EFFECT OF SIMULATING FLOODING PATTERN ON NITROGEN MANAGEMENT IN RICE (*ORYZA SATIVA* L.) PRODUCTION

QUAQUA SUMO MULBAH

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Discipline of Crop Science

School of Agricultural Sciences and Agribusiness

Faculty of Science and Agriculture

University of KwaZulu-Natal

Pietermaritzburg, South Africa

November 2010

DECLARATION

I, QUAQUA SUMO MULBAH, declare that the research reported in this thesis, except where acknowledged, is my original work. I also declare that this thesis has not been submitted for any degree or examination at any other university.

Quaqua Sumo Mulbah

I, **Joseph A. Adjetey**, supervised the above candidate in the conduct of his dissertation study.

Joseph A. Adjetey

ACKNOWLEDGEMENTS

My special and sincere thanks go to the following:

Dr. Joseph A. Adjetey for his guidance and advice as my supervisor during the entire course of this study. Above all, his availability whenever needed was the key to the timely completion of this study.

My family for their perseverance and prayer.

The Central Agricultural Research Institute of Liberia for granting me a leave to study.

The West African Council for Agricultural Research and Development (CORAF/WECARD), and the Forum for Agricultural Research in Africa (FARA) for the financial support.

Dr. Inoussa Akintayo, Africa Rice Initiative, Africa Rice Center and Mr. Ian McDonald, KwaZulu-Natal Department of Agriculture and Environmental Affairs for providing the seeds.

Mr. M. J. Erasmus and the technical staff of the Agricultural Plant Sciences (AGPS) discipline, University of KwaZulu-Natal for their technical support.

Mr. Ravin Singh of ARC-CEDERA for kindly providing the weather data from the Ukilinga weather station.

Dr. Isa Bertling for the use of her plant efficiency analyzer.

My friends and colleagues of AGPS for their assistance and moral support.

ABSTRACT

Flooding cycle in wetland rice (*Oryza sativa* L.) production systems is often subject to seasonal and cultural variations which may affect the availability and uptake of nitrogen in different ways. These factors may more or less influence the physiological and growth responses of the plant.

In an effort to improve productivity in rice cropping systems, two controlled environment studies and a field trial were conducted to evaluate the growth and yield responses of rice to different flooding regimes and nitrogen fertilizer management strategies. In the first glasshouse trial, an upland cultivar (GM-1) was used to study the effects of four flooding regimes and three nitrogen application rates on the tillering, yield components and grain yield of rice. The field study determined the applicability of the results of the glasshouse trial to out-door environmental conditions, with the aim of gaining further insight into the impact of nitrogen application strategy on tiller and grain qualities. Two wetland cultivars (FKR-19 and N-19) and GM-1 were used to evaluate the effects of two flooding regimes and two nitrogen topdressing patterns. The second glasshouse trial determined the effect of hydro-priming on the establishment of direct seeded rice, and the effect of flooding on aerenchyma formation in rice roots.

Results of the studies showed that flooding with standing water of 5 cm above the soil surface, irrespective of when it occurred, and nitrogen application increased the number of tillers and panicles, above ground dry matter, nitrogen uptake and grain yield of rice. However, late flooding and high nitrogen application rate of 220 kg ha^{-1} were found to encourage the production of late tillers, thereby reducing the efficiency of nitrogen use for grain production.

Nitrogen application in three split doses tended to increase plant nitrogen content at heading; it slightly increased the protein content of the grains at maturity, but reduced the amylose content of the starch granules. Nitrogen application in two split doses led to increased grain yield in non-flooded plants, while the three-split treatment increased nitrogen uptake and grain yield in the flooded plants.

Flooding significantly increased aerenchyma formation in the cortical tissues of rice roots, particularly at 50 mm behind the root tips. Hydro-priming seeds for 48 h improved plant establishment by shortening the germination and emergence times, and increasing the height and dry matter accumulation of seedlings, thereby ameliorating the susceptibility of rice to flooding stress.

Overall, the thesis affirmed that controlled flooding is beneficial to rice production since it enhanced the growth and yield of the plant. It further revealed that early flooding and appropriate timing of moderate nitrogen application can ensure the conservation of water and nitrogen resources, including the quality of the environment, with no significant consequence for yield and productivity of the crop.

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than half of the human race, including thousands of households in Sub-Saharan Africa (SSA). The plant belongs to the family *Poaceae* (*Gramineae*) and consists of two cultivated (*sativa* and *glabberima*) and 22 wild species (Aggarwal *et al.*, 1999; Chopra and Shyam, 2002). According to the International Rice Research Institute (IRRI), rice is grown in about 115 countries world-wide, and is only second to wheat in terms of global production (IRRI, 2007). Of the current global output of 685 million tonnes of paddy, Africa accounts for only 3.38% which is barely sufficient to meet domestic demands (FAO, 2010).

Reports by Africa Rice Center (WARDA) showed that in South Africa, rice cultivation is relatively insignificant as local demands are practically met by import from the international market (WARDA, 2007). The rising demand for the commodity in South Africa is evident by the rise in annual import value which has more than doubled since 2002 from 0.84 to 2.03 billion rands in 2007 (FAO, 2010).

Like South Africa, Liberia and other Sub-Saharan African countries rely heavily on imports in order to meet domestic consumption. Rice constitutes the principal part of the diets of the general population in Liberia; hence, the quest for self-reliance in its production, though elusive partly due to declining yield, remains a priority for the post-war nation which has experienced a steady decline in self-sufficiency from a record high of 85% in the 1970s to less than 45% by 2006 (WARDA, 2008). Additionally, the current trend of rising prices on the international rice market (Figure 1.1) has the propensity to undermine the food security of the nation, thereby rendering the need to increase domestic production more compelling.

Rice is grown in Liberia for subsistence mainly by rural farm families. The production system is completely dominated by shifting cultivation, in which farmers move from one plot to another after each harvest to allow for a reasonable fallow of the land. Due to the unsustainable nature of such practice, concerted efforts are being made by the government to encourage sedentary farming by advising the rice farmers to crop more intensively in both the wet and dry lands. The intensification of land use for rice cultivation will mean that the farmers will have to rely more on fertilizer resources in order to maximize output. Efficient fertilizer management, particularly of nitrogen resources, will therefore be required not only to increase yield, but also to safe guard the environment.

Another problem associated with the rice intensification programme is the optimization of water resources, particularly in the wetlands. Although rice is known to thrive in wetlands, the fact that the plant is not aquatic presupposes that flooding may also interfere with the normal development and yield of the crop. Since most wetlands are normally subject to unpredictable cycles of flooding, an understanding of the effects of varying durations of flooding on rice growth is important for developing effective water management strategies for the attainment of stable grain yields. Furthermore the efficiency of nitrogen fertilizer use depends on several factors, an important one of which is the moisture conditions under which it is applied. Flooded conditions which characterize the wetlands generally tend to reduce nitrogen use efficiency in most crops due to the possibility of denitrification. There is therefore the need to develop fertilizer management strategies that would avert excessive nitrogen losses taking into consideration the effect of flooding patterns in the wetlands.

The major objective of this study was to examine the effects of different nitrogen application strategies on the growth, yield and grain quality of rice grown in flooded conditions. Other specific objectives included: 1) a study of the influence of flooding regimes at different stages on growth and yield parameters, as well as nitrogen uptake and use efficiency for grain production, 2) investigations into the effects of a farmer-friendly seed invigoration treatment (hydro-priming) on rice establishment by direct seeding in wetlands, and 3) the effect of flooding on aerenchyma formation in the cortical tissues of rice roots.

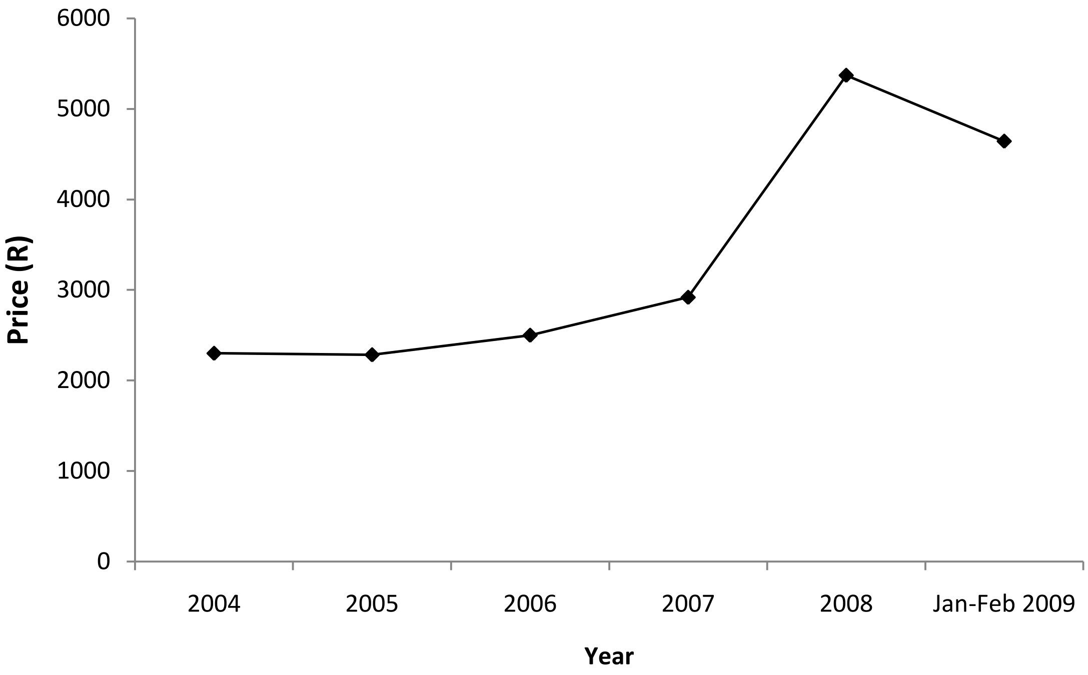


Figure 1.1: Recent trend in the price of rice on the international market since 2004. Price (R) represents the cost per tonne calculated at the parallel market exchange rate of R7.50 : US$1.00. Data source: FAO (2009).

CHAPTER 2

LITERATURE REVIEW

The following summary of past and current literature focuses on several essential aspects of rice as a crop and its production. It begins with a discussion on the growth and yield characteristics of the plant and its ecology, and extends to include the effects of various water and nitrogen management practices and their interactions on the cultivation of the crop and nitrogen use efficiency. The review ends with a discussion of the influence of agronomic practices on the quality of rice grains.

2.1. Agronomic characteristics and yield determinants

The cultivated rice species *O. sativa* (Asian rice) and *O. glabberima* (African rice) comprise thousands of varieties which are generally, more superior to their wild relatives with genes from the latter often used to improve the former (Xiao *et al.*, 1996; Maclean *et al.*, 2002). They are mainly annual or weakly perennial crops, and are well adapted to growing under semi-aquatic conditions (Sacks *et al.*, 2003). Depending on the cultivar and environmental factors, the growth duration of cultivated species may vary from 90 to 180 days. Plant height may range from 0.4 m for most dwarf varieties, to more than 5 m for deep water types.

During the vegetative phase of rice growth and development, the major visible activities are tillering and leaf development. Tillering is an intrinsic branching characteristic and important agronomic trait for productivity of plants belonging to the grass family (Moore and Moser, 1995). It consists of one or more side shoots which grow independently of the mother culm by developing their own adventitious roots (Briske, 1991). In rice and other economic grass plants such as wheat, oats and barley, tillers are specialized potential grain bearing branches whose quality and number are principal determinants of yield (Li *et al.*, 2003). Like leaves, tillers in grasses develop at the basal part of the stem where the meristems are located. As the apical meristem of a culm produces a new leaf, it also produces an axillary meristem in the axil of the previous leaf located on the opposite side of the new leaf; the axillary meristem then gives rise to a tiller (Hopkins, 2000).

Yield in rice is usually a product of the number of panicles m^{-2}, the 1000 grain weight and the percentage of spikelet fertility (Peng *et al.*, 2000). The number of panicles is directly related to the number of grain-bearing tillers, while the weight of the grains, in one respect, depends on the size of the panicle by virtue of the size of tillers which bear the panicles (Khush, 1995). The number of spikelets m^{-2} represents the sink size of cereals and therefore does not only depend on number, but also on the quality or size of the grain bearing tiller (Ying *et al.*, 1998a); hence, larger panicles tend to produce more grains than smaller ones and are therefore desirable for higher grain yield.

The number, type and time of tiller emergence are important indicators of tiller productivity. The contribution of tillers to yield correspondingly decreases in primary, secondary, and tertiary tillers with the main culm yielding a higher total grain weight (Kartick Chandra *et al.*, 2004; Awan *et al.*, 2007; Wang *et al.*, 2007). However, Counce *et al.* (1996) observed that the order of tiller emergence is more related to yield than the type of tillers. They noted that grain filling of primary and secondary tillers was respectively, 1 - 5 and 3 - 9 days later than grain filling of the main culm. After reaching the maximum, tiller number tends to decrease as the weak ones die. This explains why the maximum tiller number produced during the vegetative phase is always higher than the number of tillers that are productive at maturity.

Because tillers develop into grain-bearing panicles, an increase in the number of tillers potentially leads to an increase in grain yield. However, large tiller numbers are beneficial if only they emerge early enough and are supportable by the resources of the environment (Sheehy *et al.*, 2001). Excessive increase in the number of tillers may lead to poor yield as a result of intense inter-tiller completion in the event of low water and nutrient supply (Bulman and Hunt, 1988). Tillers that form at the later stages of growth are usually unproductive (Mohanan and Mini, 2008). They either abort or produce panicles that are too late to catch up with the ripening of the other panicles (Ritchie *et al.*, 1998). Late panicles are often weak and small with poor sink organogenesis which consequently contribute little to total yield (Mohapatra and Kariali, 2008). Late panicles may delay harvest and encourage grain shattering, or undermine grain quality by increasing the number of immature grains in the final harvest. Increasing tiller productivity can greatly increase total grain yield and will therefore require the adoption of an effective tillering control strategy that promotes early tillering while discouraging the growth of unproductive tillers.

2.2. Rice production systems

In rice production, water is the basic reference criterion used in the classification of cultivation practices and environments. For example, the terms deep water, irrigated, dry land and rainfed rice are often used to denote cultivation practices based on water utilization. These terms are also used to differentiate rice growing ecologies on the basis of surface moisture regime. Thus, four main types of rice ecological systems are universally recognized: upland, rainfed lowland, irrigated lowland, and deep water (Balasubramanian *et al.*, 2007). Also known as dry land, the upland ecosystem is usually well drained with a very rare occurrence or complete absence of flooding. Fields in the rainfed wetland ecosystem are seasonally flooded to depths as high as one meter for periods not more than 10 consecutive days during the growing season. Irrigated wetlands are like rainfed wetlands, except that they are equipped with man-made water control structures and bunds to maintain surface water when needed in the plots. The deep water ecosystem is characterized by complete inundation of fields for long periods ranging from 10 days to five months during the cropping period (Roger, 1996; Javier, 1997). The rainfed cultures depend solely on natural precipitation for the supply of water, while water in irrigated systems is provided via controlled structures in addition to rainfall.

2.3. Effect of water regime on rice production

Water is one of the most limiting factors in plant growth. It is essential to plants due to several reasons. First, water transports dissolved minerals through the soil to the roots where they are taken up by the plant, and provides physical support for plants by stimulating internal or turgor pressure within the cells (Nonami and Boyer, 1989). It is the principal medium for the many chemical and biochemical processes that support metabolism in plants; it serves as a medium for the translocation of photo-assimilates to other parts of the plant; and with evaporation through intercellular spaces, water enhances the cooling mechanism that allows plants to maintain favourable temperatures necessary for metabolic processes.

As plant cells divide, they expand by taking up water, thus causing plants to grow (Frensch and Hsiao, 1994; Proseus *et al.*, 2000). Therefore, if water is limited during growth, final cell size may be greatly reduced, thereby resulting into smaller plants with lower potential for yield. Excessive moisture on the other hand, may cause physical and chemical changes in the soil and root environments which may be deleterious to plant performance and final yield (Zaidi *et al.*, 2007).

Regardless of the ecology, the prevailing moisture condition in a rice field is known to have a profound effect on the growth, development and productivity of the crop. For example, in transplanted wetland rice, water stress during active tillering and early panicle development has been reported to reduce grain and straw yield by up to 21 and 26%, respectively (Castillo *et al.*, 1992). Similarly, Ingram and Yambao (1988) found that water stress for 5 days during the reproductive stage reduced yield by 25%; and for 15 days, yield was reduced by 88%. In upland rice, water stress imposed by controlled deficit irrigation has been found to result in yield loss of up to 50% (Lafitte *et al.*, 2004). Drought occurring during panicle development can cause delay in anthesis, reduction in the number of spikelets per panicle, and poor grain filling (Boonjung and Fukai, 1996). The reduction in seed set is reportedly due to pollen abortion or sterility arising from low water potential in the plant tissue (Nguyen and Sutton, 2009). Pronounced water deficit during flowering may lead to spikelet desiccation with the affected plants often producing no grain at all. Such symptoms may, however, be less conspicuous in some upland cultivars which have the ability to conserve moisture (Ekanayake *et al.*, 1993).

Comparatively, the practice of flooding in rice production, be it continuous or for a short duration, has been reported to contribute to higher tiller number, biomass, and grain yield than non-flooded practices (Juraimi *et al.*, 2009). However, excessive flooding resulting in complete submergence of vegetative tissues poses a real threat to growth, tillering and yield of rice plants since it undermines the very survival of the plants. Workers in South-east Asia have demonstrated that rice can withstand total submergence for even up to 15 days, but yield may be severely affected thereafter (Mackill *et al.*, 1993; Jackson and Ram, 2003). A protracted period of submergence was reported to affect the development of rice. Complete submergence at early and active tillering can lead to yield losses due to poor tillering and low panicle numbers. At the booting stage, complete submergence may cause the cessation of panicle development as well as the degeneration of spikelets (Reddy *et al.*, 1985). Nitrogen use efficiency has also been observed to decrease with increasing water depth (Sharma, 2002).

More often than not, field moisture level may be manipulated to achieve certain beneficial results. Yang *et al.* (2003a) proposed controlled water deficit as a means to increase grain filling and yield since according to them, it may encourage senescence and facilitate the remobilization of carbon reserves to the developing spikelets. They found that moderate water deficit after anthesis increased grain yield by 8 - 10 %. However, the optimum water depth has been difficult to define with certainty; but a great deal tends to depend on the degree of water control as well as how level the field is (Yoshida, 1981).

Efficient management of water has been reported to result in higher yield, and particularly in the case of irrigation, the practice has resulted in less water use. Controlled soil moisture content irrigation techniques can contribute to yield increase and reduce water consumption by 40 - 45% of that used in flooding irrigation (Shizhang *et al.,* 1994). According to these authors, the practice promotes modest rice crop water consumption by reducing transpiration, interplant evaporation and field seepage.

Alternate submergence and non submergence have been found to save water use by 15% of that used in continuously flooded culture with no effect on grain yield, provided ground water remains between 0 - 30 cm (Belder *et al.*, 2004). When imposed alternately, moderate soil submergence and drying may promote grain yield in addition to improved water use efficiency since it enhances root growth and other physiological processes (Zhang *et al.,* 2009a).

2.4. Mechanisms of rice survival in flooded environments

In well drained soils, root activities and growth are enhanced by direct aeration from the pore spaces. However, if the soil is saturated as in waterlogged and flooded terrains for a sustained period, oxygen becomes limited (Gambrell *et al.*, 1991) and root activities may be largely affected (Drew, 1992). Severe stress imposed by waterlogging reportedly leads to significant reduction in photosynthesis and net assimilation rate in rice plants (Adak and Das Gupta, 1999; Adak and Gupta, 2000), and the eventual inhibition of growth (Kozlowski, 1984; Sojka, 1992). Root activities such as active uptake and retention of water and essential nutrient elements may slow or shut down completely due to pronounced oxygen deprivation, causing many plants to show symptoms of drought (Sairam *et al.*, 2008).

Admittedly, rice is not an aquatic plant, but it is said to be well adapted to survive submergence by developing suitable anatomical features and physiological responses which alleviate the effects of hypoxia and anoxia (Das and Uchimiya, 2002). Typical morphogenic and physiological changes that occur in rice as a result of flooding are increased adventitious root formation (Visser *et al.*, 1996), rapid shoot elongation (Raskin and Kende, 1984; Fukao and Bailey-Serres, 2007), rapid coleoptile growth (Hoffmann-Benning and Kende, 1992), and the conservation of carbohydrates in submerged tissues (Setter and Laureles, 1996; Das *et al.*, 2005). Thus, the physiological adaptations of rice that enable it survive in flooded habitats may be summed up as escape or tolerance. The escape mechanism is mainly achieved by rapid shoot elongation above the surface of flood water (Bleecker *et al.*, 1986), while tolerance is expressed when plants refrain from elongating to conserve resources (Ram *et al.*, 2002; Almeida *et al.*, 2003). The escape strategy is mostly expressed by deep water rice genotypes and is mediated by high ethylene production (Kende *et al.*, 1998; Van der Straeten *et al.*, 2001; Pierik *et al.*, 2010), while tolerance is exhibited in lowland rice as it does not readily elongate when inundated; rather, it limits the consumption of carbohydrates used for cell division and elongation in response to low ethylene synthesis and sensitivity (Fukao and Bailey-Serres, 2008).

Perhaps, the most important anatomical feature of rice that ultimately facilitates the maintenance of metabolic processes in submerged tissues is aerenchyma formation (Armstrong, 1979). Aerenchyma are essentially air-filled sacs that facilitate the transport of oxygen internally from the non-submerged to submerged tissues of wetland plants, thereby creating a soil-plant-atmosphere continuum for the exchange of gases even during prolonged periods of partial inundation (Comis, 1997; Magneschi and Perata, 2009).

Aerenchyma in rice is formed by lysigeny, i.e. the death of cells (Smirnoff and Crawford, 1983; Evans, 2004). According to Clark and Harris (1981), cell lysis proceeds by cell wall separation from adjacent cells, wherein the radial walls from the collapsing cells come together and leave a large air-filled space between them. Kawai *et al.* (1998) reported that the initiation of lysis in cortical cells is defined by acidification and membrane disintegration in a specific cell location. Justin and Armstrong (1991) reported that in some rice cultivars, aerenchyma formation in root tissues may be driven by the endogenous action of ethylene.

2.5. Effects of nitrogen on rice growth and yield

Nitrogen, like other essential nutrient elements, is a critical requirement for plant growth and productivity. It is an essential component of cellular structures such as cell walls, and is required for the production of enzymes, chlorophyll, nucleic acids, and other non-structural components of the plant cell (Schrader, 1984). Correlations between nitrogen accumulation and yield have been reported in many studies on rice (Peng and Cassman, 1998; Wang *et al.*, 2001; Murchie *et al.*, 2002; Haefele *et al.*, 2003; Yang *et al.*, 2003b; Huang *et al.*, 2008). In general, about 70 – 90% of the nitrogen accumulated in rice at harvest maturity is said to be absorbed before heading (Ying *et al.*, 1998b). Leaf nitrogen plays a significant role in plant dry matter production since it is closely correlated with the rate of photosynthesis (Hirose and Werger, 1987; Evans, 1989; Leuning *et al.*, 1995). Accumulation of enough resources and the subsequent delayed senescence and efflux of nitrogen from leaves, sheaves and culms are essential for enhancing panicle weight and grain filling (Ray *et al.*, 1983). During the ripening stage of rice, the developing panicles become the major sink for nitrogen wherein large amounts of nitrogen are remobilized from the leaves and stem. At harvest, 30 – 77% of the nitrogen in vegetative plant tissue is reportedly allocated to the grains in the panicle (Witt *et al.*, 1999; Ida *et al.*, 2009).

Tillering in rice is known to be very responsive to soil fertility, particularly nitrogen nutrition. Responses may vary in accordance with the amount of nitrogen that is available during the growth period. At high nitrogen rates, a low tillering plant type tends to exhibit the advantage of higher nitrogen use efficiency, compared to high tillering varieties (Peng *et al.*, 1994). However, over application of nitrogen fertilizer at sowing and tillering has been found to result in high tiller abortion after maximum tillering, accompanied by a lowering in the percentage of effective tillers and lower grain yield per unit nitrogen uptake (De Datta and Buresh, 1989; Jiang *et al.*, 2005).

During the vegetative stage, crop plants concentrate on mobilizing sufficient resources from the soil and photosynthesis, to produce appropriate biomass that would support their reproductive phase. Excessive nitrogen nutrition is known to promote luxuriant growth, delay the reproductive phase, and encourage lodging and blast in rice (Takebe and Yoneyama, 1989). Inadequate nitrogen on the other hand, tends to retard growth, limit photosynthesis and assimilates partitioning, hasten senescence, thereby resulting in low yield (Mae, 1997). Efficient management of nitrogen resources is therefore a very critical factor for high yield and it largely depends on the choice, dosage, timing and mode of application of the nutrient carrier or fertilizer. Effective management of crop nutrition entails the provision of adequate minerals in the proper form and amount and at the right time, such that yield returns will be maximized while fertilizer cost is kept at the minimum (Prudente *et al.*, 2009).

2.6. Nitrogen sources and fate in rice cropping systems

Plants derive nitrogen nutrition from natural (e.g. biological nitrogen fixation) or artificial (e.g. chemical fertilizer) sources. Plants generally take up mineral nitrogen mainly in the form of nitrate (NO_3^-) or ammonium (NH_4^+) ions (von Wirén *et al.*, 1997). Basically, all the major forms of nitrogen fertilizers, except urea, are known to contain, ammonium or nitrate, or both (Jensen, 2006). A thorough understanding of the circumstances surrounding the availability and fate of these ions in soils is important for their efficient exploitation if crop productivity should be maximized.

Ammonium-based or NH_4^+-forming (urea) fertilizers have been deemed to be more useful as nitrogen sources in paddy rice cultures, compared to nitrate fertilizers due to the incidence of denitrification, since nitrate is largely unstable in flooded soils (Prudente *et al.*, 2009). Furthermore, rice plants are capable of directly absorbing ammonium ions under varying soil moisture conditions (Addiscott, 2005). Urea is highly soluble and contains 46% N, which is relatively high in nitrogen and therefore, cheaper compared to other sources of nitrogen except for liquid ammonia which may contain up to 82% N (Dickie, 1997). Urea can be applied as granules, or in liquid form like ammonia (Watson *et al.*, 1992). However, plants cannot use urea directly; it has to first be hydrolyzed to NH_4^+ by the enzyme urease which is usually abundant in the soil micro flora; and plants can still absorb the nitrogen when the ammonium is converted to NO_3^- (Reynolds *et al.*, 1985; Kaminskaia and Kostic, 1997).

The problem with the use of urea is that it may be lost to the atmosphere via ammonia (NH_3) volatilization when left exposed or dehydrated as in drying soil. Rao *et al*. (1983) pointed out the method of placement of urea as an important consideration if its fertilizing value is to be realized since the level of rhizosphere soil nitrogenase activity largely depends on it. In addition, gaseous ammonia may cause problems of phyto-toxicity to seed germination and growth of seedlings and may also contribute to the build-up of greenhouse gases and consequently, global warming (Bremner, 1995; Cole *et al.*, 1997; Xiao *et al.*, 2005).

Ammonia volatilization tends to increase with soil pH since the hydrolysis of urea may be accompanied by the oxidation of NH_4^+ to NH_3 under high pH conditions, wherein urease activity is effectively inhibited (Kissel and Cabrera, 1988; Sigunga *et al.*, 2002). The increase in free ammonia concentration in alkaline soils may eventually limit the transformation of urea to NH_4^+. Urea hydrolysis, however, proceeds rapidly in warm, moist soils, with most of the urea transformed to NH_4^+ in several days (Vlek and Carter, 1983; Xu *et al.*, 1993).

The nitrate from the other sources of nitrogen fertilizer can also be taken up by rice and used to produce proteins needed for growth. If not, it may be immobilized into the soil organic matter, where it will cause no problem unless it is released as nitrate by mineralizing and nitrifying bacteria (Okereke and Meints, 1985). Nonetheless, nitrate fertilizers are also subject to the same fate of nitrogen loss via leaching and denitrification (Galloway *et al.*, 2004). Nitrate may be leached from the soil and end up in ground water, fresh water or the sea, thus serving as a major pollutant (Duwig *et al.*, 2000; Duff *et al.*, 2008); or denitrified to nitric oxide (NO) or nitrous oxide (N_2O), both of which are gases that contribute to global warming like NH_3 (Davidson and Kingerlee, 1997; Amon *et al.*, 2001; Smith *et al.*, 2003). Although the complete denitrification to N_2 may represent a loss of financial resources committed to the purchase of fertilizer, loss of nitrogen in the form of N_2O is even more costly since this gas poses great danger to the environment owing to its role in the destruction of ozone in the upper atmosphere (Randeniya *et al.*, 2002).

2.7. Nitrogen management in rice cropping systems

Besides the type, quantity and placement of fertilizer material, efficient nitrogen use by rice plants may also be influenced by a number of cultural and environmental factors, prominent amongst which is moisture. Soil moisture condition may also have a huge impact on the availability and uptake of nitrogen as well as other nutrient forms irrespective of their source, often with significant consequences for biomass accumulation, tillering and yield of the rice plant. During periods of water stress, nitrogen application may have very little or no effect on growth and tillering; however, uptake may resume after the stress is relieved (Prasertsak and Fukai, 1997). Nitrogen recovery by rice plants has been found to be about 2.5 times higher under continuous flooding than the alternate flooding and drying regime (Hanif, 1987). Increased uptake of nutrients, especially nitrogen and phosphorus, has been associated with higher root growth under increasing moisture levels (Reddy and Kuladaivelu, 1992).

Usually, nitrogen uptake in well watered upland moisture regimes tends to be 1.5 – 2 times more efficient than in flooded situations (Vlek and Byrnes, 1986). This enhanced absorption of nitrogen has been attributed to the significant (by at least 50%) repression of ammonia volatilization and nitrous oxide emission (Zhou *et al.*, 2008). However, Belder (2005) and De Datta *et al.* (1983), reported that nitrogen use efficiency in flooded rice was comparatively higher than that in rice grown under continuous aerobic conditions. They showed that nitrogen acquisition by rice roots in flooded soil can be very rapid if fertilizer is applied at a time when most demanded by the crop.

The use of slow-release fertilizer can lead to increased absorption of nitrogen. Wada *et al.* (1991) found that the sink size and yield of rice were increased up to two-fold by using slow-release fertilizer as compared to equal amounts of conventional ammonia sulphate. Panda *et al.* (1988a) have suggested a single dose application of controlled-release fertilizer or prilled urea, placed in band or broadcast incorporated in the soil at sowing or early tillering, as a more efficient means of providing nitrogen nutrition for direct sown lowland rice plants. A similar practice has also been suggested for both direct-sown and transplanted rice under intermediate deep water conditions (Panda *et al.*, 1988b).

An agronomic nitrogen use efficiency of at least 15 kg of grain yield per kg of nitrogen input has been reported from the use of controlled release fertilizer band incorporated as single dose at transplanting (Horie *et al.*, 2005). Also, the rates of NH_4^+ absorption and assimilation by rice increased when NH_4^+ and NO_3^- fertilizers were mixed, engendering higher plant growth rates (Kirk and Kronzucker, 2000).

Timing of nitrogen application is another essential means by which critical growth functions and stages of the rice plant can be enhanced. In no-till water-seeded rice, 30 – 35 DAS was determined as the optimum time for applying the first nitrogen dose at 100 kg ha^{-1}; this practice promoted the production of more early tillers and higher grain yield (Stevens *et al.,* 2001). In transplanted rice, application of nitrogen at 7 days after transplanting has been found to be more beneficial than application at transplanting (Meelu *et al.*, 1987). A reduction in the time interval between nitrogen fertilizer placement and permanent flooding can lead to higher uptake and increased yield (Bacon, 1985). Single application of controlled release fertilizer (CRF) at the early seedling stage has been found to not only reduce labour requirement, but also increase the ratios of productive tillers and whole grains with lower protein content as compared to a conventional practice that involved topdressing at the panicle development stage (Miura *et al.*, 2009).

Under optimum conditions, nutrient use efficiency may be enhanced by applying fertilizer at appropriate times of the plants' need. Accordingly, a number of nitrogen management tools such as the leaf colour chat and portable chlorophyll meter have been designed to conduct *in-situ* and non-destructive assessment of crop nitrogen status, thereby assisting rice farmers to decide the optimum time to apply nitrogen fertilizer (Balasubramanian, 1998; Singh *et al.*, 2002). Significant yield increases from minimal nitrogen input (of about 50% below the traditional recommended rate) have been reported in places where these instruments were used to inform nitrogen fertilizer input decisions (Reddy and Pattar, 2006). Also, soil test and target yield concepts have been found to constitute the basic framework for fertilizer recommendation with the ultimate aim of optimizing nutrient use efficiency (Bera *et al.*, 2006).

2.8. Effects of water and nitrogen management on grain quality

Quality has not always been an easy concept to define as it is usually based on the behavior of the consumers whose judgments are often influenced by their tastes and preferences.

Accordingly, consumers have always demanded the best quality they can afford, and as nations become more self-sufficient and prosperous, the demand for better quality further increases (IRRI, 2009). Rice grain quality is influenced not only by genetics (Webb, 1991), but also by cultural factors as discussed briefly in the following sections.

2.8.1. The concept of rice grain quality

In rice production, the cooking and eating characteristics of the milled rice are essential quality issues that determine its market value and consumers' acceptance; and consequently, affects the income of farmers (Unnevehr, 1986; Cramer *et al.*, 1993). For example, the length and volume expansion, as well as the water uptake potentials of rice grains are specific characteristics of interest in the food service industry, since they determine the size of plate servings for the same amount of rice taken from different lots (Gujral and Kumar, 2003).

Quality is often based on the tastes and preferences of the consumer which may vary from one region to another or even amongst individuals from the same locality. Some consumers prefer rice that cooks soft and sticky, while others prefer soft and flaky cooked rice; yet still, others like most West Africans, prefer flaky rice with hard texture (Kaosaard and Juliano, 1991). The cooking and eating quality of rice is mainly affected by the properties of the starch, protein and lipid components of the grain which are in turn, more or less, influenced by the genotype and environment of the crop (Zhongkai *et al.*, 2002).

2.8.2. Starch properties and grain quality

Starch is the major constituent of milled rice and makes up about 90% of the dry matter (Juliano, 1993). Starch consists of a mixture of two polymers (amylose and amylopectin) that are composed entirely of glucose units (Fox and Cameron, 1982). Typically, amylose molecules constitute about 17 to 32% of the polymers in a starch granule, and the rest is amylopectin (Bennion, 1980). Structurally, amylose consists of thousands of glucose units joined together by α-1, 4 glycosidic linkages, while amylopectin is branched and bushy in appearance owing to the presence of an α-1, 6 linkage at every 15 to 30 glucose units of the chain (Alais and Linden, 1991).

The amylose-amylopectin ratio of starch granules largely determines the texture of cooked rice and is often used as an index to delineate the diversified uses of various rice types and products. For example, hard-cooking rice tends to have higher amylose-amylopectin ratios than soft-cooking types (Ong and Blanshard, 1995); and rice with lower amylose-amylopectin ratio is more suitable for products such as baby food, breakfast cereals and noodles (Whistler *et al.*, 1984). Admittedly, rice is not always eaten immediately after cooking. Rice lots that harden undesirably often require re-heating prior to consumption. The propensity of cooked rice to retrograde, or harden upon cooling, has been observed to increase proportionally with amylose content (Lin *et al.*, 2001; Yu *et al.*, 2009), and can be measured by a rapid visco-analysis (RVA) test as setback viscosity (Zhang et al., 2007).

Typically, milled rice is classified by apparent amylose content into the categories of low, intermediate and high amylose rice, if they contain 10-20%, 20-25%, and > 25% amylose, respectively. Rice is said to be waxy if it contains 0-2% amylose. However, different rice lots with the same amylose content may have different sensory attributes due to the presence of long, intermediary or short chains of amylopectin in the grain starch (Chinnaswamy and Bhattacharya, 1984; Reddy *et al.*, 1993; Ong and Blanshard, 1994). In order to distinguish various rice lots that have similar amylose contents, it may become necessary to conduct gelatinization temperature and gel consistency tests (Juliano and Villareal, 1993).

2.8.3. Influence of protein on cooking and eating qualities

Depending on the cultivar and agro-ecological conditions, the protein content of brown rice may vary from 7 to 15% (Lásztity, 1996). When milled, protein of whole kernels declines to about 7 - 9% because some of the protein-rich aleurone cells are removed during the milling process (Shih and Daigle, 2000).

Two basic types of protein bodies are known to exist in the endosperm of rice grains. One, known as type I protein body, mainly accumulates prolamin which also may account for the firmness of cooked rice since it is difficult to digest; and the other largely comprises glutelin which is easily digestible and influences the appearance of the grains (Furukawa *et al.*, 2006; Kumagai *et al.*, 2006). When proteins were removed from rice samples, Philpot *et al*. (2006) found that the gels became softer with virtually no change in firmness after cooling, indicating that protein plays very little or no role in cooked rice retrogradation. Rice lots with lower protein contents tend to be more tender and cohesive than those with intermediate protein levels (Hamaker, 1994).

Proteins render the pasting of rice flour difficult by competing with starch granules during the process of mixing; hence, rice lots with high protein contents tend to require more water for cooking (Jianping et al., 2008). This water-binding tendency of protein defines its impact on the viscosity curve. Proteins may increase the concentration of the swollen starch paste and thereby lower the peak viscosity of rice flours (Martin and Fitzgerald, 2002).

Protein embodies the very essence of the nutritional quality of rice and is often affected by the amount of storage proteins as well as their essential amino acids balance (Duan and Sun, 2005). Generally, the protein in rice is well balanced and healthy for human nutrition since it contains all of the eight essential amino acids in the proper proportion (Paraman *et al.*, 2006; Goya, 2010).

Protein ameliorates head rice content (i.e. the milling recovery of whole grains) and grain translucency, since brown rice with high protein contents are relatively less susceptible to the abrasion of milling (Perez *et al.*, 1996a). Appropriate investment in nitrogen will not only ensure higher yield, but also improved grain quality and larger returns as consumers generally prefer more head rice and translucent grains, than broken kernels and chalky grains. However, as much as increased fertility leads to the production of grains with higher protein content, the flour from such grains tend to have lower peak viscosities and consequently, poor pasting properties (Bryant *et al.*, 2009).

2.8.4. Effect of environmental factors on grain quality indices

Although the pasting behavior of rice flour is largely defined by genotype, environmental factors may cause changes in the grains and thereby influence the pasting properties of the flour (Minh-Chau Dang and Copeland, 2004). Like variety, the temperature and duration of storage are known to affect the pasting properties of rice flour (Zhou *et al.*, 2003). She *et al* (2009) also noted the adverse influence of abiotic stresses, such as high temperature, on starch synthesis and protein accumulation in developing rice grains.

Nitrogen fertilization tends to influence the proteins and amylose content, and therefore the texture of cooked rice. Under high nitrogen conditions, rice grains have been found to contain high amounts of proteins and lower amylose content (Champagne *et al.*, 2009; Zhang *et al.*, 2009b). Other studies showed that protein content increased with fertility while apparent amylose content, gelatinization temperature and lipid content were not affected by fertility, but by cultivar (Bryant *et al.*, 2008).

Although grain protein content and yield may be negatively correlated, nitrogen becomes more effective for protein accumulation in rice grains without a corresponding decrease in yield when applied at, or close to heading (Honjyo, 1971; Souza *et al.*, 1999). Studies conducted in the Senegal river valley of West Africa showed that topdressing nitrogen at the booting stage significantly increased grain yield and milling quality (Wopereis-Pura *et al.*, 2002). In IR cultivars at the International Rice Research Institute (IRRI), nitrogen application at heading have not only been reported to increase paddy yield, but also the head rice yield and protein content of the milled grains (Perez *et al.*, 1996a).

Literature on the influence of water management on grain quality is rather limited; however, Singh *et al*. (1990) reported that flooding led to an increase in the amylose content of rice grains, while decreasing its protein content.

2.9. Conclusion

The foregoing review suggested that water and nitrogen are critical to the growth and productivity of rice irrespective of ecology. However, it emphasized the need for careful management of these resources for the assurance of improved growth, yield and grain quality of the crop while at the same time ensuring that the costs of production are kept as low as possible. The major objectives of this study therefore, remained to determine the optimum rate and timing of nitrogen application for the promotion of maximum grain yield and quality, and to establish the most suitable water regime for the attainment of improved growth, efficient nitrogen uptake and use for grain production and quality of the rice crop.

CHAPTER 3

EFFECT OF TIMING OF FLOODING ON THE YIELD AND NITROGEN USE OF RICE (*Oryza sativa* L.) GROWN UNDER GREENHOUSE CONDITIONS

ABSTRACT

In lowland rice production systems, flooding may persist for varying lengths of time during the cropping period and consequently affect the availability and uptake of nitrogen. This poses a serious challenge to the productivity of the crop, particularly, if the nitrogen and water resources are not managed appropriately. A glasshouse experiment was conducted at the University of KwaZulu-Natal to evaluate the effects of nitrogen rates and flood regimes on the growth and grain yield of rice. Plants were grown in pots by direct seeding and subjected to three nitrogen levels (0, 110 and 220 kg ha^{-1}) and four water regimes, namely: continuously well watered to near field capacity, continuous flooding, early flooding, and late flooding. Tillering was monitored from emergence to heading. At heading, above ground biomass, leaf area, and nutrient uptake were determined. Yield components and grain yield were determined at harvest maturity.

Results showed that plants subjected to continuous flooding, produced more tillers and panicles and accumulated more dry matter and leaf area than those of the other water regimes, but the highest grain yield and agronomic nitrogen use efficiency were attained from early flooding accompanied by drainage at mid-tillering. Tiller number, biomass accumulation, as well as panicle and grain yield increased with nitrogen level. Nitrogen uptake was higher in plants supplied with 220 kg N ha^{-1} compared to the other two doses; but plants supplied with 110 kg N ha^{-1} were more efficient in nitrogen use. Correlation and regression analysis revealed a positive linear relationship between grain yield and the quantity of grain bearing tillers, which in turn, strongly depended on tiller development as influenced by the flooding and nitrogen regimes.

This study showed that the rice plant does not only tolerate waterlogging, but also, produces higher grain yield with increased nitrogen supply. Further experimentation will be required to verify these results, as well as their applicability under field conditions.

INTRODUCTION

Rice is the principal grain crop in Liberia, eaten at least twice daily as the major source of carbohydrates for thousands of households. The crop is produced mainly for subsistence by impoverished rural farm families with very little or no modern agricultural inputs, including fertilizers. The farming practice is dominated by shifting cultivation in upland fields where about 90% of the rice crop is produced under rain-fed conditions (Balasubramanian *et al*., 2007). As a result, yield is particularly low and seldom exceeds 1.5 t ha^{-1}. By 2006, national consumption had exceeded production by at least 50%, primarily as a consequence of declining output since the latter part of the 1980s (WARDA, 2007).

Although rice is not an aquatic plant, it is widely established that its yield can be improved with above average moisture supply (Alva and Petersen, 1979; Sarwar and Khanif, 2005). Currently, efforts are being directed at encouraging farmers in Liberia to shift towards lowland rice culture characterized by periodic flooding, with the expectation of increasing productivity with improved water supply and at the same time, promoting sedentary farming (Dolo, 2009). Due to the heterogeneous and uncertain nature of wetlands, especially in relation to flooding, there is a need for skillful management of water resources in a manner that will maximize yield and minimize wastage of inputs. Furthermore, farmers will have to adopt efficient fertilizer management strategies if they are to succeed and adjust fully to the conditions of lowland rice farming.

Of the major fertilizer resources, nitrogen is the most intensively used in rice cropping systems. When under or over applied, there may be negative consequences for yield or for the environment. Insufficient plant nitrogen results in chlorosis, reduced photosynthesis, and stunted growth. Excessive nitrogen encourages luxuriant growth, lodging, diseases, and delays maturity (Takebe and Yoneyama, 1989). If not utilized by plants or incorporated into the soil microbial biomass, excess nitrogen can increase the incidence of nitrogen loss to the environment, and therefore contribute to water pollution or greenhouse gas emission (Cole *et al*., 1997).

Just as nitrogen is known to promote plant growth and development, elevated soil moisture regimes are said to favour the growth and development of rice crops (Juraimi *et al*., 2009). Excessive soil moisture leading to flooding conditions, however, may undermine root development and nitrogen availability, and consequently interfere with the absorption of dissolved substances and eventual plant development in several crops (Pantuwan *et al.*, 1997; Zaidi *et al.*, 2007).

In most countries, rice tends to be grown in wetlands. The water conditions of such lands expected to be adopted in Liberia are not easily predictable, particularly in respect of flooding. Excessive moisture can negatively influence nitrogen use efficiency, and consequently, crop yield. This study therefore, examined the effects of diverse soil flooding patterns and different nitrogen application rates on the growth and yield of the rice crop. It also determined how water regime impacts nitrogen use in rice and what nitrogen rates may be suitable for given water management systems. Results from the study may be used to guide nitrogen and water management decisions in rice cropping systems in areas subjected to fluctuating cycles of flooding during the growth period of the rice crop.

MATERIALS AND METHODS

A pot experiment was conducted in a glasshouse (day / night temperature 30^{o}C / 20^{o}C) at the Life Sciences Campus of the University of KwaZulu-Natal in Pietermaritzburg from June to October, 2009 to evaluate the responses of rice to different soil flooding regimes and nitrogen fertilizer levels.

Plant material

A Chinese rice cultivar, “Golden Mountain # 1” (GM-1), adopted in the Republic of South Africa (RSA) for research purposes, was used in this study as it was the only available germplasm at the commencement of the experiment. Although the germplasm is reported to be adapted to upland aerobic conditions, it also has a water requirement higher than that received under normal rain-fed conditions in RSA.

Experimental design and treatments

The experimental design was a factorial combination of four water regimes and three nitrogen application rates, laid out in a completely randomized block design with four replications. The water regimes which were simulations of field moisture situations that possibly occur in wetland rice cropping included: non-flooded / continuous aerobic, but well watered control (NF), continuous flooding (CF), early flooding (EF), and late flooding (LF). The non-flooded regime was characterized by well watered, but aerobic conditions with soil moisture tension in the root zone varying between 0 and 15 kPa throughout the experimental period, whereas the flooding regimes were achieved by maintaining standing water at 5 cm from the soil surface. Commencing at 21 days after sowing (DAS), the early flooding regime lasted until 51 DAS (i.e. 30 days) when tillers had fully formed, while the continuous flooding regime lasted up to 7 days before harvest (i.e. 70 days). The late flooding commenced at 51 DAS and lasted until 7 days before harvest (40 days).

Urea (46% N) was used as the nitrogen source and applied at 0, 110, and 220 kg ha^{-1}, and in three split doses of 50% at 20 DAS; 25% at mid tillering (45 DAS) and 25 % at booting (60 DAS). The amount of fertilizer was determined on the basis of yield target (6 and 10 t ha^{-1}) and soil analysis results (Table 3.1). There was a basal application of 20 kg P ha^{-1} using single super phosphate.

Plant establishment

The volume, upper diameter and height of the pots used in the trial were 4.8 L, 30 cm and 27 cm, respectively. A double layer of clear plastic bags (15 microns thick) was used to line the inner portion of the pots containing the waterlogged treatments in order to keep water from leaking out of the drainage holes (Figure 3.1). Each pot was then filled with 10 kg air-dried soil (28% clay, 1.5% organic matter, 1.5% acid saturation) and well watered so that the control treatments were free draining. None of the flooded pots showed evidence of surface saturation due to lining with the plastic bags.

Plants were established by sowing two hills per pot with three seeds per hill. The hills were spaced 17 cm apart and the seeds planted 1.0 cm deep. Prior to sowing, seeds were washed and soaked in tap water for 24 h, following which they were incubated at 30^{0}C for another 24 h in order to stimulate vigorous germination (Vergara, 1992; Basra *et al.*, 2005). To ensure a uniform stand, the plants were thinned to one seedling per hill at two weeks after sowing.

Tensiometers (Irrometer Company, California) were used to monitor the soil water status in the root zones of the plants in order to ascertain that those subjected to the non-flooded regime, in particular, were always well watered.

A portable plant efficiency analyzer (Hansatech, UK) was used to measure the chlorophyll florescence of the plants at 30 DAS and at heading (65 DAS) in order to determine the stress level of the plants under the various flooding and nitrogen treatments. Measurements were done between 10 am and 12 mid-day on the newest fully expanded leaf on the main culm of one plant per pot. A circular spot on the upper surface of each leaf was dark adapted for 20 minutes using dark adaptation clips, and the maximum quantum yield of photochemistry (Fv/Fm) recorded.

Figure 3.1: Glasshouse trial layout. Inner portion of flooded pots were lined with 15 micro-thick plastic bags to maintain flooding.

Table 3.1: Nutrient status of soil used in pot trial and yield target based recommendations for selected nutrients. Soil sample was extracted with 1M KCl for nitrogen determination and 0.25 M NH_4HCO_3 for P, K and Zn determination.

	Nitrogen		Phosphorus			Potassium			Zinc		
Yield target (t ha^{-1})	Sample soil test (%)	Required (kg ha^{-1})	Sample soil test (mg L^{-1})	Target soil test (mg L^{-1})	Required (kg ha^{-1})	Sample soil test (mg L^{-1})	Target soil test (mg L^{-1})	Required (kg ha^{-1})	Sample soil test (mg L^{-1})	Target soil test (mg L^{-1})	Required (kg ha^{-1})
6	0.21	110	22	9	20	132	100	0	5.35	1	0
10	0.21	230	22	11	20	132	120	0	5.35	1	0
14	0.21	350	22	13	20	132	140	0	5.35	1	0

Sampling and measurements

Tiller production was monitored weekly from three weeks after sowing until heading. The main culm and side shoots bearing at least one leaf were counted as tillers.

At heading, one plant per pot was randomly chosen and harvested for leaf area, above ground biomass, and nitrogen analysis. Leaf area per plant was measured with a portable leaf area meter (LI-3000, LI-COR Biosciences) equipped with a LI-3050A belt conveyor. Above ground dry matter was measured by weighing the leaves and culms of the harvested plants on an electronic balance after oven-drying at 70^0C for 48 h to constant mass.

Plant tissue nitrogen concentration was determined by the Dumas combustion method, using a TruSpec CN – elemental analyzer (LECO Corporation). Whole plant materials (leaves and culms) were oven dried at 70^oC for 48 h and ground to powder using a ball mill (Spex Industries 8000). Sub samples of 0.125 g were weighed, sealed in tin capsules and loaded for analysis.

Nitrogen uptake at heading was determined as the product of the above ground dry mass per plant and tissue nitrogen concentration. Nitrogen use efficiency (NUE) was assessed in terms of nitrogen recovery efficiency and agronomic efficiency as shown below:

$$\text{Recovery efficiency} = \frac{\text{uptake from N fertilized pot} - \text{uptake from non fertilized pot}}{\text{N}} \times 100 \qquad (3.1)$$

$$\text{Agronomic efficiency} = \frac{\text{grain weight in N fertilized pot} - \text{grain weight in non fertilized pot}}{\text{N}} \qquad (3.2)$$

where N = the amount of nitrogen applied.

Yield components, including panicle number and spikelet fertility, and grain weight were measured at maturity. Shoots bearing heads with at least one spikelet (empty or filled) were considered as panicles.

Panicles were cut at their basal neck node from the rest of the plant. The spikelets (filled or empty) were detached from the rachis and dried in an oven at 70^oC for 48 – 72 h. Unfilled spikelets were separated from filled spikelets by water flotation. Grain weight was determined at grain moisture content of 14% wet basis (wb).

Spikelet fertility was determined as a percentage of the number of filled grains to the total number of grains per panicle. Harvest index was calculated as the ratio of economic to biological yield, i.e. grain weight per plant to total biomass yield.

Data analysis

The data were subjected to analysis of variance (ANOVA) using the windows statistical software package, GENSTAT version 12. The means of treatments showing significant difference were subjected to Fisher's Least Significance Difference (LSD) test and separated at the five percent level of significance (P = 0.05). Relationship between factors was further assessed by Pearson's correlation and regression analysis using the statistical software SPSS 15.0 for Windows.

RESULTS

Soil moisture status

Record of weekly moisture readings showed that moisture tension in the non-flooded pots was always below 15 kPa (Figure 3.2). This suggests that at no stage in the study did the plants experience water deficit.

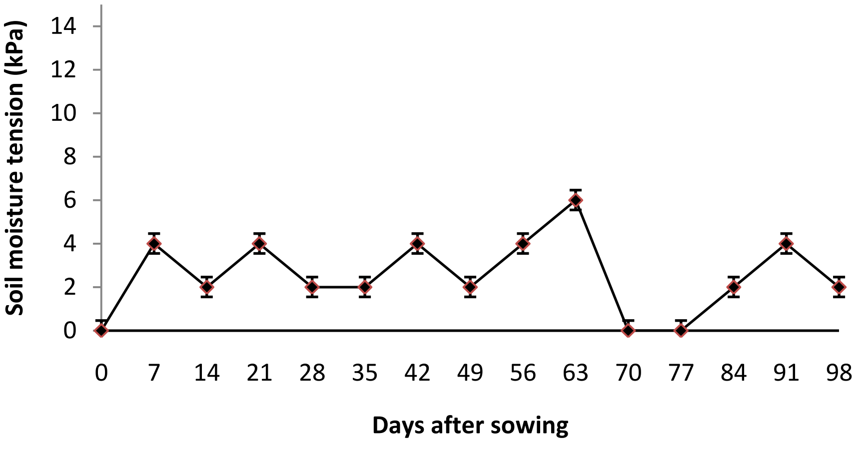


Figure 3.2: Soil moisture tension near the bottom of non-flooded pots. Data points are weekly means from 4 pots recorded each day prior to irrigation. Error bars indicate 95% CI of means.

Number of tillers

There was a positive linear relationship between nitrogen application rate and tiller production (equation 3.1).

$y = 0.025x + 6.071$; $r^2 = 0.911^{**}$ (n = 48) (3. 1),
where y = tiller number and x = nitrogen rate.

The number of tillers per pot varied significantly ($P < 0.001$) with water regime (Figure 3.3). The highest number of tillers (about 10) was produced under continuous flooding as well as under early flooding; the aerobic well-watered plants produced only 7.25 tillers on average. In effect, tiller production was limited by the absence of field saturation with water, particularly during the early growth stages. Tillers produced by late flooding were nearly as low as those produced under the non-flooded aerobic control treatments.

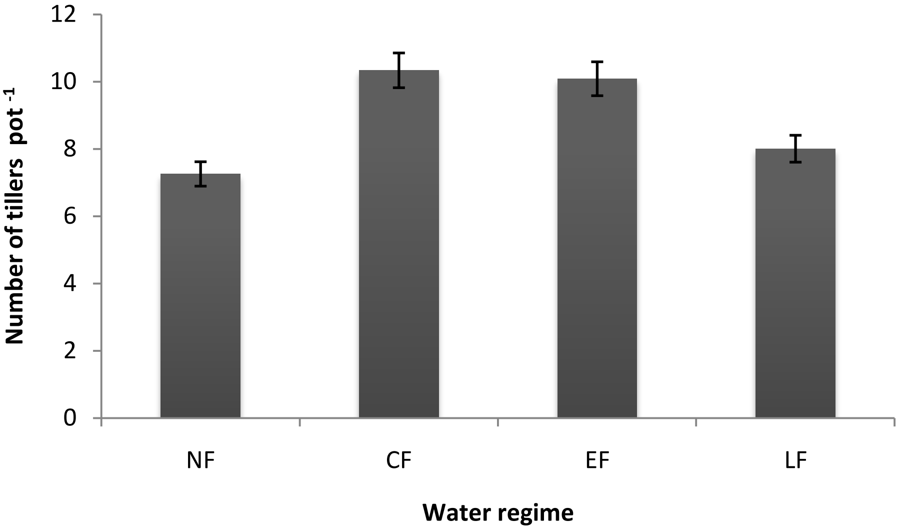


Figure 3.3: Tiller production at heading under various water regimes. Bars represent means. Error bars show 95% CI of means.

There was a significant interaction ($P < 0.001$) between nitrogen level and water regime (Figure 3.4). For all of the water regimes, growing plants without nitrogen supplement made no significant difference in tillering response. At 110 kg N ha^{-1}, only 7.5 tillers were produced for the no flooding water regime; this was significantly lower than those produced for both the early flooding (11.75) and continuous flooding (11.25) regimes.

On increasing the nitrogen rate to 220 kg ha^{-1}, tiller numbers were essentially the same (about 14) for plants grown under either early or continuous flooding, but were significantly higher than the continuous aerobic and late flooding treatments. The latter pair showed little differences compared to their numbers at the 110 kg N ha^{-1} level. Essentially, the former group produced 60% more tillers than the latter.

For the no flooding regime, the observed differences between the tiller means of all the nitrogen levels were not significant. Under the late flooding regime, the number of tillers produced by the 110 kg N ha^{-1} treatment was slightly higher than that produced by the 220 kg N ha^{-1} rate.

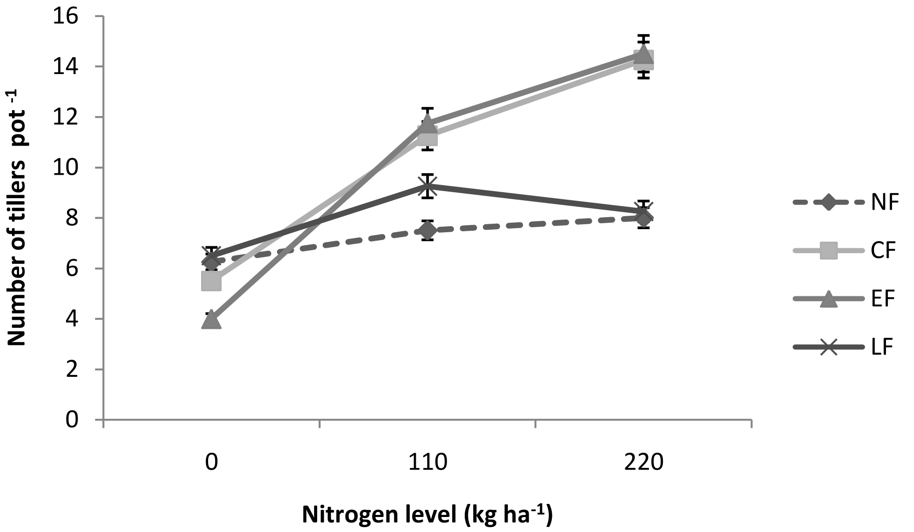


Figure 3.4: Tiller production at heading as influenced by different nitrogen rates and water regimes. Data points represent means. Error bars indicate 95% CI of the means.

Pattern of tiller production

The duration of the tillering period varied with nitrogen level and water regime (Figure 3.5 & 3.6). The days to maximum tiller production was shortest at the lowest nitrogen rate, compared to the 110 and 220 kg N ha^{-1} rates ($P < 0.01$). However, neither water nor the interaction of the two factors affected ($P > 0.05$) this period. The production of new tillers ceased about 10.5 and 4 days earlier in plants supplied with 0 and 110 kg N ha^{-1}, respectively, compared with those grown with 220 kg N ha^{-1}. Plants supplied with both 110 and 220 kg N ha^{-1} continued tillering even up to the heading period, unlike the control under which tillering essentially stopped by 37 DAS (Figure 3.5). Thus, high nitrogen application prolongs the tillering period, and hence increases their number.

For all of the water regimes, tillering essentially stopped by 51 DAS except for late flooding under which plants resumed production of new tillers with the imposition of flooding and continued, at least, up to heading (65 DAS). However, only few new tillers were formed overall in this treatment (Figure 3.6).

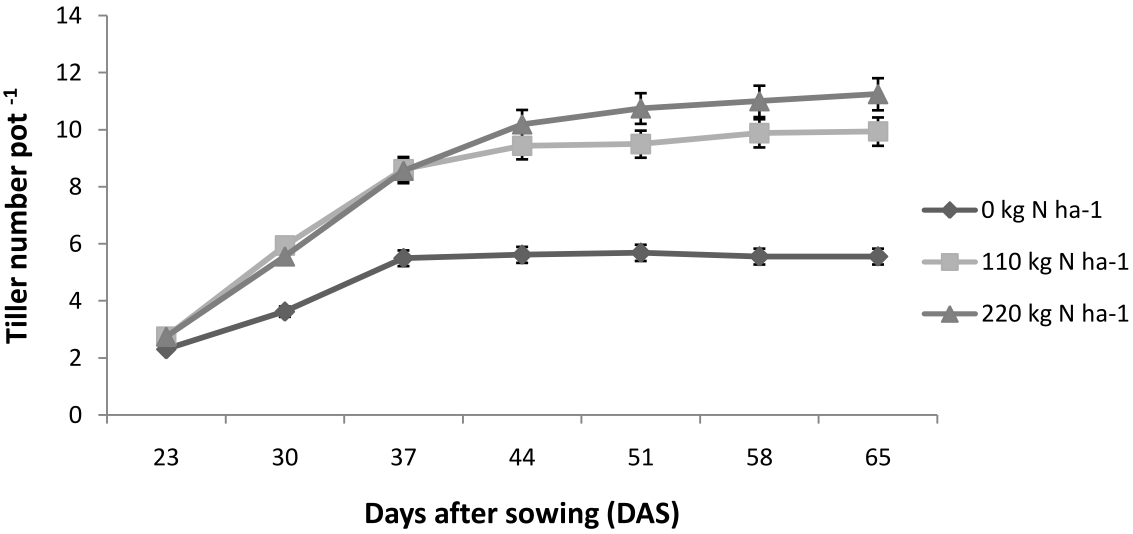


Figure 3.5: Tillering pattern as influenced by nitrogen application rate. Data points represent means. Error bars represent 95% CI of the means.

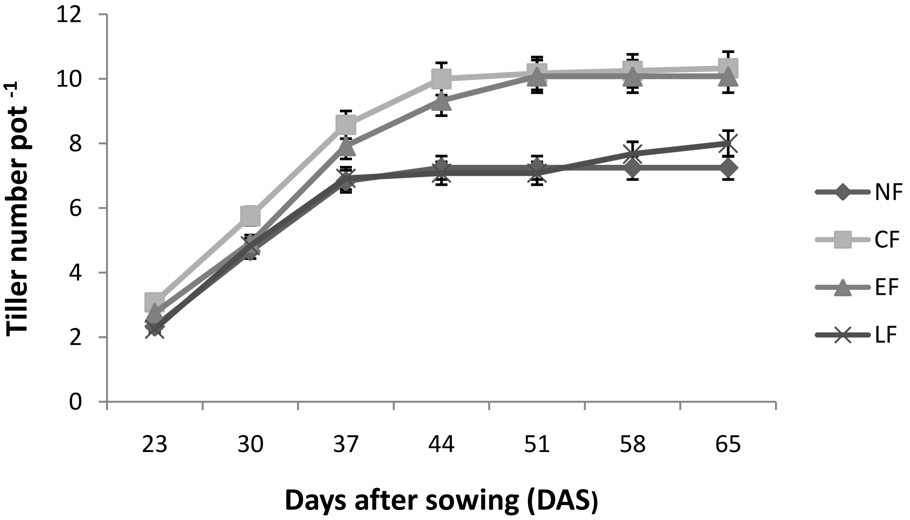


Figure 3.6: Tillering pattern as influenced by water regime. Data points represent means. Error bars indicate 95% CI of the means.

Number of panicles

The number of panicles per plant increased significantly ($P < 0.001$) with nitrogen application rate (Figure 3.7) and varied between 3.0 at 0 kg N ha^{-1} and 5.94 at 220 kg N ha^{-1}.

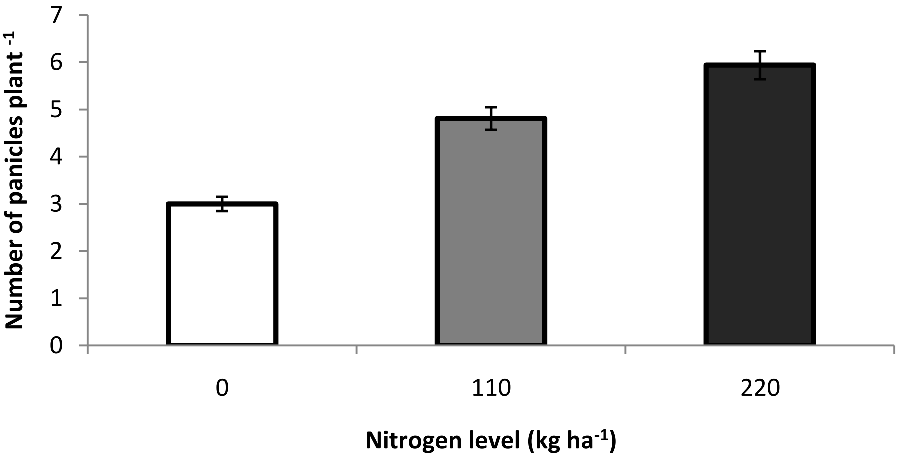


Figure 3.7: Panicle production as influenced by nitrogen application rate. Error bars indicate 95% CI of means.

The moisture conditions under which the plants were grown greatly affected panicle production ($P < 0.01$). Like tillering, plants subjected to continuous or early flooding produced more panicles than the non-flooded and late flooded plants (Figure 3.8). There was a significant interaction ($P < 0.001$) between nitrogen level and water regime. The relationship between nitrogen and panicle production was particularly strong for the early flooding regime ($r^2 = 0.945$, $P < 0.001$) under which the number of panicles increased by 0.025 for every kilogram of nitrogen applied per hectare. For the continuous and early flooding water regimes, application of 220 kg N ha^{-1} stimulated a significantly higher panicle yield than the other two nitrogen supply rates. However, under non-flooded conditions, panicle yield was not significantly affected by nitrogen dosage, although small increases were observed with increasing nitrogen supply (Figure 3.8). For late flooding, plants produced significantly more panicles with nitrogen application, but doubling the nitrogen dosage from 110 to 220 kg ha^{-1} was probably uneconomic as the difference between the two was negligible (Figure 3.8). When no nitrogen fertilizer was supplied to the plants, the difference in panicle numbers was less affected by water regime ($P > 0.05$).

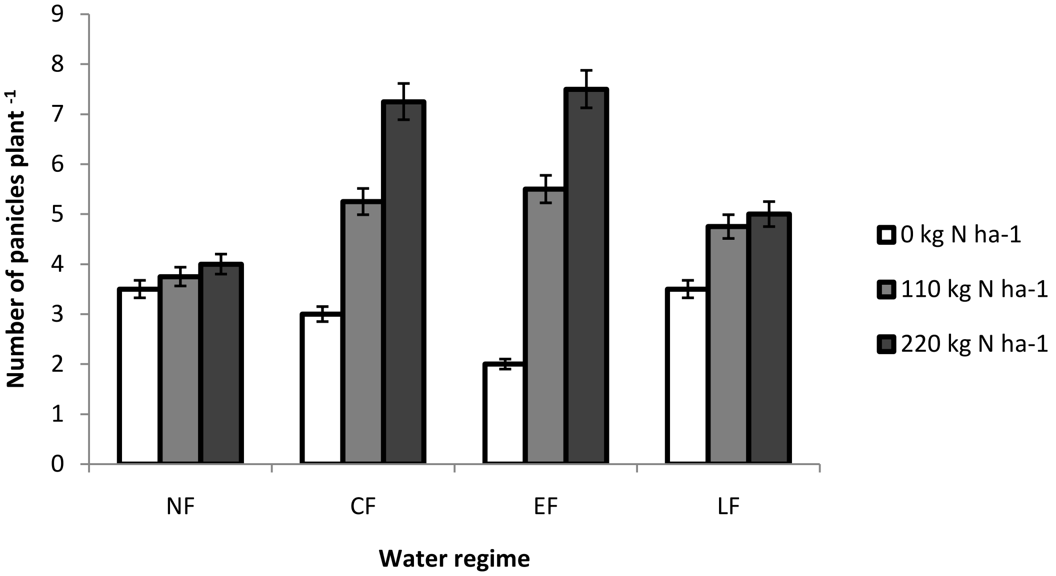


Figure 3.8: Effect of water regime on panicle yield for different nitrogen application rates. Bars represent means. Error bars indicate 95% CI of the means.

Tiller efficiency and viability

Data on two of the common indices (tiller efficiency and viability) used for the assessment of tiller productivity are presented in Table 3.2. Tiller efficiency, defined as the proportion of tillers that eventually developed into harvestable panicles was affected by water regime ($P < 0.05$), but neither by nitrogen application rate ($P > 0.05$), nor by the interaction of the two factors ($P > 0.05$). A significantly higher proportion (at least 97%) of tillers were able to develop into grain bearing heads for plants subjected to either continuous aerobic or late flooding, compared to 92% and 89% for the ones grown under early and continuous flooding, respectively.

Tiller viability, i.e. the number of tillers actually surviving to produce grain-bearing panicles, varied significantly ($P < 0.01$) with nitrogen level, but not with water regime ($P > 0.05$). At most, 3% of the tillers produced at 110 kg N ha^{-1} were lost, compared to none for plants supplied with either 220 kg N ha^{-1} or with no nitrogen fertilizer at all. Hence, with the soil autochthonous nitrogen supply alone, plants yielded just a few tillers which were all productive. But with the supply of 110 kg N ha^{-1}, the plants produced more tillers, some of which did not develop panicles.

Table 3.2: Tiller productivity for different nitrogen application rates, water regimes and their interaction.

		Tiller efficiency	CV = 9.8% LSD $_{(0.05)}$	Tiller viability	CV = 3.2% LSD $_{(0.05)}$
N level (kg ha^{-1})	0	0.96		1	
	110	0.94		0.97	
	220	0.95	0.07	1	0.02
Water regime	NF	0.98		1	
	CF	0.89		0.97	
	EF	0.92		0.99	
	LF	1	0.08	1	0.03

Relationship between tillering and panicle production

There was a positive linear relationship ($r = 0.96$, $P < 0.001$) between the number of tillers produced during the vegetative growth phase of the crop and the number of panicles at maturity (Figure 3.9). With 92% of the variances accounted for, the number of panicles tended to increase by 0.841 (t pr. < 0.001) for every vegetative tiller that was produced.

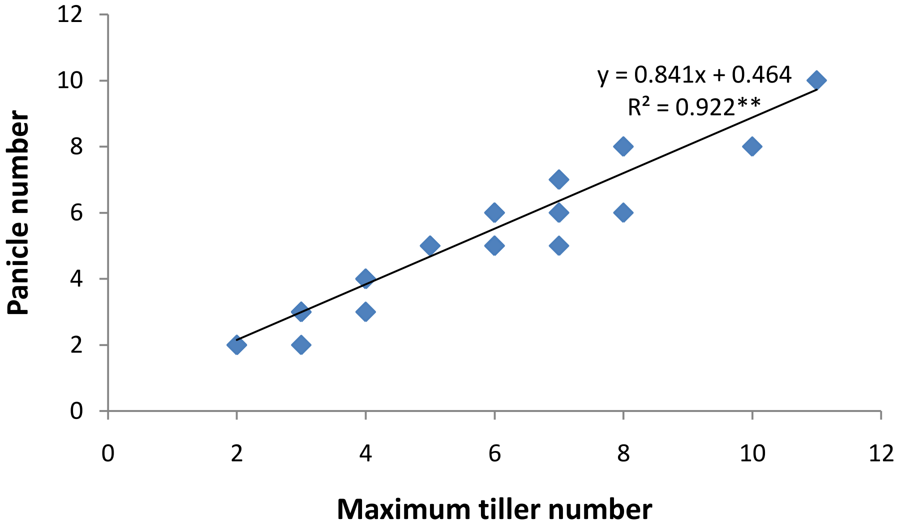


Figure 3.9: Relationship between tillering and panicle production. Data points include the effects of nitrogen dosage and water regime.

Chlorophyll florescence

The ratio of variable florescence to maximum florescence (Fv/Fm) neither changed significantly ($P > 0.05$) with nitrogen application rate, nor with water regime at both 30 DAS and at heading (65 DAS). Mean values ranged between 0.77 to 0.79 at 65 DAS, and 0.79 to 0.8 at heading (Figure 3.10), indicating that the plants were relatively free from stress. Although the maximum photochemical yield of photosystem II in healthy plants is indicated by Fv/Fm values around 0.83 (Bjorkman and Demmig, 1987), values ranging from 0.75 to 0.80 are considered to be generally high, and therefore suggestive of relatively low stress conditions in rice (Figueroa *et al.*, 1997).

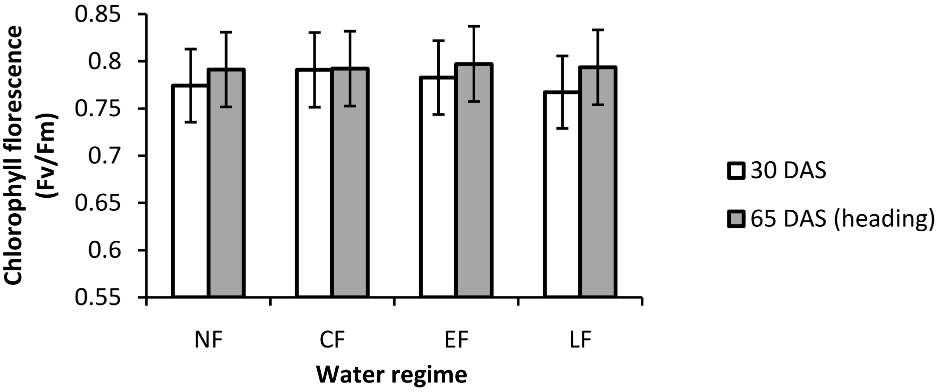


Figure 3.10: Chlorophyll florescence at early tillering and heading as affected by water regime. Error bars show 95% CI of means.

Leaf Area

Leaf area per plant increased significantly ($P < 0.001$) with increasing nitrogen rate (Table 3.3). When no nitrogen was applied, leaf area was only 354 cm^2. However, increased nitrogen rate of 110 and 220 kg ha^{-1} nearly doubled and tripled leaf area, respectively.

Water regime also had a significant impact ($P <0.001$) on leaf area development. Plants in the continuously flooded pots had the highest leaf area, while those in the non-flooded pots developed the least leaf areas (Table 3.3).

Table 3.3: Leaf area and biomass accumulation as influenced by nitrogen application rate and water regime.

		Leaf area (cm^2 plant $^{-1}$)	CV = 10.2% LSD $_{(0.05)}$	Shoot dry mass (g plant $^{-1}$)	CV = 16% LSD $_{(0.05)}$
N level (kg ha^{-1})	0	354		4.87	
	110	692		12.38	
	220	925	24	12.98	1.16
Water regime	NF	583		7.92	
	CF	740		12.38	
	EF	666		11.13	
	LF	639	55	8.88	1.34

There was a significant interaction ($P < 0.001$) between the nitrogen rates and water regimes (Figure 3.11). When no nitrogen was applied, the leaf area of plants was significantly higher for those grown under early flooding compared to the other water regimes. At 110 kg N ha^{-1}, water regime did not affect leaf area development; however, leaf area increased significantly with continuous and late flooding when the nitrogen rate was increased to 220 kg ha $^{-1}$ than with early flooding.

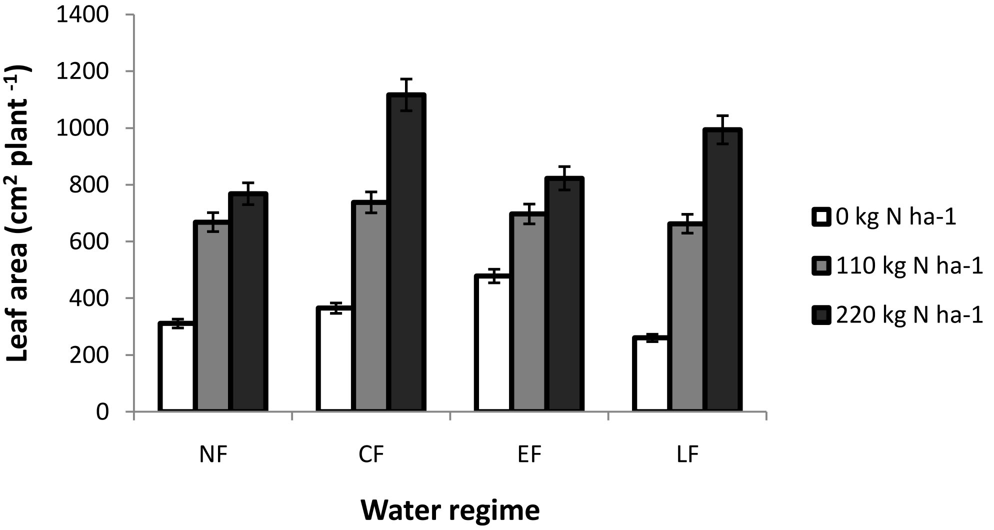


Figure 3.11: Leaf area development at heading as influenced by the interaction of water regime and nitrogen application rate. Bars indicate means. Error bars show 95% CI of means.

Above ground dry matter

By the time of heading, above ground dry matter accumulation increased significantly ($P < 0.001$) with the rate of nitrogen application (Table 3.3). Application of nitrogen fertilizer led to a two- to three-fold increase in shoot dry mass. Shoot dry mass increased significantly ($P < 0.001$) with early and continuous flooding, compared to the other two water regimes (Table 3.3). The response patterns were similar to those of leaf area and tiller number. Thus, there was a significant ($P < 0.001$) interaction between nitrogen and moisture regime for dry mass production (Figure 3.12).

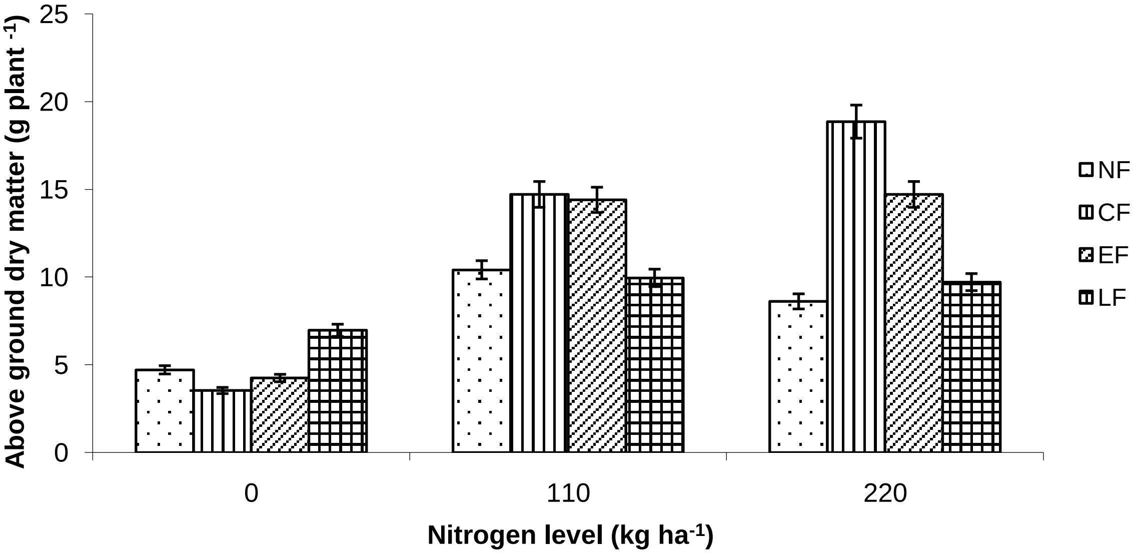


Figure 3.12: Dry matter accumulation at heading as influenced by the interaction of nitrogen application rate and moisture regime. Bars show means. Error bars show 95% CI of means.

Nitrogen concentration and uptake

Plant tissue nitrogen concentration at heading increased ($P < 0.001$) with increasing nitrogen input (Table 3.4). Plants fertilized with 220 kg N ha^{-1} had the highest nitrogen concentration (3.4%); while, those grown without nitrogen fertilizer contained only 1.6% N. Plants grown under non-flooded conditions also had the highest ($P < 0.001$) concentration of nitrogen (3.03%), compared to 2.21% for those continuously flooded.

Table 3.4: Nitrogen accumulation and nitrogen use efficiency at heading

Nitrogen level (kg ha^{-1})	Nitrogen content (%)	Nitrogen Uptake (g plant^{-1})	Nitrogen use efficiency	
			Nitrogen recovery (%)	Agronomic efficiency
0	1.66	0.08	-	-
110	2.82	0.34	35.8	8.7
220	3.47	0.44	23.9	5.9
LSD$_{(0.05)}$	0.15	0.03		
CV (%)	7.9	15.6		

There was a significant ($P < 0.01$) interaction between nitrogen and water regime. Plant tissue nitrogen concentration at heading increased with increasing nitrogen input under all, but the late flooding water regime, in which nitrogen concentration showed little increase beyond the 110 kg N ha^{-1} rate (Figure 3.13).

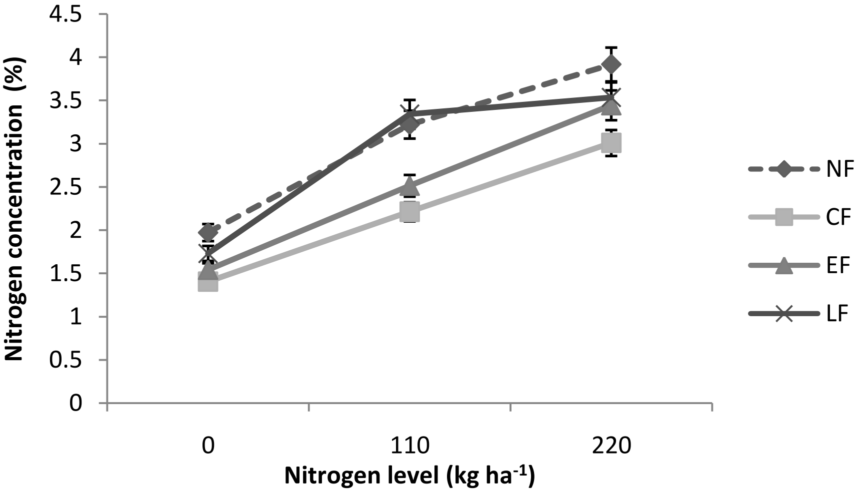


Figure 3.13: Plant tissue nitrogen concentration at heading as influenced by nitrogen application rate and water regime. Error bars indicate 95% CI of means.

Plant nitrogen uptake (the product of dry mass and nitrogen concentration) increased significantly ($P < 0.001$) with nitrogen level (Table 3.4). Uptake was about 4 - 5 times higher at 110 and 220 kg N ha^{-1} than that for the control treatment. However, uptake was only 1.3 times higher at 220 kg N ha^{-1} than that at 110 kg N ha^{-1}. That this pattern resembling that observed for nitrogen concentration and shoot dry mass is not surprising as nitrogen uptake is related to both factors.

The effect of the interaction of water regime and nitrogen dosage on nitrogen uptake (Figure 3.14) was highly significant ($P < 0.001$). When no nitrogen was applied, uptake by plants was significantly low under continuous flooding (about 0.05 g plant^{-1}), but higher under late flooding (0.12 g plant^{-1}). At 110 kg N ha^{-1}, there was no significant difference in nitrogen accumulation for all the water regimes. At 220 kg N ha^{-1}, nitrogen uptake by plants in both the continuous and early flooding regimes was significantly higher than those of the other two moisture regimes.

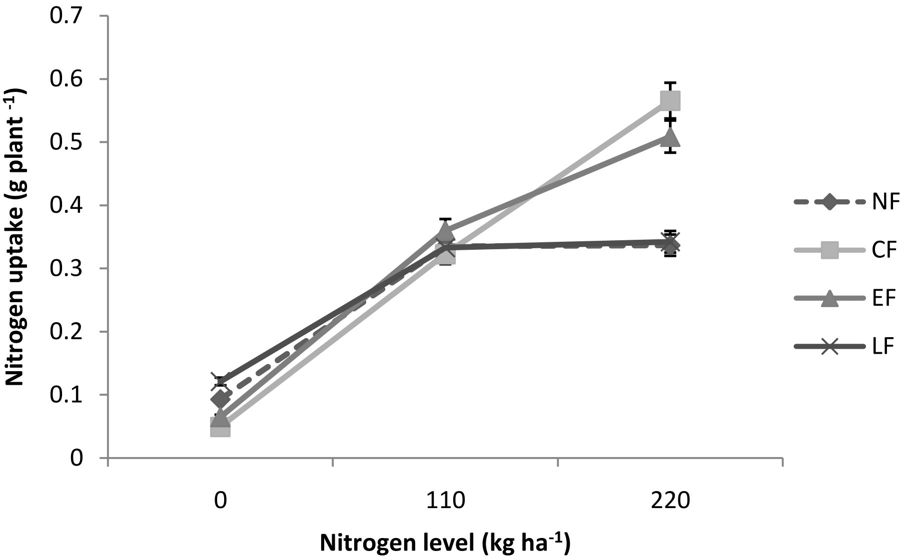


Figure 3.14: Effect of nitrogen dosage on nitrogen uptake under different water regimes. Error bars indicate 95% CI of means.

Nitrogen use efficiency

Nitrogen use efficiency (NUE), assessed by both recovery and agronomic efficiencies, differed with both nitrogen level and water regime. Fertilizer nitrogen recovery from the application of 110 kg N ha^{-1} was much higher than that from the 220 kg N ha^{-1} treatment (Table 3.4).

Nitrogen recovery at both nitrogen levels was consistently higher with continuous and early flooding compared to the non- and late flooded regimes (Figure 3.15). Under non-flooded conditions, nitrogen recovery at 220 kg N ha^{-1} was at least 50% lower than that at the 110 kg N ha^{-1} rate, suggesting waste of fertilizer resources. Both continuous and early flooding improved nitrogen use efficiency at both nitrogen input levels, but nitrogen use efficiency was poorest in the late flooding regime. Nitrogen recovery was less than 42% in all treatments.

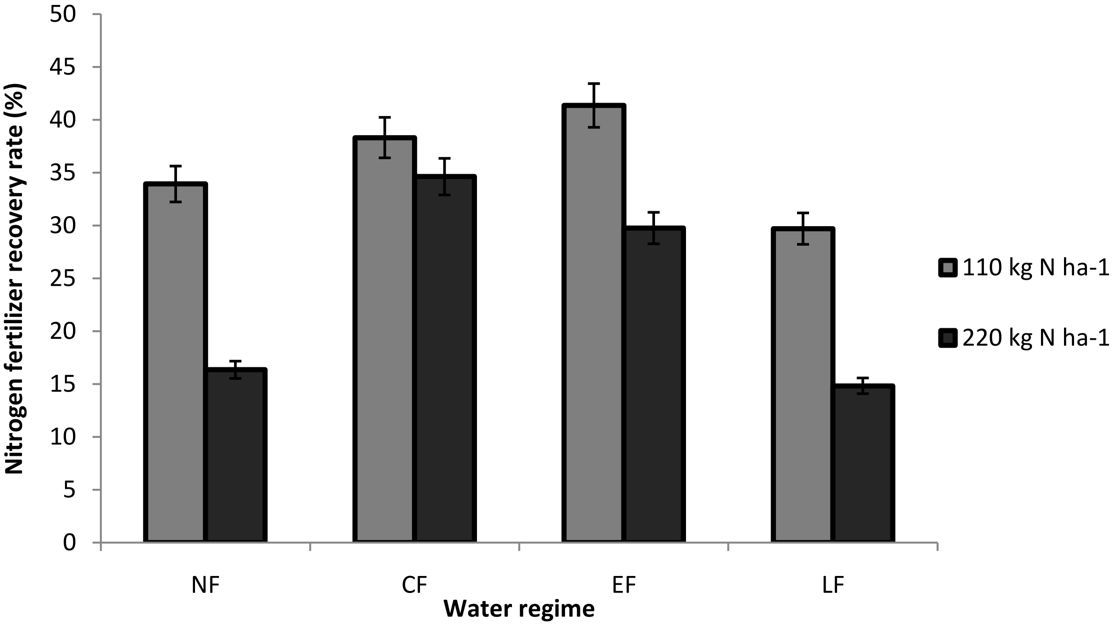


Figure 3.15: Nitrogen fertilizer recovery at heading for different water regimes. Bars indicate means. Error bars indicate 95% CI of means.

The agronomic nitrogen use efficiency – i.e. the ability of plants to increase yield in response to fertilizer input - for 110 kg N ha^{-1} was higher than that for 220 kg N ha^{-1} under all of the moisture regimes (Table 3.4). At both nitrogen levels, agronomic nitrogen use efficiency was highest for early flooding followed by continuous flooding. The non-flooded and late flooding water regimes showed the lowest agronomic nitrogen use efficiencies (Figure 3.16).

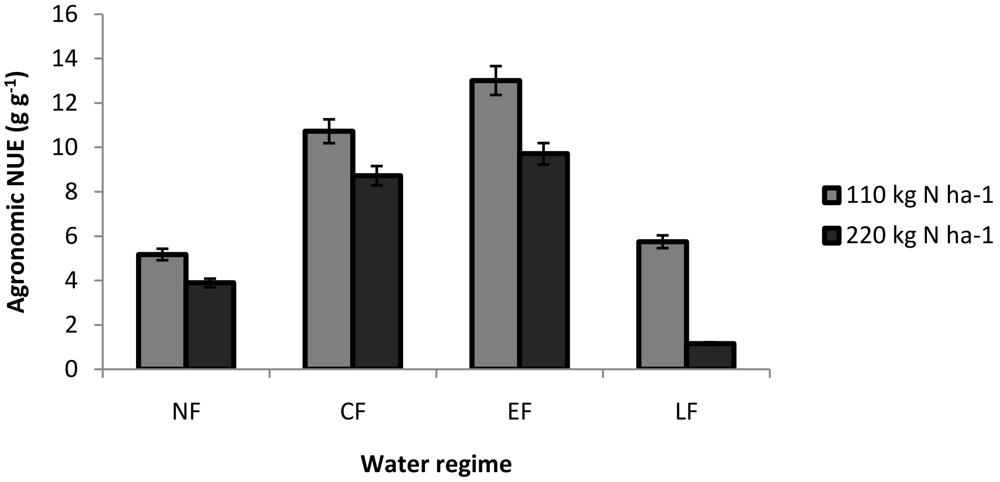


Figure 3.16: Agronomic nitrogen use efficiency for various water regimes. Bars indicate means. Error bars indicate 95% CI of the means.

Spikelet Fertility

Spikelet fertility was significantly low ($P < 0.05$) in plants grown under continuous flooding. The other treatments were however, not significantly different from one another (Figure 3.17).

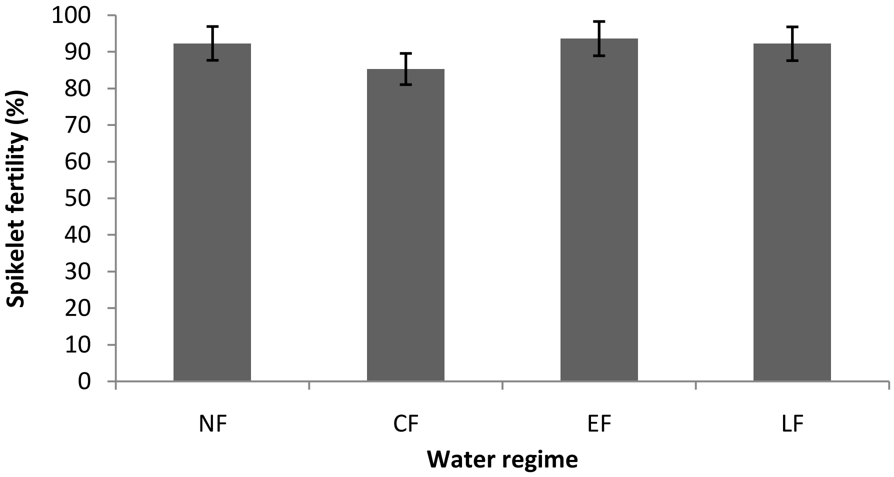


Figure 3.17: Effect of water regime on spikelet fertility. Bars represent means. Error bars show 95% CI of means.

Nitrogen application rate did not significantly affect ($P > 0.05$) spikelet fertility; however, its interaction with water regime was significant ($P < 0.05$). For all the water regimes, the difference in spikelet fertility was negligible at 0 kg N ha^{-1}. At 110 and 220 kg N ha^{-1}, spikelet fertility dramatically reduced under continuous flooding; however, under continuous flooding, spikelet fertility remained relatively high (97%) at 0 kg N ha^{-1} (Figure 3.18). Overall though, spikelet fertility was always higher than 80% irrespective of treatments.

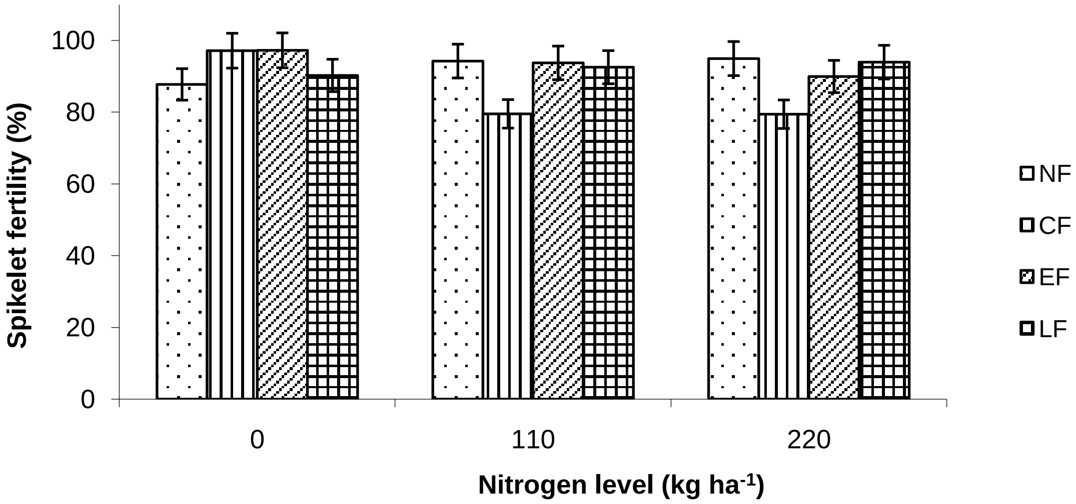


Figure 3.18: Effect of nitrogen on spikelet fertility for different water regimes. Bars represent means. Error bars show 95% CI of means.

Grain yield

Grain yield increased significantly ($P < 0.001$) with increasing nitrogen input (Figure 3.19). There was about a two-fold increase in average grain yield per plant when nitrogen application was increased from 0 to 110 kg ha^{-1}; however only a small increase was observed when nitrogen input was doubled to 220 kg ha^{-1}. A linear regression analysis accounting for about 60% of the variation in the yield response of nitrogen application rate showed a positive linear effect ($r = 0.77$) of nitrogen on grain yield, with yield estimated to rise by approximately 0.04 g for every kg ha^{-1} of nitrogen input (t pr. < 0.001).

Although a linear relationship was assumed between nitrogen input and grain yield, it was observed that a plot of the means for the nitrogen treatments (Figure 3.19) depicts a steady increase in grain yield as nitrogen input increased from 0 kg ha^{-1} to 110 kg ha^{-1}, following which the magnitude of increase diminished as the nitrogen rate increased to 220 kg ha^{-1}. The resulting curvature of that plot shows that yield may eventually stabilize or decline as nitrogen is increased.

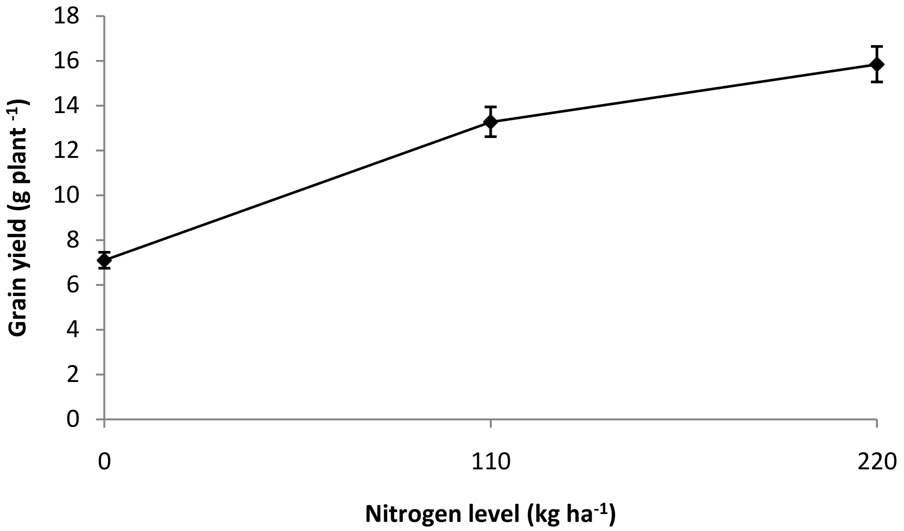


Figure 3.19: Relationship between nitrogen application rate and grain yield. Data points show means for different nitrogen application rates. Error bars show 95% CI of means.

Grain yield response varied significantly ($P < 0.01$) with the water regimes that were imposed. Plants subjected to early flooding produced the highest grain yield compared to those in the non-flooded and late flooding regimes (Table 3.5).

Table 3.5: Effect of water regime on dry matter allocation at grain maturity; values represent means.

	Water regime					
	No flooding	Continuous flooding	Early flooding	Late flooding	LSD (0.05)	CV (%)
Grain yield (g plant^{-1})	10.5	12.6	13.3	11.9	1.36	13.6
Harvest index	0.48	0.52	0.53	0.48	0.03	6.9

Grain yield was also affected significantly ($P < 0.001$) by the interaction of nitrogen and water regime (Figure 3.20). For plants grown without nitrogen, yield was consistently lower as the result of continuous and early flooding, but highest with late flooding. At 110 kg N ha^{-1}, yield significantly increased with flooding irrespective of when it occurred. When the nitrogen dosage was doubled to 220 kg ha^{-1}, yield was limited by the non- and late flooding treatments. The overall grain yield response to the treatments strongly related to the number of tillers (Figure 3.4) and nitrogen uptake (Figure 3.14).

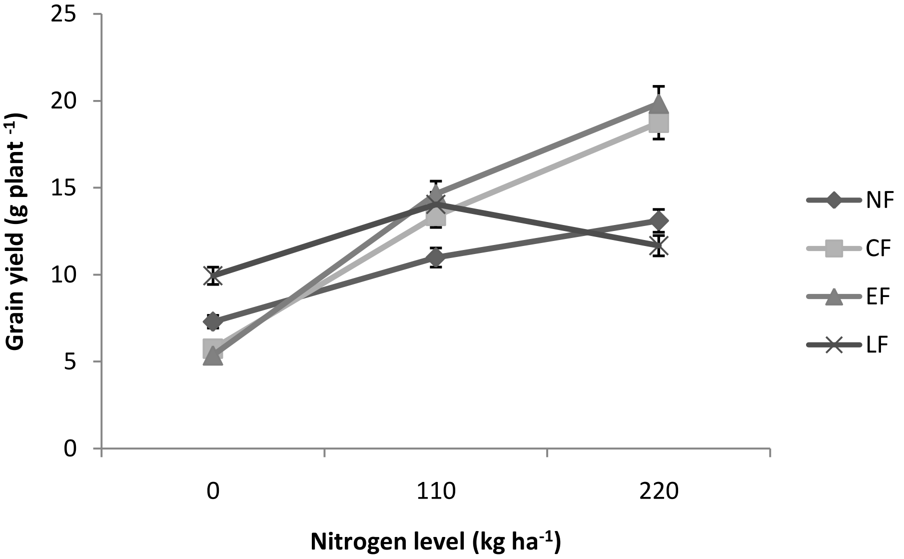


Figure 3.20: Grain yield response to nitrogen application rate and water regime. Data points represent means. Error bars show 95% CI of means.

Except for late flooding, yield increased with higher nitrogen input under all the water regimes. Under late flooding, yield increased as nitrogen input increased from 0 to 110 kg ha^{-1}; but, when nitrogen input was increased to 220 kg ha^{-1}, yield sharply decreased from 14.04 g plant^{-1} (accrued to 110 kg N ha^{-1}) to 11.67 g plant^{-1}.

Harvest index

Harvest index, the ratio of grain weight to total above ground dry matter, varied significantly ($P < 0.001$) with water regime (Table 3.5). There was no influence of nitrogen, nor was there an interaction ($P > 0.05$) between the two factors on harvest index.

The harvest indices of the plants grown under continuous and early flooding were significantly higher than those grown under the non- and late flooding regimes.

Relationship between grain yield and yield components

There was a significant positive linear relationship ($r^2 = 0.82$, $P < 0.001$) between tiller number and grain yield (Figure 3.21). Yield was estimated to rise by 2.39 g (t pr. < 0.001) for every panicle produced. Although panicle yield was strongly dependent on tillering (r = 0.96), only 77% of the variation in grain yield could be explained by the maximum number of tillers produced, while panicles accounted for an additional 5%.

The assessment of grain yield as a combined function of tillers and panicles revealed a non significant estimate of the tiller coefficient (t pr. > 0.05), suggesting that unlike tillering, panicles were the principal determinants of grain yield (Figure 3.21). Therefore, plants with higher tiller numbers out yielded their variants simply because most of their tillers eventually survived and developed grain-bearing heads.

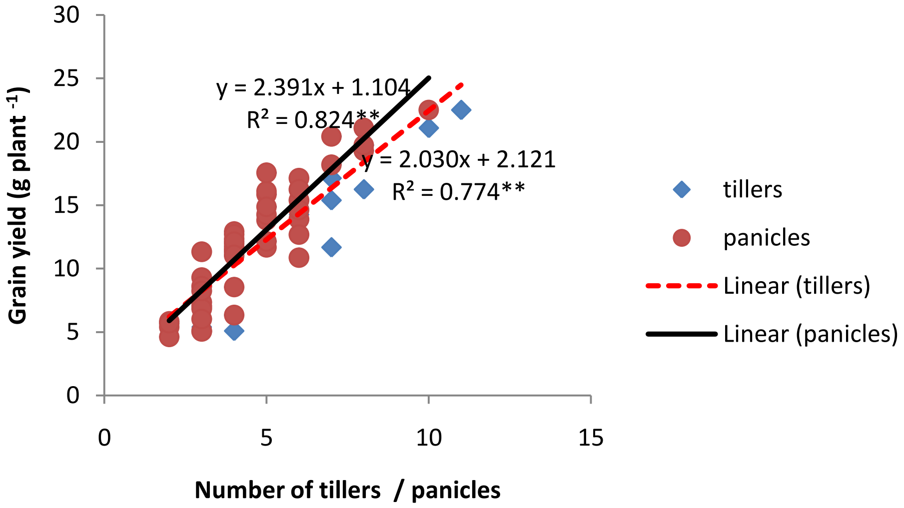


Figure 3.21: Relationships between grain yield and tillering / panicle production. Data points represent counts for both water regime and nitrogen dosage (n = 48)

Effects of biomass accumulation and nitrogen uptake on grain yield

Grain yield was positively related (r =0.85) to shoot biomass attained at heading (Figure 3.22) as well as total accumulated nitrogen in the plant tissues (r = 0.89) at that time (Figure 3.23). Grain yield increased by 0.813 g (t pr. < 0.001) for every gram of increase in above ground dry matter. Grain yield also increased by 24.9 g for every gram of nitrogen accumulated by the time of heading.

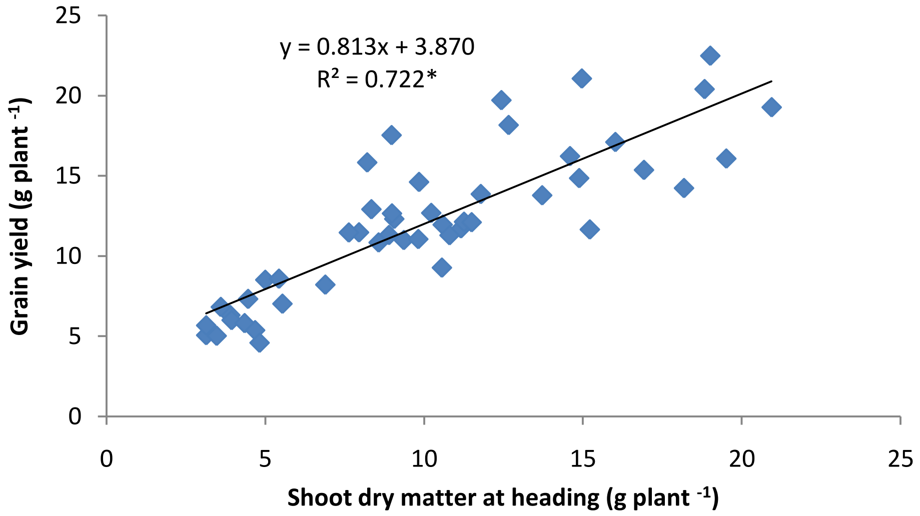


Figure 3.22: Relationship between biomass accumulation at heading and grain yield, (n = 48).

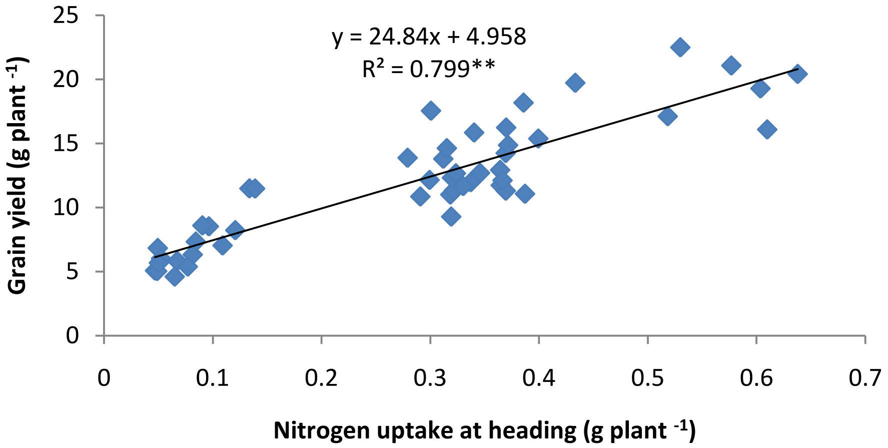


Figure 3.23: Relationship between nitrogen uptake at heading and grain yield. Data points represent counts for both water regimes and nitrogen dosage (n = 48).

DISCUSSION

The importance of nitrogen fertilizer as a tool for improving plant growth and productivity has been reported in numerous studies (De Datta *et al.*, 1968; Novoa and Loomis, 1981; Ten-Berge *et al.*, 1997; Andrews *et al.*, 2004). In the current study, the number of tillers, leaf area and dry matter accumulation at heading, as well as the number of panicles and grain yield at maturity increased with nitrogen as reported in rice and other cereal crops (Pearman *et al.*, 1978; Spiertz and De Vos, 1983; Heenan and Lewin, 1984; Kim *et al.*, 2001). However, the relatively small difference between the growth and yield responses to nitrogen at 110 and 220 kg ha^{-1}, compared to that between 0 and 110 kg ha^{-1} shows that there is a limit to which increasing nitrogen input can be exploited to improve plant growth and yield. Such diminishing returns to nitrogen reportedly becomes stronger at higher levels of nitrogen input (Cassman and Pingali, 1995; Tilman *et al.*, 2002).

Thus, increasing nitrogen dosage beyond the requirements for optimum grain yield may result more in an increased vegetative biomass as found in this study (Figure 3.12), and possibly, an increase in microbial biomass or soil organic carbon (Wang *et al.*, 2009); but the immediate benefit in terms of grain yield may be minimal. Nonetheless, a relatively high nitrogen input to high yielding rice cultivars can ensure an improved leaf nitrogen content at heading and is therefore critical for the attainment of higher grain yield (Hasegawa, 2003).

Nitrogen application rate did not affect spikelet fertility and harvest index. The plants grown without nitrogen fertilizer were expected to produce more empty spikelets. This did not happen because the number of vegetative and grain-bearing tillers produced at 0 kg N ha^{-1} was relatively small. In line with the results of this study, Mauad *et al*. (2003) observed that prolific tillering, coupled with inadequate nitrogen led to a reduction in the percentage of fertile spikelets. This was particularly true when continuous flooding led to an increase in tiller production at the 110 and 220 kg N ha^{-1} inputs, thereby causing a significant decrease in spikelet fertility. The occurrence of a negative relationship between high nitrogen rates and the fertility of rice spikelets is consistent with observations by Muirhead *et al*. (1989) and Zhang *et al.* (2000).

High nitrogen application to cereal crops is known to decrease harvest index since it encourages high dry matter accumulation (Donald and Hamblin, 1976). Even though the application of nitrogen at 110 and 220 kg ha^{-1} increased biological yield, it did not significantly change harvest index since the agronomic (grain) yields recorded at both rates were equally high (Figure 3.19). Rather than nitrogen application, flooding regime was largely responsible for the variation in harvest index (Table 3.5).

The high harvest indices recorded for both continuous and early flooding can be attributed to the high nitrogen use efficiency that ensued under the two water regimes (Figures 3.15 & 3.16). This observation is corroborated by findings that rice cultivars with large harvest index were more efficient in nitrogen use (Bufogle *et al.,* 1997). The relatively higher harvest index under continuous and early flooding suggests that plants grown under flooded conditions for the most part of their vegetative development are capable of allocating a larger proportion of their dry mass to grain yield, compared to those grown under dryer conditions. Similarly, results for a eight season trial conducted by Peng *et al.* (2006a) showed that harvest index in flooded rice was higher than that in rice grown under aerobic culture.

Several instances of declining nitrogen use efficiency in rice as a result of higher nitrogen application rates have been reported (Ohnishi *et al.,* 1999; Fageria and Baligar, 2001; Xie *et al.,* 2007). That both nitrogen recovery and agronomic nitrogen use efficiency were higher at 110 kg N ha^{-1} than they were at 220 kg N ha^{-1} (Table 3.4), raise the question of the fate of excessive nitrogen application. Some authors have attributed low efficiency of nitrogen use at higher nitrogen rates to either sustained nitrogen loss via various pathways such as NO_2, NO and N_2 emissions, and NH_3 volatilization (Bock *et al.,* 1995; Delgado, 2002), or to immobilization by soil microorganisms (Azam *et al.,* 1986; Cheshire *et al.,* 1999; Hirel *et al.,* 2007).

Since in addition to plant photosynthetic efficiency, total nitrogen uptake accounts for the level of efficiency in nitrogen use by crop plants (Nielsen, 2006), the low nitrogen use efficiency of the 220 kg N ha^{-1} treatment may be attributed to the loss of nitrogen from the pots. Even though the quantification of nitrogen loss was not a focus of this study, the elimination of surface run-off and leaching as the possible mechanisms of loss suggests that gaseous emission by volatilization or denitrification may have been responsible for most of the nitrogen loss, if any, that took place particularly in the flooding treatments. To ensure efficient agronomic use of nitrogen, Peng *et al.* (2006b) have recommended the reduction of nitrogen rate to around 120 kg ha^{-1} in high tillering plants.

Compared to the aerobic control, flooding increased the growth and yield performances of the plants as generally reported in many other studies (Sah and Mikkelsen, 1983; Eriksen *et al.*, 1985; Castillo *et al.*, 1992; Abdul-Shukor *et al.*, 2009). Like nitrogen application rates, flooding regimes differed in respect of their effects on several growth, yield and other agronomic parameters. Continuous and early flooding increased the number of tillers and panicles, above ground biomass, leaf area and nitrogen uptake at heading because they provided more water to maintain constant turgor and growth than did the non- and late flooded water regimes during the vegetative growth stage. Fukai and Inthapan (1988) found that under non-flooded conditions, rice plants exhibited stiff internal resistance to the flow of water even if the soil moisture content was high. This inadvertently led to low leaf water potential which has the propensity to retard growth (Lilley and Fukai, 1994).

Grain yield under early flooding was slightly higher than that under continuous flooding probably because of better rhizhosphere aeration that led to more panicle production and improved spikelet fertility. McHugh *et al.* (2007) noted that instead of continuous flooding, alternate flooding can be practiced in rice production without significantly offsetting yield. Because plants subjected to continuous and early flooding produced the highest grain yield response to the highest nitrogen dosage, compared to the other two water regimes, it suggests that if yield must be increased by increasing nitrogen dosage, the input of nitrogen must be done concomitantly with increased water level during both the vegetative and reproductive stages of the crop. George *et al.*(1992) affirmed that large inputs of nitrogen are usually required to support yield increment in flooded rice.

Compared to other flooding regimes, continuous flooding negatively influenced the fertility of spikelets. Although Zhang *et al.* (2008) showed that spikelet fertility is usually higher under well watered upland conditions than under flooded conditions, the suppression of spikelet fertility was probably due to inter tiller competition which is known to increase with increasing tiller numbers since late tillers were also involved. The role of late tillers in the promotion of spikelet fertility was also emphasized by Vergara (1992).

Nitrogen uptake was more efficient under continuous and early flooding than it was under non-flooding; however, this differs with findings by Vlek and Byrnes (1986), who earlier observed that nitrogen uptake in upland moisture environments may be about 50 – 60% more efficient than in flooded situations. The recovery efficiency of nitrogen under the late flooded condition was poor mainly because of the low concentration of nitrogen in the tissues of the plants, coupled with the new tiller growth that ensued. Even though nitrogen uptake slightly increased under late flooding, it was noted that flooding occurred too late to effect a significant increase in nitrogen uptake. Indications are that water may have played a pivotal role in the absorption of nitrogen by increasing the rate of uptake of the available nutrients as enhanced by transpiration, which tends to be higher under flooded than aerobic water conditions (Chen *et al.*, 2001; Kato and Okami, 2010).

Compared to the continuous aerobic and late flooding regimes, the lower shoot nitrogen concentration recorded for the continuous and early flooding regimes at heading may be attributed to the larger tiller numbers and shoot dry weights that were obtained under those moisture conditions. Nonetheless, the poor nitrogen use efficiency obtained under the late flooding and continuous aerobic conditions indicates that most of the applied nitrogen was eventually wasted. Similar incidence of low nitrogen use efficiency in rice grown under non-flooded field capacity conditions was reported by Jahan (2004) and Sariam (2004).

Grain yield in this study, depended more on the number of tillers produced than the ratio of ineffective to fertile tillers, since late tillering and tiller mortality were significantly low. The strong relationship found between tillering, panicle number and grain yield is consistent with findings from many other studies (Yoshida, 1972; Gravois and Helms, 1992; Fageria, 2009). The positive association of shoot dry weight at heading with grain yield in this study is also consistent with reports by Dingkuhn *et al.* (1998) and Fageria (2007).

CONCLUSION

Nitrogen response of rice in wetland ecologies is influenced heavily by flooding patterns. Compared to upland conditions characterized by no flooding, flooding with standing water at 5 cm above the soil surface enhanced the vegetative growth and yield of rice irrespective of when it occurred. However, continuous and early flooding led to a significant increase in grain yield. Because of the large quantity of water required for the production of flooded rice, water use can be greatly reduced if intentional flooding is halted around the time of panicle initiation to heading. Continuous and early flooding enhanced both nitrogen recovery and nitrogen use efficiency for grain production; hence, those flooding regimes are significant for economic returns, particularly in lowland areas that are fed by abundant rainfall such as in Liberia. However, further studies may be needed to quantify the optimum surface water depth for maximum rice performance.

CHAPTER 4

INFLUENCE OF SPLIT APPLICATION OF NITROGEN ON THE GROWTH, YIELD AND QUALITY OF RICE GROWN UNDER TWO CONTRASTING FIELD MOISTURE CONDITIONS

ABSTRACT

Improvement of nitrogen utilization in rice cropping systems remains a major challenge to sustainable production, especially in the wake of rising fertilizer costs. The aim of the current study was to determine the effect of different split nitrogen application strategies on the yield and quality of rice grown in flooded and non-flooded soils. A field trial was conducted from November 2009 to April 2010, utilizing a randomized split-split plot combination of two soil water regimes, two nitrogen application strategies, and three rice cultivars. Tillering, leaf area index, dry matter accumulation and nitrogen assimilation were measured at heading, and yield components, grain yield and quality were determined at maturity.

Nitrogen application in two doses increased above ground dry matter, leaf area index, as well as the number of tillers and panicles irrespective of water regime. Also, irrespective of water regime, grain protein content increased as the amylose component of its starch granules decreased in response to the three-split nitrogen treatment. However, the effect of nitrogen application strategy on nitrogen uptake, grain yield and quality varied with water regime and cultivar. In the absence of flooding, the two split nitrogen treatment increased nitrogen uptake and grain yield, while the three-dose treatment generally improved these parameters in the flooded plots. GM-1 and FKR-19 were more responsive to the two-split nitrogen treatment than N-19, whose growth and yield responses were higher at the three-dose application. Flooding increased all growth and yield responses, except spikelet fertility and grain amylose content.

The results showed that management of nitrogen application to achieve nitrogen use efficiency in rice production will depend highly on the water regime under which the crop is cultivated, as well as on cultivar. Hence, in flooded rice, three split application of nitrogen may improve yield by helping to maintain optimum nitrogen level in plant tissue

for grain development, while in non-flooded soils, it may do comparatively little to increase growth and dry matter accumulation.

INTRODUCTION

Rice yield in Sub-Saharan Africa is reportedly the lowest amongst the rice producing regions of the world (Murison, 2002; CGIAR, 2006). In order to improve yield, farmers in the region will have to consider investment in improved agricultural inputs, including nitrogen fertilizer. However, due to the limited supplies of nitrogen, coupled with the continual increase in the price of the commodity, maximizing returns from such investment will require the adoption of improved nitrogen management techniques. Considerable progress has been reported with regards to the impact of the type, depth of placement and rate of nitrogen application on rice performance in various rice cropping systems (De Datta, 1981; Mikkelsen, 1987; Ten-Berge *et al.*, 1997; Ghosh and Bhat, 1998; Bodelier *et al.*, 2000). However, based on reports from other rice producing regions, the timing of nitrogen application remains a subject for further investigations in Sub-Saharan Africa.

In addition to the limited use of nitrogen fertilizer in most Sub-Saharan African countries including Liberia, reports of investigations into the implications of the timing of its application to rice crops are usually lacking. Where fertilizer is used in rice production, the predominant practice involves the placement of fertilizer in three split doses (basal or at the commencement of tillering, at mid-tillering, and at booting). This practice seemed inefficient, particularly at high nitrogen rates as observed in the preceding study (Chapter 3). Rice requires adequate nutrition during its developmental stages to enhance its yield and productivity (Fageria *et al.*, 2006); however, supplying nitrogen at a time that is inappropriate for uptake and yield enhancement may result in wastage of the limited fertilizer resources, or poor yield and quality of the grains (Bhuiyan *et al.*, 1984; De Datta, 1987b; Fujisaka, 1993).

Appropriate timing of nitrogen application is therefore, an essential factor to consider for the improvement of rice growth, yield and grain quality. It is also important for the improvement of nitrogen use efficiency and control of nitrogen loss in rice production systems. Reports from most rice producing regions of the world suggest that the timing of nitrogen application may vary with a number of factors (Ten-Berge and Kropff, 1995;

Spiertz, 2009). In South-east Asia where the crop is pre-dominantly produced, the timing of nitrogen application is reported to vary widely with cultivar and amongst farmers (Witt *et al.*, 2005). For example, four split applications of nitrogen (basal, tillering, panicle, and grain filling) was recommended for hybrid rice production in China (Yuan *et al.*, 2003). In the Mississippi region of the United States, farmers reportedly apply a single dose of nitrogen fertilizer after flood establishment (Stevens *et al.*, 2001). Also, the glasshouse experiment reported in this thesis (Chapter 3) suggests that late application of nitrogen is of little consequence in terms of grain yield, but stimulates late tiller production which can delay harvesting. Furthermore, nitrogen uptake was very heavily dependent on the water regime adopted.

In view of the wide diversity in the timing of nitrogen application in rice cultures, the problem of nitrogen use efficiency for grain yield production remains a major issue of concern in any given rice ecology. The aim of the current study was to examine the influence of split nitrogen application strategies on the growth, yield and grain quality of three rice cultivars under two contrasting water regimes, with the view to establishing optimum practices in field conditions. Results from the study may be used as a guide to formulate a nitrogen application plan in rice cultivation.

MATERIALS AND METHODS

The field study was conducted from November 2009 to April 2010 at the Ukulinga farm (29^{o}40’ S, 30^{o}24’ E, 806 m elevation) of the University of KwaZulu-Natal, Pietermaritzburg. Based on the annual averages of long term climatic data, the location has a mean annual temperature and rainfall of 18^{o}C and 738 mm, respectively. Major climatic variables, including rainfall, around the period of the study are presented in Table 4.1 and Figure 4.1. Table 4.2 shows the basic physio-chemical properties of the soil.

Table 4.1: Monthly climatic data during the field trial at Ukulinga, PMB (November 2009 – April 2010). Data source: Agricultural Research Council (ARC), SA.

	Minimum temperature (^{o}C)	Maximum temperature (^{o}C)	Solar radiation (MJ m^{-2})	Evapo-transpiration (mm day^{-1})
November	13.67	23.68	14.00	2.85
December	14.99	24.25	14.58	2.94
January	16.57	25.95	14.92	3.03
February	17.56	28.04	17.14	3.63
March	16.24	27.12	13.47	2.84
April	14.47	25.36	11.39	2.33
Mean	15.58	25.73	14.25	2.93

Table 4.2: Physio-chemical properties of the soil used for field study (Ukulinga, October 2009)

Element	Soil test	
	0 -15 cm depth	15 – 30 cm depth
Nitrogen (%)	0.27	0.26
Phosphorus (mg L^{-1})	14	10
Potassium (mg L^{-1})	117	111
Calcium (mg L^{-1})	1887	1927
Magnesium (mg L^{-1})	1041	1071
Manganese (mg L^{-1})	7	8
Zinc (mg L^{-1})	4.1	4.1
Organic carbon (%)	1.6	1.2
Organic clay (%)	28	29
pH (KCl)	4.89	4.89
Total cations (cmol L^{-1})	18.2	18.5

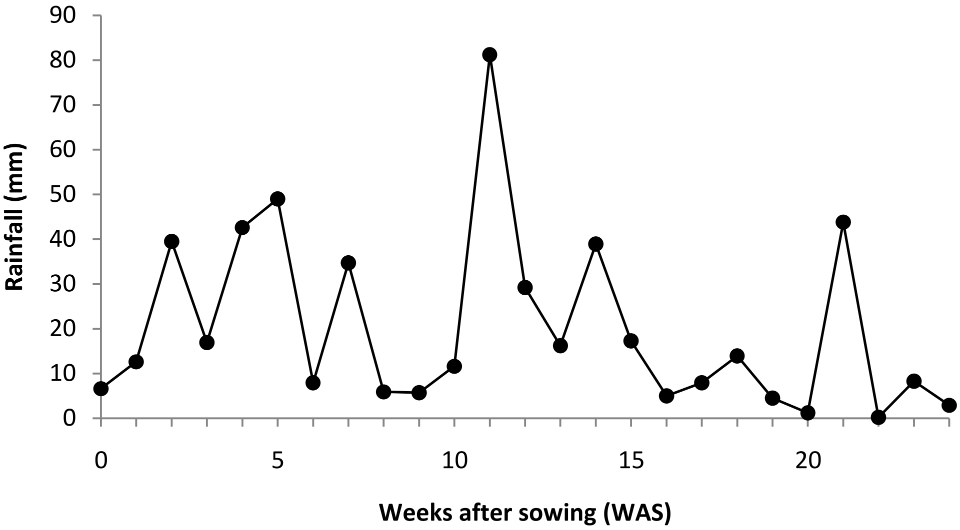


Figure 4.1: Weekly rainfall, Ukulinga (November 2009 – April 2010). Data represent averages from hourly recordings by an automatic weather station (ARC, SA).

Plant establishment

Plants were established by directly sowing three pre-soaked seeds per hill. Row and inter-row plant spacing was 15 x 15 cm. In order to obtain a uniform stand, plants were thinned to one per hill at 14 days after emergence (DAE).

Treatments and field layout

Plant materials used in the field study comprised three rice cultivars, namely, NERICA-L-19 and FKR-19 acquired from Africa Rice Centre (WARDA) and Golden Mountain #1 (GM-1) used in experiment 1 (Chapter 3). Because GM-1 is an adopted rice variety in South Africa and is well adapted to the prevailing outdoor agro-ecological conditions, it was used in this study as the check variety. The NERICAL-L-19 (hitherto referred to as N-19) and FKR-19 are prominent lowland cultivars in West Africa (Somado *et al*., 2008) and are currently being tried in Liberia under both rain-fed and irrigated conditions.

The experimental design was a randomized split-split plot combination of two water regimes, two nitrogen application strategies and three cultivars, replicated in three blocks. The water regimes (flooded and non-flooded, but well watered) were the main plots. The sub-plots were the nitrogen application strategies (two- and three-split applications), and the sub-sub plots were the cultivars. Rather than the nitrogen splits, the cultivars were used as the sub-sub plots because of the lack of space and man-power to effect an adequate sub-sub plot isolation of the two nitrogen variates.

The split nitrogen doses were as follows: 1) 50 % of the nitrogen supplied at 21 DAE and the other 50% at the mid-tillering stage, i.e., at 60 DAE ($N_{50/50}$), and (2) 50 % nitrogen at 21 DAE, 25% at the mid-tillering stage, and the other 25% at booting, i.e., at 80 DAE ($N_{50/25/25}$). Urea was used as nitrogen source and supplied at 110 kg N ha^{-1} based on results from experiment 1 (Chapter 3).

The dimensions of the main, sub- and sub-sub plots were 4.0 x 7.0 m, 2.0 x 3.5 m, and 2.0 m x 1.0 m, respectively. In order to minimize inter-plot interferences, the main plot, sub-plot and block isolations were 2.0, 2.0 and 3.0 m, respectively. The sub-sub plots were separated by 25 cm access paths. The waterlogged plots were bunded with a 46 micron-thick plastic sheet buried 30 cm deep, and the external borders clogged with soil to avoid leakage of water from the plots (Figure 4.2).

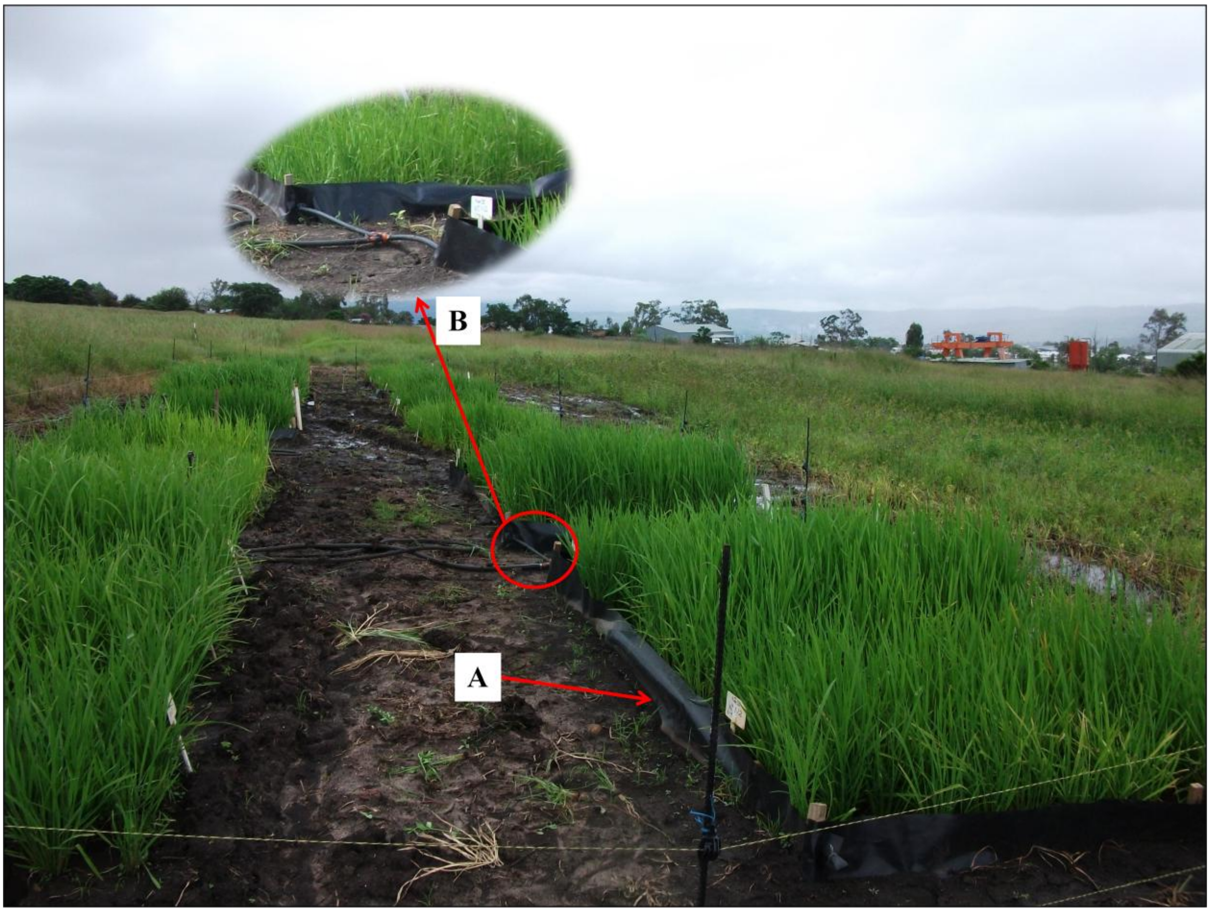


Figure 4.2: Field trial lay out showing flooded plots bunded with black plastic sheets buried into the soil (A). Valve-controlled perforated tubes were used to establish and maintain water level in the flooded plots (B).

The continuous flooding treatment was characterized by field submergence with up to 5 cm standing water commencing at 25 DAE, and lasted up to maturity. However, during the crop growth period, the flooded plots were drained two days prior to application of the nitrogen fertilizer and re-flooded three days later in order to facilitate placement and incorporation of the fertilizer into the soil. Also, to facilitate harvest, flooded plots were drained two days prior to harvest. Non-flooded plots were irrigated by sprinklers, while continuous flooding was established and maintained by drip irrigation. Each flooded plot was lined across its length with 2.0 cm poly tubing, fitted at 15 cm intervals with 6 katif drippers. The tubes were connected to ball valves (Figure 4.2) that were used to regulate the flow of water into the plots. To ensure that the non-flooded plots were free of water stress, soil moisture tension at the 30 and 60 cm profile depths of the soil was monitored three days a week using tensiometers (Irrometer Co., California). Readings from the soil moisture sensors (Figure 4.3) were also used to inform irrigation decisions.

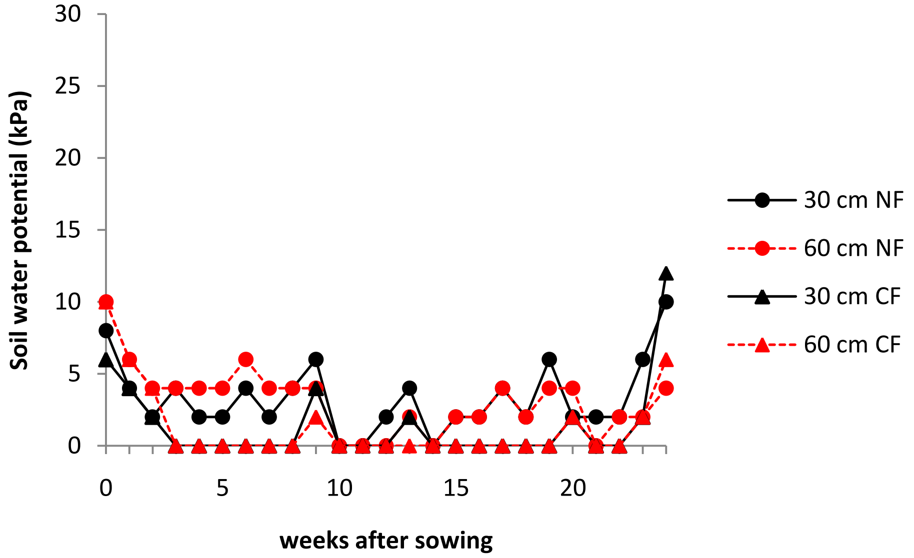


Figure 4.3: Soil water potential at 30 and 60 cm depths in flooded (CF) and non-flooded (NF) plots.

Data collection and analysis

Growth characteristics

Tiller number was counted weekly to determine maximum tiller production. Ten plants were randomly selected per plot for tiller count till heading. At heading, above ground plant materials were cut from a random sample area of 0.225 m^2 (containing 10 plants) in every sub-sub plot. Leaf area was measured using a portable leaf area meter (LI-3000, LI-COR Biosciences) equipped with an LI-3050A belt conveyor. Leaf area index was determined as the ratio of leaf area to land area covered. Leaves and culms were oven dried at 70^{0}C for 48 h to determine above ground dry matter accumulation.

Grain yield and yield components

At maturity an area of 0.225 m^2 containing 10 plants was randomly sampled for yield and yield component measurements. Tillers, including the main culm, bearing at least 10 spikelets were considered for panicle count. Straw dry weight was measured as described above for above ground dry matter accumulation at heading and used to calculate harvest index. Grain yield and 1000-grain weight were measured at 14% moisture content (wet basis) after oven drying at 70^{o}C for 3 – 5 days. Empty and filled spikelets were separated by water flotation for the measurement of spikelet fertility. Harvest index was determined as the ratio of grain weight to total above ground dry matter yield at maturity. Border row plants were excluded from all sample measurements.

Nitrogen uptake

Plant tissue nitrogen concentration at heading was determined by the Dumas combustion method using a TruSpec CN – elemental analyser (LECO Corporation) as described in Chapter 3. Nitrogen uptake was calculated as the product of above ground dry weight and plant tissue nitrogen concentration.

Irrigation and water use

Rainfall data was obtained from an automatic weather station by the site of the trial. Weekly rainfall data were calculated from daily measurements compiled from hourly readings. A measuring cylinder was used to quantify effective irrigation delivered by the irrigation system. Weekly water supplied to plots was calculated as the sum of rainfall and irrigation (Equation 4.1). Water use efficiency (WUE) was calculated according to Equation 4.2 (Israelsen and Hansen, 1962).

$$\text{Water supply per week} = \text{plot size} \times (\text{irrigation} + \text{rainfall}) \qquad (4.\ 1)$$

$$\text{WUE} = \text{grain yield (g m}^{-2}\text{)} / \text{total water supplied (L m}^{-2}\text{)} \qquad (4.\ 2)$$

Grain quality

Grain quality was assessed from measurements of grain protein and apparent amylose content at maturity. Rice flour used for protein and amylose measurements were obtained from 300 g samples of rough rice which were dehulled using a rubber-roll rice huller

(Satake, Japan). Samples weighing 100 g from the resulting brown rice were ground to produce rice flour using a cyclone mill fitted with a 0.5 mm sieve.

For protein analysis, rice flour was extracted according to the procedure described by Rao *et al*. (1993) with modification. A 1 g rice flour was extracted with 2 ml hot buffer containing 0.25 M TrisHCl (pH 8.0), 0.4% SDS, 20 mM EDTA, 2.0 mM PMSF and 5% β-mercaptoethanol and centrifuged at 12,000 x g for 10 min. The crude protein concentration of the supernatant was measured using the Coomassie brilliant blue G-250 procedure described by Bradford (1976). Bovine serum albumin (BSA) was used as protein standard.

In order to prevent interference from lipids during amylose measurement, rice starch was first purified by ethanol defatting according to the method of Chiou *et al*. (2002) with slight modifications. Rice flour samples (10 g) were incubated in 25 ml, 70% ethanol at 70^{o}C for 30 min, after which the mixture was centrifuged at 3,000 x g for 15 min. The supernatant was discarded and the procedure repeated three times. The starch-containing pellets were then collected, freeze dried and stored at 4^{o}C till ready to analyze.

Amylose was quantified according to the iodine calorimetric assay method of McGrance *et al*. (1998), modified by Hoover and Ratnayake (2001) with slight adjustments. 8 ml 90% DMSO was added to samples of defatted rice starch (0.02 g) and vigorously mixed for 2 min using a vortex mixer. The samples were then heated at 85^{o}C in a water bath for 30 min with constant shaking, after which they were allowed to cool for 45 min. No clear gel was seen after the dispersed solution cooled. The cooled samples were diluted to 25 ml with distilled water. 1 ml aliquots of the diluted solution were then added to 40 ml distilled water and 5 ml I-KI (0.0025 M I_2, 0.0065 M KI) solution, and made up to 50 ml in a volumetric flask. Colour was allowed to develop for 15 min, after which absorbance was read at 600 nm using a DU-800 spectrophotometer (Beckman Coulter). In order to maintain the traditional classification system (Perez and Juliano, 1988) the standard curve was prepared using a series of 0.014 g mixtures of purified potato amylose and amylopectin azure (Sigma Corporation) containing 0, 10, 20, 40, 50, 60, 80 and 100% amylose.

Data analysis

The data were subjected to analysis of variance (ANOVA) using the windows statistical software package, GENSTAT version 12. The means of treatments showing significant difference were subjected to Fisher's Least Significance Difference (LSD) test and separated at the five percent level of significance ($P = 0.05$).

RESULTS

Above ground dry matter accumulation

Above ground dry matter accumulation at heading differed significantly ($P < 0.001$) with cultivar, water regime and nitrogen application strategy. The cultivar N-19 yielded the largest dry matter, followed by FKR-19 (Figure 4.4). In all the cultivars, continuous flooding increased above ground dry matter significantly ($P < 0.01$) at heading irrespective of the nitrogen application strategy (Figure 4.4).

There was a significant ($P < 0.001$) interaction between nitrogen application strategy and cultivar for dry matter accumulation. In FKR-19 and N-19, there was no significant ($P > 0.05$) difference in above ground dry matter between the nitrogen application strategies; but in GM-1, application of nitrogen in two doses resulted to a significantly higher above ground dry matter accumulation, compared to application in three doses (Figure 4.4).

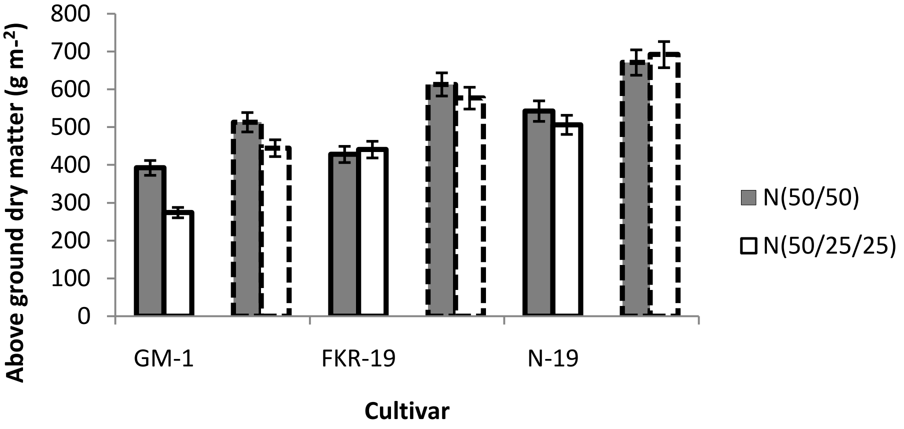


Figure 4.4: Above ground dry matter accumulation at heading for different cultivars as affect by water regime (— Non-flooded / - - - Flooded) and nitrogen application timing. Bars represent means. Error bars indicate 95% CI of means.

Leaf area index

There was a significant ($P < 0.001$) difference in leaf area index as a result of interactions between the cultivars and water regime. Under continuous flooding, the leaf area index of FKR -19 was significantly higher than that of GM-1; but under the non-flooded condition, the leaf area index of FKR-19 was significantly lower than that of the other two cultivars, whose leaf area indices were practically the same.

The response of leaf area index at heading generally resembled that of the above ground dry matter accumulation (Figure 4.5). The cultivar N-19 developed the highest leaf area index, followed by FKR-19; continuous flooding led to a significant increase in leaf area index, while the two dose nitrogen application increased leaf area index in all the cultivars (Figure 4.5).

Like above ground dry matter accumulation, the interaction between cultivar and nitrogen application strategy was significant ($P < 0.01$) for leaf area index. Application of nitrogen in two doses significantly increased the leaf area indices of GM-1 and N-19; but, it did not significantly change the leaf area index of FKR-19 (Figure 4.5).

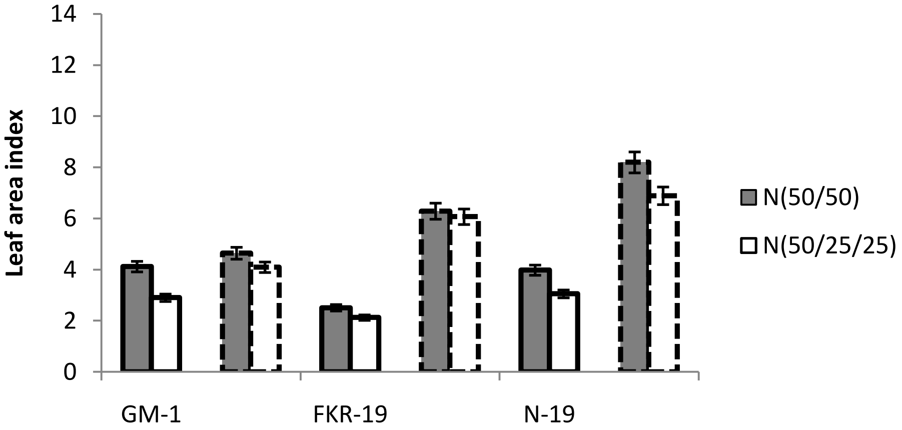


Figure 4.5: Effect of water regime (— Non-flooded / - - - Flooded) and nitrogen timing on leaf area index at heading. Bars represent means. Error bars indicate 95% CI of means.

Tiller and panicle production

The number of tillers at heading was influenced significantly ($P < 0.01$) by cultivar, nitrogen strategy and water regime (Table 4.3); but there was no significant interaction amongst any of the treatment combinations with respect to tiller production. FKR-19 had the highest number of tillers, followed by N-19. Continuous flooding led to the production of more tillers than did non-flooding, while nitrogen application in two doses promoted the production of more tillers under both water regimes, compared to the three-dose application.

Table 4.3: Tiller and panicle production as influenced by water regime and nitrogen application timing.

	Tillers m^{-2}			
	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	468	349	568	464
FKR-19	795	596	896	701
N-19	665	558	828	642
Means	643	501	764	602
				CV (%) = 7.0 $LSD_{(0.05)}$ = 73
	Panicles m^{-2}			
	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	383	318	464	408
FKR-19	437	403	510	459
N-19	418	372	459	426
Means	413	364	478	431
				CV (%) = 3.5 $LSD_{(0.05)}$ = 22

At maturity, the number of panicles m^{-2} varied significantly ($P < 0.001$) with cultivar, water regime and nitrogen application strategy. FKR-19 produced the largest number of panicles, while GM-1 produced the least. The number of panicles increased significantly with the application of nitrogen in two doses, and with continuous flooding.

For panicle production, there was a significant ($P < 0.05$) interaction between water regime and the cultivars (Table 4.3). Under continuous flooding, the number of panicles produced by FKR-19 was significantly higher than those of the other two cultivars; but the difference between the panicle numbers produced by GM-1 and FKR-19 was not significant, as was the case under no flooding.

Tiller efficiency varied significantly ($P < 0.001$) with cultivar (Table 4.4). GM-1 produced the highest proportion (85.7%) of tillers that eventually developed into matured panicles. However, the difference in tiller efficiency between FKR-19 and N-19 was not significant.

Tiller efficiency increased significantly ($P < 0.01$) with three-split nitrogen application. Although tiller efficiency increased with no flooding, the magnitude of increase was not significant ($P > 0.05$).

Table 4.4: Tiller efficiency of different cultivars as influenced by water regime and nitrogen application timing.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	0.82	0.91	0.82	0.88
FKR-19	0.55	0.68	0.57	0.66
N-19	0.63	0.67	0.55	0.66
Means	0.67	0.75	0.65	0.73
				CV (%) = 5.6 $LSD_{(0.05)}$ = 0.06

1000 grain weight

Cultivar difference in 1000 grain weight was highly significant ($P < 0.001$), with GM-1 weighing the highest (30.6 g); however, the difference between FKR-19 and N-19 (23.6 g and 23.1 g) was negligible. Flooding significantly ($P < 0.05$) increased 1000 grain weight, compared to no flooding.

In general, the nitrogen application strategy did not significantly ($P > 0.05$) affect 1000 grain weight. However, there was a significant ($P < 0.05$) interaction between cultivar and nitrogen application strategy. In GM-1, application of nitrogen in three doses significantly increased 1000 grain weight, while in N-19, 1000 grain weight increased significantly with the two-dose application (Figure 4.6).

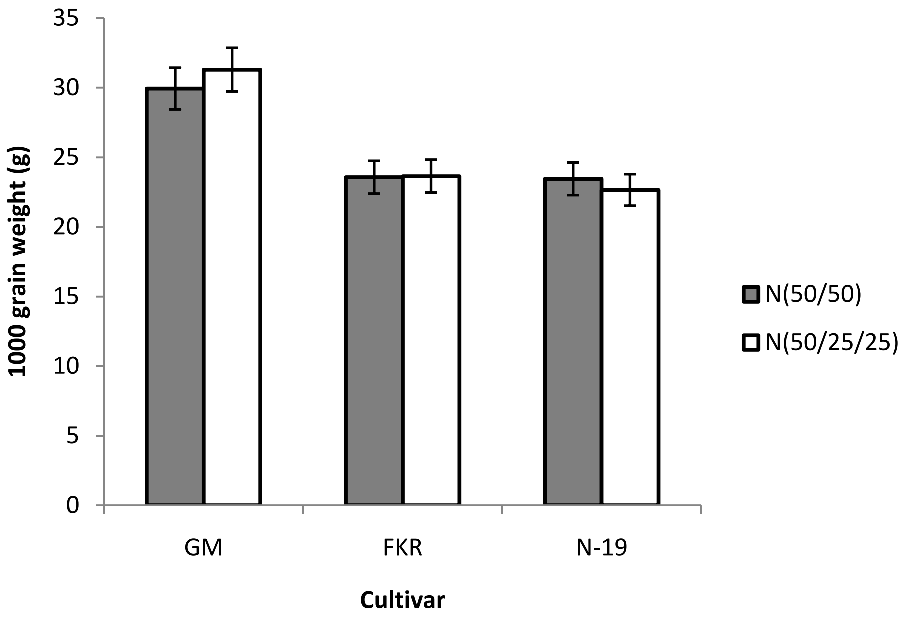


Figure 4.6: Effect of nitrogen application timing on 1000 grain weight. Bars represent means. Error bars indicate 95% CI of means.

Spikelet fertility

The nitrogen application strategy did not significantly ($P > 0.05$) affect spikelet fertility. However, spikelet fertility decreased significantly ($P < 0.001$) with flooding. There was a significant ($P < 0.001$) interaction between the cultivars and water regime (Figure 4.7). The non-flooded regime significantly increased the spikelet fertilities of GM-1 and N-19; but, the difference in spikelet fertility between the water regimes was not significant for FKR-19.

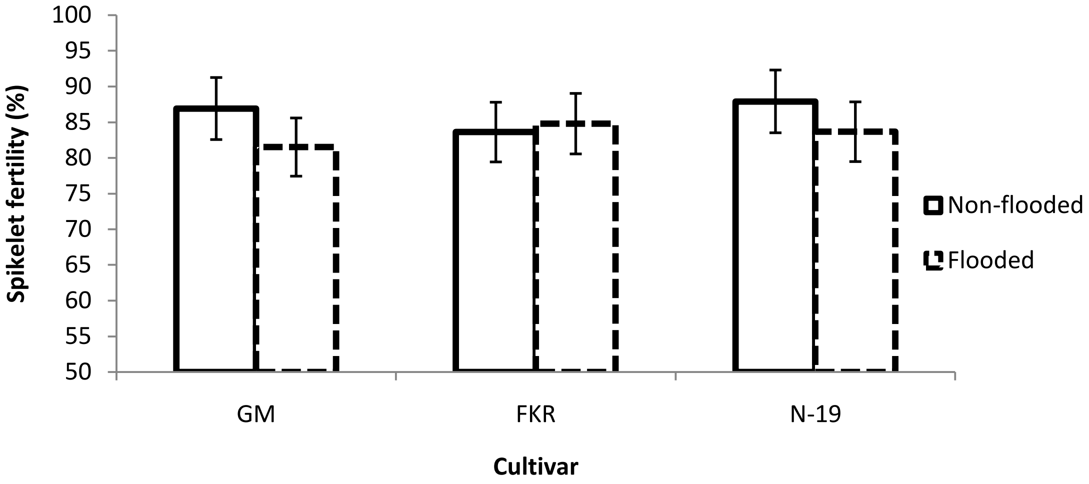


Figure 4.7: Effect of water regime on spikelet fertility. Bars represent means. Error bars indicate 95% CI of means.

Grain yield

Grain yield differed significantly (P < 0.01) with cultivar (Table 4.5). In the absence of flooding, grain yield of FKR-19 was significantly higher than those of the other two cultivars, while N-19 produced the highest grain yield under continuous flooding. Compared to the absence of flooding, continuous flooding led to a significant (P < 0.001) increase in grain yield for all the cultivars.

In the absence of flooding, the two-split nitrogen treatment led to a significant increase in grain yield, particularly for FKR-19 and N-19 (Table 4.5). Although with continuous flooding the difference between the nitrogen strategies was generally negligible, the three-split nitrogen treatment significantly increased the grain yield of N-19.

Table 4.5: Effect of nitrogen application timing on grain yield (g m^{-2})[†] under flooded and non-flooded regimes.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	799	728	1105	1117
FKR-19	1056	900	1119	1061
N-19	836	679	1077	1281
Means	897	769	1100	1153
				CV (%) = 6.8 $LSD_{(0.05)}$ = 113

[†] Grain yield values were not converted to kg ha^{-1} or t ha^{-1} basis because of the relatively small plot size harvested, since this could grossly over-estimate yields.

Harvest index

Harvest index of FKR-19 was significantly ($P < 0.001$) higher than that of both N-19 and GM-1, but there was no difference between GM-1 and N-19. Harvest index varied significantly ($P < 0.001$) with nitrogen treatment. For both GM-1 and N-19, harvest index increased significantly with the three-split application, while that of FKR-19 was virtually the same irrespective of nitrogen treatment.

Water regime did not significantly ($P > 0.05$) affect harvest index; however, its interaction with both cultivar and nitrogen strategy led to a significant ($P < 0.01$) contrast between the two water regimes. While continuous flooding increased harvest index in both GM-1 and N-19, it impacted negatively on FKR-19; also, whereas continuous flooding decreased harvest index for two-split nitrogen application, its effect was opposite with the three-split application of nitrogen (Table 4.6).

Table 4.6: Effect of nitrogen application timing and water regime on harvest index.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	0.43	0.49	0.44	0.50
FKR-19	0.58	0.56	0.51	0.52
N-19	0.45	0.44	0.45	0.53
Means	0.49	0.50	0.47	0.52
				CV (%) = 3.7 $LSD_{(0.05)}$ = 0.03

Plant nitrogen concentration and uptake

The shoot nitrogen concentration of all the cultivars was significantly ($P < 0.05$) increased by continuous flooding (Table 4.7). Although plant tissue nitrogen concentration at heading did not vary significantly ($P > 0.05$) with nitrogen application strategy, there were significant interactions ($P < 0.001$) between nitrogen strategy, flooding regime and cultivar. Specifically, the three-split nitrogen treatment significantly increased the nitrogen concentration of FKR-19 in the absence of flooding and of GM-1 under flooding conditions. The effect of the two-split dose was significantly high for GM-1 under non-flooding.

Table 4.7: Effect of nitrogen application strategy and water regime on above ground tissue nitrogen concentration (%) at heading.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	1.89	1.43	1.97	2.37
FKR-19	1.10	1.64	2.18	2.13
N-19	1.57	1.39	1.85	2.10
Means	1.52	1.49	2.00	2.20
				CV (%) = 8.7 $LSD_{(0.05)}$ = 0.33

Nitrogen uptake at heading varied significantly ($P < 0.001$) with cultivar. Uptakes of nitrogen by N-19 and FKR-19 were significantly higher than GM-1. Flooding led to a significant ($P < 0.01$) increase in the nitrogen uptake of all the cultivars. The main effect of the nitrogen application strategy was negligible at both levels of water regime; however, there was a significant ($P < 0.05$) interaction between nitrogen strategy, water regime and cultivar (Table 4.8). Under non-flooded conditions, uptake due to two-split nitrogen application was significantly higher in GM-1, while the opposite was true for FKR-19. For N-19, nitrogen uptake increased significantly with the three-split nitrogen treatment under flooding.

Table 4.8: Effect of nitrogen application timing and water regime on nitrogen uptake (g m^{-2}) at heading.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	7.41	3.93	10.06	10.47
FKR-19	4.70	7.23	13.32	12.31
N-19	8.50	7.02	12.37	14.53
Means	6.87	6.06	11.92	12.44
				CV (%) = 7.7 LSD = 1.59

Water use

Weekly irrigation water supply largely depended on rainfall. The total rainfall and irrigation supplied to flooded plots was nearly twice as much as that received by non flooded plots (Table 4.9). Under non-flooded conditions, FKR-19 was more efficient in water use than the other two cultivars, yielding about 0.92 g of grain per litre of water supplied (Table 4.9); however, under continuous flooding, it yielded only 0.6 g L^{-1}. Water use efficiency was generally low under continuous flooding.

Table 4.9: Water use of three rice cultivars as affected by water regime.

	No flooding				Continuous flooding			
[1]Cultivar	Rainfall (mm)	Irrigation (mm)	Total (mm)	WUE (g/L)	Rainfall (mm)	Irrigation (mm)	Total (mm)	WUE (g/L)
GM-1	448	445	893	0.85	448	940	1388	0.80
FKR-19	501	516	1017	0.96	501	1097	1598	0.68
N-19	504	553	1057	0.72	504	1134	1638	0.72
Mean	484	505	989	0.84	484	1057	1541	0.73

[1] Differences in rainfall and irrigation between the cultivars under the same flooding regime were due mainly to growth duration.

Grain amylose content

Grain amylose content varied significantly ($P < 0.001$) with cultivar. Under both water regimes, the amylose content of GM-1 was consistently higher than those of FKR-19 and N-19; however, the difference between FKR-19 and N-19 was negligible (Table 4.10). It is worthy to note that while GM-1 is of Chinese origin, FKR-19 and N-19 belong to the NERICA group and probably of related parentage.

Grain amylose content decreased significantly ($P < 0.001$) with continuous flooding. The three-split nitrogen treatment significantly ($P < 0.001$) decreased grain amylose content irrespective of water regime. However, under continuous flooding, while the three-split nitrogen treatment decreased the amylose contents in both FKR-19 and GM-1, it led to a significant increase in the amylose content of N-19. In the absence of flooding, the two-split nitrogen treatment significantly decreased the amylose content of GM-1, but increased those of FKR-19 and N-19.

Table 4.10: Effect of nitrogen application timing on grain amylose (%) content for different water regimes.

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	25.5	26.0	25.5	23.0
FKR-19	32.5	30.1	31.3	27.5
N-19	31.6	29.0	28.4	30.6
Means	29.9	28.4	28.4	27.0
				CV (%) = 0.5
				$LSD_{(0.05)}$ = 0.3

Grain protein content

Grain protein content varied significantly ($P < 0.001$) with cultivar (Table 4.11). FKR-19 had the highest grain protein content, followed by GM-1. Three-split application of nitrogen led to a significant ($P < 0.01$) increase in grain protein content, compared to the two-split application. The interaction of flooding regime and nitrogen timing strategy did not significantly ($P > 0.05$) affect grain protein content; nevertheless, under non flooded conditions, the protein contents of FKR-19 and N-19 significantly ($P < 0.001$) increased while that of GM-19 essentially remained unchanged by water regime (Table 4.11).

Table 4.11: Effects of nitrogen application strategy and water regime on grain protein content (%).

	Non-flooded		Flooded	
	$N_{(50/50)}$	$N_{(50/25/25)}$	$N_{(50/50)}$	$N_{(50/25/25)}$
GM-1	9.5	10.6	8.7	10.2
FKR-19	10.8	10.9	9.2	10.5
N-19	7.1	9.2	10.5	8.4
Means	9.1	10.2	9.5	9.7
				CV (%) = 7.0 $LSD_{(0.05)}$ = 1.1

DISCUSSION

The role of flooded water culture and increasing nitrogen application rates in the promotion of rice growth and yield was established from the results of the previous study (Chapter 3) and also by Khind and Ponnamperuma (1981), Sah and Mikkelsen (1983) and Ogunremi *et al.* (1986). The aim of the current study was to confirm the differences between the water regimes, and to determine the possibility of improving the effectiveness of nitrogen fertilizer by appropriate split applications in field conditions. Varietal differences in water and nitrogen responses were also investigated.

Varietal differences in above ground dry matter accumulation and leaf area index were mainly due to plant stature and tillering habit. GM-1 was nearly as tall as N-19, but had fewer tillers, FKR-19 was shorter with numerous tillers, while N-19 was tall, and produced many tillers (Table 4.3). Applying nitrogen in two doses increased tiller growth and development, thereby leading to increased biomass accumulation and leaf area index. Results obtained from other nitrogen timing studies have shown that early application of nitrogen in significant quantities before heading can lead not only to improved shoot biomass and leaf area development, but also, to higher grain yield (Ingram *et al.*, 1991). The rather sharp decline in leaf area index of FKR-19 under the non-flooded regime was mainly due to its inability to maintain most of its older leaves, and was probably an attribute of that cultivar. The loss of older leaves as a result of early senescence is reportedly a common adaptive response of plants to limiting water situations since it decreases their demand for water and at the same time allow for the redirection of limited resources to the developing sinks (Palta *et al.*, 1994; Pic *et al.*, 2002).

Because two applications of nitrogen substantially increased tiller production, it increased the number of panicles produced by the cultivars, compared to the three-split treatment. The larger percentage of productive tillers achieved by three-split nitrogen application was the result of the lower number of tillers produced by that treatment. In effect, it was the number of productive tillers that eventually contributed more to yield, rather than the percentage; hence, the two split treatment was more useful for the increment of grain yield. This finding is consistent with reports by Shahidullah *et al.* (2009) who found that the panicle number of several rice genotypes positively correlated with maximum tiller number and grain yield.

However, there are reports that the fraction of productive tillers may also account for differences in the grain yield of rice (Lafarge *et al.*, 2004; Jing *et al.*, 2010).

High grain yield in GM-1 was more a function of the 1000 grain weight, while larger sink sizes conferred by higher tiller and panicle numbers were mainly responsible for the higher grain yield in FKR-19 and N-19 as these two cultivars are of a similar genetic background (Pierik *et al.*, 2010). Superior agronomic traits including heavier 1000 grain weight reportedly resulting from high net assimilation and better dry matter partitioning during grain filling, and larger spikelet number per unit area have similarly been cited by various researchers as major determinants of grain yield (Ying *et al.*, 1998a; Jeng *et al.*, 2006). Differences in 1000 grain weight amongst the cultivars were purely due to grain size, since this observation is consistent with reports that grain size was positively correlated with the rate of grain filling and eventual grain weight (Okumoto *et al.*, 1988; Kato, 1989). Unlike the other cultivars, 1000 grain weight in GM-1 increased significantly at three-split nitrogen application because of the relatively high productivity of the fewer panicles which consequently led to increased grain filling. This finding is corroborated by reports which have associated fewer panicle numbers with larger panicle weights and better grain filling (Khush, 1995; Zhu *et al.*, 2010).

Unlike 1000 grain weight and panicle number, spikelet fertility was not a defining factor for the differences in grain yield in spite of its decline under continuous flooding. Even though spikelet fertility is known to be particularly sensitive to drought stress (Bouman and Tuong, 2001), the role of flooding in the suppression of spikelet fertility has been reported also by Jing *et al.* (2010). According to these authors, spikelet fertility of all the cultivars studied was higher in the non-flooded soil compared to the flooded regime. However, Shimono *et al.* (2002) found that sub-optimal temperature (below 20^{o}C) of the flood water during grain formation was mostly responsible for the repression of spikelet fertility in flooded soils.

With the three split nitrogen treatment, nitrogen uptake and therefore, use for grain production were higher in the flooded soil because the third application ensured that nitrogen was available during the latter growth stages for uptake and use by the plants. Similarly, Cabangon *et al.* (2004) found that nitrogen recovery increased with the number of nitrogen split applications. The finding that grain yield was higher under continuous flooding at the three-split nitrogen treatment, compared to the two-split, is consistent with the accounts of

Islam *et al.* (2009). However, in the non-flooded soil, the three split nitrogen treatment did not improve uptake and yield because it was either late, or too little to make a difference.

The higher nitrogen accumulation by N-19 and FKR-19 (Table 4.8) can be attributed to larger dry matter accumulation since nitrogen concentration of the tissues of GM-1 was significantly higher than those of FKR-19 and N-19, as nitrogen uptake is closely related to dry matter accumulation (Booij *et al.*, 1996; Plénet and Lemaire, 1999). The overall grain yield of FKR-19 and N-19 can therefore be explained by their relatively higher dry matter accumulation and nitrogen uptake.

Water use in plants may vary with cultural practices as well as plant, soil, and climatic factors (Ritchie, 1981; Hatfield *et al.*, 2001). Compared to any other crop, water use efficiency is usually low in flooded rice production (Lu *et al.*, 2000). That the use efficiency of water was higher under the non-flooded condition, it was not unexpected due to the lower total irrigation water supplied. The lower irrigation requirement was partly due to the capacity of the soil to hold sufficient moisture as indicated by records of soil moisture tension at both 30 and 60 cm below the surface (Figure 4.3).

The ability of deep soils to retain greater amounts of water for use by plants is reported to be a major contributing factor to improved water use efficiency (Qadir *et al.*, 2007). The finding that water use efficiency increased under the limited irrigation of no flooding is similar to that reported by (Kang *et al.*, 2002; Mueller *et al.*, 2005). Earlier, Jensen *et al.* (1970) found that water use is usually minimum when water supply is approximately equal to the physiological consumptive use and leaching requirements. The variation in water use amongst the cultivars was mainly due to their respective yield outputs which were mainly influenced by the water regime under which they were grown.

There was a general tendency for amylose content to decrease with three-split nitrogen application. However, that result was opposite for grain proteins content. Because three-split nitrogen application also led to an increase in plant nitrogen content at heading, particularly in the flooded soil (Table 4.7), the decrease in amylose content, accompanied by an increase in proteins, under the three split nitrogen regime suggests that the availability of high nitrogen at heading can modify the quality of rice grains by altering the amounts of proteins and carbohydrate components in the endosperm of the seeds. This is consistent with reports that high nitrogen supply can increase grain proteins, and lead to a decrease in the amylose content of rice (Kaul and Raghaviah, 1975; Bryant *et al.*, 2008; Xiong *et al.*, 2008). The role of flooding in the reduction of grain amylose was similarly reported by Borrell *et al*. (2002). Although these authors could not explain why, it is suggested that in this study, flooding decreased grain amylose content simply because it led to higher uptake of nitrogen. On the basis of the traditional classification system, the three cultivars used in this study are relatively high amylose rice types, since their amylose contents were 25% and above (Sowbhagya *et al.*, 1987; Ayres *et al.*, 1997).

As mentioned in the literature review (section 2.8.2), the amylose – amylopectin ratio of the starch component of grains is a major determinant of the cooking and eating quality of rice. Rice lots of similar amylose content may have different pasting and textural properties as a result of the different compositions of their respective grain protein. According to Zhao *et al*. (2006) a high concentration of glutelin (the largest component of grain protein) increases the adhesiveness, gel hardness and pasting temperature of rice. Protein is known to increase the resistance of rice to the abrasion of milling, and is therefore essential for the improvement of head rice yield (Mew and Misra, 1994; Francesco and Aldo, 2000). However, the effects of both amylose and protein on the eating and cooking properties as influenced by the nitrogen application strategies and water regimes was not directly determined (from rapid visco-analysis, gel consistency and gelatinization temperature tests) as desired, due to the unavailability of suitable rice processing equipment.

CONCLUSION

Nitrogen and water are critical requirements for the growth and productivity of rice. Although increasing nitrogen input can be used to improve growth and yield performances of the crop (Chapter 3), this study revealed that the effect of moderate nitrogen fertilizer input on essential grain yield determinants and grain quality can be enhanced by appropriately timing its application. It also revealed that different nitrogen fertilizer application strategies should be considered for different soil flooding regimes if efficient use of nitrogen must be realized. The results of the study suggest that application of nitrogen fertilizer in two equal splits, the first at the commencement of tillering, and the second at mid-tillering in non flooded soils, is better for the promotion of grain yield, while the alternative three-split application remains the method of choice for flooded rice cultures. The growth and yield performances of all the cultivars were rather satisfactory. Although FKR-19 and N-19 produced larger grain yields under the respective non-flooded and flooded water regimes, further studies will be required to verify the consistency of the results. The increase in grain protein content by the three-split nitrogen application strategy was important because of its implication for milling recovery. Its impact on grain amylose content is important for consumers with preferences for rice that cook more tenderly.

CHAPTER 5

STUDIES OF SEED PRIMING ON RICE PLANT ESTABLISHMENT BY DIRECT SEEDING IN WELL WATERED CONDITIONS AND AERENCHYMA FORMATION IN FLOODED CONDITIONS

ABSTRACT

Rice establishment by direct seeding in wetlands is a cost-saving alternative to transplanting which is characterized by high labour demands and water use for soil puddling operations. However, direct-seeded rice in lowlands is often limited by poor germination, emergence and uneven crop stand, and will therefore require the use of well invigorated seeds in order to improve the establishment of the crop in such environments. An understanding of the ability of rice to thrive in flooded soils was also sought by studying the formation of aerenchyma in the cortical tissues of the roots.

The effect of seed invigoration treatment on the germination and emergence of rice was determined using three replicates of three hydro-priming times (0, 24 and 48 h) and three cultivars (GM-1, FKR-19 and N-19). 25 seeds of each treatment combination were placed between double layered paper towels and germinated in a growth chamber at 25^{o}C, 65% R.H. over a 5 day period to determine the speed and percentage of germination. The days to 50% emergence, mean emergence time and final emergence were determined over a 7 day period from 25 seeds of each treatment combination sown under glasshouse conditions. Seedling height at 50% emergence and at 28 DAS, and shoot dry weight at 28 DAS were measured for 10 seedlings of each treatment combination. Aerenchyma formation in root cortical tissues of both flooded and non-flooded plants was determined from microscopic images of hand-cut sections at 5 and 50 mm behind the tips of 60 -70 mm long nodal roots.

Compared to non-primed seeds, hydro-priming for both 24 and 48 h significantly hastened germination and emergence, shortened the days to 50% emergence and increased the percentages of germination and seedling emergence. At both 50% emergence and 28 DAS, hydro-priming for 48 h produced the tallest seedlings followed by hydro-priming for 24 h.

Shoot dry weight at 28 DAS increased significantly with hydro-priming time. Flooding for 7 days significantly increased the amount of gas-filled spaces at 50 mm behind the root tips, but at 5 mm behind the root tip, only GM-1 showed minimal aerenchyma formation due to flooding.

The results showed that hydro-priming for 48 h was the best seed invigoration treatment for optimum rice establishment by direct seeding, since it significantly increased plant height at 50% emergence and increased the uniformity of emergence by shortening the mean emergence time. The ability of rice to thrive in flooded conditions can be attributed to the rapid development of aerenchyma in root cortex in response to even short episodes of flooding.

INTRODUCTION

Rice establishment by transplanting in lowlands is often demanding in terms of labour required for the puddling of paddy and transplanting of seedlings (Singh *et al.*, 1994; Singh *et al.*, 2008). Even though the manual labour required may be greatly reduced by mechanization, the additional water resources required to accomplish the process can increase over all water use for the production of the crop.

The alternative to transplanting, i.e. direct seeding as practiced in upland cultures, can eliminate the need for puddling, thereby saving costs for labour and water supply. Furthermore, direct seeding may have no adverse consequence for yield, compared to transplanting (Naklang *et al.*, 1996). Studies show that direct seeded rice matures earlier than transplanted rice, and this may increase the turn-around period for subsequent crops (Balasubramanian and Hill, 2002). Direct seeded rice has another advantage of early tillering over transplanted rice since its growth proceeds free of the damage caused by transplanting shock (Dingkuhn *et al.*, 1991). It is less prolific at tillering than transplanted rice, with the fewer tillers eventually becoming more productive due to the larger panicles they produce (Yoshida, 1981).

The advantage of direct seeding over transplanting can be fully exploited in the quest to maximize production. However, the possible gains of direct seeding in wetland ecosystems may be seriously undermined by flooding, particularly if flooding occurs and persists before the crops have fully emerged. Direct seeded rice is often limited by poor germination, emergence, and uneven crop stands (Balasubramanian and Hill, 2002). Therefore, a rapid, even and vigorous germination and establishment of seeds and seedlings will be required to minimize the incidence of poor stand in the establishment direct seeded rice. Although various seed priming techniques are known to improve seed germination and emergence (Basra *et al.*, 2003), there are limited reports regarding the use of hydro-priming as a cheap, farmer-friendly seed invigoration technique to overcome the problem of poor stand establishment in direct seeded lowland rice. Results from the previous studies (Chapters 3 and 4) suggest that the crop performs better under flooded than non-flooded (aerobic) conditions. Roots of most crop plants normally obtain oxygen required for aerobic respiration directly from the pore spaces in the soil. However, excess water due to flooding can displace gases in the soil, thereby undermining the exchange of gases between the root and its environment, and eventually, the survival of the plant (Armstrong, 1980). The rice plant does not appear to suffer this fate. Indeed, rice plants subjected to flooding reportedly develop a special anatomical feature (aerenchyma) that helps them survive by facilitating the movement of gases in their submerged tissues (Jackson and Armstrong, 1999; Drew *et al.*, 2000). A study of this gas filled spaces is important for an understanding of the crops ability to grow well in flooded conditions.

The objectives of this study were therefore: 1) to determine the effect of hydro-priming time on the germination and emergence of rice, with the view of enhancing the establishment of the crop in direct-seeded cultures, and 2) to compare gas-filled space formation in the cortical tissues of both flooded and non-flooded rice roots.

MATERIALS AND METHODS

Plant material

The three rice cultivars used in the previous study, namely GM-1, FKR-19 and N-19 were selected to study the effect of hydro-priming time on the establishment of rice by direct seeding. In order to ensure the use of good quality seeds, the specific gravity grading procedure (TNAU, 2008) for paddy rice was used to evaluate the seeds. Each seed lot was submerged in two volumes of 0.2% NaCl solution. The floating seeds were removed and discarded. Seeds that sunk to the bottom of the container were considered as good quality material, and were collected and washed three times with distilled water.

Seed priming

Seeds were hydro-primed for 0, 24 and 48 h ($P_{0,}$ $P_{24,}$ and P_{48}). The P_{24} seeds were soaked in distilled water for 12 h and incubated for another 12 h. For the P_{48} treatment, seeds were soaked in distilled water for 24 h and incubated for another 24 h. All the seeds were soaked in plastic containers at ambient temperature and subsequently incubated in a Labcon growth chamber (Labex, Ltd.) at 30^{o}C in order to stimulate uniform germination (Vergara, 1992; Basra *et al.*, 2005). To ensure uniform incubation, seeds were thinly spread between moist double layered paper towels and sealed in plastic bags to prevent desiccation. After incubation, the seeds were surface dried at ambient temperature for 24 h and sown the next day. The P_0 seeds were neither soaked, nor incubated.

Germination

Three replicates, each containing 25 seeds from every combination of cultivar and priming treatment were lined between double layers of moist paper towels, arranged in complete randomized blocks and placed in a growth chamber set at 25^{o}C. Germination was monitored at 24 h interval for 5 days and the final germination percentage recorded. Germination velocity index (Equation 5.1), calculated according to Woodstock (1976), was used to determine the speed of germination. Radicle protrusion was regarded as the criterion for germination, since it is usually the first structure to emerge from a germinating rice embryo in non-flooded environments (Counce *et al.*, 2000).

Germination velocity index (GVI) = $N1/1 + N2/2 + N3/3 + N4/4 + N5/5$ (5. 1),

N1, N2, N3, N4 and N5 = number of germinated seeds on days 1, 2, 3, 4 and 5 after sowing.

Emergence

The plant emergence test involved three replications of a split-plot combination of the three hydro-priming treatments and three cultivars. The cultivars were the main plots while the priming treatments constituted the sub-plots. 30 cm diameter pots (4.8 L) were used for the main plots, and each pot was divided into three 235.5 cm^2 sections (Figure 5.1). Twenty-five seeds of each priming treatment were sown at 2.0 cm depth, 3.0 cm apart to one of three 235.5 cm^2 sections per pot. Final emergence and the time to 50% emergence were determined by daily seedling count over a period of 7 days. Mean emergence time was calculated according to Bewley and Black (1994).

Mean emergence time (MET) = $\sum(Ni \times Di) / \sum Ni$ (5.2), where Ni = number of newly emerged seedlings at day Di.

Seedling height was measured at 50% emergence and at 28 DAS. Seedling above ground dry mass was determined at 28 DAS. Ten seedlings from each treatment combination were selected randomly for seeding height and above ground dry mass measurements.

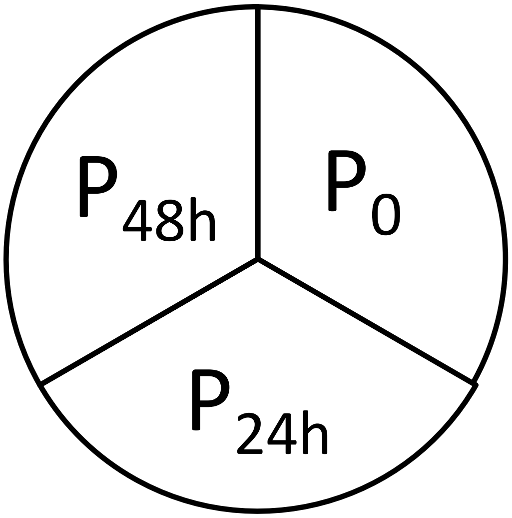


Figure 5.1: Sub plot division of 30 cm diameter pots used for plant emergence using primed seeds of three rice cultivars. Each sub plot measured 235.5 cm^2. Twenty-five seeds were sown 2.0 cm deep to each 235.5 cm^2 section.

Root anatomy

The root anatomy study involved a combination of three rice cultivars (GM-1, FKR-19 and N-19) and two water regimes (Flooded and non-flooded) established in 4.8 L (30 cm diameter) pots. Ten plants of each cultivar were grown to 235.5 cm^2 sections per pot for a period of 28 days. Flooding was established and maintained at 5 cm above the surface for 7 days beginning at 21 DAS. The non-flooded plants were watered daily, ensuring that the soil moisture tension at the bottom of the pots was kept below 15 kPa as described in Chapter 3. At 28 DAS, pots were submerged in a basin of water and the soil carefully washed off the roots.

Three seedlings of similar height per cultivar were selected for aerenchyma measurement. Hand cut sections (approximately 0.2mm thick) were taken at 5 and 50 mm behind the tips of

60 – 70 mm long nodal roots, and examined under a Nikkon light microscope. Microscopic images of the transverse root sections were photographed using a digital camera attached to the microscope. The total areas of each hand-cut section, the outer layers, cortex, gas spaces and inner layers were measured from the digital images using Image J digital imaging software for windows. The recorded measurements were used to calculate the proportion of root cortex occupied by aerenchyma (air spaces).

RESULTS

Germination and seedling emergence

The speed of germination as indicated by the germination velocity index increased significantly ($P < 0.001$) with priming time in all cultivars; however, in GM-1 the difference in germination speed between the 24 and 48 h hydro-priming times was negligible (Figure 5.2). Seed priming for both 24 and 48 h led to a significant increase in the percentage of seeds germinated by 5 DAS (Figure 5.3).

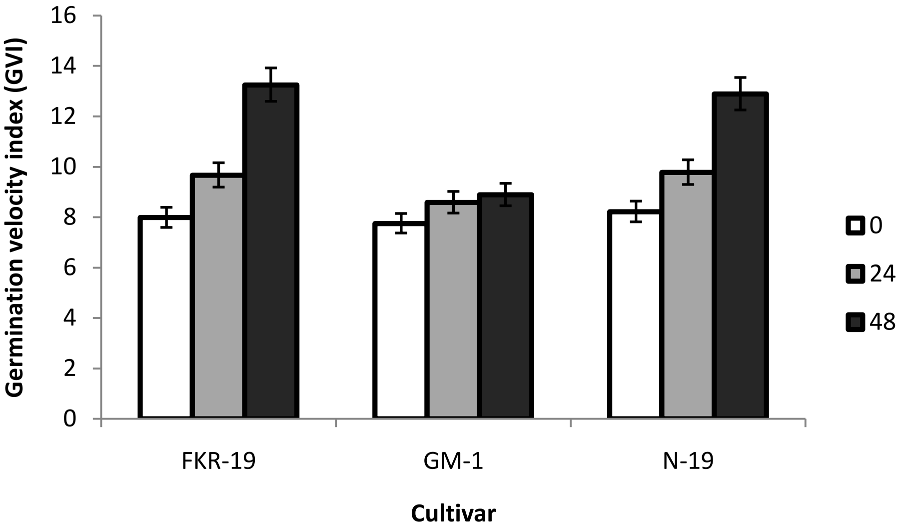


Figure 5.2: Effect of seed priming on seed germination speed. Bars indicate priming time (h). Error bars indicate 95% CI of means.

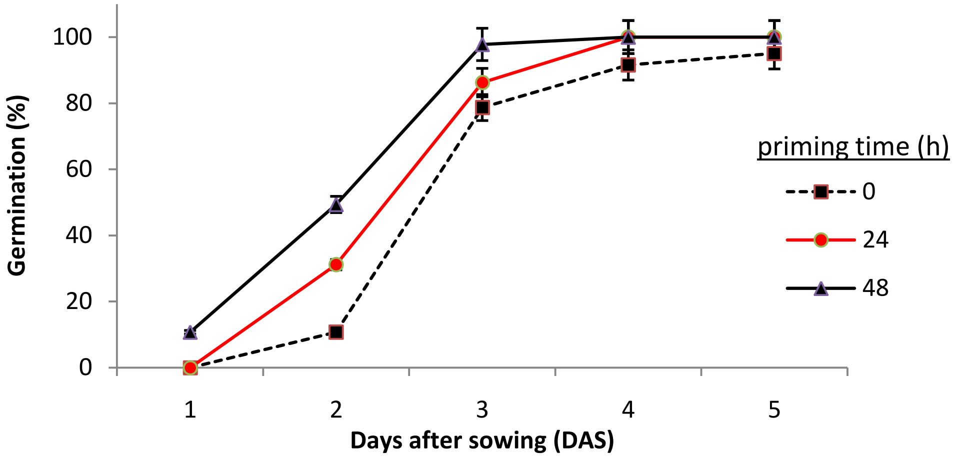


Figure 5.3: Effect of hydro-priming on the rate of seed germination. Bars show priming time (h). Error bars indicate 95% CI of means.

Seed priming for both 24 and 48 h significantly ($P < 0.001$) shortened the days to 50% emergence (Table 5.1). Similarly, primed seeds emerged significantly ($P < 0.001$) faster than the non-primed seeds (Table 5.1). However, there was no cultivar difference in emergence times. Compared to the non-primed controls, seed priming for both 24 and 48 h led to a significant ($P < 0.001$) increase in final emergence (at 7 DAS) for all the cultivars; however, the difference between the primed treatments was negligible (Figure 5.4).

Table 5.1: Effect of hydro-priming time on the emergence time and shoot dry weight of rice seedlings.

	GM-1			FKR-19			N-19			
	P_o	P_{24}	P_{48}	P_o	P_{24}	P_{48}	P_o	P_{24}	P_{48}	LSD $_{(0.05)}$
Mean emergence time (days)	4.2	3.0	2.9	3.9	2.5	2.6	4.0	2.5	2.3	1.1
Days to 50% emergence	4.0	3.0	3.0	4.0	2.0	3.0	4.0	2.0	2.0	1.4
Shoot dry weight at 28 DAS (g plant $^{-1}$)	0.20	0.22	0.24	0.17	0.22	0.22	0.19	0.20	0.22	0.02

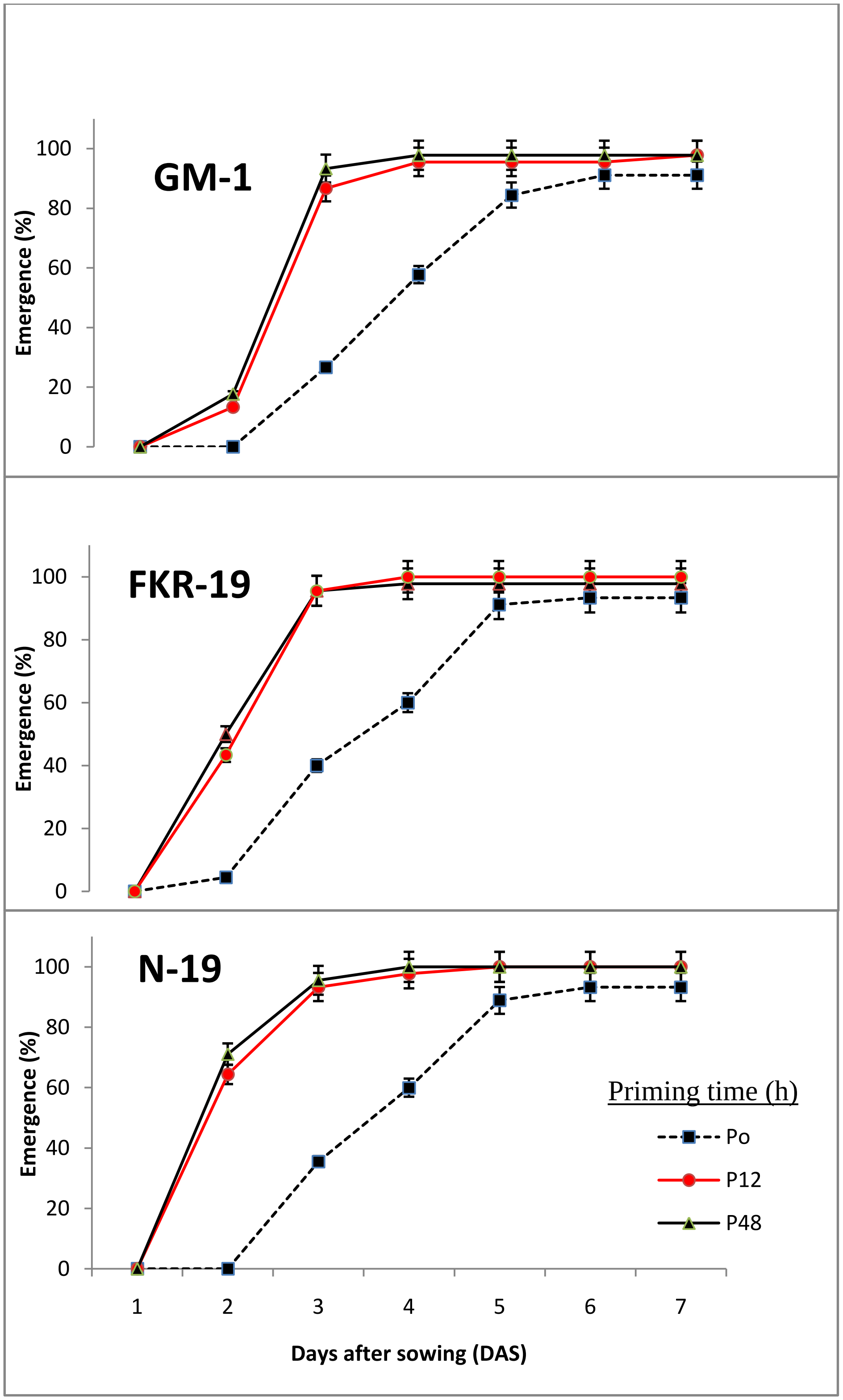


Figure 5.4: Effect of hydro-priming time on seedling emergence. Error bars show 95% CI of means.

Plant height at both 50% emergence (i.e. at 4 DAS) and at 28 DAS differed significantly ($P < 0.001$) with hydro-priming time (Figure 5.5). At 50% emergence, seedlings produced from seeds primed for 48 h were at least two times taller than those of the control. They were also significantly taller than those produced by the 24 h priming treatment. Although seedling height varied significantly ($P < 0.001$) with cultivar, there was no significant ($P > 0.05$) interaction between seed priming and cultivar at both 50% emergence and at 28 DAS. Seed priming significantly ($P < 0.001$) increased dry matter accumulation at 28 DAS (Table 5.1). Like plant height, dry matter accumulation at 28 DAS varied significantly with cultivar; but the variation was not due to the priming treatment.

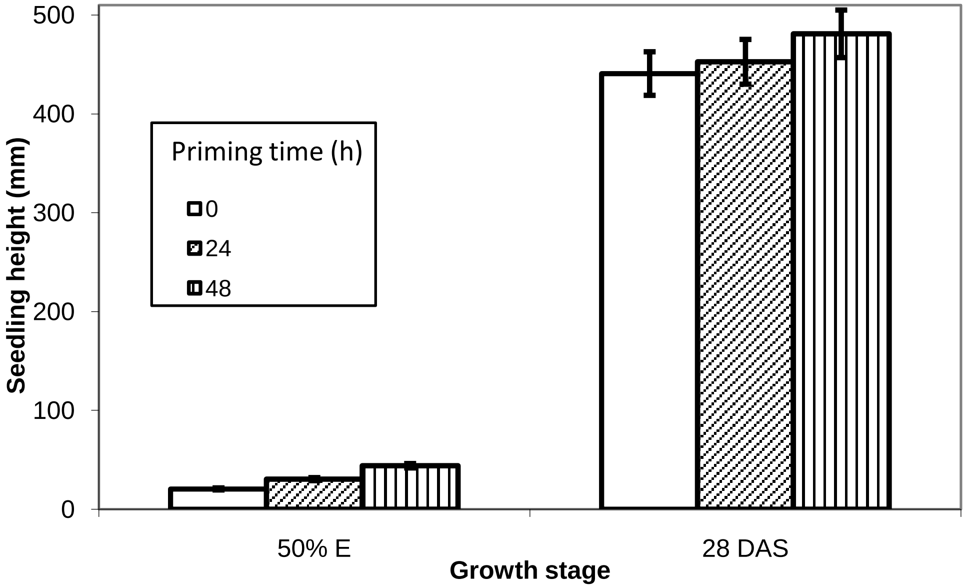


Figure 5.5: Effect of hydro-priming time on seedling height. Error bars show 95% CI of means.

Root Aerenchyma

The level of aerenchyma formation in nodal roots varied according to the positions examined behind the root tip. At 5 mm behind the root tip, only GM-1 showed a minimal (9%) air space in the root cortex when flooding was imposed for 7 days. Apart from this, no aerenchyma was seen irrespective of flooding regime or cultivar (Figure 5.6). However, at 50 mm behind the tip, aerenchyma spaces became more prominent in all the cultivars as a result of flooding, covering up to 92% of the root cortex (Figure 5.7). Thus, compared to the 5 mm position behind the root tips, significant aerenchyma developments were seen in the root cortex at the 50 mm position behind the root tips of all the cultivars (Figure 5.7 & 5.8).

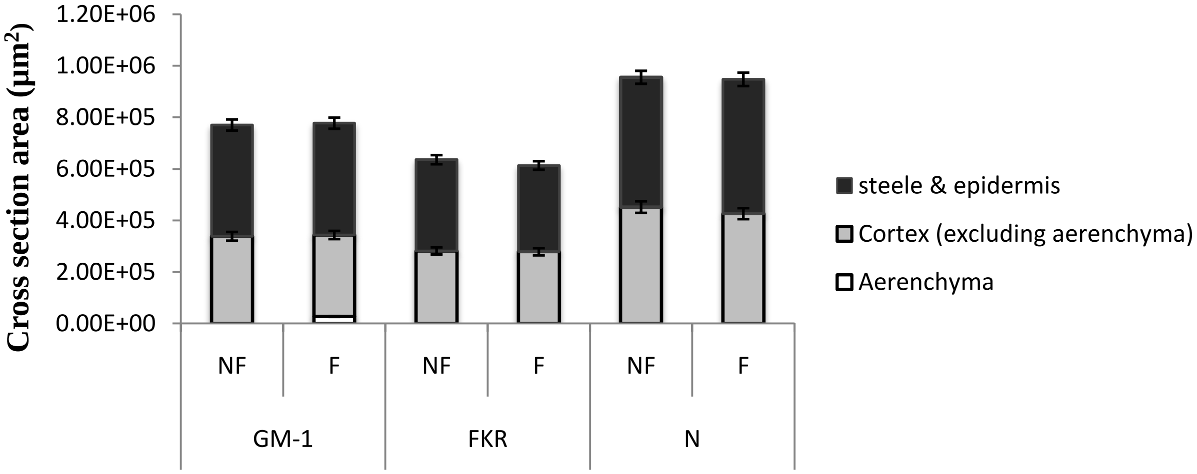


Figure 5.6: Effect of flooding regime (NF = non-flooded, F= flooded) on aerenchyma development at 5 mm behind the root tip.

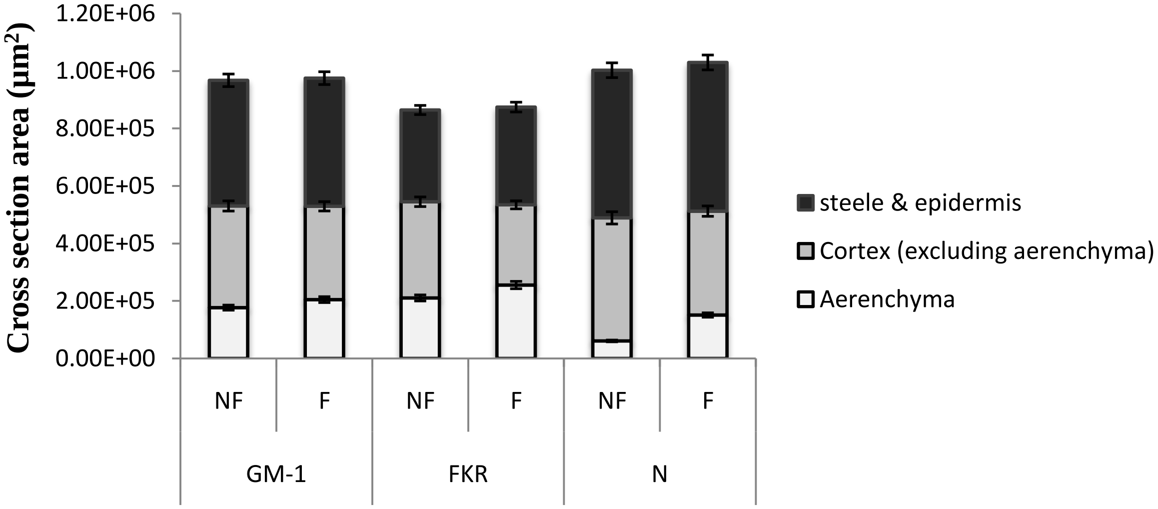


Figure 5.7: Effect of flooding regime (NF = non-flooded, F= flooded) on aerenchyma development at 50 mm behind the root tip.

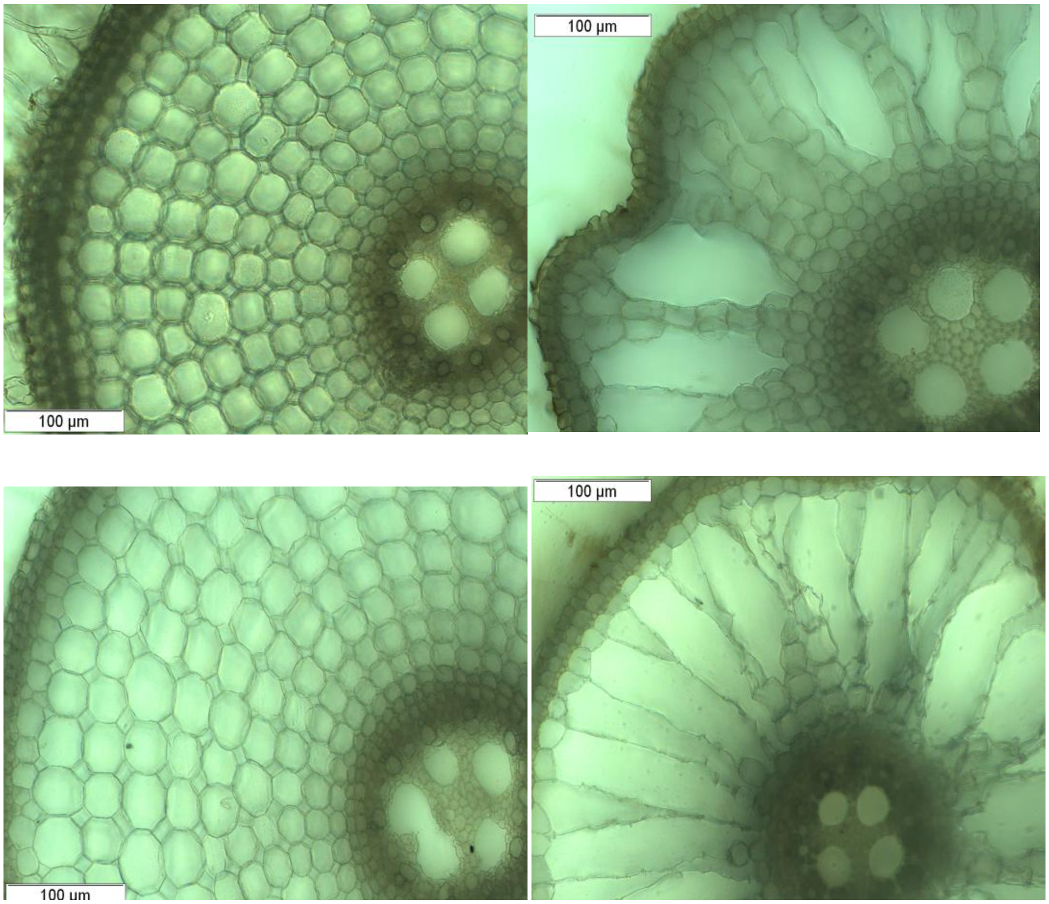


Figure 5.8: Effect of water regime on aerenchyma formation in roots of rice plants grown in flooded (below) and non-flooded (above) conditions. Hand-cut sections were taken at 5mm (left) and 50 mm (right) behind the root tips.

DISCUSSION

Rice establishment by direct seeding provides a suitable alternative to transplanting which is often demanding of labour and water use (Pandey and Velasco, 2002). The aim of the current study was to determine the effect of hydro-priming on the germination and emergence of the crop.

The results showed that hydro-priming is an important seed invigoration treatment since it increased the speed and percentage of germination of all the cultivars. Similar results have been reported for several other crops. (Rudrapal and Nakamura, 1988; Afzal *et al.*, 2004; Ghassemi-Golezani *et al.*, 2008). Seed invigoration by hydro-priming is believed to result from the metabolic repair of deterioration sustained by the seed during the hydration-dehydration process prior to actual sowing (Burgass and Powell, 1984; Thornton and Powell, 1992; 1995).

The timing of seedling emergence is known to be a major determinant for the establishment and yield of several crops (Gan *et al.*, 1992; Parera and Cantliffe, 1994; Ghassemi-Golezani *et al.*, 2010). Priming seeds for both 24 and 48 hours hastened emergence by at least one day and was significant for the early establishment of all the cultivars. About 90% emergence was achieved earlier (by 3 DAS) as the result of seed priming, compared to at most 40% for the non-primed seeds (Figure 5.4), thereby indicating the significance of seed priming in the attainment of uniform seedling emergence. Similarly, Harris *et al* (2002), Farooq and Basra (2006) and Farooq *et al.* (2007) found that seed priming did not only increase the rate of seedling emergence, but it also increased the subsequent yield of direct seeded rice. Researchers in West Africa have also reported of uniform seedling emergence and establishment in several NERICA cultivars as a result of hydro-priming for 24 h (Harris and Jones, 1997).

Aerenchyma forms a continuum which facilitates the movement of gases between submerged plant parts and the atmosphere, and therefore constitutes an essential mechanism of survival for wetland plants exposed to flooding (Bodelier, 2003; Araya, 2007). The observation of more aerenchyma formation in the roots of the flooded plants is consistent with the results of several studies (Vartapetian and Jackson, 1997; McDonald *et al.*, 2002; Mostajeran and

Rahimi-Eichi, 2008). The complete absence or low development of aerenchyma at the 5 mm position behind the root tip may be due to the proximity of that point to the apical meristematic region where most of the cells were still in their early stages of development. This is consistent with results by Raven (1996) and Malik *et al.* (2003) who reported that aerenchyma commonly forms over most parts of the rice plant body, with the exception of meristems and other tissues such as vascular bundles, sclerenchyma, and the epidermis. Compared to the non-flooded plants, aerenchyma formation, particularly at the 50 mm position behind the root tip, was more prominent in the plants subjected to flooding (Figure 5.8), and this increased root porosity and ultimately, the ability of the plants to maintain much needed oxygen in the submerged roots. The porosity of roots, defined as the area of air-filled spaces in relation to the total tissue area (Videmsek *et al.*, 2006), is normally increased by aerenchyma formation which tends to increase with hypoxia as in flooded soils (Das and Jat, 1977; Kludze *et al.*, 1994; Colmer, 2003). This observation of aerenchyma formation in the roots of the non-flooded plants in this study is certainly not uncommon. Aerenchyma formation is reportedly an integral part of normal root development in rice, since it also occurs speedily, even in well aerated media (Webb and Armstrong, 1983; Jackson *et al.*, 1985), as also was the case in this study.

CONCLUSION

The results of the current study show that seed priming can be used as a tool to improve stand establishment in rice production since it shortened the times of germination and emergence, increased seedling height and dry weight, and improved the uniformity of emergence. However, when rice is established by direct seeding in wetland cropping systems, it is essential to determine an optimum planting date such that seedlings will have sufficient time to establish ahead of flooding which may come about by intense and sustained rainfall. The prominence of aerenchyma in the cortical tissues of rice subjected to flooding represents an important anatomical adaptation that makes it possible for them to survive and flourish in wetland habitats by facilitating root aeration.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND OUTLOOK

Integrated management of water and nitrogen resources is critical for the maintenance of growth and increasing the grain yield and quality of rice. In SSA, rice is grown mostly in upland cultures where yield has been consistently low, partly as a result of poor soil fertility and water availability. Due to its potential for high yields owing to the availability of water (Inocencio and Merrey, 2003; Voortman *et al.*, 2003), the wetlands are being increasingly targeted for the expansion and intensification of the crop (Scoones, 1991). The characteristic diversity of flooding patterns in wetlands may, however, influence the growth and productivity of the crop partly due to its impact on the availability and uptake of nitrogen, and also, due to the timing of flooding *per se*. This thesis, therefore, aimed to determine an appropriate nitrogen and water management strategy that would improve the growth and productivity of rice in flooded conditions. It also determined the effect of hydro-priming on rice establishment by direct seeding, as well as the influence of flooding on aerenchyma formation in the cortical tissues of rice roots.

Results of the study on nitrogen application rates under different water regimes (Chapter 3) showed that flooding with standing water to a depth of 5 cm throughout the growth of the crop, commencing at 3 weeks after sowing, were more favorable for the growth and yield of rice, compared to continuous aerobic conditions or late flooding only. In the field study (Chapter 4), findings relating to the tendency of flooding to increase growth and yield indices of rice over non-flooding are consistent with reports by Rodriguez and Lal (1985), Neue (1993), Jearakongman *et al.* (1995) and Singh *et al.* (2001). This study therefore supports the general notion that rice can be grown in wetland conditions with better results than upland conditions and probably reinforces the need for the shift in SSA to the wetland conditions extensively used in several Asian countries where yields of up to 6 t ha^{-1} have been reported in comparison to the 1.5 t ha^{-1} for the upland conditions of SSA (Hossain, 1997; Mutert and Fairhurst, 2002; WARDA, 2008). Consistent with results from studies done in Asia (Wei and Song, 1989; Mao *et al.*, 2000; Cabangon *et al.*, 2004), the effects of early and continuous flooding were practically the same in terms of increased tillering, panicle production and grain yield, suggesting that the former flooding pattern is better than the latter in terms of water use. Wetlands prone to late flooding, on the other hand, may not provide the best yield although they may still be a better option than upland rice production.

Although grain yield increased with increasing nitrogen rates as reported by several authors (Sharma and Mittra, 1991; Alcantara and Cassman, 1996; Jiang *et al.*, 2004), the diminishing pattern of increase observed with the application of nitrogen beyond 110 kg ha^{-1} showed that the quest to increase yield by increasing nitrogen input should be approached with caution if maximum output should be obtained at a minimal cost (Herdt and Wickham, 1978; Socolow, 1999). Indeed, the 110 kg ha^{-1} rate used (Chapters 3 and 4) was based on yield target arising out of soil analysis, while the 220 kg ha^{-1} was higher than the potential requirement for the soils used. This places a significant importance on adopting realistic fertilizer rates based on soil analysis. The low efficiency of nitrogen use for grain production under late flooding and continuous aerobic conditions in comparison to the early or continuous flooding is consistent with results from other studies (De Datta *et al.*, 1983; Belder, 2005); it showed that elevated moisture level, particularly during the active tillering stages of the rice development is critical to increasing nitrogen uptake in rice, and avoiding the wastage of such a limited resource.

The results of the field study (Chapter 4) showed that depending on moisture regime, different split application strategies of nitrogen may be required to ensure optimum yield and grain quality of rice. In general, the two-dose nitrogen application favored grain yield in the non-flooded conditions, while yield in the flooded plots increased with the three-dose application. Additionally, since the three-dose application generally increased nitrogen uptake at heading, it caused a reduction in the amylose content of the grains, and an increase in grain protein as reported by several authors (Yamashita and Fujimoto, 1974; Song and Zhang, 1992; Iqbal *et al.*, 2003). Such an increase in protein is important for the milling, cooking and eating qualities of rice. Studies have shown that protein increases the percentage of whole grain that can be recovered from the processes of dehulling and polishing, since rice grains with high protein contents are usually resistant to breakage during milling (Blakeney, 1979; Jongkaewwattana *et al.*, 1993; Perez *et al.*, 1996b). Nutritionally, rice is the major source of dietary proteins for most people who rely heavily on the crop for subsistence (Juliano, 1993); therefore, the higher the protein content, the better the quality of their diet.

A further consideration of the three-dose nitrogen application for the short season cultivar (GM-1) was that at the highest nitrogen rate in greenhouse conditions, the third application led to the production and maintenance of late tillers with potentially little contribution to grain yield, but which could delay harvest, or reduce overall grain quality by increasing the amount of immature grains (Chau and Kunze, 1982; Heenan and Lewin, 1984). The three dose strategy is therefore not suitable for short season cultivars. Thus, there is the need to match the number of applications to known cultivar durations, with the two-dose application limited to shorter season cultivars and the three-dose strategy used for those of longer durations, a suggestion supported by De Datta (1987a) and Iqbal (2007).

Consistent with reports by Rabara and Ferrer (2009) and Farooq *et al.* (2010), hyrdo-priming of seeds for 48 h was found to be useful for ensuring rapid and even stand establishment of direct seeded rice, since it increased the percentages of germination and emergence, shortened the time of emergence and increased seedling height at 50% emergence, and shoot dry weight by 28 DAS under pre-flood conditions (Chapter 5). Also, aerenchyma formation in the cortical cells of rice roots increased with flooding and distance behind the root tips as reported from other studies (Ito *et al.*, 1999; Suralta and Yamauchi, 2008), and this forms the basis for the capacity of rice to not only survive, but also, to flourish in waterlogged environments (Setter *et al.*, 1997) in comparison to other field crops.

The overall grain yield increases observed in this study with nitrogen application as well as flooding have been engendered by improved tillering, panicle and shoot dry matter production, and nitrogen uptake. The positive relationships between each of these parameters and grain yield were similarly described by a number of authors (Baker *et al.*, 1992; Khush, 1995; Peng *et al.*, 1998; Kim *et al.*, 2001).

Overall, this study has demonstrated the need to supply nitrogen fertilizer to rice crops in the amount and at the time that will ensure optimum growth, grain yield and quality of the cultivars involved, while minimizing wastage and its adverse effect on the environment. It also showed that growing rice in wetlands has a considerable yield advantage compared to even well-watered aerobic conditions and, hence the proposed shift in SSA from upland to wetland conditions can only be beneficial. Although continuous flooding may have no adverse effect on the overall development and yield of rice, in the wetlands, early flooding followed by late drainage can be practised, instead of continuous flooding, to conserve water without penalty to yield.

Admittedly, a number of critical issues pertaining to nitrogen and water use efficiencies, as well as the direct effects of flooding on the physical and chemical properties of the soils could not be covered by this study due to the limitation of time. Some future work could include the issue of nitrogen loss in wetland conditions; *in situ* measurements of plant nutrient status, particularly at the commencement of tillering, active tillering, and early panicle initiation and differentiation could be considered in addition to measurements at heading and at grain filling, so as to gain insight into which phenological stage of the plant is most critical for the effectiveness of nitrogen supply and water level.

REFERENCES

ABDUL-SHUKOR, S. J., MUHAMMAD-SAIFU, A. H., MAHFUZAH, B., ANUAR, A. R. & AZMI, M. 2009. Influence of flood intensity and duration on rice growth and yield. *Pertanika Journal of Tropical Agriculture & Science,* 32**,** 195 - 208.

ADAK, M. K. & DAS GUPTA, D. K. 1999. Photosynthesis and net assimilation rate of rice cultivars as influenced by waterlogging. *Indian Journal of Plant Physiology,* 4**,** 334 - 336.

ADAK, M. K. & GUPTA, D. K. D. 2000. Prolonged waterlogging on photosynthesis and related characters in rice. *Indian Journal of Plant Physiology,* 5**,** 380 - 382.

ADDISCOTT, T. M., GOLD, A.J., OVIATT, C.A., BENJAMIN, N., AND GILLER, K.E. 2005. *Nitrate, Agriculture and the Environment,* London, CABI Publishing.

AFZAL, I., ASLAM, N., MAHMOOD, F., HAMEED, A., IRFAN, S. & AHMAD, G. 2004. Enhancement of germination and emergence of canola seeds by different priming techniques. *Caderno de Pesquisa série Biologia,* 16**,** 19 - 34.

AGGARWAL, R. K., BRAR, D. S., NANDI, S., HUANG, N. & KHUSH, G. S. 1999. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theoretical and Applied Genetics,* 98**,** 1320 - 1328.

ALAIS, C. & LINDEN, G. 1991. *Food Biochemistry,* Chichester, England, Ellis Horwood Ltd.

ALCANTARA, J. M. & CASSMAN, K. G. 1996. Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. *Cereal Chemistry,* 73**,** 556 - 560.

ALMEIDA, A. M., VRIEZEN, W. H. & VAN DER STRAETEN, D. 2003. Molecular and physiological mechanisms of flooding avoidance and tolerance in rice. *Russian Journal of Plant Physiology,* 50 743 - 751.

ALVA, A. & PETERSEN, L. 1979. Soil physical properties in relation to rice yield and water consumption under flooded and unflooded conditions. *Plant and Soil,* 52**,** 353 - 363.

AMON, B., AMON, T., BOXBERGER, J. & ALT, C. 2001. Emissions of NH_3, N_2O and CH_4 from dairy cows housed in a farmyard manure tying stall (housing, manure storage, manure spreading). *Nutrient Cycling in Agroecosystems,* 60**,** 103 - 113.

ANDREWS, M., LEA, P. J., RAVEN, J. A. & LINDSEY, K. 2004. Can genetic manipulation of plant nitrogen assimilation enzymes result in increased crop yield and greater N-use efficiency? An assessment. *Annals of Applied Biology,* 145**,** 25 - 40.

ARAYA, Y. N. 2007. Ecology of water relations in plants. *Encyclopaedia of Life Sciences.* Not Set ed.: John Wiley & Sons, Ltd.

ARMSTRONG, W. 1979. Aeration in higher plants. *In:* WOOLHOUSE, H. W. (ed.) *Advances in Botanical Research.* London: Academic Press.

ARMSTRONG, W. 1980. Aeration in higher plants. *Advances in Botanical Research,* 7**,** 225 - 332.

AWAN, T. H., AHMAD, M., ALI, I., ANWAR, M. & MANZOOR, Z. 2007. Contribution of tillers within a rice plant to yield and yield components. *Journal of Agricultural Ressearch,* 45**,** 237 - 243.

AYRES, N. M., MCCLUNG, A. M., LARKIN, P. D., BLIGH, H. F. J., JONES, C. A. & PARK, W. D. 1997. Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germplasm. *Theoretical and Applied Genetics,* 94**,** 773 - 781.

AZAM, F., MALIK, K. A. & HUSSAIN, F. 1986. Microbial biomass and mineralization-immobilization of nitrogen in some agricultural soils. *Biology and Fertility of Soils,* 2**,** 157 - 163.

BACON, P. E. 1985. The effect of nitrogen application time on Calrose rice growth and yield in south-eastern Australia. *Australian Journal of Experimental Agriculture,* 25**,** 183 - 190.

BAKER, J. T., ALLEN JR, L. H. & BOOTE, K. J. 1992. Temperature effects on rice at elevated CO_2 concentration. *Journal of Experimental Botany,* 43**,** 959 - 964.

BALASUBRAMANIAN, V. 1998. Farmer adoption of improved nitrogen management technologies in rice farming: technical constraints and opportunities for improvement. *Nutrient Cycling in Agroecosystems,* 53**,** 93 - 101.

BALASUBRAMANIAN, V. & HILL, J. E. 2002. Direct seeding of rice in Asia: emerging issues and strategic research needs for the 21st century. *In:* PANDEY, S., MORTIMER, M., WADE, L., TUONG, T. P., LOPEZ, K. & HARDY, B. (eds.) *Direct*

Seeding: Research Strategies and Opportunities. Los Banos, Philippines: International Rice Reseach Institute.

BALASUBRAMANIAN, V., SIE, M., HIJMANS, R. J. & OTSUKA, K. 2007. Increasing rice production in Sub-Saharan Africa: challenges and opportunities. *Advances in Agronomy,* 94**,** 55 - 133.

BASRA, S. M. A., FAROOQ, M. & KHALIQ, A. 2003. Comparative study of pre-sowing seed enhancement treatments in fine rce (*Oryza sativa* L.). *Pakistan Journal of Life and Social Sciences,* 1**,** 5 - 9.

BASRA, S.M.A., FAROOQ, M., & TABASSUM, R. 2005. Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (*Oryza sativa* L.). *Seed Science & Technology*, 33, 623 - 628.

BELDER, P. 2005. *Water saving in lowland rice production: An experimental and modelling study*. PhD, Wageningen University.

BELDER, P., BOUMAN, B. A. M., CABANGON, R., GUOAN, L., QUILANG, E. J. P., YUANHUA, L., SPIERTZ, J. H. J. & TUONG, T. P. 2004. Effect of water-saving irrigation on rice yield and water use in typical lowland conditions in Asia. *Agricultural Water Management,* 65**,** 193 - 210.

BENNION, M. 1980. *The Science of Food,* San Fransco, Harper & Row Publishers.

BERA, R., SEAL, A., BHATTACHARYYA, P., DAS, T. H., SARKAR, D. & KANGJOO, K. 2006. Targeted yield concept and a framework of fertilizer recommendation in irrigated rice domains of sub-tropical India. *Journal of Zhejiang University of Science,* 7**,** 963 - 968.

BEWLEY, J. D. & BLACK, M. 1994. *Seeds: Physiology of Development and Germination,* New York, Plenum Press.

BHUIYAN, N. I., CRASSWELL, E. T. & BOULDIN, D. R. 1984. Yield and crop parameters of wetland rice as influenced by soil and fertilizer nitrogen. *Plant and Soil,* 79**,** 369 - 381.

BJORKMAN, O. & DEMMIG, B. 1987. Photon yield of CO_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta,* 170**,** 489 - 504.

BLAKENEY, A. B. 1979. Rice grain quality evaluation in Australia. *Chemical Aspects of Rice Grain Quality.* Los Banos, Philippines: International Rice research Institute.

BLEECKER, A. B., SCHUETTE, J. L. & KENDE, H. 1986. Anatomical analysis of growth and developmental patterns in the internode of deepwater rice. *Planta,* 169**,** 490 - 497.

BOCK, E., SCHMIDT, I., STÜVEN, R. & ZART, D. 1995. Nitrogen loss caused by denitrifying Nitrosomonas cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor. *Archives of Microbiology,* 163**,** 16 - 20.

BODELIER, P. L. E. 2003. Interactions between oxygen-releasing roots and microbial processes in flooded soils and sediments. *In:* DE KROON, H. & VISSER, E. J. W. (eds.) *Root Ecology.* Berlin: Springer-Verlag.

BODELIER, P. L. E., ROSLEV, P., HENCKEL, T. & FRENZEL, P. 2000. Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature,* 403**,** 42 1 - 424.

BOOIJ, R., KREUZER, A. L., SMIT, A. L. & VAN DER WERF, A. 1996. Effect of nitrogen availability on dry matter production, nitrogen uptake and light interception of Brussels spouts and leeks. *Netherlands Journal of Agricultural Science,* 44**,** 3 - 19.

BOONJUNG, H. & FUKAI, S. 1996. Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Research,* 48**,** 47 - 55.

BOUMAN, B. A. M. & TUONG, T. P. 2001. Field water management to save water and increase its productivity in irrigated lowland rice. *Agricultural Water Management,* 49**,** 11 - 30.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72**,** 248 - 254.

BREMNER, J. M. 1995. Recent research on problems in the use of urea as a nitrogen fertilizer. *Nutrient Cycling in Agroecosystems,* 42**,** 321 - 329.

BRISKE, D. D. 1991. Developmental morphology and physiology of grasses. *In:* HEITSCHMIDST, R. K. & STUTH, J. W. (eds.) *Grazing Management: an ecological perspective.* Portland: Timber Press.

BRYANT, R. J., ANDERS, M. & MCCLUNG, A. M. 2009. Effect of cultural management practices on grain quality of two rice cultivars. *Cereal Chemistry,* 86**,** 405 - 409.

BRYANT, R. J., ANDERS, M. M. & MCCLUNG, A. M. 2008. Effect of nitrogen application and crop rotation on rice grain quality. *Proceedings of the 32nd Rice Technical Working Group Meetings, February 18 - 21, 2008.* San Diego, California.

BUFOGLE, A., BOLLICH, P. K., KOVAR, J. L., MACCHIAVELLI, R. E. & LINDAU, C. W. 1997. Rice variety differences in dry matter and nitrogen accumulation as related to plant stature and maturity group. *Journal of Plant Nutrition,* 20**,** 1203 - 1224.

BULMAN, P. & HUNT, L. A. 1988. Relationships among tillering, spike number and grain yield in winter wheat (*Triticum aestivum* L.) in Ontario. *Canadian Journal of Plant Science,* 68**,** 583 - 596.

BURGASS, R. W. & POWELL, A. A. 1984. Evidence for repair processes in the invigoration of seeds by hydration. *Annals of Botany,* 53**,** 753.

CABANGON, R. J., TUONG, T. P., CASTILLO, E. G., BAO, L. X., LU, G., , WANG, G., CUI, Y., BOUMAN, B. A. M., LI, Y., CHEN, C. & WANG, J. 2004. Effect of irrigation method and N-fertilizer management on rice yield, water productivity and nutrient-use efficiencies in typical lowland rice conditions in China. *Paddy and Water Environment,* 2 195 - 206.

CASSMAN, K. G. & PINGALI, P. L. 1995. Intensification of irrigated rice systems: learning from the past to meet future challenges. *GeoJournal,* 35**,** 299 - 305.

CASTILLO, E. G., BURESH, R. J. & INGRAM, K. T. 1992. Lowland rice yield as affected by timing of water deficit and nitrogen fertilization. *Agronomy Journal*, 84**,** 152 - 159.

CGIAR. 2006. *Story of the Month: September 2006* [Online]. Consultative Group on International Agricultural Research (CGIAR). Available: http://www.cgiar.org/monthlystory/september2006.html [Accessed 18 September 2010].

CHAMPAGNE, E. T., BETT-GARBER, K. L., THOMSON, J. L. & FITZGERALD, M. A. 2009. Unraveling the impact of nitrogen nutrition on cooked rice flavor and texture. *Cereal Chemistry,* 86**,** 274 - 280.

CHAU, N. N. & KUNZE, O. R. 1982. Moisture content variation among harvested rice grains. *American Society of Agricultural and Biological Engineers,* 25**,** 1037 - 1040.

CHEN, J., CHEN, M. & HE, Y. 2001. Effects of soil water condition and microclimate on transpiration rate of rice. *Journal of Applied Ecology,* 12**,** 63 - 67.

CHESHIRE, M., BEDROCK, C., WILLIAMS, B., CHAPMAN, S., SOLNTSEVA, I. & THOMSEN, I. 1999. The immobilization of nitrogen by straw decomposing in soil. *European Journal of Soil Science,* 50**,** 329 - 341.

CHINNASWAMY, R. & BHATTACHARYA, K. R. 1984. Relationship between amylose content and expansion characteristics of parboiled rice. *Journal of Cereal Science,* 2**,** 273 - 279.

CHIOU, H., MARTIN, M. & FITZGERALD, M. A. 2002. Effect of purification methods on rice starch structure. *Starch - Stärke,* 54**,** 415 - 420.

CHOPRA, V. L. & SHYAM (eds.) 2002. *Evolution and Adaptaion of Cereal Crops,* Enfield, USA: Science Publishers, Inc.

CLARK, L. H. & HARRIS, W. H. 1981. Observations on the root anatomy of rice (*Oryza sativa* L.). *American Journal of Botany,* 68**,** 154 - 161.

COLE, C. V., DUXBURY, J., FRENEY, J., HEINEMEYER, O., MINAMI, K., MOSIER, A., PAUSTIAN, K., ROSENBERG, N., SAMPSON, N. & SAUERBECK, D. 1997. Global estimates of potential mitigation of greenhouse gas emissions by agriculture. *Nutrient Cycling in Agroecosystems,* 49**,** 221 - 228.

COLMER, T. D. 2003. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Annals of Botany,* 91**,** 301 - 309.

COMIS, D. 1997. Aerenchyma: lifelines for living underwater. *Agricultural Research,* 45**,** 4 - 8.

COUNCE, P. A., KEISLING, T. C. & MITCHELL, A. J. 2000. A uniform, objective, and adaptive system for expressing rice development. *Crop Science,* 40**,** 436 - 443.

COUNCE, P. A., SIEBENMORGEN, T. J., POAG, M. A., HOLLOWAY, G. E., KOCHER, M. F. & LU, R. 1996. Panicle emergence of tiller types and grain yield of tiller order for direct-seeded rice cultivars. *Field Crops Research,* 47**,** 235 - 242.

CRAMER, G. L., WAILES, E. J. & SHUI, S. 1993. Impacts of liberalizing trade in the world rice market. *American Journal of Agricultural Economics,* 75**,** 219 - 226.

DAS, A. & UCHIMIYA, H. 2002. Oxygen stress and adaptation of a semi-aquatic plant: rice (*Oryza sativa*). *Journal of Plant Research,* 115**,** 315 - 320.

DAS, D. K. & JAT, R. L. 1977. Influence of three soil-water regimes on root porosity and growth of four rice varieties. *Agronomy Journal,* 69**,** 197 - 200.

DAS, K. K., SARKAR, R. K. & ISMAIL, A. M. 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. *Plant Science,* 168**,** 131 - 136.

DAVIDSON, E. A. & KINGERLEE, W. 1997. A global inventory of nitric oxide emissions from soils. *Nutrient Cycling in Agroecosystems,* 48**,** 37 - 50.

DE DATTA, S. K. 1981. *Principles and Practices of Rice Production,* New York, John Wiley & Sons, Inc.

DE DATTA, S. K. 1987a. Advances in soil fertility research and nitrogen fertilizer for lowland rice. *Efficiency of Nitrogen Fertilizers for Rice.* Los Banos, Philippines: International Rice Research Institute.

DE DATTA, S. K. 1987b. Nitrogen transformation processes in relation to improved cultural practices for lowland rice. *Plant and Soil,* 100**,** 47 - 69.

DE DATTA, S. K. & BURESH, R. J. 1989. Integrated nitrogen management in irrigated rice. *In:* BEAUCHAMP, E. G. (ed.) *Advances in Soil Science.* New York: Springer-Verlag.

DE DATTA, S. K., FILLERY, I. R. & CRASSWELL, E. T. 1983. Results from recent studies on nitrogen fertilizer efficiency in wetland rice. *Outlook Agriculture,* 12**,** 125 - 134.

DE DATTA, S. K., TAURO, A. C. & BALAOING, S. N. 1968. Effect of plant type and nitrogen level on growth characteristics and grain yield of indica rice in the tropics. *Agronomy Journal,* 60**,** 643 – 647.

DELGADO, J. A. 2002. Quantifying the loss mechanisms of nitrogen. *Journal of Soil and Water Conservation,* 57**,** 389 - 398.

DICKIE, D. 1997. *Using Urea and Anhydrous Ammonia on Corn Silage* [Online]. Available: http://www.omafra.gov.on.ca/english/livestock/beef/facts/urea.htm [Accessed 30 March 2009].

DINGKUHN, M., JONES, M. P., JOHNSON, D. E. & SOW, A. 1998. Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies. *Field Crops Research,* 57**,** 57 - 69.

DINGKUHN, M., SCHNIER, H. F., DE DATTA, S. K., DORFFLING, K. & JAVELLANA, C. 1991. Relationships between ripening-phase productivity and crop duration, canopy photosynthesis and senescence in transplanted and direct-seeded lowland rice. *Field Crops Research,* 26**,** 327 - 345.

DOLO, J. S. 2009. Nation-wide assessment of wetlands in large rubber concession areas. Monrovia, Liberia: Ministry of Agriculture.

DONALD, C. M. & HAMBLIN, J. 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. *Advances in Agronomy,* 28**,** 361 - 405.

DREW, M. C. 1992. Soil aeration and plant root metabolism. *Soil Science,* 154**,** 259 - 268.

DREW, M. C., HE, C. J. & MORGAN, P. W. 2000. Programmed cell death and aerenchyma formation in roots. *Trends in Plant Science,* 5**,** 123 - 127.

DUAN, M. & SUN, S. S. M. 2005. Profiling the expression of genes controlling rice grain quality. *Plant Molecular Biology,* 59**,** 165 - 178.

DUFF, J. H., TESORIERO, A. J., RICHARDSON, W. B., STRAUSS, E. A. & MUNN, M. D. 2008. Whole-stream response to nitrate loading in three streams draining agricultural landscapes. *Journal of Environmental Quality,* 37**,** 1133 - 1144.

DUWIG, C., BECQUER, T., VOGELER, I., VAUCLIN, M. & CLOTHIER, B. E. 2000. Water dynamics and nutrient leaching through a cropped ferralsol in the Loyalty Islands (New Caledonia). *Journal of Environmental Quality,* 29**,** 1010 - 1019.

EKANAYAKE, I. J., DE DATTA, S. K. & STEPONKUS, P. L. 1993. Effect of water deficit stress on diffusive resistance, transpiration, and spikelet desiccation of rice (*Oryza sativa* L.). *Annals of Botany,* 72**,** 73 - 80.

ERIKSEN, A., KJELDBY, M. & NILSEN, S. 1985. The effect of intermittent flooding on the growth and yield of wetland rice and nitrogen-loss mechanism with surface applied and deep placed urea. *Plant and Soil,* 84**,** 387 - 401.

EVANS, D. E. 2004. Aerenchyma formation. *New Pathologist,* 161**,** 35 - 49.

EVANS, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia,* 78**,** 9 - 19.

FAGERIA, N. K. 2007. Yield physiology. *Journal of Plant Nutrition,* 30**,** 843 - 879.

FAGERIA, N. K. 2009. *The Use of Nutrients in Crop Plants,* New York, CRC Press.

FAGERIA, N. K. & BALIGAR, V. C. 2001. Lowland rice response to nitrogen fertilization. *Communications in Soil Science and Plant Analysis,* 32**,** 1405 - 1429.

FAGERIA, N. K., BALIGAR, V. C. & CLARK, R. B. 2006. *Physiology of Crop Production,* New York, Haworth Press, Inc.

FAO. 2009. *FAOSTAT* [Online]. Food & Agriculture Organization. [Accessed 25 March 2009].

FAO. 2010. *FAOSTAT* [Online]. FAO Statistics Division. Available: http://www.faostat.fao.org [Accessed 5 June 2010].

FAROOQ, M. & BASRA, S. M. A. 2006. Seed priming enhances emergence, yield, and quality of direct-seeded rice. *International Rice Research Notes,* 31**,** 42 - 44.

FAROOQ, M., BASRA, S. M. A. & AHMAD, N. 2007. Improving the performance of transplanted rice by seed priming. *Plant Growth Regulation,* 51**,** 129 - 137.

FAROOQ, M., WAHID, A., AHMAD, N. & ASAD, S. 2010. Comparative efficacy of surface drying and re-drying seed priming in rice: changes in emergence, seedling growth and associated metabolic events. *Paddy and Water Environment,* 8**,** 15 - 22.

FIGUEROA, M. E., FERNÁNDEZ-BACO, L., LUQUE, T. & DAVY, A. J. 1997. Chlorophyll fluorescence, stress and survival in populations of Mediterranean grassland species. *Journal of Vegetation Science* 8**,** 881 - 888.

FOX, B. A. & CAMERON, A. G. 1982. *Food Science: A Chemical Approach,* London, Hodder and Stoughton Educational.

FRANCESCO, V. & ALDO, F. 2000. Germination behaviour of red rice (*Oryza sativa* L.) seeds in field and laboratory conditions. *Agronomie,* 20**,** 375 - 382.

FRENSCH, J. & HSIAO, T. C. 1994. Transient responses of cell turgor and growth of maize roots as affected by changes in water potential. *Plant Physiology,* 104**,** 247 - 254.

FUJISAKA, S. 1993. Were farmers wrong in rejecting a recommendation? The case of nitrogen at transplanting for irrigated rice. *Agricultural Systems,* 43**,** 271 - 286.

FUKAI, S. & INTHAPAN, P. 1988. Growth and yield of rice cultivars under sprinkler irrigation in south-eastern Queensland. 3. Water extraction and plant water relations—comparison with maize and grain sorghum. *Australian Journal of Experimental Agriculture,* 28**,** 249 - 252.

FUKAO, T. & BAILEY-SERRES, J. 2007. Ethylene-A key regulator of submergence responses in rice. *Plant Science,* 175**,** 43 - 51.

FUKAO, T. & BAILEY-SERRES, J. 2008. Ethylene- A key regulator of submergence responses in rice. *Plant Science,* 175**,** 43 - 51.

FURUKAWA, S., TANAKA, K., MASUMURA, T., OGIHARA, Y., KIYOKAWA, Y. & WAKAI, Y. 2006. Influence of rice proteins on eating quality of cooked rice and on aroma and flavor of sake. *Cereal Chemistry,* 83**,** 439 - 446.

GALLOWAY, J. N., DENTENER, F. J., CAPONE, D. G., BOYER, E. W., HOWARTH, R. W., SEITZINGER, S. P., ASNER, G. P., CLEVELAND, C. C., GREEN, P. A., HOLLAND, E. A., KARL, D. M., MICHAELS, A. F., PORTER, J. H., TOWNSEND, A. R. & VÖOSMARTY, C. J. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry,* 70**,** 153 - 226.

GAMBRELL, R. P., DELAUNE, R. D. & PATRICK, J., W. H. 1991. Redox processes in soils following oxygen depletion. *In:* JACKSON, M. B., DAVIES, D. D. & LAMBERS, H. (eds.) *Plant Life Under Oxygen Deprivation: Ecology, Physiology and Biochemistry.* Hague, Netherlands: SPB Academic Publishing.

GAN, Y., STOBBE, E. H. & MOES, J. 1992. Relative date of wheat seedling emergence and its impact on grain yield. *Crop Science,* 32**,** 1275 - 1281.

GEORGE, T., LADHA, J. K., BURESH, R. J. & GARRITY, D. P. 1992. Managing native and legume-fixed nitrogen in lowland rice-based cropping systems. *Plant and Soil,* 141**,** 69 - 91.

GHASSEMI-GOLEZANI, K., CHADORDOOZ-JEDDI, A., NASROLLAHZADEH, S. & MOGHADDAM, M. 2010. Effects of hydro-priming duration on seedling vogour and grain yield of pinto bean (*Phaseolus vulgaris* L.) cultivars. *Notulae Botanicae Horti-Agrobotanici Cluj-Napoca,* 38**,** 109 - 113.

GHASSEMI-GOLEZANI, K., SHEIKHZADEH-MOSADDEGH, P. & VALIZADEH, M. 2008. Effects of hydro-priming duration and limited irrigation on field performance of chickpea. *Research Journal of Seed Science,* 1**,** 34 - 40.

GHOSH, B. C. & BHAT, R. 1998. Environmental hazards of nitrogen loading in wetland rice fields. *Environmental Pollution,* 102**,** 123 - 126.

GOYA. 2010. *Rice- wholesome and nutritious* [Online]. New Jersey: goya.com. [Accessed 25 February 2010].

GRAVOIS, K. A. & HELMS, R. S. 1992. Path analysis of rice yield and yield components as affected by seeding rate. *Agronomy Journal,* 88**,** 1 - 4.

GUJRAL, H. S. & KUMAR, V. 2003. Effect of accelerated aging on the physicochemical and textural properties of brown and milled rice. *Journal of Food Engineering,* 59**,** 117 - 121.

HAEFELE, S. M., WOPEREIS, M. C. S., NDIAYE, M. K., BARRO, S. E. & OULD ISSELMOU, M. 2003. Internal nutrient efficiencies, fertilizer recovery rates and indigenous nutrient supply of irrigated lowland rice in Sahelian West Africa. *Field Crops Research,* 80**,** 19 - 32.

HAMAKER, B. R. 1994. The influence of protein on rice quality. *In:* MARSHALL, W. B. & WADSWORTH, J. I. (eds.) *Rice Science and Technology.* New York, USA: Marcel Dekker, Inc.

HANIF, M. 1987. Diffusion of ammonium and nitrate ions in flooded soil and nitrogen use efficiency of an irrigated rice system. *Communications in Soil Science and Plant Analysis,* 18**,** 1155 - 1172.

HARRIS, D. & JONES, M. P. 1997. On-farm seed priming to accelerate germination in rainfed, dry-seeded rice. *International Rice Research Notes,* 22**,** 30.

HARRIS, D., TRIPATHI, R. S. & JOSHI, A. 2002. On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. *In:* PANDEY, S., MORTIMER, M., WADE, L., TUONG, T. P., LOPEZ, K. & HARDY, B. (eds.) *Direct Seeding: Research Strategies and Opportunities.* Manila, Philippines: International Rice Research Institute.

HASEGAWA, H. 2003. High-yielding rice cultivars perform best even at reduced nitrogen fertilizer rate. *Crop Science,* 43**,** 921 - 926.

HATFIELD, J. L., SAUER, T. J. & PRUEGER, J. H. 2001. Managing soils to achieve greater water use efficiency: A review. *Agronomy Journal,* 93**,** 271.

HEENAN, D. P. & LEWIN, L. G. 1984. Response of Inga rice to nitrogen fertilizer rate and timing in New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry,* 22**,** 62 - 66.

HERDT, R. W. & WICKHAM, T. H. 1978. Exploring the gap between potential and actual rice yields: the Philippine case. *Economic Consequences of the New Rice Policy.* Los Banos, Philippines: International Rice Research Institute.

HIREL, B., LE GOUIS, J., NEY, B. & GALLAIS, A. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany,* 58**,** 2369 - 2387.

HIROSE, T. & WERGER, M. J. A. 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia,* 72**,** 520 - 526.

HOFFMANN-BENNING, S. & KENDE, H. 1992. On the role of abscisic acid and gibberellin in the regulation of growth in rice. *Plant Physiol.,* 99**,** 1156 - 1161.

HONJYO, K. 1971. Studies on protein content in rice grain: II. Effects of the fertilization on protein content and protein produtction in paddy grain. *Japanese Journal of Crop Science,* 40**,** 190 - 196.

HOOVER, R. & RATNAYAKE, W. S. 2001. Determination of Total Amylose Content of Starch. *Current Protocols in Food Analytical Chemistry.* John Wiley & Sons, Inc.

HOPKINS, A. (ed.) 2000. *Grass: Its Production and Utilization,* London: Blackwell Science, Ltd.

HORIE, T., SHIRAIWA, T., HOMMA, K., KATSURA, K., MAEDA, S. & YOSHIDA, H. 2005. Can yields of lowland rice resume the increases that they showed in the 1980s? *Plant Production Science,* 8**,** 259 - 274.

HOSSAIN, M. 1997. Rice supply and demand in Asia: a socioeconomic and biophysical analysis. *In:* TENG, P. S., KROPFF, M. J., TEN BERGE, H. F. M., DENT, J. B., LANSIGAN, F. P. & VAN LAAR, H. H. (eds.) *Applications of Systems Approaches at the Farm and Regional Levels.* London: Kluwer Academic Publishers.

HUANG, J., HE, F., CUI, K., BURESH, R. J., XU, B., GONG, W. & PENG, S. 2008. Determination of optimal nitrogen rate for rice varieties using a chlorophyll meter. *Field Crops Research,* 105**,** 70 - 80.

IDA, M., OHSUGI, R., SASAKI, H., AOKI, N. & YAMAGISHI, T. 2009. Contribution of nitrogen absorbed during ripening period to grain filling in a high-yielding rice variety, Takanari. *Plant Production Science,* 12**,** 176 - 184.

INGRAM, K. T., DINGKUHN, M., NOVERO, R. P. & WIJANGCO, E. J. 1991. Growth and CO_2 assimilation of lowland rice in response to timing and method of N fertilization. *Plant and Soil,* 132**,** 113 - 125.

INGRAM, K. T. & YAMBAO, E. B. 1988. Rice sensitivity to water deficit at different growth stages. *International Rice Research News Letter,* 13**,** 16 - 17.

INOCENCIO, A. & MERREY, H. 2003. Innovative approaches to agricultural water use for improving food security in Sub-Saharan Africa. International Water Management Institute.

IQBAL, A., KHALIL, I. A. & SHAH, H. 2003. Nutritional yield and amino acid profile of rice protein as influenced by nitrogen fertilizer. *Sarhad Journal of Agriculture,* 19**,** 127 - 134.

IQBAL, S. 2007. *Modeling the impact of climate change on rice (Oryza sativa) productivity in Punjab under nitrogen split application.* PhD, Faisalabad University of Agriculture.

IRRI. 2007. *Top 10 rice-producing countries* [Online]. International Rice Research Institute. [Accessed 19 May 2010].

IRRI. 2009. *Rice quatity training manual* [Online]. Los Banos, Philippines: International Rice Research Institute. Available: http://www.knowledgebank.irri.org/postproductioncourse/factsheetsNRefences [Accessed 5 June 2009].

ISLAM, S., HASANUZZAMAN, M., ROKONUZZAMAN, M. & NAHAR, K. 2009. Effect of split application of nitrogen on morphological parameters of rice genotypes. *Intenational Journal of Plant Production,* 3**,** 51 - 62.

ISRAELSEN, O. W. & HANSEN, V. E. 1962. Irrigation Principles and Practices. New York: John Wiley and Sons Inc.

ITO, O., ELLA, E. & KAWANO, N. 1999. Physiological basis of submergence tolerance in rainfed lowland rice ecosystem. *Field Crops Research,* 64**,** 75 - 90.

JACKSON, M. & ARMSTRONG, W. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology,* 1**,** 274 - 287.

JACKSON, M. B., FENNING, T. M. & JENKINS, W. 1985. Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. *Journal of Experimental Botany,* 36**,** 1566 - 1572.

JACKSON, M. B. & RAM, P. C. 2003. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. *Annals of Botany,* 91**,** 227 - 241.

JAHAN, M. S. 2004. *Rice production under different water input.* MSc., Universiti Putra Malaysia.

JAVIER, E. L. 1997. Rice ecosystems and varieties. *Rice Production in Cambodia, International Rice Research Institute, Manila, Philippines*.

JEARAKONGMAN, S., RAJATASEREEKUL, S., NAKLANG, K., ROMYEN, P., FUKAI, S., SKULKHU, E., JUMPAKE, B. & NATHABUTR, K. 1995. Growth and grain yield of contrasting rice cultivars grown under different conditions of water availability. *Field Crops Research,* 44**,** 139 - 150.

JENG, T. L., TSENG, T. H., WANG, C. S., CHEN, C. L. & SUNG, J. M. 2006. Yield and grain uniformity in contrasting rice genotypes suitable for different growth environments. *Field Crops Research,* 99**,** 59 - 66.

JENSEN, M. E., ROBB, D. C. N. & FRANZOY, C. E. 1970. Scheduling irrigations using climate-crop-soil data. *Proceedings of the American Society of Civil Engineers, Journal of the Irrigation and Drainage Division,* 96**,** 25 - 38.

JENSEN, T. 2006. Nitrogen fertilizer, forms and methods of application. *Integrated Crop Production Update 2006 Conference.* Lethbridge, Alberta.

JIANG, L., DAI, T., JIANG, D., CAO, W., GAN, X. & WEI, S. 2004. Characterizing physiological N-use efficiency as influenced by nitrogen management in three rice cultivars. *Field Crops Research,* 88**,** 239 - 250.

JIANG, L., DONG, D., GAN, X. & WEI, S. 2005. Photosynthetic efficiency and nitrogen distribution under different nitrogen management and relationship with physiological N-use efficiency in three rice genotypes. *Plant and Soil,* 271**,** 321 - 328.

JIANPING, S. U. N., CAIYUN, H. O. U. & SHAOYING, Z. 2008. Effect of protein on the rheological properties of rice flour. *Journal of Food Processing and Preservation,* 32**,** 987-1001.

JING, Y. A. N., JUN, V. U., GUANG CAN, T. A. O., JAN, V. O. S., BOUMAN, B. A, M., GUANG HUI, X. I. E., MEINKE & HOLGER 2010. Yield formation and tillering dynamics of direct-seeded rice in flooded and nonflooded soils in the Huai River Basin of China. *Field Crops Research,* 116**,** 252 - 259

JONGKAEWWATTANA, S., GENG, S., BRANDON, D. M. & HILL, J. E. 1993. Effect of nitrogen and harvest grain moisture on head rice yield. *Agronomy Journal,* 85**,** 1143 - 1146.

JULIANO, B. O. 1993. *Rice in Human Nutrition,* Rome, Food and Agriculture Orgainzation & International Rice Research Institute

JULIANO, B. O. & VILLAREAL, C. P. 1993. *Grain Quality Evaluation of World Rices,* Manila, Philippines, International Rice Research Institute.

JURAIMI, A. S., SAIFUL, M. A. H., BEGUM, M., ANUAR, A. R. & AZMI, M. 2009. Influence of flooding intensity and duration on rice growth and yield. *Pertanika Journal of Tropical Agricultural Science* 32 195 - 208.

JUSTIN, S. H. F. W. & ARMSTRONG, W. 1991. Evidence for the involvement of ethene in aerenchyma formation in adventitious roots of rice (*Oryza sativa* L.). *New Phytologist,* 118**,** 49 - 62.

KAMINSKAIA, N. V. & KOSTIC, N. M. 1997. Kinetics and mechanism of urea hydrolysis catalyzed by palladium(II) complexes. *Inorganic Chemistry,* 36**,** 5917 - 5926.

KANG, S. Z., ZHANG, L., LIANG, Y. L., HU, X. T., CAI, H. J. & GU, B. J. 2002. Effects of limited irrigation on plant biomass and water use efficiency of winter wheat in the loess plateau of China *Agricultural Water Management* 55**,** 203 – 216.

KAOSAARD, M. & JULIANO, B. O. 1991. Assessing rice quality characteristics and prices in selected international markets. *Rice Grain Marketing and Quality Issues.* Manila, Philippines: International Rice Research Institute.

KARTICK CHANDRA, S., KARIALI, EKAMBER & PRAVAT KUMAR, M. 2004. Tiller dominance in rice is dependent on assimilate concentration of the panicle during grain filling. *Indian Journal of Plant Physiology* 9**,** 402 - 406.

KATO, T. 1989. Relationship between grain-filling process and sink capacity in rice (*Oryza sativa* L.). *Japanese Journal of Breeding,* 39**,** 431 - 438.

KATO, Y. & OKAMI, M. 2010. Root growth dynamics and stomatal behaviour of rice (*Oryza sativa* L.) grown under aerobic and flooded conditions. *Field Crops Research,* 117**,** 9 - 17.

KAUL, A. K. & RAGHAVIAH, P. 1975. Influence of nitrogen fertilization on some nutritional quality characters in rice. *Plant Foods for Human Nutrition* 24**,** 391 - 403.

KAWAI, M., SAMARAJEEWA, P. K., BARRERO, R. A., NISHIGUCHI, M. & UCHIMIYA, H. 1998. Cellular dissection of the degradation pattern of cortical cell death during aerenchyma formation of rice roots. *Planta,* 204**,** 277 - 287.

KENDE, H., VAN DER KNAAP, E. & CHO, H.-T. 1998. Deepwater rice: a model plant to study stem elongation. *Plant Physiology,* 118 1105 - 1110.

KHIND, C. & PONNAMPERUMA, F. 1981. Effects of water regime on growth, yield, and nitrogen uptake of rice. *Plant and Soil,* 59 287 - 298.

KHUSH, G. S. 1995. Breaking the yield frontier of rice. *GeoJournal,* 35**,** 329 - 332.

KIM, H. Y., LIEFFERING, M., MIURA, S., KOBAYASHI, K. & OKADA, M. 2001. Growth and nitrogen uptake of CO_2 enriched rice under field conditions. *New Phytologist,* 150**,** 223 - 229.

KIRK, G. J. D. & KRONZUCKER, H. J. 2000. Nitrogen uptake by rice roots. *In:* OLK, G. J. D. K. A. D. C. (ed.) *Carbon and Nitrogen Dynamics in Flooded Soils.* . Los Banos, Philippines: International Rice Research Institute.

KISSEL, D. E. & CABRERA, M. L. 1988. Factors affecting urea hydrolysis. *In:* BOCK, B. R. & KIESSEL, D. E. (eds.) *Ammonia volatilization form urea fertilizers.* Muscle Shoals: National Fertilizer Development Center, Tennese Valley Authority.

KLUDZE, H. K., DELAUNE, R. D. & PATRICK JR., W. H. 1994. A colorimetric method for assaying dissolved oxygen loss from container-grown rice roots. *Agronomy Journal,* 86**,** 483 - 487.

KOZLOWSKI, T. T. 1984. Plant responses to flooding of soil. *BioScience,* 34**,** 162 - 167.

KUMAGAI, T., KAWAMURA, H., FUSE, T., WATANABE, T., SAITO, Y., MASUMURA, T., WATANABE, R. & KADOWAKI, M. 2006. Production of rice protein by alkaline extraction improves its digestibility. *Journal of Nutritional Science and Vitaminology,* 52**,** 467 - 472.

LAFARGE, T., TUBANA, B. & PASUQUIN, E. M. 2004. Yield advantage of hybrid rice induced by its higher control in tiller emergence. *In:* TURNER, N., ANGUS, J., MCINTYRE, L., ROBERTSON, M., BORRELL, A. & LLOYD, D. (eds.) *New directions for a diverse planet : Proceedings of the 4th International Crop Science Congress.* Brisbane, Australia: Gosford Regional Institute, International Crop Science Congress.

LAFITTE, H. R., PRICE, A. H. & COURTOIS, B. 2004. Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. *Theoretical and Applied Genetics,* 109**,** 1237 - 1246.

LÁSZTITY, R. 1996. *The chemistry of Cereal Proteins,* Boca Raton, USA, CRC Press.

LEUNING, R., KELLIHER, F. M., DE PURY, D. G. G. & SCHULZE, E. D. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell & Environment,* 18**,** 1183 - 1200.

LI, X., QIAN, Q., FU, Z., WANG, Y., XIONG, G., ZENG, D., WANG, X., LIU, X., TENG, S., HIROSHI, F., YUAN, M., LUO, D., HAN, B. & LI, J. 2003. Control of tillering in rice. *Nature,* 422**,** 618 - 621.

LILLEY, J. M. & FUKAI, S. 1994. Effect of timing and severity of water deficit on four diverse rice cultivars III. Phenological development, crop growth and grain yield. *Field Crops Research,* 37**,** 225 - 234.

LIN, Y.S., YEH, A.I. & LII, C.Y. 2001. Correlation between starch retrogradation and water mobility as determined by differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). *Cereal Chemistry,* 78**,** 647 - 653.

LU, J., OOKAWA, T. & HIRASAWA, T. 2000. The effects of irrigation regimes on the water use, dry matter production and physiological responses of paddy rice. *Plant and Soil,* 223**,** 209 - 218.

MACKILL, D. J., AMANTE, M. M., VERGARA, B. S. & SARKARUNG, S. 1993. Improved semidwarf rice lines with tolerance to submergence of seedlings. *Crop Science,* 33**,** 749 - 753.

MACLEAN, J. L., DAWE, D. C., HARDY, B. & HETTEL, G. P. (eds.) 2002. *Rice Almanac: Source Book for the Most Important Economic Activity on Earth,* Oxon, United Kingdom: CABI Publishing.

MAE, T. 1997. Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant and Soil,* 196**,** 201 - 210.

MAGNESCHI, L. & PERATA, P. 2009. Rice germination and seedling growth in the absence of oxygen. *Annals of Botany* 103**,** 181- 196.

MALIK, A. I., COLMER, T. D., LAMBERS, H. & SCHORTEMEYER, M. 2003. Aerenchyma formation and radial O_2 loss along adventitious roots of wheat with only the apical root portion exposed to O_2 deficiency. *Plant, Cell and Environment,* 26**,** 1713 - 1722.

MAO, Z., LI, Y., TUONG, T. P., MOLDEN, D. & DONG, B. 2000. Water-saving irrigation practices for rice in China. *International Rice Research Conference.* Los Banos, Philippines: International Rice Research Institute.

MARTIN, M. & FITZGERALD, M. A. 2002. Proteins in Rice Grains Influence Cooking Properties. *Journal of Cereal Science,* 36**,** 285-294.

MAUAD, M., CRUSCIOL, C. A. C., GRASSI, F. H. & CORREA, J. C. 2003. Nitrogen and silicon fertilization of upland rice. *Scientia Agricola,* 60**,** 761 - 765.

MCDONALD, M. P., GALWEY, N. W. & COLMER, T. D. 2002. Similarity and diversity in adventitious root anatomy as related to root aeration among a range of wetland and dryland grass species. *Plant, Cell & Environment,* 25**,** 441 - 451.

MCGRANCE, S. J., CORNELL, H. J. & RIX, C. J. 1998. A simple and rapid colorimetric method for the determination of amylose in starch products. *Starch-Stärke,* 50**,** 158 - 163.

MCHUGH, O. V., STEENHUIS, T. S., BARISON, J., FERNANDES, E. C. M. & UPHOFF, N. T. 2007. Farmer implementation of alternate wet-dry and nonflooded irrigation practices in the System of Rice Intensification (SRI). *Proceedings of a thematic workshop on water-wise rice production.* Los Banos, Philippines: IRRI.

MEELU, O. P., SAGGAR, S., MASKINA, M. S. & REKHI, R. S. 1987. Time and source of nitrogen application in rice and wheat. *The Journal of Agricultural Science,* 109**,** 387 - 391.

MEW, T. W. & MISRA, J. K. 1994. *A Manual of Rice Seed Health Testing,* Los Banos, International Rice Research Institute.

MIKKELSEN, D. S. 1987. Nitrogen budgets in flooded soils used for rice production. *Plant and Soil,* 100**,** 71 - 97.

MINH-CHAU DANG, J. & COPELAND, L. 2004. Genotype and environmental influences on pasting properties of rice flour. *Cereal Chemistry,* 81**,** 486 - 489.

MIURA, C., KON, K., SATOU, K., SHIBATA, S. & KANETA, Y. 2009. Character of tillering and stable productivity of high-quality and palatable grains in rice cultivar Akitakomachi cultivated by single application of fertilizer in nursery box. *Japanese Journal of Crop Science,* 78**,** 43 - 49.

MOHANAN, K. V. & MINI, C. B. 2008. Relative contribution of rice tillers of different status towards yield. *International Journal of Plant Breeding and Genetics,* 2**,** 9 - 12.

MOHAPATRA, P. K. & KARIALI, E. 2008. Time of emergence determines the pattern of dominance of rice tillers. *Australian Journal of Crop Science,* 1**,** 53 - 62.

MOORE, K. J. & MOSER, L. E. 1995. Quantifying developmental morphology of perennial grasses. *Crop Science,* 35**,** 37 - 43.

MOSTAJERAN, A. & RAHIMI-EICHI, V. 2008. Drought stress effects on root anatomical characteristics of rice cultivars (*Oryza sativa L.*). *Pakistan Journal of Biological Sciences,* 11**,** 2173 - 2183.

MUELLER, L., BEHRENDT, A., SCHALITZ, G. & SCHINDLER, U. 2005. Above ground biomass and water use efficiency of crops at shallow water tables in a temperate climate. *Agricultural Water Management,* 75**,** 117 - 136.

MUIRHEAD, W. A., BLACKWELL, J., HUMPHREYS, E. & WHITE, R. J. G. 1989. The growth and nitrogen economy of rice under sprinkler and flood irrigation in South East Australia. *Irrigation Science,* 10**,** 183 - 199.

MURCHIE, E. H., YANG, J., HUBBART, S., HORTON, P. & PENG, S. 2002. Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? *Journal of Experimental Botany,* 53**,** 2217 - 2224.

MURISON, K. (ed.) 2002. *Africa South of the Sahara 2003,* London, England: Europa Publications.

MUTERT, E. & FAIRHURST, T. H. 2002. Developments in rice production in Southeast Asia. *Better Crops,* 15**,** 12 - 17.

NAKLANG, K., SHU, F. & NATHABUT, K. 1996. Growth of rice cultivars by direct seeding and transplanting under upland and lowland conditions. *Field Crops Research,* 48**,** 115 - 123.

NEUE, H. U. 1993. Methane emission from rice fields. *Bioscience,* 43**,** 466 - 474.

NGUYEN, G. N. & SUTTON, B. G. 2009. Water deficit reduced fertility of young microspores resulting in a decline of viable mature pollen and grain set in rice. *Journal of Agronomy and Crop Science,* 195**,** 11 - 18.

NIELSEN, R. L. 2006. N loss mechanisms and nitrogen use efficiency. *2006 Purdue Nitrogen Management Workshops* [Online]. Available: www.agry.purdue.edu/ext/.../2006NLossMechanisms.pdf [Accessed 16 June 2010].

NONAMI, H. & BOYER, J. S. 1989. Turgor and growth at low water potentials. *Plant Physiology,* 89**,** 798 - 804.

NOVOA, R. & LOOMIS, R. S. 1981. Nitrogen and plant production. *Plant and Soil,* 58**,** 177 - 204.

OGUNREMI, L. T., LAL, R. & BABALOLA, O. 1986. Effects of tillage methods and water regimes on soil properties and yield of lowland rice from a sandy loam soil in Southwest Nigeria. *Soil and Tillage Research,* 6**,** 223 - 234.

OHNISHI, M., HORIE, T., HOMMA, K., SUPAPOJ, N., TAKANO, H. & YAMAMOTO, S. 1999. Nitrogen management and cultivar effects on rice yield and nitrogen use efficiency in Northeast Thailand. *Field Crops Research,* 64**,** 109 - 120.

OKEREKE, G. U. & MEINTS, V. W. 1985. Immediate immobilization of labeled ammonium sulfate and urea nitrogen in soils. *Soil Science,* 140**,** 105 - 109.

OKUMOTO, Y., SAKAI, M. & YAMAGATA, H. 1988. Studies on ripening characteristics of large-grain mutants in rice. *Rep Society of Crop Science and Breeding,* 33**,** 38 - 43.

ONG, M. H. & BLANSHARD, J. M. V. 1994. Texture determinants in cooked, parboiled rice. I: Rice starch amylose and the fine structure of amylopectin. *Journal of Symbolic Computation,* 18**,** 251 - 251.

ONG, M. H. & BLANSHARD, J. M. V. 1995. Texture determinants in cooked, parboiled rice. I: Rice starch amylose and the fine stucture of amylopectin. *Journal of Cereal Science,* 21**,** 251 - 260.

PALTA, J. A., KOBATA, T., TURNER, N. C. & FILLERY, I. R. 1994. Remobilization of carbon and nitrogen in wheat as influenced by post anthesis water deficits. *Crop science,* 34**,** 118 - 124.

PANDA, D., SAMANTARAY, R. N. & PATNAIK, S. 1988a. Management of fertilizer nitrogen for direct-sown, rainfed lowland rice (*Oryza sativa* L.). *The Journal of Agricultural Science,* 110**,** 475-479.

PANDA, M. M., GHOSH, B. C., REDDY, M. D. & REDDY, B. B. 1988b. Effect of form, method and time of urea nitrogen application on growth, yield and nitrogen uptake of direct sown and transplanted rice under intermediate deep water (15 - 50 cm) conditions. *The Journal of Agricultural Science,* 110**,** 309 - 313.

PANDEY, S. & VELASCO, L. 2002. Economics of direct seeding in Asia: patterns of adoption and research priorities. *In:* PANDEY, S., MORTIMER, M., WADE, L., TUONG, T. P., LOPEZ, K. & HARDY, B. (eds.) *Direct seeding: research issues and opportunities.* Los Banos, Philippines: International Rice Research Institute

PANTUWAN, G., FUKAI, S., COOPER, M., O'TOOLE, J. C. & SARKARUNG, S. 1997. Root traits to increase drought resistance in rainfed lowland rice. *In:* S. FUKAI, M. C., AND J. SALISBURY (ed.) *Breeding strategies for rainfed lowland rice in drought-prone environments.* Ubon Ratchathani, Thailand: Australian Centre for International Agricultural Research.

PARAMAN, I., HETTIARACHCHY, N. S., SCHAEFER, C. & BECK, M. I. 2006. Physicochemical properties of rice endosperm proteins extracted by chemical and enzymatic methods. *Cereal Chemistry,* 83**,** 663 - 667.

PARERA, C. A. & CANTLIFFE, D. J. 1994. Pre-sowing seed priming. *Horticulture Review,* 16**,** 109 - 141.

PEARMAN, I., THOMAS, S. M. & THORNE, G. N. 1978. Effect of nitrogen fertilizer on growth and yield of semi-dwarf and tall varieties of winter wheat. *The Journal of Agricultural Science,* 91**,** 31 - 45.

PENG, S., BOUMAN, B. A. S., VISPERAS, R. M., CASTA EDA, A., NIE, L. & PARK, H. K. 2006a. Comparison between aerobic and flooded rice in the tropics: Agronomic performance in an eight-season experiment. *Field Crops Research,* 96**,** 252 - 259.

PENG, S., BURESH, R. J., HUANG, J., YANG, J., ZOU, Y., ZHONG, X., WANG, G. & ZHANG, F. 2006b. Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in China. *Field Crops Research,* 96**,** 37 - 47.

PENG, S. & CASSMAN, K. G. 1998. Upper thresholds of nitrogen uptake rates and associated nitrogen fertilizer efficiencies in irrigated rice. *Agronomy Journal,* 90**,** 178 - 185.

PENG, S., KHUSH, G. S. & CASSMAN, K. G. 1994. Evolution of the new plant ideotype for increased yield potential. *In:* CASSMAN, K. G. (ed.) *Breaking the yield barrier.* Dordrecht, Netherlands International Rice Research Institute.

PENG, S., LAZA, R. C., VISPERAS, R. M., SANICO, A. L., CASSMAN, K. G. & KHUSH, G. S. 2000. Grain yield of rice cultivars and lines developed in the Philippines since 1966. *Crop Science,* 40**,** 307 - 314.

PENG, S., YANG, J., GARCIA, F. V., LAZA, R. C., VISPERAS, R. M., SANICO, A. L., CHAVEZ, A. Q. & VIRMANI, S. S. 1998. Physiology-based crop management for yield maximization of hybrid rice. *In:* VIRMANI, S. S., SIDDIQ, E. A. & MURALIDHARAN, K., eds. Advances in hybrid rice technology: proceedings of the 3rd International Symposium on Hybrid Rice, 14-16 November 1996 Hyderabad, India. Int. Rice Res. Inst.

PEREZ, C. M. & JULIANO, B. O. 1988. Grain quality and nutritional value: modified apparent amylose content (AC) of milled rice. *International Rice Research Newsletter,* 13**,** 10.

PEREZ, C. M., JULIANO, B. O., LIBOON, S. P., ALCANTARA, J. M. & CASSMAN, K. G. 1996a. Effects of late nitrogen fertilizer application on head rice yield, protein content and grain quality of rice. *Cereal Chemistry,* 73**,** 556 - 560.

PEREZ, C. M., O., J. B., LIBOON, S. P., ALCANTARA, J. M. & CASSMAN, K. G. 1996b. Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. *Cereal Chemistry,* 73**,** 556 - 560.

PHILPOT, KIM, MARTIN, MARGRIT, BUTARDO, VITO, WILLOUGHBY, DOUG, FITZGERALD & MELISSA 2006. Environmental factors that affect the ability of amylose to contribute to retrogradation in gels made from rice flour. *American Chemical Society,* 54**,** 5182 - 5190.

PIC, E., DE LA SERVE, B. T., TARDIEU, F. & TURC, O. 2002. Leaf senescence induced by mild water deficit follows the same sequence of macroscopic, biochemical, and molecular events as monocarpic senescence in pea. *Plant Physiology,* 128**,** 236 - 246.

PIERIK, R., DE WIT, M. & VOESENEK, L. A. C. J. 2010. Growth-mediated stress escape: convergence of signal transduction pathways activated upon exposure to two different environmental stresses. *New Phytologist,* no. doi: 10.1111/j.1469-8137.2010.03458.x.

PLÉNET, D. & LEMAIRE, G. 1999. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical N concentration. *Plant and Soil,* 216**,** 65 - 82.

PRASERTSAK, A. & FUKAI, S. 1997. Nitrogen availability and water stress interaction on rice growth and yield. *Field Crops Research,* 52**,** 249 - 260.

PROSEUS, T. E., ZHU, G.-L. & BOYER, J. S. 2000. Turgor, temperature and the growth of plant cells: using Chara corallina as a model system. *Journal of Experimental Botany,* 51**,** 1481 - 1494.

PRUDENTE, J. A., SIGUA, G. C., KONGCHUM, M. & PRUDENTE, A. D. 2009. Improving yield and nutrient uptake potentials of Japonica and Indica rice varieties with nitrogen fertilization. *World Journal of Agricultural Sciences,* 4**,** 326 - 332.

QADIR, M., SHARMA, B. R., BRUGGEMAN, A., CHOUKR-ALLAH, R. & KARAJEH, F. 2007. Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries. *Agricultural Water Management,* 87**,** 2 - 22.

RABARA, R. C. & FERRER, M. C. 2009. Hydropriming of aged rice seeds to improve germination. *Philippine Journal of Crop Science,* 34**,** 111.

RAM, P. C., SINGH, B. B., SINGH, A. K., RAM, P., SINGH, P. N., SINGH, H. P., BOAMFA, I., HARREN, F., SANTOSA, E., JACKSON, M. B., SETTER, T. L., REUSS, J., WADE, L. J., PAL SINGH, V. & SINGH, R. K. 2002. Submergence tolerance in rainfed lowland rice: physiological basis and prospects for cultivar improvement through marker-aided breeding. *Field Crops Research,* 76**,** 131 - 152.

RANDENIYA, L. K., VOHRALIK, P. F. & PLUMB, I. C. 2002. Stratospheric ozone depletion at northern mid latitudes in the 21 st century- the importance of future concentrations of greenhouse gases nitrous oxide and methane. *Geophysical Research Letters,* 29**,** 10 - 15.

RAO, A. H., KARUNA SREE, B. & REDDY, A. R. 1993. Water stress-responsive 23-kDa polypeptide from rice seedlings is boiling stable and is related to RAB 16 family of proteins. *Journal of Plant Physiology,* 142**,** 83 – 93.

RAO, J. L. N., REDDY, B. B. & RAJARAMAMOHAN RAO, V. 1983. Rhizosphere soil nitrogenase (C_2H_2 reduction) as influenced by the nitrogen management in intermediate deep water rice. *The Journal of Agricultural Science,* 101**,** 547-551.

RASKIN, I. & KENDE, H. 1984. Role of Gibberellin in the Growth Response of Submerged Deep Water Rice. *Plant Physiol.,* 76**,** 947-950.

RAVEN, J. A. 1996. Into the voids: The distribution, function, development and maintenance of gas spaces in plants. *Annals of Botany,* 78**,** 137 - 142.

RAY, S., MONDAL, W. A. & CHOUDHURI, M. A. 1983. Regulation of leaf senescence, grain-filling and yield of rice by kinetin and abscisic acid. *Physiologia Plantarum,* 59**,** 343 - 346.

REDDY, B., GHOSH, B. & PANDA, M. 1985. Flood to lerance of rice at different crop growth stages as affected by fertilizer application. *Plant and Soil,* 83**,** 255 - 263.

REDDY, B. G. M. & PATTAR, P. S. 2006. Leaf colour chart-a simple and inexpensive tool for nitrogen management in transplanted rice (*Oryza sativa*). *Indian Journal of Agricultural Science,* 76**,** 289 - 292.

REDDY, R. K., ALI, S. Z. & BHATTACHARYA, K. R. 1993. The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice. *Carbohydrate Polymers,* 22**,** 267 - 275.

REDDY, T. Y. & KULADAIVELU, R. 1992. Root growth of rice (*Oryza sativa*) as influenced by soil moisture regime and nitrogen. *Indian Journal of Agronomy,* 37**,** 694 - 700.

REYNOLDS, C. M., WOLF, D. C. & ARMBRUSTER, J. A. 1985. Factors related to urea hydrolysis in soils. *Soil Science Society of America,* 49**,** 104 - 108.

RITCHIE, J. T. 1981. Water dynamics in the soil-plant-atmosphere system. *Plant and Soil,* 58**,** 81 - 96.

RITCHIE, J. T., SINGH, U., GODWIN , D. C. & BOWEN, W. T. 1998. Cereal growth, development and yield. *In:* TSUJI, G. Y., HOOGENBOOM, G., THORNTON, P. K. & THORNTON, P. (eds.) *Understanding options for agricultural production.* London: Business & Economics - Kluwer Academic Publishers

RODRIGUEZ, M. S. & LAL, R. 1985. Growth and yield of paddy rice as affected by tillage and nitrogen levels. *Soil and Tillage Research,* 6**,** 163 - 178.

ROGER, P. A. 1996. *Biology and Management of the Floodwater Ecosystem in Rice Fields,* Phillipines, International Rice Research Institute.

RUDRAPAL, D. & NAKAMURA, S. 1988. The effect of hydration-dehydration pre-treatment on egg plant and radish seed viability and vigor. *Seed Science and Technology,* 16**,** 123 - 130.

SACKS, E. J., ROXAS, J. P. & CRUZ, M. T. S. 2003. Developing perennial upland rice I: field performance of *Oryza sativa / O. rufipogon* F1, F4, and BC1F4 progeny. *Crop Science,* 43**,** 120 - 128.

SAH, R. N. & MIKKELSEN, D. 1983. Availability and utilization of fertilizer nitrogen by rice under alternate flooding: II. Effect on growthy and nitrogen use efficiency. *Plant and Soil,* 75**,** 227 - 234.

SAIRAM, R., KUMUTHA, D., EZHILMATHI, K., DESHMUKH, P. & SRIVASTAVA, G. 2008. Physiology and biochemistry of waterlogging tolerance in plants. *Biologia Plantarum,* 52**,** 401 - 412.

SARIAM, O. 2004. *Growth performance of rice under different water and nitrogen management.* PhD, Universiti Putra Malaysia.

SARWAR, M. J. & KHANIF, Y. M. 2005. Effect of different water levels on rice yield and Cu and Zn concentration. *Journal of Agronomy* 4**,** 116 - 121.

SCHRADER, L. E. 1984. Functions and transformations of nitrogen in higher plants. *In:* HAUCK, R. D., BEATON, J. D., GORING, C. A. I., HOEFT, R. G., RANDALL, G. W. & RUSSELL, D. A. (eds.) *Nitrogen in Crop Production.* Madison, Wisconson: American Society of Agronomy.

SCOONES, I. 1991. Wetlands in drylands: key resources for agricultural and pastoral production in Africa. *Ambio,* 20**,** 366 - 371.

SETTER, T. L., ELLIS, M., LAURELES, E. V., ELLA, E., SENADHIRA, D., MISHRA, S. B., SARKARUNG, S. & DATTA, S. 1997. Physiology and genetics of submergence tolerance in rice. *Annals of Botany,* 79**,** 67 - 77.

SETTER, T. L. & LAURELES, E. V. 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. *Journal of Experimental Botany,* 47**,** 1551 - 1559.

SHAHIDULLAH, S. M., HANAFI, M. M., ASHRAFUZZAMAN, M., ISMAIL, R. M. & SALAM, M. A. 2009. Tillering dynamics in aromatic rice genotypes. *International Journal of Agriculture & Biology* 11**,** 509 - 514.

SHARMA, A. R. 2002. Agronomic practices for increasing nitrogen-use-efficiency and productivity of flood-prone lowland rice in Eastern India. *Fertilizer News,* 47**,** 27 - 44.

SHARMA, A. R. & MITTRA, B. N. 1991. Effect of different rates of application of organic and nitrogen fertilizers in a rice-based cropping system. *The Journal of Agricultural Science,* 117**,** 313 - 318.

SHE, K. C., KUSANO, H., KOIZUMI, K., YAMAKAWA, H., HAKATA, M., IMAMURA, T., FUKUDA, M., NAITO, N., TSURUMAKI, Y. & MATSUMOTO, K. 2009. A novel superior factor widely controlling the rice grain quality. *Paper for Nature,* 6**,** 1 - 17.

SHEEHY, J. E., DIONORA, M. J. A. & MITCHELL, P. L. 2001. Spikelet numbers, sink size and potential yield in rice. *Field Crops Research,* 71**,** 77 - 85.

SHIH, F. & DAIGLE, K. 2000. Preparation and characterization of rice protein isolates. *Journal of the American Oil Chemists' Society,* 77**,** 885 - 889.

SHIMONO, H., HASEGAWA, T. & IWAMA, K. 2002. Response of growth and grain yield in paddy rice to cool water at different growth stages. *Field Crops Research,* 73**,** 67 - 79.

SHIZHANG, P., SHOUSHENG, L., GOULANG, X. & ZIJING, W. 1994. New water consumption pattern of rice under water - saving irrigation. *Irrigation and Drainage Systems,* 8**,** 97 - 102.

SIGUNGA, D. O., JANSSEN, B. H. & OENEMA, O. 2002. Ammonia volatilization from vertisols. *European Journal of Soil Science,* 53**,** 195 - 202.

SINGH, B., SINGH, Y., LADHA, J. K., BRONSON, K. F., BALASUBRAMANIAN, V., SINGH, J. & KHIND, C. S. 2002. Chlorophyll meter- and leaf color chart-based nitrogen management for rice and wheat in Northwestern India. *Agronomy Journal,* 94**,** 821 - 829.

SINGH, K. B., GAJRI, P. R. & ARORA, V. K. 2001. Modelling the effects of soil and water management practices on the water balance and performance of rice. *Agricultural Water Management,* 49**,** 77 - 95.

SINGH, N., SEKHON, K. S. & KAUR, A. 1990. Effect of pre-harvest flooding of paddy on the milling and cooking quality of rice. *Journal of the Science of Food and Agriculture,* 52**,** 23 - 34.

SINGH, R. K., SINGH, V. P. & SINGH, C. V. 1994. Agronomic assessment of 'beushening' in rainfed lowland rice cultivation in Bihar, India. *Agriculture, Ecosystems & Environment,* 51**,** 271 - 280.

SINGH, V. P., SINGH, G., SINGH, S. P., KUMAR, A., SINGH, Y., JOHNSON, D. E. & MORTIMER, A. M. 2008. Direct seeding and weed management in the irrigated rice-wheat production system. *In:* SINGH, Y., SINGH, V. P., CHAUHAN, B., ORR, A., MORTIMER, A. M., JOHNSON, D. E. & HARDY, B. (eds.) *Direct Seeding of Rice and Weed Management in the Irrigated Rice-Wheat Cropping System of the Indo-Gangetic Plains.* Los Banos: International Rice Research Institute.

SMIRNOFF, N. & CRAWFORD, R. M. M. 1983. Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Annals of Botany,* 51**,** 237 - 249.

SMITH, K. A., BALL, T., CONEN, F., DOBBIE, K. E., MASSHEDER, J. & REY, A. 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science,* 54**,** 779 - 791.

SOCOLOW, R. 1999. Nitrogen management and the future of food: Lessons from the management of energy and carbon. *Proceedings of the National Academy of Sciences of the United States of America,* 96**,** 6001.

SOJKA, R. E. 1992. Stomatal closure in oxygen-stressed plants. *Soil Science,* 154**,** 269 - 280.

SOMADO, E. A., GUEI, R. G. & KEYA, S. O. (eds.) 2008. *NERICA: The New Rice for Africa–a Compendium,* Cotonou, Benin: Africa Rice Center (WARDA).

SONG, W. C. & ZHANG, Y. H. 1992. Rice tetraploidy and its effect on agronomic traits and nutritional constituents. *Acta Agronomica Sinica,* 18**,** 137 - 144.

SOUZA, S. R., STARK, E. M. L. M. & FERNANDES, M. S. 1999. Foliar spraying of rice with nitrogen: effect on protein levels, protein fractions, and grain weight. *Journal of Plant Nutrition,* 22**,** 579 - 588.

SOWBHAGYA, C. M., RAMESH, B. S. & BHATTACHARYA, K. R. 1987. The relationship between cooked-rice texture and the physicochemical characteristics of rice. *Journal of Cereal Science,* 5**,** 287 - 297.

SPIERTZ, J. H. J. 2009. Nitrogen, sustainable agriculture and food security: A review. *Sustainable Agriculture,* 6**,** 635 - 651.

SPIERTZ, J. H. J. & DE VOS, N. M. 1983. Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. *Plant and Soil,* 75**,** 379 - 391.

STEVENS, G., HEFNER, S. & GLADBACH, T. 2001. Effect of nitrogen timing and rates on tillering and yield of no-till water-seeded rice. *Communications in Soil Science and Plant Analysis,* 32**,** 421 - 428.

SURALTA, R. R. & YAMAUCHI, A. 2008. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and waterlogging. *Environmental and Experimental Botany,* 64**,** 75 - 82.

TAKEBE, M. & YONEYAMA, T. 1989. Measurement of leaf color scores and its implication to nitrogen nutrition of rice plants. *Japan Agricultural Research Quarterly,* 23**,** 86 - 93.

TEN-BERGE, H. F. M. & KROPFF, M. J. 1995. Founding a systems research network for rice. *In:* BOUMA, J. B., KUYVENHOVEN, A., BAUMAN, B. A. M., LUYTEN, J. C. & ZANDSTRA, H. G. (eds.) *Eco-regional Approaches for Sustainable Land Use and Food Production.* The Hague: Springer.

TEN-BERGE, H. F. M., THIYAGARAJAN, T. M., SHI, Q., WOPEREIS, M. C. S., DRENTH, H. & JANSEN, M. J. W. 1997. Numerical optimization of nitrogen application to rice. Part I. Description of manage-N. *Field Crops Research,* 51**,** 29 - 42.

THORNTON, J. M. & POWELL, A. A. 1992. Short-term aerated hydration for the improvement of seed quality in Brassica oleracea L. *Seed Science Research,* 2**,** 41 - 49.

THORNTON, J. M. & POWELL, A. A. 1995. Prolonged aerated hydration for improvement of seed quality in Brassica oleracea L. *Annals of Applied Biology,* 127**,** 183 - 189.

TILMAN, D., CASSMAN, K. G., MATSON, P. A., NAYLOR, R. & POLASKY, S. 2002. Agricultural sustainability and intensive production practices. *Nature,* 418**,** 671 - 677.

TNAU. 2008. Farmer friendly specific gravity grading in paddy [Online]. *TNAU Agritec Agritec Portal.* Available: http://agritech.tnau.ac.in/seed_certification/seed%20treatments_Special. html. [Assessed 29 March 2010].

UNNEVEHR, L. J. 1986. Consumer demand for rice grain quality and returns to research for quality improvement in Southeast Asia. *American Journal of Agricultural Economics,* 68**,** 634 - 641.

VAN DER STRAETEN, D., ZHOU, Z., PRINSEN, E., VAN ONCKELEN, H. A. & VAN MONTAGU, M. C. 2001. A Comparative Molecular-Physiological Study of Submergence Response in Lowland and Deepwater Rice. *Plant Physiology,* 125**,** 955 - 968.

VARTAPETIAN, B. B. & JACKSON, M. B. 1997. Plant adaptations to anaerobic stress. *Annals of Botany,* 79**,** 3 - 20.

VERGARA, B. S. 1992. *A Farmer's Primer on Growing Rice,* Los Banos, Philippines, International Rice Research Institute.

VIDEMSEK, U., TURK, B. & VODNIK, D. 2006. Root aerenchyma - formation and function. *Acta Agriculturae Slovenica,* 87**,** 445 - 453.

VISSER, E. J. W., BÖGEMANN, G. M., BLOM, C. & VOESENEK, L. 1996. Ethylene accumulation in waterlogged Rumex plants promotes formation of adventitious roots. *Journal of Experimental Botany,* 47**,** 403 - 410.

VLEK, P. L. G. & BYRNES, B. H. 1986. The efficacy and loss of fertilizer N in lowland rice. *Nutrient Cycling in Agroecosystems,* 9**,** 131 - 147.

VLEK, P. L. G. & CARTER, M. F. 1983. The effect of soil environment and fertilizer modifications on the rate of urea hydrolysis. *Soil Science,* 136**,** 56.

VON WIRÉN, N., GAZZARRINI, S. & FROMMER, W. B. 1997. Regulation of mineral nitrogen uptake in plants. *Plant and Soil,* 196**,** 191 - 199.

VOORTMAN, R., SONNEVELD, B. & KEYZER, M. 2003. African land ecology: Opportunities and constraints for agricultural development. *Ambio: A Journal of the Human environment,* 32**,** 367 - 373.

WADA, G., ARAGONES, R. C. & ANDO, H. 1991. Effect of slow release fertilizer (meister) on the nitrogen uptake and yield of the rice plant in the tropics. *Japanese Journal of Crop Science,* 60**,** 101 - 106.

WANG, F., CHENG, F. & ZHANG, G. 2007. Differences in grain yield and quality among tillers in rice genotypes differing in tillering capacity. *Rice Science,* 14**,** 135 - 140.

WANG, G., DOBERMANN, A., WITT, C., SUN, Q. & FU, R. 2001. Performance of site-specific nutrient management for irrigated rice in Southeast China. *Agronomy Journal,* 93**,** 869 - 878.

WANG, H., MO, J., LU, X., XUE, J., LI, J. & FANG, Y. 2009. Effects of elevated nitrogen deposition on soil microbial biomass carbon in major subtropical forests of southern China. *Frontiers of Forestry in China,* 4**,** 21 - 27.

WARDA 2007. Africa Rice Trends 2007 Brief. Cotonou, Benin: Africa Rice Center (WARDA).

WARDA 2008. 2007 Africa Rice Trends. 5th ed. Cotonou, Benin: Africa Rice Center (WARDA).

WATSON, C. J., STEVENS, R. J., LAUGHLIN, R. J. & POLAND, P. 1992. Volatilization of ammonia from solid and liquid urea surface-applied to perennial ryegrass. *The Journal of Agricultural Science,* 119**,** 223 - 226.

WEBB, B. D. 1991. Rice quality and grades. *Rice Utilization,* 2**,** 89–116.

WEBB, J. & ARMSTRONG, W. 1983. The effects of anoxia and carbohydrates on the growth and viabiliy of rice, pea and pumpkin roots. *Journal of Experimental Botany,* 37**,** 579 - 603.

WEI, Z. & SONG, S.-T. 1989. Irrigation model of water saving-high yield at lowland paddy field. *International Commission on Irrigation and Drainage, Seventh Afro-Asian regional Conference.* Tokyo, Japan.

WHISTLER, R. L., BEMILLER, J. N. & PASCHALL, E. F. (eds.) 1984. *Starch: Chemistry and Technology,* Orlando, Florida: Academic Press, Inc.

WITT, C., DOBERMANN, A., ABDULRACHMAN, S., GINES, H. C., GUANGHUO, W., NAGARAJAN, R., SATAWATANANONT, S., THUC SON, T., SY TAN, P. & VAN TIEM, L. 1999. Internal nutrient efficiencies of irrigated lowland rice in tropical and subtropical Asia. *Field Crops Research,* 63**,** 113 - 138.

WITT, C., PASUQUIN, J. M. C. A., MUTTERS, R. & BURESH, R. J. 2005. New leaf color chart for effective nitrogen management in rice. *Better Crops* 89 36 - 39.

WOODSTOCK, W. L. 1976. Seed Vigor Testing Handbook. *Association of Official Seed Analysts of America (AOSA) Newsletter,* 50.

WOPEREIS-PURA, M. M., WATANABE, H., MOREIRA, J. & WOPEREIS, M. C. S. 2002. Effect of late nitrogen application on rice yield, grain quality and profitability in the Senegal River valley. *European Journal of Agronomy,* 17**,** 191 - 198.

XIAO, H., ZHOU, Q. & MA, L. 2005. Joint effects of acetochlor and urea on germinating characteristics of crop seeds. *Science in China Series C: Life Sciences,* 48**,** 1 - 6.

XIAO, J., GRANDILLO, S., AHN, S. N., MCCOUCH, S. R., TANKSLEY, S. D., LI, J. & YUAN, L. 1996. Genes from wild rice improve yield. *Nature,* 384**,** 223 - 224.

XIE, W., WANG, G., ZHANG, Q. & GUO, H. 2007. Effects of nitrogen fertilization strategies on nitrogen use efficiency in physiology, recovery, and agronomy and redistribution of dry matter accumulation and nitrogen accumulation in two typical rice cultivars in Zhejiang, China. *Journal of Zhejiang University of Science,* 8**,** 208 – 216.

XIONG, F., WANG, Z., GU, Y.-J., CHEN, G. & ZHOU, P. 2008. Effects of nitrogen application time on caryopsis development and grain quality of rice variety Yangdao 6. *Rice Science,* 15**,** 57 - 62.

XU, J. G., HEERAMAN, D. A. & WANG, Y. 1993. Fertilizer and temperature effects on urea hydrolysis in undisturbed soil. *Biology and Fertility of Soils,* 16**,** 63 - 65.

YAMASHITA, K. & FUJIMOTO, T. 1974. Studies on fertilizer and quality of rice. IV. Relationship between protein content of milled rice as affected by nitrogen fertilization and eating quality. *Tohoku Agricultural Experiment Station,* 48**,** 91 - 96.

YANG, J., ZHANG, J., WANG, Z., LIU, L. & ZHU, Q. 2003a. Postanthesis water deficits enhance grain filling in two-line hybrid rice. *Crop Science,* 43**,** 2099 - 2108.

YANG, W.H., PENG, S., HUANG, J., SANICO, A. L., BURESH, R. J. & WITT, C. 2003b. Using leaf color charts to estimate leaf nitrogen status of rice. *Agronomy Journal,* 95**,** 212 - 217.

YING, J., PENG, S., HE, Q., YANG, H., YANG, C., VISPERAS, R. M. & CASSMAN, K. G. 1998a. Comparison of high-yield rice in tropical and subtropical environments: I. Determinants of grain and dry matter yields. *Field Crops Research,* 57**,** 71 - 84.

YING, J., PENG, S., YANG, G., ZHOU, N., VISPERAS, R. M. & CASSMAN, K. G. 1998b. Comparison of high-yield rice in tropical and subtropical environments II. Nitrogen accumulation and utilization efficiency. *Field Crops Research,* 57**,** 85 - 93.

YOSHIDA, S. 1972. Physiological aspects of grain yield. *Annual Review of Plant Physiology,* 23**,** 437 - 464.

YOSHIDA, S. 1981. *Fundamental of Rice Crop Science,* Los Banos, Philippines, International Rice Research Institute.

YU, S., MA, Y. & SUN, D.-W. 2009. Impact of amylose content on starch retrogradation and texture of cooked milled rice during storage. *Journal of Cereal Science,* 50**,** 139 - 144.

YUAN, L., X., W., LIAO, F., MA, G. & XU, Q. 2003. *Hybrid Tice Technology,* Beijing, China, China Agriculture Press.

ZAIDI, P. H., MANI SELVAN, P., SULTANA, R., SRIVASTAVA, A., SINGH, A. K., SRINIVASAN, G., SINGH, R. P. & SINGH, P. P. 2007. Association between line per se and hybrid performance under excessive soil moisture stress in tropical maize (*Zea mays* L.). *Field Crops Research,* 101**,** 117 - 126.

ZHANG, H., XUE, Y., WANG, Z., YANG, J. & ZHANG, J. 2009a. An alternate wetting and moderate soil drying regime improves root and shoot growth in rice. *Crop Science,* 49**,** 2246 - 2260.

ZHANG, Y.-X., DING, Y.-F., U, G.-H., WANG, Q.-S., HUANG, K.-S. & WANG, S.-H. 2007. Starch structure and paste property of rice with different amylose content. *Acta Agronomica Sinica* 33 1201 - 1205.

ZHANG, Y., CHEN, Y., YAN, G., DU, B., ZHOU, Y. & YANG, J. 2009b. Effects of nitrogen nutrition on grain quality in upland rice Zhonghan 3 and paddy rice Yangjing 9538 under different cultivation methods. *Acta Agronomica Sinica,* 35**,** 1866 - 1874.

ZHANG, Y. J., ZHOU, Y. R., DU, B. & YANG, J. C. 2008. Effects of nitrogen nutrition on grain yield of upland and paddy rice under different cultivation methods. *Acta Agronomica Sinica,* 34**,** 1005 - 1013.

ZHANG, Z., TEIJI, N., MASAHIRO, C. & IWAO, N. 2000. Correlation between spikelet fertility and relative root amount in rice plants exposed to cool temperature at the young microspore stage. *Japanese Journal of Crop Science,* 69**,** 175 - 181.

ZHAO, J., BAXTER, G. & BLANCHARD, B. 2006. How do endosperm proteins affect the pasting and texture of rice? – A study of the physiochemical properties of rice using a model system. *13th World Congress of Food Science & Technology*. Nantes, France: IUFoST EDP Sciences.

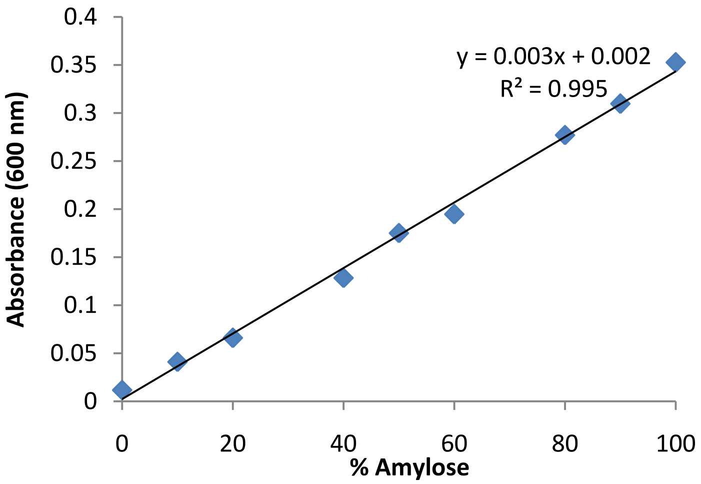
ZHONGKAI, Z., KEVIN, R., STUART, H. & CHRIS, B. 2002. Composition and functional properties of rice. *International Journal of Food Science & Technology,* 37**,** 849 - 868.

ZHOU, S., HOU, H. & HOSOMI, M. 2008. Nitrogen removal, N_2O emission, and NH_3 volatilization under different water levels in a vertical flow treatment system. *Water, Air, & Soil Pollution,* 191**,** 171 - 182.

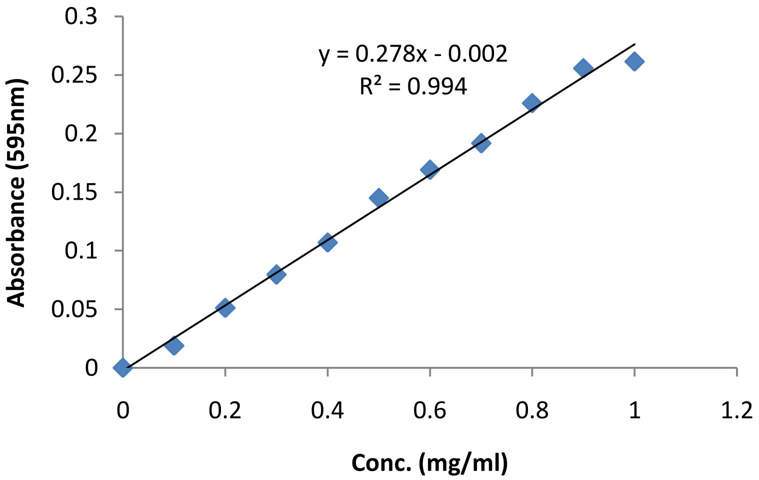
ZHOU, Z., ROBARDS, K., HELLIWELL, S. & BLANCHARD, C. 2003. Effect of rice storage on pasting properties of rice flour. *Food Research International,* 36**,** 625 - 634.

ZHU, J., ZHOU, Y., LIU, Y., WANG, Z., TANG, Z., YI, C., TANG, S., GU, M. & LIANG, G. 2010. Fine mapping of a major QTL controlling panicle number in rice. *Molecular Breeding,* 10.1007/s11032-010-9420-6**,** 1 - 10.

APPENDIX

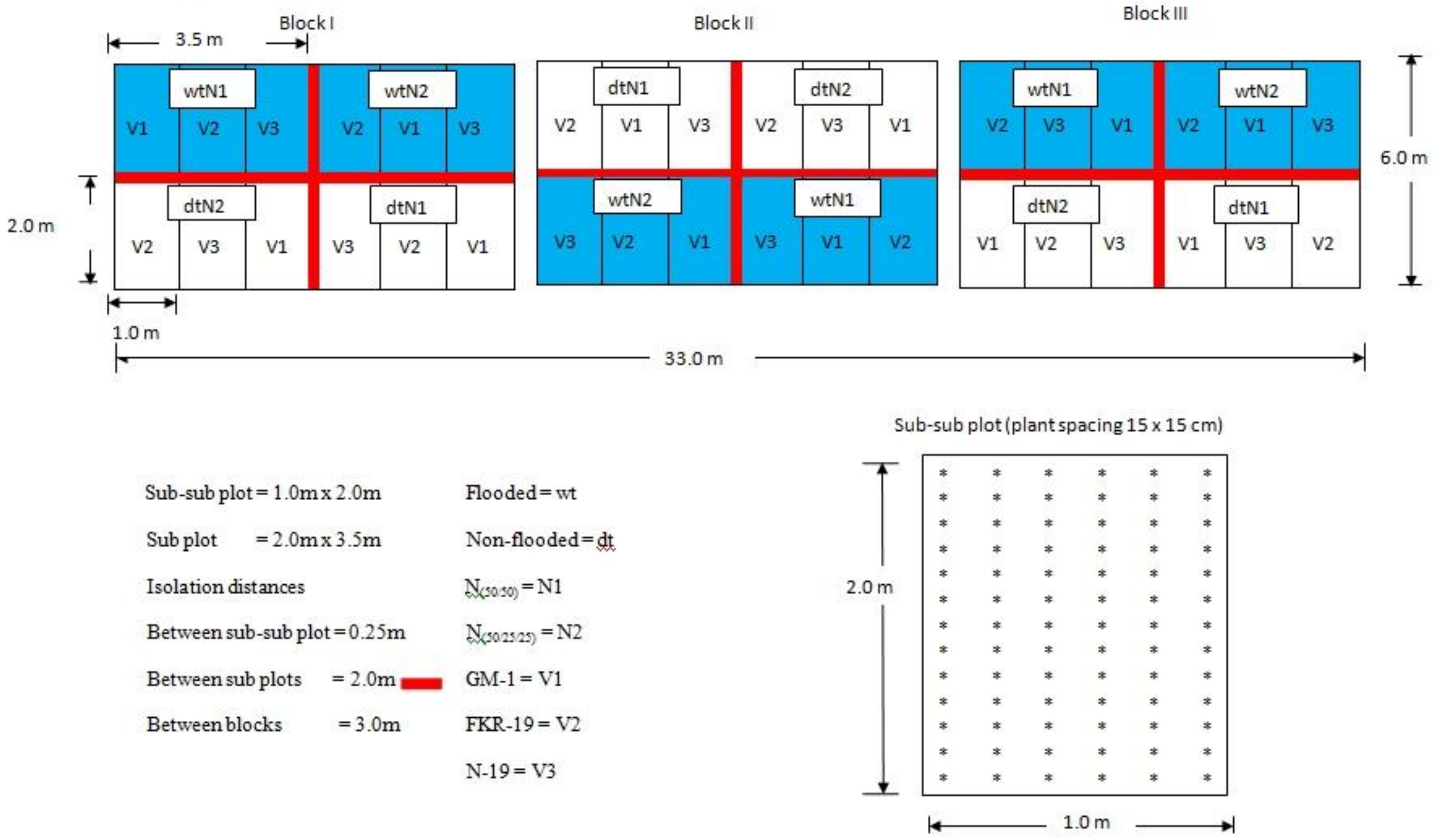


Appendix 1: Amylose standard curve



Appendix 2: Protein standard curve

Field trial plan



Sub-sub plot = 1.0m x 2.0m

Sub plot = 2.0m x 3.5m

Isolation distances

Between sub-sub plot = 0.25m

Between sub plots = 2.0m 🟥

Between blocks = 3.0m

Flooded = wt

Non-flooded = dt

$N_{(50/50)}$ = N1

$N_{(50/25/25)}$ = N2

GM-1 = V1

FKR-19 = V2

N-19 = V3

Appendix 3: Field trial layout