after irrigation	3	1	7	0.16	ns
Recovery score at 10 days after irrigation	2	1	5	0.10	*

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

Tests for homogeneity of variance were calculated for each of the traits examined and significant differences were not observed among the populations and their parents. Therefore, a pooled selection index was constructed (Figure 3). Selection index classes were constructed of which each class had five values. There were four classes with positive selection indices and class selection index 20 compared two genotypes, which are WBK 150-B-B-B and WBK 39-B-B-B with selection index of 20.7 and 15.9, respectively (Table 6). Selection index class 10 had the highest genotypes, which comprised two parents WAB 450-I-B-P-103-HB and NERICA 3 with selection index of 9.2 and 6.8, respectively. The best progenies in this class based on selection index value were WBK 78-B-B-B with selection index of 10.5 followed by WBK 136-B-B with 9.7. The resistant and susceptible checks belong to selection index class -10 with selection index values of -7.74 and -10.8,

respectively. The worst selection index class was selection index class -45 , which had only one genotype WBK 03-B-B-B with selection index of -42.7 (Figure 3; Table 6). A majority of the parents belong to the selection index class -5 and the worst parent was RAM 24 with a selection index class of -30 (Figure 3; Table 6).

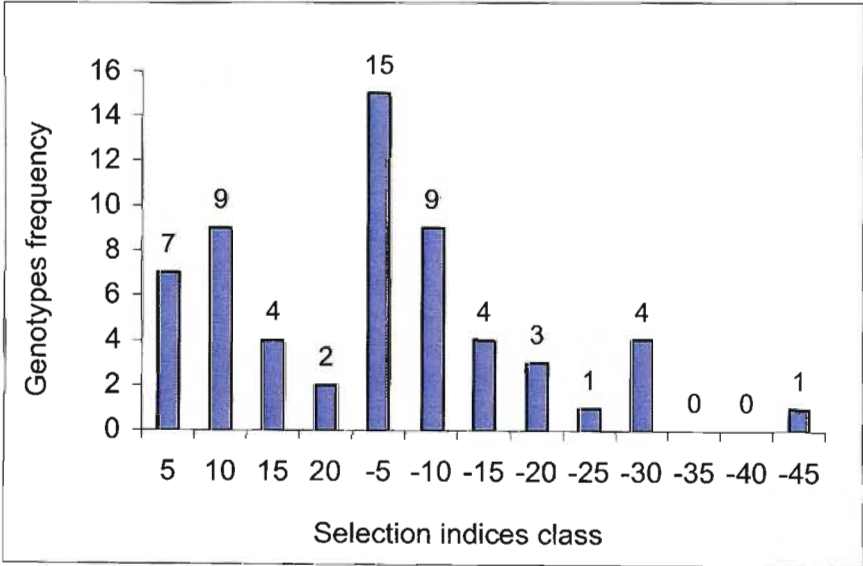


Figure 3: Selection indices for segregating progenies and parents under drought stress during 2005 dry season experiment based on 14 traits of rice.

Table 6: Progenies tested and their selection indices

Progenies / parental lines*	Pouulations	Generation	Selection Index
WBK 150-B-B-B	WBK 150	BC1F4	20.70
WBK 39-B-B-B	WBK 39	F4	15.91
WBK 41-B-B-1	WBK 41	F4	14.26
WBK 41-B-B	WBK 41	F3	13.39
WBK 42-B-B-2	WBK 42	F4	12.73
WBK 30-B-B-B	WBK 30	F4	11.59
WBK 78-B-B-B	WBK 78	BC1F4	10.49
WBK 136-B-B	WBK 136	BC1F3	9.74
WBK 149-B-B	WBK 149	BC1F3	9.63
WAB 450-I-B-P-103-HB	Parent	Parent	9.23
WBK 35-B-B-1	WBK 35	F4	9.11
WBK 02-B-B-B	WBK 02	F4	6.87
NERICA 3	Parent	Parent	6.78
WBK 30-B-B	WBK 30	F3	6.17
WBK-28-B-B-1	WBK 28	F4	6.05
WBK 42-B-B	WBK 42	F3	5.59
WBK 03-B-B-1	WBK 03	F4	4.71
WBK 35-B-B-B	WBK 35	F4	4.01
WBK 34-B-B	WBK 34	F3	1.87
WBK 32-B-B-B	WBK 32	F4	1.15
WBK 87-B-B-B	WBK 87	BC1F4	0.47
WBK 150-B-B	WBK 150	BC1F3	0.27
WBK 70-B-B	WBK 70	BC1F3	-0.06
WBK 39-B-B	WBK 39	F3	-0.42
WBK 114-B-B-B	WBK 114	BC1F4	-0.90
WBK 64-B-B	WBK 64	BC1F3	-1.26
WBK 110-B-B-B	WBK 110	BC1F4	-2.07
WBK 106-B-B	WBK 106	BC1F3	-2.25
WBK 28-B-B-B	WBK 28	F4	-2.52
WBK 28-B-B-4	WBK 28	F4	-3.69
NERICA 2	Parent	Parent	-3.89
WAB 450-I-B-P-6-1-1	Parent	Parent	-4.12
WBK 64-B-1	WBK 64	BC1F3	-4.58
WBK 15-B-B-2	WBK 15	F4	-4.84
WAB 375-B-9-H3-2	Parent	Parent	-4.84
WAB 365-B-1-H1-HB	Parent	Parent	-5.29
WAB 880-1-38-13-1-P1-HB	Parent	Parent	-5.63
WBK 41-B-B-B	WBK 41	F4	-6.71
WBK 100-B-B-B	WBK 100	BC1F4	-6.99
OS 6 (resistant check)	Check	Check	-7.74
WBK 28-B-B	WBK 28	F3	-7.90
WBK 39-B-3	WBK 39	F3	-8.29
WBK 40-B-B-B	WBK 40	F4	-8.56
WBK 34-B-B-B	WBK 34	F4	-9.33
WBK 78-B-B	WBK 78	BC1F3	-9.75
IR 20 (Susceptible check)	Check	Check	-10.77
WBK 42-B-B-B	WBK 42	F4	-13.30
WBK 39-B-B-1	WBK 39	F4	-13.40
WBK 15-B-B-1	WBK 15	F4	-14.51
WBK 136-B-B-B	WBK 136	BC1F4	-14.51
WBK 184-B-B	WBK 184	BC1F3	-17.87

Progenies / parental lines*	Populations	Generation	Selection Index
WBK 64-B-B-B	WBK 64	BC1F4	-18.76
RAM 118	Parent	Parent	-19.12
WBK 40-B-B	WBK 40	F3	-21.46
RAM 24	Parent	Parent	-26.98
WBK 35-B-B	WBK 35	F3	-29.25
WBK 35-B-1	WBK 35	F3	-29.96
WBK 75-B-B	WBK 75	BC1F3	-30.00
WBK 03-B-B-B	WBK 03	F4	-42.74

*See Table 3, appendix iv, v and vi for more information;

6.6.3 Tolerance indices of some rice characters at the vegetative stage of plant development

Tolerance indices for progenies and parents with a negative value for each character performed better under drought stress compared to fully irrigated conditions (Table 7). High tillering progenies like RAM 24, WBK 100-B-B-B, and IR 20 had a greater reduction in tiller number than low tillering progenies. However, some high tillering progenies, RAM 118 and WBK 42-B-B-B, had less reduction. Reduction in tiller number ranged from 26.7 to 69.7% with a mean of 56.66% (Table 7).

High tillering progenies also had greater reduction in plant height as compared to less tillering progenies (Table 7). The resistant check was better than the progenies in relation to tiller reduction, while most of the progenies had lesser tiller reduction than the susceptible check. Reduction in plant height ranged from 3.51 to 41.45 % with a mean of 19.76%. Only WBK 41-B-B and WBK 41-B-B-B showed no height reduction (Table 7).

Drought stress caused delay in days to flowering. The delay in flowering was more pronounced in early flowering progenies compared to late flowering ones. Progenies with positive values for “days to 50% flowering” flowered much earlier in the drought stressed experiment than the fully irrigated experiments and they comprise about 45.0 % of the total progenies. The mean of -4.54% for “days to 50% flowering” indicated that more progenies in the drought stressed experiment flowered earlier than in the fully irrigated experiment. The susceptible check IR 20 flowered earlier and had no delay in days to flowering as compared to OS 6 the tolerant check (Table 7)

Table 7: Drought tolerance indices in the segregating populations and parents of some rice traits

Germplasm*	Populations	Generation	Tiller number	Plant height	Days to 50% flowering	LAI	SLDW	RLWC
WBK 114-B-B-B	WBK 114	BC1F4	69.7	30.3	3.4	5.1	19.5	11.2
WBK 28-B-B-B	WBK 28	F4	65.0	23.0	3.4	-76.8	14.7	0.8
WBK 40-B-B-B	WBK 40	F4	64.7	16.9	2.3	43.4	17.2	10.9
WBK 32-B-B-B	WBK 32	F4	64.6	26.8	-0.7	-148.7	-9.8	-0.6
RAM 24	Parent	Parent	64.5	26.1	-18.5	-52.8	20.6	1.1
WBK 78-B-B-B	WBK 78	BC1F4	64.3	22.6	-3.2	-92.4	19.8	4.3
WAB 880-1-38-13-1-P1-HB	Parent	Parent	63.9	38.5	4.4	-40.8	30.9	-3.3
WBK 28-B-B	WBK 28	F3	63.9	39.8	-3.4	27.0	37.8	2.1
WBK 40-B-B	WBK 40	F3	63.5	19.7	-1.0	-27.8	14.4	8.8
WBK 100-B-B-B	WBK 100	BC1F4	63.4	8.7	10.3	-5.6	0.4	4.2
IR 20 (susceptible check)	Check	Check	63.3	12.8	NA	-72.3	-7.9	8.4
WBK 106-B-B	WBK 106	BC1F3	63.2	27.5	1.5	9.3	21.0	-11.6
WBK 64-B-B	WBK 64	BC1F3	62.6	41.5	-23.2	16.4	27.7	3.6
WAB 450-I-B-P-103-HB	Parent	Parent	60.5	25.0	10.3	-6.8	18.7	-8.0
WBK 41-B-B-1	WBK 41	F4	60.4	22.2	-23.2	34.8	12.8	4.3
WAB 365-B-1-H1-HB	Parent	Parent	60.0	15.8	1.4	8.0	6.2	-5.7
WBK 03-B-B-B	WBK 03	F4	59.6	28.2	6.1	35.1	30.7	-6.7
WBK 42-B-B	WBK 42	F3	57.8	14.6	-1.0	3.6	4.0	-13.6
WBK 30-B-B	WBK 30	F3	57.3	30.3	1.4	-201.7	17.0	3.9
WBK 28-B-B-4	WBK 28	F4	57.0	25.3	1.4	34.1	33.1	-13.6
WBK 110-B-B-B	WBK 110	BC1F4	55.9	21.1	9.8	6.8	43.8	-15.4
NERICA 2	Parent	Parent	55.6	30.6	-23.2	5.3	28.9	-4.1
WBK-28-B-B-1	WBK 28	F4	54.1	6.91	4.4	-350.7	-70.3	-5.2
WBK 150-B-B-B	WBK 150	BC1F4	53.5	32.7	9.8	-14.7	29.3	-21.9
WBK 64-B-1	WBK 64	F3	53.3	25.4	1.5	-39.8	-9.3	4.1
WBK 35-B-1	WBK 35	F3	52.7	23.3	-5.7	27.7	-1.0	-30.3
WBK 39-B-B	WBK 39	F3	51.4	27.0	-3.2	47.7	12.2	7.3
WBK 35-B-B-1	WBK 35	F4	51.2	19.0	5.8	-34.2	32.8	-46.8
WBK 136-B-B	WBK 136	BC1F3	51.2	8.4	-41.5	-691.1	-51.7	13.9
WBK 39-B-3	WBK 39	F3	50.6	20.3	3.7	4.4	13.4	-26.6
WBK 136-B-B-B	WBK 136	BC1F4	50.6	20.6	-32.9	66.6	48.5	-5.3
WBK 150-B-B	WBK 150	BC1F3	50.0	37.8	-75.6	-17.4	20.0	11.1
WBK 39-B-B-1	WBK 39	F4	50.0	9.3	1.3	-48.0	8.5	-6.5
WBK 41-B-B	WBK 41	F3	49.4	-7.1	7.2	-152.2	-2.2	-25.7
WBK 39-B-B-B	WBK 39	F4	47.1	16.7	-1.0	-29.3	10.1	-5.8
WBK 41-B-B-B	WBK 41	F4	46.8	-1.2	-11.6	26.9	30.8	25.4
WAB 450-I-B-P-6-1-1	Parent	Parent	46.2	14.3	6.1	40.0	19.2	2.7
WBK 42-B-B-2	WBK 42	F4	46.0	10.0	3.7	4.3	3.2	-47.1
WBK 35-B-B-B	WBK 35	F4	44.4	15.5	-1.0	-9.8	0.5	0.7
WBK 34-B-B	WBK 34	F3	44.4	4.1	-2.0	-652.5	-79.7	-26.4
OS 6 (resistant check)	Check	Check	41.6	3.5	-21.0	-160.6	2.6	-8.8
RAM 118	Parent	Parent	41.5	13.1	-23.2	-103.9	0.4	10.9
WBK 42-B-B-B	WBK 42	F4	41.5	4.5	3.7	30.4	11.4	-1.8
NERICA 3	Parent	Parent	26.7	18.6	18.1	-74.4	-2.5	-6.8
Mean			56.66	19.76	-4.54	-59.72	9.71	-4.72

*See Table 3, appendix iv, v and vi for more information; NA = no data

The mean tolerance index for leaf area index (LAI) was -59.72% , which showed that drought stress effect was lesser as compared to other rice characters examined (Table 7). However, the LAI for susceptible check was reduced unlike the OS 6. The percentage reduction in specific leaf dry weight (SLDW) ranged from 0.4 to 48.5 % with a mean of 9.71% reduction. More than 54 % of the progenies maintained high water status (negative values) excluding the susceptible check during drought stress. The susceptible check (IR 20) had 8.4 % reduction in relative leaf water content (RLWC), while the resistant check (OS 6) had -8.8 % increases in relative leaf water content under stressed condition. The best performed progeny was WBK 41-B-B based on plant height, LAI, SLDW and RLWC (Table 7).

6.6.4 Correlation analyses

Drought tolerance (little leaf drying) and leaf rolling were significantly positively correlated with recovery ability at 3 d and 10 d after stress relief (Table 8). Plant height was significant and negatively correlated with plant recovery ability at 3 d and 10 d after stress relief. Tiller number and root dry weight were also significantly ($P \leq 0.05$) and negatively associated with plant recovery ability at 3 d and 10 d after stress relief. Significant relationship between root-to-shoot ratio and plant recovery was not observed both at 3 d and 10 d after stress relief (Table 8).

Table 8: Correlation coefficient of rice characters with recovery ability at 3 and 10 days after stress relief in segregating populations and their parents

Traits	3 days after stress relief	10 days after stress relief
Drought score	0.400***	0.531***
Leaf rolling score	0.266**	0.352***
Leaf area index	-0.093 ns	-0.162 ns
Plant height	-0.207*	-0.291**
Tiller number	-0.202*	-0.091 ns
Relative leaf water content	0.037 ns	-0.026 ns
Specific leaf dry weight	-0.089 ns	0.0367 ns
Shoot dry matter	0.013 ns	-0.111 ns
Root dry matter	-0.035 ns	-0.197*
Root / shoot ratio	-0.017 ns	-0.093 ns
Days to 50% flowering	0.167 ns	0.147 ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

There was a significant ($P \leq 0.001$) positive correlation between drought tolerance score (Dt) and leaf rolling (LR). Significant and negative correlation were observed between Dt and leaf area index (LAI), specific leaf dry weight (SLDW), plant height (PH), root dry weight (RD) and shoot dry weight (SD) (Table 9). Significant and negative correlation was also observed between leaf rolling and leaf area index and plant height. Time to flower (FLW) was significantly ($P \leq 0.001$) positively correlated with leaf area index and negatively correlated with tiller number (TN); while a significant positive correlation was observed between shoot and root dry weight. Relative leaf water content (RWC) had significant negative correlation only with root dry weight. Specific leaf dry weight (SDW) had significant positive correlation between LAI and PH. Significant and positive correlation was observed between plant height and RD and SD (Table 9).

Table 9: Linear correlation coefficients among traits in the segregating progenies and their parents in water stressed experiment

Traits	Dt	LR	LAI	PH	TN	RD	SD	RWC	SDW
LR	0.483***								
LAI	-0.434***	-0.206*							
PH	-0.410***	-0.209*	0.470***						
TN	-0.023 ns	0.012 ns	-0.264**	0.033 ns					
RD	-0.419***	-0.150 ns	0.289**	0.227*	0.073 ns				
SD	-0.260**	-0.013 ns	0.188*	0.275**	0.270**	0.461***			
RWC	0.118 ns	-0.013 ns	0.135 ns	0.074 ns	-0.189 ns	-0.287**	0.0245 ns		
SDW	-0.215*	-0.087 ns	0.453***	0.193*	-0.092 ns	0.081 ns	-0.186 ns	0.032ns	
FLW	-0.170 ns	-0.097 ns	0.373***	0.068 ns	-0.329***	0.050 ns	-0.005 ns	-0.049 ns	0.140ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

6.7 Discussions

6.7.1 Consistency of progenies responses to water stress conditions

Progenies showed different drought (based on ratings for leaf drying) responses under water stressed and non-stressed conditions. Progenies with drought score of 1 to 3 could be promising sources of drought tolerance as they exhibited less leaf rolling under drought stressed condition (Appendix IV). Drought score has long been used to identify drought tolerant genotypes in the field and greenhouse (Chang *et al.*, 1974; O'Toole and Maguling, 1981). There was a wide range of progenies with different tillering abilities. The low tillering progenies could be adaptable to upland land ecologies, while the high tillering progenies could be adaptable to lowland rice ecologies. Chang *et al.* (1974) earlier reported that low and high tiller number was characteristic of upland and lowland rice ecologies, respectively. High leaf area index and specific leaf dry weight were observed amongst the progenies. These traits could assist with the maintenance of high transpiration rates and photosynthetic activities of the plant. This is similar to the reports (Cabuslay *et al.*, 1999; Lawlor, 1995). Therefore, progenies with high leaf area indices and specific leaf dry weight could be promising for grain yield production as they enhanced plant plant photosynthetic activities.

Specific leaf dry weight could be a useful selection criterion, because it was reported to be relatively stable across environments (WARDA, 1996).

Progenies with high selection indices such as WBK 150-B-B-B and WBK 39-B-B-B were identified under drought stressed condition. These progenies were drought tolerant and later flowering. These progenies could be promising for early season drought environments. A report showed that late flowering genotypes, which flowered after the water had disappeared from the field, suffered large yield reduction due to late season drought (Fukai, 1999).

This study identified an early flowering progenies such as WBK 41-B-B-1, WBK 41-B-B, WBK 136-B-B and WBK 149-B-B (Appendix IV) and selection index values of 14.26, 13.39, 9.74, and 9.63, respectively. These progenies could be promising for late season drought environments as early maturity genotypes could provide an escape mechanism, as they are very likely to complete their life cycle before the late season drought occurs.

6.7.2 Recovery ability and associated characters

The per centage of recovery of rice plants after stress relief varied with the progenies both at 3 and 10 days after stress relief (Table 8). Significant relationships were observed between recovery scores at 3 and 10 days for drought and leaf rolling scores and plant height. Reaction of progenies to drought stress varied with time and stress intensity, therefore, progenies with fast recovery ability for the characteristics observed could be regarded as drought tolerant (Table 8). The ability of a genotype to recover fast from drought stress, enables more access to the favourable environments and this could results in greater yield than slow recovering genotypes. These progenies could be promising for high yield under drought stressed conditions. Recovery ability of a genotype has been reported as a determinant of grain yield (Chang *et al.*, 1974; De Datta *et al.*, 1975). Less growth immediately after stress relief could be attributed to death of plant tissues particularly in the dried portion of the plant.

6.7.3 Relationships between plant characters and consistency

A significant correlation was observed between early flowering and small leaf area index (Table 9). This could be regarded as ideal for efficient resource distribution as leaf area

adjustment is one of the mechanisms for plants moderate water use; this was also observed by Blum (2005). An ideal plant proposed by WARDA (1996) is a plant with high specific leaf dry weight during early growth but with rapid decline at maturity. The study showed significant positive correlation ($r = 0.453^{***}$) between LAI and SLDW. Thus, genotypes showing high SLDW such as WBK 41-B-B with early flowering (Appendix IV and VI) could be regarded as ideal for late season drought tolerance.

Efficient use of plant resources at maturity is very important, especially if it will enhance high grain yield. The significant relationship between early flowering and high tiller number could enhance high grain yield by increasing nutrient translocation from the soil to the plant shoot. A significant relationship between drought tolerance and high biomass production was observed (Table 9), which could suggest that drought tolerant genotypes were efficient at using limited water. Similar reports showed that the production of plant biomass was a function of water use efficiency (Puckridge and O'Toole, 1981). High biomass could also be regarded as indirect way of competitive ability with weeds. Thus, progenies such as WBK 35-B-B-1, WBK 136-B-B-B, WBK 150-B-B-B and WBK 87-B-B-B with high biomass production at an early growth stage could possess the ability to compete with weeds. This is consistent with the experiments in which high amounts of aboveground biomass was observed to be significantly correlated with competitiveness with weeds in rainfed rice ecologies (Fofana *et al.*, 1995 and Dingkuhn *et al.*, 1998).

Progenies such as WBK 41-B-B and WBK 41-B-B-B maintained plant height under drought stressed. They could be promising for rice farmers both in the upland and lowland ecologies, as tall plants ranked higher in trait preferences in Sikaso region of Mali. The best progeny was WBK 41-B-B and maintained plant height, leaf area index, specific leaf dry weight and relative leaf water content under drought stressed condition. These progeny could be used in population improvement for these traits under drought stressed conditions.

Conclusion

Progenies with high selection indices such as WBK 150-B-B-B could be promising for a wide range of rice ecologies and exhibit high tolerance to drought stressed conditions. Drought stress is an important factor in rice production. Drought stress tolerance is difficult

to measure. Putative secondary traits of rice have been identified to be significantly associated with drought stress. Significant correlation was observed between drought tolerance with less leaf rolling, large leaf area index, taller plants, high root dry weight and high shoot dry weight. These traits could be used as drought stress indicators. The early and late flowering progenies identified here could be promising for late and early season drought environments. Drought tolerance, taller plants and less leaf rolling were significantly correlated with fast recovery ability hence; these traits could be used in drought breeding programmes.

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APPENDIX IV

The effect of water stress in some rice characteristics in segregating populations during 2005 dry season experiment

Progenies / parental lines	Populations	Generation	Drought score	Leaf rolling score	Days to 50 % Flowering	Recovery score 3 days after stress relief	Recovery score 10 days after stress relief
WBK 39-B-B-B	WBK 39	F4	2	2	151	1	1
WBK 30-B-B	WBK 30	F3	3	3	144	2	1
WBK 32-B-B-B	WBK 32	F4	3	3	144	2	1
WBK 70-B-B	WBK 70	BC1F3	3	3	144	3	1
WBK 150-B-B	WBK 150	BC1F3	3	3	144	2	1
WBK 78-B-B-B	WBK 78	BC1F4	3	3	144	2	1
WBK 28-B-B-1	WBK 28	F4	3	3	151	1	1
WBK 28-B-B-4	WBK 28	F4	3	2	138	5	2
WBK 42-B-B-2	WBK 42	F4	3	3	144	2	1
WAB 450-I-B-P-103-HB	Parent	Parent	3	3	131	4	2
WBK 28-B-B	WBK 28	F3	3	3	138	4	2
WBK 34-B-B	WBK 34	F3	3	4	131	2	1
WBK 39-B-B	WBK 39	F3	3	2	148	3	2
WBK 41-B-B	WBK 41	F3	3	3	109	3	2
WBK 42-B-B	WBK 42	F3	3	3	148	2	1
WBK 87-B-B-B	WBK 87	BC1F4	3	3	138	3	1
WBK 110-B-B-B	WBK 110	BC1F4	3	3	120	3	1
WBK 114-B-B-B	WBK 114	BC1F4	3	3	141	3	2
WBK 136-B-B	WBK 136	BC1F3	3	3	116	1	1
WBK 30-B-B-B	WBK 30	F4	3	3	131	1	1
WBK 35-B-B-B	WBK 35	F4	3	3	148	1	1
WBK 40-B-B-B	WBK 40	F4	3	2	151	4	2
WBK 150-B-B-B	WBK 150	BC1F4	3	2	120	3	1
WBK 03-B-B-1	WBK 03	F4	3	3	116	2	1
WBK 35-B-B-1	WBK 35	F4	3	3	138	3	1
NERICA 3	Parent	Parent	3	3	109	2	1
WAB 450-I-B-P-6-1-1	Parent	Parent	3	4	131	3	1
WAB 880-I-38-13-1-P1-HB	Parent	Parent	3	3	151	3	1
WBK 02-B-B-B	WBK 02	F4	4	2	138	2	1
WBK 100-B-B-B	WBK 100	BC1F4	4	3	131	3	2
WBK 106-B-B	WBK 106	BC1F3	4	3	131	3	1
WBK 149-B-B	WBK 149	BC1F3	4	3	101	2	1
WBK 28-B-B-B	WBK 28	F4	4	3	141	3	1
WBK 39-B-3	WBK 39	F3	4	3	144	4	2
WBK 64-B-1	WBK 64	BC1F3	4	3	131	1	1
NERICA 2	Parent	Parent	4	3	101	3	2
RAM 118	Parent	Parent	4	5	101	1	1
OS 6 (resistant check)	Check	Check	4	3	161	3	2
WBK 64-B-B	WBK 64	BC1F3	4	3	101	2	1
WBK 75-B-B	WBK 75	BC1F3	4	4	148	6	4
WBK 184-B-B	WBK 184	BC1F3	4	4	144	5	3
WBK 34-B-B-B	WBK 34	F4	4	4	138	3	2
WBK 41-B-B-B	WBK 41	F4	4	3	120	1	1
WBK 42-B-B-B	WBK 42	F4	4	3	144	2	1
WBK 41-B-B-1	WBK 41	F4	4	2	101	1	1

Progenies / parental lines	Populations	Generation	Drought score	Leaf rolling score	Days to 50 % Flowering	Recovery score 3 days after stress relief	Recovery score 10 days after stress relief
WAB 365-B-1-H1-HB	Parent	Parent	4	4	144	3	2
WBK 40-B-B	WBK 40	F3	5	4	151	4	3
WBK 64-B-B-B	WBK 64	BC1F4	5	4	138	2	1
WBK 136-B-B-B	WBK 136	BC1F4	5	3	109	2	1
WBK 35-B-1	WBK 35	F3	5	4	148	6	4
WBK 39-B-B-1	WBK 39	F4	5	2	148	4	2
IR 20 (susceptible Check)	Check	Check	5	4	v.late	4	3
WBK 03-B-B-B	WBK 03	F4	6	4	131	5	4
RAM 24	Parent	Parent	6	5	109	4	2
LSD			1.6	1.4	13.9	3.04	1.82
Probability			**	*	***	ns	*

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

APPENDIX V

The effect of water stress in some rice characteristics in segregating populations during 2005 dry season experiment

Progenies / parental lines	Populations	Generation	LAI	SLDW (g)	Plant height (cm)	% RLWC	% Establishment	Tiller number
WBK 150-B-B-B	WBK 150	BC1F4	6.34	10.43	41.7	86.37	81.82	4
WBK 30-B-B	WBK 30	F3	6.29	11.52	40	72.57	71.82	4
WBK 42-B-B	WBK 42	F3	5.43	13.47	34.2	85.69	57.28	4
WBK 114-B-B-B	WBK 114	BC1F4	5.38	12.61	35.25	75.13	64.55	3
WBK 39-B-B-B	WBK 39	F4	5.34	12.9	42.5	86.02	47.28	5
WBK 78-B-B-B	WBK 78	BC1F4	5.33	11.83	45	80.07	80	3
WAB 450-I-B-P-103-HB	Parent	Parent	5.28	11.43	43.65	85.12	86.37	3
WBK 35-B-B-1	WBK 35	F4	5.14	11.13	42.3	86.38	88.19	4
WBK 41-B-B	WBK 41	F3	5.07	18.22	35.14	86.11	86.37	5
WBK 39-B-3	WBK 39	F3	5.07	11.71	35.95	81.66	81.82	4
WBK 02-B-B-B	WBK 02	F4	5.05	10.53	44.05	70.42	63.64	4
WBK-28-B-B-1	WBK 28	F4	4.98	14.04	35	73.61	69.09	5
WBK 28-B-B-B	WBK 28	F4	4.96	10.63	37.5	75.55	84.55	4
OS 6 (resistant check)	Check	Check	4.9	14	41.8	66.35	80	5
WBK 42-B-B-2	WBK 42	F4	4.76	11.85	49.6	84.03	86.37	4
WBK 70-B-B	WBK 70	BC1F3	4.65	12.85	41	65.52	52.73	4
WBK 34-B-B	WBK 34	F3	4.59	10.89	43.75	83.56	62.73	5
WBK 149-B-B	WBK 149	BC1F3	4.52	13	35.5	84.87	77.27	4
WAB 365-B-1-H1-HB	Parent	Parent	4.4	11.93	39.9	81.97	95.46	3
WBK 40-B-B	WBK 40	F3	4.35	12.42	34.28	69.03	74.55	4
WAB 880-1-38-13-1-P1-HB	Parent	Parent	4.35	9.41	29.1	81.62	89.09	4
WBK 34-B-B-B	WBK 34	F4	4.34	11.16	41.95	82.95	74.55	4
WBK 150-B-B	WBK 150	BC1F3	4.12	10.88	37.6	75.93	75.46	4
NERICA 3	Parent	Parent	3.95	9.9	44.85	74.54	85.46	6
WBK 32-B-B-B	WBK 32	F4	3.88	12.48	35.5	74.67	70	4
WBK 39-B-B-1	WBK 39	F4	3.87	11.9	32.25	84.15	81.82	4
WBK 87-B-B-B	WBK 87	BC1F4	3.81	10.9	37.1	77.72	40	4
WBK 03-B-B-1	WBK 03	F4	3.8	10.72	37.78	85.45	60	5
WBK 28-B-B-4	WBK 28	F4	3.64	10.41	38.48	84.72	80	6
WBK 106-B-B	WBK 106	BC1F3	3.58	9.52	37.4	81.5	88.18	4
WBK 30-B-B-B	WBK 30	F4	3.57	11.63	48.22	84.38	72.73	4
WBK 136-B-B	WBK 136	BC1F3	3.56	10.89	38.15	74.11	66.37	4
WAB 450-I-B-P-6-1-1	Parent	Parent	3.56	9.83	37.55	74.32	89.09	4
WBK 184-B-B	WBK 184	BC1F3	3.51	10.48	40.8	82.34	79.1	4
WBK 35-B-B-B	WBK 35	F4	3.49	13.33	37.9	75.64	44.55	4
WBK 100-B-B-B	WBK 100	BC1F4	3.41	12.27	44	71.98	76.36	5
WBK 39-B-B	WBK 39	F3	3.4	10.72	39.55	73.5	87.27	4
WBK 75-B-B	WBK 75	BC1F3	3.35	16.16	29.1	74.86	59.09	4
WBK 64-B-B	WBK 64	BC1F3	3.24	9.92	29.1	81.39	73.64	4
WBK 40-B-B-B	WBK 40	F4	3.16	10.87	34.55	70.61	69.09	4
WBK 35-B-1	WBK 35	F3	3.16	10.98	39.98	83.34	83.64	4
WBK 42-B-B-B	WBK 42	F4	3.15	10.2	33.4	72.71	76.37	5
WBK 28-B-B	WBK 28	F3	2.87	10.3	35.5	81.28	61.82	3
WBK 64-B-B-B	WBK 64	BC1F4	2.77	10.15	34.65	77.24	33.64	3
NERICA 2	Parent	Parent	2.77	10.22	34.38	80.31	79.09	4
WBK 64-B-1	WBK 64	BC1F3	2.76	10.5	29.5	78	80.91	4

Progenies / parental lines	Populations	Generation	LAI	SLDW (g)	Plant height (cm)	% RLWC	% Establishment	Tiller number
WBK 41-B-B-1	WBK 41	F4	2.75	12.46	40.5	83.86	92.73	4
WBK 110-B-B-B	WBK 110	BC1F4	2.52	8.68	39	82.49	77.27	5
RAM 24	Parent	Parent	2.46	9.75	39.15	80.36	92.73	6
WBK 03-B-B-B	WBK 03	F4	2.39	8.83	26.85	76.04	44.55	4
WBK 41-B-B-B	WBK 41	F4	2.27	11.56	31.9	69.74	69.09	5
RAM 118	Parent	Parent	2.08	11.15	41.35	72.47	68.18	10
WBK 136-B-B-B	WBK 136	BC1F4	1.2	8.7	29.15	74.67	43.64	5
IR 20 (susceptible check)	Check	Check	1.18	7.59	25.82	72.92	88.18	7
LSD			2.343	3.973	8.924	17.574	22.619	1.992
Probability			**	*	***	ns	***	***

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

APPENDIX VI

Mean value of analysis of variance of rice characteristics in fully irrigated experiment in segregating populations in 2005 dry season experiment

Progenis / parental lines	Populations	Generation	Days to 50 % Flowering	LAI	SLDW (g)	Plant height (cm)	Tiller number	% Establishm-ent	% RLWC
WBK 64-B-B	WBK 64	BC1F3	82.00	3.88	13.73	49.70	10.70	80.00	84.43
WBK 136-B-B	WBK 136	BC1F3	82.00	0.45	7.18	41.65	8.20	51.82	86.04
WBK 136-B-B-B	WBK 136	BC1F4	82.00	3.59	16.89	36.70	9.10	29.09	70.89
WBK 150-B-B	WBK 150	BC1F3	82.00	3.51	13.61	60.40	8.00	70.91	85.45
WBK 41-B-B-1	WBK 41	F4	82.00	4.22	14.28	52.05	10.10	86.37	87.59
WBK 41-B-B-2	WBK 41	F4	82.00	4.24	11.56	43.50	9.50	62.73	78.81
NERICA 2	Parent	Parent	82.00	2.93	14.38	49.50	9.00	70.91	77.15
RAM 118	Parent	Parent	82.00	1.02	11.19	47.57	17.10	37.28	81.37
RAM 24	Parent	Parent	92.00	1.61	12.28	53.00	15.50	90.00	81.26
WBK 41-B-B-B	WBK 41	F4	107.50	3.11	16.71	31.51	9.40	68.18	93.54
WBK 136-B-3	WBK 136	BC1F3	112.50	2.81	13.25	42.54	9.00	68.19	82.40
WBK 150-B-1	WBK 150	BC1F3	114.00	5.33	9.33	39.04	8.14	52.73	79.32
WBK 41-B-B	WBK 41	F3	117.50	2.01	17.82	32.81	8.90	51.82	68.53
WBK 34-B-B	WBK 34	F3	128.50	0.61	6.06	45.60	8.10	26.37	66.09
WBK 28-B-B	WBK 28	F3	133.00	3.93	16.56	59.00	8.30	42.73	83.04
WBK 106-B-B	WBK 106	BC1F3	133.00	3.95	12.06	51.60	9.50	75.46	73.04
WBK 110-B-B-B	WBK 110	BC1F4	133.00	2.71	15.43	49.40	10.20	66.36	71.52
WBK 150-B-B-B	WBK 150	BC1F4	133.00	5.53	14.76	62.00	8.60	81.82	70.86
WBK 64-B-1	WBK 64	BC1F3	133.00	1.98	9.61	39.54	7.50	72.73	81.30
WBK 35-B-B-4	WBK 35	F4	133.00	2.84	12.22	50.90	8.20	76.37	62.34
WBK 42-B-B-3	WBK 42	F4	133.00	3.28	12.81	59.11	8.30	68.19	73.91
WBK 136-B-1	WBK 136	BC1F3	133.00	2.00	17.78	47.50	8.00	41.82	70.65
WBK 136-B-4	WBK 136	BC1F3	133.00	5.51	12.30	44.29	7.50	67.28	70.95
NERICA 3	Parent	Parent	133.00	2.27	9.66	55.10	7.50	61.82	69.78
OS 6 (resistant check)	Check	Check	133.00	1.88	14.38	43.32	7.70	70.00	60.99
WBK 03-B-B-B	WBK 03	F4	139.50	3.68	12.74	37.39	9.90	50.00	71.27
WBK 78-B-B-B	WBK 78	BC1F4	139.50	2.77	14.75	58.10	8.40	53.64	83.65
WBK 28-B-B-4	WBK 28	F4	139.50	5.52	15.55	51.54	12.80	67.28	74.60
WBK 35-B-1	WBK 35	F3	139.50	4.37	10.87	52.10	7.40	71.82	63.97
WBK 35-B-2	WBK 35	F3	139.50	3.17	14.77	49.81	8.10	72.73	63.51
WBK 35-B-3	WBK 35	F3	139.50	3.27	13.74	46.01	6.60	79.10	65.02
WBK 40-B-B-1	WBK 40	F4	139.50	0.77	12.36	37.70	7.00	60.91	75.28
WBK 106-B-2	WBK 106	BC1F3	139.50	NA	NA	30.75	7.95	13.64	72.92

Progenies / parental lines	Populations	Generation	Days to 50 % Flowering	LAI	SLDW (g)	Plant height (cm)	Tiller number	% Establishm-ent	% RLWC
WBK 35-B-B-6	WBK 35	F4	139.50	4.08	11.92	49.40	8.00	69.09	66.46
WBK 42-B-B-5	WBK 42	F4	139.50	4.48	14.79	49.70	9.70	59.10	71.86
WBK 64-B-B-4	WBK 64	BC1F4	139.50	4.10	12.61	41.80	8.00	28.18	60.78
WAB 375-B-9-H3-2	Parent	Parent	139.50	1.68	16.79	36.43	10.50	27.28	71.66
WAB 450-I-B-P-6-1-1	Parent	Parent	139.50	5.93	12.16	43.80	6.50	49.10	76.35
WBK 32-B-B-B	WBK 32	F4	143.00	1.56	11.37	48.50	11.30	30.91	74.26
WBK 39-B-B	WBK 39	F3	143.00	6.50	12.22	54.14	7.20	71.82	79.29
WBK 39-B-1	WBK 39	F3	143.00	5.05	14.94	53.97	9.10	73.64	72.48
WBK 28-B-B-B	WBK 28	F4	146.00	2.81	12.46	48.70	10.00	61.82	76.19
WBK 30-B-B	WBK 30	F3	146.00	2.09	13.88	57.40	8.20	67.28	75.49
WBK 35-B-B	WBK 35	F3	146.00	2.85	11.99	41.65	7.05	20.00	74.60
WBK 35-B-B-B	WBK 35	F4	146.00	3.18	13.39	44.83	7.20	30.91	76.16
WBK 42-B-B	WBK 42	F3	146.00	5.63	14.04	40.04	8.30	37.27	75.42
WBK 100-B-B-B	WBK 100	BC1F4	146.00	3.23	12.32	48.19	12.30	41.82	75.11
WBK 114-B-B-B	WBK 114	BC1F4	146.00	5.67	15.66	50.60	9.90	50.00	84.60
WBK 28-B-B-2	WBK 28	F4	146.00	5.25	12.35	50.55	7.60	53.64	80.88
WBK 35-B-B-1	WBK 35	F4	146.00	3.83	16.55	52.19	8.20	75.46	58.85
WBK 35-B-B-3	WBK 35	F4	146.00	NA	NA	52.71	7.50	18.19	66.77
WBK 42-B-B-1	WBK 42	F4	146.00	5.25	14.66	50.88	6.70	82.73	60.23
WBK 35-B-B-5	WBK 35	F4	146.00	4.12	16.00	60.10	7.10	74.55	75.56
WBK 40-B-2	WBK 40	F3	146.00	7.11	13.14	50.85	12.60	77.27	82.67
WBK 42-B-1	WBK 42	F3	146.00	3.27	15.37	36.52	9.30	30.91	84.09
WBK 64-B-B-1	WBK 64	BC1F4	146.00	6.39	13.44	48.50	6.80	17.28	72.10
WAB 365-B-1-H1-HB	Parent	Parent	146.00	4.78	12.72	47.40	7.50	62.73	77.56
WAB 450-I-B-P-103-HB	Parent	Parent	146.00	4.95	14.06	58.20	7.60	69.09	78.85
WBK 39-B-B-2	WBK 39	F4	148.00	4.80	12.81	46.61	8.30	48.19	73.70
WBK 39-B-B-B	WBK 39	F4	149.50	4.13	14.35	51.00	8.50	36.37	81.31
WBK 40-B-B	WBK 40	F3	149.50	3.41	14.51	42.67	9.60	45.46	75.69
WBK 42-B-B-B	WBK 42	F4	149.50	4.53	11.51	34.97	8.55	48.18	71.45
WBK 35-B-B-2	WBK 35	F4	149.50	5.03	15.46	36.33	6.90	43.64	78.97
WBK 39-B-3	WBK 39	F3	149.50	5.31	13.52	45.11	8.10	80.00	64.52
WBK 39-B-B-1	WBK 39	F4	149.50	2.62	13.00	35.57	8.00	74.55	79.03
WBK 42-B-B-2	WBK 42	F4	149.50	4.98	12.25	55.09	7.40	73.64	57.13
WBK 28-B-B-5	WBK 28	F4	149.50	4.72	16.55	47.80	10.30	70.00	74.72
WBK 39-B-4	WBK 39	F3	149.50	4.30	12.75	43.32	7.60	76.36	71.58
WBK 39-B-5	WBK 39	F3	149.50	2.55	15.56	48.70	6.80	90.00	79.45
WBK 64-B-3	WBK 64	BC1F3	149.50	3.35	16.73	32.23	8.30	46.37	71.17

Progenies / parental lines	Populations	Generation	Days to 50 % Flowering	LAI	SLDW (g)	Plant height (cm)	Tiller number	% Establishm-ent	% RLWC
WBK 40-B-B-B	WBK 40	F4	154.50	5.58	13.12	41.59	9.90	48.18	79.21
WBK 28-B-B-3	WBK 28	F4	154.50	4.05	12.96	43.10	7.50	44.55	73.10
WBK-28-B-B-1	WBK 28	F4	158.00	1.11	8.25	37.60	9.80	48.19	69.99
WAB 880-1-38-13-1-P1-HB	Parent	Parent	158.00	3.09	13.62	47.35	9.70	77.28	79.01
WBK 40-B-1	WBK 40	F3	163.00	4.86	14.73	46.65	11.30	60.91	78.67
IR 20 (susceptible check)	Check	Check	V.late	0.69	7.04	29.60	17.70	63.64	79.59
LSD			23.119	3.323	3.900	16.228	3.554	32.144	19.857
Probability			***	*	***	**	***	***	ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant; V.late =late flowering; NA =not available

CHAPTER 7

Genetic studies of morpho-physiological traits in segregating populations of rice under stress and non-stress conditions

Abstract

The genetic variations in quantitative characteristics in plant populations are a primary concern in any plant-breeding programme. Therefore, a great deal of attention should be paid to their measurement and characterisation. The objectives of this study were to investigate the genetic effects of morpho-physiological traits controlling stress tolerance and determine the gene action for drought stress tolerance. The genetic materials used in this study include five *Oryza glaberrima* genotypes and four interspecific inbred lines as female parents and two interspecific hybrids and two *O. sativa* lines as male parents. The parents were crossed using a North Carolina II mating design. The populations were advanced by selfing to the F3 and F4 generations. Two experiments comprising drought stressed and fully irrigated treatments were established simultaneously with F3 and F4 progenies and their parents. Experiments were laid as 8 x 8 and 9 x 9 lattice designs with two replications, under drought stress and fully irrigated conditions, respectively. The resistant and susceptible checks were OS 6 and IR 20, respectively. Overhead sprinkler irrigation was applied for 40 days after sowing, three times weekly, to maintain field water capacity. Irrigation was stopped in the stressed experiment for 50 days to simulate stress and then full irrigation was resumed until maturity. During the stressed period, data on morpho-physiological traits were collected and recovery ability of the progenies when stress was relieved. Highly significant differences were observed between general combining ability (GCA) variance for females and males for the characteristics examined. Females GCA and specific combining ability (SCA) were significant for tiller number, plant height and specific leaf dry weight, indicating the importance of additive and non-additive genetic effects for these traits. Heritability estimates based on female parents ranged from 47% to 67% for plant height, leaf area, specific leaf dry weight and SPAD, while it was not significant for tiller number, which was under the control of overdominance. Heritability estimates based on regression were significant and ranged from 32 % to 72% for drought tolerance scores, and ranged from 22% to 56% for leaf rolling scores. Highly significant positive relationships were observed between F3 and F4 generations for drought tolerance and leaf rolling scores for all the populations examined. Both additive and non-additive genetic effects were prominent in some of the traits, which could assist breeders' selections.

7.1 Introduction

Drought is one of the major constraints for rice production in the rain fed ecology in sub-Saharan Africa (SSA). It occurs not only in upland but also in lowland rice ecologies. Rice consumption in SSA continues to grow at a rapid pace, faster than any other staple food (FAO, 2001). Therefore, a major challenge for research and development activities in the sub-Saharan African region are to develop drought tolerant lines with wide adaptability

across rice growing ecologies. These challenges involve improvement of local landraces of rice through introduction of new genes from other rice species, thus creating wide genetic variation for the selection of rice with good agronomic characters. Genetic variation in quantitative characteristics in plant populations is a primary concern of plant-breeding programmes. Therefore, a great deal of attention should be paid to their measurement and characterisation. The benefits from this characterisation will assist in designing the best and most appropriate breeding programme.

Knowledge of the heritability estimates of a character is very important since it determines the extent to which plant improvement through selection is possible. Falconer and Mackay (1996) have described the use of parent-offspring regression analysis as an estimator of narrow sense heritability estimates. A high parent-offspring correlation implies higher heritability estimates and provides a measure of the general combining ability of parents for the concerned trait. Estimates of broad and narrow sense heritability for grain yield and its components were high using classical methods of quantitative genetics have been reported by many researchers (Smith *et al.*, 1998; Montagnon *et al.*, 2003).

Information on genetic variances and their effects have contributed to rice improvement and to the understanding of the gene action involved in the expression of heterosis and economically important quantitative traits. A procedure for estimating the components of genetic variance of a population which has undergone random mating with no inbreeding has been suggested (Comstock and Robinson, 1948; Sahagun-Castellanos, 1999).

Progress has been made in introgressing genes from *O. glaberrima* into *O. sativa*. Jones *et al.* (1997) reported that some interspecific progenies of *O. sativa* x *O. glaberrima* crosses combined high spikelet number of *O. sativa*, with many useful vegetative traits of *O. glaberrima*. These included rapid vegetative growth, droopy lower leaves, high tillering ability, and superior grain quality. Leaf area index, specific leaf area and leaf chlorophyll content were intermediate between the parents (Dingkuhn *et al.*, 1998), suggesting that additive gene action controlled their inheritance.

The work done on the introgression of genes into interspecific hybrids of rice provided little information on genetic analysis, especially the morpho-physiological traits under stressful conditions. Information obtained from this study may be helpful in identifying parents with outstanding combining ability for these traits that can be used in breeding for drought tolerance. Knowledge of the additive portion of the genetic variation relative to the phenotypic variance would also be useful as it reflects the degree to which the progenies are likely to resemble the parents.

7.2 Objectives

The objectives of this study were to investigate the genetic effects of the morpho-physiological traits associated with drought stress tolerance in rice genotypes.

7.2.1 Hypothesis

There is adequate genetic variation in morphological and physiological traits associated with drought tolerance in rice genotypes.

7.3 Materials and Methods

7.3.1 Soil properties and environmental parameters

The segregating progenies were established at ICRISAT research station, Samanko, Mali.

The soils are acidic and deficient in organic matter and total nitrogen (Table 1). The soil texture is silty clay loam with very low cation exchange capacity. The soil was of low fertility. This resulted from inadequate levels of essential nutrients especially Nitrogen (N) content, which was 0.058 %. The soil organic matter was low, 0.480 % (Table 1).

Table 1: Soil properties in 0-20 cm soil depth for the experimental site at Samanko, during 2005 dry season.

Property	2005
pH (1:1 water)	4.964
Organic matter (%)	0.480
Total N (%)	0.058
P (ppm)	9.600
K (ppm)	0.232
Ca (ppm)	2.776
Mg (ppm)	0.896
CEC (cmol (+)/kg)	9.740
Sand (%)	21.200
Silt (%)	60.800
Clay (%)	17.800
Soil texture	Silty clay loam

The monthly means of maximum and minimum air temperature, pan evaporation, rainfall and air relative humidity during the experiments were as shown (Table 2).

Table 2: Monthly means maximum and minimum air temperature, pan evaporation, rainfall and air relative humidity during the experiments

Month	Maximum Temperature (° C)	Minimum Temperature (° C)	Pan Evaporation (mm day ⁻¹)	Rainfall (mm)	% air relative humidity
October	38.6	25.5	3.3	2.5	79.0
November	38.9	22.8	4.7	0.0	75.8
December	37.1	23.1	6.2	0.0	69.1
January	37.4	20.2	6.5	0.0	58.7

The 2.5 mm rainfall received during the experimental period fell once on the 4th of October 2005. The gradual decrease in temperature and relative humidity was due to severe Harmantan haze with very dry and cool air. Pan evaporation had a monthly mean of 5.18 mm day⁻¹. This season experienced lower temperature and Pan evaporation as well as higher relative humidity as compared to the previous seasons' drought experiment (Table 2).

7.3.2 Genetic materials used in crosses

Genetic materials used in the crosses comprised five *Oryza glaberrima* genotypes and NERICA 2 and NERICA 3, interspecific inbred lines that have been released as varieties grown in many West and Central Africa (WCA) countries. Other parents were interspecific inbred lines: WAB450-I-B-P-103-HB, WAB450-I-B-P-6-1-1, WAB450-I-B-P-105-HB, and WAB880-1-38-13-1-P1-HB. They were selected from Observational / Replicated Yield Trials (OYT/RYT) based on their drought tolerance and yield performance. Finally, WAB 365-B-1-H1-HB and WAB 375-B-9-H3-2 which are improved *O. sativa* lines selected from Elite Varietal Trials (EVT) at WARDA as high yielding varieties were also used as parents.

7.3.3 Cross combinations and pollination method

The crosses were classified into four groups: group A (*O. glaberrima* x *O. sativa*), group B (*O. glaberrima* x Interspecific), group C (Interspecific x *O. sativa*) and group D (Interspecifics x Interspecific) and a total of 36 cross combinations were made (Table 3). Five *Oryza glaberrima* genotypes and four inbred interspecific genotypes (derived from crosses between *O. sativa* and *O. glaberrima*) were used as female parents (seed parents) and crossed with two improved *O. sativa* L. subsp (*japonica*), and two inbred interspecific lines as male parents (pollen parents) using the North Carolina design II mating scheme (Comstock and Robinson, 1952).

The populations that were generated using the North Carolina design II mating scheme (Table 2, Chapter 5) were advanced to F3 and F4 generations for seed increase by single plants selection. A total of four populations were advanced to F3 generation and eight populations to F4 generation only, while twelve populations were advanced to both F3 and F4 generations (Table 3). These populations and their parents accounted for the genetic materials used in this study. Pedigree and agronomic characteristics of the parents used in the crosses shown in (Table 1, Chapter 5). Selection of these progenies was based on availability of enough seed to conduct two trials simultaneously as well as for seeds harvest. Therefore the number of progenies in the fully irrigated (non-stressed) experiment was greater than the stressed experiment. All the progenies in stressed experiment were represented in the fully irrigated experiment for comparison.

Table 3: Parental lines and groupings used in the North Carolina II mating design.

Group/line code	<i>Sub-group-1 (female)</i>	<i>Sub-group-2(male)</i>
Group A	<i>O. glaberrima</i>	<i>O. sativa</i>
A1	RAM 85	WAB365-B-1-H1-HB
A2		WAB375-B-9-H3-2
A3	RAM 86	WAB365-B-1-H1-HB
A4		WAB375-B-9-H3-2
A5	RAM 118	WAB365-B-1-H1-HB
A6		WAB375-B-9-H3-2
A7	CG14	WAB365-B-1-H1-HB
A8		WAB375-B-9-H3-2
A9	TOG 5681	WAB365-B-1-H1-HB
A10		WAB375-B-9-H3-2
Group B	<i>O. glaberrima</i>	Interspecific
B1	RAM 85	NERICA 2
B2		NERICA 3
B3	RAM 86	NERICA 2
B4		NERICA 3
B5	RAM 118	NERICA 2
B6		NERICA 3
B7	CG14	NERICA 2
B8		NERICA 3
B9	TOG 5681	NERICA 2
B10		NERICA 3
Group C	Interspecific	<i>O. sativa</i>
C1	WAB450-IBP-103-HB	WAB375-B-9-H3-2
C2		WAB365-B-1-H1-HB
C3	WAB450-IBP-6-1-1	WAB375-B-9-H3-2
C4		WAB365-B-1-H1-HB
C5	WAB450-IBP-105-HB	WAB375-B-9-H3-2
C6		WAB365-B-1-H1-HB
C7	WAB880-1-38-13-1-P1-HB	WAB375-B-9-H3-2
C8		WAB365-B-1-H1-HB
Group D	Interspecific	Interspecific
D1	WAB450-IBP-103-HB	NERICA 2
D2		NERICA 3
D3	WAB450-IBP-6-1-1	NERICA 2
D4		NERICA 3
D5	WAB450-IBP-105-HB	NERICA 2
D6		NERICA 3
D7	WAB880-1-38-13-1-P1-HB	NERICA 2
D8		NERICA 3

Rice seeds were sown in a pot filled with sandy loam soil in the greenhouse during the month of January 2004. There were four sets of plantings staggered at weekly intervals to

synchronize flowering. *Oryza glaberrima* genotypes are photoperiod-sensitive and require short day length to flower. A darkroom was constructed, which reduced the day-length by about eight hours by putting the plants outdoor from 7.0 hr to 15.0 hr each day. Emasculation was done only on a plant whose panicle had emerged about half way from the boot. The spikelets at the middle portion of the panicle were selected to be the best for emasculation. The spikelet was cut two-thirds from the base with a sterilized scissors to expose the anthers and then the anthers were removed by a micropipette attached to vacuum emasculator. The emasculated panicle was then covered with a pollinating bag and closed with paper clips or stapler. The optimum time to pollinate was midday to 15.00 hours. The pollen was removed from the male plant into a petri dish containing a little water. Bags were gently removed from the seed parent and pollen was transferred onto the sigma by an office pin or forceps. The bag was replaced on the female plant and the date and the parents of the cross were written on the back of the bag. Mature seeds were harvested when they lost their green colour between 25 d to 30 d after pollination. The F₁ seed was naked without glum. Dormancy of the naked F₁ seed was broken by placing the seeds in a dry air oven at 55⁰ C for five days and germinated on moist filter paper in petri dishes in the laboratory. The seedlings were transplanted when the radicle was about 5.0 mm to 10 mm into pots in the glasshouse. These F₁ seedlings and their parents were sown in randomised complete block design in two replications.

7.3.4 Cultural details and experimental design

The F₁ populations that were derived from the North Carolina II mating design were advanced to F₃ and F₄ generations. Two experiments comprising drought stressed and fully irrigated (non-stressed) treatments were established simultaneously with F₃ and F₄ progenies and their parents (Table 4). These will enable to identify at which generation of the population may be suitable for drought stress screening and to accelerate their advancement in the breeding programme. The drought stressed experiments contained 64 entries including OS 6 and IR 20 as resistant and susceptible checks, respectively. Entries were sown on the 20th October 2005 in (8 x 8) lattice design with two replications. The fully irrigated experiments, which acted as a control had 81 entries reasons as explained (See

7.3.3) were sown on the 18th October 2005 in (9 x 9) lattice design with two replications, including OS 6 and IR 20 as resistant and susceptible checks, respectively.

The dried seed were dibbled on shallow holes at the rate of three seeds per hole with a spacing of 20 cm within plants and 20 cm between rows and thinned to one plant per hole after 15 d of seedling emergence and a total of 55 plants per plot. The experiments were hand weeded at 15 d and 35 d after sowing prior to fertilizer application. Basal fertilizer was applied at the rate of 200 kg ha⁻¹ of 17-17-17, N-P-K and urea (46% N) was top-dressed at the rate of 100 kg ha⁻¹ at 35 d after sowing. There was no insecticide applied. The observed characters from F3 and F4 progenies and their parents were used in the estimation of their heritability estimates (Table 4)

7.3.5 Water management

Overhead sprinkler irrigation was applied for 40 days after sowing, three times weekly, to maintain field capacity. Irrigation was stopped in the stressed experiment for 50 days at maximum tillering stage to simulate stress and full irrigation resumed again until maturity. Simulation of drought at maximum tillering stage is advisable for rice crop (Singh and Mackill, 1991).

7.4 Data collection

The Standard Evaluation System (SES) for Rice reference manual (IRRI, 1996) was used for all trait measurements except where stated. Measurements were taken at weekly intervals on all traits in both stressed and fully irrigated-control experiments for six weeks during the stress period. At maturity, twelve populations with complete sets of crosses (six females and two males) were used in the calculation of genetic components. Three F1 plants were randomly selected in each of the population and were evaluated for tiller number, plant height, leaf area and specific leaf dry weight (SLDW) derived as leaf area per leaf dry weight. Leaf chlorophyll content (SPAD) was measured with a SPAD chlorophyll meter (Minolta, Tokyo) and the average of repeated measurement was recorded. This information was used for the calculations of genetic variance components. Morphological and physiological characters were also measured on the F3 and F4 progenies for heritability

study. The following measurements were taken as observed for the whole plot, otherwise, were stated:

Table 4: Genetic materials used in the drought stressed and non-stressed experiments

Populations / parental lines	Pedigree	Generations
Populations		
WBK 02	RAM 24 x NERICA 2	F4
WBK 03	RAM 24 x NERICA 3	F4
WBK 15	RAM 118 X WAB 375-B-9-H3-2	F4
WBK 28	WAB 450-IBP-103-HB x WAB375-B-9-H3-2	F3, F4
WBK 30	WAB 450-IBP-103-HB x NERICA 3	F3, F4
WBK 32	WAB 450-IBP-105-HB x WAB375-B-9-H3-2	F4
WBK 34	WAB 450-IBP-105-HB x NERICA 3	F3, F4
WBK 35	WAB 450-IBP-6-1-1 x WAB365-B-1-H1-HB	F3, F4
WBK 39	WAB 880-1-38-13-1-P1-HB x WAB365-B-1-H1-HB	F3, F4
WBK 40	WAB 880-1-38-13-1-P1-HB x WAB375-B-9-H3-2	F3, F4
WBK 41	WAB 880-1-38-13-1-P1-HB x NERICA 2	F3, F4
WBK 42	WAB 880-1-38-13-1-P1-HB x NERICA 3	F3, F4
WBK 64	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // WAB365-B-1-H1-HB	F3, F4
WBK 70	WAB 450-IBP-105-HB x NERICA 3 // WAB365-B-1-H1-HB	F3
WBK 75	WAB 880-1-38-13-1-P1-HB x WAB365-B-1-H1-HB // WAB365-B-1-H1-HB	F3
WBK 78	WAB 880-1-38-13-1-P1-HB x NERICA 3 // WAB365-B-1-H1-HB	F3, F4
WBK 87	RAM 118 X WAB365-B-1-H1-HB // WAB375-B-9-H3-2	F4
WBK 100	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // WAB375-B-9-H3-2	F4
WBK 106	WAB 450-IBP-105-HB x NERICA 3 // WAB375-B-9-H3-2	F3
WBK 110	WAB 450-IBP-6-1-1 x NERICA 3 // WAB375-B-9-H3-2	F4
WBK 114	WAB 880-1-38-13-1-P1-HB x NERICA 3 // WAB375-B-9-H3-2	F4
WBK 136	WAB 450-IBP-103-HB x WAB375-B-9-H3-2 // NERICA 2	F3, F4
WBK 149	WAB 880-1-38-13-1-P1-HB x NERICA 2 // NERICA 2	F3
WBK 150	WAB 880-1-38-13-1-P1-HB x NERICA 3 // NERICA 2	F3, F4
WBK 184	WAB 880-1-38-13-1-P1-HB x WAB375-B-9-H3-2 // NERICA 3	F3
Parents of populations		
NERICA 2		Parent
NERICA 3		Parent
WAB 365-B-1-H1-HB		Parent
WAB 375-B-9-H3-2		Parent
WAB 450-I-B-P-103-HB		Parent
WAB 450-I-B-P-105-HB		Parent
WAB 450-I-B-P-6-1-1		Parent
WAB 880-1-38-13-1-P1-HB		Parent
RAM 24		Parent
RAM 118		Parent
OS 6		Resistant check
IR 20		Susceptible check

Drought score: Drought score (Dt) was rated weekly based on the leaf drying symptoms observed. Rating of drought score on a scale of 0-9 was done as shown below (De Datta *et al.*, 1988):

Score	0	No symptoms of stress
	1	Slight drying of leaf tips
	2	25% of the length of 25% of all leaves (apart from older leaves that are normally dry)
	3	At least 25% of the length and 26% to 50% of all leaves are dry.
	4	At least 25% of the length of 50% of all leaves are dry; 25% of leaves are fully dried.
	5	50% of all leaves are fully dried
	6	51-69% of all leaves are fully dried
	7	70% of all leaves are fully dried
	8	More than 70% of all leaves are fully dried
	9	All plants are apparently dead

Leaf rolling: A leaf rolling score was adapted from O'Toole and Moya (1978). Drought and leaf rolling scores were taken at weekly intervals throughout the period of stress between 1300 and 1500 hr to assess the effects of water stress.

Score	0	No leaf rolling
	1	Leaves slightly rolled
	2	Leaves rolled into Slight V-shape
	3	Leaves rolled into deep V-shape
	4	Leaves rolled and leaf margins touching
	5	All leaves completely rolled

Plant height and tiller number: Plant height (cm) was measured from soil surface to the tip of the shoot and tiller number per plant recorded within each hill as described by IRR (1996).

Relative leaf water content (RLWC): Relative leaf water content was determined between 1300 and 1500 hr by the method suggested by Barrs and Weatherly (1962). Three young fully expanded leaves from the main stem were randomly selected in each plot and placed in a plastic bag and transported to the laboratory immediately. A rectangular metal cock was used in sample taken from the middle portion of each leaf and weighed to determine the fresh weight. Turgid weight was determined by weighing the leaf samples put in petri dishes containing water for four hours. They were then oven dried at 60^o C for 24 hours and weighed for dry weight determination. Relative leaf water content was calculated as:

$$\text{RLWC} = \{(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})\} \times 100$$

Plant biomass (fresh and dry): Plant biomass was taken at weekly intervals. Two plants were randomly selected per plot and the aboveground part of the plant was cut off and fresh weight recorded. For the root sampling, a hole of 30 cm depth was dug about 10 cm diameter around the plant. Roots were gently removed into a 2 mm mesh screen and washed free of soil with water to ensure minimum root lost for fresh weight determination. The samples were oven dried at 65^o C for 72 hours and weighed for shoot and root dry weight.

Leaf area index and specific leaf dry weight: Two young fully expanded leaves from the main stem were randomly selected in each plot and leaf area (LA) was determined using a leaf area meter (li-3100, Lincoln, NE USA). Leaf area index (LAI) was calculated as described by Yoshida (1981):

$$\text{LAI} = (\text{sum of the leaf area of all leaves} / \text{unite area where the leaves have been collected}).$$

The specific leaf dry weight (SLDW) was calculated as the area of leaf per leaf dry weight (Jones *et al.*, 1997; Zaharieva *et al.*, 2001).

7.5 Statistical analysis

Variance components were estimated by the method of moments using PROC VARCOMP procedure in SAS computare software version 9.1 (SAS Institute, 2003). General combining

ability (gca) and specific combining ability (sca) for the parents and crosses were estimated for each trait using the following model:

$$Y_{ijk} = \mu + r_k + f_i + m_j + (fm)_{ij} + e_{ijk}$$

Y_{ijk} = phenotypic observation on the progenies, r_k = replication effect, f_i = female parent GCA effects, m_j = male parent GCA effects $(fm)_{ij}$ = interaction between female and male parents in the crosses (SCA) and e_{ijk} = experimental error due to environmental effects.

Additive genetic variances (δ^2A) and variance due to dominance (δ^2D) and other parameters were estimated by equating to the their expected mean square (Comstock and Robinson 1948; Wolf *et al.*, 2000), using the following equations:

$$(i) \delta^2F \text{ and } \delta^2M = \text{Cov. (HS)} = \delta^2A / 4$$

$$(ii) \delta^2F \times \delta^2M = \text{Cov. (FS)} - 2 \text{Cov. (HS)} = \delta^2D / 4$$

$$(iii) \delta^2P = \delta^2A_m + \delta^2A_f + \delta^2D + \delta^2E + \delta^2R$$

$$(iv) h^2 \text{ (half sibs of line)} = \delta^2A_f / \delta^2P$$

$$(v) h^2 \text{ (half sibs of tester)} = \delta^2A_m / \delta^2P$$

$$(vi) d \text{ (degree of dominance)} = (2 \delta^2D / \delta^2A)^{1/2} \text{ for female and male}$$

Where δ^2F and δ^2M represents variation among female parents and male parents (GCA), respectively and $\delta^2F \times \delta^2M$ represents variation of interaction between female parent and male parent in the crosses (SCA); While δ^2E and δ^2R were variations due to environment and replications, respectively, at $F = 0$ (inbreeding coefficient). Comstock and Robinson (1952) suggested that degree of dominance “d” could be regarded as complete dominance if the value of “d” is equal to 1.0 and over dominance if greater than 1.0.

Parent-offspring regression was performed to estimate heritability as described by Falconer and Mackay (1996). The F4 population means were regressed on their F3 parent values for each trait. Linear phenotypic correlations were also calculated for each character of the population using a model described by Gomez and Gomez (1984) as: $Y = \alpha + b X$, where:

Y = phenotypic value for F4 population, X = phenotypic value for F3 population, α = intercept and b = coefficient of linear correlation.

7.6 Results

7.6.1 Monitoring the drought stress condition

Soil moisture content (SMC) of each plot was monitored at 0-20 cm soil depth every five days throughout the stress period using the gravimetric soil analysis method. A graduated soil auger was used for sampling the soil. The weight of fresh soil (Fwt) and soil oven dried for 48 hours at 70⁰ C (Dwt) were taken. Per cent moisture content was calculated as: $Fwt - Dwt / Fwt * 100$. The soil moisture content decreased gradually and by the 21st day of stress, 1.3% of soil moisture was lost (Figure 1), as cracks were observed on the soil surface.

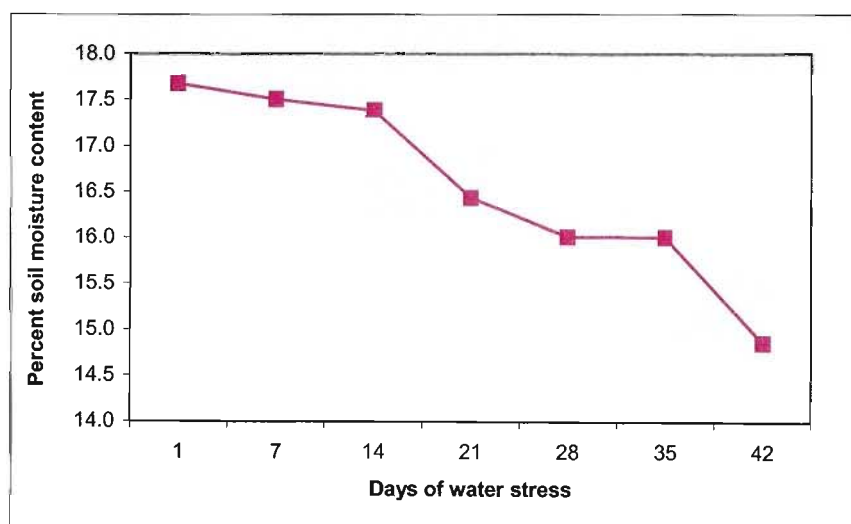


Figure 1: Per cent moisture content monitored in the top 20cm of soil depth

7.6.2 Estimation of variance components

Estimation of variance components was derived from non-stressed conditions because the experiment was under normal conditions. The mean square values showed a significant variation for plant height, chlorophyll content (SPAD), number of tillers, leaf area and specific leaf dry weight (Table 5). The female parent and the interaction between male and female were significant for all the traits except SPAD. The male sources were not significant for SPAD and leaf area (Table 5).

Table 5: Analysis of variance for plant height, SPAD, leaf area, SLDW and tiller number in non-stressed condition

Source of variation	df	Mean square value				
		Plant height	SPAD‡	Leaf area	SLDW‡	Tiller number
Replication	1	140.17 ns	2.98 ns	0.45 ns	0.001 ns	0.92 ns
Male (GCA)	1	7704.17**	2.05 ns	45.07 ns	7.17***	245.12**
Female (GCA)	5	6025.35**	10.25**	443.41***	74.34***	89.75*
Female x Male (SCA)	5	1682.59*	3.45 ns	97.60***	26.33***	89.61*
Experimental error	11	486.83	1.12	9.92	0.001	19.04

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant ; ‡SPAD = chlorophyll content; SLDW = specific leaf dry weigh

Estimates of GCA (females) variances were significant for all characters examined and as well as for SCA variances, but not significant for SPAD. The GCA (males) variances were significant for tiller number, plant height and specific leaf dry weight (Table 6). GCA variance for female was higher than that of SCA in all the characteristics, except tiller number and specific leaf dry weight (Table 6).

Table 6: GCA , SCA variances and their ratio for plant height, SPAD, leaf area, SLDW and tiller number in F1 generations in non-stressed conditions.

Rice character	GCA (females)	GCA (males)	SCA	SCA/GCA (females)	SCA/GCA (males)
Tiller number	0.035**	12.96**	35.28**	100.00	2.72
Plant height	1085.70***	501.80**	597.88*	0.55	1.19
Leaf area	86.53***	-4.38 ns	43.84***	0.51	-10.00
Specific leaf dry weight	12.00***	-1.60***	13.17***	1.10	-8.23
SPAD	1.89**	-0.16 ns	1.18 ns	0.62	-7.37

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively; ns = non significant

Generally, the SCA/GCA ratio for females was higher than that of males except plant height. The ratio of SCA/GCA variance for both females and males was highest in tiller number as compared to other characters examined. The ratio of SCA/GCA for males was negative for leaf area, specific leaf dry weight and SPAD (Table 6).

Analysis of genetic components of variance showed greater differences between additive variance of female versus male parents for all the characters examined (Table 7). Additive variance for female parents was positive for the characteristics, while only tiller number and plant height had positive additive variance for males. Additive variance for males was lower than that for females for all characters examined except tiller number.

Table 7: Estimates of genetic parameters and heritability estimates for plant height, SPAD, leaf area, SLDW and number of tillers in F1 generations under non-stressed condition

Components of variance	Tiller number	Plant height	Leaf area	Specific leaf dry weight	SPAD
δ^2A (half sibs of male)	51.84	2007.20	0.00	0.00	0.00
δ^2A (half sibs of female)	0.14	4342.80	346.12	48.00	7.56
δ^2D	141.12	2391.52	175.36	52.68	4.72
δ^2P	210.63	9199.47	513.09	94.28	12.92
h^2 (half sibs of male)	0.25	0.22	0.00	0.00	0.00
h^2 (half sibs of female)	0.00	0.47	0.67	0.51	0.59
d.(degree of dominance male)	2.33	1.54	18.72	10.26	3.07
d.(degree of dominance female)	44.90	1.05	1.00	1.48	1.12

Gene effects from both the female and male parents could be regarded as over dominant for characters examined except for leaf area where the gene effects from the female parents expressed complete dominance. Degree of dominance was higher in the males for all characters than in the females except for tiller number (Table 7). Heritability estimates for tiller number was higher in male parents than the female parents. However, female parents had higher heratibility estimates for plant height, chlorophyll content, leaf area and specific leaf dry weight than the male parents (Table 7).

7.6.3 Heritability estimates of individual cross of some rice characteristics.

Heritability estimate was highly significant for drought tolerance scores for all the crosses examined and it ranged from 0.32 to 0.72 (Table 8). Five populations had heritability estimates higher than the mean estimates for the experiments. Leaf rolling score was significantly ($P \leq 0.001$) highly heritable in some populations (WBK 28, WBK 41 and WBK 42). These populations also had a high level of drought tolerance. The analysis of variance under drought stressed conditions for RLWC was not significant amongst genotypes. Thus, no significant difference in the heritability estimate of RLWC in the populations could be expected. However, a significant ($P \leq 0.05$) heritability estimate value for RLWC was observed in WBK 28 and WBK 42 (Table 8).

Table 8: Heritability estimates by regression of F_4 populations means on F_3 parent values for drought tolerance scores, leaf rolling scores and relative leaf water content under stress conditions

Populations	Drought tolerance scores		Leaf rolling ability scores		Per centage relative leaf water content	
	b-value	s.e	b-value	s.e	b-value	s.e
WBK 28	0.57***	± 0.088	0.56***	± 0.075	0.25*	± 0.081
WBK 30	0.49**	± 0.133	0.40**	± 0.112	- 0.04 ns	± 0.296
WBK 34	0.59***	± 0.115	0.38**	± 0.110	0.24 ns	± 0.180
WBK 35	0.34***	± 0.052	0.22 ns	± 0.111	0.15 ns	± 0.113
WBK 39	0.32**	± 0.087	0.48**	± 0.133	0.13 ns	± 0.125
WBK 40	0.35***	± 0.069	0.26**	± 0.069	0.19 ns	± 0.094
WBK 41	0.44**	± 0.135	0.53***	± 0.065	0.20 ns	± 0.121
WBK 42	0.64***	± 0.102	0.42***	± 0.070	0.43*	± 0.154
WBK 64	0.49***	± 0.074	0.44**	0.123	- 0.00 ns	± 0.100
WBK 136	0.72***	± 0.077	0.42**	± 0.106	0.47 ns	± 0.270
WBK 150	0.52**	± 0.121	0.34*	± 0.109	0.29 ns	± 0.237
Mean (h^2)	0.497		0.405		0.212	

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level, respectively and ns = non significant;

One of the main purposes of this experiment was to test the reliability of F3 characters as predictors of later generation performance under stressed and non-stressed (fully irrigated) conditions. Heritability estimates for plant biomass showed a significant variation amongst the populations and responded to stress conditions. The pattern of shoot and root dry weight inheritance varied in response to environmental conditions. The heritability estimates of shoot and root dry weights were significant under stressed conditions for WBK 64, however, no significant differences were observed under non-stressed conditions (Table 9). In WBK 35, heritability estimate of shoot weight was significant under stressed and non-stressed conditions but its root dry weight showed no significant difference under stressed conditions. In one of the drought tolerant populations, WBK 150, only shoot dry weight heritability estimate was significant under stressed and non-stressed conditions (Table 9).

Table 9: Heritability estimates by regression of F4 populations means on F3 parents' values for rice biomass under stressed and non-stressed conditions

Populations	Shoot dry weight (g)				Root dry weight (g)			
	Stressed		Non-stressed		Stressed		Non-stressed	
	b-value	s.e	b-value	s.e	b-value	s.e	b-value	s.e
WBK 28	0.34 ns	± 0.217	0.13 ns	± 0.100	0.70*	± 0.280	0.13 ns	± 0.065
WBK 35	0.70*	± 0.274	0.290**	± 0.052	1.0 ns	± 3.154	0.28***	± 0.030
WBK 39	0.50 ns	± 0.353	0.24*	± 0.088	0.13 ns	± 0.371	0.29**	± 0.098
WBK 40	0.38**	± 0.097	0.79 ns	± 0.422	0.22*	± 0.081	0.85 ns	± 0.406
WBK 41	0.30 ns	± 0.197	0.65***	± 0.085	0.16 ns	± 0.166	0.51*	± 0.186
WBK 42	0.19 ns	± 0.103	0.11 ns	± 0.126	0.04 ns	± 0.089	0.093ns	± 0.093
WBK 64	0.49**	± 0.109	0.22 ns	± 0.211	0.62***	± 0.104	0.30 ns	± 0.133
WBK 136	0.44 ns	± 0.329	0.98 ns	± 0.370	0.39*	± 0.170	0.70**	± 0.155
WBK 150	0.47*	± 0.161	0.39**	± 0.128	- 0.04 ns	± 0.205	0.19 ns	± 0.107
Mean (h^2)	0.423		0.422		0.361		0.376	

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level, respectively and ns = non significant.

Heritability estimates of leaf area index and specific leaf dry weight were very low amongst the populations compared to other traits. However, WBK 35 had significant heritability estimate for leaf area index at ($P \leq 0.05$) and ($P \leq 0.01$) under stressed and non-stressed

conditions, respectively (Table 10). Two populations had significant heritability estimates for leaf area index under stressed and two had the same in non-stressed conditions (Table 10). Significant heritability estimates were not observed for specific leaf dry weight for all the populations except WBK 64 under non-stressed condition (Table 10)

Table 10: Heritability estimates by regression of F4 populations means on F3 parents' values for rice leaf area index and specific leaf dry weight under stressed and non-stressed conditions

Populations	Leaf area index				Specific leaf dry weight (g)			
	Stressed		Non-stressed		Stressed		Non-stressed	
	b-value	s.e	b-value	s.e	b-value	s.e	b-value	s.e
WBK 28	0.30 ns	± 0.193	0.24 ns	± 0.200	0.48*	± 0.207	-0.24 ns	± 0.249
WBK 35	0.72*	± 0.303	0.53**	± 0.138	0.11 ns	± 0.219	0.01 ns	± 0.143
WBK 39	-0.03 ns	± 0.300	0.30*	± 0.135	0.23 ns	± 0.114	0.12 ns	± 0.127
WBK 40	0.36**	± 0.092	0.25 ns	± 0.263	0.19 ns	± 0.142	0.11 ns	± 0.184
WBK 41	0.10 ns	± 0.083	0.28 ns	± 0.145	0.04 ns	± 0.180	-0.63 ns	± 0.285
WBK 42	0.28*	± 0.097	0.13 ns	± 0.163	0.47 ns	± 0.276	0.25 ns	± 0.220
WBK 64	0.27 ns	± 0.188	0.00	± 0.00	0.03 ns	± 0.375	0.51*	± 0.127
WBK 136	-0.07 ns	± 0.086	-0.09 ns	± 0.277	0.09 ns	± 0.222	0.14 ns	± 0.321
WBK 150	0.473 ns	± 0.215	0.19 ns	0.126	0.38*	± 0.154	0.07 ns	± 0.156
Mean (h^2)	0.279		0.213		0.224		0.136	

All the populations examined showed highly significant heritability estimates for tiller number under non-stressed conditions, while only WBK 28, WBK 35 and WBK 41 had significant heritability estimate for tiller number under stressed conditions (Table 11). For plant height, significant heritability estimate was observed for all the populations except WBK 28 under non-stressed condition. Only populations WBK 41, WBK 42, WBK 64 and WBK 136 had significant heritability estimates under stressed condition (Table 12).

Table 11: Heritability estimates by regression of F4 populations means on F3 parents' values for rice tillering ability and plant height under stressed and non-stressed conditions

Populations	Tiller number				Plant height (cm)			
	Stressed		Non-stressed		Stressed		Non-stressed	
	b-value	s.e	b-value	s.e	b-value	s.e	b-value	s.e
WBK 28	0.23***	± 0.061	0.40**	± 0.118	0.01 ns	± 0.048	0.02 ns	± 0.073
WBK 35	0.31***	± 0.057	0.21**	± 0.077	0.08 ns	± 0.062	0.20**	± 0.077
WBK 39	0.11 ns	± 0.160	0.47***	± 0.063	0.12 ns	± 0.092	0.20***	± 0.058
WBK 40	0.06 ns	± 0.088	0.41***	± 0.091	0.11 ns	± 0.093	0.16*	± 0.072
WBK 41	0.36***	± 0.074	0.45***	± 0.047	0.251**	± 0.059	0.28***	± 0.057
WBK 42	0.08 ns	± 0.115	0.15***	± 0.035	0.14*	± 0.056	0.24***	± 0.65
WBK 64	0.06 ns	± 0.365	0.25***	± 0.047	0.20***	± 0.053	0.20**	± 0.065
WBK 136	0.11 ns	± 0.124	0.41***	± 0.113	0.18*	± 0.082	0.30***	± 0.065
WBK 150	0.13 ns	± 0.082	0.34***	± 0.086	-0.02 ns	0.087	0.17*	± 0.077
Mean (h^2)	0.163		0.342		0.121		0.197	

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant

7.6.4 Correlation analysis of individual crosses under stressed and non-stressed conditions

High significant positive relationships were observed between F3 and F4 generations for drought tolerance and leaf rolling scores for all the populations. Only WBK 28 and WBK 42 had significant relationships for relative leaf water content (Table 12).

Table 12: Phenotypic correlation between F₃ and F₄ populations for drought tolerance, leaf rolling ability and per cent relative leaf water content of rice under stressed conditions.

Populations	Drought tolerance scores		Leaf rolling ability scores		Per centage relative leaf water content	
WBK 28	0.96	***	0.92	***	0.70	**
WBK 30	0.76	**	0.75	**	-0.05	ns
WBK 34	0.85	***	0.74	**	0.40	ns
WBK 35	0.90	***	0.53	ns	0.39	ns
WBK 39	0.76	**	0.75	**	0.30	ns
WBK 40	0.85	***	0.77	***	0.54	ns
WBK 41	0.71	**	0.93	***	0.45	ns
WBK 42	0.89	***	0.88	***	0.66	*
WBK 64	0.91	***	0.77	**	-0.01	ns
WBK 136	0.95	***	0.78	**	0.48	ns
WBK 150	0.81	**	0.70	**	0.36	ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant

The relationship between F₃ and F₄ populations under stressed conditions varied significantly in relation to plant biomass. Drought stress significantly affected trait relationships. Traits that were correlated in non-stressed conditions were also not correlated under stressed conditions. Significant ($P \leq 0.01$) correlation was observed for shoot dry weight under stressed conditions in WBK 40 and WBK 64, while significant ($P \leq 0.001$) correlation was observed in WBK 35 and WBK 41 under non-stressed conditions. Only WBK 35 had significant correlation for shoot dry weight both in stressed and non-stressed conditions and root dry weight under non-stressed conditions (Table 13). Significant differences were not observed for root-to-shoot ratio under stressed and non-stressed conditions amongst the populations examined except WBK 28 (data not shown).

Table 13: Phenotypic correlation between F₃ and F₄ populations for plant biomass of rice under stressed and non-stressed conditions.

Populations	Shoot dry weight (g)				Root dry weight (g)			
	Stressed		Non-stressed		Stressed		Non-stressed	
WBK 28	0.45	ns	0.55	ns	0.62	*	0.72	ns
WBK 35	0.63	*	0.87	***	0.11	ns	0.95	***
WBK 39	0.42	ns	0.67	*	0.10	ns	0.71	*
WBK 40	0.78	**	0.53	ns	0.64	*	0.57	ns
WBK 41	0.43	ns	0.95	***	0.28	ns	0.72	*
WBK 42	0.50	ns	0.30	ns	0.14	ns	0.33	ns
WBK 64	0.86	**	0.47	ns	0.93	***	0.75	ns
WBK 136	0.40	ns	0.80	ns	0.61	*	0.92	**
WBK 150	0.68	*	0.69	**	-0.06	ns	0.48	ns

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant

In comparison with other characters examined, the relationship between F₃ and F₄ generations were not significant in most of the populations for leaf area index and specific leaf dry weight. Root to shoot ratio had significant difference for heritability estimate and correlation coefficient between F₃ and F₄ generations at ($P \leq 0.05$) only in WBK 28 at stressed and non-stressed conditions (Data not shown).

There were more populations that had significant relationship for tiller number and plant height under non-stressed conditions as compared to stressed conditions (Table 14). Three populations had significant ($P \leq 0.001$) correlation between F₃ and F₄ generations for tiller number and two for plant height under stressed conditions. WBK 41 was the only line whose performance could be predicted in any of the environments for tiller number and plant height (Table 14)

Table 14: Phenotypic correlation between F₃ and F₄ populations for tillering ability and plant height of rice under stressed and non-stressed conditions.

Populations	Tiller number				Plant height (cm)			
	Stressed		Non-stressed		Stressed		Non-stressed	
WBK 28	0.45	***	0.53	**	0.03	ns	0.04	ns
WBK 35	0.59	***	0.35	**	0.16	ns	0.33	**
WBK 39	0.09	ns	0.70	***	0.17	ns	0.41	***
WBK 40	0.09	ns	0.51	***	0.16	ns	0.28	*
WBK 41	0.55	***	0.78	***	0.49	***	0.55	***
WBK 42	0.09	ns	0.48	***	0.31	*	0.44	***
WBK 64	0.23	ns	0.57	***	0.45	***	0.37	**
WBK 136	0.11	ns	0.57	***	0.29	*	0.66	***
WBK 150	0.21	ns	0.46	***	-0.04	ns	0.28	*

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non significant;

7.7 Discussion

7.7.1 Genetic components of variance

The significance of general combining ability (GCA) of females indicates a prominent additive genetic effect for tiller number, plant height, leaf area, specific leaf dry weight and chlorophyll content (Table 6). However, due to few numbers of parents used in this study, the results obtained pertain to this set only. Additive effects are very important in plant breeding and they indicate level of resemblance between relatives (Falconer and Mackay, 1996). There could be a greater predictability of progeny performance if female parents in this study are used in the development of rice for these characters. Male parents had significant GCA only for tiller number, plant height and specific leaf dry weight. The male parents in this study could well be used for rice population improvement for these characters. The specific combining ability (SCA), which represents non-additive genetic effects were significant for tiller number, plant height, leaf area and specific leaf dry weight, but not for chlorophyll content (Table 6). These results were similar to the work of

Majumder *et al.* (1990) who reported high GCA and SCA for tiller number at the vegetative stage of *O. sativa*.

The GCA and SCA indicate the importance of additive and non-additive genetic effects for these traits, respectively. The SCA/GCA ratio was higher for tiller number and specific leaf dry weight. This is consistent with work of Sarker *et al.* (2002) who reported high SCA/GCA ratio for tiller number. These results indicated that additive genetic effects are more important for plant height, leaf area and SPAD, while non-additive variance is more important for tiller number and specific leaf dry weight. Non-additive genetic effects may be important for chlorophyll production in interspecific hybrids. Low chlorophyll content was found to be significantly associated with high specific leaf dry weight (Dingkuhn *et al.*, 1998). The presence of significantly positive GCA for female parents for all the characters examined indicates that rapid selection could be possible through breeding for these characters using the female parents in the future crosses. The same could be said for male parents except for specific leaf dry weight that had significant negative GCA.

The additive variance (δ^2A) for half sibs of female parents were much greater than those for half sibs of the male parents for all the characters except tiller number (Table 8). Narrow sense heritability (h^2) estimates for half sibs of female parents were more than double those for male parents except for tiller number. The degree of dominance for the male parents was much greater than female in all the characters except tiller number. Generally, the dominance level was greater than 1, indicating the importance of overdominance. These results indicate that response to selection could be accelerated for these traits as female parents could be regarded as good combiners. At times, the high level of overdominance are as a result of an upward bias by linkage disequilibrium, especially in F2 single crosses of inbred lines (Wolf *et al.*, 2000). However, in this study, F3 to F4 generation progeny were used, hence linkage disequilibrium was not expected to be a big problem. A high tillering male parent could accelerate the breeding for tillering in rice. Jones *et al.* (1997) reported that in the interspecific crosses involving *O. glaberrima* as the male parent, the progenies resembled *O. glaberrima* more than *O. sativa* (female parents).

7.7.2 Heritability estimates

Heritability estimates of some traits were estimated by regressing F4 populations means on F3 parents' values. Environmental influence on the inheritance of some traits was observed. Some traits that were highly heritable under non-stressed (fully irrigated) conditions were not heritable under stressed conditions, suggesting that selection under non-stress conditions will not identify genotypes that performed under stress. In other words, the two environments were not correlated; hence selection should be performed under both environments. Therefore, populations with highly heritable traits under stressed conditions could be used as donors for those traits in the breeding programme. These traits could be useful to improve selection efficiency when grain yield has been reduced. Traits such as leaf area index and tiller number could be used in indirect selection for grain yield under drought stressed conditions. The effectiveness of a drought screening procedure is best measured by the heritability estimates achieved for target traits, whether the focus is conventional or marker-assisted plant breeding (Bidinger, 2002). Populations such as WBK 35 and WBK 150 had significant heritability estimates for shoot dry weight under stressed and non-stressed conditions. While WBK 41 had significant heritability estimates for plant height and tiller number both under stressed and non-stressed conditions. Significant heritability estimates and correlation coefficient were observed for root to shoot ratio and tiller number in WBK 28 both under stressed and non-stressed conditions.

Significant heritability estimates were observed for drought and leaf rolling scores for most of the populations examined. Thus indicating that drought and leaf rolling scores are putative traits for drought response, thus corroborating the earlier reports by O'Toole (1982). The magnitude of heritability estimates is useful in estimating selection progress in segregating populations. Blum (1988) reported that genetic variance and heritability for grain yield decline under stress. Therefore, he stressed the importance of secondary traits as predictors of grain yield under stress conditions.

7.7.3 Relationships between F3 and F4 populations

Any method that is fast and efficient in screening segregating populations in the breeding programme is always welcome. Significant phenotypic correlations were observed between

traits of F3 and F4 populations both under stressed and non-stressed conditions for some populations. Therefore, correlated traits could be used in predicting performance in the next generations. Highly significant positive relationships were observed between F3 and F4 generations for drought tolerance and leaf rolling scores for all the populations (Table 13). Two populations, WBK 28 and WBK 42, had significant correlations for relative leaf water content. The performance for relative leaf water content may not be predictable at early generations of the breeding programme for most of the populations examined (Table 13). Only WBK 35 had significant correlation for plant biomass both in stressed and non-stressed conditions. Significant correlation for tiller number and plant height were observed only WBK 41 under stressed and non-stressed conditions. Therefore the performance of WBK 41 could be predicted in both stressed and non-stressed conditions for tiller number and plant height. Regression analysis between F2 and F3 populations of rice, Ekanayake *et al.* (1985) reported significantly positive correlation between root characteristics and plant height, tiller number and shoot dry weight. Thus the method should be effective predicting performance of populations especially for most secondary traits as shown in the current study.

Conclusion

Parents that exhibit high combining abilities in hybridisation programmes could be more useful to plant breeders. The significance of general combining ability of female parents in this study indicates a prominent additive genetic effect for tiller number, plant height, leaf area, specific leaf dry weight and chlorophyll content. The additive effect for these traits would enhance population improvement of rice. Specific combining ability was also high for some traits indicating non-additive genetic effects, which could assist breeders in population improvement of rice. This study observed high heritability estimates for drought score and leaf rolling score in most of the populations examined. Populations such as WBK 35 had significant heritability estimate for leaf area index under stressed and non-stressed condition. While WBK 28 had significant heritability estimates and correlation coefficient for root to shoot ratio and tiller number in both stressed and non-stressed conditions. Traits that are highly heritable are simpler to breed for because they respond to selection. This study has identified some populations with highly heritable traits both under stressed and non-stressed conditions. These populations could be used in population improvement of rice in any of the

water regimes. This study has also identified some traits that could be used in predicting performance of a population based on early generation performance. The populations identified in this study could be advanced rapidly in the breeding programme for these traits.

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CHAPTER 8

Developing drought tolerant rice germplasm for upland ecologies in the tropics of Africa: An overview

Introduction

Dwindling of water resources to agriculture is a major concern globally. This is more pronounced in the developing countries, particularly in Sub-Sahara Africa (SSA). Apart from global warming that is generally believed to be one of the major contributing factors causing dwindling of water resources to agriculture, desertification and desert-encroachment are major factors in SSA. Water insufficiency, is adversely affecting agricultural activities with a great impact on all aspects of human societies and animal life. This therefore emphasises the need to look for the most efficient and reliable ways for using available water. Efficient use of available water will hopefully increase agricultural production in the region. One of the major options is genetic improvement of crops for drought stress tolerance. Thus, advocate for more resources to be channelled into agricultural research for development of drought tolerant crops particularly major cereals like rice. These staple and therefore food security crops should be adaptable to the increasingly water limiting production ecologies in SSA. The goal of this current study is therefore to contribute to increasing food security in West Africa through breeding adaptable and drought stress tolerant rice cultivars, which are adoptable by farmers.

The specific objectives of this current study were therefore:

- (1) to determine farmers' varietal trait preferences and perceptions on drought and management practices in Sikasso region in Mali,
- (2) to identify genotypes with good crossability as parents for use in interspecific hybrid production,
- (3) to study the efficacy of gametocides as chemical hybridising agents for use in rapid hybridisation of rice genotypes,
- (4) to identify drought tolerant interspecific genotypes at the vegetative stage, and
- (5) to identify the gene action and heritability of morpho-physiological traits under stress and non-stress conditions.

Findings from the study

Participatory rural appraisal (PRA) is being used as a tool for involving communities in the varietal development processes. In Sikasso region, many upland rice varieties have been introduced but with low adoption by the farmers. The reasons could stem from lack of farmers' preferred traits and their perceptions were not considered in the development of varieties. Some varieties that were adopted are poor yielding due to susceptibility to drought stress. This study identified farmer preferences for rice traits in different and across ecologies. In upland and lowland rice ecologies, the most preferred rice characteristics were short duration and tall plants, respectively. Short duration trait was ranked second in lowland rice ecology. Short duration cultivars could escape late season drought by completion of their life cycle before water becomes limiting in the soil. While long duration was ranked second as the most preferred trait after yield in the irrigated ecology. Late maturing genotypes were promising in early season drought environments, as they would probably recover when favourable conditions resumed. Most farmers in the upland and lowland rice ecologies experienced drought at the vegetative stage, while most farmers experienced drought at the reproductive stage in the irrigated rice ecology. Therefore, different duration of rice genotypes would be adaptable to these ecologies. The results from the PRA indicated that specific breeding is required for different rice ecologies and with different trait preferences in Sikasso. Farmers' preferences and perceptions will be considered in devising a breeding strategy for Sikasso region. Above all, genotypes with drought tolerance at both vegetative and reproductive stage should be developed and deployed into this region. Farmers' preference for tall plants was in sharp contrast from the requirements for dwarf rice that gave rise to green revolution in Asia. This presents breeding challenges, as tall varieties are more likely to have lodging problems. This also shows how breeders' objectives might fail to coincide with the farmers' preferences.

Significant variation exists among genotypes for response to drought stress. This response depends on the severity, season and timing of the drought stress. Therefore selection indices were used to identify the best performing genotypes under drought stressed conditions for use as parental lines in the breeding for drought stress tolerance in this current study. In 2004, genotypes with high selection indices were WAB 450-I-B-P-103-HB, WAB 880-1-

38-13-1-P1-HB and NERICA 2. They were drought tolerant (little leaf drying) and showed less leaf rolling. They also had fast recovery ability and early flowering. While in 2005, genotype with the highest selection index was WAB 450-I-B-P-103-HB. Other genotypes with high selection indices were WAB 450-I-B-P-105-HB, NERICA 3 and TOG 5681. These genotypes could be promising as a gene pool in breeding for drought tolerance, which is required in varieties to be deployed in all three ecologies in Sikasso region where grain yield is reduced by about 60% due to drought stress at both vegetative and reproductive stages. Although these results were based on a case study of Sikasso region, they are likely to be applicable in similar ecologies in Sub-Saharan Africa.

The *O. glaberrima* genotypes exhibited higher tillering ability such as RAM 86, RAM 118 and CG 14, although with low selection indices, they were used as parents, because of their tillering ability and high plant biomass. Genotypes suffered considerable reduction in most of the rice traits examined under drought stressed conditions. The study found that some genotypes such as CG 14, TOG 5681 and WAB 375-B-9-H3-2 maintained reasonable increases in LAI thus exhibiting a drought tolerance mechanism. Significant relationships were observed between drought tolerance (little leaf drying) with less leaf rolling, lower tiller numbers, larger leaf area index and tall plants. Fast recovery ability after stress relief was significantly associated with drought tolerance, less leaf rolling and larger leaf area index. Leaf area index, plant height and tiller number under fully irrigated conditions were significantly positively correlated with leaf area index, plant height and tiller number, respectively under stressed condition. These relationships among morpho-physiological traits could be used for breeding strategies that target secondary traits for indirect selection for grain yield under drought stress conditions, as they indirectly affect the pathways of high yield in rice genotypes.

Genotype-environment (G x E) interaction is an important factor in selection of good material and is of utmost importance to plant breeders. Wide adaptability and stability of genotypes are considered as the most important in breeding for drought tolerance. Identification of putative traits in rice genotypes that are stable in diverse environments will provide good sources or donors of drought tolerance to breeding programmes. This study

observed that G x E interaction was significant for tiller number, relative leaf water content and root dry weight, indicating that these characters were highly influenced by changes in environment. Amongst the genotypes examined, RAM 3 was identified to be stable for tiller number and relative leaf water content. Improvement of rice for these characters should be location specific, as they are sensitive to change in environment.

Breeders face challenges in creating hybrids in natural self-pollinating crops like rice. The appraisal of gametocides as chemical hybridising agents showed inconsistent results between seasons. Per cent empty spikelets and male sterility increased with concentration level of ethrel. The ethrel concentration at 2000 ppm had 41.5% and 42.8% male sterility induction in 2004 and 2005, respectively, while at 4000 ppm had 40.5 and 46.1 male sterility induction in 2004 and 2005, respectively. The effect was most apparent when ethrel was applied both at panicle primordium differentiation and spikelet primordium differentiation stage of plant growth and 46.6% male sterility induction was obtained in 2004. In 2005, ethrel application only at panicle primordium differentiation had the highest male sterility induction of 49.2%. Ethrel application was phytotoxic to the rice plant causing a reduction in plant height and panicle length. It also caused increased tillering. In the case of GA₃, it caused increased plant height, which resulted in increased lodging, hence, male sterility data were not obtained. Ethrel and gibberellic acid were not effective in inducing male sterility in hybrid rice production in this study, because a 100% male sterility, which is desired in hybridisation was not achieved. The current methods of using hand pollination and vacuum emasculators could be expensive for national agricultural services (NAS) therefore, further research efforts are urgently required to develop some alternative ways of inducing male sterility in hybrid rice production.

Interspecific hybridization is a cross between two, sometimes, distinctly related species. Plant breeders undertake interspecific crosses because of the need to resolve some breeding problems. Some of these reasons include the need to transfer one or more genes from one species to another and developing new character expression. Interspecific hybridisation for plant improvement is complicated by the presence of incompatibility barriers, such as hybrid seed sterility, which hinder heterogenetic recombinations. High sterility of hybrid seeds in

the crosses between *O. glaberrima* x *O. sativa*, are usually very high. In this study, 9.29 % seed set in F₁ hybrids in the crosses involving *O. glaberrima* x *O. sativa* genotypes was obtained. Three crosses with high per cent seed set were CG 14 x WAB 365-B-1-H1-HB (20.5%), RAM 86 x WAB 375-B-9-H3-2 (14.8%) and TOG 5681-x WAB 365-B-1-H1-HB (14.5%). Higher success rate was achieved in the crosses involving interspecific x interspecific as compared to other crosses examined in this study. The best female parents that combined significantly based on per centage seed set were CG 14, WAB 450-IBP-105-HB and WAB 450-IBP-103-HB, while the best male parents were NERICA 3 and NERICA 2. These genotypes could be promising parents in interspecific hybridisation programme. Higher sterility was observed in backcrosses involving *O. glaberrima* cytoplasm as compared to single crosses, except with the CG 14 cytoplasm. In fertility restoration, backcrossing that involved *O. glaberrima* cytoplasm may not be promising as compared to *O. sativa* cytoplasm.

Selection indices were used to identify the best progenies in early generation of the breeding programme under drought stressed conditions. Fifteen progenies were identified with high selection indices: such as WBK 149-B-B, WBK 136-B-B, WBK 41-B-B, WBK 41-B-B-1. These progenies were also early flowering and they could be promising for deployment in late season drought environments as early maturity genotypes could provide an escape mechanism. Late flowering progenies with high selection indices such as WBK 150-B-B-B, WBK 39-B-B-B, WBK 30-B-B, WBK 30-B-B-B and WAB 450-I-B-P-103-HB could be promising for early season drought environments as they could recover when favourable conditions resumed in the environments. Progenies identified with tall plant height were WBK 42-B-B-2; WBK 30-B-B-B; WBK 78-B-B-B; WBK 02-B-B-B and WBK 100-B-B-B. This trait was ranked highest by farmers in the lowland and second in the upland ecologies in Sikasso region, Mali (see Chapter 2), hence, this germplasm should be considered in breeding for Sikasso and similar ecologies elsewhere.

Progenies with low drought score (little leaf drying) were considered to be drought tolerant. Progenies identified to be drought tolerant were WBK 39-B-B-B; WBK 30-B-B; WBK 32-B-B-B; WBK 70-B-B; WBK-150-B-B; WBK 28-B-B-1; WBK 28-B-B-4; WBK 42-B-B-2 and WAB 450 -I-B-P-103-HB. A wide range of progenies with high to less leaf rolling were

identified in this study. Progenies that were identified to have less leaf rolling under drought stress are WBK 39-B-B-B, WBK 30-B-B; WBK 32-B-B-B; WBK 70-B-B, WBK-150-B-B; WBK 28-B-B-1 and WBK 28-B-B-4. Leaf area index (LAI) is an important yield component. Plants with high LAI could intercept more solar radiation due to their large surface leaf area as compared to plants with small LAI. This study has identified the following genotypes with high LAI and specific leaf dry weight: WBK 42-B-B; WBK 30-B-B; WBK 114-B-B-B and WBK 35-B-B-B. These progenies would be considered for use as source germplasm in breeding that target similar ecologies.

Knowledge of a heritability estimate of characters is very important since it indicates extent to which plant improvement is possible through selection. Selection of parents is one of the most important steps towards developing drought tolerant lines. Unfortunately, genetic variance and consequently heritability estimates for most characters decline under drought stress. Progenies with highly heritable putative traits for drought stress tolerance are likely to perform better as compared to progenies with low heritable traits under drought stress conditions. Heritability estimates for drought tolerance (little leaf drying) and leaf rolling were highly significant for all the populations examined and it ranged from 0.32 to 0.72 and 0.22 to 0.56, respectively. This study has identified populations such as WBK 41 with significantly high heritability estimate for plant height and tiller number both under drought stress and non-drought stress conditions. Thus it is highly possible to improve drought tolerance, leaf rolling, tiller number and plant height through selection in almost all populations examined in the current study.

Some rice traits such as drought tolerance, leaf rolling, leaf area index, plant height and tiller number were identified to be significantly associated both under drought stress and non-stress conditions. Rice population improvement or selection for these traits could be done under any of these production conditions. Highly significant positive relationships were observed between F3 and F4 generation populations for some traits such as drought tolerance and leaf rolling scores for all the populations examined. These traits could be used in predicting performance of a population in the early generation of breeding, thus rapid screening can be conducted at F3 generation and reduce costs of carrying many genotypes to

the F4 generation. Effectively, this study confirmed that early generation selection was a viable strategy.

Highly significant differences were observed between general combining ability (GCA) variance for females and males for the characteristics examined. Female GCA and specific combining ability (SCA) were significant for tiller number, plant height and specific leaf dry weight, indicating the importance of additive and non-additive genetic effects for these traits. Degree of dominance was higher in the males for all characters examined than in the females except for tiller number, suggesting that breeding strategy should involve inbreeding and crossing breeding. Heritability estimates for plant height and tiller number were higher in male parents than the female parents. However, female parents had higher heritability estimates for chlorophyll content, leaf area and specific leaf dry weight than the male parents. High heritability estimates confirmed that in addition to overdominance, additive effects also played a role in influencing the morpho-physiological traits in rice genotypes. These could be exploited in the development of drought tolerant rice.

Conclusion and recommendations

The relationships between traits under drought stressed conditions are very important for drought stress breeding programme. A good knowledge of their interaction and complimentary effects under drought stress would enhance breeding for drought tolerant genotypes. This area requires further investigation for an efficient drought stress-breeding programme to be realised. Future studies should investigate whether there is a significant relationship between these morpho-physiological traits and grain yield in rice. Progenies identified to be drought tolerant were WBK 39-B-B-B; WBK 30-B-B; WBK 32-B-B-B; WBK 70-B-B; WBK-150-B-B; WBK 28-B-B-1; WBK 28-B-B-4; WBK 42-B-B-2 and WAB 450 -I-B-P-103-HB. These progenies should be evaluated further in the three ecologies and select the best performed lines.

As farmers' preferences for rice varied from ecology to ecology, this study recommends specific breeding for different rice ecologies. Screening for secondary traits that have been identified to be significantly associated with rice grain yield could also provide important information for indirect selection for grain yield under drought stressed conditions.

Genotype response to drought stress existed among rice species. Genotypes responded differently in different drought stress regimes. This study observed large genotype by environment (G x E) interactions for tiller number, relative leaf water content and root dry weight. This therefore emphasised the importance of screening environments to be a true representative of the target environments or the farmers' environments. Success of the selected genotypes would depend on the true representation of the target environments. This study therefore recommends characterization of environments as priority before commencement of drought stress screening. As shown in the current study, involvement of farmers in breeding would help to identify the target environments with a high genetic correlation with the breeding environment. Thus, well characterised breeding and target environments, coupled with easy identification of direct and indirect putative traits for grain yield potential would enhance efficiency in breeding for drought stress tolerance. Overall it is indicated that participatory research approaches that involve farmers, breeders and plant physiologists would be valuable in identifying and generating drought tolerant and adoptable rice cultivars in the tropics of Africa with considerable impact on food security.