Genetic, physiological and modelling approaches towards tolerance to salinity and low nitrogen supply in rice (*Oryza sativa* L.)

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Genetic, physiological and modelling approaches towards tolerance to salinity and low nitrogen supply in rice (*Oryza sativa* L.)

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Abstract

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Meeting the world's requirement for rice production in the future needs a dual approach: 1. the theoretical yield potential of rice should increase and 2. the yield gaps should be reduced especially in marginal areas where stresses such as salinity, low supply of plant nutrients, droughts and flooding often limit yields. Rice breeding used to be geared towards developing cultivars for high-input management conditions and these cultivars may not be suited to low input environments. In regions such as Africa, where most of the rice is produced under low-input conditions, breeding should be targeted at rice cultivars that are tolerant to the main stresses encountered in the rice agro-ecosystems. Soil salinity and inadequate supply of plant nutrients (especially nitrogen) are two major stresses limiting rice yields in The Gambia where this study was undertaken. The aim of this study was to determine appropriate selection methods for rice across a range of environments and to identify options at the level of yield components and physiological traits to increase rice yield potentials under both high and low input conditions. For this purpose, a segregating population of rice comprising Recombinant Inbred Lines (RILs) developed from the cross of a high-yielding, semi-dwarf, salt-sensitive cultivar, IR29 and a tall, traditional, salt-tolerant cultivar, Pokkali, was grown in fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 or 100 kg ha^{-1} nitrogen.

Analyses of variance revealed significant genotype × environment interaction for yield and for the four yield components, number of panicles m^{-2}, total number of grains per panicle, thousand grain weight and percent spikelet fertility, across the range of test environments. Inter-environmental correlations for grain yield between the 0 and 100 kg ha^{-1} N fertilizer regimes were high and significant in both fresh and saline water signifying that in the lowland, rice can be bred for general adaptability to different N fertilizer levels. However, the inter-environmental correlations for yield between fresh water and saline conditions, especially for the highest yielding cultivars in either environment, were low. This suggests that different sets of rice cultivars should be bred for cultivation in fresh water or saline environments. Analysis of the relationships between yield and yield components by means of regression revealed that generally in fresh water environments, yield was sink-limited and that grain number attributes (comprising number of panicles m^{-2} and total number of grains per panicle) of rice should be enhanced in order to boost yield potential under fresh water conditions. In saline environments, however, salt stress strongly limits assimilate production and translocation. To increase rice yield potential in saline environments, cultivars with better grain filling attributes (comprising grain weight and spikelet fertility) should be developed. The N fertilizer regime influenced the relative importance of panicles m^{-2} and total number of grains per panicle in fresh water and of individual grain weight and spikelet fertility in saline environments, for yield determination.

Through molecular marker analysis, putative quantitative trait loci (QTLs) were detected for grain yield and yield components in all four test environments. Overall, markers accounted for 23%-60% of the variation in yield and yield components. Markers associated with more than one trait had either similar or opposite effects on the traits. For all five traits studied, most markers were expressed in only one environment implying strong environmental specificity in expression of the QTLs for rice yield and yield components. Marker-assisted selection, based on AFLP markers, was successfully conducted for grain yield of rice in all four test environments over two years.

A study of the physiological basis of yield formation recognized the importance of high biomass production in all test environments for high yielding ability. High biomass production in fresh water environments was associated with high leaf area index and high leaf N concentration while in saline environments, high biomass production was better associated with leaf area index than with leaf N concentration. Salt stress reduced leaf area index, biomass production and yield but increased leaf N concentration and prolonged growth. In saline environments, differences in leaf area index and biomass between salt-tolerant and salt-sensitive genotypes were larger during the pre-flowering growth phase than after anthesis. Around flowering time, high stem weights and allocation of more dry matter to shoots instead of roots, reduced yields in fresh water environments but increased yields in saline water environments. High leaf weights and late flowering increased yields in fresh water but in saline environments high leaf weights decreased yields in the zero N fertilizer regime and increased yields in the high N fertilizer regime. In saline environments, late flowering generally reduced yields.

The ORYZA1 model gave good predictions of yield and biomass in all test environments although grain yield was better predicted than biomass production and predictions were better in fresh water than in saline environments. The model revealed, through sensitivity analysis, the yield increment that can be achieved by improving leaf area index, specific leaf N and fraction of dry matter allocated to panicles during grain filling, for diverse rice genotypes in different environments. For saline environments, inclusion of effects of salinity on specific leaf N and spikelet fertility in the model are expected to improve its performance.

A thorough understanding of the genetic and physiological basis of yield formation in different environments would help breeders develop rice cultivars with high yield potentials under low- and/or high input cultivation environments. Integrating knowledge from different scientific disciplines such as statistics, physiology, biotechnology and systems modelling would facilitate this process.

Key words: Rice, O*ryza sativa*, yield potential, yield gap, salinity, nitrogen supply, agro-ecosystems, Recombinant Inbred Line, genotype × environment interaction, yield components, adaptability, molecular markers, QTL, biomass, leaf area, leaf N, dry matter allocation, ORYZA1, modelling.

Preface

The work reported in this thesis was funded by Wageningen University through a Sandwich PhD Fellowship, the Laboratory of Plant Breeding Group (WUR), the Crop and Weed Ecology Group (WUR), the National Agricultural Research Institute (NARI), The Gambia, The Netherlands Foundation for the Advancement of Tropical Research (WOTRO) and the International Foundation for Science (IFS), Sweden. I acknowledge their support in enabling me to pursue this multi-facetted approach towards identifying possibilities of improving the genetic yield potential of rice under both stressed and non-stressed conditions. As anyone who has worked with lowland rice would confirm, the lowland is a difficult environment to work in. I therefore unreservedly express my appreciation of the efforts by farmers who produce rice in the lowlands and also in other environments, especially low-resource farmers, who put so much effort into their fields only to get very little in return. I hope the findings in this thesis can help in even a small way to improve your earnings.

The completion of this thesis owes a lot to many individuals who have been instrumental in providing me with much needed assistance and support during the course of my studies. First and foremost I wish to thank my supervisors, Professors Piet Stam and Paul Struik and Dr. Fred van Eeuwijk for their patience and limitless support. I learned a lot under their tutelage. I truly appreciate your guidance and the interest you all took in both my work and personal well being. I am also grateful to Professor Martin Kropff and Dr. Johan Dourleijn who both started this work with us. Their roles were taken up by Professor Struik and Dr. Fred van Eeuwijk, respectively.

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Developing a population of recombinant inbred lines (RILs) is a long and tedious process. The RIL population used in this study was developed at the International Rice Research Institute (IRRI), Philippines, by Dr. Glenn Gregorio and his colleagues. I, therefore, owe a profound depth of gratitude to IRRI for providing the seeds of this RIL population and also generating the AFLP marker data. Glenn, you always addressed my requests with urgency for which I cannot thank you enough. Unfortunately we only 'met' through the magic of the internet but never physically. I am sure time will remedy that.

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Finally, I thank the Almighty Allah for giving me the strength and determination to successfully complete this phase of my education.

Baboucarr Manneh Wageningen, March 2004

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CHAPTER 1

General introduction

General introduction

Importance of rice

Rice (*Oryza sativa* L.) is the most important food crop in the world considering the area under cultivation and the number of people depending on it. It is the principal food of nearly half of mankind. It is estimated that 40% of the world's population use rice as a major source of energy. Globally, rice ranks second only to wheat in terms of area harvested, but in terms of importance as a food crop, rice provides more energy per hectare than any other cereal crop. At average world yields, a hectare of rice could sustain 5.7 persons for a year compared to 5.3 for maize and 4.1 for wheat (De Datta, 1981).

In order to meet the projected demand for rice, it has been estimated that global annual rice production needs to be increased from the present 560 million tons to 850 million tons by 2025 (Khush, 1997). This additional rice production has to come from either an expansion of the rice area or increasing yields or both. Expanding the area under rice cultivation is not an option for increasing rice production in many areas due to the pressures of urbanization, industrialization, crop diversification and other economic factors (Tyagi and Mohanty, 2000). In Asia where 90% of the world's rice is produced, land area under rice is actually declining (Papademetriou, 2000). It is only in Africa and Latin America that suitable areas for rice production still remain unutilized. Hence most of the additional rice production has to come from land already under rice cultivation through yield improvement. Thus, increasing the efficiency of rice production systems through the judicious use of agrochemicals, irrigation and cultivars of rice better adapted to their cultivation environments may offer the only possibilities of meeting the forecast demand for rice in the future.

This thesis looks into the possibilities of improving the genetic potential of rice for high-yielding ability in marginal environments characterized by salt stress and poor nitrogen supply. Such areas predominate in the rice production systems of most farmers in developing countries such as The Gambia, in West Africa.

Production systems of rice and their associated abiotic stresses

Rice is cultivated in a wide range of ecosystems under varying temperatures and moisture regimes. The majority of rice ecotypes are semi-aquatic plants adapted to saturated soil conditions where it is difficult for other crop species to survive. Four major agro-ecosystems are generally recognized (Khush, 1997): (1) irrigated lowland rice, (2) rainfed lowland rice, (3) upland rice, and (4) flood-prone rice. Irrigated lowland rice is grown in lowlands with an assured supply of water and the rice is grown in bunded fields

with water control. Rainfed lowland rice, on the other hand, is grown in lowlands characterized by alternate flooding and drying as a result of irregular rainfall patterns. Most of the rainfed lowlands are underdeveloped and lack proper water control. In contrast to lowland rice, upland rice is grown in free-draining soils where the water table is always below the rooting depth. Moisture comes entirely from rainfall. Flood-prone rice is grown in low-lying lands close to rivers. In West Africa, flood-prone rice that is produced in coastal tidal swamps where the dominant vegetation is mangrove, is called mangrove swamp rice (WARDA, 1994). Salinity and other soil stresses restrict mangrove swamp rice production to small areas in some West African countries. The relative importance of each of these production systems (irrigated lowland rice, rainfed lowland rice, upland rice and flood-prone rice) varies from one rice-producing region to the other (Table 1). All these production systems are associated with different abiotic stresses such as drought, flooding, weed infestation, salinity, soil acidity and poor nutrient supply. More inputs are used in producing irrigated rice than in any of the other systems. As a result of this, the highest rice yields are recorded in irrigated fields. The lowest yields are found in upland rice production systems where high weed infestation, frequent droughts and poor nutrient supplies greatly limit rice yields.

Salinity stress

Salinity is one of the major problems limiting rice production in the world. Some of the main causes of salinization in agricultural fields are improper irrigation practices and salt water intrusion into crop fields. The demand for water for both agricultural and non-agricultural purposes from an ever-expanding world population is expected to

Table 1. Distribution of rice area over different production systems in different regions of the world.

Region	Rice area in 1000 ha			
	Irrigated lowland	Rainfed lowland	Upland	Flood-prone
South Asia	24120	10488	7309	5589
Southeast Asia	14924	16073	2381	4361
East Asia	34372	1888	778	0
Latin America	2046	427	3685	108
Africa	1107	280	2800	1327
Other countries	2144	0	127	0
Total	79210	40553	17152	11451

Adapted from Khush, 1997.

place great strains on water supply systems leading to use of water of marginal quality for irrigation and also possibly to conflicts over water in the future (Chaudhary, 2000). Continued use of irrigation especially in semi-arid environments often leads to problems of increased soil salinity. Removal of natural vegetation such as mangroves from coastal regions and river deltas has led to intrusion of saline water into productive croplands. This problem is expected to be aggravated by climate change which is predicted to bring about increases in sea level, frequency of storms and rising temperatures (Yeo, 1999). It is, thus, essential to develop and employ technologies that will reduce the spread of salinization, reduce salinity levels in crop fields or increase the salt tolerance of crops.

Electrical conductivity (EC) measurements are used as indications of total quantities of soluble salts in soils. Values of EC used to be quoted in mmho cm^{-1} (Landon, 1996) but are nowadays given in mS cm^{-1} or dS m^{-1}. A distinction can be made between salt stress and ion stress (Levitt, 1980). Salt stress occurs only when the concentration of a salt is high enough to lower the water potential appreciably (0.5-1.0 bar). If the salt (or acid or base) concentration is not high enough to lower the water potential appreciably, then the stress is termed an ion stress. Salt stresses can be ascribed to two salts - calcium salts and sodium salts although most of the salt stresses in nature are due to Na salts, particularly NaCl. Salinity effects can be classified as osmotic, toxic or nutritional. Salt stress causing toxicity could be termed primary salt injury and that causing osmotic stress and nutritional stress (inducing deficiency of other nutrients) is secondary salt-induced stress.

The initial effects of salt stress on plants are due to osmotic stress but over long periods of time, salts accumulate to toxic levels in actively transpiring leaves thereby enhancing the rate of leaf senescence. Both the initial and long-term effects of salt stress lead to a reduced photosynthetic capacity of the plant and eventually low biomass production. Salt tolerance mechanisms in plants fall into two main categories: those involved in minimizing salt entry into the plant and those involved in minimizing salt concentration in cytoplasm (Munns, 2002). Thus, research into salt tolerance of crops has been conducted at different levels of organization from molecular to crop level. In rice, some of the approaches taken to study salinity tolerance include comparing sensitive and tolerant cultivars for:

- dry matter production and accumulation of Na^+, K^+, Ca^{2+} and Mg^{2+} at different growth stages (Gill, 1990);
- height, grain yield, biomass production, tissue content of Na^+, K^+, Cl^- and SO_4^{2-} (Gill and Singh, 1989);
- yield and yield components (Narayanan and Rangasamy, 1991);
- levels of free proline and total protein in the leaf and nitrogen in the shoot portion

(Krishnamurthy *et al.*, 1988);

- protein and chlorophyll concentrations, membrane permeability and chlorophyll fluorescence in young and old leaves (Lutts *et al*., 1996);
- low sodium transport to the shoot (Garcia *et al*., 1995);
- shoot sodium concentration, compartmentation of salt in older rather than in younger leaves, tolerance to salt within leaves and plant vigour (Yeo *et al*., 1990); and
- level of indole acetic acid (IAA) in plants (Nilsen and Orcutt, 1996).

However, salt tolerance is a whole plant phenomenon and it is, therefore, necessary to assess the effect of any tolerance mechanism at the crop level.

Low nitrogen supply

Nutrient influx takes place across the soil-root interface including the complex of materials and organisms in the rhizosphere. For cereals, nitrogen is often the most limiting element for crop production. Cereals account for about 55% of global nitrogen fertilizer applied to crops (Gilland, 2002). In rice, most of these nitrogenous fertilizers (50-70%) are lost to the environment causing pollution in water systems (Chaudhary, 2000). Furthermore, due to unavailability and relatively high costs of fertilizers, many farmers in developing countries apply little or no fertilizers to their crops. Thus, in both high- and low-input environments, it is essential to use cultivars more efficient than present cultivars at extracting essential inputs from their environments and with improved input utilization efficiencies.

The degree of heritability and gene action involved in the efficient utilization of essential elements has not been intensively investigated. For some crops, the levels of segregation of efficient and inefficient strains are significant enough to justify selection in breeding programmes (Hale and Orcutt, 1987). In rice, Collins and Creswell (1986) reported finding varietal differences for efficiency of translocation of carbon and nitrogen. Dey and Rao (1989) also reported discovering differences among rice varieties with regards to tolerance to nutrient stress (N, P and K). The possibility of exploiting genetic variation for improved nitrogen recovery from the soil and improved nitrogen use efficiency holds great promise of increasing rice yields in both high- and low-potential environments with the added benefit of minimizing nitrogen losses to the environment. This will increase the economic efficiency of added fertilizers and reduce environmental pollution. This varietal intervention should be coupled with an improved management of inputs with regards to quantities applied, and timing and methods of application.

Crop yield improvement

Breeding for increased yielding ability of crops in target environments, usually follows

one of two methods. The first method attempts to increase the potential yield of the crop. Potential yield is the theoretical yield of a crop that can be obtained. In this case, crop growth is determined solely by crop characteristics and the radiation and temperature of the particular location (van Ittersum *et al.*, 2003). Another approach to increase crop yields through breeding is to breed cultivars that will help narrow the yield gap. The yield gap is the difference between maximum attainable yields and actual yields in farmers' fields. The maximum attainable yield is defined as the yield at experimental stations/on-farm plots with no physical, biological or economic constraints and with the best known management practices for a given time in a given ecology (FAO, 2003).

Increasing potential yields

In the effort to increase rice production, breeders have placed much emphasis on raising the potential yield of rice. This has proven to be quite successful in the past especially with the introduction of dwarfing genes into modern cultivars of rice. In the middle part of the last century, breeders at national and international research institutes crossed tall traditional varieties with semi-dwarf varieties and succeeded in producing semi-dwarf progeny that were very responsive to nitrogen fertilizers (Chang, 1989). Subsequently, several semi-dwarf *indica* inbred cultivars were released. The most well-known of these, IR8, was released by IRRI (International Rice Research Institute) in 1966 (Peng *et al.*, 1999) and due to its adaptedness to tropical environments and high-yielding ability it was quickly adopted by many farmers in Asia, Africa and Latin America. IR8 and its derivatives spread rapidly in rice-growing regions of the world and due to their higher-yielding ability over local varieties their introduction had strong economic impacts on the livelihood of rice farmers in many parts of the world (Chang, 1989).

However, since the spectacular yield increases achieved by the introduction of the semi-dwarf characteristic into modern rice cultivars, yield potentials have almost levelled off (Morgan *et al.*, 2002) at 10 t ha^{-1} for current high-yielding inbred cultivars, while the theoretical potential has been estimated at 15.9 t ha^{-1} in tropical environments (Peng *et al.*, 1999). In order to break through this apparent yield barrier, several researchers advocated for more breeding work to be targeted at those characteristics of the rice plant that contribute to final grain yield. Researchers at the International Rice Research Institute (IRRI), embarked on producing a New Plant Type (NPT) of rice, which was defined mainly on the basis of physiological yield components (Yin *et al.*, 2000) with the expectation that it would raise the potential yield of rice in the tropics. Rice genotypes developed by IRRI based on the NPT concept are expected to yield 12.5 t ha^{-1}. Development of hybrids using the NPT concept could raise yields to a further 14 to 15 t ha^{-1} (Balasubramanian *et al.*, 2000). Unfortunately poor grain filling and low biomass production of prospective plants with

the new plant type may hamper the realization of this increased yield potential (Peng *et al*., 1999).

Bridging yield gaps

In many countries, actual rice yields are only 4 to 6 t ha^{-1} although some progressive farmers are able to attain yields of 8.0 to 8.5 t ha^{-1} (Chaudhary, 2000). There is often a yield gap of 10% to 60% or more between maximum attainable yields and farm-level yields (FAO, 2003). The yield gap is highest in rainfed, flood-prone and problem soil ecologies where the presence of abiotic stresses such as drought, flooding, salinity and poor nutrient supplies result in limited rice yields. Thus, the yield gap differs from one rice-producing region to the other (Table 2) closely following the distribution patterns of rice ecosystems (Table 1). For example, the yield gap in Asia, where a high proportion of rice is produced in irrigated lowlands, is estimated at 54.2%, while in Africa, where irrigated rice forms a small proportion of the total rice area, the yield gap stands at 73.5% (Balasubramanian *et al*., 2000).

Raising the yield potential often leads to an increase in the yield gap. Hence, more breeding effort is needed to produce rice cultivars better able to narrow the exploitable gaps between maximum attainable yields and farm-level yields through breeding for tolerance to the stresses responsible for the yield gaps.

Research tools used for genetic improvement of crops

Nowadays, researchers combine knowledge from many disciplines in their quest to improve the genetic potential of crop cultivars. Recent advances in molecular biological techniques allowing scientists to probe the plant at very low levels of biological organization and to reveal minute sub-cellular and molecular modifications in response to stress have greatly raised expectations of the capabilities of these new techniques (Blum, 1994). Improvements in computing techniques also allow breeders to use powerful statistical approaches to quickly evaluate large data sets containing

Table 2. Mean rice yields in different regions (1994).

Region	Yield (t ha^{-1})	Yield gap (%)
Europe	5.2	37.3
Asia	3.8	54.2
Latin America and Caribbean	3.2	61.4
Africa	2.2	73.5
World	3.7	55.4

Adapted from Balasubramanian *et al*., 2000.

information on many genotypes. Use of these new technologies would be particularly relevant for breeding cultivars for marginal environments where cultivation environments are highly variable. These marginal environments have not received as much attention from breeders as environments with optimum or near-optimum growing conditions. Traditionally, most breeders prefer to select for high yielding cultivars under high-input conditions where field conditions have been made uniform by applications of large amounts of inputs and also because of the apparently higher heritabilities than under low input conditions (Ceccarelli, 1997).

Statistical approaches

Yields of high-yielding cultivars of rice are often variable because of a high degree of genotype × environment (G × E) interaction (Chaudhary, 2000). G × E interaction is the differential performance of genotypes across a range of environments. This results partly from the fact that selection of high-yielding cultivars is usually practised under favourable growth conditions and partly as a result of the differential response of cultivars to stress. Thus, when improved cultivars are grown in low-potential environments where abiotic stresses limit crop-yielding ability, they often yield poorer than traditional cultivars with inherent low-yield potentials. It is, therefore, necessary to understand the mechanisms underlying G × E interaction in crops and the inheritance patterns of the genes responsible for yield stability/instability. This raises the need to expand breeding work into breeding rice with improved tolerance to stresses in cropping environments coupled with an improved input utilization efficiency. In this way, the new cultivars can better express their potentials even under sub-optimal growing conditions.

To minimize G × E interaction in new cultivars, breeders either select for adaptation to a wide range of, sometimes, contrasting environments (general adaptability) or adaptation to a well-defined narrow range of environments (specific adaptability). There is no general consensus among breeders as to which approach is superior to the other. Some researchers advocate for breeding to be targeted at specific adaptation when significant G × E interaction occurs so as to exploit the full genetic potential of crops in target environments (van Oosterom *et al*., 1993; Ceccarelli, 1997). Other researchers argue that it is possible to combine yield stability with input responsiveness in crop cultivars thereby enabling new cultivars to yield high in both low- and high-input environments (Rajaram *et al*., 1997; WARDA, 2002). Numerous stability statistics are employed to effect such selection (Lin *et al*., 1986; Eskridge *et al*., 1991). However, for breeders the main interest is to select cultivars that would rank high among the topmost yielding cultivars in prospective cultivation environments.

Molecular markers

The mapping of plant genomes was driven by the realization that genetic maps could facilitate indirect selection based on associations between desired traits and more easily determined traits (van den Berg, 1997). Morphological markers and then biochemical markers were used in the past to select for desirable traits in crops. However, the infrequency of these markers together with other associated drawbacks have limited their widespread use in plant breeding. Biochemical markers reveal polymorphisms at the protein level. The most commonly used protein markers are isozymes, which are variant forms of the same enzyme (Kumar, 1999).

Recent advances in molecular biological techniques have led to the development of DNA-based markers that are well-distributed in plant genomes. With the advent of molecular marker technology, researchers are now also able to gain insight into the inheritance patterns of genes controlling several important plant traits. A whole array of DNA-based markers is now available including RFLPs (restriction fragment length polymorphisms), VNTR (variable number tandem repeats), RAPD (randomly amplified polymorphic DNA) and AFLPs (amplified fragment length polymorphisms) (Kumar, 1999). Reproducibility, small quantities of sample required for analysis, high number of polymorphisms generated per primer combination, ease of use and cost effectiveness, make AFLPs the markers of choice for many researchers attempting to gain insight into the inheritance patterns of quantitative traits through the use of DNA-based markers (Loh *et al.*, 1999; Mueller and Wolfenbarger, 1999). The association of molecular markers with traits of interest is studied to reveal QTLs (quantitative trait loci), that is genomic regions associated with differences in trait values. In this way, QTLs influencing plant traits in various cultivation environments can be identified, thus, elucidating the mechanisms of G × E interaction in crop plants. The expression patterns of QTLs in different environments will give an idea about the extent of QTL × environment (QTL × E) interaction occurring within the population under study, for a given trait. Those QTLs detected across a range of environments would be indications of possible locations on the genome, of genes conferring broad adaptability while QTLs detected in only a subset of environments may correspond to genomic locations where genes conferring specific adaptability to certain growing conditions, are located.

Physiological studies

In order to increase basic productivity in cereals, Morgan *et al.* (2002) recommended that changes be made in their development and metabolism. Physiological studies provide a way to dissect complex traits, such as yield, into simpler components that might be under separate genetic control (Yin *et al.*, 2002). However, so far not much success has been achieved in modifying physiological traits at low levels of

organization to produce new cultivars of crops with higher yield potentials mainly due to compensatory mechanisms in the pathways leading to yield formation or effects of environmental factors on the modified trait. Thus, the closer a physiological trait is to the crop, in level of organization, the more likely that its modification will impact upon yield (Richards, 1996). Hence, the possible effects of the improvement of a physiological trait on yield can only be ascertained by assessing the impact at plant and crop levels. It is, thus, essential to integrate knowledge from several disciplines in order to breed new rice cultivars that will help tackle the dual problems of how to increase rice yield potentials and at the same time reduce yield gaps.

Crop simulation models

Biological systems, such as crops, are the result of a complex of processes. The study of these processes is complicated by the fact that they interact with each other and the environment. Thus, the final yield realized from a crop will be the outcome of the interaction between the various genes controlling traits contributing to yield and between the genes and the environment. It is, therefore, important to identify these traits if one is to fully understand the genetic control of yield and the interaction between the genes controlling yield and the environment.

Crop modelling enables researchers to integrate knowledge from different disciplines in a quantitative way. This helps researchers to understand the underlying processes that determine complex agricultural systems. Simulation models can be used to identify how representative a particular environment is relative to the range of target environments or to identify physiological traits required for high-yielding ability of crop cultivars under different environments (Slafer, 2003). Crop simulation models could, thus, help reduce costs of breeding new cultivars for different environments. Once the effects of major environmental variables on key physiological traits are known, researchers could engage in ideotype breeding by designing crop cultivars with optimum combinations of key physiological traits that would confer high-yielding ability in target environments.

Rationale of the thesis

Rice in Africa

Most of the rice in Africa, except for irrigated paddy, is produced by traditional methods (WARDA, 1994). Rice yields in these traditional production systems are much lower than international averages. Despite an increase in rice production in Africa from the 1980s to the present, rice production has not been able to keep up with demand, resulting in continued importation of rice into Sub-Saharan Africa (Duwayri *et al.*, 2000). The

increase in rice production in Sub-Saharan Africa over the last two decades was mostly because of an expansion in rice area. However, due to environmental concerns continued expansion of the rice area is not feasible. Large areas in Africa never benefited from high-input agriculture, which was responsible for the massive increase in agricultural production in the world over the past decades (Ceccarelli, 1997). Since most breeding work is targeted towards high external input agriculture, many farmers in Africa have not enjoyed the full benefits of modern cultivars. It is, therefore, essential that breeding efforts be aimed at producing cultivars adapted to farming conditions prevailing in farmers' fields in Africa in order to help raise their rice yields.

Rice in The Gambia

Rice is grown in 110 countries, including to varying degrees, every country in West Africa. In The Gambia rice is the main staple food. The per capita consumption of rice in The Gambia has been estimated to be 110 kg per person per annum, one of the highest in Africa (WARDA, 1996). Of the 106,000 tonnes of rice consumed per annum in The Gambia only 20,000 tonnes is produced in the country. The rest is imported. The average yield of rice produced in the country is 1.1 t ha^{-1}. The main cause of this low yield is the minimal use of external inputs such as fertilizers and pesticides because of their high costs. Other contributory factors to this low yield are the widespread use of low-yielding traditional varieties, poor land preparation practices and the erratic rainfall. As there is no end in sight to the stringent economic austerity measures that have been implemented in most developing countries, the cheapest and most accessible option left open for increased rice production is to adapt varieties to their growing environments. This means breeding rice for tolerance/resistance to both biotic (pests and diseases) and abiotic stresses (drought, salinity, acidity, high and low temperatures, and low nutrient supply).

All these abiotic stresses affect rice production in The Gambia to varying degrees some time during the year. Drought stress is particularly serious in the uplands but also in rainfed lowland areas when long dry spells occur during the rainy season or the rains cease prematurely. Soil salinity and soil acidity stresses are confined to the mangrove swamp ecology. Sylla (1994) identified salinity as being the dominant factor limiting rice production in The Gambia river basin. Rice produced in the mangrove zone comprises a significant fraction of the rice-producing area in The Gambia – about 52% contributing 54% of the country's total rice production (WARDA, 1993). Low temperature stress usually occurs in the irrigated lowlands when rice is grown during the relatively cooler months from November to March in the dry season. During this period, night temperatures can sometimes be as low as 10 °C. However, the problem of low soil nutrient supply is the most widespread and is endemic in all the various rice-growing ecologies of the country, that is, the uplands, rainfed lowlands, irrigated lowlands and

mangrove swamps. This is mainly due to the high cost of fertilizers in The Gambia relative to the per capita income of Gambians. Few farmers can afford to apply the recommended rates of fertilizer at the present fertilizer prices. In addition, rice is grown mainly for subsistence in The Gambia and rarely as a commercial crop. Hence, most farmers are unwilling to invest in costly inputs in rice production. Thus, low-cost strategies such as the use of adapted varieties stand a better chance of being adopted by farmers and helping in increasing rice production in the country. Salinity and low N supply are two of the major stresses affecting rice production in The Gambia. Hence, the focus of this research on these two soil stresses.

Research objectives

The main goal of undertaking the research presented in this thesis was to determine the possibility of exploiting genetic variation in rice for tolerance to salinity and low nitrogen supply to increase rice yields in marginal environments. Among the more specific objectives, the first was to study the extent of G × E interaction for yield and its components in a segregating population of rice grown in fresh and saline water with or without N fertilizer application. This would enable us to determine the efficacy of breeding for specific or general adaptability in rice within the range of environments under study. The second objective was to assess the relative contributions of yield components to the variation in yield of rice in different environments. In the presence of significant G × E interaction, selection can be aimed at increasing the levels of yield components that contribute most positively to yield in each test environment. A third objective of the research was to use AFLP markers to identify QTLs associated with yield and yield components of rice in both stressed and non-stressed environments.

Variations in growing conditions are not expected to have any effects on DNA-based markers. However, the degree of association between DNA-based markers and traits under study will be influenced by changes in environmental conditions. This will be revealed by QTL × E interaction occurring for a particular trait. Thus, studying the expression patterns of QTLs in different environments can help researchers identify and select QTLs for yield and/or its components conferring general or specific adaptability. Following the identification of QTLs for yield and its components, the next objective is to explore the possibility of using the identified QTLs in a marker-assisted selection (MAS) scheme to predict and select for yield and yield components of rice in different environments. The final objective is to determine the physiological properties of rice responsible for high-yielding ability in different environments through classical physiological studies and use of the ORYZA1 crop growth simulation model.

Thesis outline

To meet the demand for increased rice production, it is necessary to understand the level of variability in rice yields between different cultivation environments and the changes in the rice plant causing this yield variability. In Chapter 2, the extent of G × E interaction for yield and yield components in a RIL (Recombinant Inbred Line) population of rice grown under different combinations of salinity and N fertilizer application is discussed. Also included in the chapter is a discussion on the possibility to breed for yield stability and/or adaptability in rice in different environmental conditions. The relative importance of four yield components for yield determination in each of four different environments is also presented.

Chapter 3 deals with the identification of QTLs for specific and general adaptability for yield and yield components of rice in environments with varying combinations of salinity and N fertilizer application. The possibility of using marker-assisted selection to select for high-yielding ability in rice is also explored.

The possible effects on yield and yield components of rice, in different environments, of modifications in physiological traits collected at flowering stage are discussed in Chapter 4. As yield components are formed at different stages of crop development, the extent of association between physiological traits collected at different growth stages and yield components of rice in different environments is also discussed.

Yield differences between genotypes of rice arise from differences in physiological properties. In Chapter 5, a comparison is made of the differences in LAI, leaf N concentration, dry matter production and partitioning between plant organs, and growth duration between the highest- and lowest-yielding rice genotypes in four different environments to explain differences in yields.

In Chapter 6, the ORYZA1 crop growth model is used to simulate the yield and biomass production of 15 genotypes of rice in different salinity and nitrogen fertilizer regimes. Then, a sensitivity analysis was performed on each genotype in each environment with regards to LAI, specific leaf N and fraction of dry matter allocated to panicles between flowering and maturity. The physiological traits found to be most relevant for yield and biomass formation in each environment are discussed.

Chapter 7 discusses the possibility of breeding for general or specific adaptability in rice when significant G × E interaction for yield occurs. The discussion also includes an examination of options at the level of yield components and crop physiological characteristics for the genetic improvement of rice in order to better adapt new rice cultivars to the diverse environmental conditions under which rice is cultivated.

CHAPTER 2

Devising selection strategies for rice in the event of significant genotype × environment interaction for yield

Devising selection strategies for rice in the event of significant genotype × environment interaction for yield

Abstract

Most of the rice breeding efforts in the past had been targeted at developing cultivars for high input conditions. Rice cultivars bred under such conditions may not be adapted to low input environments where the presence of abiotic stresses such as salinity and poor nutrient supply may necessitate the development of separate breeding goals for low and high input environments. Differential response of crop genotypes to different growing conditions, also known as genotype × environment interaction (G × E), requires breeders to select for general or specific adaptability in crops, to avoid mismatches of cultivars with environments. To gain insight into the causes of G × E in rice, a segregating population comprising Recombinant Inbred Lines (RILs) from the cross, IR29 × Pokkali, was grown under lowland conditions with salt stress (EC of 8 dS m^{-1}) or without salt stress (EC of 0.15 dS m^{-1}). Under both fresh water and saline conditions, two N fertilizer levels were tested: 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer applied as urea. Analysis of variance and inter-environmental correlations were computed to determine the extent of G × E for yield and four yield components: number of panicles per square metre, total grains per panicle, spikelet fertility and thousand grain weight. Multiple regression of yield on yield components was performed to evaluate the relationships between yield and its components under different environmental conditions. Inter-environmental correlations between fresh and saline water environments, especially for high yielding genotypes, were low and therefore implied that selection should be aimed at specific adaptability of rice for fresh or saline water environments. When environments were differentiated solely by differences in N fertilizer application, inter-environmental correlations of grain yield were high although these correlations were much higher in fresh water environments than in saline environments. This signified that lowland rice could be bred for general adaptability to environments with variable N fertilizer regimes. Genotypic variation in yield was highest in environments where N fertilizer was applied, both in fresh and saline water conditions. Hence, selection for general adaptability in rice should be conducted in high N fertilizer environments. For the yield components, inter-environmental correlations were generally lowest for panicle number and highest for grain weight. Thus, most of the G × E in yield might be due to the sensitivity of panicle number to environmental variability. Generally, associations between yield and grain number attributes (comprising panicle number and total grains per panicle) were higher in fresh water than in saline water whilst associations between yield and

grain filling attributes (comprising spikelet fertility and grain weight) were higher in saline water than in fresh water. These varying relationships between yield and its components in different environments could be exploited to improve genetic yield potentials of rice for different environments when significant G × E in yield is observed.

Introduction

It has been estimated that 60% of global agriculture is practised by farmers in marginal environments (Alexandratos, 1995). The agricultural systems of these farmers are characterized mainly by great variability in the agro-ecosystem both within and between farms and low use of inputs due to either inefficient supply systems or high costs relative to the earning capacities of most farmers. Increasing yields in the fields of these farmers will have far-reaching positive social and economic impacts because agriculture is the main employer and chief source of livelihood for many people in the developing world. These farmers have not benefited much from the spectacular yield increases achieved by the combination of modern breeding technologies and use of external inputs. The main reason for this is that much of modern plant breeding has been practised in and aimed at optimum or near optimum growing conditions where environmental noises can be kept under control, error variances are small and response to selection is high (Richards, 1996; Ceccarelli, 1997). Consequently most of the new crop cultivars released are found to yield well in favourable environments and poorly in the unfavourable conditions that are symptomatic of most of the agricultural lands in the world. This ensues from genotype × environment (G × E) interaction encountered by breeders in many crops. Genotype × environment interaction is the differential performance of genotypes across a range of environments. Significant G × E interaction in yield has been reported for many crops as diverse as, for example, barley (Aastveit and Aastveit, 1993; May and Kozub, 1995; Nurminiemi and Rognli, 1996; Voltas *et al*., 1999), maize (Chapman *et al*., 1997; Crossa *et al*., 1999), chickpea (Singh *et al*., 1996), pigeon pea (Chauhan *et al*., 1998), wheat (Annicchiarico and Mariani, 1996) and rice (Yan *et al*., 1999; Pantuwan *et al*., 2002).

To tackle G × E interaction, breeders usually follow one of two main approaches to produce genotypes that will be suited to their growing environments. One approach is to breed for specific adaptability. Such a strategy will aim at producing genotypes that are suited to a well-defined, narrow range of environments with either favourable or unfavourable growing conditions (Lin and Binns, 1994). Another possibility is to breed for general adaptability, that is, adaptation to a wide range of environments, where genotypes are developed that would give high yields relative to other cultivars in both favourable and unfavourable environments (Eskridge *et al*., 1991). Both these

approaches require clear descriptions of target environments. Researchers at the International Maize and Wheat Improvement Centre (CIMMYT) have pursued the path of breeding for wide adaptability with regards to wheat. Despite the concerns of some breeders that different cultivars are needed for different levels of production, breeders at this institute cite several cases where cultivars of wheat were produced that gave high yields in favourable environments and were also superior under drought to other test cultivars (Rajaram *et al*., 1997). The West Africa Rice Development Association (WARDA) also successfully bred many cultivars of rice for irrigated lowland ecologies and some of these cultivars were found to perform well in rainfed lowland ecologies where the water supply was erratic (WARDA, 2002). It is thus argued that it should be feasible to breed for input efficiency and input responsiveness, that is, high performance at low and high levels of input use, respectively (Rajaram *et al.,* 1997).

Other breeders advocating the practice of specific adaptability have cited several instances where breeding could contribute to increased yields in marginal environments when these environments are targeted directly. Ceccarelli (1997) found that a selection of Syrian barley landraces yielded significantly higher than a selection of modern barley cultivars under high stress conditions and the modern cultivars yielded significantly higher than the landraces in non-stress conditions. At intermediate stress levels there was no significant difference between the yields of the landraces and modern cultivars. The level of stress in those trials was defined by different amounts of inputs and rainfall. In a separate study to determine the response of rice genotypes to drought stress, Pantuwan *et al*. (2002) reported that among a range of populations of recombinant inbred lines of rice grown under different conditions of water supply, flowering date was an important determinant of grain yield. Early-flowering lines were suited to areas with a high likelihood of late drought occurring but under irrigated conditions these lines would not yield as high as late-flowering lines. Jiang *et al*. (1999) also found that in a segregating population of maize derived from a cross of highland and lowland tropical cultivars there was a high amount of G × E interaction for most traits. They concluded that when large differences exist between target environments distinct sets of genes or alleles of genes would be required for specific adaptation. In pearl millet, van Oosterom *et al*. (2003) reported that high-tillering landraces yielded higher than low-tillering modern cultivars under stress whilst in favourable environments modern cultivars out-yielded the landraces. Thus cultivars selected in high input (non-stress) environments may not necessarily be suited for low input agriculture (van Oosterom *et al*., 1993; Ceccarelli, 1997).

In this pursuit of producing high-yielding crop cultivars, breeders have used different stability statistics to determine which cultivars will be best suited to the target environments (Lin *et al*., 1986). These various statistics fall into three groups. One group

of statistics basically determines the biological stability of cultivars, that is, the ability of cultivars to maintain similar yield levels across all test environments. The second group of statistics determines the performance of cultivars between environments relative to the other cultivars being assessed. The last group of stability statistics regresses the residual mean square from the regression model on an environmental index. The first two groups of stability statistics are to be preferred to the third one because the third group estimates the unpredictable response of genotypes to environments and therefore has no predictive value unless environmental index can be replaced by actual environmental factors such as temperature or rainfall (Lin *et al*., 1986; Eskridge *et al*., 1991).

Breeding for stress tolerance has always been a difficult proposition because of the polygenic nature of its inheritance and the high level of environmental variation often involved in terms of intensity and timing of stress and their interaction with other environmental factors. In addition, a higher level of stress may nullify any level of plant stress tolerance (Raymond *et al*., 1994). Salinity is one of the most common stresses present in rice fields around the world (Zeng and Shannon, 2000a). This problem may be worsened by the increased demand (as a result of population pressure) for irrigation in semi-arid climates, which has been identified to be a major cause of secondary salinization (i.e., that due to human activity) in those areas (Yeo, 1999). Measures need urgently to be found that would either decrease salinity levels in agricultural fields or use cultivars of crops that are more tolerant to salinity stress. Use of interventions such as liming (Sylla, 1994) and irrigation and fertilizer application (Rahman *et al*., 1995) to alleviate salinity stress are expensive and not available to many poor farmers. Thus the use of salt-tolerant cultivars of rice might be the cheapest and most accessible option for increasing yields in saline rice fields. Various researchers have reported genotypic variation in rice for salt tolerance (e.g., Gregorio, 1997; Mandal *et al*., 1999; Lefèvre *et al*., 2001; Asch and Wopereis, 2001).

Salt stress in soils is often associated with other abiotic stresses such as mineral deficiencies, flooding, soil alkalinity or drought. It is therefore important to consider breeding for multiple stress tolerance when breeding rice for saline environments (Gregorio *et al*., 2002). In crops salinity has been reported to reduce nitrogen mineralization through biological activity in the soil (van Hoorn *et al*., 2001) and inhibit the transport of nitrate from the roots to the shoots ((Rios-Gonzalez *et al*., 2002). Breeding simultaneously for several traits is difficult to achieve due to the fact that many traits of agronomic interest are under the control of several genes some of which interact with each other positively or negatively. In addition, sometimes genes with opposite effects on a trait may be located closely on the same chromosome segment and thus segregate together during mating. Despite these apparent difficulties several researchers at the International Rice Research Institute (IRRI) have reported cases in rice where

success was achieved in breeding for tolerance to salinity as well as other attendant soil stresses (Neue *et al*., 1998; Gregorio *et al*., 2002; Quijano-Guerta and Kirk, 2002).

Nitrogenous fertilizers usually have the most marked effects on yield of cereals because nitrogen is usually the most limiting nutrient in soils. Under favourable conditions increasing applications of 'N' increase dry matter production and grain production to a maximum, beyond which yield may decline. This decline is frequently associated with lodging, and may not occur if lodging is prevented (Arnold and Austin, 1989). Older and usually taller varieties lodge more readily than modern cultivars most of which are semi-dwarf to medium in height. However, even modern cultivars differ in their response to nitrogen. Prasertsak and Fukai (1997) found differences in yield between two cultivars of rice grown at different combinations of N supply and water stress. Consistent genotypic variation for utilization efficiency of N and P has also been reported for rice across several environments (Fukai *et al.*, 1999). Differences in physiological efficiency of N use for grain production among different cultivars of rice have also been reported (Ohnishi *et al*., 1999). The use of crop cultivars with improved nitrogen recovery and utilization efficiencies will be useful in both low and high input agricultural systems. In low input systems such cultivars can help farmers increase their crop yields with minimal use of external inputs. In high input systems these cultivars will reduce nitrogen losses to the environment, which causes pollution to the atmosphere and water systems (Ghosh and Bhat, 1998).

This research was undertaken with the aim of determining the possibility of increasing rice yields under different environmental conditions through genetic improvement of cultivars. This was done by (i) determining the extent of G × E interaction for grain yield and yield components of a population of Recombinant Inbred Lines (RILs) of rice in four different environments and (ii) determining the options for increasing yields in different environmental conditions by considering the extent of relationships existing between yield and yield components in the various test environments.

Materials and methods

Plant material

A segregating population of rice (*Oryza sativa* L.) comprising 276 F8 recombinant inbred lines (RILs) developed at IRRI (International Rice Research Institute, Los Baños, Philippines) from the cross IR29 × Pokkali (both *indica* varieties) was used as the plant material. IR29 is a short, high yielding modern cultivar released by IRRI and is known to be very sensitive to salinity (Gregorio, 1997). Pokkali is a tall, traditional variety from India known to be tolerant to salinity (Yeo and Flowers, 1986; Garcia *et al*., 1995; Gregorio, 1997). As the RILs were randomly selected and one of the two parents is a

short, high-yielding, modern cultivar and the other is a tall traditional variety, it is expected that the RIL population will also be segregating for response to nitrogen. This is due to the fact that modern rice cultivars have been bred for response to high levels of mineral fertilizer application while traditional varieties have usually been selected by farmers in environments with sub-optimal levels of nutrient supply.

Field experiments

One hundred (100) RILs were randomly selected from the RIL population and used in the trial of 1999. In 2000, four extra RILs were included. These RILs together with the parents were grown during the rainy season (June-October) in 1999 and 2000 in a series of experiments at Sapu (13.55° N latitude), in The Gambia. A split-split plot experimental design was used with salinity as the main plot factor, rate of nitrogen fertilizer application as the sub-plot factor and genotype as the sub-subplot factor. Two levels of salinity and two levels of nitrogen fertilizer application (giving a total of four treatment combinations or environments) were tested. Three replications were maintained in each year of the trials. Each sub-subplot plot measured 2.6 m × 3.0 m in both years. Additional information on the trials is given in Table 1. The following treatment combinations also referred to later as test environments, were obtained:

S1N1 – Fresh water (river water) at an electrical conductivity (EC) of 0.15 dS m^{-1} and 0 kg N ha^{-1};

S1N2 – Fresh water (river water) at an EC of 0.15 dS m^{-1} and 100 kg N ha^{-1} as urea;

S2N1 – Salt water at an EC of 6 or 8 dS m^{-1} and 0 kg N ha^{-1};

S2N2 – Salt water at an EC of 6 or 8 dS m^{-1} with 100 kg N ha^{-1} as urea.

Pre-germinated rice seeds were sown in a nursery that was well watered and regularly weeded. The seedlings were transplanted to the field following the experimental design described above. After transplanting the trial plots were kept continuously flooded by irrigating with river water till all RILs and cultivars were close to physiological maturity. Salinity was imposed by manually broadcasting measured amounts of granular table salt in standing water to attain the required salinity.

Data collection

Salinity of the ponded-water was measured two days after every significant rainfall or after a protracted period without rains (more than four consecutive days). An ES-421 salt meter (Atago Co. Ltd., Japan) was used to measure salinity levels. When the salinity level was too low more salt was added to raise the salinity and when the salinity was too high the saline plots were irrigated with fresh water to reduce the salinity to the desired level.

Data was also collected on grain yield and yield components. Four yield components were assessed – number of panicles per square meter, total grains per panicle, thousand grain weight, and spikelet fertility. A peg was randomly placed between four plant hills towards the middle of each plot. At maturity these four hills were harvested separately from the rest of the plot. Yield component data was determined from these four hill samples from each plot.

Number of panicles m^{-2} (panicles m^{-2})

All the panicles in four hill samples were counted for each plot. As the area covered by these four hills was 0.16 m^2 (0.4 m × 0.4 m) the number counted was multiplied by 6.25 (1.0 m^2 / 0.16 m^2) to convert it to number of panicles m^{-2} for that plot.

Total number of grains per panicle (grains/panicle)

The four hill samples were threshed separately for each plot and then winnowed to separate filled from empty grains. These two classes were counted separately, added together and then the sum was divided by the total number of panicles in the four hill samples to give the total number of grains per panicle.

Thousand grain weight (g)

All the filled grains counted from the four hill samples were weighed and thousand-kernel weight was calculated using the following formula:

Thousand grain weight (g) = (Weight of filled grains from four hill samples (g) / total number of filled grains from four hill samples) × 1000

Table 1. Details of environmental conditions and field experiments conducted in 1999 and 2000 at Sapu Research Station, The Gambia.

	1999	2000
Sowing date	24 May	8 June
Transplanting date	2 July	4 July
Target salinity (dS m^{-1})	~ 6	~ 8
Total rainfall (mm)	1784	1326
N fertilizer (kg ha^{-1})	70	100
Basal fertilizers (kg ha^{-1})		
N (Diammonium Phosphate)	15.7	0
P (Triple Super Phosphate)	40	40
K (Muriate of Potash)	40	40

Spikelet fertility (%)

This was determined by dividing total number of filled grains in the four-hill samples by total number of grains per panicle and then multiplying by 100.

Grain yield (kg ha^{-1})

With the exception of plants in the outermost rows and the four-hill samples, all the plants in each plot were harvested upon reaching maturity. The harvested plants were then threshed, dried, cleaned and weighed. The weight of the grain harvested from the four-hill samples used for yield component analysis was added to that of the grain harvested from the rest of the plot to give the total grain yield from a plot. The yield (g) from this net plot was then converted to yield in kg ha^{-1}.

Data analysis

Analysis of variance models were fitted to yield and its components using the Mixed Procedure (Proc Mixed) of SAS (1999). Preliminary analyses revealed interaction between the factor year on the one hand and all terms involving genotypes on the other hand (genotypic main effect and all genotype-by-environment interactions). For ease of presentation, it was therefore decided to perform and present analyses per year (1999 and 2000). One of the differences between 1999 and 2000 was the occurrence of bird damage in 1999, leading to the introduction of a qualitative co-variable for bird damage. Bird damage was scored following the Standard Evaluation System of IRRI (IRRI, 1996). Interpretations of genotype by environment interactions was based on least squares means (LSMeans) for line × salinity level × nitrogen level combinations.

Relations between yield and yield components in different environments were studied on the basis of the line × salinity × nitrogen means, where the combinations of salinity (fresh or saline) and nitrogen (0 kg ha^{-1} or 100 kg ha^{-1}) determined the environment. Performance for the same trait in two environments was analysed by various means. Firstly, a scatter plot was constructed, on which a fitted regression line, either linear or quadratic, was superimposed as a rough description of the general trend. The most important part of genotype-by-environment interaction for breeders consists in genotypic rank changes between environments. For that reason, Spearman rank correlations were calculated between performances in two environments to help interpret the scatter plots, whenever the trend was non-linear. In addition, standard deviations per environment were calculated.

Univariate relations between yield and its components were investigated by simple regression and correlation. For a multivariate study of yield in dependence on its components, use was made of the logarithmic relation:

(i) log [Yield (kg ha^{-1})] = log [NPM (panicles ha^{-1})] + log [TGP (grains $panicle^{-1}$)] + log [WTG (kg)] + log [SFERT (%)/100]

that follows from the multiplicative relationship between yield components and yield:

(ii) Yield = NPM × TGP × WTG × SFERT

where, NPM is the number of panicles m^{-2} (panicles m^{-2}); TGP the total number of grains per panicle (grains $panicle^{-1}$); WTG the thousand grain weight (g); and SFERT the spikelet fertility (%). Please note that in the equations (i) and (ii) all units are standardized.

The logarithmic expression is amenable to an analysis by regression that allowed us to assess the relative importance of the various components for final yield. We performed a forward regression of log[Yield] on the logarithms of the four components and registered the order in which the components were included and their contribution in terms of percentage of sum of squares in yield explained.

We proceeded to predict grain yield from the four yield components using the above equations relating yield components to yield. Taking R^2 to be analogous to heritability, we regressed predicted yields on observed yields to determine the predictability of yield from its components in each separate environment. Heritability is essentially the regression of genotype on phenotype, which is similar to the regression of predicted yield on observed yield.

Results

Analysis of variance

Inspection of Tables 2a and 2b reveals that of the terms important to breeders, namely all the terms involving line, the line main effect in both 1999 and 2000 clearly dominated. Genotype-by-environment interactions were quite small in 1999, but did reach an appreciable level in 2000. Because of the significance of the line × salinity × nitrogen interaction in 2000, an interpretation of the results should be based on the further description of corresponding least squares means. Figure 1 visualizes these means in 1999 and 2000, where the means for 1999 have been included for completeness.

Genotype × Environment interaction (G × E) for grain yield

There were clear differences between yields in 1999 and 2000 with regards to the behaviour of the lines in their response to the combined effects of salinity and nitrogen

Table 2(a). ANOVA table for yield in 1999.

Source	DF	Sums of Squares ($\times 10^6$)	% Total variation	F value	Pr > F
Corrected Total	1162	515.427	81.3		
BIRDS	1	14.608	2.9	39.58	**
REP	2	1.446	0.3	0.49	n.s.
SLEV	1	2.699	0.6	0.94	n.s.
Error1	2	33.003	6.5		
NLEV	1	15.703	3.1	12.43	*
SLEV×NLEV	1	9.163	1.8	2.28	n.s.
Error2	4	15.701	3.1		
LINE	99	266.005	51.7	16.57	**
LINE×SLEV	99	21.707	4.3	1.67	**
LINE×NLEV	99	21.344	4.2	1.28	*
LINE×SLEV×NLEV	99	17.165	3.4	0.97	n.s.
Error3	754	96.882	18.7		

* Significant ($P < 0.05$); ** significant ($P < 0.0001$); n.s. not significant ($P > 0.05$).

(Note: BIRDS – bird damage score; REP – replication effect; SLEV – salinity effect; NLEV – N fertilizer effect; LINE – effect of genotype).

Table 2(b). ANOVA table for yield in 2000.

Source	DF	Sums of Squares ($\times 10^6$)	% Total variation	F value	Pr > F
Corrected Total	1213	779.451	84.0		
REP	2	36.547	4.7	0.68	n.s.
SLEV	1	96.105	12.4	3.67	n.s.
Error1	2	53.475	6.9		
NLEV	1	1.700	0.3	0.18	n.s.
SLEV×NLEV	1	14.724	1.9	1.43	n.s.
Error2	4	39.061	5.1		
LINE	103	200.222	25.7	12.14	**
LINE×SLEV	103	133.863	17.2	8.06	**
LINE×NLEV	103	50.030	6.5	3.01	**
LINE×SLEV×NLEV	103	28.512	3.7	1.75	**
Error3	790	125.209	16.1		

** Significant ($P < 0.0001$); n.s. not significant ($P > 0.05$).

(Note: REP – replication effect; SLEV – salinity effect; NLEV – N fertilizer effect; LINE – effect of genotype).

(Figs. 1a-h). In 1999, very few lines showed differential responses across salinity × nitrogen regimes. Rank orders for genotypes hardly changed between environments, and neither could changes in yield range be observed. There was a linear relationship between the yields of lines in fresh and saline water both at zero and optimum nitrogen fertilizer rates in 1999. The highly significant Spearman correlation coefficients ($R > 0.75$ in all cases) between yields in different environments (Table 4) also confirmed our observations that treatment effects were not strong enough that year to adequately identify lines which showed differential performance across environments.

In 2000, differential genotypic responses to salinity × nitrogen regimes were obvious and will therefore form the main basis of discussion for this chapter. In this trial, there was significant G × E interaction for yield and yield components because either some or all of the following interactions – line × salinity level, line × nitrogen level and line × salinity level × nitrogen level – were significant for the measured traits (Table 2b). The interaction of salinity level × nitrogen level was not significant.

(i) Effect of salinity on yields at different rates of applied nitrogen fertilizer Salinity decreased rice yields strongly compared to the fresh water situation. However, the response of the lines to salinity differed according to the level of N fertilizer level used. Mean environmental yields were significantly reduced at the zero N fertilizer

Table 3. Mean values for yield and yield components of RILs in four test environments in 2000.

Environment	Traits				
	Yield (kg ha^{-1})*	NPM (panicles m^{-2})	SFERT (%)	TGP (grains/panicle)	WTG (g)
S1N1	2770 ^{b}	192 ^{a}	92 ^{b}	50^{b}	34 ^{ab}
S2N1	2008 ^{a}	233 ^{a}	87 ^{ab}	35^{a}	31^{a}
S1N2	2599 ^{ab}	209 ^{a}	86 ^{ab}	47 ^{ab}	38^{b}
S2N2	2308 ^{ab}	272 ^{a}	83 ^{a}	38 ^{ab}	31^{a}
Mean	2421	227	87	43	34
$LSD_{0.05}$	638	87	8	14	5

* In each column, figures followed by the same letters were not significantly different from each other (at $P < 0.05$).

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

Table 4. Spearman rank correlation coefficients for yields of RILs between different test environments in 1999 (n=98).

Environments	S1N2	S2N1	S2N2
S1N1	0.824*	0.827	0.760
S1N2		0.759	0.762
S2N1			0.817

* All coefficients significant at $P < 0.0001$.

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively).

Table 5. Spearman rank correlation coefficients for yield and yield components of RILs (n=97) between different test environments in 2000.

Environments	Yield (kg ha^{-1})	NPM (panicles m^{-2})	SFERT (%)	TGP (grains/panicle)	WTG (g)
S1N1/S2N1	0.298**	0.212*	0.473***	0.580***	0.769***
S1N1/S1N2	0.680***	0.454***	0.634***	0.621***	0.698***
S1N1/S2N2	0.489***	0.284**	0.461***	0.503***	0.801***
S2N1/S1N2	0.231*	0.082n.s.	0.467***	0.549***	0.617***
S2N1/S2N2	0.510***	0.441***	0.668***	0.668***	0.781***
S1N2/S2N2	0.533***	0.326**	0.457***	0.512***	0.703***

* Significant ($P < 0.05$); ** significant ($P < 0.01$); *** significant ($P < 0.0001$); n.s. not significant ($P > 0.05$).

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively).

level but not at the high N fertilizer level (Table 3) by salinity stress. The relationship between yields in the fresh and saline water environments at both levels of N fertilizer application was curvilinear (Figs. 1e and 1f). The regression line curved downward away from the 1:1 line in both cases but this downward movement of the regression line was more severe at zero than at high N rate. The weaker agreement between yields in the fresh and saline water environments at zero N dosage relative to the high N dosage was also shown by the lower Spearman correlation coefficients for the rankings of yields in fresh water and saline environments at zero N rate compared to when high N was applied (Table 5).

At both levels of N fertilizer application, the highest yielding lines in fresh water were among the most sensitive to salinity, as evidenced by their very low yields under

salinity (Figs. 1e and 1f). With zero N fertilizer application most lines yielded significantly higher in fresh water than in saline water ($LSD_{0.05}$ = 638 kg ha^{-1}). Few lines were able to yield higher in salt water than they did in fresh water but these yield differences were not significant between the two salt levels (P > 0.05). With high N supply, however, some lines yielded significantly better in salt water than in fresh water. In addition, with high N application, it was mostly the highest yielding lines in fresh water that produced significantly lower yields in salt water than in fresh water. Apparently the tolerance of most lines to salinity was improved with the addition of extra nitrogen fertilizer because there were no significant yield differences for most lines between fresh and saline water when a high N dosage was given (Fig. 1f). Among the lines tested, within the same N fertilizer regime, the variation in yield was much smaller in saline than in fresh water environments (Table 6).

(ii) Effect of nitrogen fertilizer on yields in fresh and saline water The response of the lines to added N fertilizer differed strongly depending on the presence or absence of salt stress. In fresh water mean yields were higher in the zero N fertilizer regime than in the high N fertilizer regime while in saline water mean yields were lower in the zero N fertilizer regime than in the high N fertilizer regime (Table 3). However, in both fresh and saline water environments, mean yields in the zero N fertilizer regime were not significantly different from those in the high N fertilizer regime. The regression line in the plot of yields in the high N fertilizer regime against yields in the zero N fertilizer regime in fresh water, described an upward curve moving away from the 1:1 line towards the high N fertilizer axis: most of the highest yielding lines in zero N fertilizer regime responded more positively to applied N fertilizer than the poor or average yielding lines while most of the poorest yielding lines in the zero N fertilizer regime responded negatively to N fertilizer application. However, despite this curvilinear relationship the yields of the lines in these environments were highly correlated with each other as can be seen in the relatively high Spearman correlation coefficient in Table 5 (R = 0.68) implying that the rankings of the lines with regards to yields were similar between these two environments. In saline water, the relationship between yields at high and zero N fertilizer rates was linear but with a lower Spearman correlation coefficient (R = 0.51) implying that in this RIL population, genotype × nitrogen interaction was stronger in saline water than in fresh water environments. In saline water, there was no improvement in R^2 over the linear equation when a quadratic equation was fitted to the scatter plot (Fig. 1h). Within the fresh water and saline water environments the variation in yield was higher in the high N fertilizer regime than in the zero N fertilizer regime (Table 6).

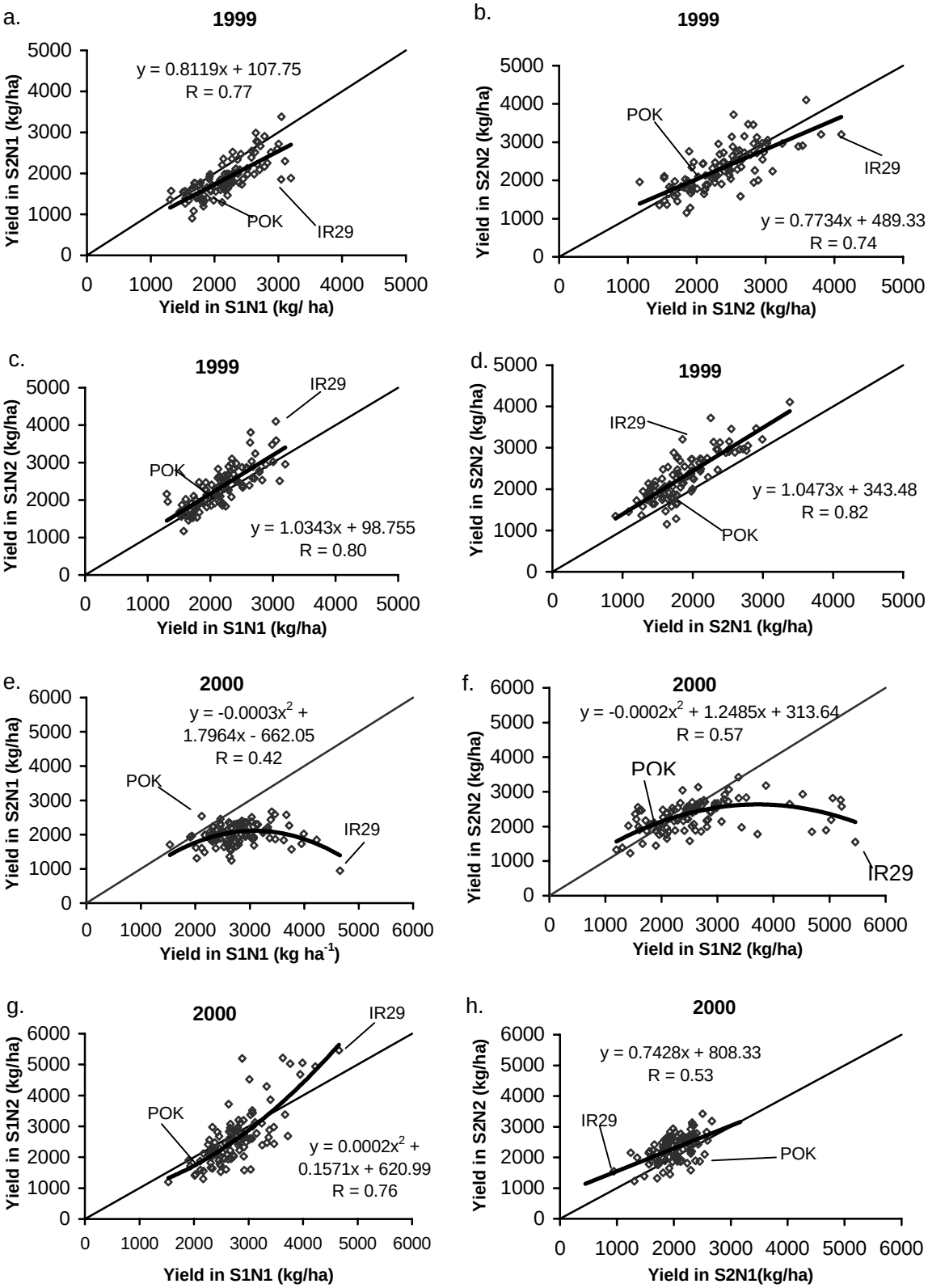

Fig. 1. Scatter plots of rice yields in various environments during two years of trials showing positions of two parents (IR29 and Pokkali – POK) relative to RILs and inter-environmental correlations for yield. (For 1999, $LSD_{0.05}$= 574; for 2000, $LSD_{0.05}$ = 638; environments S1N1, S1N2, S2N1 and S2N2 are explained in the text).

Table 6. Standard deviations of yield and yield components in four different environments during the 2000 trial (n=97).

Environment	Traits				
	Yield (kg ha^{-1})	NPM (panicles m^{-2})	SFERT (%)	TGP (grains panicle^{-1})	WTG (g)
S1N1	509.01	35.96	3.58	9.27	2.50
S2N1	310.74	34.72	6.93	7.09	2.98
S1N2	903.67	50.45	6.25	9.73	2.84
S2N2	430.11	47.06	10.74	6.55	3.07

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

In both fresh and saline water some lines yielded significantly better with no N fertilizer than with high N fertilizer ($LSD_{0.05}$ = 638 kg ha^{-1}). These lines responded quite negatively to extra N fertilizer usage. This was more apparent in fresh water than in salt water. There were also lines that showed a significantly positive response to added N fertilizer under both fresh and saline water although there were more such lines in saline than in fresh water conditions. In fresh water, lines that had the strongest response to applied N were also among those with the highest yields at zero applied N. Lines that yielded around 3000 kg ha^{-1} or more in fresh water with zero N application, showed strong positive or negative response to applied N fertilizer. In salt water most of the lines that showed strong positive or negative response to applied N fertilizer had yields around 2200 kg ha^{-1} when no N fertilizer was applied.

The two parents, IR29 and Pokkali, showed markedly different properties. IR29 was very sensitive to salinity and its yield was significantly reduced ($P < 0.05$) by salinity at both zero (by 3713 kg ha^{-1}) and high N (by 3912 kg ha^{-1}) application rates. However, this cultivar showed a positive response to N fertilizer under both fresh water and saline conditions and this response was significant ($P < 0.05$) only in fresh water (804 kg ha^{-1}) but not under saline conditions (604 kg ha^{-1}). Pokkali, on the other hand, performed better under saline conditions than in fresh water at both zero (by 427 kg ha^{-1}) and high (by 230 kg ha^{-1}) N fertilizer rates and responded negatively to applied N under both fresh (–242 kg ha^{-1}) and saline (–438 kg ha^{-1}) water conditions. This traditional cultivar gave low but stable yields under all four test environments because these yield differences were rather small and not statistically significant ($P > 0.05$).

Genotype × Environment (G × E) interaction for yield components

Effect of salinity at different rates of applied nitrogen fertilizer Salt stress affected the yield components differently in the two N regimes (Fig. 2). In the RIL population that we used there was more genotype × salinity interaction in both the zero and high N environments for number of panicles per m^2 than for any of the other yield components. This was shown by the Spearman correlation coefficients in Table 5 between the rankings of yield components of RILs in saline water against those in fresh water at zero (S1N1/S2N1) and high (S1N2/S2N2) N fertilizer usage. With the exception of a few lines that were highly sensitive to salinity, the ranking of most lines with regards to thousand grain weight hardly changed between the fresh and saline water environments at both levels of N fertilizer usage.

For most genotypes, the number of panicles was higher under saline stress than in fresh water environment, at both levels of N fertilizer application (Table 3). This increment in panicle number, however, was significant ($LSD_{0.05}$ = 87.36) for more lines in the high N fertilizer regime than in the low N fertilizer regime. Few lines had higher panicle numbers in fresh water than in salt water and only for one line was this increment significant at both levels of N fertilizer application (Figs. 2a(i), 2a(ii)). The variation in panicle number between fresh and saline water environments at both levels of N fertilizer use was quite similar although the variation was slightly higher in fresh water than in saline water (Table 6).

On the other hand salinity depressed spikelet fertility at both levels of N fertilizer usage (Table 3) compared to the fresh water environment. This reduction was significant for more lines at the zero N than at the high N fertilizer application rate ($LSD_{0.05}$ = 8.18%). Few lines had higher spikelet fertilities in the saline than in the fresh water environment at the zero N fertilizer rate but these differences were not statistically significant. In the high N regime, however, more lines had higher spikelet fertility values in saline water than in fresh water and these differences were actually significant for some lines.

Salinity caused a strong reduction in total grains/panicle relative to the fresh water environment for most lines at both levels of N fertilizer usage. This reduction was significant ($P < 0.05$) for more lines in the zero than in the high N fertilizer regimes. Certain lines produced more grains per panicle in saline water than in fresh water but these were significant only in the high N fertilizer regime ($LSD_{0.05}$ = 14.12) and not in the zero N fertilizer regime. At the high N fertilizer rate, differences in total grains per panicle between the fresh and saline water environments were not significant for most lines.

Similarly for thousand grain weight also, there was a reduction in mean values in

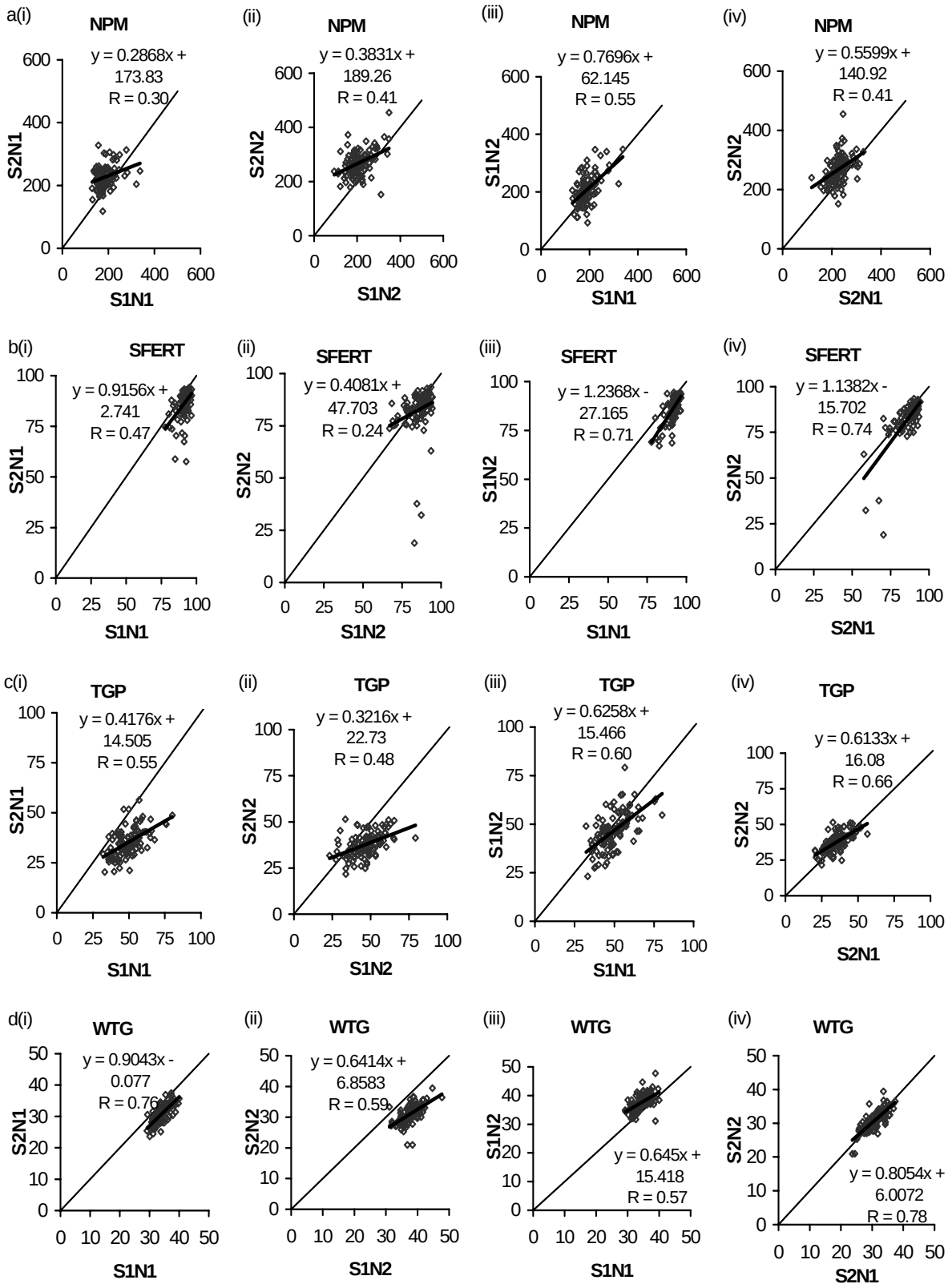

Fig. 2. Scatter plots for four yield components of rice in different environments in 2000 (n = 104); (for NPM, $LSD_{0.05}$ = 88 panicles m^{-2}; SFERT, $LSD_{0.05}$ = 8.2%; TGP, $LSD_{0.05}$ = 14 grains $panicle^{-1}$; WTG, $LSD_{0.05}$ = 4.6 g. See the text for explanations of acronyms).

saline environments compared to fresh water environments at both levels of N fertilizer usage (Table 3). However, this reduction was significant for more lines in the high N regime than in the low N regime ($LSD_{0.05}$ = 4.55g). At both levels of N fertilizer usage some lines had slightly higher grain weights in saline water than in fresh water but these differences were not significant.

Effect of N fertilizer application in fresh and saline water The effect of N fertilizer on the yield components varied between the fresh and saline water environments. However, generally for all the yield components there was more genotype × salinity interaction than genotype × nitrogen interaction (Fig. 2 and Table 6).

In fresh water, there was more genotype × nitrogen level interaction for panicles m^{-2}, than for total grains per panicle, spikelet fertility or thousand grain weight. Similarly in the saline environment, the ranking of the RILs changed between N fertilizer regimes more for number of panicle m^{-2} than for the other yield components (Table 5). For these other traits, there was a comparable level of genotype × nitrogen level interaction for spikelet fertility and total grains per panicle and these were higher than that for grain weight.

In both fresh and saline water there was an increase in panicle number in the high N regime over the zero N regime (Table 3) and this increment was significant for some lines in fresh as well as in saline water (Figs. 2a(iii) and 2a(iv)). Some RILs, however, produced more panicles m^{-2} at zero than at high N fertilizer rates although this difference was significant for only two lines in the fresh water environment ($LSD_{0.05}$ = 87.36). There was more variation in number of panicles m^{-2} at high N than at zero N fertilizer rates in both fresh water and saline water environments (Table 6).

On the other hand, N fertilizer application in both fresh and saline water decreased spikelet fertility of most genotypes. This was significant for more lines in fresh than in saline water ($LSD_{0.05}$ = 8.18%). Some RILs, however, had higher spikelet fertilities in the high N fertilizer regime than in the low N fertilizer regime but more lines showed this behaviour in saline than in fresh water, although there was only one instance in the saline environment where this was actually significant (Figs. 2b(iii) and 2b(iv)). The level of variation in spikelet fertility was higher in saline environments than in fresh water in both N regimes and the variation was also higher in the high N regime than in the zero N regime in both fresh and saline water (Table 6).

The effect of N fertilizer application on total grains per panicle was markedly different between the fresh and saline water environments. In fresh water, N fertilizer application on average led to a reduction, although not significant ($P > 0.05$), in total grains per panicle compared to the zero N fertilizer regime (Table 3). There were some lines, however, that produced more grains per panicle at the high N than at low N

fertilizer rate (Fig. 2b(iii)) although more RILs showed a significant reduction in total grains per panicle than those with a significant increase in total grains per panicle when N fertilizer was applied ($LSD_{0.05} = 14.12$). Conversely, in saline water most lines showed an increase in total grains per panicle with N fertilizer use but only for two RILs was this increase significant (Fig. 2c(iv)). In salt water also some lines produced fewer grains per panicle in the high N fertilizer regime than in the zero N fertilizer regime and this was significant for some lines. The RILs exhibited more variation for total grains per panicle in fresh water than in saline water and the variation was similar between the N fertilizer regimes.

With regards to thousand grain weight, application of N fertilizer led to an increase in mean values over the zero N fertilizer environment in fresh water (Table 3) and this increment was significant for many lines. Fewer lines showed a reduction in grain weight when N fertilizer was applied compared to the zero N fertilizer environment and this reduction was significant for only one line in fresh water (Fig. 2d(iii)). In saline water, however, there were no significant differences between grain weights at zero and at high N fertilizer rates for most RILs (Fig. 2d(iv)). Only one RIL showed a significant reduction in grain weight and two RILs showed significant increases in grain weight with N fertilizer application compared to the zero N fertilizer situation in saline water. Similar to spikelet fertility, thousand grain weight also showed more variation in saline water than in fresh water and also more variation in the high N fertilizer regime than in the zero N fertilizer regime (Table 6).

Relationships between yield and yield components in different environments

The yield components were all positively correlated to yield in all four test environments. However, these correlations between pairs of yield components and yield were not always significant in the different environments (Table 7). The strength and sign of the correlations between yield components themselves varied from one environment to the other (Table 7).

Regression analysis (Table 8) showed that among the four yield components considered here, number of panicles m^{-2} accounted for most of the variation in yield in all test environments except the highly stressful zero N fertilizer regime in saline water, where spikelet fertility was apparently more relevant to yield variation than any other yield component. The number of panicles m^{-2} accounted for more yield variation at the high N fertilizer level in both fresh and saline water than at the zero N fertilizer level. Spikelet fertility, on the other hand, contributed more to yield variation at the high N fertilizer level than at the low N fertilizer level in fresh water while in saline water it contributed more to yield variation at the low N fertilizer regime than at the high N fertilizer level. In contrast to spikelet fertility, total grains per panicle and

Table 7. Pearson correlation coefficients between yield components of rice in fresh and saline water environments. Figures above the diagonal are correlation coefficients in the zero N fertilizer environments and those below the diagonal are for the 100 kg ha^{-1} N fertilizer environments (n = 104).

	Fresh water environments					Saline water environments				
	Yield	NPM	SFRT	TGP	WTG	Yield	NPM	SFERT	TGP	WTG
YLD		0.438**	0.176	0.218*	0.079		0.196	0.477**	0.173	0.177
NPM	0.635**		0.039	–0.332*	–0.173	0.425**		0.071	–0.421**	0.039
SFERT	0.380**	0.311*		–0.005	–0.039	0.400**	0.169		0.148	–0.006
TGP	0.152	–0.051	0.069		0.048	0.007	–0.305*	–0.093		–0.035
WTG	0.074	–0.001	–0.123	0.044		0.190	0.010	–0.025	–0.067	

* Significant at $P < 0.05$, ** significant at $P < 0.0001$

(Note: NPM – no. of panicles $m^{-}2$; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

Table 8. Relative contributions of yield components to the variation in grain yield of rice in four different environments in 2000.

Trait	S1N1	S1N2	S2N1	S2N2
NPM	0.1919*** a	0.4034*** a	0.0264n.s. c	0.1722*** a
TGP	0.1486*** b	0.0282* c	0.0024n.s. d	0.0296 * d
WTG	0.0259* c	0.0080n.s. d	0.0313* b	0.0478* c
SFERT	0.0261** d	0.0371** b	0.2651*** a	0.1084 *** b

* Significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

[a] R^2 for first added component; [b] R^2 for second added component; [c] R^2 for third added component; [d] R^2 for fourth added component.

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

thousand grain weight both accounted for more yield variation at the zero N fertilizer level than at the high N fertilizer level in fresh water and accounted for more yield variation at high N fertilizer level than at zero N fertilizer level in saline water. In all environments grain weight accounted for small proportions of the total variation in yield relative to the more significant yield components in the various environments.

Fresh water with 0 kg ha^{-1} N fertilizer

In this environment all yield components contributed significantly to the variation in yield. However, panicle number and total grains per panicle accounted for more of the variation in yield than grain weight and spikelet fertility although panicle number accounted for a greater proportion of the variation than total grains per panicle did (Tables 7 and 8). Spikelet fertility and grain weight were not significantly correlated to observed yield in this environment ($P > 0.05$). Among the yield components, panicle number had a significant negative correlation with total grains per panicle ($P < 0.05$).

Fresh water with 100 kg ha^{-1} N fertilizer

Here, variation in panicle number ($P < 0.0001$) accounted most significantly for the variation in yield (Tables 7 and 8). Spikelet fertility ($P < 0.01$) and total grains per panicle ($P < 0.05$) also accounted for small but significant proportions of yield variation while grain weight made no significant contribution to the total variation ($P > 0.05$) in yield in this environment (Table 8). With regards to yield components, panicle number and spikelet fertility were significantly (positively) correlated ($P < 0.05$).

Saline water with 0 kg ha^{-1} N fertilizer

In this environment, only variation in spikelet fertility ($P < 0.0001$) and grain weight ($P < 0.05$) significantly accounted for the variation in yield. Panicle number and total grains per panicle made no significant contributions to the total variation in yield in this environment ($P > 0.05$). For yield components, panicle number had a significant negative correlation with total grains per panicle ($P < 0.0001$).

Saline water with 100 kg ha^{-1} N fertilizer

Here, all four yield components made significant contributions to the variation in yield. However, panicle number and then spikelet fertility accounted for most of the variation in yield ($P < 0.0001$ in both cases) while grain weight and then total grains per panicle also made significant but small contributions to yield variation in this environment ($P < 0.05$). With regards to correlations between yield components, panicles per m^2 had a significant negative correlation with total grains per panicle.

Predicting grain yield from yield components

The agreement between observed yield and yield predicted from the four yield components in Table 8 varied between the four test environments. At both N fertilizer levels, agreement between predicted and observed yields was higher in fresh water than in saline water (Fig. 3). This was reflected in the higher R^2 values in Figs. 3a and 3b than in Figs. 3c and 3d. This is understandable in view of the fact that the four yield

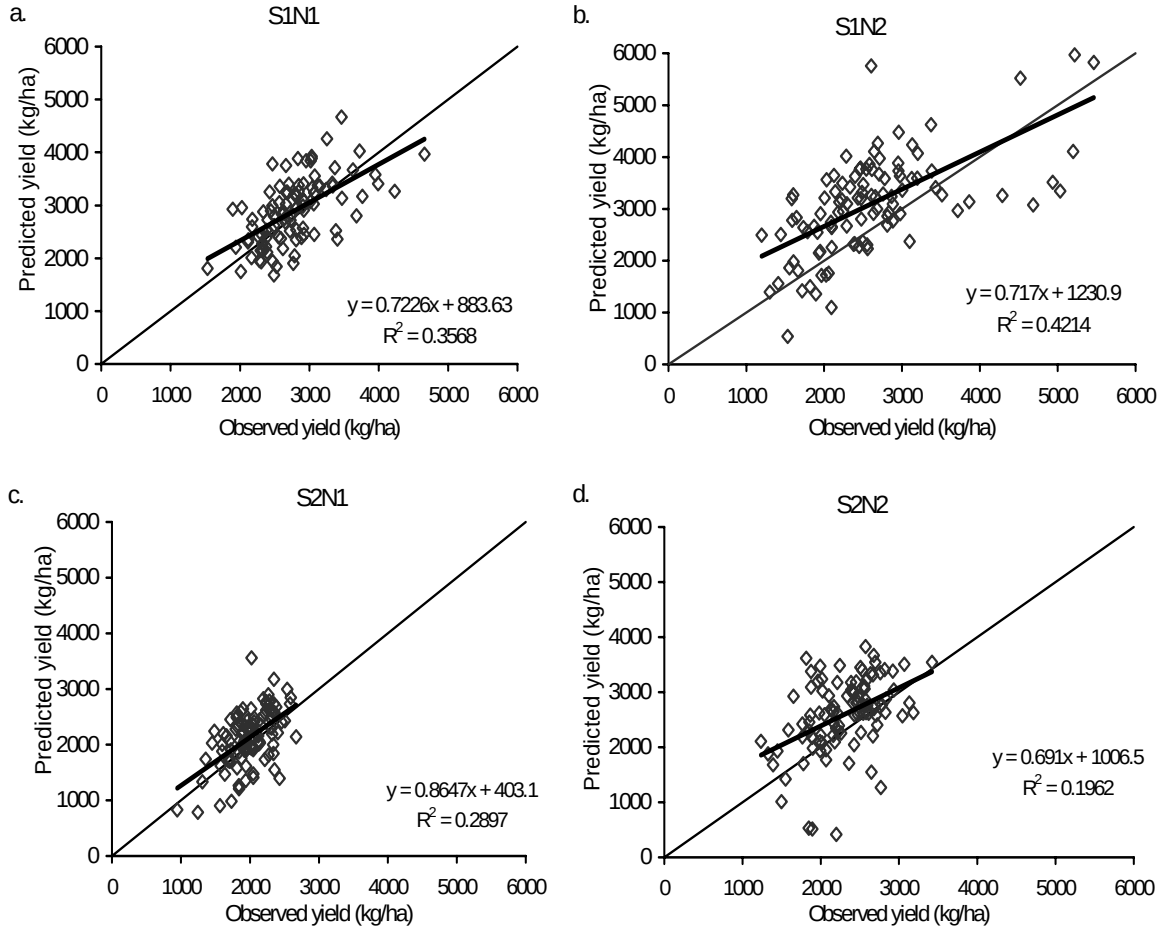

Fig. 3. Scatter plots of yield (predicted from yield component values) against observed yields across four test environments in year 2000 (n = 104). S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively.

components accounted for more yield variation in fresh water than in saline water (Table 8). Between the two N fertilizer levels in fresh water, yield predictions were better at the high N level than at the zero N fertilizer level (Figs. 3a and 3b) while in saline water, yields were better predicted at the zero N fertilizer level than at the high N fertilizer level (Figs. 3c and 3d).

In both fresh and saline water, there was an apparent over-estimation of yield for most lines in the high N environments because in these environments most of the points were above the 1:1 line (Figs. 3b and 3d).

Discussion

Yield differences between years

Some of the causes for the differing qualities of the data of 1999 and 2000 could be attributed to several important differences between the trial conditions of 1999 and 2000. In 1999, the first experimental year, the target salinity was ~ 6 dS m^{-1}. This salinity was maintained for only a little over one month when there was a heavy downpour (more than 200 mm of rainfall in one day). After this downpour all the trial plots were flooded for 3 days but fortunately the rice plants were not completely submerged. From then on it was not possible to regulate salinity anymore and no salt was added to the plots again. In 2000 salinity was increased to 8 dS m^{-1} and it was possible to maintain salinity in the necessary plots up to harvest time. Furthermore the total rainfall was much higher in 1999 than in 2000 (Table 1). The significance of this was that salinity was diluted much quicker by rainfall in 1999 than in 2000.

In addition, different planting densities were used in the two years. Due to the unavailability of adequate seedlings, one seedling per hill was planted in 1999 at a spacing of 25 × 28cm as opposed to 3 seedlings per hill at a spacing of 20 × 20cm in 2000 and 2001.

In 1999, the main effect of nitrogen level (NLEV) was significant but not in 2000. A possible explanation for this could be that in 1999 much older seedlings were transplanted and at a lower density than in 2000. Thus addition of N fertilizer to N treatments in 1999 would lead to a much higher tillering in these plots than in the untreated plots. This would then lead to a larger difference between the mean yields of genotypes in zero and high N fertilizer regimes in 1999 than in 2000 when the optimum planting density was used.

Effects of treatments on grain yield and yield components

Generally salinity caused a decline in yield in both N fertilizer regimes. Addition of nitrogen fertilizer on the other hand caused an increase in yield of most lines in both fresh and saline water environments. However, the main effects of these two factors and their interaction had no significant effects on yield because we worked with a population which was segregating for tolerance to salinity and which we expected to segregate also for response to nitrogen fertilizer. Thus, with regards to main effects of salinity and nitrogen fertilizer the responses of good and bad lines tended to cancel each other out. Consequently the mean performance of lines in fresh (2707 kg ha^{-1}) and saline water (2153 kg ha^{-1}) and zero (2393 kg ha^{-1}) and 100 kg ha^{-1} N (2468 kg ha^{-1}), was very similar and thus their differences were statistically not significant ($P > 0.05$).

The difference between fresh and saline water environments in mean yield response of lines to added N fertilizer was mainly due to lodging (data not shown). Taller genotypes lodged more than semi-dwarf genotypes and this lodging was worse with added N fertilizer. Thus yield loss due to lodging in fresh water was greater in the high N regime than in the zero N fertilizer regime. This caused the lower mean yields in the high N fertilizer regime compared to the zero N fertilizer regime in fresh water. In the saline environment, however, plant height was reduced by salinity and this led to less lodging of tall genotypes. Thus the confounding effect of lodging on the utilization efficiency of RILs of added N fertilizer was reduced under salinity. Hence RILs were better able to express their inherent potential N utilization efficiencies in salt water as opposed to fresh water. Consequently mean yields increased with added N fertilizer in saline water.

The significant G × E interaction especially that of the three-way interaction of line × salinity × nitrogen level (Table 2b) meant that the performance of lines in a fresh water or saline environment depended on the level of nitrogen fertilizer applied. It is clearly evident from the Spearman correlation coefficients in Table 5 and the 1:1 charts for yield in Fig. 1, that the highest yielding lines in one environment did not usually yield so high in other environments. However, the RIL population used here expressed more genotype × salinity interaction for grain yield than genotype × nitrogen fertilizer interaction. Although rank correlations of yields between combinations of environments were all significant, the correlations were weakest in the comparison of yields in the saline environment without N fertilizer (S2N1) against yields in the two fresh water environments (S1N1 and S1N2). At the same salinity level the rank correlation of yields between N levels were relatively high. These correlations were highest in the fresh water environment. Thus when environmental differences are characterized solely by levels of N fertilizer usage, selections in high N fertilizer environments can be expected to perform well under non-fertilized conditions also. Similar findings were reported by Fukai *et al*. (1999) and Sinebo *et al*. (2002) who also found that selection in high fertilizer environments was adequate for yield improvement in both low and high fertilizer environments. However, when the range of target environments encompass extremely different environmental conditions such as fresh water and saline environments, it might be worthwhile to breed cultivars specifically adapted to saline environments with no N fertilizer application. Hence the decision as to whether to use specific or general adaptability in breeding programmes should be determined by the distance between environmental variables and the variability of the variables (Casanova *et al*., 2002). It is thus imperative for breeders to clearly describe target environments and then select genotypes (with either specific or general adaptability) that best suit the various subdivisions of the target

environments (Chapman *et al*., 1997; Ceccarelli, 1997; Fukai *et al*., 1999).

There was more G × E for number of panicles m^{-2} than for yield and the other three yield components with regards to rankings of RILs between different environments. This implied that panicle number is an unstable trait that is very susceptible to environmental variation. Haefele *et al.* (2002), in a study of the effects of fertilizer treatments on yield components of rice, also found that treatment effects were more pronounced on panicle number m^{-2} than on other yield components. The other yield components (spikelet fertility, total grains per panicle and thousand grain weight) were more stable. Thus depending on the strengths of the relationships between these latter three yield components and grain yield, breeders could register more success at breeding for general adaptability through improvements in spikelet fertility, total grains per panicle and grain weight than by breeding for yield directly.

Relationships between yield and yield components

The relationships between yield and yield components varied from one environment to the other. At both levels of N fertilizer application, panicle number appeared to be the most important of the four yield components determining grain yield in fresh water while in the saline environments spikelet fertility accounted for most of the variation in yield (Table 8). The significance of the contribution of the other yield components to grain yield in fresh water depended on the strengths of their correlations with panicle number m^{-2} in fresh water but this was not the case in saline water (Table 7).

The complementary and sometimes conflicting relationships among yield components have been reported before (Peng *et al*., 1999; Zeng and Shannon, 2000b). Under fresh water conditions and high N fertilizer rates several researchers reported finding stronger associations between grain yield of rice and panicle number than with other yield components. In a survey of rice yields from 40 farms in Spain, Casanova *et al.* (2002) found that only panicles per square meter and spikelets per square meter (comprising panicles per square meter and spikelets per panicle) made significant contributions to yield while spikelet fertility, thousand grain weight and number of spikelets per panicle (*per se*) made no significant contributions to grain yield. Similarly, Samonte *et al.* (1998), using path analysis of yield and yield components, reported higher contributions to grain yield of rice from panicles per square meter than from spikelets per panicle, which also made a larger contribution than grain weight. Kato and Ttakeda (1996) also found no genetic association between yield sink capacity per plant and single grain weight but strong associations between yield sink capacity and spikelet number per panicle and panicles per plant.

At both levels of N fertilizer application, introduction of salinity caused a general reduction in spikelet fertility, total grains per panicle and grain weight but increased

the number of panicles per square meter compared to the fresh water environment. Zeng and Shannon (2000a) in a greenhouse experiment found that seedling survival rate, tiller number per plant and spikelet number per panicle were the major causes of yield loss under salinity for rice cultivar M-202. In a later greenhouse study, Zeng *et al.* (2001) found that the negative effect of salinity on tiller number of rice was only significant when plants were salinized for a 20-day duration before panicle initiation while the reduction in spikelets per panicle and seed weight were most pronounced when salt stress was imposed between the 3-leaf and panicle initiation stages or between panicle initiation and booting stages. Asch and Wopereis (2001), however, found that in a field experiment carried out using high fertilizer rates, salt stress strongly reduced spikelet number per panicle, grain weight, and spikelet fertility regardless of development stage. The increase in panicle number of rice grown under saline conditions was also reported earlier by Sylla (1994). Tillering determines maximum panicle number and increased tillering by the rice crop under saline stress is a response by the plant to dilute salt concentration in the shoots. However, when salt stress is accentuated by dry weather such as dry season weather, tillering is greatly reduced by salt stress (Asch *et al*., 1997).

Thus, it might be possible to breed for increased rice yields in the fresh water environments where high N fertilizer rates are used by selecting lines that produce more panicles and more filled grains. Panicle number and spikelet fertility were positively correlated with each other and with yield in the high N fertilizer regime in fresh water (Table 7). These two traits also exhibited a high amount of variation in this environment thereby increasing the chances of attaining breeding success for increased levels of these traits in such environments. This would mean selecting lines of rice with more fertile tillers or ability to tolerate high planting densities and also produce more filled grains. Peng *et al.* (1999) recommended that in order to increase the yield potentials of the new plant type of rice, grain filling and biomass production of the new lines have to be improved.

In fresh water environments where N fertilizer is usually not applied, high emphasis should be placed on selecting for high grain number and intermediate panicle number. This is due to the fact that even though both panicle number m^{-2} and total grains per panicle made significant positive contributions to yield in the zero N fertilizer regime in fresh water, the correlation between these two yield components was negative. The negative correlation between panicle number and total grains per panicle of rice has been reported before (Yoshida, 1981; Zeng and Shannon, 2000b). However, Kato (1997) reported finding no strong genetic correlation between panicles per plant and number of spikelets per plant in rice. Thus very high panicle numbers may not be necessary for high yielding ability of rice in certain environments that are similar to

zero N fertilizer regime in fresh water included in our trial.

In highly stressful environments where salt stress is accompanied by inadequate supplies of N fertilizer, selection should be aimed at producing heavier and more filled grains. The negative effects of salinity on spikelet fertility are well documented (Zeng and Shannon, 2000b; Asch and Wopereis, 2001). The higher variation in spikelet fertility and grain weight in saline environments relative to fresh water environments alludes to the fact that it is feasible to breed for increased spikelet fertility and grain weight and subsequently, increased grain yield, in this highly stressful environments.

When high N fertilizer rates are applied in saline environments, however, selection should change then and be targeted at raising panicle number, spikelet fertility and grain weight. This should apparently be possible because despite the negative correlation between panicle number m^{-2} and total grains per panicle in the high N fertilizer regime in saline water, we saw in Table 8 that total grains per panicle contributed very little to total yield variation in this environment. In addition, the correlation between total grains per panicle and grain yield was highly insignificant ($P > 0.05$).

Conclusions

Significant G × E interaction can be exploited to select for specific or general adaptation in rice. Rice cultivars can then be bred for different field situations - optimum, near-optimum or difficult environments. Considering the vast hectarages of rice that are exposed to abiotic stresses, production of new cultivars of rice that are able to increase yields in marginal environments could make big impacts on improving the quality of life for many people in the developing countries who are dependent on rice production from these environments.

The RIL population used here has shown that it is possible to breed for high yields and general adaptability but not high yields and yield stability. This was seen in Fig. 1 that across the range of environments compared there were always genotypes that gave high yields in contrasting environments even though these genotypes were few. Genotypes that gave stable yields across environments were not amongst the highest yielding lines in either of the environments contrasted. However, when environmental differences are large and the aim is to select rice cultivars for maximum yields in target environments then selections should be made for specific adaptability.

Better description of cultivation environments would enable breeders to be more successful in producing cultivars of rice that are able to increase yields under potential conditions as well as under more marginal conditions. The extent of competition among yield components in yield formation is strongly dictated by environmental conditions. The negative relationships that exist between some yield components

should not retard breeding progress when the target environment is adequately described. Then, breeders can prioritize selection for the particular yield components that are more likely to lead to cultivars with the potential to raise yield levels in their target environments.

CHAPTER 3

Use of marker-assisted selection for yield and yield components of rice

Use of marker-assisted selection for yield and yield components of rice

Abstract

Use of DNA-based markers in plant breeding can accelerate the cultivar development process in both stressed and non-stressed environments. Often crop genotypes respond differently to different growing conditions and this complicates cultivar selection for different environments. In order to elucidate the genetic basis of genotype-by-environment interaction (G × E) for yield as well as the variable relationships between yield and its components in rice, we determined the associations between 139 AFLP markers and grain yield and its components in fresh water (EC of 0.15 dS m^{-1}) and saline environments (EC of 8 dS m^{-1}) with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, during the years 2000 and 2001. AFLP markers were generated on a population of Recombinant Inbred Lines of rice, developed from the cross of a semi-dwarf, salt-sensitive, modern cultivar, IR29, and a tall, salt-tolerant, traditional variety, Pokkali. Through multiple regression analysis, several markers were found to be significantly associated with yield and yield components in the four different environments – 36 for grain yield, 51 for number of panicles per m^2, 41 for percent spikelet fertility, 29 for total grains per panicle and 33 for thousand grain weight. For all these traits, more than 70% of the significant markers were detected in only one environment thus implying strong environmental specificity of most quantitative trait loci (QTLs) for yield and yield components of rice. Marker-assisted selection (MAS), using markers significantly associated with grain yield, was successful in identifying superior yielding genotypes of rice in fresh and saline water environments with or without the addition of 100 kg ha^{-1} N fertilizer, in both 2000 and 2001. Indirect selection for yield using MAS performed with markers for yield components also successfully identified superior genotypes in all environments in year 2000 but only in zero N fertilizer regimes in fresh and saline water during year 2001. The accuracy of these predictions based on yield component markers was less than those performed using markers for yield. More than 86% of markers associated with grain yield were also detected for one or more yield component although not necessarily in the same environment. Several markers were also significantly associated with two or more yield components. Significant associations of markers with more than one trait might be due to pleiotropy or close linkage of some QTLs for yield and yield components. Some of the pleiotropic or closely linked QTLs had opposite effects on the traits for which they were identified and this might explain the often-reported negative correlations between some yield components under certain environmental conditions. For all five traits studied, both

parents possessed superior alleles although Pokkali possessed the superior alleles at most of the significant marker loci detected in this study.

Introduction

As with the majority of economic traits in crop plants, crop yield is quantitative in nature and under the control of many genes or gene complexes that are described as quantitative trait loci. Improving such traits through conventional breeding is slow and difficult due to pleiotropy and epistatic interaction of the genes and the large genotype-by-environment interaction associated with these quantitative traits. The use of molecular techniques that can accurately predict crop performance in diverse environments holds much promise for overcoming these problems encountered when breeding for quantitative traits.

Nowadays, various DNA-based markers are available such as RFLPs (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism), that can be used as genetic markers. The association of these markers with agronomic traits of interest is studied to identify QTLs (quantitative trait loci) – regions of the genome where genes controlling quantitative traits are located. Once such an association is established indirect selection can be practised targeted at the presence or absence of markers of interest in breeding lines. This method of selection, also known as marker-assisted selection, can greatly speed up breeding programmes and eventually reduce the costs of breeding a new crop variety. Using this approach large numbers of plants can be screened at the same time and it may not be necessary to grow crops till maturity before selection is practised. Another potential use of this technique is in map-based cloning where the identified QTLs are fine-mapped, cloned and then inserted through transformation techniques into the genome of a crop variety or breeding line that lacks this gene.

Application of molecular techniques to rice improvement will help to achieve better yields (Goff, 1999). Apart from its economic and nutritional benefits, rice has become a model system for cereal genomics (Delseny *et al*., 2001). Rice is a very suitable model system for molecular genetic studies in monocots. It is a diploid with twelve gametic chromosomes, has one of the smallest genomes of any monocot known, has a large collection of cultivated varieties and wild species, can be regenerated from protoplasts (McCouch and Doerge, 1995) and has a high transformation efficiency and ease of transformation compared to other cereal species (Izawa and Shimamoto, 1996). Well-saturated molecular linkage maps of rice are available that provide researchers with 2000 mapped molecular markers, or approximately one marker every 0.9 cM (McCouch and Doerge, 1995). The completion of a draft sequence of the rice genome,

which was achieved through private and public research, is expected to trigger research that will lead to further improvements in this important crop (IRGSP, 2003).

Rice has also been important in comparative mapping in other Gramineae species, such as wheat, barley, rye, oats and maize. The identification of corresponding chromosome segments in modern crop varieties suggests that gene mapping in one species could provide clues as to the possible location of orthologous genes in related species. Izawa and Shimamoto (1996) reported that the order of DNA markers is well conserved over species and that QTLs determining certain agronomic traits correspond closely in rice, sorghum and maize. Thus, locating genes of agronomic importance in rice can be expected to have a positive impact on several of the most important cereal crops in the world (Havukkala, 1999).

There are numerous reports in literature on QTLs identified in several crops controlling various traits (Koornneef and Stam, 2001). For example, for drought and blast resistance in rice (McCouch and Doerge, 1995); for Na/K ratio in tissues of rice subjected to salinity stress (Gregorio, 1997); salt-tolerance in tomato (Foolad and Jones, 1993); salt tolerance at germination and the seedling stage in barley (Mano and Takeda, 1997); drought avoidance/tolerance in rice (Champoux *et al.,* 1995); phosphorus deficiency tolerance in rice (Wissuwa *et al.*, 1998); and the ability to stimulate nitrogen fixation in the rhizosphere of rice (Wu *et al*., 1994 and 1995). Some success has already been reported in breeding methods employing these new techniques. For example, Stuber (1995) reported the production of 'enhanced' hybrids of maize some of which out-yielded check hybrids by more than 15% through the use of DNA-based markers. Cho *et al.* (1994) have also used molecular markers to select for the semi-dwarf characteristic in rice. In tomato, Frary *et al.* (2000) used molecular marker technology to identify QTL alleles that increased fruit size and successfully introgressed one QTL into large fruited cultivars. However, reports on such successful applications of marker-assisted selection in increasing rice yields or in selecting for abiotic stress tolerance in rice are lacking in literature.

Due to the complexity of grain yield and the many problems encountered in its study, Slafer (2003) suggested that the identification and localization of genes controlling key traits related to yield may help narrow the gap between genotype and phenotype of crops. Moreover there may be less genotype × environment interaction for yield components compared to yield. Component analysis may also help to identify QTLs with more precision and confidence. Such an approach aiming at studying the inheritance of traits underlying yield have been undertaken by researchers for different crops. Results from many of these studies found clustering of QTLs for yield and several yield components at the same chromosome regions in rice (Zhuang *et al.*, 1997; Li *et al.*, 2000), barley (Bezant *et al.*, 1997) and maize (Ribaut *et al.*, 1997; Jiang *et al.*, 1999). The possible

causes of these could be pleiotropy or tight linkage of genes controlling the different traits (Hittalmani *et al*., 2002). However, some yield QTLs did not coincide with a QTL for any yield component indicating that possibly other yield components not taken into consideration in the studies might also be contributing to yield (Yin *et al*., 2002). Often more QTLs are detected for yield components than for yield (Teulat *et al*., 2001; Yin *et al*., 2002). Thus, other regions of the genome affecting yield, may not be detected directly for yield possibly because their contributions are too small to be detected at the selected threshold level. Hence studying yield components can help breeders improve yields of cultivars by including QTLs for yield components in their breeding programmes that would otherwise be left out.

The objectives of carrying out this research were to (i) identify molecular markers that can significantly account for variation in yield and yield components of rice under diverse environmental conditions, (ii) find out to what extent markers for yield coincide with markers for yield components in different environments, and (iii) assess the accuracy of predictions when yield is predicted based on molecular marker information for yield *per se* and also for yield components (marker-assisted selection).

Materials and methods

Plant material

A segregating population of rice (*Oryza sativa* L.) comprising 276 recombinant inbred lines (RILs) was used. The RIL population was developed at the International Rice Research Institute (IRRI) in Philippines, from the cross IR29 × Pokkali (both *indica* varieties) by single seed descent. IR29 is a short, high yielding modern cultivar released by IRRI and is known to be very sensitive to salinity (Gregorio, 1997). Pokkali is a tall, traditional variety from India known to be tolerant to salinity (Yeo and Flowers, 1986; Garcia *et al.*, 1995; Gregorio, 1997). As one of the two parents is a short, high-yielding, modern cultivar and the other is a tall traditional variety, it is expected that the RIL population will also be segregating for response to nitrogen fertilizer. This is due to the fact that modern rice cultivars have been bred for response to high levels of mineral fertilizer application while traditional varieties have usually been selected by farmers in environments with sub-optimal levels of nutrient supply.

The RIL population, together with the parents, was grown during the rainy season (June-Oct.) in 1999 and 2000 in a series of genetic experiments at Sapu (13.55° N latitude), in The Gambia (see Chapter 2). One hundred and sixty RILs were selected from the population of 276 RILs for use in our study. From these 160 RILs 98 were used in the trials of 1999 and 2000 (see Chapter 2). Yield and yield component data were collected from these 98 RILs and their parents. After exhaustive analyses of the two-year

yield data, 38 RILs were selected from the 160 RILs for high-, medium- and low-yielding ability in the different test environments based on the molecular marker signature of the different RILs. Of these 38 RILs, 22 were present in the 98 RILs grown in 1999 and 2000 while the remaining 16 RILs had not been grown before in our trials. In 2001, these selected 38 lines together with the two parents and two improved cultivars were then grown at the same experimental site using the same split-split plot design.

Field experiments

A split-split plot experimental design was used with salinity as the main plot factor, rate of nitrogen fertilizer application as the sub-plot factor and genotype as the sub-subplot factor. Each sub-subplot plot measured 2.6 m × 3.0 m and a spacing of 20 cm × 20 cm within and between rows was used. Two levels of salinity and two levels of nitrogen fertilizer application (giving a total of four treatment combinations) were tested. Three replications were maintained in each year of the trials. Additional information on the trials is given in Table 1. The following treatment combinations also referred to later in the text as test environments, were obtained:

S1N1 – Fresh water (river water) at an electrical conductivity (EC) of 0.15 dS m^{-1} and 0 kg N ha^{-1};

S1N2 – Fresh water (river water) at an EC of 0.15 dS m^{-1} and 100 kg N ha^{-1} as urea;

S2N1 – Salt water at an EC of 8 dS m^{-1} and 0 kg N ha^{-1};

S2N2 – Salt water at an EC of 8 dS m^{-1} with 100 kg N ha^{-1} as urea.

Table 1. Details of environmental conditions and field experiments conducted in 2000 and 2001 (Sapu Research Station, The Gambia).

	2000	2001
Sowing date	8 June	9 July
Transplanting date	4 July	31 July
Salinity (dS m^{-1})	~8	~8
Total rainfall (mm)	1326	754
Average minimum temp. (°C)	22.4	22.5
Average maximum temp. (°C)	34.0	34.0
Basal fertilizers (kg ha^{-1})		
P (Triple Super Phosphate)	40	40
K (Muriate of Potash)	40	40

Pre-germinated rice seeds were sown in a nursery that was well watered and regularly hand-weeded. Around 22-27 DAS (days after sowing) the seedlings were transplanted to the field following the experimental design described above. After transplanting the trial plots were kept continuously flooded by irrigating with river water till all RILs and cultivars were close to physiological maturity. Salinity was imposed by manually broadcasting measured amounts of granular table salt in standing water to attain the required salinity.

Data collection

Salinity of the ponded-water was measured two days after every significant rainfall or after a protracted period without rains (more than four consecutive days). An ES-421 salt meter (Atago Co. Ltd., Japan) was used to measure salinity levels. When the salinity level was too low more salt was added to raise the salinity and when the salinity was too high the saline plots were irrigated with fresh water to reduce the salinity to the desired level.

Data were also collected on grain yield and yield components. These were recorded following the Standard Evaluation System of IRRI (IRRI, 1996). Four yield components were assessed – number of panicles per square meter, total grains per panicle, spikelet fertility and thousand grain weight. A peg was randomly placed between four plant hills towards the middle of each plot. At maturity these four hills were harvested separate from the rest of the plot. Yield component data were determined from these four hill samples from each plot.

Number of panicles per m^2 (panicles/m^2) – All panicles in four hill samples were counted for each plot. As the area covered by these four hills was 0.16 m^2 (0.4 m × 0.4 m) the number counted was multiplied by 6.25 (1.0 m^2 / 0.16 m^2) to convert it to number of panicles/m^2 for that plot.

Total grains per panicle (grains/panicle) – Four hill samples were threshed separately for each plot and then winnowed to separate filled grains from empty grains. These two classes were counted separately, added together and the sum was divided by total number of panicles in the four hill samples to give total number of grains per panicle.

Thousand grain weight (g) – All filled grains counted from the four hill samples were weighed and thousand-kernel weight was calculated using the following formula:

Thousand grain weight = (Weight of filled grains from four hill samples (g)/total number of filled grains from four hill samples) × 1000

Spikelet fertility (%) – This was determined by dividing total number of filled grains in the four-hill samples by TGP and then multiplying by 100.

Grain yield (kg ha^{-1}) – With the exception of plants in the outermost rows and the four-hill samples, all plants in each plot were harvested upon reaching maturity. The harvested plants were then threshed, dried, cleaned and weighed. The weight of the grain harvested from the four-hill samples used for yield component analysis was added to that of the grain harvested from the rest of the plot to give the total grain yield from a plot. The yield (g) from this net plot was then converted to yield in kg ha^{-1} by multiplying with 1.7482.

Statistical analysis

The SAS statistical package was used to perform ANOVA on yield and yield components. The Mixed Procedure (Proc MIXED) of SAS (1999) was used to perform the ANOVA analyses and generate least squares means (LSMeans) of the line × salinity level × nitrogen combination for the above traits. One RIL was dropped from the data set due to ambiguous labelling of the RIL. Subsequent QTL analysis was performed using LSMeans for 97 RILs.

Molecular marker analysis

A molecular marker map was developed at IRRI with the same RIL population to map QTLs for salt tolerance. The map contained 205 AFLP markers, 3 STS (sequence tagged sites) markers and one phenotypic marker (for salt tolerance) distributed over all the 12 chromosomes of rice and was generated using 32 primer combinations (Gregorio, 1997). The AFLP map was prepared using DNA collected from 80 RILs selected through selective genotyping for salinity tolerance (38 very tolerant and 42 very sensitive).

For our research, we selected 98 RILs at random from the 276 RILs. These 98 RILs consisted of 25 fingerprinted (Gregorio, 1997) and 73 non-fingerprinted RILs. These 98 RILs were grown in the field experiments of 1999 and 2000. Extra 7 RILs were selected from the non-selected 178 RILs. We then requested IRRI to generate AFLP markers on these new 7 and the initial 73 non-fingerprinted RILs (80 in total) using 25 primer combinations that we determined from the earlier map to give good coverage of the genome. One hundred and thirty-nine (139) AFLP markers were generated from these 80 RILs (different from the 80 already genotyped by Gregorio). The other 70 markers did not show any polymorphisms in our set of RILs. This new AFLP data set was combined with the earlier data set that contained 209 molecular markers. Thus the combined marker data set contained 209 markers of which 80 RILs had scores for all markers (Gregorio, 1997) and the other 80 RILs (selected by us) had scores for only 139 markers.

We analysed this new marker data set for the combined 160 RILs and tried to produce

a linkage map using JoinMap (Stam and van Ooijen, 1995). Most of the 209 markers showed highly skewed segregation ratios and estimated recombination frequencies between certain markers were unrealistically high. Only 78 markers could be assigned to 11 of the 12 linkage groups of rice. Linkage group 4 could not be constructed because out of the 5 markers located on this linkage group by Gregorio (1997), in our tentative map, only one marker had a segregation ratio within the range (40:60) expected of RILs. We eventually decided not to construct a linkage map in view of these severe deficiencies in the tentative map we had constructed.

We then determined the scores of the 209 markers and 70 markers that were found to have missing scores in more than half of the 160 RILs were discarded. These 70 markers included the 3 STS markers and one phenotypic marker. The missing scores for the remaining 139 markers were estimated using information about correlations of the affected markers with other markers. A new data file was generated in which all 160 RILs had scores for the 139 markers.

QTL detection

We used Genstat 6.0 to perform stepwise regression of individual markers on yield and yield components of 97 RILs from year 2000 trials to identify markers that were significantly associated with grain yield and its components in the various test environments. A significant association was declared at a variance ratio of 4 or above ($P < 0.05$).

Marker-assisted selection (MAS)

Additive effects of all significant markers for a particular trait in each environment were summed up to give the predicted trait values of RILs in any particular year. Grain yield was predicted through two different approaches. In the first method, yield was predicted from estimates of additive effects of markers for yield *per se*. This was labelled Yield1. In the second approach, yield was predicted from estimates of additive effects of markers for yield components using the equation:

$$\text{Yield (kg ha}^{-1}) = \text{NPM} \times 10{,}000 \text{ (no. ha}^{-1}) \times \text{TGP (grains/panicle)} \times \text{WTG (kg)} / (1{,}000 \times 1{,}000) \times \text{SFERT (\%)} / 100$$

where, NPM is panicles per m^2; TGP is total grains per panicle; WTG the thousand grain weight and SFERT the spikelet fertility. Yield predicted through this second approach was called Yield2.

Then to determine the accuracy of MAS, scatter plots of predicted against observed traits were made for each trait in each environment in 2000 and the coefficient of

determination (R^2) was calculated for each chart separately. Similar scatter plots were drawn for grain yield in 2001. As breeders are most interested in how well rank orders of genotypes are predicted with regards to agronomic traits, Spearman rank correlations were calculated to determine genotypic rank changes between predicted and observed values for each trait in each environment in 2000 and 2001.

For grain yield, we decided to test how well mean yields will be predicted at different selection pressures. This was done by calculating the realized co-heritability between observed and predicted yields. We computed this co-heritability for Yield1 only. Co-heritability describes the correlated response of two or more traits to selection. That is, response to selection in one trait resulting from selection on a correlated trait. In analogy to the response to selection on a single trait, the equation for the correlated response reads:

$$\frac{R_Y}{\sqrt{\sigma_Y^2}} = \text{coh}^2_{\text{x,y}} \frac{S_X}{\sqrt{s_X^2}}$$

This above formula can be rewritten as:

$$\frac{y_S - y}{\sqrt{\sigma_Y^2}} = \text{coh}^2_{\text{x,y}} \frac{x_S - x}{\sqrt{\sigma_X^2}}$$

Thus realized co-heritability was computed as:

$$\text{coh}^2_{\text{x,y}} = \frac{Y_S - Y}{X_S - X} \times \sqrt{\frac{s_X^2}{s_Y^2}}$$

where,
R_y = response in trait *y* to selection on trait *x*,
S_x = selection differential,
σ_y^2 = variance in *y*,
σ_x^2 = variance in *x*,
Y_s = mean of observed yields (of selected RILs),
Y = grand mean of observed yields,
X_s = mean of predicted yields (of selected RILs),
X = grand mean of predicted yields,
s_y^2 = estimated variance of observed yield,
s_x^2 = estimated variance of predicted yield.

Assuming observed and predicted yields are perfectly co-heritable traits then the co-heritability between the traits would be 1.0. This would mean that yield could be

predicted perfectly from estimates of marker effects. Realized co-heritability was computed at different selection pressures (highest yielding 5%-20% of RILs) in each environment for years 2000 and 2001.

Note that in computing realized co-heritability, RILs are selected for their marker-based predicted yields whereas the response is for observed yield. So this realized co-heritability is a measure for the efficiency of marker-based selection.

Results

Genotype × Environment interaction for Yield in 2000 and 2001

ANOVA analysis revealed significant genotype × salinity × nitrogen fertilizer interaction for grain yield in both 2000 and 2001. Yield data for the 22 RILs grown both in 2000 and 2001 were then pooled and analysed to determine the year effect on the three-way interaction of genotype × salinity × nitrogen fertilizer. This year effect was found not to be significant. Fig. 1 shows that the response patterns of the genotypes to different combinations of salinity and nitrogen fertilizer were similar. However, the effect of salt stress on yield was more acute in 2001 than in 2000. This resulted in a stronger shift of the points away from the 1:1 lines towards the fresh water axes in 2001 (Figs. 1b (i), 1b (ii)) than in 2000 (Figs. 1a (i), 1a (ii)). Another noticeable difference between the comparative yields of the RILs in contrasting environments is that in the plots of yields in fresh water environments against yields in saline environments, R values were higher in 2000 than in 2001 while in the plots of yields in zero N fertilizer regimes against yields in high N fertilizer regimes, R values were lower in 2000 than in 2001.

Segregation of AFLP markers

One hundred and thirty-nine AFLP markers were generated for our study. Sixty-one of these 139 markers had segregation ratios beyond the maximum of 40:60 ratio expected in a RIL population segregating beyond the eighth filial generation. The segregation was skewed in favour of Pokkali at 33 of these marker loci (> 60% of offspring) and in favour of IR29 at another 28 marker loci (> 60% of offspring). The segregation ratios for the other 78 markers were within the expected 40:60 ratio.

Molecular markers significantly associated with agronomic traits

Through the use of multiple regression of molecular marker scores on agronomic traits, 108 markers were identified that had significant associations with grain yield and/or its four components – number of panicles m^{-2}, total grains per panicle, spikelet fertility and grain weight, in four test environments. Thirty-seven of these markers

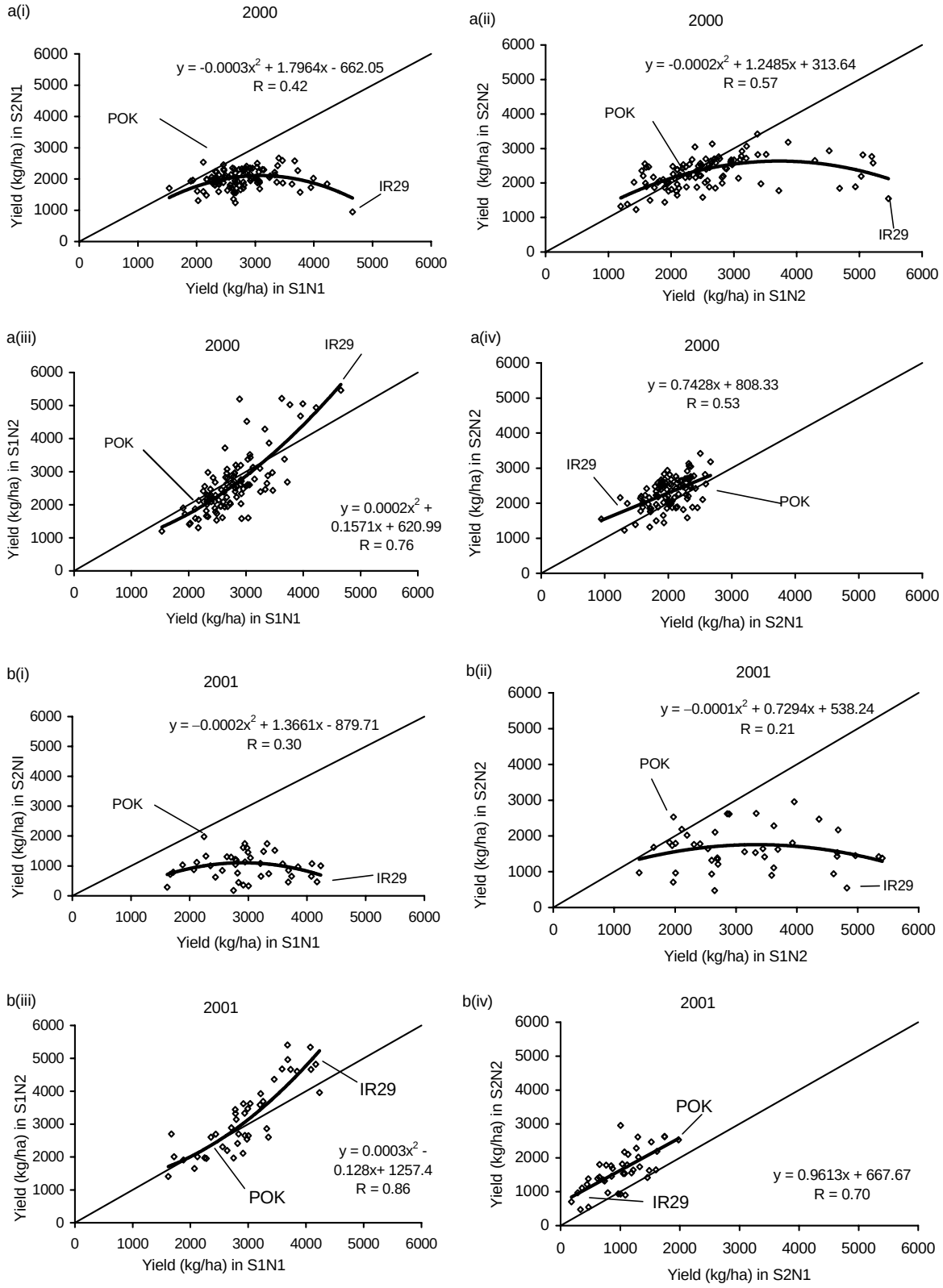

Fig. 1. Scatter plots showing inter-environmental correlations for yields of RILs and their parents, IR29 and Pokkali (POK) in years 2000 and 2001. S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively.

were significant for only one trait in one environment and the other 71 were either significantly expressed in at least one environment for more than one trait or were significantly expressed in two or more environments for the same or different traits. Table 2 shows the number of markers that had significant associations with each of the five studied traits in more than one environment.

The IR29 alleles at some of the identified markers had similar effects on all the traits for which they were significant while at other marker loci, the IR29 alleles had increasing effects on some traits and reducing effects on others. For instance, out of nine markers that were detected for both number of panicles m^{-2} and total grains per panicle, in this study, only two had similar effects on the two traits whilst the other seven had opposite effects on the two traits (See Addendum 1). Around half (five out of eleven) of the markers significantly associated with spikelet fertility and grain weight in this RIL population had similar effects on both traits. In addition, the IR29 alleles at markers that were significantly expressed for the same trait in more than one

Table 2. Number of markers detected in more than one environment for yield and yield components. Figures in parentheses refer to the proportion (%) of markers associated with a trait that are consistently expressed in more than one environment.

Environments	Trait				
	Yield	NPM	SFERT	TGP	WTG
S1N1 / S2N1	1	1	2	0	1
S1N1 / S1N2	3	2	0	2	0
S1N1 / S2N2	1	2	1	1	2
S2N1 / S1N2	0	1	3	1	0
S2N1 / S2N2	1	2	2	3	1
S1N2 / S2N2	1	2	2	0	0
S1N1 / S2N1 / S1N2	1	0	0	0	0
S1N1 / S2N1 / S2N2	0	1	0	0	0
S1N1 / S1N2 / S2N2	0	0	1	0	0
S2N1 / S1N2 / S2N2	0	0	0	0	0
S1N1 / S2N1 / S1N2 / S2N2	0	0	0	0	0
Subtotal (% total markers)	8 (22)	11 (22)	11 (27)	7 (24)	4 (12)
Total no. of markers	36	51	41	29	33

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

environment usually had consistent effects on the particular trait in these environments with the exception of two markers – P3/M7-6 and P1/M2-4 (CH-3). The IR29 allele at P3/M7-6 had a negative effect on panicles m^{-2} in the zero N fertilizer regime in fresh water and a positive effect in the high N fertilizer regime in saline water while the IR29 allele at P1/M2-4 (CH-3) had a negative effect on spikelet fertility in the high N fertilizer regime in fresh water and a positive effect on the same trait in the zero N fertilizer regime in saline water.

Thirteen of the 36 markers found to be significantly associated with yield in the four environments were also significantly expressed for a yield component in the same environment. The IR29 allele of eleven of these markers had similar effects on yield and the particular yield component concerned while the IR29 alleles at two marker loci, P3/M5-2 in the zero N fertilizer regime in fresh water and P4/M3-4 in the high N fertilizer regime in saline water, reduced yield and increased total grains per panicle at the same time in these two environments. Another 18 markers for yield were also detected for one or more yield component(s) in a separate environment. The other 5 markers identified for yield did not coincide with any yield component in any of the environments.

In all four environments the tall, salt-tolerant parent, Pokkali, supplied the superior alleles for most of the markers found to be significantly associated with grain yield (Table 3). Furthermore, Pokkali contributed the superior alleles at most of the markers with relatively large effects on grain yield such as P2/M10-1, P3/M9-3 and P2/M3-2(CH-2). The proportion of significant marker loci at which the IR29 allele was

Table 3. Totality of markers linked to putative QTLs for yield and yield components under different environmental conditions. Figures in parentheses refer to proportion of markers (%) at which IR29 possessed the superior allele for a trait in a particular environment.

Environment	Trait				
	Yield	NPM	SFERT	TGP	WTG
S1N1	13 (23)	14 (21)	8 (14)	12 (67)	9 (33)
S2N1	11 (27)	16 (38)	14 (43)	8 (88)	8 (75)
S1N2	12 (42)	19 (32)	16 (23)	5 (60)	16 (44)
S2N2	9 (33)	15 (53)	16 (69)	12 (83)	5 (60)

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

superior to the Pokkali allele was higher in the high N fertilizer regime than in the zero N fertilizer regime in fresh and saline water for grain yield. Between the fresh and saline water environments, the proportion of marker loci with superior IR29 alleles was higher in the saline environment than in the fresh water environment with zero N fertilizer application but this proportion was higher in fresh water than in saline water with high N fertilizer application.

For all four yield components, the proportion of marker loci at which IR29 possessed the superior alleles was higher in the saline environment than in the fresh water environment at both levels of N fertilizer application (Table 3). Regarding panicles m^{-2} and spikelet fertility, Pokkali possessed the superior alleles at most of the markers detected in all test environments except the high N fertilizer regime in saline water where IR29 possessed the superior alleles for most of the markers significantly associated with these two traits. The proportion of marker loci with superior IR29 alleles, for these two traits, was higher in the high N fertilizer regime than in the zero N fertilizer regime in both fresh and saline water.

Pokkali possessed few superior alleles at marker loci significantly associated with total grains per panicle. The high yielding, semi-dwarf cultivar, IR29, supplied most of the superior alleles for this trait. For total grains per panicle, IR29 supplied proportionately fewer superior alleles in the high N fertilizer regime than in the zero N fertilizer regime in both fresh water and saline environments. With regards to grain weight, Pokkali supplied the superior alleles for most of the markers detected in saline environments while in fresh water environments IR29 supplied most of the superior alleles. The proportion of marker loci with superior IR29 alleles, for grain weight, was lower in fresh water than in saline water in both the zero N and high N fertilizer regimes.

QTL × Environment interaction

QTL x environment interaction (QTL × E) could be envisaged in different forms, all of which were detected in this study (Addendum 1). When there is significant QTL × E we would expect that for the same trait evaluated in different environments, markers identified would be significant in only a subset of the environments. For example, more than 70% of the markers significantly associated with yield in our trials were expressed in only one environment. In the second form of QTL × E the size of the effect of significant markers would vary from environment to environment. A clear example of this is marker P2/M10-1, the IR29 allele of which had an effect of −315 kg ha^{-1} on yield in the zero N fertilizer regime and a much larger effect (−1042 kg ha^{-1}) in the high N fertilizer regime in fresh water. In the most extreme form of QTL × E the sign of the effect of an allele, at a marker locus, on the same trait would change

Table 4. Percentage variance accounted for by the totality of markers detected for yield and yield components in four environments.

Environment	Trait				
	Yield	NPM	SFERT	TGP	WTG
S1N1	48.1	50.0	46.0	38.6	26.7
S2N1	40.0	58.9	59.5	32.7	35.5
S1N2	48.2	59.6	58.4	22.8	50.4
S2N2	36.9	50.6	80.3	39.5	30.1

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

between environments. No such marker was found for yield but as explained above, two markers where the IR29 alleles had such effects were detected for panicles per m^{-2} and spikelet fertility (P3/M7-6 and P1/M2-4 (CH-3), respectively).

Despite this apparently high degree of QTL × E interaction in this RIL population for the traits studied, some markers were also detected in more than one environment for each of the five traits. For grain yield, more markers showed consistency across fresh water environments than did across saline environments (Table 2). Markers with consistent effects across fresh water environments were also detected for panicles per m^{-2} and total grains per panicle. Only one such marker was detected for spikelet fertility and none for grain weight (Table 2). More markers with consistent effects across saline environments or between a fresh water environment and saline environment were detected for yield components than for yield.

Variation in traits accounted for by markers

The proportion of variation accounted for by markers differed between traits and environments (Table 4). With regards to yield, the markers identified in fresh water (with zero or 100 kg ha^{-1} N fertilizer) accounted for more of the variation in yield than those identified in saline water (with zero or 100 kg ha^{-1} N fertilizer). At each level of N fertilizer application, the amount of variation in grain yield accounted for by markers was comparable between the fresh and salt water environments.

In fresh water, markers associated with panicles per m^{-2}, spikelet fertility and grain weight, accounted for more variation in the high N fertilizer regime than in the zero N fertilizer regime. However, in saline water, markers associated with panicles per m^2 and grain weight accounted for less variation in the high N fertilizer regime than they

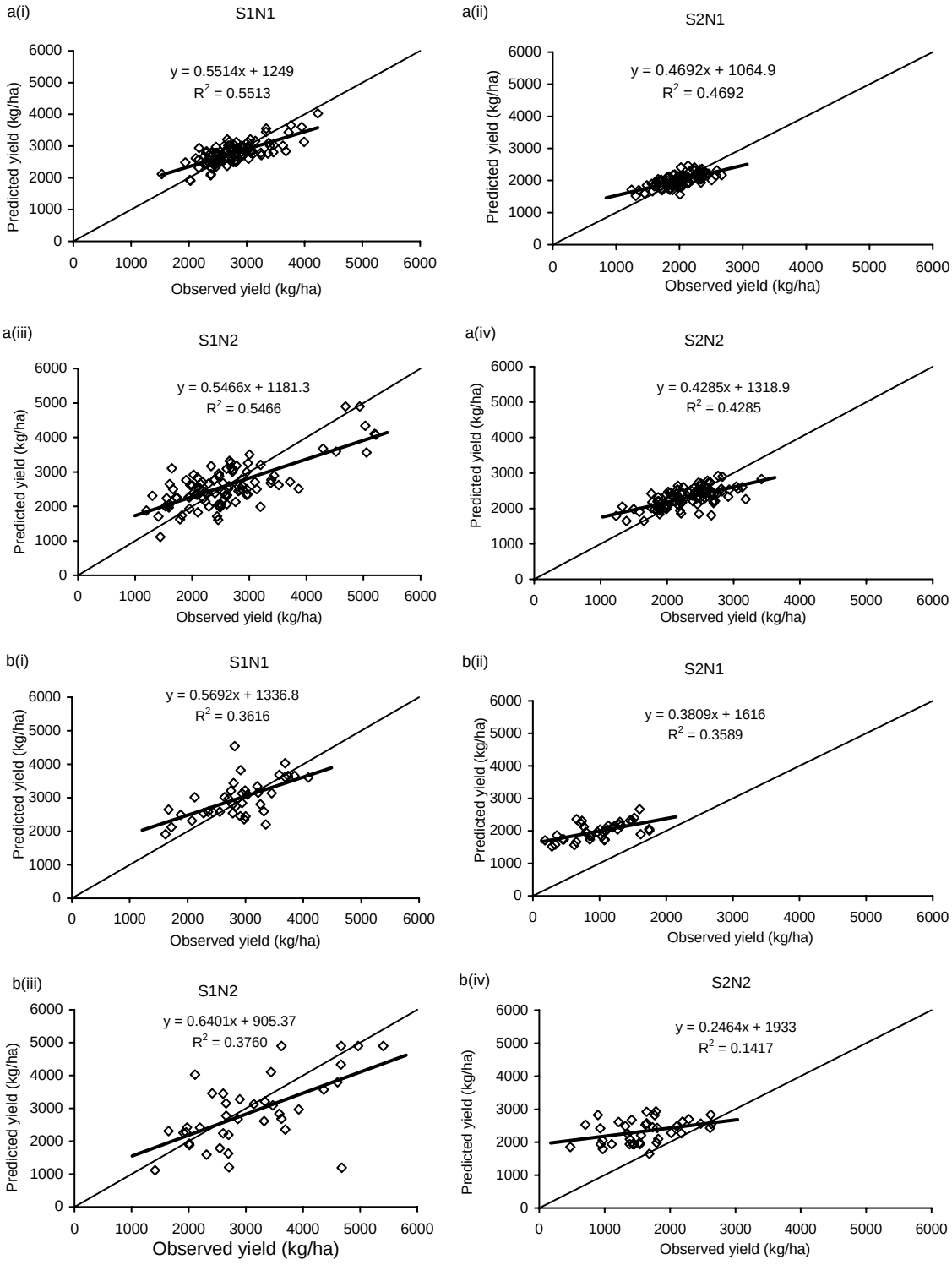

Fig. 2. Scatter plots of predicted against observed yield in year 2000 (a(i)-a(iv) and 2001 (b(i)-b(iv). Yields predicted based on sums of estimates of additive effects of markers for grain yield *per se* (n = 97 in 2000 and n = 38 in 2001). Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively.

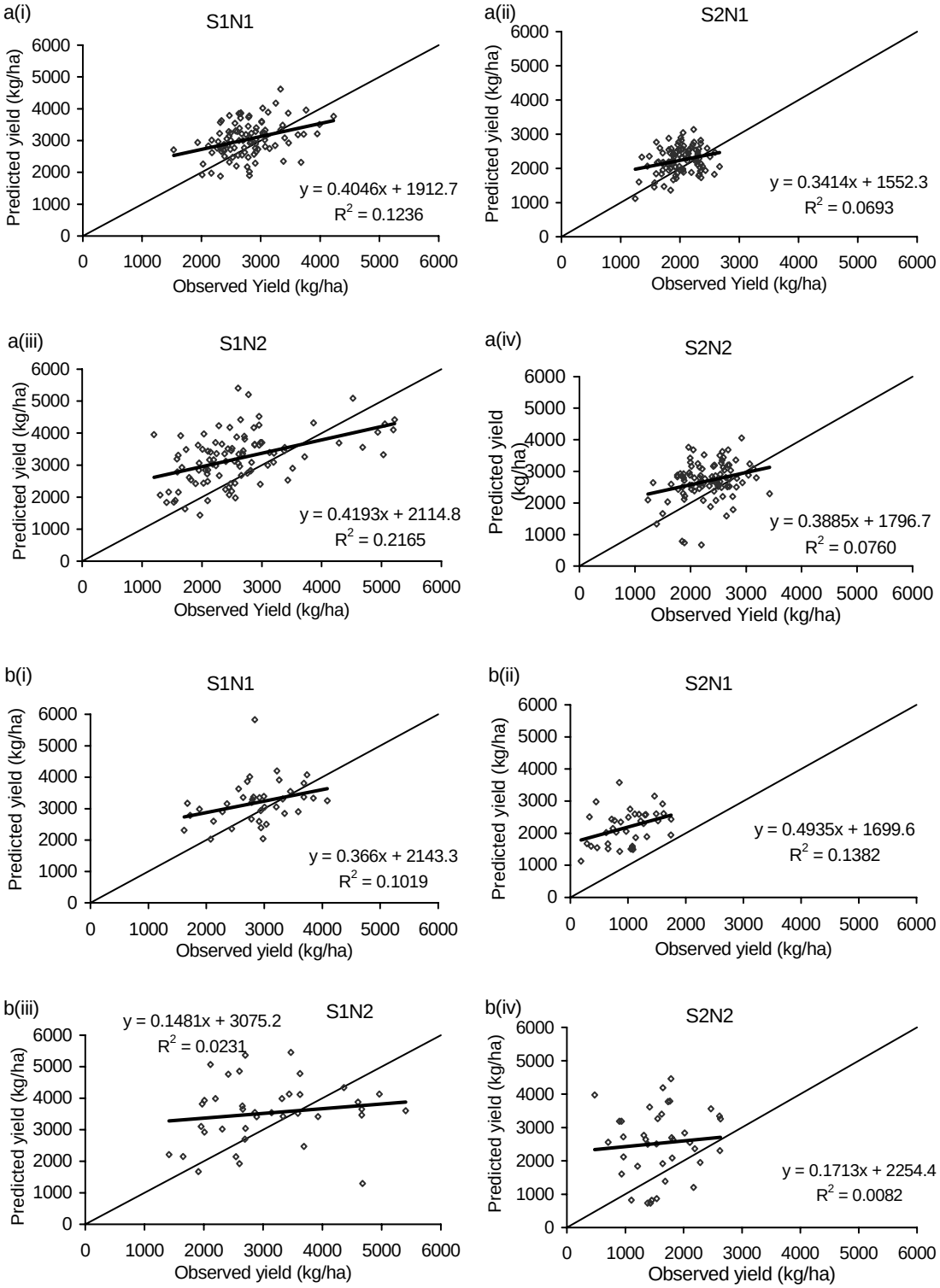

Fig. 3. Scatter plots of predicted against observed yield in year 2000 (a(i)-a(iv) and 2001 (b(i)-b(iv). Yields predicted based on sums of marker estimates for four yield components explained in the text (n = 97 in 2000 and n = 38 in 2001). Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively.

Table 5. Correlation of rankings of genotypes between predicted and observed traits in 2000 as revealed by Spearman rank correlation coefficients. Predicted traits were estimated from additive effects of markers detected for yield and yield components in year 2000 in different environments (n=97).

Environment	Yield1	Yield2	NPM	SFERT	TGP	WTG
S1N1	0.686	0.338**	0.657	0.505	0.681	0.558
S2N1	0.698	0.196 n.s.	0.781	0.674	0.487	0.608
S1N2	0.572	0.466***	0.844	0.755	0.503	0.764
S2N2	0.636	0.222*	0.755	0.599	0.656	0.437

All values except for Yield2, significant at $P < 0.0001$; for Yield2: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, n.s. not significant ($P > 0.05$);

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; Yield1 – predicted yield estimated from markers for yield *per se;* Yield2 – predicted yield estimated from markers for yield components; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

did in the zero N fertilizer regime while markers associated with spikelet fertility still accounted for more variation in the high N fertilizer regime than in the zero N fertilizer regime. Markers expressed for total grains per panicle in fresh water environments accounted for less variation in the high N fertilizer regime than in the zero N fertilizer regime while in saline water, markers associated with total grains per panicle accounted for more variation in the high N fertilizer regime than they did in the zero N fertilizer regime.

Marker-assisted selection (MAS)

The accuracy of MAS was better in 2000 than in 2001 for all studied traits. This was reflected in the higher R^2 values of the regression lines when predicted yields were plotted against observed yields in 2000 (Figs. 2a, 3a) than in 2001 (Figs. 2b, 3b). In addition, rank correlation coefficients between predicted and observed traits were higher for all traits in 2000 than in 2001 with the sole exception of Yield2 (Tables 5 and 6). Rank orders of RILs by their yields were well predicted for Yield1 in all four environments and the predictions were consistently better for Yield1 than for Yield2 both in 2000 and 2001. The prediction of Yield2 in 2001 was poor, and it was only in zero N fertilizer regime under fresh and saline water conditions, that rank orders were significantly conserved between observed and predicted yields while in the high N fertilizer regimes in fresh and saline water, the rank orders of observed and predicted

Table 6. Correlation of rankings of genotypes between predicted and observed traits in 2001 as revealed by Spearman rank correlation coefficients. Predicted traits were estimated from additive effects of markers detected for yield and yield components in year 2000 in different environments (n=97).

Environment	Yield1	Yield2	NPM	SFERT	TGP	WTG
S1N1	0.569**	0.357*	0.206 n.s.	0.207 n.s.	–0.010 n.s.	0.198 n.s.
S2N1	0.593***	0.366*	0.076 n.s.	0.596***	0.050 n.s.	0.435**
S1N2	0.556**	0.220 n.s.	0.233 n.s.	0.153 n.s.	–0.079 n.s.	0.269 n.s.
S2N2	0.397*	0.109 n.s.	0.302 n.s.	0.430**	–0.386 n.s.	0.342*

* significant ($P < 0.05$), ** significant ($P < 0.01$), *** significant ($P < 0.0001$), n.s. not significant ($P > 0.05$);

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} or 100 kg ha^{-1} N fertilizer, respectively; Yield1 – predicted yield estimated from markers for yield *per se;* Yield2 – predicted yield estimated from markers for yield components; NPM – no. of panicles m^{-2}; SFERT – percent spikelet fertility; TGP – total grains per panicle; WTG – thousand grain weight).

yields were significantly different from each other (Table 6).

With regards to the four yield components, rank orders were significantly conserved between observed and predicted values in 2000 (Table 5). However, the prediction of yield components from estimates of marker effects was poor in 2001. In none of the four test environments were rank orders significantly correlated between predicted and observed values of panicles per m^2 and total grains per panicle in 2001. For spikelet fertility and grain weight, rank correlation coefficients were significant in only the saline environments, (with zero or 100 kg ha^{-1} N fertilizer), but not in the fresh water environments, (with zero or 100 kg ha^{-1} N fertilizer) in 2001. Consequently the quality of prediction of Yield2 was also poor in 2001.

Selection efficiency

The realized co-heritability between predicted and observed yields in 2000 and 2001 for Yield1 at 5%-20% selection pressure, was better in 2000 than in 2001 (Fig. 4). This confirmed our earlier observation that rank correlations between predicted and observed yields were stronger in 2000 than in 2001. Generally, the realized co-heritability was higher in fresh water than in saline water in both years. Furthermore, the realized co-heritability between predicted and observed yields at different selection pressures varied between test environments. These differences in co-heritability at different selection pressures implied that the relationship between predicted and

observed yields was not linear over the whole range of the yield data. However, in both 2000 and 2001, MAS was successful in identifying superior genotypes in all test environments as the realized co-heritability at 20% selection pressure was generally greater than 0.20 in all environments.

Discussion

The RIL population used in our study exhibited stronger genotype × salinity interaction than genotype × nitrogen fertilizer interaction. This was expected because this population was developed from a cross of a salt-sensitive (IR29) and a salt-tolerant parent (Pokkali). However, the level of genotype × nitrogen interaction was strong enough to be significant in both 2000 and 2001.

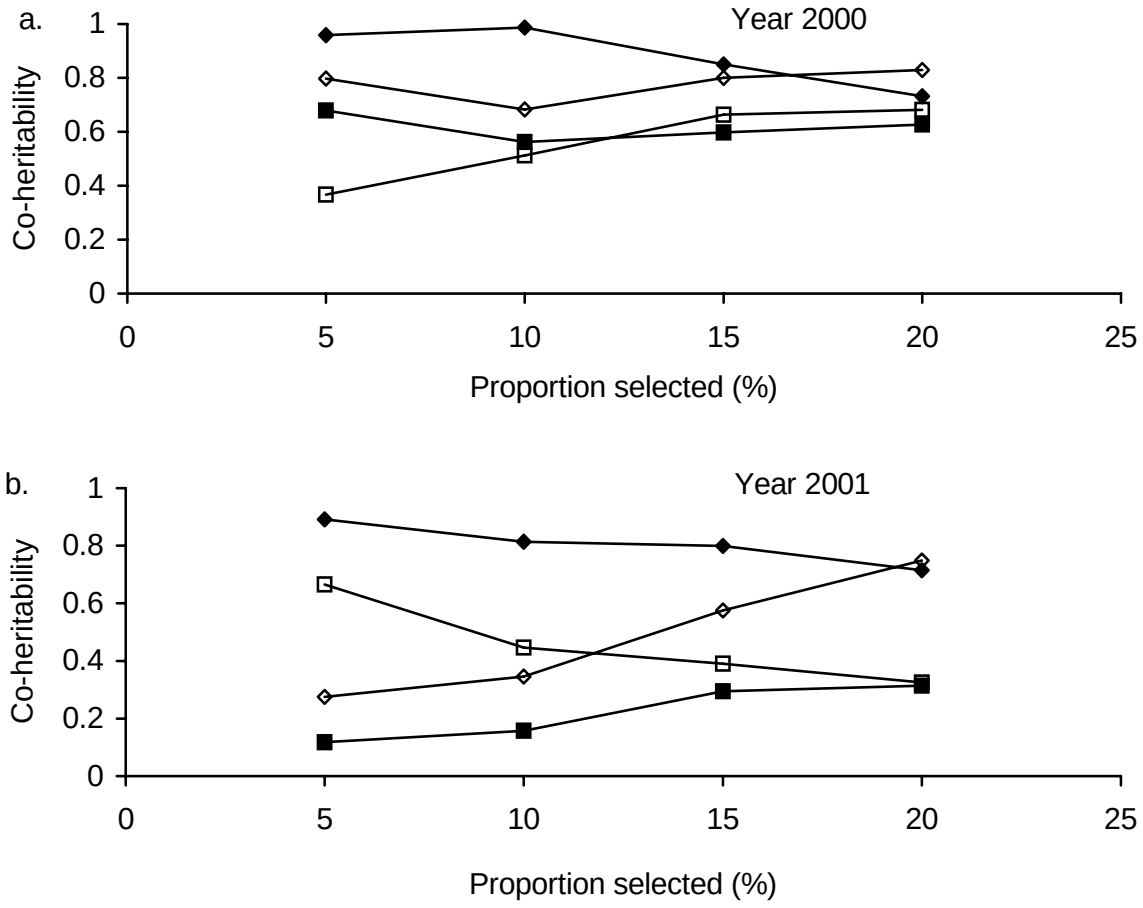

Fig. 4. Efficiency of MAS as reflected in the co-heritability between observed and predicted yields at different selection pressures in years 2000 and 2001 (n = 97 in 2000 and n = 38 in 2001). ◊ Fresh water environment with 0 kg ha^{-1} N fertilizer; ♦ fresh water environment with 100 kg ha^{-1} N fertilizer; □ saline environment with 0 kg ha^{-1} N fertilizer; ■ saline environment with 100 kg ha^{-1} N fertilizer.

Fewer polymorphic markers were generated in our study than in the research of Gregorio (1997) using the same RIL population. Common *indica* rices belong to the same group (group I) on the basis of genetic affinity following the isozyme classification system of Glaszmann (Khush, 1997). The two parents, IR29 and Pokkali, are both *indica* varieties and belong to this group. This close relatedness of many rice cultivars leads to the generation of relatively low numbers of polymorphic markers per primer combination in segregating populations of rice. In the earlier work of Gregorio (1997) 32 primer combinations were used to generate 205 AFLP markers. We used 25 primer combinations and were able to produce only 139 polymorphic markers. In other crops such as barley (Schut, 1998; Qi *et al.*, 1998) and tomato (Haanstra, 2000) relatively fewer primer combinations are sufficient to generate many polymorphic AFLP markers.

Skewed segregation was detected in 44% of the 139 markers generated for our study. This distorted segregation in molecular markers is common in rice (Gregorio, 1997). Distorted segregation in segregating populations of rice is attributed to the possible association between distorted markers and genes responsible for gametophytic incompatibility (He *et al*., 2001) or selection in earlier generations against traits such as shattering (Thompson *et al*., 2003). The RIL population used in this study was developed through single seed descent without selection. With regards to plant height, the population is skewed towards the tall parent, Pokkali (Gregorio, 1997). Thus the distorted segregation we observed in some of the markers could be due to association of the concerned markers with genes for gametophytic incompatibility or to plant height.

For all the traits studied, markers were detected in all environments that had significant associations with them. There was apparently a high degree of environmental specificity with regards to expression of these markers with significant associations to grain yield and yield components. Due to the lack of a molecular linkage map it was difficult to ascertain whether some of the identified markers were actually linked to the same QTLs or not. Stepwise regression has the deficiency that only the 'best' subset regression model is identified while there could be several equally good models (Montgomery and Peck, 1982). As the selection of markers through stepwise regression is performed randomly, when two or more markers are closely linked and all are significantly related to a trait under study, one marker may be selected in one environment and not in another environment because in the other environment another closely linked marker was selected first and it accounted for most of the observed variation in the trait.

Markers were detected for yield and yield components that showed consistent effects in more than one environment for a particular trait. Such markers would be

good candidates for inclusion in breeding programmes aimed at wide adaptation. In addition, most of the markers associated with grain yield in this population of RILs were also expressed for one or more yield components either in the same environment or in a different environment. Yet most markers associated with yield and yield components were detected in only one environment for the particular trait. As noted earlier, due to the lack of a molecular marker map we could not confirm whether the expression of markers in only one environment was the result of QTL × environment interaction or was caused by stepwise regression picking up different markers linked to the same QTL in different environments. Other forms of QTL × environment interaction, especially change in size, and for panicles m^{-2} and total grains per panicle, also a change in sign of QTL effects between environments, were detected in our study.

Differential expression of QTL, also known as QTL × environment interaction (QTL × E) has been reported in other studies, for example, for plant type traits of rice at two different locations (Yan *et al*., 1999), root characteristics of rice in contrasting water-deficit regimes (Price *et al*., 2002), for grain quality traits of maize in different years (Séne *et al*., 2000) and for light response in *Arabidopsis* (Borevitz *et al*., 2002). In these studies, QTLs that were expressed across a broad range of environments have also been reported. Hence in breeding programmes employing the technique of MAS to increase rice yields, the best approach would be to select QTLs for wide adaptability supplemented with QTLs specifically expressed in the range of environments targeted by the breeding programme. Such an approach is already being used to transfer QTLs for root morphological traits between two improved rice cultivars (Price *et al*., 2002).

As most of the markers associated with yield were also detected for one or more yield component(s) in the same or different environments, this implies either pleiotropy or close-linkage of QTLs controlling these traits (Li *et al*., 2000). Thus component analysis is useful in QTL studies since it can indicate possible locations on the genome affecting yield in environments where the QTL effects on yield are too small to be detected at these locations.

Superior alleles appeared to be dispersed over the parents (depending on the environment) indicating that this cross offers the opportunity for improvement in yield and yield components by pyramiding the relevant QTL alleles. This dispersal of superior alleles between the two parents could explain the transgressive segregation observed for all traits in this study (data not shown). For grain yield, the tall traditional cultivar, Pokkali, supplied most of the superior alleles in all environments. This was actually unexpected for it is commonly believed that high yielding modern cultivars would have accumulated more superior alleles for yield through selection, than traditional cultivars. In a cross between *O. sativa* and wild *O. rufipogon*, Moncada *et al.* (2001) also found that 56% of QTLs with positive effects on yield and yield

components were supplied by *O. rufipogon*. Thus QTL studies are very useful in uncovering new alleles that can be introgressed into modern high yielding cultivars raising the possibility of increasing the yields of modern cultivars still further (through better adaptation) in diverse environments. Introgression of new alleles from wild or traditional varieties would help to broaden the genetic base of rice cultivars leading to potential improvements in pest and disease resistance.

The quality of yield predictions in terms of R^2, rank correlation coefficients and co-heritability were better in 2000 than in 2001. This might possibly be due to marginal year effects on yield. The year × genotype × salinity × nitrogen effect was not significant for grain yield. Yet, Table 1 shows that the amount of rainfall received in 2000 was almost twice that of 2001. This would then dilute both the applied salt in saline treatments and tend to flush away applied N fertilizer earlier. Nevertheless marker assisted selection was successful in predicting yields in both 2000 and 2001. For yield components, MAS was only successful in estimating component values in 2000 while in 2001 MAS could only successfully predict spikelet fertility and thousand grain weight in saline environments.

In addition, for grain yield and yield components, the amount of variation accounted for by the significant markers varied between the different test environments. This would then affect the quality of predictions made from estimates of effects of these markers. Total variation in yield accounted for by markers was lower in environments where salt was applied in both N regimes (Table 3). Soil salinity under natural field conditions is quite variable (Russell, 1978; Sylla, 1994). This variability would be further accentuated when the salinity was imposed manually as it was done in our trial. Gomez and Gomez (1984) pointed out that non-uniform application of treatments may result in higher variability in treated plots than in untreated plots. Thus in the N fertilizer regime in saline water where both salt and N fertilizer were applied, the amount of yield variation accounted for by markers was lowest. This trend was not seen in any of the four yield components. Generally, markers for the two components panicles per m^2 and spikelet fertility accounted for more variation in all environments than markers for yield did while markers for total grains per panicle and grain weight accounted for less variation than markers for yield did.

Furthermore, only a few markers were common to yield and yield components in the same environment (See Addendum 1). Thus when yield was predicted from markers for yield components in 2000 and 2001 the predictions were less accurate compared to when yield was predicted from markers for yield *per se* (Figs. 2, 3; Tables 5, 6). Markers for total grains per panicle and grain weight generally explained less variation than markers for yield did. This would then affect the quality of yield predicted from estimates of markers for the four components – panicles m^{-2}, spikelet

fertility, total grains per panicle and grain weight. Yin *et al.* (2002) also found that yield predictions from QTLs for yield *per se* of barley were better than those from QTLs for yield components. Additionally, some of the markers detected for yield in this study had opposite effects on some yield components. Amongst the yield components themselves, some markers that were expressed for more than one component had opposite effects on the components. Thus using markers for yield components alone in a MAS breeding programme would not be a good approach (Ribaut *et al*., 1997). It would be more meaningful to select markers for yield first and then augment these with markers for yield components. The markers for yield components can be included in the breeding programme when they are found to act in same direction on yield as the selected markers for yield.

Conclusions

In this study we saw that MAS can successfully be used to predict yield and yield components of rice in diverse environments although the quality of predictions varied between environments and years in which the trial was conducted. However, using markers for yield *per se,* yield was well predicted under most circumstances.

Most of the markers detected for yield were also associated with one or more yield components although some markers detected for yield components were not significantly associated with yield. Thus component analysis could help unearth new regions of the genome with indirect effects on grain yield. Among yield components, certain markers were expressed for only some yield components and not others. This raises the possibility of breaking the negative associations found to exist between some yield components, which could eventually lead to higher yield potentials in rice.

Both parents possessed superior alleles for all traits. This explained the transgressive segregation observed in yield and yield components and offers the opportunity to improve these traits of rice in different environments through a pyramiding approach. Pokkali contributed many favourable alleles to the cross and could therefore be a source of alleles for IRRI's new plant type.

Addendum 1. Putative QTLs for yield and yield components detected using a RIL population of rice grown in four test environments (n=97). Numbers in the table refer to estimated effects of putative QTLs in units of the trait. (S1N1, S1N2, S2N1, S2N2 refer to the test environments described in the Materials and Methods section. Y: yield (kg ha^{-1}); N: no. of panicles m^{-2}; S: spikelet fertility (%); T: total grains/panicle; W: thousand grain weight (g)).

Marker	S1N1					S2N1					S1N2					S2N2				
	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W
P1/M1-1(CH-12)						-127	-17.30													
P1/M2-7(CH-8)								-2.93				-20.20	-3.48			-246				
P2/M10-1	-315										-1042				-2.16		-21.90			
P2/M10-2-1						81			3.42						-1.16				4.39	
P2/M10-3			-1.43																	
P2/M10-5												-33.60								
P3/M1-1(CH-8)	161					160									-0.87					
P3/M1-2(CH-12)							20.91								-0.94					
P3/M1-3									3.46					5.65						
P3/M1-4													2.01							
P1/M2-10													-2.00							
P3/M1-5											-307					-165				
P3/M1-6															-1.44					
P3/M1-7															2.53					
P3/M4-1	-223																	6.12		
P3/M4-4				-5.23								31.58								
P3/M5-2	-171			6.52		-196					-562								3.13	
P3/M5-3																			-3.19	
P3/M5-4																153		2.96		
P3/M7-1(CH-12)	-208	-20.20								2.01		-30.60	-3.29					-2.17		
P3/M7-2														-4.62			15.87			
P3/M7-6		-13.8															21.73	2.71		
P3/M7-7(CH-6)										1.90		-39.30								
P3/M9-2								2.94												
P3/M9-3	-694																	6.50		
P1/M3-2(CH-3)	-172							3.145							-1.06					
P3/M9-4			-4.12																	
P3/M9-7				7.10				-3.44									28.92			
P3/M9-8															-1.28			5.55		
P4/M3-1					-1.40						340									-1.30
P4/M3-2					-1.26			1.86										3.64		
P4/M3-3													-2.75							
P4/M3-4					-0.88				4.05				-2.16			-160			2.50	
P4/M4-1		-17.1	-2.09																2.45	

Addendum 1. Continued.

Marker	S1N1					S2N1					S1N2					S2N2				
	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W
P1/M3-4		-13.2		7.6								-29.20								
P1/M3-5(CH-1)				6.47																
P1/M3-6(CH-3)	-670			-8.09														2.38		
P1/M3-7																	-16.90			
P1/M3-8								1.18												
P1/M3-9						-202							-3.40							
P1/M1-2		-15.70					-10.40										-35.90			
P1/M4-1											-546									
P1/M4-2(CH-1)				4.56								-28.10		3.98						
P1/M4-6	-208																			
P1/M5-2(CH-11)	-135	-14.70														-300				
P1/M5-4												15.32								
P1M5-6											-420									
P1/M1-3							21.10										18.21			
P1M5-8(CH-5)									2.43						-1.45		-23.2			
P1/M5-10							-16.30													
P1/M5-11																203			3.91	
P1/M6-1				-3.28			15.14													
P1/M6-2(CH-8)	185										709	22.91								
P1/M6-3												49.65	8.44				51.64			
P1/M6-4																	32.32			
P1/M6-6(CH-11)																	-20.10			
P1/M1-4(CH-1)		-21.70				-181								-3.77			-23.60			
P1/M6-8(CH-6)												-17.50								
P1/M6-9										-1.39	333									
P1/M7-1							-11.40					-17.10								
P1/M7-2															1.41					
P1/M7-3						-142	-15.00						-2.50							
P1/M7-6												38.59								
P1/M7-7																				-1.25
P1/M7-8(CH-10)				3.17	1.45			-3.37												
P1/M1-5(CH-11)						-147														
P1/7-9									3.50											
P1/M7-10													-2.20							
P1/M7-13												-41.90	-4.28						2.31	
P1/M9-2													-3.34							
P1/M9-3		-14.80					-26.20													

Addendum 1. Continued.

Marker	S1N1					S2N1					S1N2					S2N2				
	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W	Y	N	S	T	W
P1/M10-1				-5.18							400									
P1/M10-3(CH-2)											366						38.99			
P1/M10-4		12.77																-2.05		
P1/M2-1															1.47					
P2/M1-1															1.14			-4.96		
P2/M1-2			-1.27						-1.50											
P2/M1-6			1.69		-0.90					-1.24		49.18	2.30				16.64	2.67		
P2/M1-7							25.86								0.93					
P2/M2-4							-44.50													3.07
P1/M2-4(CH-3)								14.28					-2.12							
P2/M2-5							25.10			2.99			-9.61					-15.00		
P2/M2-7		14.16																	-3.04	
P2/M3-2(CH-2)		-24.90							3.55		-841							2.06	4.23	
P2/M3-3						-117										-259				
P3/M3-4			-3.90		-1.55													-5.13		
P2/M3-5							-13.00										-29.20		4.48	
P2/M3-6		-60.10				299		5.06		5.90								20.75		5.22
P2/M4-2																			2.2	
P2/M4-4					1.96		-20.30													3.30
P1/M2-5(CH-3)								-17.20												
P2/M4-5															3.34			14.81		
P2/M4-6									4.89											
P2/M5-3								-2.79												
P2/M5-5(CH-8)				6.10								-21.10		3.63	1.43					
P2/M6-1(CH-3)		33.02																		
P2/M6-1-1					-0.57	-124						-33.50								
P2/M6-2(CH-7)										1.08		-12.40								
P2/M6-3		-19.50								2.28									2.55	
P1/M2-6																190				
P2/M6-4	-325										-487									
P2/M7-1			-1.84					-3.00												
P2/M7-2															-1.23					
P2/M8-1							-17.80													
P2/M8-2			-1.55		1.18			-2.97								-167				
P2/M9-1	160						17.21													
P2/M9-2CH-12)				3.59								-23.80								
P2/M9-4(CH-12)								-2.59					-3.43							

CHAPTER 4

Understanding the physiology of yield determination of rice in different environments through path analysis

Chapter 4

Understanding the physiology of yield determination of rice in different environments through path analysis

Abstract

Understanding yield formation in rice under different growing conditions requires a thorough knowledge of the underlying processes. Analysis of yield components has often been undertaken by researchers to study the process of yield formation. Frequently, negative correlations were found between some yield components under certain growing conditions. These negative associations hinder the success of indirect selection for yield through yield components. Hence, the need to look more closely into the processes involved in the formation of yield components themselves, as these components are determined at different developmental stages. We used the method of path coefficient analysis to investigate the relationships between yield and its components – panicles per square metre, total grains per panicle, spikelet fertility and thousand grain weight – on the one hand, and the relationships between physiological traits collected around flowering time and yield components and yield, on the other hand, in different environments. The physiological traits studied were shoot biomass, leaf weight, stem weight, partitioning coefficient of total dry matter allocated to shoots, LAI, leaf N concentration and temperature sum (°Cd) between sowing to flowering. Fifteen genotypes of rice, comprising 11 Recombinant Inbred Lines (RILs), together with their parents, IR29 and Pokkali, as well as two improved rice cultivars ROK5 and ITA212, were grown under fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer. In the path analysis, grain number attributes (product of panicles per square metre and total grains per panicle) were more influential in yield determination than grain filling attributes (product of spikelet fertility and thousand grain weight) in fresh water environments while in saline environments grain filling attributes influenced grain yield more than grain number attributes. In fresh water, total grains per panicle had a larger effect on grain yield in the zero N fertilizer regime than when 100 kg ha^{-1} N fertilizer was applied. Effect of panicles per square metre on yield, however, was larger in the high N fertilizer regime than in the zero N fertilizer regime. Thousand grain weight had negative effects on grain yield in fresh water environments and this effect was much larger at the high N fertilizer rate than at the zero N fertilizer rate. In saline environments, both spikelet fertility and thousand grain weight had larger effects on yield at the zero N fertilizer rate than at the 100 kg ha^{-1} N fertilizer rate. Under both fresh and saline water conditions, high biomass production had a positive effect on grain yield due to its effects on key yield components (increasing grain number attributes in fresh

water and increasing grain filling attributes in saline water). Stem weight and fraction of dry matter allocated to shoots both had negative effects on yield in fresh water environments and positive effects on yield in saline environments. On the other hand, high leaf weight had positive effects on grain yield in all environments except the saline environment without N fertilizer application. Delayed flowering had a positive effect on panicles per square metre in fresh water environments and therefore a positive effect on yield while in saline environments, delayed flowering had a negative effect on grain filling attributes and hence a negative effect on grain yield. LAI and leaf N concentration both had positive effects on grain yield although their effects were smaller than those of biomass-related traits.

Introduction

Quantification of processes that lead to grain production in common cultivars of a crop is valuable for identifying key traits for selecting high yielding cultivars in target environments (Kiniry *et al*., 2001). Yield sink capacity, defined as the maximum size of the organs of a plant to be harvested, has been recognized by breeders as a primary objective to increase yield. For rice, yield sink capacity comprises number of panicles per plant, number of spikelets per panicle, and filled grain weight (Kato and Takeda, 1996). These yield components could be further broken down into their respective sub-components to gain insight into the mechanisms controlling the expression of these traits. According to Yin *et al*. (2002), crop seed yield can be dissected into various physiological or agronomic traits that might be under separate and probably simpler genetic control.

The critical developmental stages at which rice yield components are initiated and differentiated occur sequentially (Zeng *et al*., 2001). Panicles per square metre is largely determined by two weeks after maximum tillering while total spikelets per panicle is determined during the reproductive growth stage, that is, between panicle initiation and heading (Yoshida, 1981). Percent filled spikelets is determined before, at, and after flowering. Grain weight is largely determined by the grain size, which is controlled by the size of the hull, and grain filling ability. The size of the hull, the structure containing the grain, is a stable varietal characteristic but could be slightly influenced by solar radiation during the two weeks before heading (Yoshida, 1981).

Determining the physiological traits most involved in the formation of a yield component could give insight into the possibilities of manipulating the size or number of the component. Researchers could then strive to obtain an optimum combination of yield components that would best suit the requirement for high yielding ability in rice cultivars for any particular environment. Such an approach aimed at identifying key

physiological traits involved in the determination of yield components could generate information on how to minimize or eliminate the phenomenon of yield component compensation that is widely reported for many crops. Yield component compensation is the phenomenon where a high production of one component leads to the low production of another component. In rice, Gravois and Helms (1992) found a negative relationship between panicle density and filled grain percentage. Similarly, Kato and Takeda (1996) reported finding a negative correlation between grain weight and number of spikelets per plant. Yield component compensation has been reported in other crops such as field beans (Duarte and Adams, 1972) and millet (van Oosterom *et al*., 2003). The extent of yield component compensation varies across populations and environments (Li *et al*., 1998). It is thus essential to study the relationships between yield and yield components in each environment when significant genotype-by-environment interaction in yield is observed.

Researchers have often used the method of path coefficient analysis to study the relationships between yield and other plant characteristics in order to identify key traits for improvement, for example, in rice (Reuben and Katuli, 1989; Gravois and Helms, 1992), barley (Puri *et al*., 1982) and Crested Wheatgrass (Dewey and Lu, 1959). In path analysis, path diagrams are drawn in which the variables under study are arranged into predictor and response variables. The correlation between predictor variables and response variables is partitioned into direct and indirect effects (Samonte *et al*., 1998). The total correlation between a predictor variable and a response variable would be different from their Pearson correlation when there is strong correlation between predictor variables as is known to occur for yield components of rice (Yoshida, 1981).

In this research, grain yield, yield components and physiological traits of 11 Recombinant Inbred Lines from the cross IR29 × Pokkali and four cultivars of rice were subjected to multiple regression and path coefficient analyses with the objectives of finding out (i) the key yield components for yield determination in different environments, (ii) the extent of the association between yield components and physiological traits at different growth stages in each test environment (iii) the relationship between physiological traits, yield components and yield of rice in different environments and (iv) the modifying effect of the cultivation environment on the relationships between physiological traits, yield components and yield of rice.

Materials and methods

Plant material

A segregating population of rice, comprising 276 recombinant inbred lines (RILs), developed at IRRI from the cross IR29 × Pokkali (both *indica* varieties) was used. IR29

is a short, high yielding modern cultivar released by IRRI (International Rice Research Institute) and is known to be very sensitive to salinity (Gregorio, 1997). Pokkali is a tall, traditional variety from India known to be tolerant to salinity (Yeo and Flowers, 1986; Garcia *et al*., 1995; Gregorio, 1997). As one of the two parents is a short, high-yielding, modern cultivar and the other is a tall traditional variety, it is expected that the RIL population will also be segregating for response to nitrogen fertilizer. This is because modern rice cultivars have been bred for response to high levels of mineral fertilizers especially N fertilizer while traditional varieties have usually been selected by farmers in environments with sub-optimal levels of nutrient supply.

The RIL population, together with the parents, was grown during the rainy season (June-Oct.) in 1999 and 2000 in a series of genetic experiments at Sapu (13.55° N latitude), in The Gambia (see Chapter 2). One hundred and sixty RILs were randomly selected from the population of 276 RILs for use in our study. From these 160 RILs, 100 were used in the trials of 1999 and 2000 (see Chapter 2). Yield and yield component data were collected from these 100 RILs and their parents. After exhaustive analyses of the two-year yield data, 38 RILs were selected from the 160 RILs for high-, medium- and low-yielding ability in the different test environments based on the molecular marker signatures of the different RILs. The results of these genetic studies are presented elsewhere (see Chapter 2). Of these 38 RILs, 22 were grown before in our trials of 1999 and 2000 while the remaining 16 RILs had not been grown before in our trials.

In 2001, these selected 38 lines together with the two parents were grown at the same experimental site using the same split-split plot design. Two rice cultivars were introduced into the trial in 2001 – ROK5 and ITA212. Thus a total of 42 RILs and cultivars were grown in 2001. ROK5 is a tall, moderately salt-tolerant cultivar bred in Sierra Leone (WARDA, 1994) and has been used in the saline swamps of West Africa for many years. ITA212 is a semi-dwarf, high-yielding West African tropical *japonica* bred by researchers at the International Institute of Tropical Agriculture (IITA), in Ibadan, Nigeria.

Field experiments

A split-split plot experimental design was used with salinity as the main plot factor, level of nitrogen fertilizer application as the sub-plot factor and genotype as the sub-subplot factor. Each sub-subplot measured 2.6 m × 3.0 m and a spacing of 20 cm × 20 cm within and between rows was used. Two levels of salinity and two levels of nitrogen fertilizer application (giving a total of four treatment combinations) were tested. Three replications were maintained in each year of the trials. Additional information on the trials is given in Table 1. The following treatment combinations also referred to later in the text as test environments, were obtained:

Table 1. Details of environmental conditions and field experiments conducted in years 2000 and 2001 (Sapu Research Station, The Gambia).

	2000	2001
Sowing date	8 June	9 July
Transplanting date	4 July	31 July
Salinity (dS m^{-1})	~8	~8
Total rainfall (mm)	1326	754
Average minimum temp. (°C)	22.4	22.5
Average maximum temp. (°C)	34.0	34.0
Basal fertilizers (kg ha^{-1})		
P (Triple Super Phosphate)	40	40
K (Muriate of Potash)	40	40

S1N1 – Fresh water (river water) at an electrical conductivity (EC) of 0.15 dS m^{-1} and 0 kg N ha^{-1};
S1N2 – Fresh water (river water) at an EC of 0.15 dS m^{-1} and 100 kg N ha^{-1} as urea;
S2N1 – Salt water at an EC of 8 dS m^{-1} and 0 kg N ha^{-1};
S2N2 – Salt water at an EC of 8 dS m^{-1} with 100 kg N ha^{-1} as urea.

Pre-germinated rice seeds were sown in a nursery that was well watered and regularly weeded. Around three weeks after emergence (22 DAS – days after sowing) seedlings were transplanted to the field following the experimental design described above. After transplanting, trial plots were kept continuously flooded by irrigating with river water till all RILs and cultivars were close to physiological maturity. Salinity was imposed by manually broadcasting measured amounts of granular table salt in standing water to attain the required salinity.

Data collection

Physiological data were collected on 11 RILs plus the two parents and ROK5 and ITA212. The physiological data collected on these 15 genotypes included dry weights of leaves, stems and panicles, leaf area index (LAI) and leaf nitrogen concentration (%). Shoot biomass was estimated by taking the sum of dry weights of leaves, stems and panicles (where present).

Physiological data were collected on the same days for all 15 genotypes at roughly two-week intervals (71, 85, 98, 111, 127 and 133 DAS). Most genotypes flowered around 98 DAS and matured around 127 DAS. On each sampling date, four plant hills were uprooted and separated into roots, leaves, stems and panicles depending on the

developmental stage. Only green leaves were measured and then only up to flowering. Dead leaves were removed but not weighed. LAI was measured with an AccuPAR model PAR80 Ceptometer (Decagon Devices, Inc., USA).

Dry matter was determined by air-drying the separate organs under shade followed by oven drying at 70 °C till a constant weight was attained. Leaf nitrogen concentration (%) was obtained from dried leaf samples, which were processed using the micro-Kjeldahl process. Partitioning coefficients were derived by calculating the fraction of new dry matter (DM) production distributed to each plant organ between subsequent sampling stages (Kropff *et al*., 1994). Five different partitioning coefficients were calculated:

FRT – partitioning coefficient of total dry matter allocated to the root
FSH – partitioning coefficient of total dry matter allocated to the shoot
FLV – partitioning coefficient of shoot dry matter allocated to the leaves
FST – partitioning coefficient of shoot dry matter allocated to the stems
FSO – partitioning coefficient of shoot dry matter allocated to the panicles (storage organs).

LAI and leaf N concentration were measured up to two weeks after most lines flowered. No observed values were available for these parameters at maturity in both years.

The dates of sowing, transplanting, flowering and physiological maturity were recorded for all lines. Weather data including daily minimum and maximum temperatures, were collected from the local weather station. The dates of sowing, transplanting, flowering and maturity together with the weather data, were entered into the computer program DRATES (Kropff *et al*., 1994) which then used this information to compute temperature sum, in heat units (°Cd), required by each line of rice to complete development stages from sowing to flowering and then maturity. The temperature sum required during grain filling was determined by computing the difference between the temperature sum from sowing till maturity and the temperature sum from sowing to flowering. Using DRATES we also computed the developmental stages that each sampling date coincided with.

Data was also collected on yield components. Four yield components were assessed: number of panicles per m^2, thousand grain weight, spikelet fertility and total grains per panicle. A peg was randomly placed between four plant hills towards the middle of each plot and at maturity these four hills were harvested separately from the rest of the plot. Yield component data was determined from these four hill samples from each plot.

Number of panicles m^{-2} (panicles m^{-2})
All panicles in four hill samples were counted for each plot. As the area covered by these four hills was 0.16 m^2 (0.4 m × 0.4 m) the number counted was multiplied by 6.25 (1.0 m^2 / 0.16 m^2) to convert it to number of panicles m^{-2} for that plot.

Total grains panicle^{-1} (grains panicle^{-1})
The four hill samples were threshed separately for each plot and then winnowed to separate filled from empty grains. These two classes were counted separately, added together and the sum was divided by the total number of panicles in the four-hill samples to give the total number of grains per panicle.

Thousand grain weight (g) – All filled grains counted from four hill samples were weighed and thousand-kernel weight was calculated using the following formula:

Thousand grain wt. = (Weight of filled grains from four hill samples (g) / total number of filled grains from four hill samples) × 1000

Spikelet fertility (%) – This was determined by dividing total number of filled grains in the four-hill samples by total number of grains per panicle and then multiplying by 100.

Grain yield (kg ha^{-1}) – At maturity net plots were harvested for each sub-subplot leaving one border row at each of the four sides of the plots. The harvested grain was threshed, cleaned, dried and weighed to determine grain yield in kg ha^{-1}.

Data analysis
A logarithmic transformation was performed on yield, yield components and physiological traits followed by simple linear correlation and multiple regression analyses. Correlation analysis was performed for yield and yield components in each of the four test environments to find out the simple linear associations between yield and yield components and amongst the yield components across different environments.

To assess the extent of association between physiological traits and yield components, each yield component was individually regressed on the totality of physiological traits collected at each of the first three sampling stages (71, 85 and 98 DAS). At the fourth sampling stage (111 DAS) only thousand grain weight and spikelet fertility were regressed on physiological traits because it is expected that panicles per square metre and total grains per panicle would have been determined prior to flowering. For each yield component, the R^2 values were plotted against sampling dates to illustrate the changes in the extent of the association between physiological traits and yield components over time in different environments.

At each sampling stage, the following physiological traits were used as regressors to determine the strength of their associations with yield components at each sampling stage in each of the four different test environments.

First sampling stage The following traits collected at this stage were used in the regression models: green leaf weight, stem weight, shoot biomass, leaf N concentration (%), LAI, partitioning coefficient of total dry matter allocated to shoots, and partitioning coefficients of shoot dry matter allocated to leaves and stems.
Second sampling stage With the exception of LAI, all the physiological traits used in the regression models for the first sampling stage were collected at the second sampling stage. Thus in the regression models used at this stage, LAI collected at the first sampling stage was used together with the other physiological traits collected during the second sampling.
Third sampling stage At this stage, data was collected on the following traits which were subsequently used in the regression equations: green leaf weight, stem weight, shoot biomass, leaf N concentration (%), LAI, partitioning coefficient of total dry matter allocated to shoots, and temperature sum from sowing to flowering (°Cd).
Fourth sampling stage Physiological traits were regressed on only thousand grain weight and spikelet fertility at this stage. The following physiological traits collected at this stage were used in the regression equations: green leaf weight, stem weight, shoot biomass, leaf N concentration (%), LAI and temperature sums (°Cd) from sowing to flowering, maturity and during the grain filling period (between flowering and maturity).

Yield, yield components and physiological traits collected at the third sampling stage (around flowering time for most genotypes in this study) were standardized as follows:

$$X = \frac{x - \bar{x}}{\sigma_X}$$

where, X = standardized variable, x = mean value for each genotype, $\bar{x}$ = Grand mean of all 15 genotypes, and σ_x = standard deviation of variable.

We then used stepwise regression to identify the two or three physiological traits that were most strongly associated with individual yield components around flowering time (third sampling stage). In each test environment, these standardized values of yield, yield components and selected physiological traits at the third sampling stage were subjected to path coefficient analysis using the CALIS procedure of SAS (1999). Path analysis was performed following the methods of Duarte and Adams (1972) and Li (1975). This analysis was done following the premise that yield is the product of

yield components which are themselves determined from physiological traits. For each yield component, only the physiological traits most strongly associated with it around flowering time were included in its model. As the physiological traits found to be most closely associated with yield components were sometimes different in different test environments, a separate path diagram was drawn for each of the four environments.

Path analysis allowed us to sequentially determine the relevance of physiological processes for yield formation through yield components. The direct effects of predictor variables were the path coefficients computed through the CALIS procedure of SAS. A path coefficient is a standardized regression coefficient (Li, 1975). Indirect effects were computed as the product of the correlation coefficient between two variables and the path coefficient from the second variable to the response variable. Thus the total effect of a predictor variable X_1 correlated with another predictor variable X_2, on a response variable Z, would be given by:

$$r_{ZX1} = p_{X1Z} + r_{X1X2}p_{ZX2}$$

where, r_{ZX1} = total correlation between Z and X_1, p_{X1Z} = path coefficient from X_1 to Z, r_{X1X2} = correlation coefficient between X_1 and X_2, p_{X2Z} = path coefficient from X_2 to Z.

For example, the total correlation between panicle number per square metre (NPM) and yield in Fig. 5 was calculated as:

$$r_{YN} = 0.29 + (–0.25 \times 0.62) + (–0.21 \times –0.33) + (0.20 \times –0.15) = 0.174$$

where, r_{YN} = total correlation between yield (Y) and panicle number per square metre (N or NPM). The difference between this figure (0.174) and 0.177 in Table 3 is due to rounding up errors.

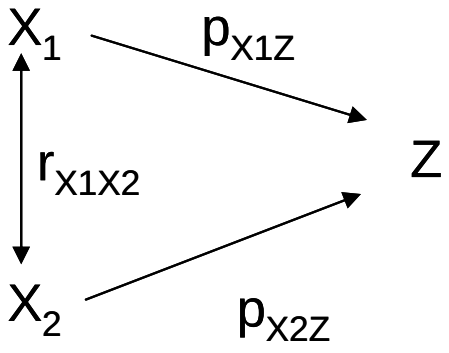

Fig. 1. Simple path diagram showing the relationship between two correlated predictor variables, X_1, X_2 and a response variable, Z. Single-headed arrows denote path coefficients and double headed arrows denote correlations between predictor variables.

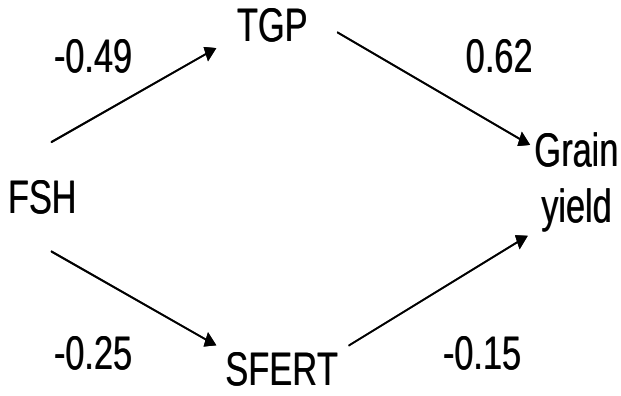

Fig. 2. Illustration of the total effect of a physiological trait, FSH (fraction of total dry matter allocated to shoots), on grain yield through two intermediary variables (total grains/panicle (TGP) and spikelet fertility (SFERT)) in the zero N fertilizer regime in fresh water.

The effect of a physiological trait on yield was computed as the sum of the products of path coefficients of all paths joining that physiological trait to yield. For example, in the zero N fertilizer regime in a fresh water environment, fraction of total dry matter allocated to shoots (F or FSH), which acts on yield (Y) through two yield components total grains/panicle (T or TGP) and spikelet fertility (S or SFERT) (Fig. 2 and Fig. 5) would have a total correlation with yield computed as:

$$r_{YF} = p_{FT}p_{TY} + p_{FS}p_{SY}$$

where, r_{YF} = correlation between yield and FSH, p_{FT} = path coefficient from FSH to TGP (–0.49), p_{TY} = path coefficient from TGP to yield (0.62), p_{FS} = path coefficient from FSH to SFERT (–0.25), p_{SY} = path coefficient from SFERT to yield (–0.15).

Thus, the total effect of fraction of total dry matter allocated to shoots (FSH) on grain yield in the zero N fertilizer regime in fresh water (see Table 4), was:

$$r_{YF} = (-0.49 \times 0.62) + (-0.25 \times -0.15) = -0.3038 + 0.0375 = -0.2663$$

As with regression coefficients, path coefficients can be larger than 1.0 since no limitation was set on the magnitude of the standard partial regression coefficient (Sokal and Rohlf, 1981). In our study, the presence of path coefficients larger than 1.0 was due to the inclusion of highly correlated physiological traits, such as shoot biomass, leaf weight and stem weight, in some of our path models. Removal of one of the highly correlated variables led to poor model agreement with the data and we thus decided to include the correlated traits in the model. In all four environments, the χ^2 relative to its degrees of freedom was low with $P > 0.1$ thus confirming that the path models used agreed well with the data (Carey, 1998).

Results

Simple correlations between yield and yield components

Correlations between yield and yield components and amongst yield components varied from one environment to the other both in terms of signs and magnitude of the correlation coefficients and significance levels. For example, the correlation between number of panicles per square metre and grain yield was significant and positive in fresh water with high N fertilizer (S1N2) but significant and negative in saline water with high N fertilizer (S2N2). In fresh water with zero N fertilizer application, yield had a stronger correlation with total grains per panicle than with number of panicles per square metre but at high N fertilizer application yield was more strongly associated with number of panicles per square metre than with total grains per panicle (Table 2). Grain yield was actually negatively correlated with thousand grain weight and spikelet fertility in fresh water although this correlation was only significant between yield and thousand grain weight in fresh water without N fertilizer (S1N1). The correlations between yield and thousand grain weight and yield and spikelet fertility in saline

Table 2. Simple linear correlation involving yield and yield components in different environments.

Comparison	Environment			
	S1N1	S1N2	S2N1	S2N2
NPM/TGP	0.214	0.265	–0.021	–0.311
NPM/WTG	–0.368	–0.510	–0.091	–0.569*
NPM/SFERT	–0.074	0.198	0.208	–0.503
NPM/Yield	0.475	0.715**	0.191	–0.545*
TGP/WTG	–0.188	–0.326	–0.215	0.229
TGP/SFERT	0.024	–0.654**	–0.175	–0.221
TGP/Yield	0.700**	0.490	–0.161	0.249
WTG/SFERT	0.210	0.137	0.743**	0.661**
WTG/Yield	–0.545*	–0.489	0.774**	0.574*
SFERT/Yield	–0.208	–0.159	0.726**	0.720**

* significant ($P < 0.05$); ** significant ($P < 0.01$).

(Note: S1N1, S1N2 – Fresh water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer, respectively; NPM – number of panicles per square metre (panicles m^{-2}); TGP – Total grains per panicle (grains/panicle); SFERT – spikelet fertility (%); WTG – Thousand grain weight (g)).

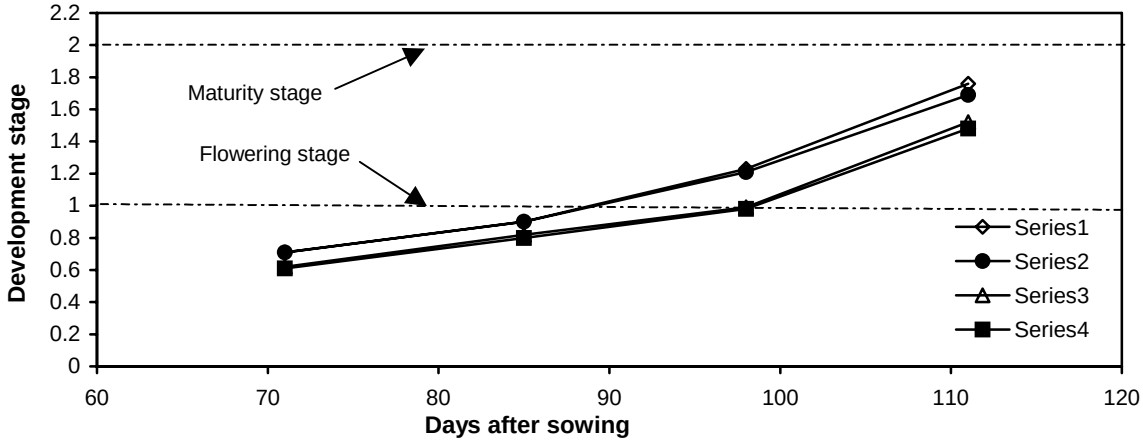

Fig. 3. Mean developmental stages of 15 genotypes of rice at which physiological samples were collected in four different environments (Series 1 and 2 are fresh water environments with 0 kg ha^{-1} and 100 kg ha^{-1} N fertilizer, respectively; Series 3 and 4 are salt water environments with 0 kg ha^{-1} and 100 kg ha^{-1} N fertilizer, respectively).

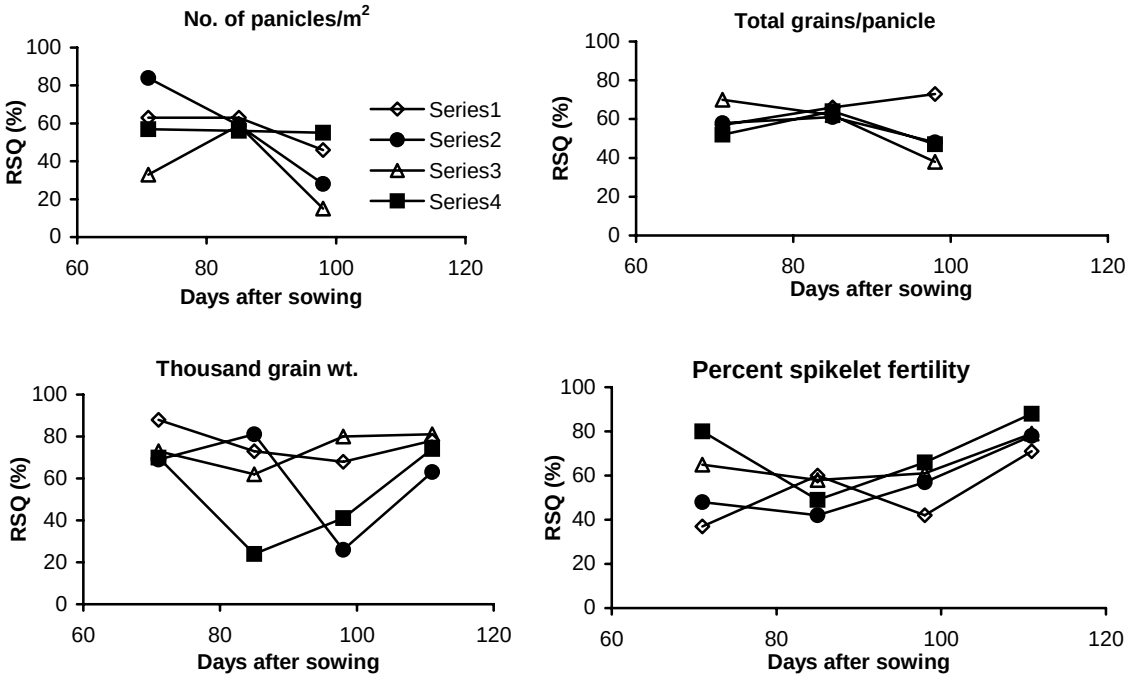

Fig. 4. Strength of associations (RSQ (%) – R^2) between physiological traits collected at different growth stages and yield components in four test environments. See Materials and Methods for exact physiological samples used in the regression analysis at each sampling stage (Series 1 and 2 are fresh water environments with 0 kg ha^{-1} and 100 kg ha^{-1} N fertilizer, respectively; Series 3 and 4 are salt water environments with 0 kg ha^{-1} and 100 kg ha^{-1} N fertilizer ha^{-1}, respectively).

environments were similar at the zero N fertilizer level but in the high N fertilizer environment yield was more strongly associated with spikelet fertility than with thousand grain weight.

Determination of yield components by physiological traits at different growth in different environments

Mean developmental rates of genotypes were slower under salt stress than in fresh water. Application of N fertilizer also reduced developmental rates but this reduction was much less than the reduction in development rate caused by salt stress. Thus the different sampling dates coincided with different developmental stages of rice genotypes in the four test environments (Fig. 3).

The proportion of the variation in yield components accounted for by physiological traits was found to differ between sampling stages and between test environments. In all four environments the association between panicles number and physiological traits was stronger at the first and second sampling stages, as reflected in the higher R^2 values, than at the third sampling stage. Furthermore, at the first and second sampling stages, the association between number of panicles per square metre and physiological traits was stronger in fresh water than in saline water at both levels of N fertilizer application. The association between physiological traits and panicle number varied little in the zero N fertilizer regime in fresh water (46%-63%) and in the high N fertilizer regime in saline water (55%-57%) (Fig. 4). In the high N fertilizer regime in fresh water, however, physiological traits at the first sampling stage accounted for more than 80% of the variation in panicle number in fresh water and this then dropped sharply in the second (59%) and third sampling stages (28%). In saline water without N fertilizer application, the association between physiological traits and panicle number was highest at the second sampling stage (59%) while this association was much lower in the first (33%) and third sampling stages (15%).

At the zero N fertilizer level in fresh water, the proportion of variation in total grains per panicle accounted for by physiological traits increased progressively from the first to third sampling stages while in saline environments, this proportion declined from the first to third sampling stages. The association between physiological traits and total grains per panicle in the high N fertilizer regime in both fresh and saline water followed similar patterns and the associations were also similar in the two environments. In these two environments this association was slightly stronger at the second sampling stage than at the first and third sampling stages.

With the exception of the second sampling stage in the high N fertilizer regime in fresh water, physiological traits were more strongly associated with thousand grain weight at the first and fourth sampling stages than at the second and third stages (Fig.

4). Furthermore this association between physiological traits and thousand grain weight was consistently stronger in the zero N fertilizer regime in both fresh and saline water than in the high N fertilizer regime at all four sampling stages. However, the difference between the zero and high N fertilizer regimes regarding the proportion of variation in thousand grain weight accounted for by physiological traits was larger at the second and third sampling stages than at the first and fourth stages.

In all four test environments, spikelet fertility had a stronger association with physiological traits at the fourth sampling stage than at the three earlier stages. Except for the second sampling stage in the zero N fertilizer regime in fresh water, the strength of the association between physiological traits and spikelet fertility followed a consistent pattern in the other environments. The proportion of variation in spikelet fertility accounted for by physiological traits, declined from the first to second sampling stages and then increased from the second to the fourth sampling stages.

Relationships between yield and yield components in different environments

The relative importance of yield components for yield determination was found to differ between environments. In fresh water environments, panicle number and total grains per panicle appeared to be the most important components for yield determination whilst in saline environments, grain weight and spikelet fertility were more important for yield determination (Table 3). Within fresh water environments, the effect of one yield component clearly dominated. In the zero N fertilizer regime in fresh water this was total grains per panicle and in the high N fertilizer regime in fresh water, it was panicle number per m^2. Grain weight had relatively strong negative total effects on grain yield in fresh water environments, and these effects were larger at the high N fertilizer level than at the zero N fertilizer level. In saline environments, the relative contributions of grain weight and spikelet fertility to the variation in grain yield in either the zero N fertilizer rate or the high N fertilizer rate, were similar.

Changes in both the sign and magnitude of total correlations between yield and yield components due to indirect effects via other yield components were observed. For correlations between yield and panicle number per m^2 and yield and total grains per panicle, indirect effects only affected the magnitude of correlation coefficients. With regards to thousand grain weight and percent spikelet fertility, however, indirect effects altered the signs of their total correlations with yield in the high N fertilizer regime in saline water (for thousand grain weight) and in the zero N fertilizer regime in fresh water (for percent spikelet fertility). The change in sign of the negative direct effect to a positive total correlation between grain weight and yield in the high N fertilizer regime in saline water was due to the strong positive indirect effect via percent spikelet fertility. For spikelet fertility in the zero N fertilizer regime in fresh

Table 3. Path coefficient analysis of direct and indirect effects of yield components on grain yield of rice in different environments.

Trait	Environment			
	S1N1	S1N2	S2N1	S2N2
No. of panicles m^{-2}				
Direct effect	0.293	0.719	0.178	0.019
Indirect effects via				
TGP	–0.155	0.048	–0.002	–0.048
WTG	0.069	0.027	0.058	0.053
SFERT	–0.030	–0.043	0.096	0.096
Total correlation	0.177	0.751	0.330	0.120
Total grains/panicle				
Direct effect	0.615	0.220	0.045	0.531
Indirect effects via				
NPM	–0.073	0.158	–0.009	0.002
WTG	–0.043	0.020	0.122	–0.004
SFERT	–0.048	0.085	0.046	–0.284
Total correlation	0.451	0.483	0.204	0.245
Thousand grain weight				
Direct effect	–0.326	–0.060	0.641	–0.103
Indirect effects via				
NPM	–0.061	–0.324	0.016	–0.011
TGP	0.081	–0.073	0.008	0.021
SFERT	0.014	–0.013	0.120	0.535
Total correlation	–0.292	–0.470	0.785	0.442
Spikelet fertility				
Direct effect	–0.147	–0.160	0.198	0.808
Indirect effects via				
NPM	0.058	0.194	0.086	–0.007
TGP	0.198	–0.117	0.009	–0.186
WTG	0.030	–0.005	0.384	–0.066
Total correlation	0.139	–0.088	0.677	0.549

(Note: S1N1, S1N2 – fresh water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer respectively; S2N1, S2N2 – saline water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer, respectively).

water, the sign change from a negative direct effect to a positive total effect was mainly caused by the positive indirect effect due to the positive correlation with total grains per panicle.

With regards to correlations between yield and individual yield components, panicle number per m^2 was found to have a higher correlation with yield in the high N fertilizer regime in fresh water than in any of the other three test environments. For total grains per panicle, its total correlation with yield was higher in fresh water than in saline environments. The total correlations between yield and total grains per panicle were similar between the two N fertilizer regimes in both fresh and saline water (Table 3). Grain weight, on the other hand, had negative correlations with yield in fresh water while in saline water it was positively correlated with yield. The negative correlation between grain weight and yield was stronger at the high N fertilizer level than at the zero N fertilizer level while in saline water the positive correlation between grain weight and yield was stronger at the zero N fertilizer level than at the high N fertilizer level. The strong negative effect of grain weight on yield in the high N fertilizer regime in fresh water was mainly due to the strong negative indirect effect as a result of the correlation with panicle number per m^2. The strong positive effect of grain weight on grain yield in saline environments was mainly caused by the strong positive indirect effect due to the correlation with spikelet fertility. Regarding, spikelet fertility, it was only weakly correlated with yield in fresh water environments but in saline environments it had strong positive correlations with yield (Table 3).

Relationships between physiological traits collected around flowering stage, yield and yield components in different environments

Around flowering stage of most of the rice genotypes used in this study, the physiological traits found to be most important in the determination of individual yield components were usually different across the studied environments. However, certain physiological traits had significant effects on more than one yield component in some environments.

Fresh water with 0 kg ha^{-1} N fertilizer (S1N1)

In this environment, among the physiological traits most significantly associated with yield components around flowering, shoot biomass and leaf weight had the largest positive effects on grain yield (Table 4). Leaf N concentration and LAI also had positive effects on yield although their effects were smaller than those of shoot biomass and leaf weight. Shoot biomass had a positive effect on panicle number per m^2 (Table 5) and the contribution of the path from shoot biomass to yield through panicle number was also positive (Fig. 5). Hence the effect of shoot biomass around

Table 4. Path coefficient analysis of total effects of physiological traits collected around flowering stage on grain yield of rice in different environments.

Physiological trait	Environment			
	S1N1	S1N2	S2N1	S2N2
Shoot biomass	0.249	0.253	0.440	N.A.
Stem weight	–1.337	–0.889	0.005	0.275
Leaf weight	1.386	0.670	–0.142	0.266
FSH	–0.266	–0.029	0.406	0.210
Leaf N%	0.074	N.A.	0.020	N.A.
LAI	0.030	N.A.	N.A.	N.A.
TSUMF	N.A.	0.121	0.045	–0.497

N.A. – Physiological trait not included in regression model in the particular environment.
(Note: S1N1, S1N2 – fresh water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water environment with 0 kg ha^{-1} N and 100 kg ha^{-1} N fertilizer, respectively; FSH - fraction of total dry matter allocated to shoots; TSUMF – temperature sum (°Cd) between sowing and flowering).

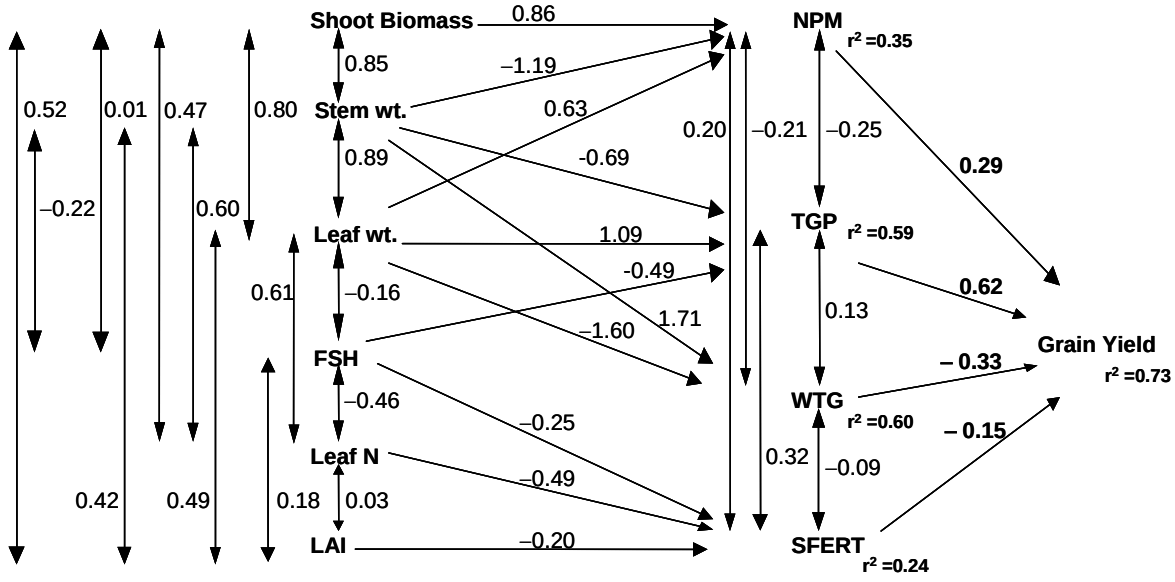

Fig. 5. Path analysis of the effects of physiological traits on yield through yield components in a fresh water environment with 0 kg ha^{-1} N fertilizer (S1N1). (NPM – number of panicles per square metre; TGP – total grains per panicle; SFERT – percent spikelet fertility; WTG – thousand grain weight; FSH – fraction of dry matter allocated to shoots).

flowering time on yield was positive. The positive effect of leaf weight on yield was mainly due to its large positive effect on total grains per panicle and comparatively large effect on panicle number and these two yield components were earlier on found to be the most important components for yield determination in this environment.

Concerning the negative effects of stem weight on yield, this was due to the negative effect of this trait on number of panicles per square metre. In addition, the products of the paths from stem weight to yield through panicle number, total grains per panicle and grain weight were all negative even though stem weight actually had positive effects on total grains per panicle and thousand grain weight in this environment. Fraction of total DM allocated to shoots around flowering time also negatively affected yield but in this case this was largely as a result of the strong negative effect of this trait on total grains per panicle (Table 5) which was the most important yield component for yield determination in this environment. Thus based on our results from the selection of genotypes used in this study, around flowering time, high yielding cultivars in this environment should have high shoot biomass, high leaf weight, high leaf N, large LAI, low stem weight and a low partitioning coefficient of DM to shoots.

Fresh water with 100 kg ha^{-1} N fertilizer (S1N2)

Similar to the above situation in zero N fertilizer regime, in this environment also shoot biomass and leaf weight had the largest positive effects while stem weight had the largest negative effect on grain yield (Table 4). The fraction of DM allocated to shoots had a negative effect on yield as in the zero N fertilizer regime described earlier although the total effect of fraction of DM allocated to shoots on yield was much smaller in this environment compared to zero N fertilizer regime. Shoot biomass had a positive effect on yield due to its positive effect on total grains per panicle, which made a strong positive contribution on yield, second only to the contribution of number of panicles per m^2. Although leaf weight had negative effects on number of panicles per m^2, grain weight and spikelet fertility (Table 6), the overall effects of the paths from leaf weight to yield through these components were positive. The path through number of panicles per m^2 was strongly positive and as panicle number accounted for more variation in yield than the other components, eventually the effect of a high leaf weight on yield was positive.

Temperature sum (°Cd) between sowing and flowering had a positive effect on yield due to the positive effect of delayed flowering on total grains per panicle. With regards to stem weight, it decreased panicle number and increased total grains per panicle and grain weight. However, the paths joining stem weight to yield through these components all had negative effects on yield with the path through number of

Table 5. Path coefficient analysis of direct and indirect effects of physiological traits collected around flowering stage on yield components of rice in S1N1.

Physiological traits	Yield components			
	Number of panicles m^{-2}	Total grains per panicle	1000 grain weight (g)	Spikelet fertility (%)
Shoot biomass				
Direct effects	0.864			
Indirect effects via				
Stem weight	–1.012			
Leaf weight	0.504			
Total correlation	0.353			
Stem weight				
Direct effects	–1.191	–0.687	1.705	
Indirect effects via				
Shoot biomass	0.731			
Leaf weight	0.0561	0.970	–1.424	
Shoot DM fraction		0.108		
Total correlation	–0.404	0.391	0.281	
Leaf weight				
Direct effects	0.627	1.089	–1.596	
Indirect effects via				
Shoot biomass	0.688			
Stem weight	–1.059	–0.614	1.522	
Shoot DM fraction		0.078		
Total correlation	0.256	0.553	–0.074	
Shoot DM fraction				
Direct effects		–0.489		–0.249
Indirect effects via				
Stem weight		0.152		
Leaf weight		–0.174		
Leaf N%				0.225
LAI				–0.036
Total correlation		–0.511		–0.060
Leaf N (%)				
Direct effects				–0.492
Indirect effects via				
Shoot DM fraction				0.115
LAI				–0.006
Total correlation				–0.381
LAI				
Direct effects				–0.199
Indirect effects via				
Shoot DM fraction				–0.045
Leaf N%				–0.015
Total correlation				–0.259

Table 6. Path coefficient analysis of direct and indirect effects of physiological traits collected around flowering stage on yield components of rice in S1N2.

Physiological traits	Yield components			
	Number of panicles m^{-2}	Total grains per panicle	1000 grain weight (g)	Spikelet fertility (%)
Shoot biomass				
Direct effects		1.150		
Indirect effects via				
Stem weight		–0.855		
TSUMF		0.330		
Total correlation		0.625		
Stem weight				
Direct effects	–0.889	–0.940	0.568	
Indirect effects via				
Shoot biomass		1.047		
Leaf weight	0.629		–0.510	
TSUMF		0.424		
Total correlation	–0.260	0.531	0.058	
Leaf weight				
Direct effects	0.740		–0.601	–0.630
Indirect effects via				
Stem weight	–0.757		0.485	
Shoot DM fraction				–0.101
Total correlation	–0.017		–0.116	–0.731
Shoot DM fraction				
Direct effects				0.183
Indirect effects via				
Leaf weight				0.353
Total correlation				0.536
TSUMF				
Direct effects		0.548		
Indirect effects via				
Shoot biomass		0.690		
Stem weight		–0.724		
Total correlation		0.514		

panicles per m^2 having the largest negative effect (Fig. 6). Fraction of DM allocated to shoots had a positive effect on spikelet fertility but this yield component had only a small negative effect on yield in this environment. Furthermore, the effect of the path joining fraction of DM allocated to shoots to yield, through spikelet fertility, was negative. Hence, the overall effect of fraction of DM allocated to shoots on yield was small and negative. High yielding genotypes in this environment should possess the

ability to produce high amounts of biomass around flowering time, have high leaf weights and flower late. These should be complemented with a low stem weight and less importantly a small allocation of DM to shoots.

Saline water with 0 kg ha^{-1} N fertilizer (S2N1)

In this environment, shoot biomass and the fraction of DM allocated to shoots were the most important physiological traits around flowering time for yield determination (Table 4). These traits had strong positive effects on thousand grain weight and percent spikelet fertility, the two yield components that accounted for most of the variation in yield in this environment (Table 7). The paths from shoot biomass and fraction of DM allocated to shoots to yield through grain weight and spikelet fertility also had positive effects on yield. The combined positive effects of shoot biomass and fraction of DM allocated to shoots on grain weight and spikelet fertility and the positive effects of the paths through these two components surpassed the negative effects of shoot biomass and fraction of DM allocated to shoots on total grains per panicle and the paths from shoot biomass and fraction of DM allocated to shoots to yield through total grains per panicle.

Leaf N concentration and temperature sum (°Cd) between sowing and flowering also had positive effects on yield although their effects were smaller than those of shoot biomass and fraction of DM allocated to shoots. Contrary to the situations in

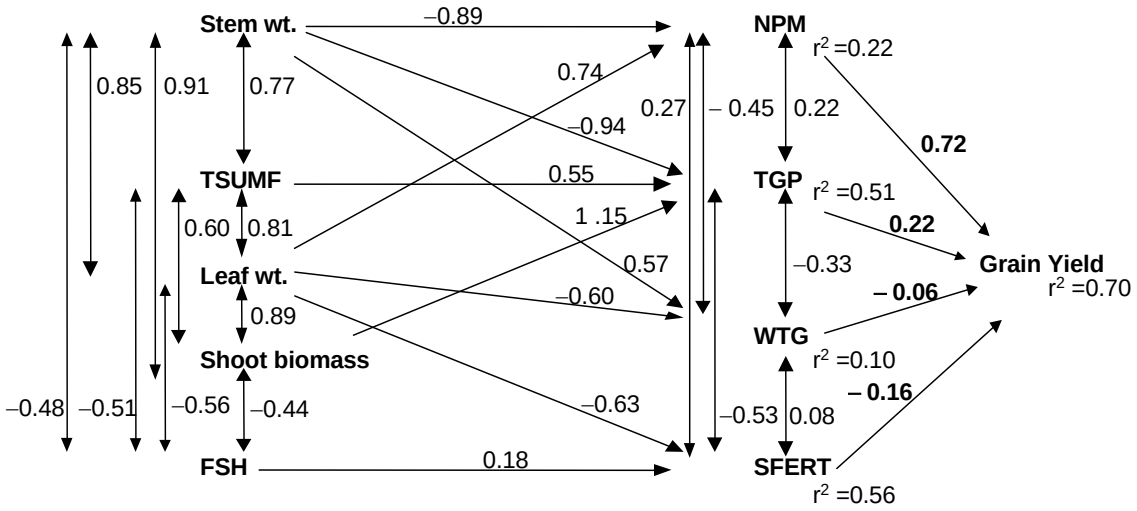

Fig. 6. Path analysis of the effects of physiological traits on yield through yield components in a fresh water environment with 100 kg ha^{-1} N fertilizer application (S1N2). (NPM – number of panicles per m^2; TGP – total grains per panicle; SFERT – percent spikelet fertility; WTG – thousand grain weight; FSH – fraction of dry matter allocated to shoots; TSUMF – temperature sum (°Cd) between sowing and flowering.)

zero and high N fertilizer regimes in fresh water, leaf weight had a negative effect on yield in this environment despite its small positive effect on thousand grain weight. The path from leaf weight to yield passing through grain weight was actually negative (Fig. 7) and this caused the negative influence of this trait on yield. In this highly stressful environment, based on results from rice genotypes used in this study, the most important selection criteria around flowering time for producing high yielding genotypes of rice would be high biomass production, ability to allocate more DM to shoots instead of roots and low leaf weights.

Saline water with 100 kg ha^{-1} N fertilizer (S2N2)

Stem weight, leaf weight and fraction of DM allocated to shoots all had positive effects on yield while temperature sum (°Cd) between sowing and flowering (equivalent to delayed flowering) had a strong negative effect on yield in this environment (Table 4). The positive effects of leaf weight and fraction of DM allocated to shoots on yield were mainly due to the large positive effects of these traits on total grains per panicle. High stem weight around flowering time promoted grain filling (high spikelet fertility) and thus had a positive effect on grain yield (Table 8) since spikelet fertility accounted for more variation in yield in this environment than the other components.

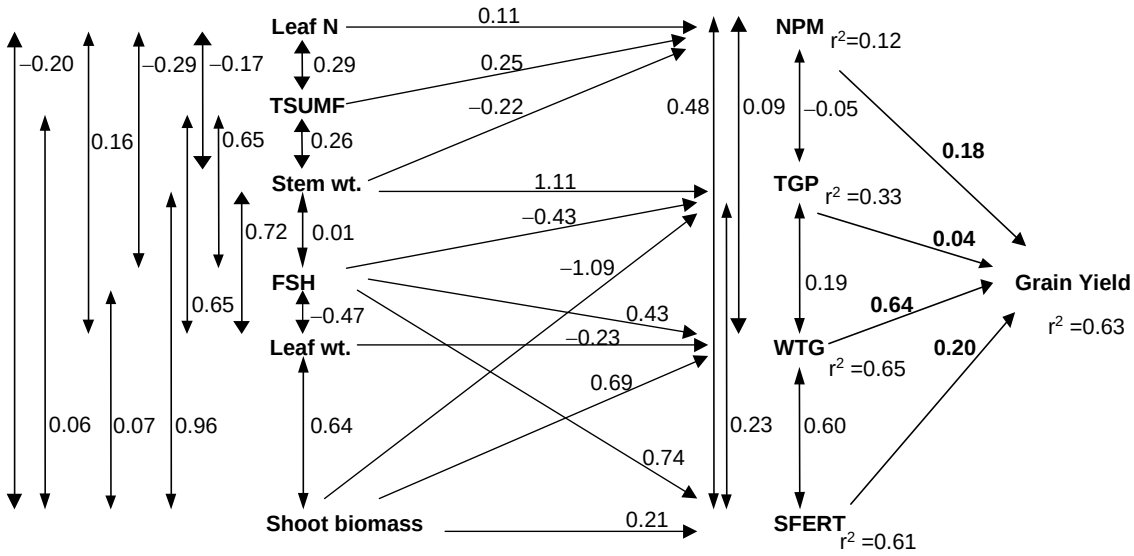

Fig. 7. Path analysis of the effects of physiological traits on yield through yield components in a saline environment with 0 kg ha^{-1} N fertilizer (S2N1). (NPM – number of panicles per m^2; TGP – total grains per panicle; SFERT – percent spikelet fertility; WTG – thousand grain weight; FSH – fraction of dry matter allocated to shoots).

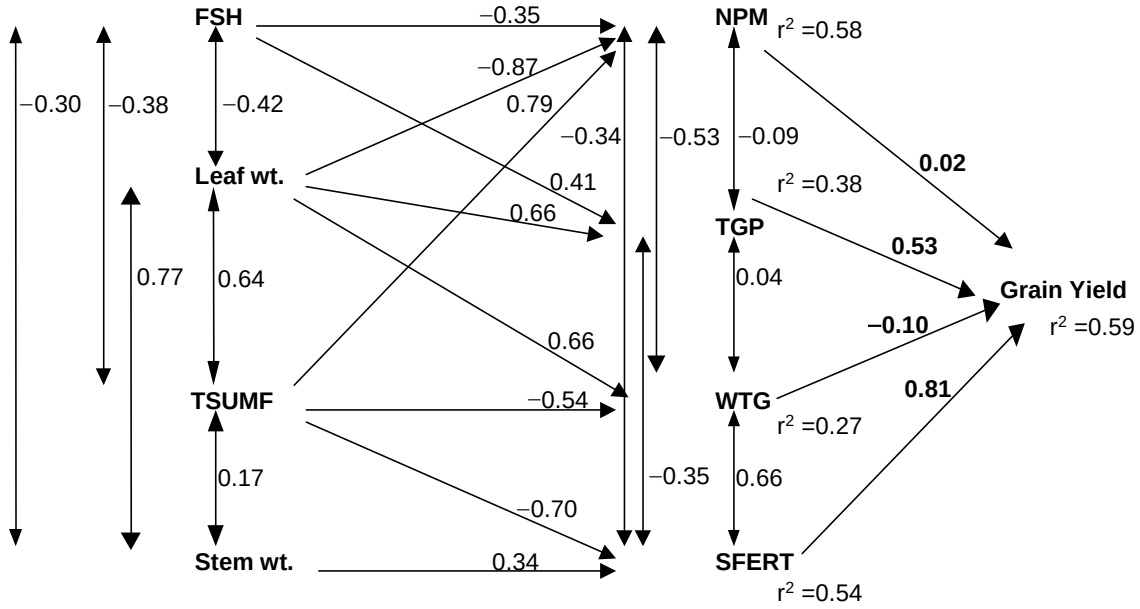

Fig. 8. Path analysis of the effects of physiological traits on yield through yield components in a saline water environment with 100 kg ha^{-1} N fertilizer application (S2N2). (NPM – number of panicles per m^2; TGP – total grains per panicle; SFERT - percent spikelet fertility; WTG – thousand grain weight; FSH – fraction of dry matter allocated to shoots).

The large negative effect of delayed flowering (TSUMF) on yield was mainly due to the large negative contribution of the path from TSUMF passing through spikelet fertility (Fig. 8). In addition delayed flowering also reduced thousand grain weight and spikelet fertility and these two accounted for more yield variation than the other yield components in this environment. Thus on the basis of rice genotypes used in our study, in this environment, having high stem and leaf weights coupled with a high allocation of DM to shoots at the expense of roots, around flowering time and early flowering should enable rice genotypes to give higher yields.

Discussion

Through path analysis we gained better insight into the relationships between yield and yield components in different environments than through simple linear correlation analysis. The advantage of path analysis over simple linear correlation analysis in this study was exemplified by those situations where the total effects of predictor variables on response variables were found to be quite different from the direct effects due to the indirect effects through some other correlated predictor variables. For instance, the simple linear correlation between yield and total grains per panicle in the zero N

Table 7. Path coefficient analysis of direct and indirect effects of physiological traits collected around flowering time on yield components of rice in S2N1.

Physiological Traits	Yield components			
	Number of panicles m^{-2}	Total grains per panicle	1000 grain weight (g)	Spikelet fertility (%)
Shoot biomass				
Direct effects		–1.086	0.688	0.210
Indirect effects via				
Stem weight		1.066		
Leaf weight			–0.147	
Shoot DM fraction		–0.030	0.030	0.052
Total correlation		–0.050	0.571	0.262
Stem weight				
Direct effects	–0.215	1.105		
Indirect effects via				
Shoot biomass		–1.046		
Shoot DM fraction		–0.004		
Leaf N%	–0.019			
TSUMF	0.065			
Total correlation	–0.169	0.055		
Leaf weight				
Direct effects			–0.230	
Indirect effects via				
Shoot biomass			0.442	
Shoot DM fraction			–0.202	
Total correlation			0.010	
Shoot DM fraction				
Direct effects		–0.426	0.434	0.739
Indirect effects via				
Stem weight		0.011		
Leaf weight			0.108	
Shoot biomass		–0.076	0.048	0.015
Total correlation		–0.491	0.590	0.754
Leaf N%				
Direct effects	0.111			
Indirect effects via				
Stem weight	0.037			
TSUMF	0.073			
Total correlation	0.221			
TSUMF				
Direct effects	0.247			
Indirect effects via				
Stem weight	–0.057			
Leaf N%	0.032			
Total correlation	0.222			

Table 8. Path coefficient analysis of the direct and indirect effects of physiological traits collected around flowering stage on yield components of rice in S2N2.

Physiological Traits	Yield components			
	Number of panicles m^{-2}	Total grains per panicle	1000 grain weight (g)	Spikelet fertility (%)
Stem weight				
Direct effects				0.341
Indirect effects via				
TSUMF				–0.119
Total correlation				0.222
Leaf weight				
Direct effects	–0.866	0.663	0.657	
Indirect effects via				
Shoot DM fraction	0.147	–0.172		
TSUMF	0.506		–0.346	
Total correlation	–0.213	0.488	0.311	
Shoot DM fraction				
Direct effects	–0.355	0.409		
Indirect effects via				
Leaf weight	0.365	–0.277		
TSUMF	–0.300			
Total correlation	–0.290	0.132		
TSUMF				
Direct effects	0.795		–0.543	–0.704
Indirect effects via				
Stem weight				0.058
Leaf weight	–0.557		0.422	
Shoot DM fraction	0.133			
Total correlation	0.371		–0.121	–0.646

fertilizer regime in saline water, and yield and panicle number m^{-2} in the high N fertilizer regime in saline water, were negative but the total effects of each of these components on yield in the respective environments computed through path analysis were positive. Hence, proper estimation of the contribution of any yield component for yield determination requires the consideration of the modifying effects of the correlations with other yield components on the influence of the particular component on yield.

The hierarchy of importance of yield components in yield determination, as revealed by path analysis, differed between environments. These differences in the relative importance of yield components in yield determination between environments might be due to differences in growing conditions at the time of maximum determination of yield components. Panicle number and total spikelets per panicle are determined before flowering while grain weight and spikelet fertility will largely be determined by the ability to fill the spikelets from flowering to maturity. In fresh water, total grains per panicle and panicle number m^{-2} were the most important yield components for yield determination at both levels of N fertilizer application due to their larger positive effects on grain yield compared to thousand grain weight and spikelet fertility. Thus it was more important for rice genotypes to produce more spikelets before flowering than to be able to have high filled grain weight. Apparently in fresh water environments, the genotypes were able to produce sufficient assimilates during grain filling to fill the sinks (spikelets) produced before flowering. Thus yield was sink limited in fresh water environments. Hence for high yielding ability in fresh water environments, rice genotypes should invest more assimilates into producing spikelets and less into grain filling ability. The method used to increase grain number would be dictated by the N fertilizer regime being used in target environments. Based on our findings, when no N fertilizer is applied in the fresh water environments being targeted, then breeders should seek to increase spikelet number primarily through total grains per panicle and then through panicle number. However, in high N fertilizer regimes under fresh water conditions, increase in spikelet number should be attempted primarily through an increase in panicle number m^{-2} and then total grains per panicle.

The importance of increased spikelet production and high number of panicles for high yield performance in fresh water environments has been reported elsewhere (Reuben and Katuli, 1988; Gravois and McNew, 1993). In our trials, grain weight, on the other hand, was negatively correlated with grain yield in fresh water environments. Different researchers have reported positive (Suarez *et al*., 1989; Zeng *et al*., 2000b), negative (Vlek *et al*., 1979; De and Rao, 1988) and insignificant (Panwar *et al*., 1989) relationships between grain weight and grain yield of rice in fresh water environments. Similar conflicting findings have been reported about the relationship between spikelet fertility and grain yield of rice in fresh water. Sarawgi *et al.* (1997) found a strong positive relationship between spikelet fertility and grain yield while Gravois and Helms (1992) reported that filled grain per panicle was not an important contributor to yield in fresh water conditions. Vlek *et al.* (1979) found a negative correlation between percentage of spikelets maturing and yield at zero, medium and high levels of N fertilizer usage. In our trials, spikelet fertility had a weak correlation with grain yield in fresh water environments. These conflicting reports on the relationships between

yield and grain weight and yield and spikelet fertility could be due to the use of different rice populations (Dewey and Lu, 1959) or different environmental conditions between trial sites.

In saline environments, all yield components contributed positively to grain yield in our trials. However, at both levels of N fertilizer application in the saline environments, grain weight and spikelet fertility contributed more towards yield determination than panicle number m^{-2} and total grains per panicle. The limited compensation between earlier and later-formed yield components of rice under salt stress conditions has been reported before (Zeng *et al.*, 2001). Assimilate production is suppressed under salt stress due to the inhibitory effects of high salt concentrations in the soil on nutrient uptake, LAI and photosynthesis (Khan *et al.*, 1997) leading to source limitation of yield. This low production of assimilates would mean that at the specific period when a particular component is determined, assimilate supply would not be sufficient to enable the component to reach such a high level that it would exert a negative influence on the level of other correlated components. The reduced total spikelet production (comprising panicle number m^{-2} and total grains per panicle) means there would be less physiological competition between total spikelets and grain filling attributes (comprising weight and spikelet fertility) under salt stress. Thus, in saline environments all yield components were positively correlated with yield. Furthermore, the accelerated leaf senescence due to accumulation of salts in crop leaves under salt stress, negatively influences assimilate production during the grain filling period and when source limitation is experienced after flowering grain filling will be affected (Yoshida, 1981). Hence under salt stress, rice genotypes with better grain-filling abilities should be able to yield higher than those with poor grain-filling.

The physiological traits subjected to path analysis in our study were those collected around flowering time. Path analysis gave us strong indications about the effects of physiological traits collected around flowering time on yield and yield components in different environments. However, as we saw earlier, the degree of association between physiological traits and yield components varies between developmental stages and also between environments. Thus to determine the key physiological traits influencing the size and/or number of yield components requires a detailed knowledge of the phenology of crop plants in different environments complemented by knowledge of times of determination of individual yield components.

In fresh water environments, among the physiological traits collected around flowering stage, shoot biomass, stem weight, leaf weight and dry matter allocation to shoots had the strongest influences on grain yield of rice. The positive associations between shoot biomass and panicle number m^{-2} in S1N1 and shoot biomass and total grains per panicle in S1N2 as well as the positive contributions of the paths connecting

shoot biomass to yield through panicle number m^{-2} and total grains per panicle (in S1N1 and S1N2, respectively) would enable genotypes with high shoot biomass at flowering time to produce high yields. Regarding the effect of leaf weight on yield, production of more green leaves in fresh water would be beneficial for the rice crop for this would enable it to produce more assimilates through photosynthesis. This increased assimilate production by the crop would eventually lead to high yields because more dry matter will be available for storage in the grains.

Delayed flowering in fresh water environments enables the rice crop to accumulate more dry matter, which will eventually be available for grain production. Thus for rice genotypes differing only in flowering duration, late flowering genotypes in such environments should be able to yield higher than early flowering genotypes.

Stem weight had a strong negative correlation with yield in fresh water environments due to its large negative direct effects on panicle number and total grains per panicle. The 15 genotypes of rice assessed in this study differed in plant height and in both fresh water environments tall genotypes lodged more than the semi-dwarf genotypes (data not shown). Lodging decreases yield as a result of reduced light penetration into the canopy, loss of grains from panicles lying in water and possibly increased disease incidence due to the high humidity within the lodged crop. Furthermore, among the 15 genotypes used in our study, taller genotypes generally had heavier stems than shorter genotypes around flowering. As stems comprise mostly structural material it is desirable to have low stem weights at flowering and high leaf and panicle weights because these latter two would contribute more towards yield than stems.

Both leaf N concentration and LAI were positively associated with yield in the zero N fertilizer regime in fresh water although their contributions were much smaller than those of shoot biomass and leaf weight. This was expected as leaf N concentration and LAI act on yield and yield components through the vegetative and reproductive structures. The limitations of the analytical models used in path analysis restricted us to treat all physiological traits as acting directly on yield components. However, both leaf N concentration and LAI had strong positive correlations with shoot biomass and leaf weight, both of which had positive effects on yield in the zero N fertilizer regime in fresh water. Hence leaf N concentration and LAI also had positive effects on grain yield in this environment.

The strong negative effect of the fraction of DM allocated to shoots on yield in the zero N fertilizer regime in fresh water could be understood from the fact that this coefficient is the complement of the fraction of DM allocated to roots. Since N supply to crops would be limiting in fresh water environments without N fertilizer, it is desirable to have a large root system in such a situation in order for the rice plant to absorb more N from the soil (Yoshida, 1981) and thus increase its photosynthetic

activity. With the application of N fertilizer in fresh water conditions the negative effect of fraction of DM allocated to shoots on yield diminished apparently because even genotypes with smaller root systems were able to absorb sufficient N from the soil. Thus according to our results, for high yield performance in fresh water environments, rice genotypes should possess high shoot biomass, high leaf weights, low stem weights and allocate proportionately more biomass to roots instead of shoots. Additionally, at zero N fertilizer levels in fresh water a high leaf N concentration and high LAI are advantageous and at high N fertilizer levels late flowering can also lead to increased yields.

In saline environments, among the studied physiological traits only fraction of DM allocated to shoots had a consistent, strong and positive effect on yield at both levels of N fertilizer application. The effects of other physiological traits on yield varied strongly between the two levels of N fertilizer application. In the zero N fertilizer regime, a high shoot biomass and high allocation of dry matter to shoots around flowering time, were more important for yield determination than other physiological traits considered. This was due the positive effects these two traits had had on grain weight and spikelet fertility, which together comprise grain filling ability. Grain filling ability has been reported to be a major determinant of rice yield under saline stress conditions (Buu and Truong, 1988). Reduced grain filling under salt stress is caused by insufficient production of DM as a result of decreased photosynthetic activity (Li *et al*., 1998) and reduced translocation of assimilates from the sources (leaves) to the sink (grain) (Asch *et al*., 1997; Abdullah *et al*., 2001). Rice genotypes that are able to produce more shoot biomass under such highly stressful environments can then allocate more biomass to grains during yield formation. Furthermore, salinity decreases shoot growth proportionately more than root growth thereby causing an increase in the root/shoot ratio (Orcutt and Nilsen, 2000). Thus allocating more biomass to shoots instead of roots would enable plants to have smaller root systems which would reduce the detrimental effects of a saline growth medium on the roots (Lin and Kao, 1999) and at the same time increase shoot biomass.

The negative effect of leaf weight around flowering time on yield in the zero N fertilizer regime in saline water was due to the negative correlation between leaf weight and grain weight. Indeed, leaf weight around flowering time had negative direct effects on grain weight in all test environments except in the high N fertilizer regime in saline water. Cui *et al.* (2002) also found a negative relationship between leaf area at flowering time and both grain filling percentage and grain yield. This implies that under certain growth conditions, leaves and grains might possibly be competing for assimilates around flowering time. Assimilate production under saline conditions without N fertilizer application, would be very low due to the combined

effects of salt stress and low N supply. Thus, possibly, competition between leaves and grain filling attributes for scarce assimilates caused the negative total effect of leaf weight on grain yield in the zero N fertilizer regime in saline water. Furthermore, leaf weight had a significant direct correlation only with grain weight and not with any other yield component in the zero N fertilizer regime in saline water, hence its effect on yield was eventually negative. This was a result of the fact that grain weight accounted for more variation in yield in the zero N fertilizer regime in saline water than any other yield component did. Thus based on our findings, in saline environments where no N fertilizer is applied, the key physiological traits around flowering time to be considered for inclusion in breeding for high yielding genotypes are high shoot biomass production, high allocation of dry matter to shoots and low leaf weights.

In saline environments with high N fertilizer application, leaf weight had a positive effect on yield as opposed to the negative effect it had on yield in the zero N fertilizer regime. The increased supply of N to the crop in the high N fertilizer regime would lead to more open leaves and a higher LAI than in zero N fertilizer regime thus enabling the crop to produce more assimilates and eventually higher yields. High leaf weight in this environment, increased total grains per panicle and grain weights, both of which contributed positively to yield. In both saline environments, stem weight had a positive effect on yield although this effect was much larger at the high N fertilizer level than at the zero N fertilizer level. Remobilization of assimilates from stems to panicles is said to be an important source of assimilates for grain production under abiotic stress conditions since under such situations yield is source-limited. Up to 40% of the weight of stems could be translocated to grains when assimilate production is insufficient to fill the grains (Yoshida, 1981; Samonte *et al*., 2001) during the period of grain filling. The positive effect of a high stem weight on spikelet fertility in this environment that we saw in our research is in conformity with these reports. Late flowering, on the other hand, had a strong negative effect on yield due to its strong negative effect on spikelet fertility in this environment. Hence based on our findings, when N fertilizer is applied in saline environments, high yielding genotypes of rice should possess high stem and leaf weights, high allocation of dry matter to shoots and flower early.

Conclusions

The main yield components involved in yield determination differed between test environments especially between fresh and saline water environments. In fresh water, production of more spikelets (comprising panicle number m^{-2} and total grains per panicle considered together) was more important for yield determination while in saline environments grain filling attributes (comprising grain weight and spikelet

fertility) were more important in yield determination. In fresh or saline water the relative importance of one of the two most important components is determined by the N fertilizer regime. In fresh water and no N fertilizer, total grains per panicle accounted for more variation in yield than panicle number m^{-2} but in the high N fertilizer regime total grains per panicle accounted for less variation in yield than panicle number m^{-2}. In saline environments, with zero N fertilizer application, grain weight accounted for more variation in yield than spikelet fertility while in the high N fertilizer regime, grain weight accounted for less variation in yield than spikelet fertility.

Yield levels in different environments can be increased through breeding or agronomic practices by manipulating the physiological traits that most influence key components for yield determination in the particular cultivation environment. Increased biomass production is desirable in all cultivation environments because this is positively associated with key yield components in all four test environments. Stem weight should be reduced in fresh water environments and increased in saline environments in order to increase yields. Leaf weight had a complex relationship with yield although its effects on yield were largely positive. According to our results, leaf weight at flowering should be increased in fresh water environments and in saline environments with high N fertilizer application but in zero N fertilizer regimes under salt stress, leaf weight at flowering should be reduced for increased yield performance.

The fraction of biomass allocated to shoots (FSH) is less important as a selection criterion when a high amount of biomass is produced than when the crop cultivation environment limits biomass production. In fresh water, a low FSH should be selected for, especially in zero N fertilizer regimes because this will favour larger root system development and hence better nutrient absorption. In saline environments a high FSH should be selected, because this will reduce root/shoot ratio and increase grain filling thus leading to higher yields. Delayed flowering is useful in fresh water environments because it promotes higher panicle number m^{-2}, which is important for yield determination. In saline environments early flowering should be encouraged because high TSUMF has a strong negative effect on grain filling in saline environments.

Leaf N concentration and LAI at flowering should both be increased although their effects on yield are rather limited compared to those of biomass-related plant characteristics. Leaf N concentration had an effect on yield only in the zero N fertilizer regimes in both fresh and saline water environments. LAI had a significant association with yield only in the fresh water environment where no N fertilizer was applied.

Altogether, it is concluded that the potential exists to increase rice yields in both high and low potential environments when a better understanding is gained of the relationships between physiological traits, yield and yield components in target environments.

CHAPTER 5

Phenological and physiological differences between high- and low-yielding genotypes of rice

Phenological and physiological differences between high- and low-yielding genotypes of rice

Abstract

Yield differences between rice genotypes arise from differences in phenology and physiology under variable growing conditions. To understand the physiological traits that enable high-yielding ability of rice in different environments, a collection of diverse genotypes of rice were grown over a two-year period under fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer. Significant genotype × salinity × nitrogen fertilizer interaction was expressed for grain yield by two different sets of Recombinant Inbred Lines (RILs) from the same population together with their parents, IR29 and Pokkali, in years 2000 and 2001 as well as for two improved cultivars ROK5 and ITA212 grown only in year 2001. Subsequent to this, LAI, specific leaf N, dry matter production, growth duration and partitioning of assimilates between organs, were compared between high- and low-yielding genotypes (comprising five RILs, IR29, Pokkali, ROK5 and ITA212) at different growth stages in different environments in year 2001. Salt stress of 6 d Sm^{-1} reduced LAI, biomass production and yield while increasing specific leaf N and growth duration of most genotypes of rice. Application of 100 kg ha^{-1} N fertilizer, on the other hand, generally increased LAI, biomass production and yield, and slightly delayed maturity of most genotypes assessed. The apparent response of genotypes to applied N fertilizer was stronger in saline water than in fresh water conditions and there was large variability for this trait among the nine genotypes of rice studied. In fresh water environments, rice genotypes that had large leaf areas and high specific leaf N produced more biomass than those with smaller levels of these two traits. Under saline conditions, however, high biomass production was more closely associated with a large leaf area than with high specific leaf N. Furthermore, in both fresh water and saline environments, late-maturing genotypes produced more biomass than early-maturing genotypes with similar LAI and specific leaf N. High biomass production was associated with high yielding ability in both fresh and saline water conditions although the fraction of dry matter allocated to panicles determined the yield level attained in the different environments from the biomass produced. In saline environments, early vigour was found to be more relevant for high yielding ability than high final biomass production. In addition, salt-tolerant genotypes of rice allocated proportionately less biomass to roots and more to shoots than salt-sensitive genotypes.

Introduction

In the developing world, rice is the most important staple food. According to projections, the population in the Less Developed Countries (LDCs) will reach 6.4 billion by the year 2020 (App, 1986). This necessitates a huge increase in world rice production in order to meet the food requirements of this burgeoning population. Realising this increased rice production would require improvements in the productivity of present rice lands through the use of more efficient cultivars of rice and better agronomic practices. Another possible solution is to expand the area of rice production into new areas where environmental stresses had precluded rice growing earlier. Both these approaches demand the use of cultivars of rice that are better able to withstand the environmental stresses in present or potential rice fields.

Salinity is one of the major obstacles to increasing production in rice growing areas worldwide. An estimated 1.5 million ha of cultivable mangrove swamps in West Africa is affected by salinity (Jones, 1986). Although there are extensive studies of salinity effects on rice, our understanding of the quantitative effects of salinity on rice and critical thresholds of responses, especially with respect to modern, commonly used cultivars is still limited (Zeng *et al*., 2000a). Many attempts by national and international research institutes to introduce improved rice production technology failed, partly because of the highly variable soil salinity (Sylla, 1994).

The tolerance of rice to salinity stress varies with the growth stage. The germination stage is the most tolerant to salinity (Levitt, 1980; Gill and Singh, 1989). Rice is least tolerant to salinity during the seedling stage but its tolerance increases with age (Gill and Singh, 1989) although the flowering stage is also another sensitive stage (Lutts *et al*., 1996; Gill and Singh, 1989). It is thus important to consider the salinity tolerance of rice at different growth stages in order to gain insight into the mechanism of salinity tolerance in rice and the contribution of the various components of salinity tolerance to final grain yield. This stress is often associated with an insufficient supply of nitrogen and therefore it is interesting to study the response of rice to both factors.

Nitrogen holds the key to productivity of all cereal crops (Ponnamperuma and Deturck, 1993). It is required in comparatively large quantities by plants and is usually the most limiting nutrient in soils for cereal production. Hence, to be able to achieve and sustain the higher crop yields necessitated by an ever-expanding world population, without causing environmental problems, would require the use of more efficient cultivars better able to extract essential nutrients from their environments. This translates to an improved nutrient recovery from the soil, and improved nutrient utilisation efficiency. Such cultivars will increase the efficiency of crop production systems. In high-potential environments such cultivars would increase the economic efficiency of chemical fertilizers while at the same time reducing environmental pollution especially

with highly soluble and volatile nutrients such as nitrogen. As varietal intervention represents an easy and usually environmentally friendly way of improving crop production, these cultivars would also be useful in low-potential environments because they will help resource-poor farmers to increase their yields under low-input conditions.

Salt stress affects the growth and development of crops and since low nitrogen supply to crops also affects growth and development of crops it will be interesting to study the relationship between the response of rice to these two stresses. Salinity stress in crops leads to a reduced effective leaf area and dry matter production (Ashraf and Ali, 1998). Furthermore, salt stress has been reported to increase the protein and total nitrogen content of rice cultivars (Krishnamurthy *et al*., 1988). Jadav *et al.* (1976) also reported finding higher concentrations of total N but lower concentrations of nitrate-N in salinized wheat plants at maturity than in non-salinized ones although the uptake of N from the soil was reduced under salinity. These findings point to the fact that salinity affects the nitrogen status and other physiological parameters of crop plants.

Photosynthesis provides the raw materials for all plant products from which yield is eventually derived (Richards, 2000) and the longer crops maintain photosynthetic activity through a longer growth duration, the higher the potential yield (Ying *et al*., 1998). Crop photosynthesis itself is influenced by incident solar radiation, photosynthetic rate per unit leaf area, leaf area index and leaf orientation (Yoshida, 1981). The photosynthetic apparatus is also sensitive to leaf nitrogen status for most of the leaf nitrogen is present in photosynthetically active compounds (Dreccer *et al*., 2000; Makino *et al*., 2000). The decision to allocate much of the biomass formed from photosynthesis to harvested organs (a high harvest index) instead of vegetative structures is very crucial in yield formation. Many modern high yielding rice cultivars have high harvest indices (Yoshida, 1981). An understanding of the genetic differences in crop-related photosynthetic parameters between cultivars and the effect of cultivation environment on these traits can help crop breeders select more effectively the combination of physiological traits that can lead to higher yields in different environments. The contributions of physiological studies to breeding so far, however, have been modest to small. One reason for this could be a predefined or different focus in most physiological studies, using cultivars with a limited genotypic range in a wide range of environments (Yin *et al*., 2000). Thus for a better integration of physiological research with breeding, it is necessary to work with relevant populations (Jackson *et al*., 1996).

The presence of abiotic stresses in farmers' fields is one of the reasons why crop yields from farmers' fields are usually so much lower than those from experimental fields leading to the so-called yield gap. In experimental fields these stresses are controlled to a large extent through improved management practices. Reducing this yield gap would necessitate the development of crop cultivars capable of producing

high yields in farmers' fields (Peng *et al*., 1999). One possible way of attaining this is to augment traditional breeding methods with related disciplines such as crop physiology. It is presumed that with better knowledge of crop physiology, deficiencies in cultivars and options for improvement may be identified, so that breeding targets can be defined more effectively. Furthermore, expression of physiological traits depends on genotype × environment interaction, and may show considerable variation. Thus the importance of physiological traits may not be evident at different locations or even at the same location, under different management practices. However, selection based on physiological traits with high heritabilities and major impacts on yield could assist in obtaining genetic gain (Bindraban, 1997).

The objective of this study was to find out the physiological and phenological differences between high and low yielding genotypes of rice that might have implications for breeders in their efforts to improve rice yields under different environmental conditions.

Materials and methods

Plant material

A segregating population of rice, comprising 276 recombinant inbred lines (RILs), developed at IRRI from the cross IR29 × Pokkali (both indica varieties) was used. IR29 is a short, high yielding modern cultivar released by IRRI (International Rice Research Institute) and is known to be very sensitive to salinity (Gregorio, 1997). Pokkali is a tall, traditional variety from India known to be tolerant to salinity (Yeo and Flowers, 1986; Garcia *et al*., 1995; Gregorio, 1997). As one of the two parents is a short, high-yielding, modern cultivar and the other is a tall traditional variety, it is expected that the RIL population will also be segregating for nutrient use efficiency. This is due to the fact that modern rice cultivars have been bred for response to high levels of mineral fertilizer usage while traditional varieties have usually been selected by farmers in environments with sub-optimal levels of nutrient supply (see Chapter 2).

The RIL population, together with the parents were grown during the rainy season (June-Oct.) in 1999 and 2000 in a series of genetic experiments at Sapu (13.55° N latitude), in The Gambia (see Chapter 2). One hundred and sixty RILs were randomly selected from the population of 276 RILs for use in our study. From these 160 RILs, 100 were used in the trials of 1999 and 2000 (see Chapter 2). Yield and yield component data were collected from these 100 RILs and their parents. After exhaustive analyses of the two-year yield data, 38 RILs were selected from the 160 RILs for high-, medium- and low-yielding ability in the different test environments based on the yields and molecular marker signatures of the different RILs. The results of these genetic studies are shown

elsewhere (see Chapter 2). Of these 38 RILs, 22 were grown before in our trials of 1999 and 2000 while the remaining 16 RILs had not been grown before in our trials.

In 2001, these selected 38 RILs together with the two parents were then grown at the same experimental site using the same split-split plot design. Two rice cultivars were introduced into the trial in 2001 – ROK5 and ITA212. Thus a total of 42 RILs and cultivars were grown in 2001. ROK5 is a tall, moderately salt-tolerant cultivar bred in Sierra Leone (WARDA, 1994) and has been used in the saline swamps of West Africa for many years. ITA212 is a semi-dwarf, high-yielding Tropical *japonica* cultivar bred at the International Institute of Tropical Agriculture (IITA), in Ibadan, Nigeria.

Field experiments

A split-split plot experimental design was used with salinity as the main plot factor, rate of nitrogen fertilization as the sub-plot factor and genotype as the sub-subplot factor. Each sub-subplot plot measured 2.6 m × 3 m and a spacing of 20 cm × 20 cm within and between rows was used. Two levels of salinity and two levels of nitrogen fertilization (giving a total of four treatment combinations) were tested. Three replications were maintained in each year of the trials. Additional information on the trials is given in Table 1. The following treatment combinations also referred to later in the text as test environments were obtained:

S1N1 – Fresh water (river water) at an electrical conductivity (EC) of 0.15 dS m^{-1} and 0 kg N ha^{-1};

S1N2 – Fresh water (river water) at an EC of 0.15 dS m^{-1} and 100 kg N ha^{-1} applied as urea;

S2N1 – Salt water at an EC of 8 dS m^{-1} and 0 kg N ha^{-1};

S2N2 – Salt water at an EC of 8 dS m^{-1} with 100 kg N ha^{-1} applied as urea.

Pre-germinated rice seeds were sown in a nursery that was well watered and regularly weeded. Around three weeks after emergence (22 DAS – days after sowing) the seedlings were transplanted to the field following the experimental design described above. After transplanting the trial plots were kept continuously flooded by irrigating with river water till all RILs and cultivars were close to physiological maturity. Salinity was imposed by manually broadcasting measured amounts of granular table salt in standing water to attain the required salinity.

Salinity of the ponded-water was measured two days after every significant rainfall or after a protracted period without rains (more than four consecutive days). An ES-421 salt meter (Atago Co. Ltd., Japan) was used to measure salinity levels. When the salinity level was too low more salt was added to raise the salinity and when the

Table 1. Details of environmental conditions and field experiments conducted in years 2000 and 2001 (Sapu Research Station, The Gambia).

	2000	2001
Sowing date	8 June	9 July
Transplanting date	4 July	31 July
Salinity (dS m^{-1})	~8	~8
Total rainfall (mm)	1326	754
Average minimum temp. (°C)	22.4	22.5
Average maximum temp. (°C)	34.0	34.0
Basal fertilizers (kg ha^{-1})		
P (Triple Super Phosphate)	40	40
K (Muriate of Potash)	40	40

salinity was too high the saline plots were irrigated with fresh water to reduce the salinity to the desired level.

Data collection

Physiological data were collected on 11 RILs plus the two parents and ROK5 and ITA212. The physiological data collected on these 15 genotypes included dry weights of roots, stems, leaves and panicles, leaf area index (LAI) and leaf nitrogen concentration (%). In this chapter, the physiological discussions will focus on five (L15, L57, L87, L96 and L146) out of the 11 RILs, the two parents (IR29 and Pokkali) and the two improved cultivars (ITA212 and ROK5). In the results and discussion sections, physiological and phenological properties of high-yielding L15, L87, L146, IR29 and ITA212 will be contrasted against those of poor-yielding L96 and POKKALI in fresh water environments. Likewise the same properties of salt-tolerant L15, L57, POKKALI and moderately tolerant ROK5 will be contrasted against those of salt-sensitive L96, L146 and IR29 in the saline environments. IR29, ITA212, L15, L87, and L146 are semi-dwarf lines and Pokkali, L57, L96 and ROK5 are tall lines (Fig. 2).

Physiological data were collected on the same days for all 15 genotypes at roughly two-week intervals (71, 85, 98, 111, 127 and 133 DAS). Most genotypes flowered around 98 DAS and matured around 127 DAS. On each sampling date, four plant hills were uprooted and separated into roots, leaves, stems and panicles depending on the developmental stage. Only green leaves were measured. Dead leaves were removed but not weighed. LAI was measured with the AccuPAR model PAR80 Ceptometer, (Decagon Devices, Inc., USA).

Dry matter was determined by air-drying the separate organs under shade followed

by oven drying at 70 °C till a constant weight was attained. Leaf nitrogen concentration (%) was obtained from dried leaf samples, which were then processed using the micro-Kjeldahl process. Partitioning coefficients were calculated following the method used by Kropff *et al.* (1994). Five different partitioning coefficients were calculated:

FRT – partitioning coefficient of total dry matter allocated to the root
FSH – partitioning coefficient of total dry matter allocated to the shoot
FLV – partitioning coefficient of shoot dry matter allocated to the leaves
FST – partitioning coefficient of shoot dry matter allocated to the stems
FSO – partitioning coefficient of shoot dry matter allocated to panicles (storage organs).

LAI and leaf N concentration were measured up to two weeks after most lines flowered. No observed values were available for these parameters at maturity.

The dates of sowing, transplanting, flowering and physiological maturity were recorded for all lines. Weather data including daily minimum and maximum temperatures, were collected from the local weather station. The dates of sowing, transplanting, flowering and maturity together with the weather data, were entered into the computer program DRATES (Kropff *et al*., 1994) which then used this information to compute temperature sum, in heat units, required by each line of rice to complete development stages from sowing to flowering and then maturity. The temperature sum required during grain filling was determined by computing the difference between the temperature sum from sowing till maturity and the temperature sum from sowing to flowering.

At maturity, net plots were harvested for each sub-subplot leaving one border row at each of the four sides of the plots. The harvested grain was then threshed, cleaned, dried and then weighed to determine grain yield in kg ha^{-1}.

Data analysis

The SAS (1999) statistical package was used to perform analysis of variance and compute standard errors of mean organ weights, LAI and leaf N concentration for the 15 RILs and cultivars. Afterwards Microsoft Excel was used to plot graphs of sampling date against organ dry weights and partitioning coefficients for L15, L57, L96, L146, Pokkali, IR29 and ROK5 in saline environments and L15, L87, L96, L146, Pokkali, IR29 and ITA212 in fresh water. The yield response of these genotypes to salinity and N fertilizer were estimated as:

$$SxN\ (\%) = \frac{SxN2 - SxN1}{SxN1} \times 100 \qquad \text{(i)}$$

where, *SxN* is the apparent response to N fertilizer at '*x*' level of salinity; *x* has two levels – 1 and 2; *x* = 1 refers to fresh water environments and *x* = 2 refers to saline environments.

$$SNx\ (\%) = \frac{S1Nx - S2Nx}{S1Nx} \times 100 \qquad \text{(ii)}$$

where, *SNx* is the salt tolerance index (percent yield loss in salt water relative to fresh water) at '*x*' level of N fertilizer application; *x* has two levels – 1 and 2; *x* = 1 refers to 0 kg ha^{-1} and *x* = 2 refers to 100 kg ha^{-1} N fertilizer application.

Relationship between yield and physiological traits

A simplification of the sequence of events leading to yield formation is depicted in Fig. 1. Leaf N status has a strong influence on photosynthesis due to its effects on radiation use efficiency (amount of canopy photosynthesis per unit radiation absorbed), LAI and the length of time that leaves can maintain their greenness (Dreccer *et al*., 2000). Incident solar radiation is intercepted by the leaves, which are the seat of photosynthetic activity in plants. Thus a high LAI is advantageous in that it would allow plants to intercept more sunlight and then effect more photosynthesis than plants with smaller LAI values. A long growth duration allows plants to maintain photosynthetic activity longer thereby leading to more biomass production. The partitioning of biomass to different organs is dictated by the phenology of the crop. Leaf N concentration, LAI and growth duration of the genotypes of rice cited above were compared to determine whether differences in these during the growth season corresponded with high or low biomass production in different environments. The partitioning coefficients of DM to different organs and weights of organs were also compared between the genotypes to help explain yield formation from biomass produced.

Results

Yield response to salinity and N fertilizer

Significant yield differences were observed between genotypes when grown in environments with different levels of salinity and nitrogen fertilizer in both 2000 and 2001 (Table 2). This implied that differences in yielding ability among the tested genotypes in either fresh or saline water depended on whether the genotypes were grown under N limiting or high N supply conditions. Henceforth yield and physiological properties of the nine genotypes mentioned above will be discussed for the four combinations of salinity × nitrogen studied in this research, which were, S1N1, S1N2, S2N1 and S2N2.

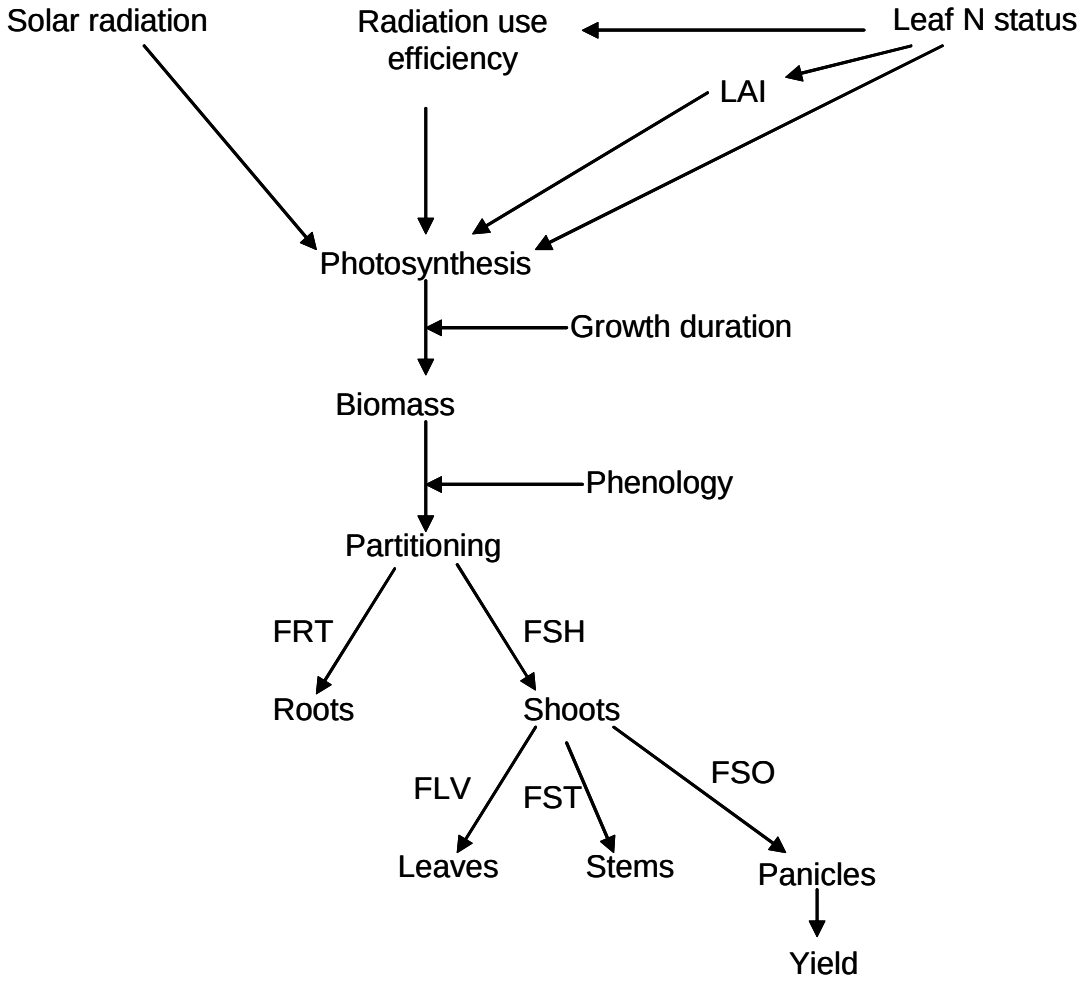

Fig. 1. Schematic representation of processes leading to yield formation in rice. (Partitioning coefficients of dry matter to roots (FRT), shoots (FSH), leaves (FLV), stems (FST) and panicles (FSO)).

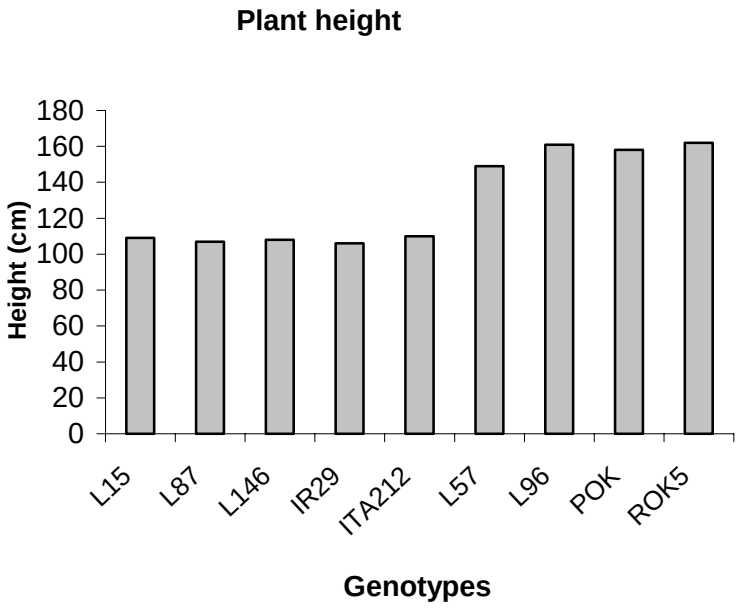

Fig. 2. Plant height (cm) of different genotypes of rice in fresh water.

The general patterns of yield response to salinity and N fertilizer of the 42 genotypes grown in 2001 were similar to the patterns observed for the 100 genotypes grown in 2000 (Fig. 3). However, these responses were more pronounced in 2001 than in 2000 due to the selection practised and the higher rainfall in 2001 compared to 2000.

There were marked differences between the genotypes concerning their yield response to salinity and N fertilizer. Pokkali and ROK5 responded negatively to N fertilizer in fresh water whereas IR29, L57, L87 and L96 gave positive but poor responses to N fertilizer in fresh water (Table 2). L146 and ITA212 showed the strongest positive responses to applied N fertilizer in fresh water while L15 showed a moderate response to applied N fertilizer in the same environment. The response of the genotypes to N fertilizer was higher in saline water than in fresh water. In saline water Pokkali and IR29 gave small positive responses to N fertilizer. L15, L57 and L87

Table 2. Yields and responses (%) to salinity and N fertilizer of 5 RILs (L15, L57, L87, L96 and L146) and 4 cultivars (Pokkali, IR29, ITA212 and ROK5) of rice grown in different environments in 2001.

Genotype	Yield (kg ha^{-1}) in different environments				Response (%) to salt and N			
	S1N1*	S1N2	S2N1	S2N2	S1N	S2N	SN1	SN2
Parents								
POKKALI	2247a	1975a	1981c	2530cd	–13	28	12	–29
IR29	4172cd	4823ef	467a	543a	16	17	89	89
RILs								
L96	2440a	2694b	622a	1387b	11	123	75	49
L57	2940ab	3334c	1744c	2634cd	14	52	41	21
L15	3452bc	4364de	1521bc	2469cd	27	63	56	44
L146	3683cd	5404f	463a	1382b	47	199	88	75
L87	4087cd	4665e	1076ab	1537b	15	43	74	68
Cultivars								
ITA212	4076cd	5344f	654a	1423b	32	118	84	74
ROK5	4234d	3958cd	1006ab	2954cd	–7	194	77	26
Mean	3482	4063	1060	1874	17	77	70	54

* In each of the columns S1N1, S1N2, S2N1 and S2N2, figures followed by different letters are significantly different from each other ($P < 0.05$).

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S1N, S2N – apparent response to N fertilizer in fresh and saline water, respectively; SN1, SN2 – salt tolerance index at zero and 100 kg ha^{-1} N fertilizer application, respectively).

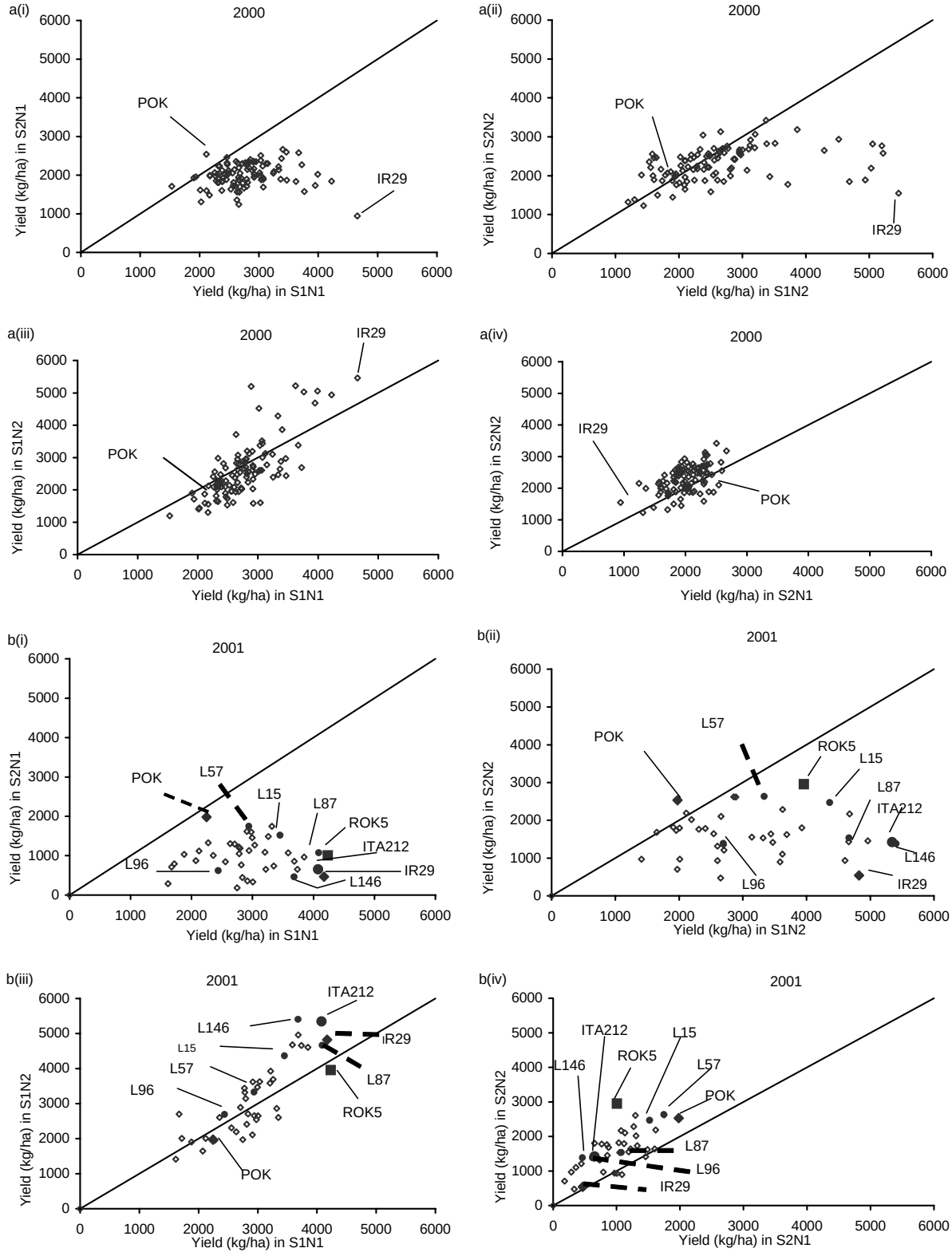

Fig. 3. Scatter plots showing G × E for yield in a RIL population and their parents, IR29 and Pokkali, in year 2000 (n = 104) plus yields of selected genotypes of rice in year 2001 (n = 42). See text for explanation of acronyms.

responded moderately to N fertilizer compared to other genotypes whilst L146, L96, ITA212 and ROK5 gave strong positive responses to N in saline water (Table 2).

With regards to the yield responsiveness to salinity, Pokkali, L57 and L15 tolerated salt stress better than the other genotypes in the zero N fertilizer regime due to the lower yield losses they suffered compared to IR29, L87, L96, L146, ITA212 and ROK5 all of which showed more than 70% yield loss under saline conditions in the zero N fertilizer regime compared to their yields in fresh water. Salt tolerance of all lines was higher in the high N fertilizer regime than in the zero N fertilizer regime. At the high level of N fertilizer application, Pokkali yielded higher in saline water than it did in fresh water, thus indicating a strong level of salt tolerance at this level of N fertilizer application. L57 and ROK5 showed good tolerance to salinity in the high N fertilizer regime whereas L15 and L96 were moderately tolerant to salinity at the high level of N fertilizer application. IR29, L87, L146 and ITA212 still showed strong sensitivity to salinity in the high N fertilizer regime although their yield losses were smaller than in the zero N fertilizer regime (Table 2).

Physiological responses

Growth duration

Salt stress delayed flowering and maturity of most lines under both zero and high N fertilizer conditions although flowering was delayed more than maturity. This led to a general reduction in the grain filling duration for most lines under saline conditions compared to fresh water conditions (Fig. 4). With the sole exception of IR29, N fertilizer application in fresh water had the effect of increasing the grain filling duration by either delaying flowering, maturity or both. In saline water also, N fertilizer application increased grain filling duration of most lines, except for L15 and IR29, by delaying flowering and maturity. For IR29 flowering was delayed more strongly than maturity and this led to a short grain filling duration for this line.

LAI, leaf N concentration, dry weights of organs and partitioning coefficients

Compared to the fresh water environment, salt stress led to reductions in weights of plant organs (roots, stems, leaves and panicles) and LAI but increased leaf N concentration of all lines at both the zero N and high N fertilizer rates. On the other hand, application of N fertilizer was found to increase dry weights of organs of all lines in both fresh water and saline environments.

Fresh water with 0 kg ha^{-1} N fertilizer In this environment, L87, L96 and ITA212 clearly produced more biomass than IR29, Pokkali, L15 and L146 did (Fig. 5a(v)).

The former three lines maintained higher levels of leaf N concentration during the growth season especially around flowering (DAS 98) and two weeks after flowering (DAS 111) than the latter lines (Fig. 5a(i)). With regards to LAI, L87 and ITA212 both had large leaf areas throughout the growth season although L87 had a higher LAI than ITA212 (Fig. 5a(iii)). L96 initially had a low LAI but around two weeks after flowering (DAS 111) it had the highest LAI of all the lines compared. In addition L96 and ITA212 matured later than the other lines (Fig. 4a.) and this would allow them to maintain their photosynthetic activities longer than the earlier maturing lines. These properties considered together would enable L87, L96 and ITA212 to produce more biomass than the other lines. Pokkali had a low LAI, low leaf N concentration and

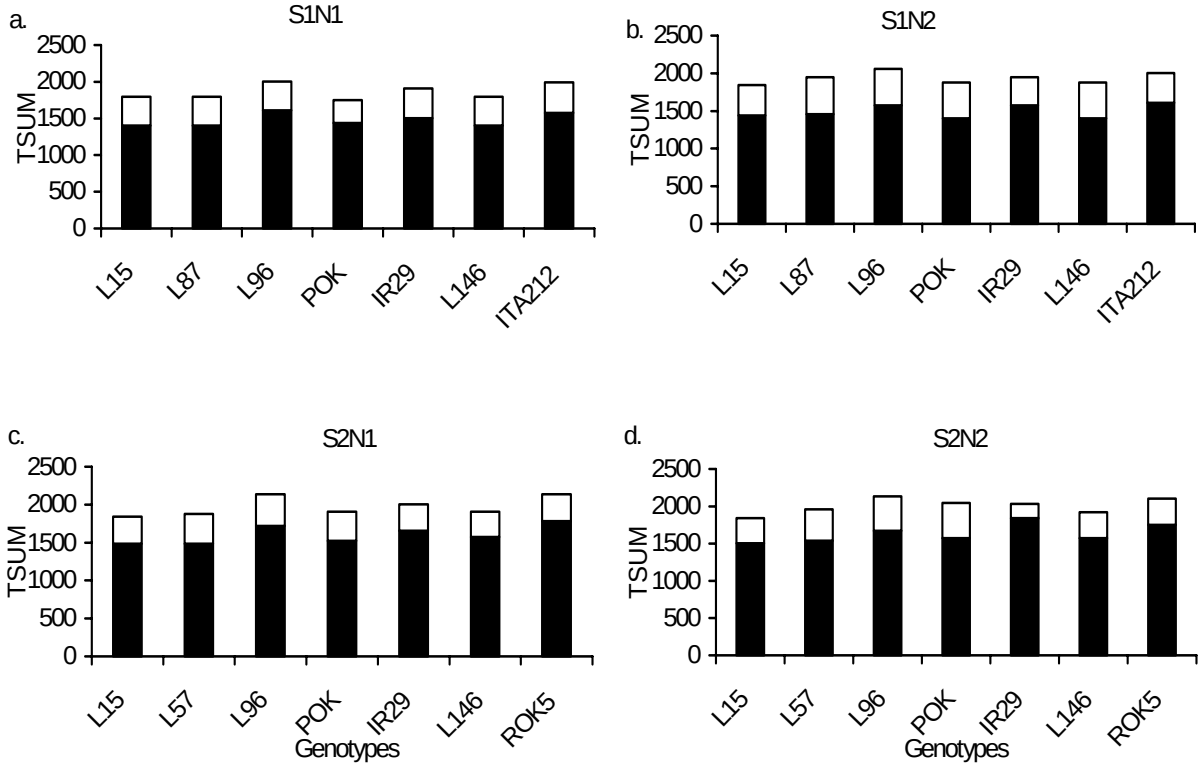

Fig. 4. Temperature sum (TSUM – °Cd) from sowing to flowering and maturity of rice genotypes across environments. Rice genotypes L87 and ITA212 in fresh water environments were replaced by L57 and ROK5, respectively, in saline environments. (Full height of bars represent temperature sum from sowing to maturity; filled portions represent temperature sum from sowing to flowering; and blank portions represent temperature sum during grain filling. S1N1, S1N2 – fresh water with or without application of 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with or without application of 100 kg ha^{-1} N fertilizer, respectively).

matured early. It thus produced the lowest amount of biomass in this environment compared to other lines. IR29 on the other hand had a large leaf area around flowering and after flowering but leaf N levels comparable to those of Pokkali at these growth stages and thus produced less biomass than L87, L96 and ITA212. L15 and L146 both had low LAI, low leaf N concentrations and matured early and could therefore only produce low quantities of biomass in this environment.

The three lines L87, L96 and ITA212 also maintained higher leaf (Fig. 5b(iii)) and stem weights (Fig. 5b(v)) than the other lines although there were no clear patterns of differences between genotypes regarding partitioning coefficients of dry matter to leaves (Fig. 5c(iii)) and stems (Fig. 5d(i)). The lines L87, L96, IR29 and ITA212 had higher root weights between flowering and maturity which allowed them to absorb more nutrients from the soil than the other lines with lower root weights (Fig. 5b(i)). These lines thus had higher leaf N concentrations at these growth stages than the rest although the leaf N concentration of IR29 was only slightly higher than that of Pokkali which yielded poorly in this environment. However, with regards to partitioning of DM to panicles (FSO), L87 allocated proportionately more shoot dry matter to panicles than L96 and ITA212 although ITA212 had a slightly higher partitioning coefficient of shoot dry matter to panicles than L96 (Fig. 5d(iii)). L87 and ITA212 also had higher panicle weights than the other genotypes whereas L96 had very low panicle weights. Thus L87 and ITA212 yielded highly in this environment whilst L96 gave a poor yield. IR29, L15 and L146 all partitioned high amounts of DM to panicles and therefore yielded highly in this environment because this high partitioning of dry matter to panicles was coupled with a biomass production that was not much lower than those of L87, L96 and ITA212. Pokkali on the other hand allocated proportionately high amounts of shoot dry matter to panicles but due to the low quantity of biomass it produced and a short grain filling duration, its yield was subsequently low.

Fresh water with 100 kg ha^{-1} N fertilizer The genotypes L96, L146 and ITA212 produced more biomass in this environment than other genotypes included in the study (Fig. 5a(vi)). In this second fresh water environment also L96 had a high leaf N concentration around flowering and after flowering (Fig. 5a(ii), a high LAI after flowering and it matured late (Fig. 4b.). ITA212 had high LAI (Fig. 5a(iv)) and leaf N concentration throughout the growth season while L146 had a low LAI and low leaf N concentration during most of the growth season and it also matured relatively early. The high biomass production of L96 and ITA212 can be understood from their LAI and leaf N values together with their long growth durations but for L146 these cannot provide an adequate explanation of its ability to produce such a high amount of biomass. After flowering, L146 had a leaf N concentration only slightly higher than

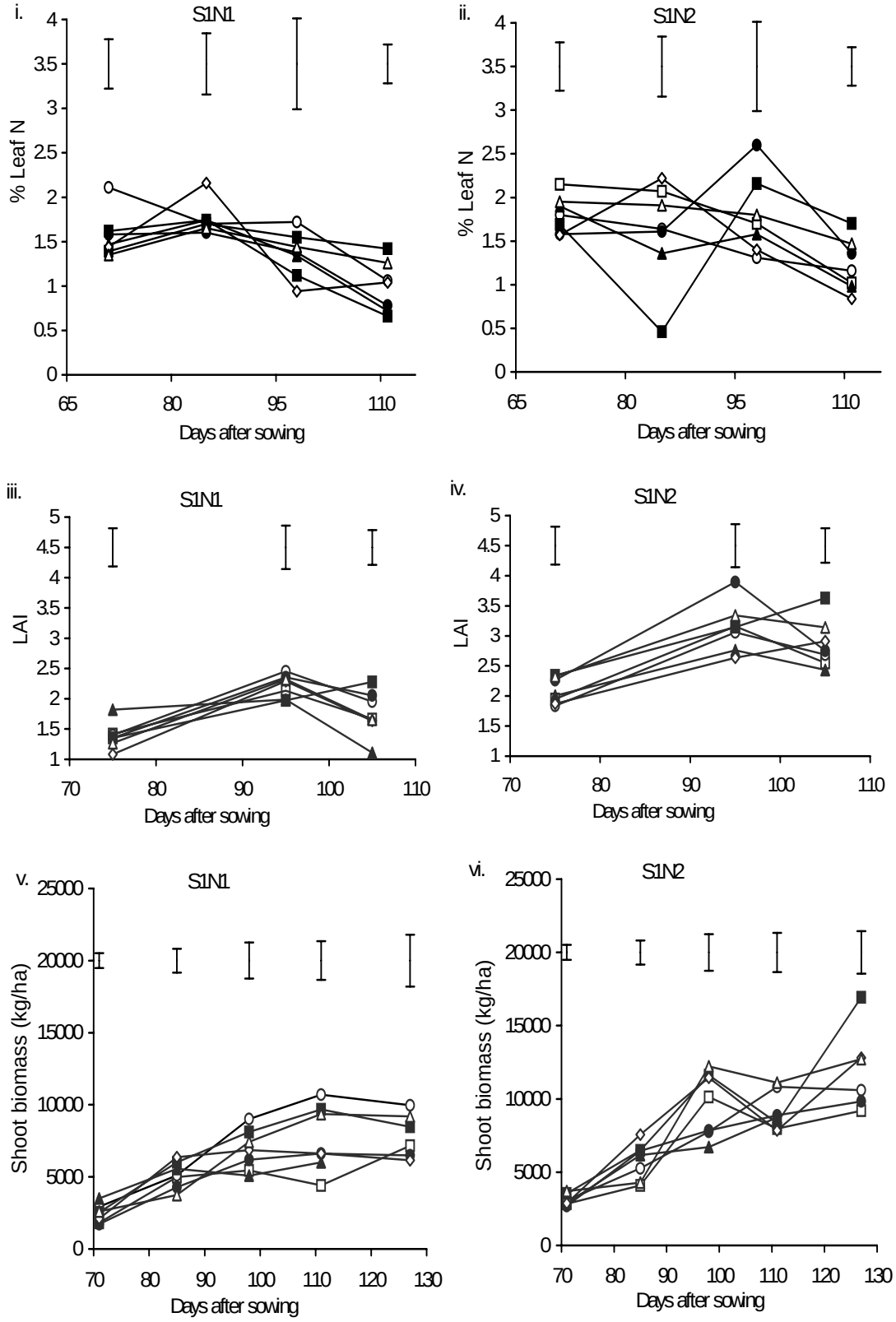

Fig. 5a. Leaf N concentration (%), LAI, and shoot biomass (kg ha^{-1}) of seven rice genotypes in fresh water environments with 0 kg ha^{-1} N fertilizer (S1N1) and 100 kg ha^{-1} N fertilizer (S1N2). Vertical bars: ± standard error of the mean; (□ L15; ○ L87; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ITA212).

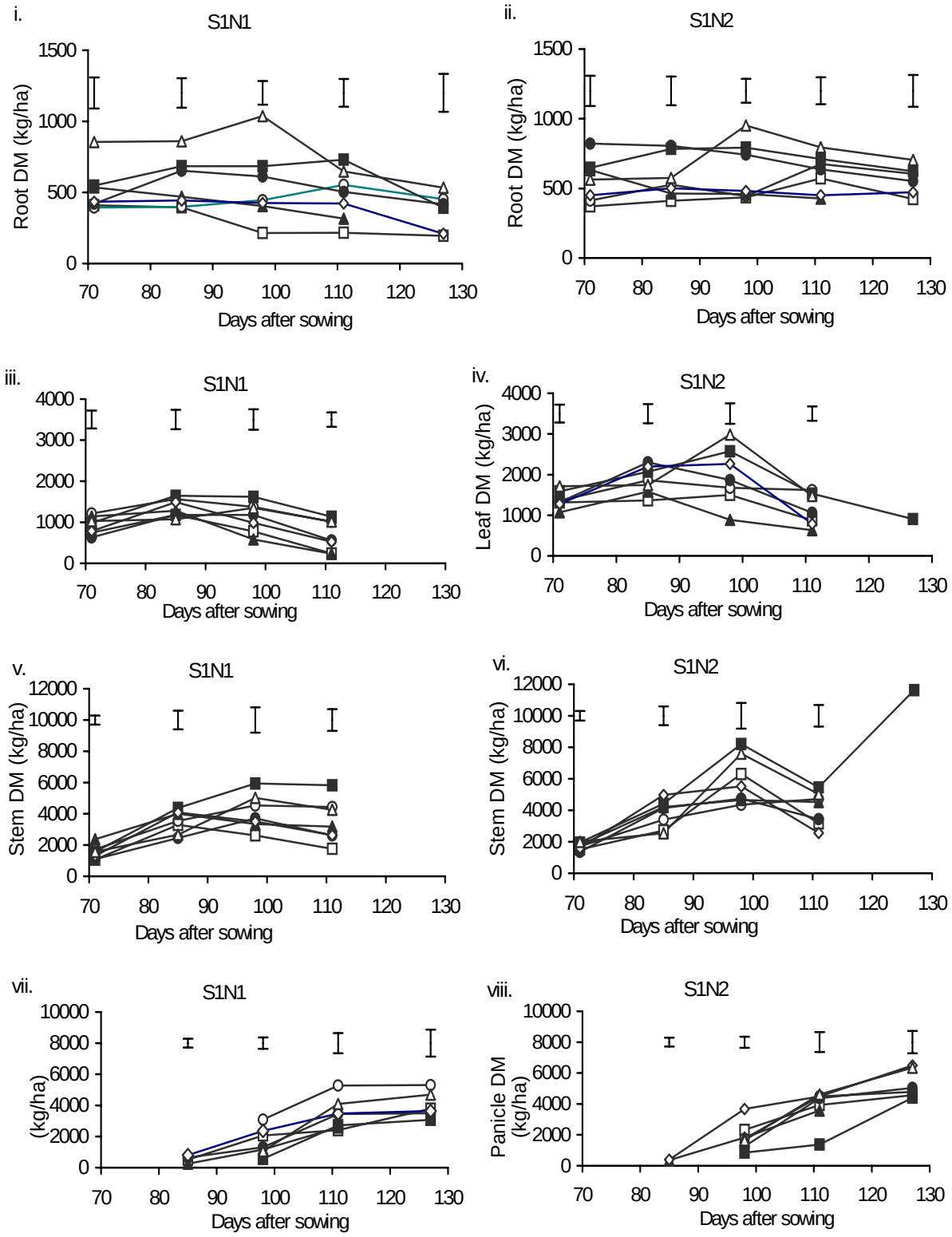

Fig. 5b. Dry matter content of different organs (kg m^{-2}) of seven rice genotypes in fresh water environments with 0 kg ha^{-1} N fertilizer (S1N1) and 100 kg ha^{-1} N fertilizer (S1N2). (Vertical bars: ± standard error of the mean; (□ L15; ○ L87; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ITA212).

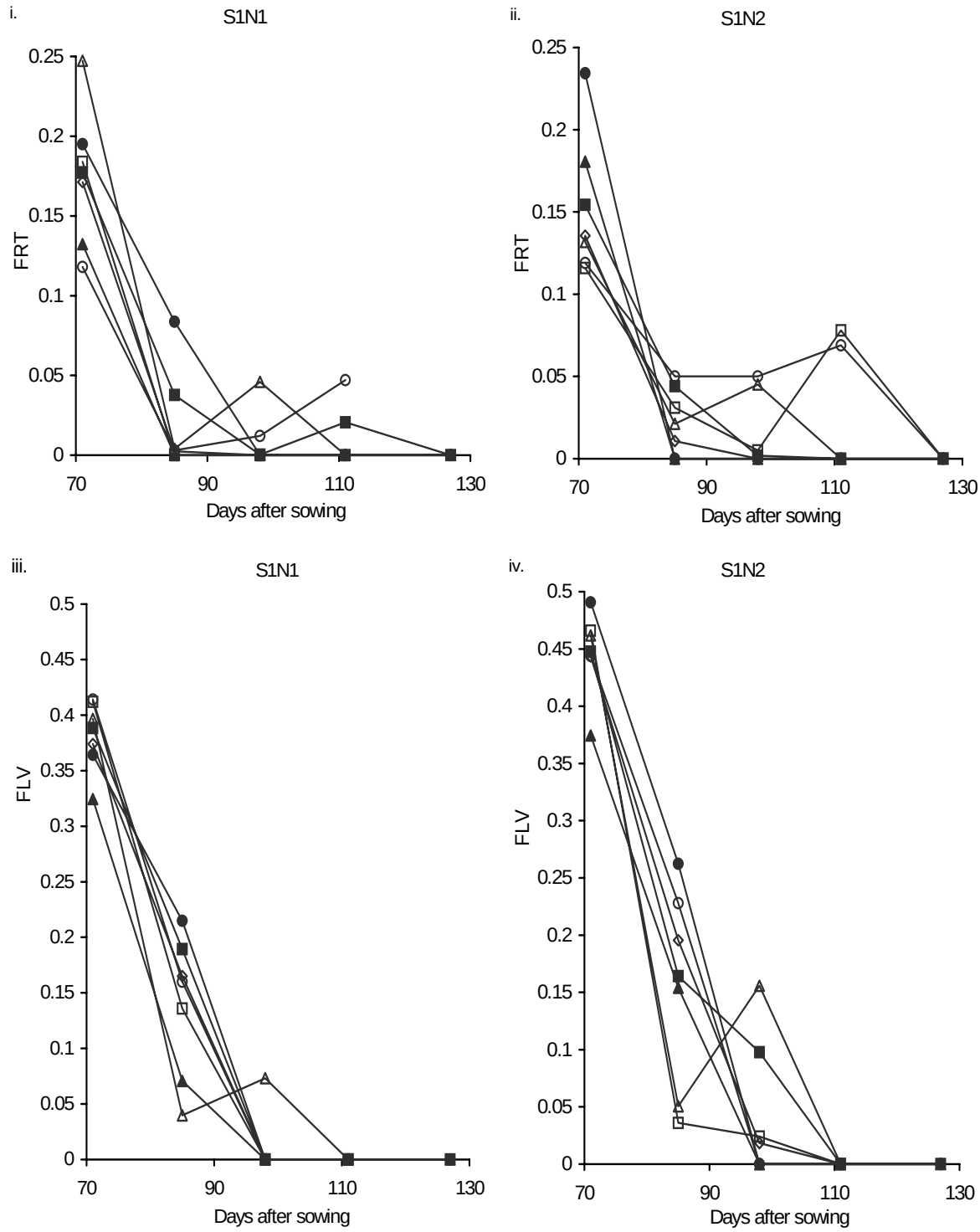

Fig. 5c. Partitioning coefficient of total dry matter allocated to roots (FRT) and partitioning coefficient of shoot dry matter to leaves (FLV) on different sampling dates for seven rice genotypes in fresh water environments with 0 kg ha^{-1} N fertilizer (S1N1) and 100 kg ha^{-1} N fertilizer (S1N2). (□ L15; ○ L87; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ITA212).

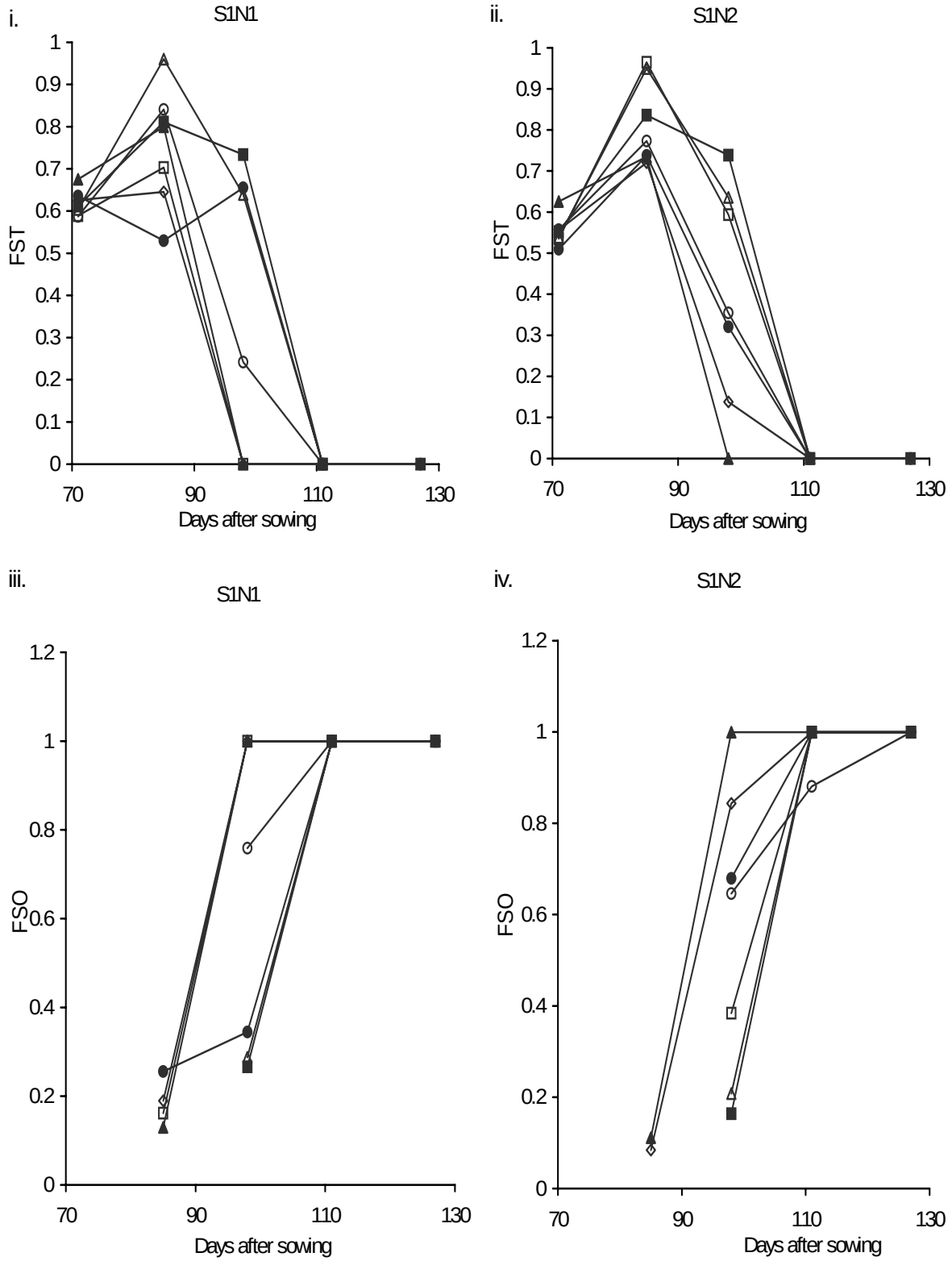

Fig. 5d. Partitioning coefficients of shoot dry matter allocated to stems (FST) and panicles (FSO) on different sampling dates for seven rice genotypes in fresh water environments with 0 kg ha^{-1} N fertilizer (S1N1) and 100 kg ha^{-1} N fertilizer (S1N2). (□ L15; ○ L87; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ITA212).

those of genotypes that produced lower amounts of biomass in this environment. Thus one can ascribe the high biomass production of L146 possibly to a better utilization efficiency for absorbed N fertilizer than the other tested genotypes. Pokkali had a low LAI, low leaf N concentration and matured only slightly later than some of the lines. These properties enabled it to produce a small amount of biomass in this environment. IR29 had a high LAI, variable leaf N concentration and intermediate growth duration and its biomass production was thus lower than those of L96 and ITA212. L15 and L87 had intermediate values of LAI and leaf N concentration although during early growth L15 had high leaf N concentrations. These two RILs thus produced less biomass at maturity than did the other genotypes that had better combinations of LAI and leaf N concentration.

With regards to weights of specific organs, L96, L146 and ITA212 had clearly higher leaf weights at 98 DAS (Fig. 5b(iv)) than the other lines whereas only L96 and ITA212 had distinctly higher stem weights than the other lines on this sampling date (Fig. 5b(vi)). With regards to root weights L87, L96, IR29 and ITA212 all had high root weights between flowering and maturity (Fig. 5a(ii)). This translated to a higher leaf N concentration for L96, IR29 and ITA212 only and not L87 that had a lower leaf N concentration than L96, IR29 and ITA212 despite its high root weight during this period. L146 and ITA212 had higher panicle weights (Fig. 5b(viii)) than the other lines although L146 allocated proportionately more shoot dry matter to panicles than ITA212 (Fig 5d(iv)). Furthermore, L146 had a relatively long grain filling duration compared to the other lines. These two lines thus gave high yields due to the combination of a high biomass production, a high panicle weight and in the case of L146, a long grain filling duration. The RIL L96 allocated proportionately the least amount of shoot dry matter to panicles compared to other genotypes included in the study (Fig. 5d(iv)) and thus yielded poorly in this environment despite its high biomass production (Fig. 5a(ii)) and long grain filling duration (Fig. 4). Pokkali had a high partitioning coefficient of shoot dry matter to panicles in this environment also but due to the low amount of biomass it produced its yield was subsequently low. The RILs L15 and L87 allocated proportionately moderate amounts of shoot dry matter to panicles and thus gave average yields whereas IR29 allocated slightly more shoot dry matter to panicles than these two RILs and thus yielded higher than they did.

Saline environment with 0 kg ha^{-1} N fertilizer The lines that produced high quantities of biomass at maturity in this environment were L57, L96, Pokkali and ROK5, all tall lines (Fig. 2). Pokkali had a high initial LAI, which dropped drastically in later growth and L96 had a consistently low LAI (Fig. 6a(iii)) but a long growth duration (Fig. 4c). L57 and ROK5 had high leaf areas from early growth till around flowering (DAS 98).

Pokkali maintained a high leaf N concentration throughout its growth and L96 had average leaf N concentration in early growth but this increased to a high level around flowering (Fig. 6a(i)). L57 on the other hand, had low leaf N concentrations throughout growth and ROK5 had intermediate leaf N concentrations in early growth that decreased to low levels in later growth. Thus for high final biomass production, a large LAI in early growth appears to be crucial in this environment although L96 seems to offset a low initial LAI by possessing intermediate to high leaf N concentrations from early growth till around flowering (Fig. 6a(i)) coupled with a long growth duration (Fig. 4c). One of the RILs, L15, also had a high LAI from early growth till around flowering but low leaf N concentration in early growth that increased around flowering and also a shorter growth duration than the rest of the lines. The short growth duration (Fig. 4c) limited the ability of this line to accumulate biomass in this environment. IR29 and L146 both possessed lower LAI in early growth than L15, L57, Pokkali and ROK5. IR29 and L146 had low to intermediate leaf N concentrations (Fig. 6a(i)) and thus these two lines produced low amounts of biomass in this environment (Fig. 6a(v)).

Salt tolerant cultivars had distinctly higher LAI, leaf weights, stem weights and total biomass than salt sensitive lines from early growth till around flowering in this environment. However, the clearest distinction between salt tolerant and sensitive lines was observed in panicle weights. Tolerant lines had much higher panicle weights than salt sensitive lines (Fig. 6b(vii)). L57 and Pokkali both allocated high amounts of shoot dry matter to panicles (Fig. 6d(iii)) and this coupled with the high amount of biomass they produced (Fig. 6a(v)) allowed them to yield higher in this environment than the other genotypes. L15 had a high partitioning coefficient of shoot dry matter to panicles and thus despite its low final biomass production comparable to those of salt-sensitive IR29 and L146, it was still able to give a relatively high yield in this environment. ROK5 had a low partitioning coefficient of shoot dry matter to panicles (Fig. 6d(iii) but due to the high amount of biomass it produced (Fig. 6a(v)) its yield was still higher than those of sensitive genotypes.

With regards to root characteristics, salt tolerant lines L15, L57 and POKKALI had low root weights (Fig. 6b(i)) and low root partitioning coefficients in this zero N fertilizer regime under saline conditions (Fig. 6c(i)) while salt-sensitive IR29 and L96 had high root weights and high root partitioning coefficients. The moderately salt-tolerant ROK5 had a high root biomass in this environment but low partitioning coefficient of total dry matter roots. Salt-sensitive L146 also maintained a low root biomass (Fig. 6b(i)) in this environment although it partitioned more biomass to the roots (Fig. 6c(i)). With regards to root biomass, the differences in weight between sensitive and tolerant lines was more evident from 111 DAS to maturity with the

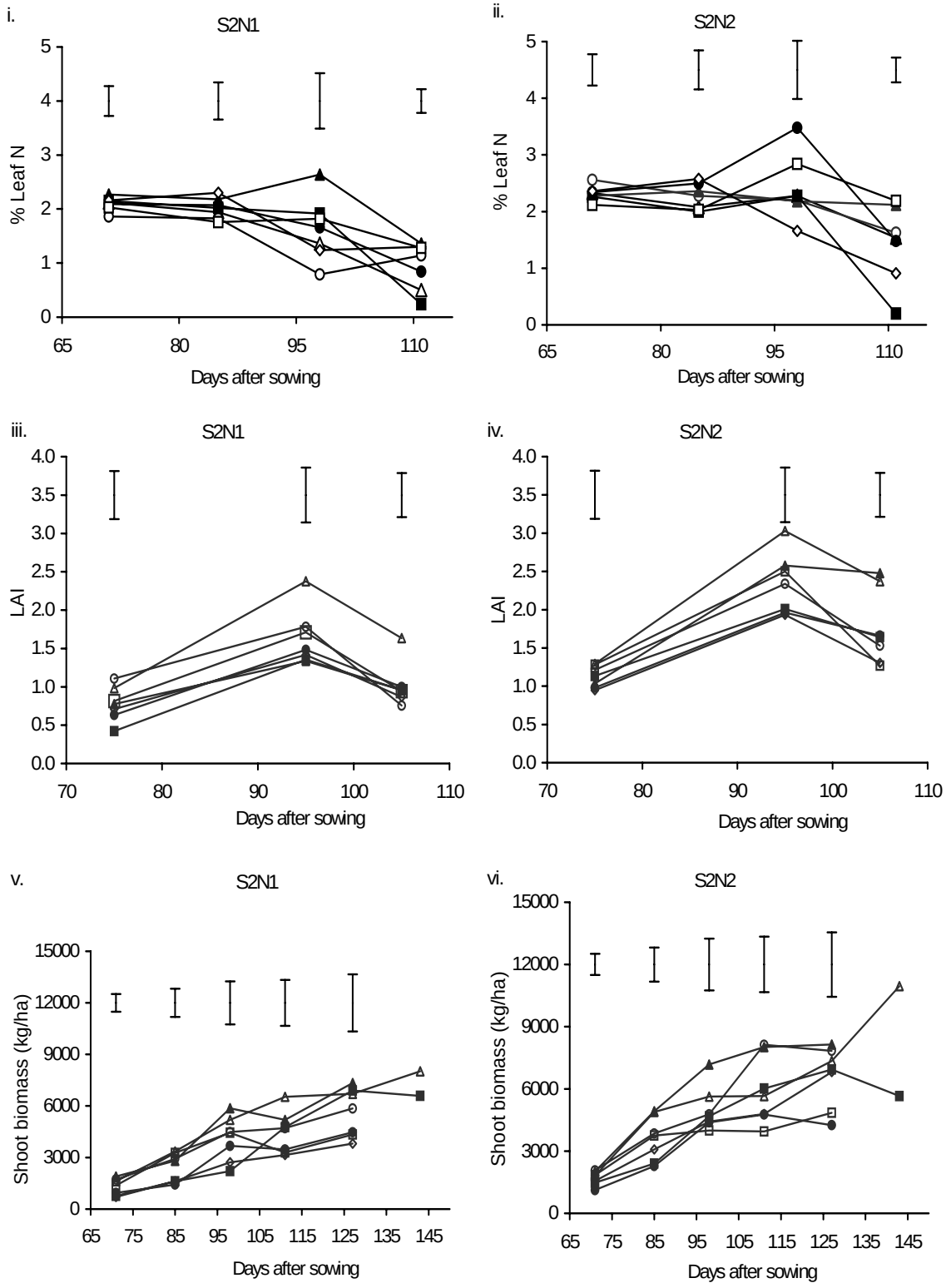

Fig. 6a. Leaf N concentration (%), LAI and shoot biomass (kg ha^{-1}) of seven rice genotypes, on different sampling dates grown under saline conditions with 0 kg ha^{-1} N fertilizer (S2N1) and 100 kg ha^{-1} N fertilizer (S2N2). (□ L15; ○ L57; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ROK5).

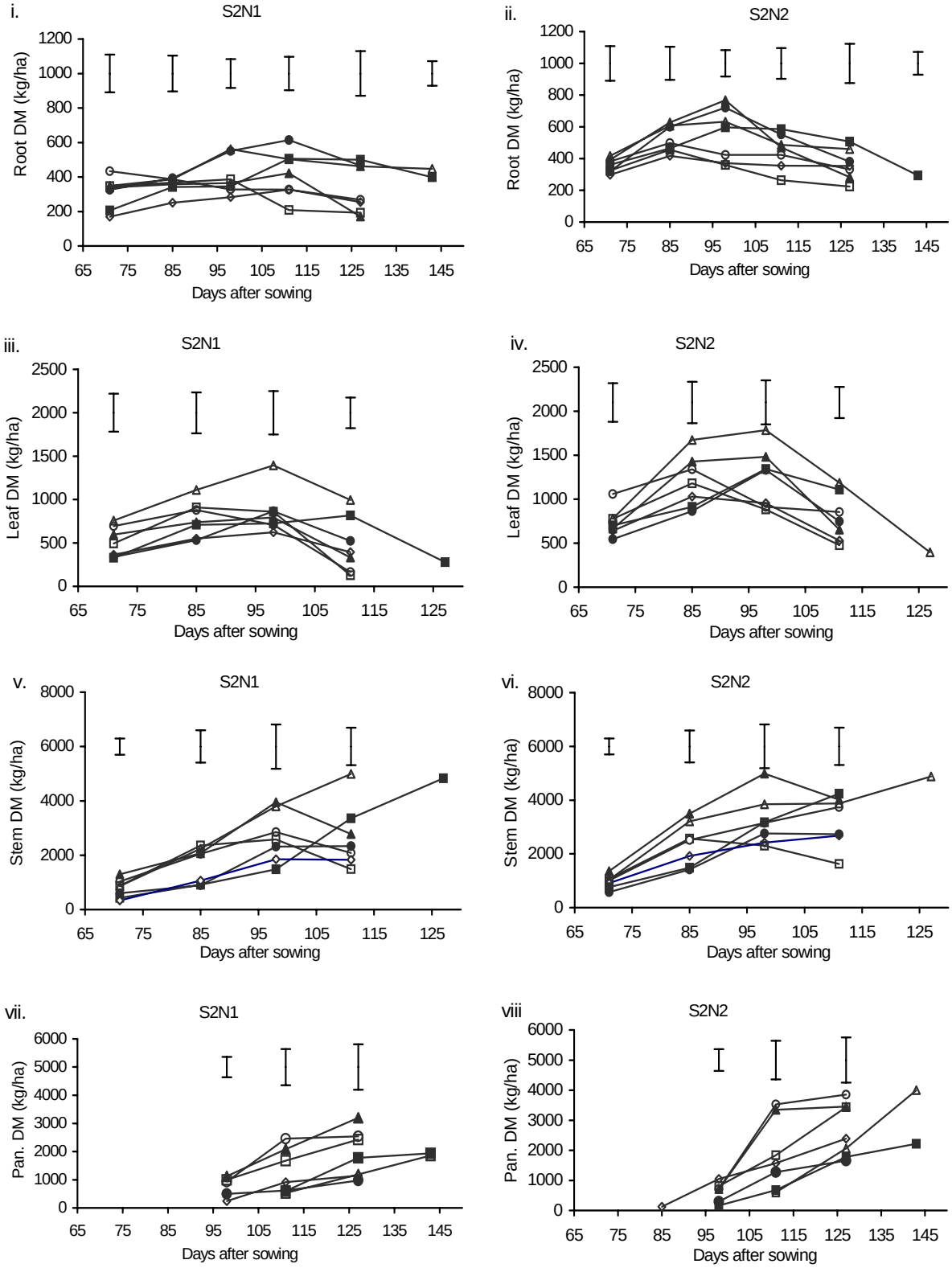

Fig. 6b. Dry matter content (kg ha^{-1}) of different organs on different sampling dates of seven rice genotypes grown under saline conditions with 0 kg ha^{-1} N fertilizer (S2N1) and 100 kg ha^{-1} N fertilizer (S2N2). (Vertical bars: ± standard error of the mean. (□ L15; ○ L57; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ROK5).

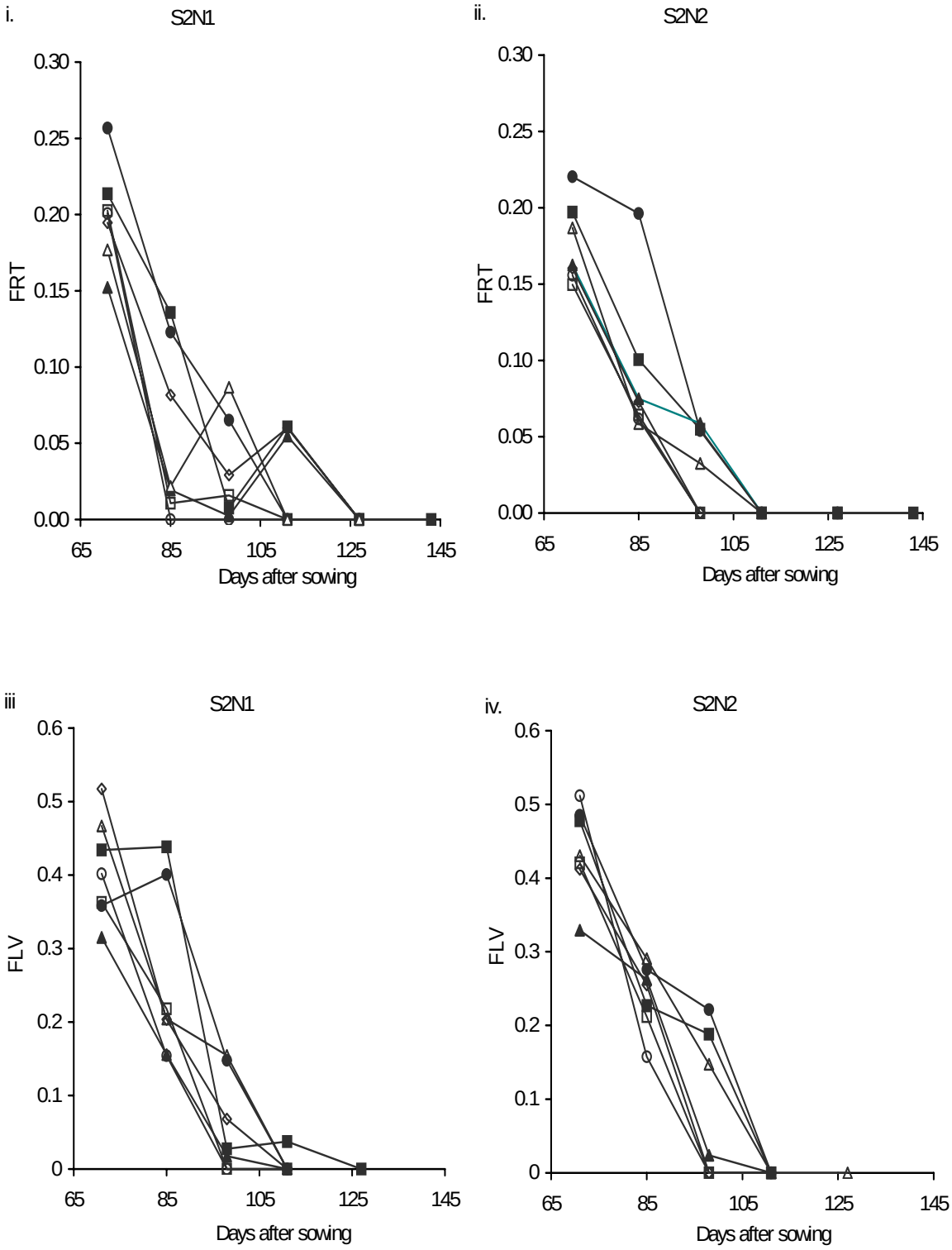

Fig. 6c. Partitioning coefficient of total dry matter allocated to roots (FRT) and partitioning coefficient of shoot dry matter allocated to leaves (FLV) on different sampling dates for seven rice genotypes grown under saline conditions with 0 kg ha^{-1} N fertilizer (S2N1) and 100 kg ha^{-1} N fertilizer (S2N2). (□ L15; ○ L57; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ROK5).

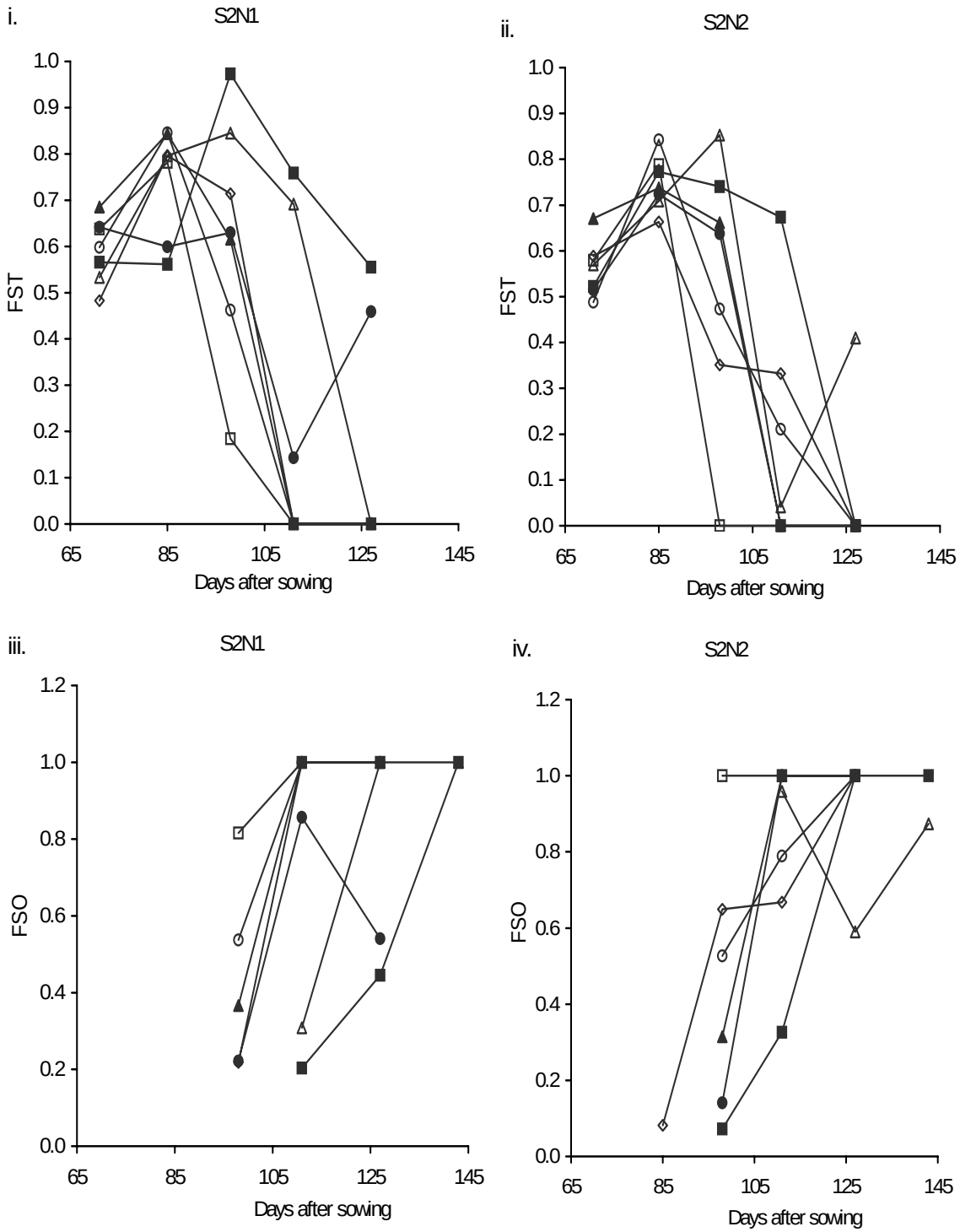

Fig. 6d. Partitioning coefficients of shoot dry matter allocated to stems (FST) panicles (FSO) on different sampling dates for seven rice genotypes grown under saline conditions with 0 kg ha^{-1} N fertilizer (S2N1) and 100 kg ha^{-1} N fertilizer (S2N2). (□ L15; ○ L57; ■ L96; ▲ Pokkali; ● IR29; ◊ L146; Δ ROK5).

exception of L146. Thus in general the sensitive lines partitioned more DM to roots in this environment than the more tolerant lines. Moderately tolerant ROK5 maintained a higher root biomass and allocated proportionately more dry matter to roots than the more tolerant lines (Fig. 6c(i)). Root weight did not seem to be related to leaf N content for most lines in this environment because IR29 and ROK5 which had high root weights from flowering till maturity had intermediate to low levels of leaf N.

Saline environment with 100 kg ha^{-1} N fertilizer In this environment, L57, L146, Pokkali and ROK5 produced higher biomass than L15, L96 and IR29 (Fig. 6a(vi)). L96 had a high initial LAI but this decreased in later growth (Fig. 6a(iv)). In this environment also L96 and ROK5 had longer growth durations than the other lines included in the study (Fig. 4d). Genotypes L15, L57, Pokkali and ROK5 all had high LAI throughout their growth duration although Pokkali had a low LAI in early growth which increased in later growth and L15 and L57 had low leaf areas at two weeks after flowering (Fig. 6a(iv)). The leaf N concentration of lines (Fig. 6a(ii)) did not correspond with biomass production because some lines that produced high quantities of biomass at maturity had high leaf N concentrations while some had low leaf N concentrations. Thus in this saline environment also a high LAI in early growth appears to be important for high biomass production at maturity. L146, which showed the strongest yield response to N fertilizer in saline water, had high initial leaf N but this declined in later growth. In late growth, L146 had a very low leaf N concentration (Fig. 6a(ii)). This line thus appears to have a high efficiency of utilization efficiency for N fertilizer because it produced a high biomass in this environment despite its low LAI (Fig. 6a(iv)) and leaf N concentrations in early growth that were not much higher than those of other lines that produced less biomass.

Salt tolerant genotypes had higher LAI, leaf weights, stem weights and total biomass in early growth than salt sensitive lines although the difference in LAI was more evident around flowering while for weights of vegetative organs (Fig. 6b(iv), 6b(vi)) the differences were clearer around two weeks before flowering (DAS 85). With regards to panicle weights, salt tolerant lines had higher panicle weights than salt sensitive lines (Fig. 6b(viii). From flowering till maturity L15 allocated all new biomass produced to panicles (Fig. 6d(iv)) and was thus able to yield highly in this environment despite producing a lower amount of final biomass compared to L146, L57, Pokkali and ROK5 (Fig. 6a(vi)). L96 allocated proportionately low amounts of shoot dry matter to panicles and thus despite its high biomass production, its final yield was low. The dwarf parent, IR29 had an initially low partitioning coefficient of shoot dry matter to panicles and this coupled with a low biomass production meant its grain yield was low. L146 flowered earlier (Fig. 4d) than all other lines in this environment

but it allocated low amounts of shoot dry matter to panicles around two weeks after flowering and thus gave a low yield even though it produced a high amount of biomass. Pokkali partitioned moderate amounts of biomass to panicles at flowering but later allocated all new DM produced to panicles (Fig. 6d(iv)) and this together with a high biomass production (Fig. 6a(vi)) enabled it to give a high yield.

L57 allocated high amounts of shoot dry matter to panicles and thus gave a high yield because it also produced a high amount of biomass. ROK5 had a low partitioning coefficient of shoot dry matter to panicles but because of the high amount of biomass it produced it was able to yield highly in this environment.

Regarding root weights (Fig. 6b(ii)), differences between salt-tolerant and salt-sensitive lines were more evident at maturity (127 DAS). Salt-sensitive L96, L146 and IR29 had higher final root weights than the more salt-tolerant L15, L57 and POKKALI (Fig. 6b(ii)). ROK5, however, which had the highest yield in this environment, had a high final root weight similar to that of L96. The differences between the fractions of dry matter allocated to roots between tolerant and sensitive lines did not follow a clear pattern although salt-sensitive IR29 and L96 allocated proportionately more DM to roots than salt-tolerant L15 and L57 (Fig. 6d(ii)). POKKALI, the salt-tolerant parent also allocated low amounts of biomass to roots on the first two sampling dates. Salt-sensitive L146 and moderately tolerant ROK5 both allocated low amounts of dry matter to roots. Thus, these two lines that showed strong positive responses to N fertilizer under saline conditions appeared to partition more DM to shoots instead of roots in this environment.

Discussion

In the saline environments due to the higher rainfall in 2000 compared to 2001 (Table 1), the measured salinity levels in the saline treatments were lower in 2000 than in 2001. Hence in 2001 salinity in the field lasted for longer periods before it was diluted by rainfall than in 2000. Thus the effect of salt stress on the lines was stronger in 2001 than in 2000 (Figs. 3a(i), 3a(ii) and 3b(i), 3b(ii)). This affected the performance of the lines as reflected in the higher mean yield of saline treatments in 2000 (2026 kg ha^{-1}) than in 2001 (1376 kg ha^{-1}) [data not shown].

Grain yield and physiological properties of the lines were both influenced by the level of salinity imposed and by the fertilizer regime. The genotypes examined in this study exhibited considerable genetic variation in the yield response of rice to different levels of salinity and N supply. Generally, salt stress reduced yields and organ weights while N fertilizer application increased yields and organ weights of rice. Yield response to N fertilizer was higher in saline water than in fresh water and the yield loss due to salt stress was higher in the zero N than in the high N fertilizer regime. Quantity

of biomass produced influenced yields in all four test environments although the proportion of the biomass that was finally allocated to panicles was a key determinant of final yield. In turn, differences in total biomass production of the various lines of rice arose largely as a result of differences in LAI, leaf N concentration, growth duration and possibly differences in utilization efficiency of applied N fertilizer.

Under fresh water conditions, having a large root system is advantageous in that it enables crops to absorb more nutrients including N, which are then translocated to different plant parts but mainly the leaves. Nutrient content has a strong influence on the photosynthetic activity of plants because essential nutrients are directly or indirectly involved in photosynthesis and respiration (Yoshida, 1981). Increased levels of N in the leaves would lead to increased photosynthetic potential (Mader *et al*., 1982) because much of the leaf N is present in Rubisco protein (Ribulose diphosphate carboxylase), the key protein involved in photosynthesis in C_3 higher plants (Makino *et al*., 2000). The importance of a high leaf N concentration for high biomass production was particularly evident under fresh water conditions where N supply was limiting. Under such conditions, genotypes that had high leaf N concentrations and high leaf areas produced more biomass than other genotypes with lower leaf N concentrations and LAI. In fresh water conditions with high levels of N supply, LAI was particularly important for differentiating between genotypes that produced high amounts of biomass and genotypes that produced less biomass because apparently N supply was not limiting. The increased supply of N through fertilizer application increased LAI and growth duration of most lines of rice in this study leading to higher biomass production in this environment over the zero N fertilizer regime. Weerakoon *et al.* (1999) also reported increases in root and shoot biomass and tiller number of rice with increased N supply. However, in both fresh water environments the proportion of total biomass formed that was allocated to panicles was what eventually determined yield levels of the genotypes investigated in this study.

Compared to fresh water environments, leaf N concentrations of all genotypes increased in saline environments under both zero and high N fertilizer regimes. Over short periods of time, salt stress exposes plants to secondary osmotic stress (Levitt, 1980) but when the stress continues over a longer duration of time then salts accumulate in transpiring leaves to toxic levels inhibiting growth of younger leaves (Munns, 2002). The increased salt concentration in the leaves leads to the accumulation of N-containing compounds involved with stress response (Lefèvre *et al*., 2001; Roy and Wu, 2001; Chattopadhayay *et al*., 2002). Some of these nitrogenous compounds such as proline inhibit root growth thus limiting the capacity of plants to absorb nutrients under salt stress (Lin and Kao, 1999).

Salinity also reduces photosynthetic rate and photosynthesis-related parameters

(Chatrath *et al*., 2000; Loreto *et al*., 2003) thus leading to low accumulation of assimilates in leaves (Sultana *et al*., 2001) although these effects are more pronounced on salt-sensitive than on salt-tolerant genotypes (Tiwari *et al*., 1997). Some of the direct negative effects of salt stress on photosynthesis are due to its reduction of LAI (Faustino *et al*., 1996; Zeng *et al*., 2003), hastening leaf senescence (Lutts *et al*., 1996) and reduction of Rubisco content (Cho *et al*., 1995). Thus maintaining a higher leaf area and leaf weight under salt stress would enable tolerant genotypes to produce more assimilates as a result of a larger photosynthetic capacity than salt-sensitive lines. In our study, a high LAI was apparently more important for high biomass production than leaf N concentration under salt stress although one of the tall lines, L96 was able to produce a high amount of biomass under salt stress despite possessing low LAI possibly as a result of its longer growth duration than most of the genotypes in this study. A large LAI would be particularly useful in the early growth of rice as rice is least tolerant to salinity during seedling stage but its tolerance increases with age (Gill and Singh, 1989; Lutts *et al*., 1996).

The reduction in yield and biomass of all lines in this experiment by salt stress was similar to the findings of other researchers (e.g., Krishnamurthy *et al.*, 1988; Shannon *et al*., 1998; Asch *et al*., 2000). As salt stress impedes nutrient uptake thereby leading to salt-induced nutrient deficiency (Russell, 1978) application of N fertilizer partially alleviated salt stress (Osman *et al*., 1982). Sultana *et al.* (2001) reported observing large reductions in the effects of salt stress on rice when a foliar spray of nutrients was administered.

With regards to root biomass, maintaining low root biomass and/or root partitioning coefficient would signify that there would be less root surface exposed to osmotic or toxic injury. Under salt stress the ability of tolerant lines to partition less DM to roots might help them minimize salt injury to roots. Consequently, there would then be more assimilates available for shoot growth. Thus despite differences in height (Fig. 2) salt-tolerant lines had higher shoot weights during early vegetative growth than salt-sensitive lines. Only later in the growing season, did the tall and salt-sensitive line L96, attain high shoot biomass levels similar to those of salt-tolerant genotypes. This relationship between yield and early biomass production has also been found by researchers for crops growing under drought stress (Richards, 1996). According to Munns (2002) a high shoot:root ratio (a low root weight relative to shoot weight) and high growth rate will reduce the accumulation of salts in the shoot thereby minimizing the damaging effects of salt stress on the plant.

To achieve salt tolerance, the ability to produce high stem biomass in early growth is important. After flowering, stem biomass usually starts declining because most of the new and some of the previously produced assimilates are channelled to panicles.

Rice lines with higher stem weights at flowering can thus remobilize more stem reserves to the panicles despite the reduced photosynthetic capacity of the senescing leaves. Under post-anthesis stress conditions, reserves, mainly from the stem have been said to be an important source of assimilates. Total translocated amounts may be as high as 2 to 3 t ha^{-1} (Bindraban, 1997).

Salt-tolerant genotypes allocated more biomass to the panicles than salt-sensitive lines under both zero and high N fertilizer rates. This allowed the tolerant lines to attain higher yields under saline conditions than the sensitive lines. Sylla (1994) cited several workers who found that grain yield of rice was more affected by salinity than vegetative characters. Similarly, Gill and Singh (1989) found dry weights of panicles of rice to be decreased with an increase in salinity and variety × salinity interaction was significant with the more tolerant varieties having higher weights. In our trial, ROK5, the moderately tolerant cultivar had low FSO values in both saline environments. Thus despite the ability of this cultivar to produce a lot of DM under saline conditions its poor ability to translate this into yield as evidenced by its low FSO under saline conditions (Fig. 6d(iii) and 6d(iv)) limited it to yield only moderately in saline soils.

Under high N fertilizer conditions in both fresh and saline water, one of the RILs, L146, produced high amounts of biomass despite possessing low LAI and low leaf N concentrations during most of its growth and a growth duration similar to the majority of the other lines. This RIL apparently possessed a higher N utilization efficiency under high N fertilizer conditions than the other genotypes included in this study. Genotypic variation in nitrogen utilization efficiency in rice was also reported by Borrell *et al.* (1998). Another line L96, which possessed a large root system under both fresh water and saline conditions, increased its leaf N concentration with N fertilizer application possibly as a result of a larger uptake ability for applied N fertilizer over other genotypes with smaller root systems. Thus the response of rice genotypes to N fertilizer with regards to yield and biomass production could arise as a result of different physiological mechanisms all of which can be exploited by breeders to increase the yield potential of rice in different environments.

Conclusions

High LAI and high leaf N concentrations are essential for high biomass production in fresh water environments but in saline environments a high LAI appeared to be more important for high biomass production than a high leaf N concentration. Production of a high amount of biomass was important for high yielding ability in both fresh and saline water environments but this has to be taken in conjunction with a high partitioning coefficient of dry matter to panicles in order to produce rice genotypes

with higher yield potentials. A high biomass production in early growth had a more direct effect on grain yield than final biomass production in saline environments.

Level of crop N supply influences the physiological response of rice to salinity. This was seen in Figs. 6a-d, where the rankings of genotypes with regards to organ weights or LAI varied with the N treatment. Thus when breeding for salinity tolerance consideration should be given to the N supply status of the target environment.

Differences in yielding ability of rice genotypes in different environments are due to inherent differences in basic physiological properties such as LAI, leaf N concentration, growth duration and possibly differences in N utilization efficiency. As the genotypes in this study exhibited differences in these physiological properties this raises the possibility of increasing the yield potential of rice cultivars under both optimum and marginal environments by breeding for favourable combinations of these traits in new cultivars of rice.

CHAPTER 6

Simulating yield and biomass production of diverse genotypes of rice

Simulating yield and biomass production of diverse genotypes of rice

Abstract

To expand the use of crop simulation models in plant breeding, models must incorporate reliable genetic coefficients for key physiological traits coupled with data for key environmental variables, so as to enable accurate prediction of crop growth and yield of genotypes in diverse environments. In a study to determine the potential of a dynamic crop growth model, ORYZA1, to predict rice yield and uncover physiological options for increasing rice yield potential, a collection of 15 divergent genotypes of rice were grown under fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer. The rice genotypes comprised 11 Recombinant Inbred Lines (RILs), together with their parents, IR29 and Pokkali, as well as two improved rice cultivars ROK5 and ITA212. For most of the rice genotypes included in the study, ORYZA1 estimated yields and biomass production well in all four test environments. However, generally, ORYZA1 estimated yields better than biomass production in all environments and yield predictions were better in fresh water environments than in saline water environments. Performing sensitivity analysis using LAI, specific leaf N and fraction of dry matter allocated to panicles revealed the potential gains in biomass production and yield to be realized through manipulations of these traits. Comparison of estimated biomass production of the various rice genotypes under fresh water conditions also revealed possible differences in N use efficiency within the collection of rice genotypes. Improving the performance of ORYZA1 and similar models in saline environments, requires consideration of effects of salinity on model input traits especially specific leaf N and spikelet fertility.

Introduction

Simulation models have been used to describe crop growth and yield processes for several decades (Boote *et al.*, 1998). The applicability of early crop models was limited by the fact that those models focused mainly on predicting potential production and often did not consider the effects of growth limiting factors such as biotic and abiotic stresses. However, with the massive improvement in computer technologies and better understanding of physiological growth processes, researchers have since developed more complex models that can predict crop performance based on environmental variables and genotype-specific coefficients. Nowadays, simulation models are available that can reliably describe/predict the growth and yield formation for a large number of crops (Matthews, 2002) such as rice (Kiniry *et al.*, 2001; Dingkuhn, 1996), sorghum (Wade, 1995), wheat (Bindraban, 1997), barley (Yin *et al.*, 2000) and millet (Samake, 2003) under different growth conditions.

Differences in crop performance arise as a result of either genetic or environmental differences. Crop simulation models can be used to determine the effect of differences in key physiological traits (identified through empirical research) on yield. Models can therefore be used to identify physiological characteristics of crop cultivars that limit yields in a particular environment. For those situations where target environments of crops are diverse, effects of main environmental factors on key physiological traits are determined first. Then simulation models can be used to identify crop genotypes that are likely to perform well in the various sub-divisions of the target environment. Thus models offer a way of designing genotypes with optimal characteristics for particular growth conditions (Donald, 1968). Crop models have been used in designing the New Plant Type (NPT) of rice at the International Rice Research Institute (IRRI). This NPT rice is expected to increase the yield potential of present tropical semi-dwarf rice cultivars by up to 50% (Khush *et al.*, 1995). However, the successful application of simulation models in cultivar improvement requires effective collaboration between breeders, physiologists, agronomists and modellers (Aggarwal *et al.*, 1995; Matthews, 2002).

Crop simulation models would be particularly useful in the diverse and low-input conditions that characterize most farmers' field conditions in developing countries where strong genotype-by-environment (G × E) interaction is common. Traditionally, in the presence of G × E interaction, researchers use costly and time-consuming multi-locational trials to select genotypes with adaptability to specific environments or to the entire test region (Lansigan and Aggarwal, 1996). Use of simulation models under these variable growing conditions will help reduce research costs and considerably shorten the time span involved in the cultivar development process (Palanisamy *et al.*, 1995).

The ORYZA1 model, developed jointly by researchers at Wageningen University and Research Centre in the Netherlands and the International Rice Research Institute

(IRRI) in the Philippines (Kropff *et al*., 1994), is an explanatory dynamic mathematical model to simulate growth and development of irrigated rice under potential production conditions (Casanova *et al*., 2000). The model was developed in the Philippines and parametrized using physiological parameters of improved semi-dwarf rice cultivars under high input conditions. However, the model can also be used to simulate rice growth under sub-optimal growing conditions when leaf N content and other varietal characteristics are determined throughout the growing season and then used as model input parameters (Kropff *et al*., 1994). The ORYZA1 model was used by Dingkuhn (1996) to simulate yields of the rice cultivar IR64 in variable N environments. In a separate study, Wu and Wilson (1998) also successfully used another model, RIDEV, to simulate yields of recombinant inbred lines of rice in environments varying in mean ambient temperature and the presence of rice pests.

The objectives of this study were: (i) to determine the ability of ORYZA1 to predict yields of diverse genotypes of rice under different environmental conditions and (ii) to identify physiological options for increasing yield potential of rice genotypes in different environments.

Materials and methods

Plant material

A segregating population of rice, comprising 276 recombinant inbred lines (RILs), developed at IRRI from the cross IR29 × Pokkali (both *indica* varieties) was used. IR29 is a short, high yielding modern cultivar released by IRRI and is known to be very sensitive to salinity (Gregorio, 1997). Pokkali is a tall, traditional variety from India known to be tolerant to salinity (Yeo and Flowers, 1986; Garcia *et al*., 1995; Gregorio, 1997). As one of the two parents is a short, high-yielding, modern cultivar and the other is a tall traditional variety, it is expected that the RIL population will also be segregating for response to N fertilizer. This is due to the fact that modern rice cultivars have been bred for response to high levels of mineral fertilizer usage while traditional varieties have usually been selected by farmers in environments with sub-optimal levels of nutrient supply (see Chapter 2).

The RIL population, together with the parents were grown during the rainy season (June-Oct.) in 1999 and 2000 in a series of genetic experiments at Sapu (13.55 °N latitude), in The Gambia (see Chapter 2). One hundred and sixty RILs were randomly selected from the population of 276 RILs for use in our study. From these 160 RILs 100 were used in the trials of 1999 and 2000 (see Chapter 2). Yield and yield component data were collected for these 100 RILs and their parents. After exhaustive analyses of the two-year yield data, 38 RILs were selected from the 160 RILs for high, medium and low-

yielding ability in the different test environments based on the yields and molecular markers present in the different RILs. The results of these genetic studies are shown elsewhere (see Chapter 2). Of these 38 RILs, 22 were grown before in our trials of 1999 and 2000 while the remaining 16 RILs had not been grown before in our trials.

In 2001, these selected 38 RILs together with the two parents were then grown at the same experimental site using the same split-split plot design. Two rice cultivars were introduced into the trial in 2001 – ROK5 and ITA212. Thus a total of 42 RILs and cultivars were grown in 2001. ROK5 is a tall, moderately salt-tolerant cultivar bred in Sierra Leone (WARDA, 1994) and has been used in the saline swamps of West Africa for many years. ITA212 is a West African tropical *japonica* cultivar of rice, bred by the International Institute of Tropical Agriculture (IITA), in Ibadan, Nigeria.

Field experiments

A split-split plot experimental design was used with salinity as the main plot factor, rate of nitrogen fertilization as the sub-plot factor and genotype as the sub-subplot factor. Each sub-subplot plot measured 2.6 m × 3.0 m and a spacing of 20 cm × 20 cm within and between rows was used. Two levels of salinity and two levels of nitrogen fertilization (giving a total of four treatment combinations) were tested. Three replications were maintained in each year of the trials. Additional information on the trials is given in Table 1 of Chapter 5. The following treatment combinations, also referred to later in the text as test environments were obtained:

S1N1 – Fresh water (river water) at an electrical conductivity (EC) of 0.15 dS m^{-1} and 0 kg N ha^{-1};

S1N2 – Fresh water (river water) at an EC of 0.15 dS m^{-1} and 100 kg N ha^{-1} as urea;

S2N1 – Salt water at an EC of 8 dS m^{-1} and 0 kg N ha^{-1};

S2N2 – Salt water at an EC of 8 dS m^{-1} with 100 kg N ha^{-1} as urea.

Pre-germinated rice seeds were sown in a nursery that was well watered and regularly weeded. Around three to four weeks after emergence (22-27 days after sowing) the seedlings were transplanted to the field following the experimental design described above. After transplanting the trial plots were kept continuously flooded by irrigating with river water till all RILs and cultivars were close to physiological maturity. Salinity was imposed by manually broadcasting measured amounts of granular table salt in standing water to attain the required salinity.

Salinity of the ponded-water was measured two days after every significant rainfall or after a protracted period without rains (more than four consecutive days). An ES-421 salt meter (Atago Co. Ltd., Japan) was used to measure salinity levels. When the salinity level was too low more salt was added to raise the salinity and when the

salinity was too high the saline plots were irrigated with fresh water to reduce the salinity to the desired level.

Data collection

In 2000, physiological data were collected on only the two parents IR29 and Pokkali but in 2001 physiological data were collected on 11 RILs plus the two parents and ROK5 and ITA212. The physiological data collected in both 2000 and 2001 included dry weights of roots, stems, leaves and panicles, leaf area index (LAI) and leaf nitrogen concentration (%). Total above-ground biomass was determined by adding the weights of dried leaves, stems and panicles (where present) together.

As the two parents flowered and matured on different days between treatments, sample collection was done on different days in year 2000. The first sample was collected at 45 days after sowing (DAS), the second at 71 DAS, the third coinciding with flowering at 98, 101 and 110 DAS and the fourth coinciding with maturity at 122, 125 and 128 DAS. In 2001, however, due to the larger number of genotypes on which physiological data was collected, samples were taken on the same dates for all lines of rice in all treatments at each sampling stage. In 2001, physiological data were collected at roughly two-week intervals: 71 DAS, 85 DAS, 98 DAS, 111 DAS, 127 DAS and 133 DAS. Most lines flowered at around 98 DAS and matured at around 127 DAS. Only L96 and ROK5 reached physiological maturity after 127 DAS.

In both years, on each sampling date, four plant hills were uprooted, washed thoroughly to free the roots from soil and separated into roots, leaves, stems and panicles depending on the developmental stage. Only green leaves were measured. Dead leaves were removed but not weighed.

LAI, leaf nitrogen concentration and weights of stems and green leaves, were measured up to flowering in 2000 and two weeks after most genotypes flowered in 2001. No observed values were available for these parameters at maturity in both years. At maturity, in both years, the total vegetative matter was weighed and recorded for each sample but not separated into green leaves and stems as done in earlier sampling stages.

In 2000, LAI was determined by manually measuring the length and width of green leaves of the two parents and then multiplying these with a leaf shape factor of 0.7 (Yoshida, 1981). In 2001, LAI was measured with the AccuPAR model PAR80 Ceptometer (Decagon Devices, Inc., USA). Specific leaf area (SLA) was calculated using LAI and leaf dry weight values for the lines in the different environments.

Dry matter was determined by air-drying the separate organs under shade followed by oven-drying at 60-70 °C till a constant weight was attained. Leaf nitrogen concentration (%) was obtained from dried leaf samples, which were then processed

using the micro-Kjeldahl process. Partitioning coefficients were derived by calculating the fraction of new dry matter (DM) production distributed to each plant organ between subsequent sampling stages (Kropff *et al*., 1994). Five different partitioning coefficients were calculated:

FRT – partitioning coefficient of total dry matter allocated to the root
FSH – partitioning coefficient of total dry matter allocated to the shoot
FLV – partitioning coefficient of shoot dry matter allocated to the leaves
FST – partitioning coefficient of shoot dry matter allocated to the stems
FSO – partitioning coefficient of shoot dry matter allocated to the panicles (storage organs).

Percent leaf nitrogen was obtained from leaf samples dried at 70 °C for 24 h and then processed using the micro-Kjeldahl process. Specific leaf nitrogen content (g m^{-2}) was calculated from leaf nitrogen concentration and SLA. Data was also collected on grain yield, temperature sum from sowing to flowering and from sowing to maturity.

The dates of sowing, transplanting, flowering and physiological maturity were recorded for all lines. Weather data, including daily minimum and maximum temperatures, was collected from the local weather station. The dates of sowing, transplanting, flowering and maturity together with the weather data were entered into the computer program DRATES (Kropff *et al.*, 1994) to compute the temperature sum required for each line of rice from sowing to flowering and maturity.

Grain yield (kg ha^{-1})

With the exception of plants in the outermost rows all plants in each plot were harvested upon reaching maturity. The harvested plants were then threshed, dried, cleaned and weighed. Net area harvested was determined and the yield (g) from this net plot was then converted to yield in kg ha^{-1}.

Data analysis

The SAS statistical package was used to perform ANOVA on grain yield. The Mixed Procedure (Proc MIXED) of SAS (SAS Inst., 1999) was used to perform the ANOVA and generate least squares means (LSMeans) of the line × salinity level × nitrogen level combination for yield. With regards to physiological traits, the arithmetic means from the three replications were computed for each genotype and then used as model input parameters in ORYZA1.

Simulating grain yield and biomass production

To simulate rice yields and biomass production in different environments, we used the

ORYZA1 model (version 1.3; Kropff *et al*., 1994). ORYZA1 describes rice growth and development as affected by light, temperature and cultivar characteristics for phenological, morphological and physiological processes. Model input requirements of ORYZA1 are: geographical latitude, daily weather data (radiation, minimum and maximum temperature), plant density and dates of seeding and transplanting. Parameter values that describe the morpho-physiological characteristics of genotypes are also required including phenological development rates, relative growth rate of leaf area, specific leaf area, spikelet growth factor, potential grain weight, leaf N content and fraction of stem reserves. In this research, data were collected over two growing seasons. In the first year, data were collected on only two genotypes, but in the second year data were collected on 15 diverse genotypes. Sampling frequency and the developmental stages at which samples were collected, were different between the two years. Thus model calibration and evaluation were done separately for each year's data.

Model parametrization and calibration

(i) Year 2000 – In this year, we parametrized ORYZA1 using data for the two parents, IR29 and Pokkali. The computer program DRATES was used to calculate developmental rates of vegetative and reproductive phases and developmental stages corresponding to each sampling date. Observed values for the following were input into the ORYZA1 model:

- Developmental rates during vegetative and reproductive phases
- Developmental stage at each sampling
- Weight of roots, stems, green leaves and storage organs (panicles)
- Partitioning coefficients for roots, shoots, stems, leaves and storage organs
- LAI and SLA up to flowering
- Specific leaf nitrogen content up to flowering
- Number of plants per hill
- Number of hills m^{-2}

Because of lack of measured data at maturity, as mentioned earlier, LAI, SLA and specific leaf nitrogen content at maturity had to be assumed.

The choice of these assumed values was done taking into consideration the values for IR72 at similar developmental stages (Kropff *et al.*, 1994), the trial environment and the cultivar under consideration. For example, for IR72 at maturity under 225 kg N ha^{-1}, LAI, SLA and specific leaf N content were 2.45 ha ha^{-1}, 0.0017 ha leaf kg^{-1} leaf and 0.83 g N m^{-2}, respectively. For IR29 and POK, lower values of LAI and specific leaf N content were used at maturity because in our N fertilizer treatments

only 100 kg N ha^{-1} was used. For POK, a lower LAI was used in the high N fertilizer regime in fresh water because of its serious lodging. In the selection of SLA at maturity, the selected LAI was divided by the selected green leaf weight. Under saline conditions, lower final LAI and specific leaf N content values were used but the values chosen for IR29 were lower than those for POK due to the fact that POK was more tolerant to salinity than IR29.

The relative growth rate of leaf area during the exponential growth phase (RGRL) was changed in conditions where no urea was applied, that is, the zero N fertilizer regimes in fresh and saline environments. The default value for conditions under which N fertilizer was applied is 0.008 $(°Cd)^{-1}$ and the authors of the model advised using 0.005 $(°Cd)^{-1}$ when the rice crop is growing under nitrogen-limiting conditions (Kropff *et al*., 1994). Default values were maintained in the model for the other model input parameters.

For each of the two parents in each test environment, ORYZA1 was calibrated by first running the parametrized models and then replacing the assumed values with values estimated by ORYZA1.

(ii) Year 2001 – For this year, observed physiological data was available for more growth stages than in 2000 including one stage between flowering and maturity for the eight observed model input parameters used in 2000. This necessitated the use of a different set of assumed values in year 2001 from those of 2000. Hence at maturity in year 2001, the following assumed values were used in the ORYZA1 model:

A final specific leaf N of 0.21 g N m^{-2} because many genotypes had specific leaf N values less than 0.20 g N m^{-2} in some environments at the fourth sampling stage, which was a stage between flowering and maturity of the rice genotypes used.

A final LAI of 1.5 in the zero N fertilizer regime in fresh water, 2.0 in the high N fertilizer regime in fresh water, 0.8 in the zero N fertilizer regime in saline water and 1.0 in the zero N fertilizer regime in saline water.

Final weight of green leaves and weight of stems for the different rice genotypes were chosen based on the values used in 2000 for the two parents, IR29 and Pokkali. The values of these parameters used in ORYZA1 models for semi-dwarf genotypes (height < 120 cm) were those of IR29 in 2000. For taller genotypes, the values used were similar to those of Pokkali in 2000.

Once again DRATES was used to estimate developmental rates for each genotype: In the next step, we calibrated the ORYZA1 model by making the following changes in model input parameters:

(a) We assumed that all new assimilates produced after flowering will be channelled to the panicles (FSO = 1.0 from flowering to maturity).

(b) We assumed a final LAI of 0.50 for tall genotypes (130-150 cm), 0.30 for very tall genotypes (> 150 cm) in fresh water environments (S1N1 and S1N2).
(c) To accommodate the effect of salinity on accelerating leaf senescence, a final LAI of 0.20 in S2N1 and 0.40 in S2N2 were used. Lodging of genotypes taller than 130 cm in S2N2 was assumed and a final LAI of 0.20 was used in those situations.

The versions of ORYZA1 used in this second step are henceforth referred to as calibrated model.

Model evaluation

Evaluation procedure was done differently between years.

(i) Year 2000 – The calibrated models were run for both IR29 and Pokkali and the simulated yield and biomass production were plotted against observed values in 1:1 scatter plots.

(ii) Year 2001 – Model evaluation in year 2001 was done in two stages. In the first stage, we used observed and assumed model input parameters in the ORYZA1 model and then ran the model for each genotype in each test environment. From the output, simulated yield and biomass production for each combination of genotype and environment were recorded. These values were then plotted against observed values and R^2 was computed to determine the accuracy of predictions made by the ORYZA1 model.

In the second stage of model evaluation in 2001, we ran the calibrated ORYZA1 model and recorded simulated yield and biomass production for each genotype in each environment. We then plotted simulated yield and biomass production against observed values and once again computed R^2 to determine any improvements in model predictions over the non-calibrated model.

In order to gain insight into the dynamics of biomass production, we plotted the simulated and observed biomass for the two parents, IR29 and Pokkali, from sowing till maturity in all four environments in 2001.

Sensitivity analysis

To determine the physiological differences between rice genotypes that determine their ability to produce biomass and eventually yield in different environments, we studied the effects of maximizing LAI and specific leaf N at all sampling stages, and partitioning coefficient of shoot dry matter to panicles between flowering and maturity on yield and biomass production of the 15 genotypes. Maximum values for these three traits within the range of values observed in the 15 genotypes at each sampling stage,

were substituted in the calibrated model for each genotype in each environment for 2001 and the ensuing model was then run. The percent increase in yield and biomass over those of the calibrated ORYZA1 model by maximizing LAI, specific leaf N or fraction of shoot dry matter allocated to panicles, were computed. The stronger the percent improvement in yield or biomass of a genotype, with a maximum increase in one of the three traits, the more deficient that genotype was with regards to that trait in the particular environment.

Results

Model evaluation

(i) *Year 2000* ORYZA1 simulated grain yield better than biomass production for the two parents in 2000 (Figs. 1a and b). Generally, the model simulated yield and biomass production of IR29 rather well and much better than it did for Pokkali in the four test environments in 2000. Between environments, variation in yield and biomass production was much smaller for Pokkali than for IR29. Biomass production of IR29 was slightly over-estimated in all four environments but for Pokkali, biomass production was under-estimated by ORYZA1.

Grain yield of Pokkali, was well estimated at the zero N fertilizer rate in both fresh and saline water but over-estimated at the high N fertilizer rate in fresh and saline water. On the other hand, biomass production of Pokkali was better estimated at the high N fertilizer rate than at the zero N fertilizer rate in both fresh and saline water in the year 2000.

(ii) *Year 2001*

Grain yield In 2001, the initial yield estimations of the 15 rice genotypes using their individual genotypic means of physiological traits as model input parameters, were rather poor in all four test environments (Table 1). However, when we calibrated the ORYZA1 model by mimicking the effect of lodging on LAI of genotypes taller than 130 cm (Fig. 2), the strong effect of salinity on LAI of sensitive genotypes and also the improved DM partitioning to panicles for other genotypes, yield predictions were much improved especially in the high N fertilizer regimes under both fresh water and saline conditions (Table 1). The calibrated model gave good estimates of observed minimum and maximum yields among the fifteen rice genotypes, from model input parameters in fresh water environments but in saline environments minimum yields were over-estimated. Yield predictions from these calibrated versions of ORYZA1 were better in high N fertilizer environments than in the zero N fertilizer environments in both fresh and saline water (Fig. 3). Yields of most genotypes were under-estimated

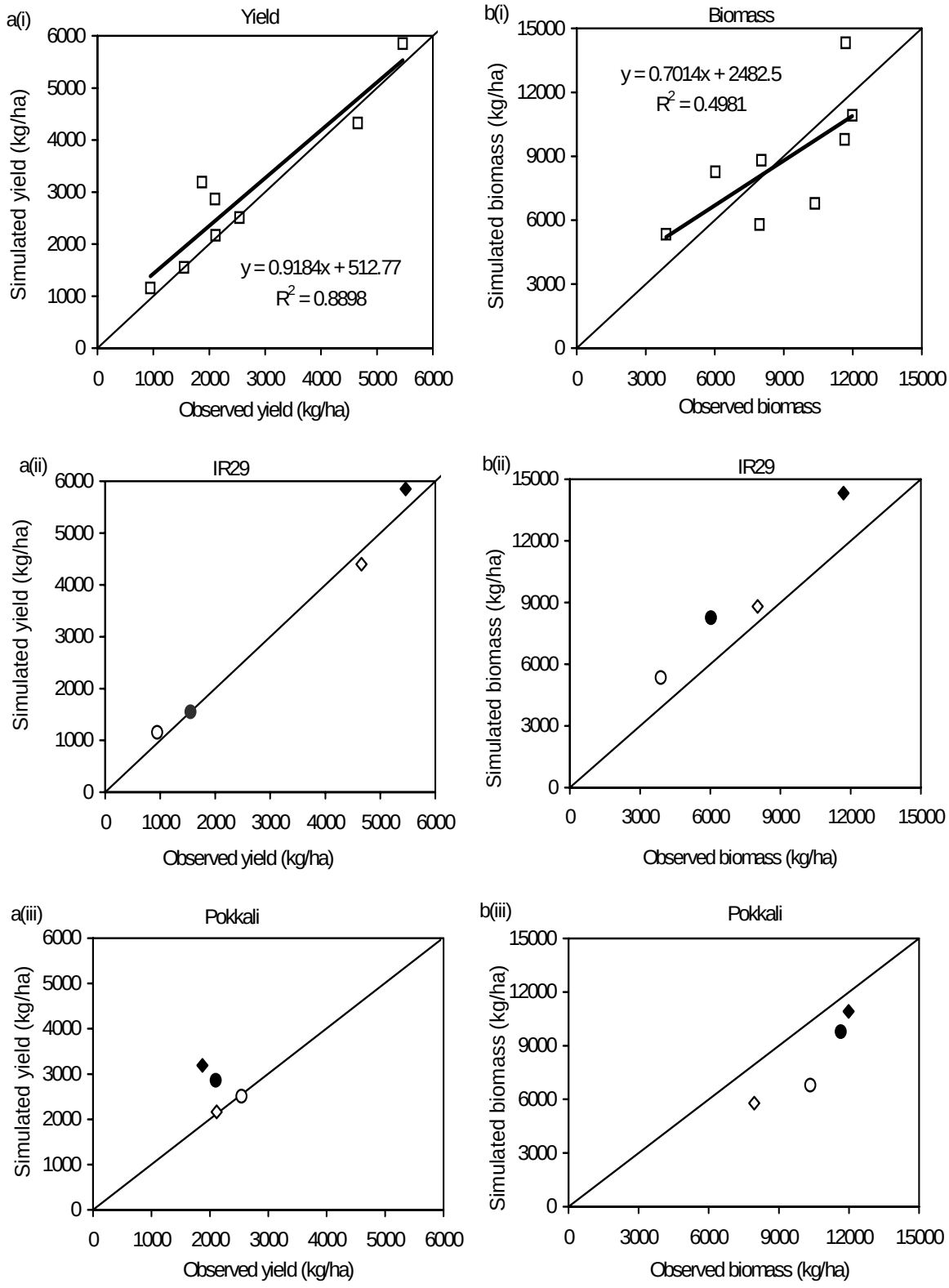

Fig. 1. Plots of observed against simulated yield and shoot biomass of IR29 and Pokkali in year 2000. □ Pooled data for IR29 and Pokkali across four environments; ◊ fresh water with 0 kg ha^{-1} N fertilizer; ♦ fresh water with 100 kg ha^{-1} N fertilizer; ○ saline water with 0 kg ha^{-1} N fertilizer; ● saline water with 100 kg ha^{-1} N fertilizer.

in the zero N fertilizer regime in fresh water in the output from the calibrated versions of the ORYZA1 model (Fig. 3a). In saline environments, on the other hand, the ORYZA1 model over-estimated yields of most genotypes, especially for salt-sensitive genotypes (Figs. 3c and d).

Biomass production Accuracy of model prediction, as expressed by R^2 values of the relationships between observed and predicted values, of biomass production of the 15 genotypes based on observed model input parameters, was similar in the four test environments. Using observed model input parameters, ORYZA1 predicted biomass production of the 15 rice genotypes better than grain yield, in most of the four test environments in 2001 in the output from the non-calibrated versions of ORYZA1 (Tables 1 and 2). Modifications of final LAI and FSO values made no significant improvements of R^2 values in the plots of simulated against observed biomass production in outputs of the calibrated ORYZA1 models over those of the non-calibrated versions (Table 2), in 2001. Maximum biomass production was under-estimated by ORYZA1 in all four test environments but these under-estimations were worse in fresh water environments than in saline water environments (Fig. 4). Minimum biomass production was, however, well-estimated in all environments except the fresh water environment with high N fertilizer application, where minimum biomass production from model input traits was strongly over-estimated.

There was a good agreement between simulated and observed biomass production in fresh water environment without N fertilizer for all genotypes except for RIL L57, for which the model grossly under-estimated biomass production (Fig. 4a). In the fresh water environment with high N fertilizer application, the model under-estimated biomass production for genotypes that produced high quantities of biomass and over-estimated biomass production of genotypes that produced less biomass. The worst under-estimation of biomass production was made in the high N fertilizer regime in fresh water for the RILs L57 and L96 (Fig. 4b), both tall genotypes (Fig. 2). However, the model made serious under-estimations of biomass production for both semi-dwarf and tall genotypes in the zero and high N fertilizer regimes in fresh water. On the other hand, in the two fresh water environments, large over-estimations of biomass production by ORYZA1 were made only in the high N fertilizer regime in fresh water (Fig. 4b) and then only for semi-dwarf genotypes.

In the two saline environments, model prediction of biomass production generally agreed well with observed data for genotypes that produced low to average quantities of biomass without any N fertilizer application but were much lower for genotypes that produced high amounts of biomass in this environment (Fig. 4c). These serious under-estimations of biomass production were made for the RILs, L57, L101, L156

and the salt-tolerant parent, Pokkali. In the high N fertilizer regime, however, biomass production was over-estimated for most genotypes that produced low quantities of biomass and under-estimated for genotypes that produced high quantities of biomass (Fig. 4d).

Table 1. Regression parameters reflecting the agreement between simulated and observed yields of 15 genotypes of rice grown under four different environmental conditions at Sapu, The Gambia, in 2001 (n = 15 in each of the environments S1N1, S1N2, S2N1 and S2N2; n = 60 for the pooled data).

Environment	Initial model		Calibrated model	
	Regression equation	R^2	Regression equation	R^2
S1N1	y = 0.124x + 2209.10	0.01 $^{n.s.}$	y = 0.430x + 1623.50	0.24 $^{n.s.}$
S1N2	y = 0.435x + 1818.00	0.19 $^{n.s.}$	y = 0.683x + 1177.20	0.77**
S2N1	y = –0.289x + 1583.90	0.10 $^{n.s.}$	y = 0.356x + 847.47	0.26 $^{n.s.}$
S2N2	y = 0.518x + 1267.60	0.18 $^{n.s.}$	y = 0.658x + 998.80	0.45**
Pooled data	y = 0.581x + 944.59	0.46**	y = 0.779x + 614.33	0.82**

$^{n.s.}$ not significant (P > 0.05); ** significant at P < 0.01.

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively).

Table 2. Regression parameters reflecting the agreement between simulated and observed biomass production of 15 genotypes of rice grown under four different environmental conditions at Sapu, The Gambia, in 2001 (n = 15 in each of the environments S1N1, S1N2, S2N1 and S2N2; n = 60 for the pooled data).

Environment	Initial model		Calibrated model	
	Regression equation	R^2	Regression equation	R^2
S1N1	y = 0.258x + 4681.30	0.21 $^{n.s.}$	y = 0.241x + 4748.60	0.23 $^{n.s.}$
S1N2	y = 0.166x + 7609.10	0.18 $^{n.s.}$	y = 0.141x + 7752.30	0.17 $^{n.s.}$
S2N1	y = 0.296x + 3142.60	0.27*	y = 0.248x + 3199.60	0.24 $^{n.s.}$
S2N2	y = 0.333x + 4388.90	0.20 $^{n.s.}$	y = 0.316x + 4293.10	0.22 $^{n.s.}$
Pooled data	y = 0.468x + 3270.90	0.49**	y = 0.460x + 3178.60	0.50**

$^{n.s.}$ not significant (P > 0.05); * significant at P < 0.05; ** significant at P < 0.01;

(Note: S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – Saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively).

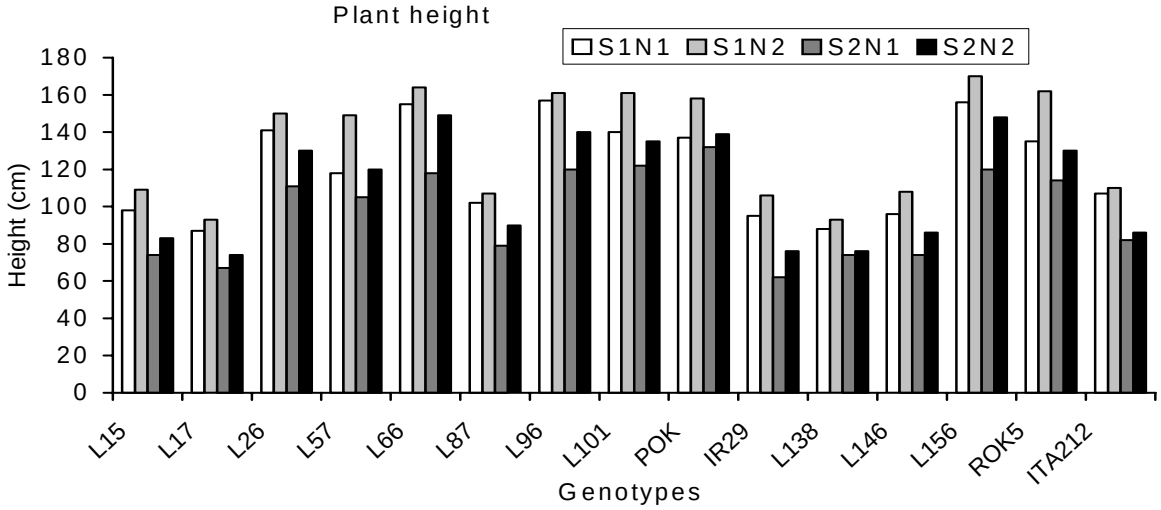

Fig. 2. Plant height (cm) of 15 genotypes of rice in four environments: S1N1- fresh water with 0 kg ha^{-1} N fertilizer; S1N2 – fresh water with 100 kg ha^{-1} N fertilizer; S2N1 – saline water with 0 kg ha^{-1} N fertilizer; S2N2 – saline water with 100 kg ha^{-1} N fertilizer.

Dynamics of biomass production The ORYZA1 model simulated biomass production of the two parents, IR29 and Pokkali, better during early growth than in later growth. Biomass production from sowing to maturity was better simulated for IR29 (Fig. 5a) than for Pokkali (Fig. 5b). For IR29, the pattern of biomass production was well simulated in all environments except during late growth in the saline environment with high N fertilizer application, when the model grossly over-estimated its biomass production (Fig. 5a(iv)). With regards to Pokkali, the ORYZA1 model generally under-estimated its biomass production. This under-estimation was worse under saline conditions (Figs. 5b(iii), 5b(iv)) compared to fresh water environments (Fig. 5b(i), Fig. 5b(ii)).

Sensitivity analysis

The 15 rice genotypes used in this study differed in specific leaf N and LAI at the different sampling stages. In addition, between flowering and maturity, the fraction of DM allocated to panicles also varied between the 15 genotypes. Considering the crucial role that leaf N status and LAI play in photosynthesis and eventually in biomass production, we decided to investigate the effects of substituting observed genotypic values for specific leaf N, LAI and partitioning coefficient of shoot dry matter to panicles, with maximum observed values in each environment. In this way, we were able to determine the physiological deficiencies for each of the 15 rice

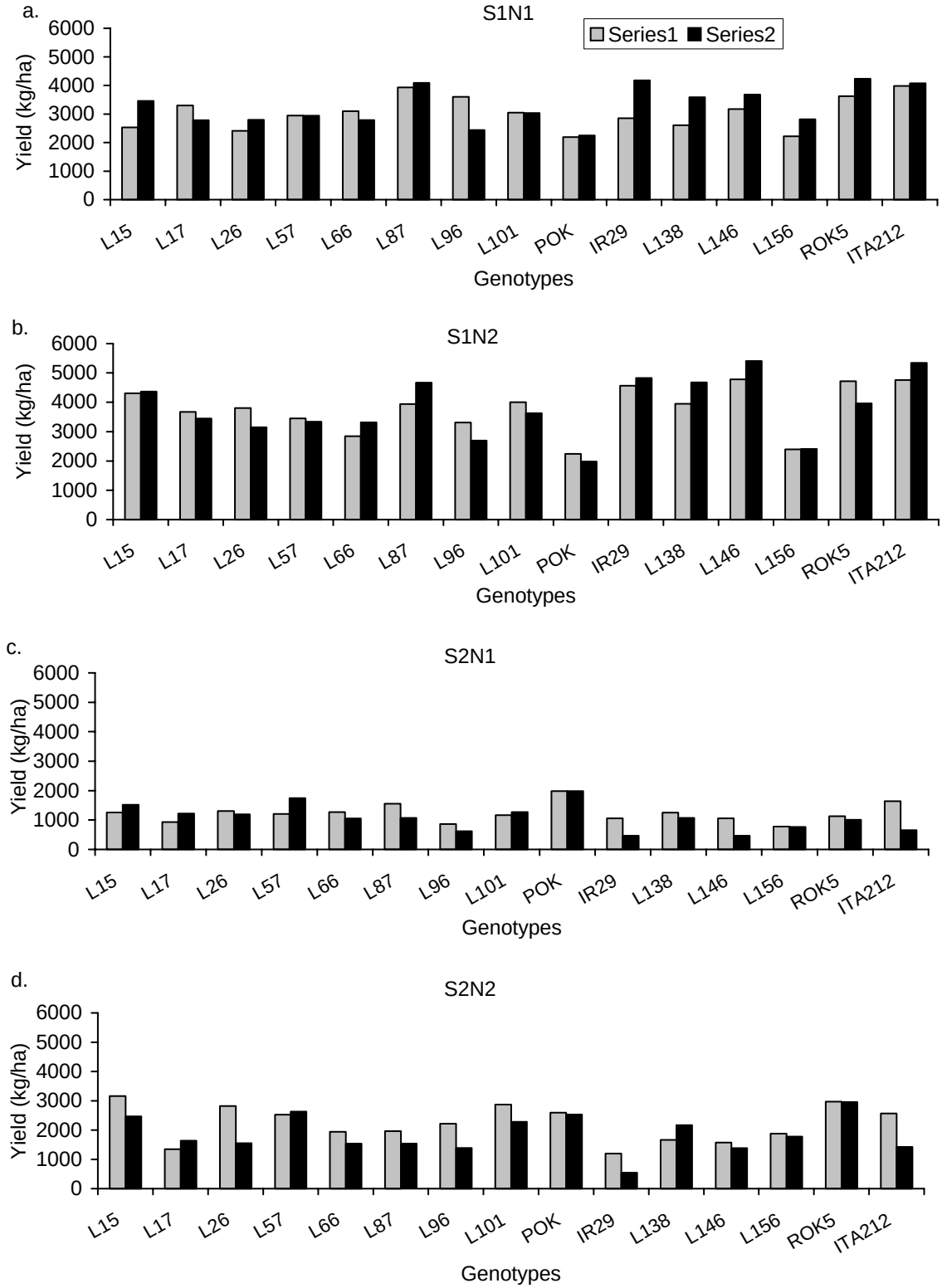

Fig. 3. Simulated and observed yields of 15 rice genotypes grown under different conditions in year 2001. S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively. Series 1 – simulated yield (kg ha^{-1}); Series 2 – observed yield (kg ha^{-1}).

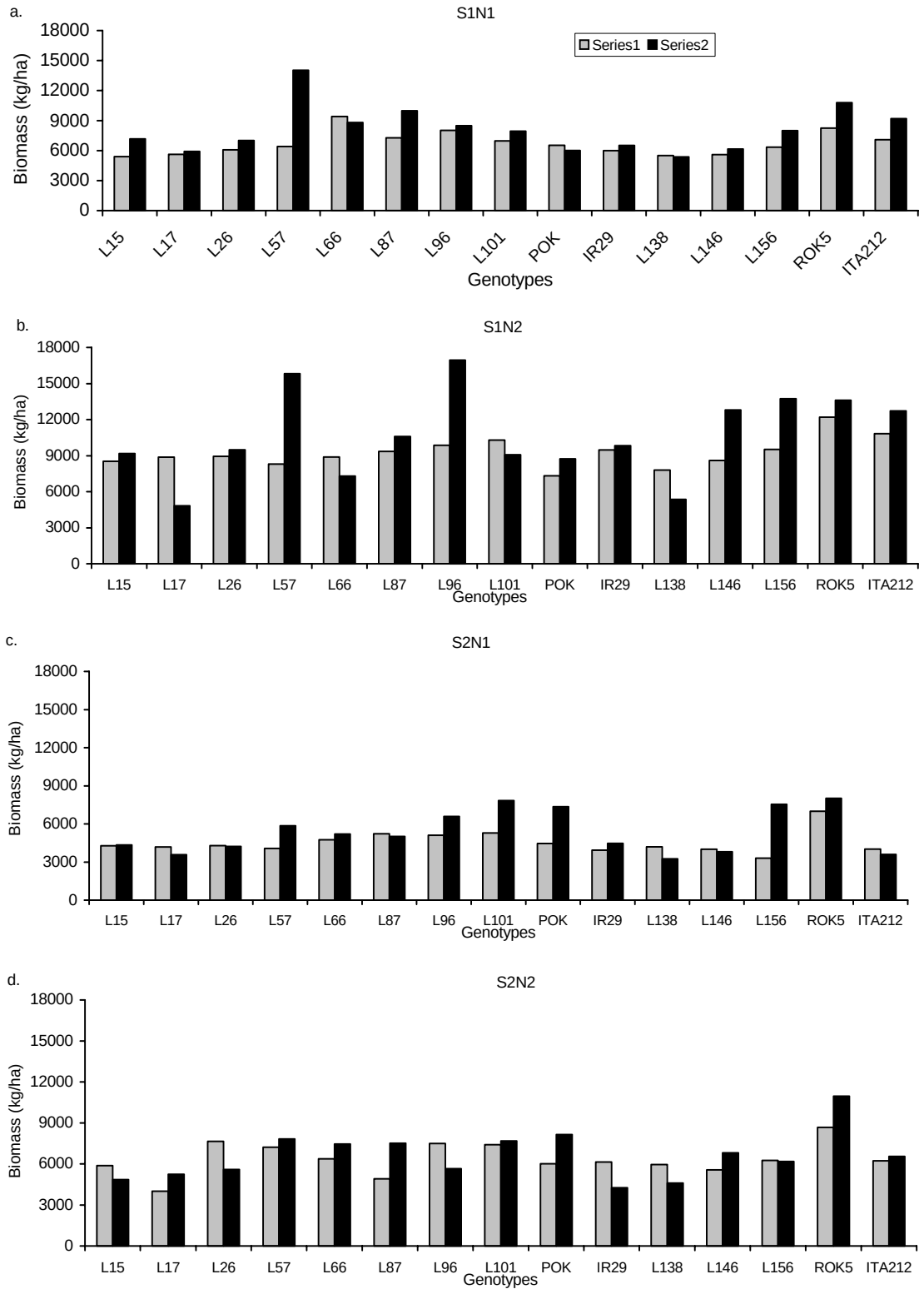

Fig. 4. Simulated and observed biomass production of 15 rice genotypes grown under different conditions in year 2001. S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively. Series 1 – simulated biomass production (kg ha^{-1}); Series 2 – observed biomass production (kg ha^{-1}).

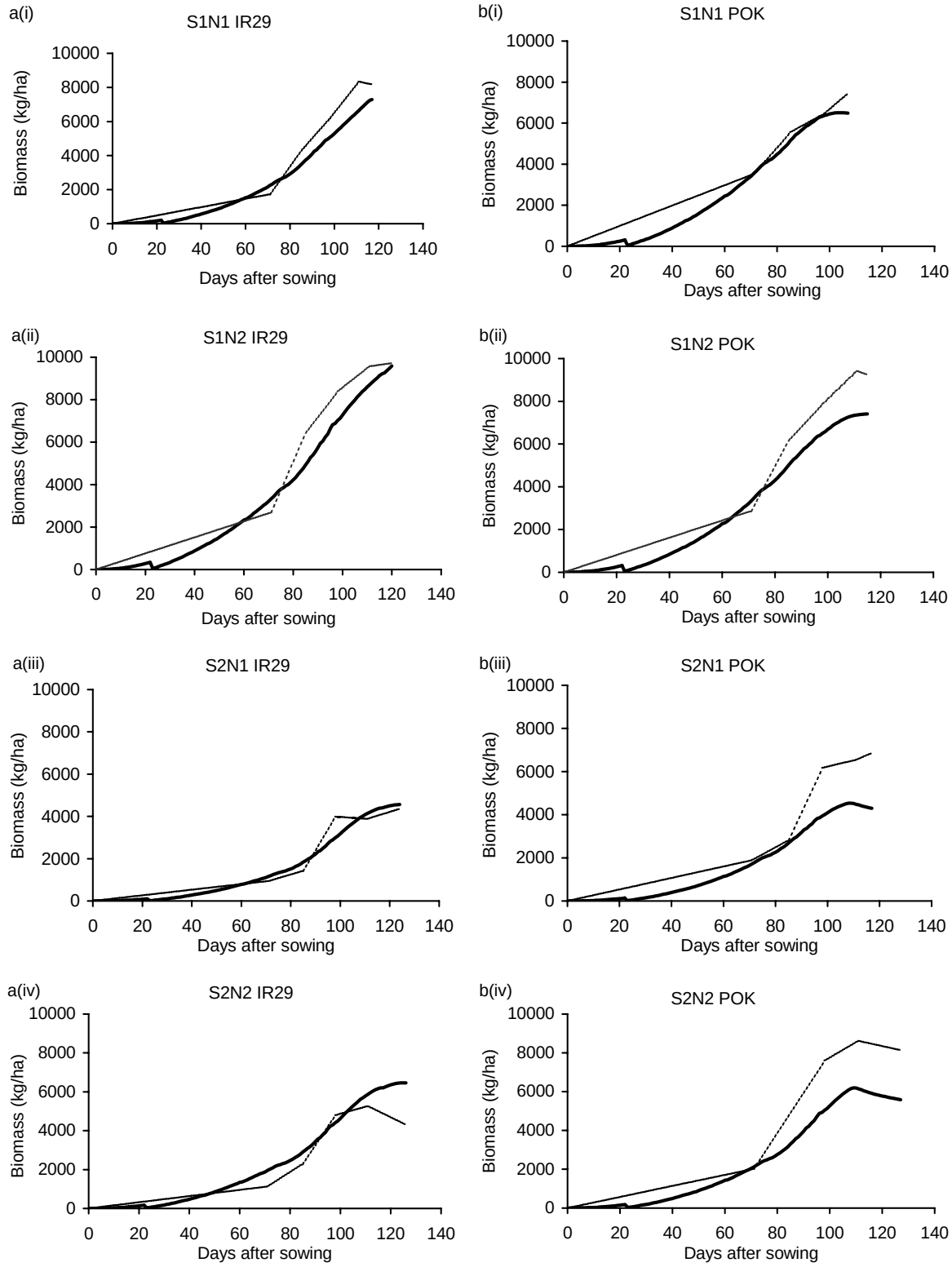

Fig. 5. Comparison of simulated against observed biomass production of IR29 and Pokkali (POK) between sowing and maturity in year 2001. Solid line: simulated biomass (kg ha^{-1}); dotted line: observed biomass (kg ha^{-1}); DAS – days after sowing. S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively.

genotypes with regards to specific leaf N, LAI and FSO, limiting biomass production and yield in each test environment.

Grain yield ORYZA1 was successful in differentiating between high and low yielding genotypes in all test environments. The potential increase in yield by maximizing the fraction of dry matter allocated to panicles, specific leaf N and LAI varied among the 15 genotypes between the four test environments. Fraction of DM allocated to panicles between flowering and maturity was identified as the key trait determining whether a genotype would attain a high or low yield level in both fresh water environments and in the saline environment without N fertilizer application. The highest yielding genotypes in these three environments allocated all new DM produced after flowering to panicles. Lowest yielding genotypes continued to allocate DM to vegetative structures after flowering thus reducing yielding ability. Effects of maximizing LAI or specific leaf N on grain yield in these three environments become relevant only after considering the fraction of shoot dry matter allocated to panicles between flowering and maturity.

In addition to having high partitioning coefficients of shoot dry matter to panicles values, the highest yielding genotypes in the fresh water environment without N fertilizer, also had high specific leaf N and relatively high LAI and thus showed little potential increase in yield after maximizing their specific leaf N and LAI values in the model (Fig. 6a). On the other hand, highest yielding genotypes in the fresh water environment with high N fertilizer application, allocated high amounts of shoot dry matter to panicles, had high LAI values and moderately high specific leaf N (Fig. 6b). After considering the fraction of shoot dry matter allocated to panicles for any genotype in the zero N fertilizer regime under saline conditions, its combination of specific leaf N and LAI would determine its yield (Fig. 6c). In the high N fertilizer regime under saline conditions, the combination of fraction of shoot dry matter allocated to panicles, specific leaf N and LAI determined yields of rice genotypes (Fig. 6d).

Biomass production The effect of maximizing leaf N concentration or LAI on simulated biomass production of the 15 rice genotypes differed between genotypes and between environments. ROK5, the moderately salt-tolerant improved cultivar showed little increase in simulated biomass production with maximum LAI and specific leaf N values in all four test environments. This cultivar was among the genotypes that produced the highest quantities of biomass in the four test environments and this was also predicted by ORYZA1 (Fig. 4a-d). Apparently on each sampling date, ROK5 had LAI and specific leaf N values close to maximum levels within the 15 rice genotypes studied in the four test environments.

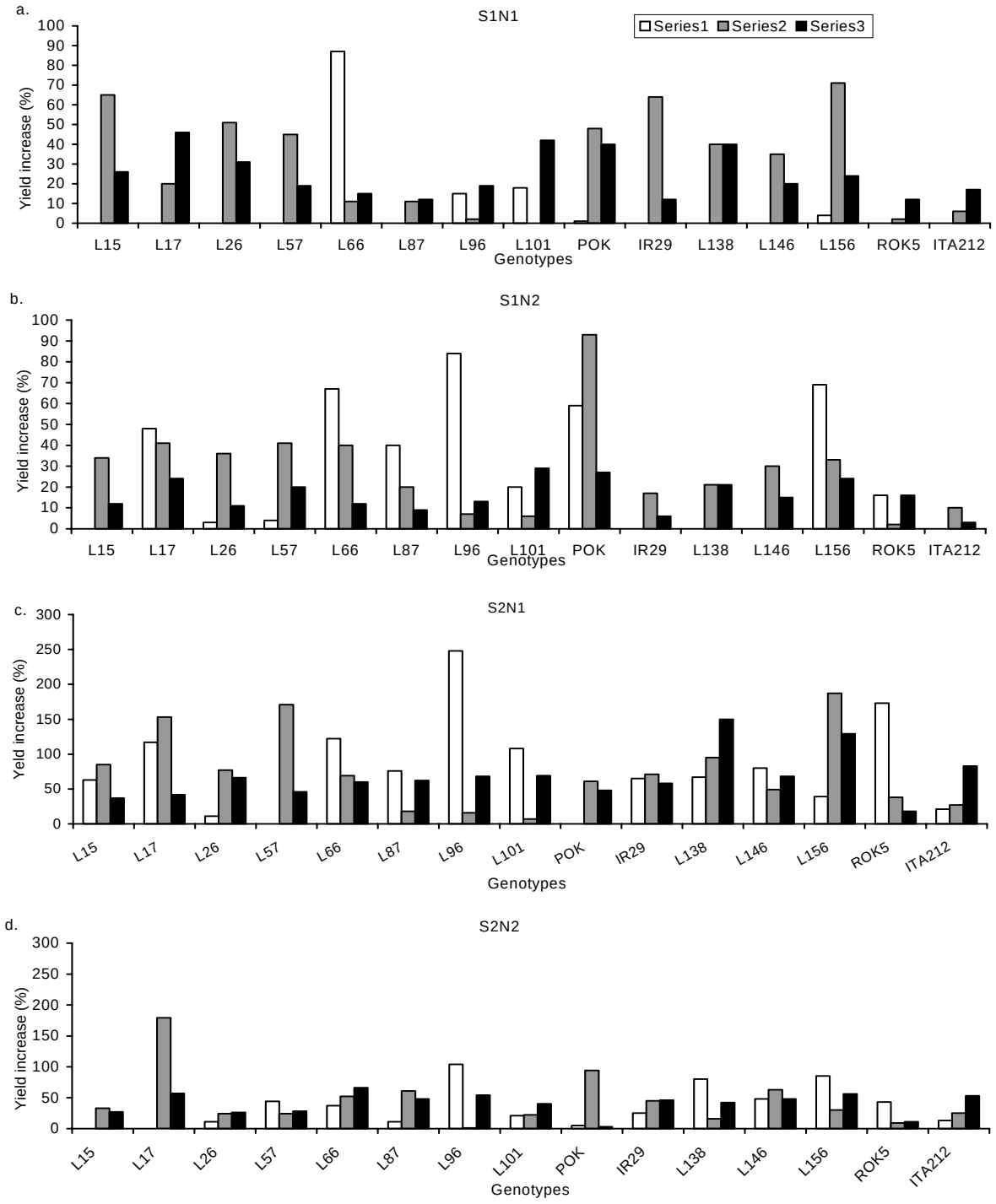

Fig. 6. Potential yield increase (%) for 15 genotypes of rice after substituting maximum genotypic values in each environment for observed values of fraction of shoot dry matter allocated to panicles between flowering and maturity, LAI and specific leaf N at all sampling stages. Series 1 – Maximum allocation of shoot dry matter to panicles; Series 2 – maximum specific leaf N; Series 3 – maximum LAI. S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively.

In the different test environments, sensitivity analysis with ORYZA1 revealed different potentials to increase the biomass production of the 15 genotypes of rice used in this study through modifications of their specific leaf N or LAI. Maximizing LAI had a larger effect on potential biomass production than maximizing specific leaf N for most of the genotypes in all four test environments. In addition, for most genotypes, maximizing LAI had larger effects on biomass production in the zero N fertilizer regimes than in the high N fertilizer regimes (Fig. 7). This implied that the range in observed LAI among the 15 rice genotypes was larger in the zero N fertilizer regimes than in the high N fertilizer regimes in both fresh and saline water.

Rice genotypes of which high biomass production in the zero N fertilizer regime in fresh water was well predicted by ORYZA1, showed less than 20% increase in biomass production when their specific leaf N values were maximized in the model. Among genotypes that exhibited 20% or more biomass increase with maximum levels of specific leaf N, those with the lowest LAI had the lowest biomass production (Fig. 7a). Thus the RILs L17, L138 and L146 had low specific leaf N and extremely low LAI and thus produced the least biomass in this environment.

In the high N fertilizer regime in fresh water, the combination of specific leaf N and LAI determined level of biomass production for the genotypes of which biomass production was well predicted. Thus ROK5 and ITA212, whose high biomass production were well predicted by ORYZA1, showed little increase in biomass production with maximum levels of LAI and specific leaf N (Fig. 7b).

Under saline conditions without N fertilizer, biomass production was determined more by LAI than by specific leaf N for those genotypes of which biomass production was well predicted by ORYZA1. Thus, ROK5 produced a high quantity of biomass in the saline environment without N fertilizer, mainly due to its high LAI and less to a moderately high specific leaf N (Fig. 7c).

For those genotypes of which biomass production in the high N fertilizer regime in saline water, was well estimated by ORYZA1, level of biomass production was determined by the combination of specific leaf N and LAI that they possessed. Thus, a combination of relatively high specific leaf N and high LAI enabled L57, L101 and ROK5 to produce high quantities of biomass in this environment (Fig. 7d) while L15 and L17 produced less biomass due to their combinations of low specific leaf N and low LAI (Fig. 7d).

Discussion

In both years 2000 (Fig. 1a(i), Fig. 1b(i)) and 2001 (Tables 1 and 2), when yield and biomass data of the genotypes were pooled together, the calibrated ORYZA1 model estimated grain yield better than biomass. For the pooled data sets, the model

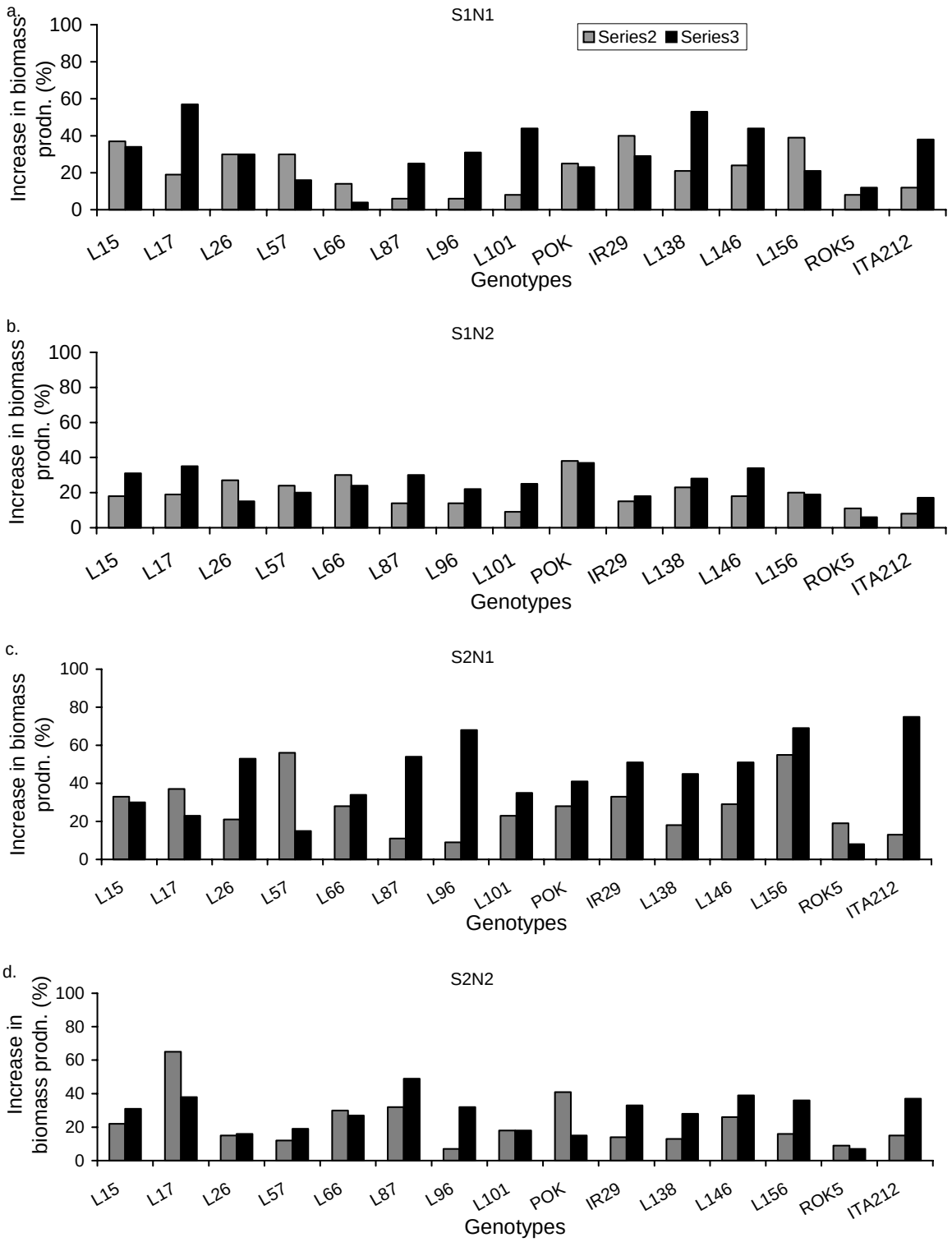

Fig. 7. Potential increase in biomass (%) for 15 genotypes of rice after substituting maximum genotypic values in each environment for observed values of LAI and specific leaf N at all sampling stages. Series 2 – Maximum specific leaf N; Series 3 – maximum LAI. S1N1, S1N2 – fresh water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively; S2N1, S2N2 – saline water with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer, respectively.

estimated yield and biomass production of genotypes well. Thus averaged over environments and genotypes, ORYZA1 successfully estimated yield and biomass production of the diverse genotypes tested in this study. The modifications of fraction of shoot dry matter allocated to panicles and LAI that we made in the calibrated ORYZA1 model, to improve model performance, both affected the estimated yield whilst only modifications of LAI would affect biomass production. Thus in 2001 we saw that the initial ORYZA1 model estimated biomass production better than yield but in the calibrated model, grain yield was estimated better than biomass production.

Despite the fact that the authors of ORYZA1 parameterized the model using values for semi-dwarf, high-yielding cultivars under a high N fertilizer regime in fresh water, where conditions for growth were close to the optimum for such cultivars, for most genotypes used in this study, yield was estimated rather well in all test environments in both 2000 and 2001. In our research too, during 2001, the calibrated ORYZA1 model made the best estimates of grain yield in the fresh water environment with high N fertilizer application where growing conditions most closely resembled those of the conditions under which the model was originally calibrated.

In saline environments, yields of more sensitive genotypes were generally over-estimated. This could arise as a result of the fact that salinity stress induces spikelet sterility and this is worse in salt-sensitive genotypes than in salt-tolerant genotypes (Abdullah *et al.*, 2001). ORYZA1 estimates spikelet sterility based solely on ambient temperatures at the time of flowering (Kropff *et al.*, 1994). Thus an under-estimation of spikelet sterility in salt-sensitive genotypes could lead to over-estimations of their yields by ORYZA1under salt stress.

Salt stress causes other physiological changes in rice such as enhancing synthesis of N-containing compounds in leaves (Faustino *et al.*, 1996) and reducing photosynthesis (Chattopadhayay *et al.*, 2002), which would affect the quality of predictions of yield and biomass production for rice genotypes made by ORYZA1. These salinity-induced physiological changes affect salt sensitive cultivars of rice more than salt tolerant cultivars. Thus, ORYZA1 generally over-estimated yields and biomass production of salt sensitive genotypes in saline environments. Through sensitivity analysis we saw that the salt-sensitive genotypes for which these over-estimations were made all maintained high levels of specific leaf N. ORYZA1 estimated yields and biomass production of salt-tolerant cultivars well although for some genotypes that maintained low levels of specific leaf N, yield and biomass production were under-estimated. To improve the performance of models such as ORYZA1 in variable environments, Dingkuhn (1996) recommended that more detailed models be developed that can simulate the underlying traits of adaptive phenotypic plasticity.

Estimates of biomass production in all environments also agreed well with observed

data for the limited data set containing data from the two parents, IR29 and Pokkali, in 2000. However, in 2001, the estimated biomass production of some of the 15 genotypes differed widely from actual observed biomass production. The 15 genotypes assessed in this study differed in many physiological traits, some of which could not be modelled by ORYZA1. Rice has been reported to show variation for N use efficiency (Fukai *et al*., 1999; Ohnishi *et al*., 1999). As explained earlier in the Materials and Methods section, the two parents and eleven RILs used in this study were expected to show variation for N use efficiency. Thus the large under-estimations and over-estimations of biomass production of some genotypes in fresh water environments might reflect differences in N use efficiency of the genotypes. Hence for similar leaf N concentrations, ORYZA1 would over-estimate biomass production of less N efficient genotypes of rice and under-estimate biomass production of more N efficient genotypes.

ROK5, the tall, moderately salt-tolerant cultivar, appeared to be well adapted in terms of biomass production, to growing conditions in the four environments studied in this trial. This cultivar was developed from a cross between a traditional cultivar and an improved cultivar (WARDA, 1994). Thus, it might have inherited genes conferring adaptation to local environments from its traditional parent. Sensitivity analysis revealed that inability to allocate sufficient dry matter to panicles was what most limited the yield potential of this cultivar especially in saline environments.

For the other rice genotypes evaluated in this study, sensitivity analysis revealed different options to increase their yield potentials in the four test environments. Thus using models such ORYZA1 can help breeders identify physiological traits, improvements in which would help increase yield potentials of rice in target environments.

Conclusions

The crop growth model ORYZA1 can adequately estimate yields and biomass production of divergent genotypes of rice in contrasting environments using limited physiological data as model input parameters. However, the model generally estimated grain yield better in high N fertilizer environments in both fresh and saline water. In environments with sub-optimal growing conditions, the ability of ORYZA1 to estimate yield and biomass production will be improved when coefficients are included in the model to account for the effects of key environmental factors on biomass production and yield formation.

The model also gave indications of possible differences in N use efficiency, amongst the 15 genotypes of rice assessed, for biomass production in fresh water. Furthermore, performing sensitivity analysis enabled us to determine the options for increasing yield potentials and biomass production of different rice genotypes through

manipulations of key physiological traits.

For a more complete study, it is essential to conduct sensitivity analysis on key physiological traits at different growth stages. This would allow researchers to assess the effects on yield and biomass production of modifications in key physiological traits on yield during different phases of crop development. With ORYZA1 these traits were evaluated using a smaller collection of germplasm than are normally screened by breeders when selecting parents in breeding programmes. Thus use of simulation models in breeding can greatly help to reduce the costs of the breeding programme.

CHAPTER 7

General discussion

General discussion

Rice production has been growing faster than the world population from the 1960s to 1990 (Duwayri *et al.*, 2000). This was largely due to the widespread use of improved cultivars possessing the semi-dwarf characteristic and disease resistance genes together with the adoption of intensive management techniques such as irrigation, use of agrochemicals and mechanization, for rice production. This intensification of rice production has placed great strains on natural resources and the environment. Since 1990, rice production has grown slower than the world population due to declining productivity of rice production systems (Duwayri *et al.*, 2000). This is a cause for alarm for all stakeholders concerned with meeting the food requirements of a rapidly expanding world population. Most of the increased rice production was realized in irrigated rice production systems where high levels of inputs are normally used for rice production. Soil salinity, high development costs, water scarcity and environmental concerns limit the possibilities of expanding the irrigated rice area (Papademetriou, 2000). The preponderance of abiotic stresses such as poor soil nutrient supply, soil salinity, droughts, weed infestation and flooding, in other rice production systems (rainfed lowland, upland, and flood-prone rice) has greatly limited rice yields in these systems. The bulk of the rice in many African countries, such as The Gambia, is produced in the uplands, rainfed lowlands and flood-prone areas where field conditions are often variable and yields are low. This results in large rice imports by countries in this region thereby stretching their already weak economies. Increasing rice yields in these marginal environments would make big impacts on the livelihoods of people in these areas. Improving the cultivation environment through fertilizer application, liming or irrigation is not feasible for many of these farmers due to the high costs of these technologies relative to their incomes, or due to unavailability or lack of sufficient knowledge. The improvement of the genetic yield potential of rice for marginal environments remains the cheapest and most accessible option for most of these low resource farmers. Thus, more research is needed to produce a combination of rice cultivars and management packages that would help increase yields in the various rice production systems found around the world.

In the past, breeders put more emphasis on increasing rice yields by increasing yield potential of rice under high input conditions. The reported stagnation in yield potential of *indica* inbred lines of rice under high input conditions in the tropics, has highlighted the need to revisit the technologies that sustained the huge increases in rice production in the last century. Furthermore, rice cultivars selected under high input conditions may not necessarily yield well in marginal environments. This then raises the need for

breeders to invest more effort into developing rice cultivars that would either maintain high yield levels in both high and low input environments (general adaptation) or yield high under only high input or low input conditions (specific adaptation). The decision as to whether to breed rice cultivars with specific or general adaptation depends to a large part on the extent of genotype × environment interaction occurring in rice yields within the range of target environments. In this thesis, G × E interaction was determined for grain yield, as expressed in a segregating population of rice grown under fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer. The rice population comprised Recombinant Inbred Lines (RILs) developed from the cross of a salt-sensitive, high yielding, semi-dwarf, improved cultivar, IR29 and a tall, salt-tolerant, traditional variety, Pokkali. The cultivation environments were differentiated by the presence or absence of salt stress and N fertilizer. To gain insight into the causes of G × E in rice yields across the range of environments included in this study, the relationships between yield, yield components and physiological traits of rice in the various test environments were examined using different research tools. QTL analysis was also conducted to study the genetic relationships between yield and yield components followed by marker-assisted selection for yield under stressed and non-stressed growing conditions. Finally, a crop growth model was used to explore the possibilities of yield improvement of rice, grown under different environmental conditions, through manipulations of LAI, specific leaf N and partitioning coefficient of shoot dry matter allocated to panicles. The studies revealed several sources of genetic variation in rice, all of which can be utilized through a holistic approach to increase grain yield under stressed and/or non-stressed conditions.

G × E in for grain yield in rice

From the results presented in Chapter 2 and Chapter 3, it was seen that significant G × E for yield occurred in this RIL population in the range of environments studied. The extent of G × E for yield depended on the environments being contrasted. When the trial environments were differentiated by only the presence or absence of N fertilizer, rankings of genotypes with regards to yield between the two environments did not change much. However, the rankings of genotypes with regards to yield between the two N fertilizer levels, as revealed by Spearman rank correlations, were better conserved in fresh water environments than in saline environments. There was less genotype × nitrogen interaction for yield in fresh water environments compared to saline environments. Based on these findings, it was therefore concluded that in lowland rice production systems characterized mainly by differences in N fertilizer usage, selection in high N fertilizer environments should succeed in identifying genotypes

that have general adaptability to the different N regimes. Furthermore, in Chapter 2, we also saw that variation in yield was higher in environments where N fertilizer was applied than those without N fertilizer. Since the chances of registering breeding success are higher in environments where more genetic variation is expressed for a trait, the results indicate that selection for high yields in fresh or saline environments should be practised in high N fertilizer environments. These findings support the common practice amongst plant breeders to select for improved yield under high input conditions. More caution should be exercised in saline environments when selecting for broad adaptation to different N fertilizer regimes. When resource availability permits, separate selection schemes should be devised in saline environments aimed at developing different sets of rice cultivars for low and high N fertilizer environments.

When target environments encompassed ecologies where salinity stress was experienced, it was found that G × E for the highest yielding genotypes in either fresh or saline water environments was strong enough to warrant selection for adaptability to specific growing conditions. Despite the fact that some of the RILs in the IR29 × Pokkali population showed good yield stability (maintained similar yield levels and rankings in all test environments), most such genotypes achieved only average yields in the various environments. The majority of the highest yielding genotypes in either fresh or saline water exhibited strong rank order changes for grain yield between fresh and saline water environments. Hence when breeding goals are driven by the need to produce rice cultivars that would help in avoiding disasters such as crop failure under salt stress, then breeders could try to select more stable genotypes and sacrifice the extra yield to be realized from the use of specifically adapted genotypes. Figure 1 in Chapter 2 showed that the yield penalty associated with recommending similar rice genotypes for fresh and saline environments was rather high. Furthermore, cultivation environments where salt stress is high enough to seriously depress rice yields are often well known to farmers and researchers. Thus, for the mangrove rice ecologies in West Africa and similar cultivation environments elsewhere, where salinity is a major problem to rice production, the development of salt tolerant cultivars of rice by breeders would greatly help in narrowing the yield gap in these environments.

Large, significant differences were observed between maximum yields in fresh water environments and those in saline environments. This implied that the level of salt tolerance for yield within this RIL population was not high enough to enable the most salt-tolerant genotypes to attain the high yields realized in fresh water environments. This highlights the importance of improving cultivation environments through cultural interventions such as liming, irrigation and fertilizer application to alleviate abiotic stress on crops in order to increase yields. Nonetheless, for those farmers who cannot access such interventions, use of salt tolerant rice cultivars alone would still

enable them to increase rice yields above present levels. Researchers are also looking into other avenues to increase crop yields under salt stress such as using biotechnology. To enhance its salt tolerance, some researchers have already used transformation techniques to introduce genes from either foreign sources into improved rice cultivars (Garg *et al*., 2002), the tolerance level of which is much higher than is known to exist naturally in available rice germplasm. However, in many parts of the world, much controversy still surrounds the use of genetically modified crops. Before these issues are resolved, rice breeders have to continue to look for adaptive genes conferring salt tolerance in traditional sources of genes such as local cultivars, wild species and related species. Better combinations of adaptive genes that would raise salt tolerance levels in rice germplasm need to be explored.

Application of QTL studies to explain and exploit G × E for yield in rice

Use of DNA-based markers in plant breeding can greatly accelerate breeding programmes by reducing the need to grow crops till maturity before effecting selection. In addition, the phenotypic plasticity associated with yield in many environments especially in saline environments, can hamper breeding advance when direct selection for yield is practised (Zeng *et al.*, 2003). DNA-based markers represent genotypic values and should therefore be more robust at identifying superior genotypes than methods based on phenotypic selection alone. Yield QTLs give indications of possible locations on the genome where genes controlling yield are located. Selection schemes can be designed to introgress interesting QTLs into improved cultivars. This raises the possibility of introducing novel gene combinations into improved rice cultivars, which could help increase both potential yield and confer greater tolerance to biotic and abiotic stresses.

In Chapter 3, results were presented on several putative yield QTLs in the rice genome, which were used to reliably predict yields under both stressed and non-stressed conditions. Estimated heritabilities for yield, deduced from regressions of yield predicted from molecular markers on actual observed yields, were high under all tested environmental conditions. Marker-assisted selection (MAS) not only succeeded in identifying superior genotypes but it also reliably predicted mean yields in all environments. Similar to the situation where phenotypic selection for yield was practised, MAS with yield QTLs was also generally more successful in fresh water than in saline water environments. Nonetheless, the reliability of MAS to identify superior rice genotypes under salt stress would help breeders overcome the reported problem of low selection efficiency through phenotypic selection for yield in saline environments (Zeng *et al*., 2003). With regards to selections in different N fertilizer regimes, reliability of MAS with yield QTLs was comparable between N fertilizer

regimes in fresh water while in saline environments, yield QTLs produced better estimates of yield in the zero N fertilizer regime than in the high N fertilizer regime.

Rank order changes for yield amongst the highest yielding genotypes between fresh and saline environments at either zero or high N fertilizer application levels, might signify the presence of different sets of genes controlling yield in fresh or saline water. The expression patterns of yield QTLs found in the IR29 × Pokkali RIL population in the range of tested environments also implied strong environmental specificity of the genes controlling yield in rice. As I explained in Chapter 3 this could have been caused by artefacts due to multiple regression identifying separate markers all linked to the same QTL in different environments. I could therefore not confirm from these results the extent of QTL × environment (QTL × E) interaction for yield within the range of environments studied. Many other researchers have also reported the apparent environmental specificity of most yield QTLs (Schut, 1998; Li *et al.*, 2000). QTL × E interaction has been cited as one of the main deficiencies limiting the application of QTL results to environments where the studies had not been conducted (Slafer, 2003). All the same I found three yield QTLs that were consistently expressed in fresh water at both levels of N fertilizer application while only one or no such QTL was found in other environmental contrasts. These QTLs are indications of possible locations of wide adaptability genes in this RIL population. In fresh water, the effects of consistent yield QTLs were larger in the high N fertilizer regime than in the zero N fertilizer regime. These findings are in agreement with our earlier observation that, for rice grown in fresh water environments, breeding success can be achieved by selecting in high N fertilizer regimes, for general adaptation to different N fertilizer environments.

In the various test environments, the two parents, IR29 and Pokkali both donated positive and negative yield QTL alleles. This shows that the capacity exists to increase rice yield potential in different environments beyond present levels by pyramiding interesting genes that control yield, in new cultivars. Pokkali, the tall, traditional cultivar, supplied most of the superior alleles for yield QTLs detected in all four test environments in this study (Table 3 in Chapter 3) although the contribution of superior yield QTL alleles from Pokkali was higher in the three stress environments and least under favourable growth conditions (S1N2). This emphasizes the important role of germplasm collections containing traditional cultivars and wild relatives as potential sources of novel genes or alleles of genes, for breeding programmes especially those aimed at sub-optimal growing conditions. Often tight linkage between desirable and undesirable genes in traditional cultivars or wild relatives requires breeders to make several backcrosses to the recurrent parent after performing the initial cross before a cultivar with acceptable agronomic properties is produced. Thus, using phenotypic selection to introduce new genes into crop cultivars from distantly related genotypes

either of the same or different species is usually slow. With QTL studies, researchers can already identify yield QTLs of interest and compute the probabilities of obtaining recombinants possessing desirable combinations of yield QTLs. Software packages are available that can be used to scan mapped populations and produce estimates of the amount of unwanted donor genome found in potential donors of desirable genes (van Berloo, 2000). Use of these kinds of software could accelerate breeding progress by reducing the number of backcrosses needed to produce cultivars with acceptable agronomic values. Such an approach requires the construction of reliable genetic maps showing precise locations of yield QTLs in the rice genome. Much progress has already been made in this direction. Researchers working in the International Rice Genome Sequencing Project (IRGSP), have created a database in which the physical map, genetic map and sequencing information of the rice genome, have been integrated (Anonymous, 2003). This integrated map would help breeders determine the genes involved in identified QTL regions thereby increasing the potential applications of QTL techniques in plant breeding.

Can component analysis explain G × E of rice yields?

Crop yield is the end product of a whole process of biological events, which take place in a plant during its growth cycle. Some of these processes are inter-linked and their interactions produce complex results, which could have additive, antagonistic or synergistic effects on crop yield. In small grain cereals, such as rice, barley and wheat, the phenomenon of yield component compensation is widely reported and is one of the reasons why many researchers shy away from attempting to increase yield potential through yield components for these crops. Direct selection for yield, ignoring these relationships, can only be expected to produce modest improvements in genetic potential of crops. Most of the large increases in cereal yield potentials in the past arose as a result of either conscious or unconscious selection of some key trait(s) that had huge implications for yield. For instance, use of heterotic genes in breeding, semi-dwarfing genes, which greatly improved the harvest index and subsequently the yield potential of cereals (Richards, 1996) and the New Plant Type of rice designed mainly on the basis of physiological yield components (Peng *et al*., 1999). Thus, to make the next big leap in cereal yield potentials for both stressed and unstressed conditions, researchers need to use more intrusive techniques to dissect the process of yield formation.

From studies conducted in year 2000 and 2001, the results of which were presented in Chapter 2 and Chapter 4, it was described how G × E for rice yield could be explained by changes in the strengths of associations between yield and yield components in different environments. Four yield components were studied – number of panicles per m^2, total grains per panicle, spikelet fertility and thousand grain weight.

Despite the differences in size and composition of rice populations used in the regressions of yield on its components in years 2000 and 2001, the general indication was that in fresh water environments grain number (a product of panicles per m^2 and total grains per panicle) was more relevant for yield determination whilst in saline environments, grain filling attributes (a product of spikelet fertility and grain weight) were more important for yield determination. These relationships can be understood from the point of view of source/sink relationships in rice in response to changing environmental conditions. Apparently in fresh water, yield is sink limited because the rice plant is able to produce enough assimilates after flowering to fill most of the grains formed. In saline environments, on the other hand, salinity restricts assimilate production and translocation due to its negative effects on various plant processes thereby leading to source limitation of yield. After flowering, assimilate production under salt stress is not able to fill the grains sufficiently to give high yields. Thus, in fresh water environments, attempts at improving rice yield potential through its components should be aimed at increasing grain number while in saline environments such efforts should be directed at selecting genotypes with high grain filling abilities.

Supply of N fertilizer under either fresh or saline water environments had a modifying effect on the relative importance of the sub-components of grain number or grain filling attributes in yield formation. The year of experimentation also affected these relationships as well as the inter-relationships amongst yield components. These year effects might have been due to differences in composition and size of populations studied and/or weather conditions especially rainfall. The amount of rainfall in 2000 was almost double that of 2001 at the experiment site, in The Gambia. Hence salt stress lasted longer before it was diluted by rainfall in 2000 relative to 2001. In addition, the higher rainfall also implied that humidity in 2000 was higher than in 2001. The combination of low rainfall and low humidity in 2001 relative to 2000 meant that the effects of salt stress on the rice crop were greater in 2001 than in 2000. This was reflected in the lower mean yields from saline treatments in 2001 compared to 2000.

The number of total grains per panicle was more strongly associated with grain yield in fresh water environments without N fertilizer application whilst panicles per m^2 accounted for most of the variation in yield in fresh water environments where 100 kg ha^{-1} N fertilizer was applied. Application of N fertilizer to rice promotes tillering and hence production of a higher number of panicles than under non-fertilized conditions. In both years 2000 and 2001, spikelet fertility was more strongly associated with grain yield in saline environments without N fertilizer application than in any other environment. Thousand grain weight, however, had small but significant effects on yield in year 2000 and these effects were smaller at the zero N fertilizer level than at the high N fertilizer level. In year 2001, grain weight had large effects on yield in

saline environments and its effects were larger in the zero N fertilizer regime than in the high N fertilizer regime.

With regards to inter-environmental correlations, these were higher for grain yield than for most yield components (Figs 1 and 2 of Chapter 2). Among the yield components, a comparison of inter-environmental correlations revealed that, of the four yield components examined, panicles per m^2 was generally the least stable component. Apparently G × E for grain yield in rice was mainly due to the high sensitivity of panicles per m^2 to changing environmental conditions. In the past, breeders selected rice cultivars that responded well to high doses of N fertilizer application. In that attempt, breeders might inadvertently have been selecting against genes controlling other yield components that are not as responsive to N fertilizer as panicles per m^2. For instance, in this study grain weight was generally more stable between environments than all other yield components. Moreover, there was less variation for grain weight in the IR29 × Pokkali RIL population than for the other yield components in all test environments. Despite this limited variation of grain weight, in all environments, several QTLs were detected for grain weight, which could potentially be used in breeding programmes to increase grain weight of rice beyond present maximum levels.

Analysis of yield components is useful in uncovering the underlying causes of G × E for yield across a range of environments so as to identify possible options for cultivar improvement in each environment. Nonetheless, attempts at improving yield potential through yield components alone are often not as efficient as direct selection for yield. Some of the reasons for this are that yield components can never perfectly account for the variation in yield and that compensatory relationships exist between yield components under certain growing conditions. In Chapters 2 and 3, reliable estimates of rice grain yield were produced from yield components directly and also from yield component QTLs under most growing conditions in years 2000 and 2001. However, these yield predictions made either through phenotypic selection of yield components or through yield component QTLs were always less efficient than when yield was selected for directly through phenotypic selection or through yield QTLs. Several instances of yield component compensation were observed in this research as explained in Chapters 2, 3 and 4, at both the phenotypic and genotypic levels in the various test environments. At the phenotypic level, it was found that in certain environments, some yield components were positively correlated whilst in other environments either the same or other yield components, were negatively correlated. Path coefficient analysis was particularly useful under such situations for it considered the effects of inter-relationships amongst yield components in computing the total contribution of a yield component to yield. At the genotypic level, several QTLs were identified which were expressed for more than one yield component and these QTLs

either increased or decreased both components or increased one and reduced the other. The majority of yield QTLs identified in this research were also detected for one or more yield component in either the same or in separate environments. This information can be combined with knowledge of the relative importance of yield components for yield determination in a particular environment thereby allowing breeders to design crosses aimed at pyramiding the relevant component QTLs in prospective rice cultivars.

Physiological options for increasing rice yield potential under stressed and non-stressed conditions

The complex relationships that exist between yield and its components and amongst the yield components themselves, illustrate some of the difficulties faced by breeders in their efforts to increase crop yield potentials for different growing conditions. Much physiological research was conducted in the past aimed at unravelling these relationships in order to increase yield potentials. Most of these research efforts failed to produce the expected increase in yield potential because the researchers often overlooked the inter-relationships amongst physiological traits and also because the traits were usually evaluated under controlled conditions that were not representative of actual field situations (Slafer, 2003). However, integrating the effects of modifications of physiological processes over the crop growth cycle could enable researchers to assess the impact on yield of manipulations of physiological traits. In this regard, use of analytical techniques such as path coefficient analysis and systems modelling are useful in determining the effects of changes made in physiological characteristics at different development stages, on grain yield.

Under the various environmental conditions of this study, the research results identified several physiological options, at different levels of organization, for improvement of rice yield potential in different environments. In Chapters 4 and 5, a discussion was made on the need to increase biomass production of rice in all test environments for increased yield potential. Ideally this should be complemented with a high allocation of assimilates to panicles. In the results presented in Chapter 4, we saw that among the selection of genotypes used in this research, increasing the proportion of dry matter allocated to shoots at flowering time could only increase yields under saline conditions but would have little or negative effects on yield in fresh water environments. There was apparently large genotypic variation among the 15 rice genotypes studied, for the fraction of dry matter allocated to panicles although the highest yielding genotypes in all four test environments generally had good abilities to partition dry matter to panicles, as explained in Chapters 5 and 6. According to Slafer (2003), increasing harvest index alone without increasing biomass has implications on

other growth processes, which could affect yield negatively.

The results presented in Chapter 4 indicated that manipulation of the stem/leaf ratio of rice genotypes could also affect rice yield potential in different environments. In fresh water environments, a reduction of stem weights and an increase in leaf weights around flowering time are expected to increase yield potential. In saline environments, however, increasing stem weights is expected to cause slight but positive increases in yield while increasing leaf weights would reduce yield potential in zero N fertilizer regimes and increase yield potential in high N fertilizer regimes. With regards to roots, a high root weight was associated with high yielding genotypes in fresh water environments without N fertilizer application (Chapters 4 and 5). Under such N limiting conditions genotypes with larger root systems would be able to absorb more N from the soil and therefore be able to produce more biomass through photosynthesis which could then be used for yield formation. A large root size is apparently less important in fresh water environments with high N fertilizer application because possibly most genotypes are able to absorb sufficient amounts of N from the soil under such conditions. In saline environments, however, the ability to partition less dry matter to roots and more to shoots would allow rice plants to expose less root surface to salt injury while at the same time maintain relatively high amounts of biomass in shoots thereby diluting the effects of absorbed salts on plant tissues.

Classical physiological studies comparing certain physiological attributes of high and low yielding genotypes of rice in stressed and non-stressed environments revealed that differences in LAI, leaf N concentration, growth duration and possibly N use efficiency, could explain yield differences in the various environments (Chapter 5). Results from sensitivity analysis of yield presented in Chapter 6 revealed that yield potentials of rice in each of the four test environments could be increased by improvements of LAI, leaf N concentration and fraction of dry matter allocated to panicles. Of these three physiological traits studied, high-yielding genotypes usually had high levels of one or two traits but showed potential for yield increase through modifications of the other trait(s). In Chapters 2, 5 and 6, it was explained that indications of differences in N use efficiency were found within the collection of rice genotypes used in both 2000 and 2001. This could also be another option for increasing rice yield potential in both low and high N fertilizer environments. Modifications of growth duration could also be expected to produce increments in rice yield potential. As explained in Chapter 5, long growth duration favoured high biomass production in fresh water environments although this does not necessarily lead to high yields when the genotype(s) had low coefficients for dry matter partitioning to panicles. A long growth duration would only be desirable in saline environments when it is coupled with salt tolerance. Over long periods of growth in a saline medium, salts

accumulate in rice leaves to toxic levels thereby accelerating leaf senescence and resulting in reduced photosynthetic capacity. Thus generally, to increase rice yield potential for saline environments, breeders should select for early to medium duration. The precise developmental stages at which modifications of physiological traits are expected to make most impact on yield need to be explored further through simulation studies.

The findings of the various studies, results for which are presented in this thesis, corroborate the view of Perera *et al*. (1998) that there is still abundant genetic variation in *indica* rice which can be exploited to increase rice yield potentials under different growing conditions.

Main conclusions

In lowland rice breeding programmes, selection schemes should be aimed at general adaptability in fresh water environments characterized mainly by differences in N fertilizer application. This is the ability of genotypes to rank high in all target environments. Such selection should be practised under high N fertilizer conditions. When the range of target environments includes both fresh and saline water environments, separate selection schemes should be used to identify genotypes adapted specifically to either fresh or saline water environments. In saline environments with variable application of N fertilizer, selection of rice genotypes with general adaptability to different soil N statuses is feasible but may not be as successful as in fresh water environments.

There is potential to increase the genetic yield potential of rice in different environments when proper understanding of the relationships between yield and its components and between the components themselves, is obtained. In fresh water environments, the yield potential of rice could be increased by improving its grain production, which comprises panicle number and grains per panicle. In saline environments, yield potentials of rice could be increased by improving grain-filling ability of genotypes. Sub-components of grain filling attributes are grain weight and fertility of spikelets. The level of N fertilizer usage in fresh or saline water would dictate the relative importance of a particular sub-component of grain number production or grain filling ability for yield determination.

In order to exploit the genetic variation in yield components for increasing rice yield potential, it is essential to study the physiological traits involved in their formation. These physiological traits could then be manipulated through either agronomic practices or through breeding, so as to favour the best combination of components required for higher yield potential in a particular environment.

Marker-assisted selection has the potential to improve rice breeding programmes for

it can successfully identify superior genotypes of rice under both stressed and non-stressed conditions. QTL studies can accelerate breeding programmes by identifying possible locations of useful genes and estimating the amount of unwanted donor genome that needs to be removed through back-crossing. QTL methodology also has the potential of enabling researchers to pyramid alleles of useful genes that act in the same direction on a trait of interest thereby giving the possibility of increasing both potential yield and tolerance to environmental stresses.

The ORYZA1 model can be used to simulate growth and yield of diverse genotypes of rice in both stressed and non-stressed conditions. The model can also identify physiological options for increasing rice yield potential in different environments. For stressed conditions, model performance could be further improved by incorporating effects of key environmental variables in model subroutines.

Future challenges

The need for increased food production around the world is more acute now than ever before due to the rapidly increasing world population and the high pressure this exerts on limited natural resources. Rice, being the most important food cereal for human consumption, needs to see a dramatic increase in production. As expanding the land area is not an option for increased rice production in most areas, new options should be explored to help increase rice yield potential. There is a need to improve the genetic yield potential for both low and high input cultivation environments.

The earlier reluctance of most breeders to effect selection in low input environments due to low heritabilities and gains to selection could now be overcome when greater use is made of the advances made in computing technologies and biotechnology over recent decades to create synergies in research. Already, farmers have started reaping the benefits of such an integration of technologies in plant breeding. Researchers at the West Africa Rice Development Association (WARDA) used biotechnology techniques to overcome sterility barriers that are often encountered by breeders when they cross different subspecies of rice. Researchers at this institute crossed high yielding Asian rice (*Oryza sativa*) with traditional African rice (*Oryza glaberrima*) and used embryo rescue techniques to get viable offspring (WARDA, 2001). The inter-specific progeny developed from this cross were found to combine high yielding traits of their *sativa* parents with the stress tolerance derived from their *glaberrima* background. Many African farmers have already adopted these inter-specific hybrids and have reported markedly improved yields over their usual *sativa* and *glaberrima* cultivars under both high- and low-input conditions. One main property of the inter-specifics is their high grain production. Usually, *sativa* cultivars produce an average of 250 grains per panicle and the *glaberrimas* around 75-100 grains per panicle (WARDA, 2001). The

inter-specifics can produce more than 400 grains per panicle. Many African governments are already instituting policies to help in the widespread use of the inter-specific cultivars across the whole range of rice production systems in their countries. This shows the potential benefits that can be realized through greater collaboration between scientific disciplines.

The application of molecular marker technology holds great promise in improving the efficiency of cultivar development process. However, despite the abundance of reports on quantitative trait loci (QTLs) found for different traits in crop plants, most of the reports of successful applications of QTL methodology in plant breeding have been with regards to introgression of major genes (Anonymous, 1998). Marker-assisted selection (MAS) had been shown in our study to be effective at identifying superior genotypes of rice under both fresh and saline water conditions. Its more widespread use in plant breeding could be expected when index maps with high densities of markers, are developed for crops (Mazur *et al*., 1995). Further research is also needed to increase the efficiency of QTL detection techniques and accuracy of parameter estimates. Most QTL detection techniques, as the one I used in this study, ignore epistatic interactions between yield QTLs. As was seen in Chapter 3, additive effects of QTLs generally explained less than 60% of the variation in yield and its components. Inclusion of epistatic effects in QTL models would improve the power of QTL analysis (Yi and Xu, 2002). Unfortunately, at present, most of the readily available computer programmes for detecting QTLs are not able to accommodate calculations of epistatic effects due to the complex computations involved.

More research is also needed to determine the genes controlling key physiological traits at different developmental stages. Plant breeders have often overlooked the significance of development stage-dependent expression of genes in yield formation. Crop simulation models can be used to first identify the growth stages at which improvement of morpho-physiological traits are expected to make the largest impact on yield. QTL analysis could then be employed to identify the genomic regions controlling the traits at the particular stage of crop development. The relevant QTLs could then be introgressed into promising genotypes through marker-assisted breeding or by the use of genetic transformation.

To increase use of crop simulation models in rice breeding, there is a need to undertake more empirical research to determine genetic coefficients of rice for key physiological traits contributing to its yield formation under low and high input conditions. In addition, rice researchers also need to determine coefficients for key environmental variables in rice growing ecologies. These environmental coefficients should be incorporated into simulation models together with genetic coefficients for various physiological traits of rice. The resultant models can then be used in ideotype

breeding for different environmental conditions.

Thus combining knowledge from different scientific disciplines will give rice researchers a better understanding of the processes underlying yield formation in variable growing conditions, enable them to identify options to increase yield potential and devise more efficient ways of detecting and pyramiding relevant genes in prospective rice cultivars.

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Summary

Most of the rice breeding effort in the past was geared towards increasing yield potential of rice under high input management systems. The use of high yielding cultivars of rice under high input conditions in irrigated lowland rice production systems has succeeded in meeting the world's requirements for rice production for the larger part of the second half of the last century. However, towards the end of the century, world rice production was noticed to be growing less than the world population. This worrying new trend was attributed to declining productivity of rice production systems especially in irrigated lowlands, which produce most of the world's rice. Production constraints such as soil salinity, poor soil nutrient supply, droughts and flooding, in other rice ecosystems (uplands, rainfed lowlands and flood-prone areas) limit rice yields in those systems. Irrigated rice area comprises a small proportion of the total rice area in Africa. Most of the rice in Africa is produced under low input conditions resulting in poor yields. Rice cultivars bred for high input environments may not be suited to these low input environments as many rice cultivars are not bred for tolerance to the abiotic stresses present in marginal environments. This results in a large yield gap between average farm yields and maximum attainable yields of rice in Africa. In The Gambia, soil salinity and low soil supply of nutrients especially nitrogen are two of the major constraints limiting rice production. Provision of rice of cultivars that are tolerant to the major stresses in rice ecosystems would greatly help improve productivities of the systems with a resultant improvement in the livelihoods of poor farmers.

Meeting the requirement for increased rice production in the future due to an ever-expanding world population demands a dual approach of increasing rice yield potentials for high input environments and reducing yield gaps, especially in marginal areas where the potential for increased production is highest. Several researchers have recently reported a stagnation of rice yield potentials in tropical environments at 10 tonnes ha^{-1}. New breeding approaches are needed to break through this apparent yield ceiling. Furthermore, researchers also need to put more effort into breeding for stress tolerance in rice so as to increase the genetic yield potential of rice under marginal conditions. The objectives for carrying out the research presented in this thesis were to determine appropriate selection strategies when significant genotype-by-environment (G × E) interaction of rice yield is observed across a range of environments and also to identify options, at the level of yield components and physiological traits, for increasing the genetic yield potential of rice under both high and low input conditions.

For this purpose, a series of experiments were conducted in which a collection of

divergent rice genotypes were grown under fresh water (EC of 0.15 dS m^{-1}) and saline (EC of 8 dS m^{-1}) conditions with 0 kg ha^{-1} N or 100 kg ha^{-1} N fertilizer in a lowland environment in The Gambia, West Africa. Significant G × E interaction was observed in grain yield and yield components as revealed by analysis of variance. Comparisons of grain yields between environments, showed high inter-environmental correlations between yields in zero and 100 kg ha^{-1} N fertilizer regimes in both fresh and saline water environments although the environmental correlations between yields at different N fertilizer levels were higher in fresh water than in saline water environments (Chapter 2). This shows that it is possible to breed rice cultivars that have general adaptability to both low and high levels of N fertilizer application in lowland rice ecosystems. However, the environmental correlations for yields between fresh and saline water environments, especially for the high yielding genotypes, were low, signifying that different sets of rice genotypes should be selected for cultivation in either fresh or saline water environments.

Analyses of relationships between yield and yield components in the different environments gave insights into the causes of G × E in yield at the yield component level (Chapters 2 and 4). The relative contributions of two major aspects of yield - grain number (a product of panicles m^{-2} and total grains per panicle) and grain filling ability (a product of grain weight and spikelet fertility) – differed between fresh and saline environments. In fresh water environments, yield was generally sink limited as the rice crop was able to fill most of the grains formed. Thus, increasing grain number of rice genotypes for fresh water environments should boost yield potentials. In saline environments, however, salt stress limits assimilate production and translocation. Under such conditions, rice yield potential would be improved by selecting genotypes with high grain filling abilities. The level of N fertilizer usage modified the relative importance of panicles m^{-2} or total grains per panicle in fresh water and grain weight or spikelet fertility in saline environments, for yield determination.

The genetic basis of these complex relationships between yield and yield components leading to G × E in yield across the range of environments was examined through analyses of the associations between DNA-based markers (AFLPs) and yield and yield components (Chapter 3). Significant associations between traits and DNA-based markers should indicate possible quantitative trait loci (QTLs) for the traits, which are regions of the genome where genes controlling the traits are located. Overall, markers accounted for between 23% and 60% of the variation in yield and yield components across four environments. More than 86% of the markers found to be associated with grain yield, were also associated with one or more yield component in either the same or in different environments. Markers expressed for more than one trait had either similar or opposite effects on the traits. This could explain the positive

and negative correlations between yield and yield components and amongst yield components across the range of environments. Thus, use of QTL methodology especially when coupled with yield component analysis can give insights into the genetic basis of G × E for rice yield across a range of environments. The study also revealed options to increase rice yield potential in different environments through a pyramiding approach by accumulating QTLs, which act in the same direction on yield, in potential rice cultivars.

Indirect selection of agronomically important plant traits through molecular markers (marker-assisted selection – MAS) would accelerate breeding programmes by reducing the need to grow crops till maturity before effecting selection. Use of MAS was shown to be successful at identifying superior genotypes of rice in both stressed and non-stressed environments. However, MAS was more successful at identifying superior rice genotypes when markers for yield *per se* were used to make selections than when yield was selected for by using markers for yield components. Hence, attempts to increase rice yield potential through yield components by either phenotypic or genotypic selection, should carefully consider the relationships between yield and yield components in target environments.

In Chapter 4, path coefficient analysis was conducted to determine how physiological traits, collected around flowering time, influenced grain yield through yield components, in different environments. The results suggested different plant type designs for rice in fresh or saline water environments. Similar plant types are suggested in fresh water environments for both zero and high N fertilizer regimes while in saline water environments the plant type designed for zero and high N fertilizer regimes should be slightly different. The positive associations between high shoot biomass and grain yield were found to be due to the positive effect of a high shoot biomass on grain number attributes (product of panicles m^{-2} and grains per panicle) in fresh water environments and grain filling attributes (product of grain weight and spikelet fertility) in saline environments. Allocation of more dry matter to shoots instead of roots reduced grain yield in fresh water environments but increased grain yield in saline environments. Thus in fresh water environments, rice genotypes with large root systems should be selected and this effect will be more beneficial under low than under high N fertilizer conditions. In saline environments, there is a high need to dilute absorbed salts in shoots so as to minimize salt injury to tissues especially the leaves where most of the salts accumulate. Hence in saline environments, selected rice genotypes should be able to partition proportionately more biomass to shoots than roots.

Stem weight had a negative effect on panicles m^{-2} and also grain yield, because panicles m^{-2} had a strong influence on yield in fresh water environments. In saline

environments, however, stem weight had positive effects on grain yield especially in the high N fertilizer regime. High leaf weights, LAI and late flowering all had positive effects on grain yield in fresh water due to their effects on key yield components. In saline environments, however, high leaf weight had a negative effect on yield in the zero N fertilizer regime and a positive effect on yield in the high N fertilizer regime. Rice genotypes selected in saline environments should be early to medium flowering types due to the negative effect of a delayed flowering time on grain filling attributes. These associations between physiological traits and yield components and yield should be examined at different growth stages of rice in order to gain a fuller understanding of physiological mechanisms involved in yield formation under stressed and non-stressed conditions.

In Chapter 5, comparisons of physiological traits (LAI, leaf N concentration, weights of leaves, stems, panicles and total shoot biomass, and partitioning coefficients of dry matter to different plant organs) were made between low and high yielding genotypes of rice at different growth stages under four environmental conditions. The objective of this study was to identify the physiological traits that enable high yielding ability in different environments. Differences in physiological traits between high and low yielding genotypes were more evident in saline environments than in fresh water environments. In both fresh water and saline environments, yield levels were found to be strongly influenced by the total biomass production in conjunction with the fraction of dry matter allocated to panicles. In fresh water environments, rice genotypes that had large leaf areas and high leaf N concentrations produced more biomass than those with smaller levels of these two traits. Under saline conditions, however, high biomass production was more closely associated with a large leaf area than with high leaf N concentration. Salt stress reduced LAI, total biomass and yield but increased leaf N concentration and growth duration of most rice genotypes. However, the reduction of LAI, biomass and yield under salt stress was more acute on salt sensitive than salt tolerant genotypes of rice. The differences in LAI and biomass between salt tolerant and salt sensitive genotypes of rice were larger during the pre-flowering period than in the post-flowering period.

Chapter 6 looked at the possibility to use an eco-physiological crop growth model, ORYZA1, to simulate biomass production and grain yield of diverse genotypes of rice and also to identify physiological options to increase the yield of each genotype across a range of environments. ORYZA1 estimated grain yields better than biomass production and these predictions were better in fresh water than in saline water environments. Nonetheless, the model was generally able to differentiate between high and low yielding genotypes of rice in all environments based on model input traits. In all test environments some rice genotypes produced high quantities of biomass despite having

relatively low to medium LAI, specific leaf N and growth duration. This could signify that the genotypes assessed in this study also differed with regards to N use efficiency, and other researchers had reported that genetic variation exists in rice for this trait. In saline environments, it is expected that inclusion of salinity effects on leaf N concentration and spikelet fertility of rice, in the appropriate subroutines will improve model performance. The potential yield gains for each rice genotype, to be obtained through improvements of LAI, specific leaf N and fraction of dry matter allocated to panicles, differed between genotypes and between environments.

In Chapter 7, the overall results of the study are integrated and discussed. The results show that the genetic yield potential of rice could be increased in both stressed and non-stressed environments through a better understanding of the processes involved in yield determination from yield components and physiological traits. Furthermore, it was explained how QTL analysis could be used to elucidate the often-reported compensatory mechanisms between yield components of rice and also the variable relationships between yield and yield components across different environments. An understanding of the genetic basis of these mechanisms would guide breeders in their design of crosses that would help pyramid useful alleles of genes in rice cultivars so as to increase yield potentials in different environments. Suggestions for increased application of advances made in other scientific disciplines such as biotechnology, statistics and systems modelling, in plant breeding, are also discussed.

Samenvatting

De veredeling van rijst heeft zich in het verleden voornamelijk gericht op het verkrijgen van rassen die een hoge opbrengst geven onder gunstige omstandigheden, d.w.z. bij optimale watervoorziening en voldoende beschikbaarheid van nutriënten. Dankzij het gebruik van hoog-opbrengende rassen, in combinatie met bemesting, is het tot voor kort gelukt om in geïrrigeerde laaglandgebieden voldoende rijst te produceren. Gedurende de laatste jaren echter blijkt de mondiale rijstproductie minder snel te groeien dan de wereldbevolking. Deze verontrustende trend is waarschijnlijk toe te schrijven aan afnemende opbrengsten in geïrrigeerde laaglandgebieden, die het leeuwendeel van de wereldrijstproductie voor hun rekening nemen. In andere gebieden (o.a. hoogland, regenafhankelijk laagland) zijn factoren zoals bodemverzilting, lage bodemvruchtbaarheid, droogte en regelmatige overstromingen de oorzaak van opbrengsttekorten. In Afrika vormt de geïrrigeerde rijstteelt slechts een gering aandeel van het totale rijstareaal. Rijst wordt daar voor het merendeel geproduceerd in landbouwsystemen die zich kenmerken door een laag input-niveau, hetgeen resulteert in lage opbrengsten. De moderne rijstrassen, die zijn ontwikkeld voor en onder optimale teeltomstandigheden, zijn doorgaans niet geschikt voor dergelijke lage-input milieus omdat zij niet bestand zijn tegen de abiotische stress die in dergelijke milieus veelvuldig optreedt. Het resultaat hiervan is dat in Afrika het verschil tussen de gemiddelde opbrengst bij de boer en de maximaal haalbare opbrengst erg groot is. In Gambia zijn bodemverzilting en geringe beschikbaarheid van nutriënten (met name stikstof) de belangrijkste factoren die de rijstopbrengst beperken. Als er rassen beschikbaar zouden komen die goed bestand zijn tegen de meest voorkomende abiotische stress in de rijstteelt aldaar, dan zou dat de rijstproductie in Gambia ten goede komen en de levensomstandigheden van de kleine, arme boeren aanzienlijk verbeteren.

De wereldbevolking groeit snel en daarmee neemt ook de behoefte aan een hogere rijstproductie toe. Om de opbrengsten van rijst per oppervlakte-eenheid te laten stijgen, is een tweeledige benadering nodig. Ten eerste moet de opbrengstpotentie van rijst onder omstandigheden bij optimale watervoorziening en voldoende beschikbaarheid van nutriënten omhoog. Ten tweede moet de kloof tussen potentiële en werkelijke opbrengst bij de boer worden verkleind. Dit laatste is met name belangrijk voor marginale gebieden waar de mogelijkheden voor het verkleinen van de kloof het grootst zijn.

Verschillende onderzoekers hebben recentelijk gerapporteerd dat er sprake is van een stagnatie in de stijging van opbrengstpotentie van rijst onder tropische

omstandigheden. Er lijkt een opbrengstplafond van zo'n 10 t per ha te bestaaan. Slechts met behulp van nieuwe veredelingsstrategieën kan dit plafond mogelijk worden doorbroken. Voor marginale teeltomstandigheden is bovendien veel aandacht nodig voor het veredelen op stresstolerantie in rijst. Op die manier kan de voor boeren haalbare opbrengst voor rijst onder marginale condities worden verhoogd.

De doelstellingen van het in dit proefschrift beschreven onderzoek waren dan ook:

- Het ontwikkelen van geschikte selectiestrategieën voor verschillende milieus wanneer er sprake is van significante genotype × milieu (G × E) interactie ten aanzien van de rijstopbrengst;
- Het identificeren van mogelijkheden om de genetische haalbare opbrengst van rijst bij zowel hoge als lage inputs te verhogen, door te bezien in hoeverre selectie op bepaalde opbrengstcomponenten en fysiologische eigenschappen soelaas biedt.

Om deze doelstellingen te realiseren werd een serie proeven uitgevoerd waarin een verzameling divergente rijstgenotypes werd geteeld onder zoet water (met een EC van 0.15 dS m^{-1}) en zilt water (EC van 8 dS m^{-1}) condities met een stikstofgift van 0 of 100 kg ha^{-1}. Deze proeven werden uitgevoerd in een laaglandmilieu in Gambia. Een ANOVA toonde aan dat de G × E interactie significant was voor korrelopbrengst en voor een aantal opbrengstcomponenten. Voor 0 en 100 kg N ha^{-1} bleken de korrel-opbrengsten van de genotypen goed gecorreleerd te zijn. Dit gold voor zowel het zoet-water als het zout-water milieu. De correlaties tussen opbrengsten van de verschillende N trapen waren evenwel hoger in het zoet-water milieu dan in het zout-water milieu (Hoofdstuk 2). Het lijkt derhalve mogelijk rassen te maken die breed zijn aangepast aan zowel lage als hoge N niveaus in agro-ecosystemen waarin laaglandrijst wordt verbouwd. De correlaties tussen de opbrengsten in zoet- en zout-water milieus waren echter laag. Dit werd vooral veroorzaakt door de hoog-productieve genotypen. Voor de teelt in zoet of zilt water lijken dan ook geheel verschillende rijstgenotypen nodig.

Analyses van de relaties tussen korrelopbrengst en opbrengstcomponenten in de veschillende milieus verschaften inzicht in de oorzaken van de G × E interactie ten aanzien van korrrelopbrengst en opbrengstcomponenten (Hoofdstukken 2 en 4). Het belang van de twee belangrijkste elementen van opbrengst, te weten korrelaantal (een product van het aantal pluimen per m^2 en het aantal korrels per pluim) en het vermogen om de korrels te vullen (bestaande uit aartjesfertiliteit en individueel korrelgewicht) verschilden tussen het zoete en het zilte milieu. In het zoet-water milieu werd de opbrengst in het algemeen sink-gelimiteerd aangezien het rijstgewas in staat was de meeste gevormde korrels ook inderdaad te vullen. Daarom zou een toename in het aantal korrels van de rijstrassen onder zoet-water condities moeten leiden tot een hogere opbrengstpotentie. In zilte milieus, echter, beperkt zoutstress de assimilaten-productie en het assimilatentransport. Onder dergelijke omstandigheden zou de

opbrengstpotentie van rijst kunnen worden verbeterd door te selecteren op een grote sinksterkte, dat wil zeggen voldoende korrels en voldoende grote korrels. Het niveau van stikstofbemesting had een effect op de invloed die het aantal pluimen per m^2 en het totaal aantal korrels per pluim op de opbrengst hadden in het zoet-water milieu en had tevens een effect op de invloed van het individueel korrelgewicht en de vruchtbaarheid van de aartjes op het opbrengend vermogen.

De complexe relaties tussen opbrengst en opbrengstcomponenten hangen samen met de G × E interactie voor opbrengst die over het brede spectrum van milieus werd aangetoond. Deze complexe relaties hebben deels een genetische basis. Daarom werd voor de kwantitatieve eigenschappen opbrengst en de opbrengstcomponenten een genetische analyse uitgevoerd met behulp van moleculaire merkers (AFLP) (Hoofdstuk 3). Significante associaties tussen eigenschappen en moleculaire merkers kunnen mogelijk aangeven op welke posities van het genoom de genen zich bevinden die dergelijke kwantitatieve eigenschappen bepalen (quantitative trait loci, QTLs). Merkers verklaarden voor 23-60% de variatie in opbrengst en in opbrengst-componenten over de vier milieus. Meer dan 86% van de merkers die bleken te associëren met korrelopbrengst, waren ook geassocieerd met één of meerdere opbrengstcomponenten, hetzij in hetzelfde milieu hetzij in een ander milieu. Merkers die met meer dan één eigenschap associeerden konden gelijkgerichte maar ook tegengestelde effecten op de eigenschappen hebben. Hiermee zou kunnen worden verklaard waarom – afhankelijk van het milieu – de relaties tussen opbrengst en opbrengstcomponenten en tussen opbrengstcomponenten onderling zowel positief als negatief konden zijn. De QTL methode geeft enig inzicht in de genetische achtergrond van de G × E interactie voor rijst, vooral wanneer ook de QTLs voor opbrengst-componenten worden meegenomen. Het onderzoek toonde ook aan dat het mogelijk is om de opbrengstpotentie van rijst in verschillende milieus te verhogen door QTLs te stapelen die de opbrengst in dezelfde richting beïnvloeden.

Indirecte selectie van agronomisch relevante planteigenschappen door gebruik te maken van moleculaire merkers (merkerondersteunde selectie, MOS) zou het veredelingsproces kunnen versnellen. Bij het toepassen van MOS is het immers niet langer nodig de gewassen voor selectie te laten afrijpen. MOS bleek nuttig bij het identificeren van superieure genotypen van rijst, zowel onder omstandigheden met stress als onder stressvrije omstandigheden. MOS bleek echter meer succes te hebben bij het identificeren van de superieure genotypen wanneer merkers voor opbrengst werden gebruikt voor de selectie dan wanneer geselecteerd werd met behulp van merkers voor opbrengstcomponenten. Het is daarom van belang dat bij de pogingen om de opbrengstpotentie van rijst te verhogen door middel van selectie op opbrengstcomponenten (of dat nu is op basis van fenotype of op basis van genotype)

goed rekening te houden met de relaties tussen opbrengst en opbrengstcomponenten in het milieu waarvoor geselecteerd wordt.

In Hoofdstuk 4 werd een padcoëfficiëntanalyse uitgevoerd. Doel hiervan was vast te stellen hoe in verschillende milieus de fysiologische eigenschappen die rond de bloei werden waargenomen, de korrelopbrengst beïnvloedden via hun invloed op de opbrengstcomponenten. De resultaten gaven aan dat het optimale planttype van rijst verschilt voor zoet-water en zilte milieus. Bij teelt in zoet water zijn dezelfde fysiologische eigenschappen relevant voor de beide stikstoftrappen (0 en 100 kg N per ha). Voor de zilte milieus zijn voor de beide stikstofbemestingen toch enigszins verschillende eigenschappen nodig. De positieve koppeling tussen bovengrondse biomassa en korrelopbrengst bleek in zoet-water milieus samen te hangen met het positieve effect van een hoge bovengrondse massa op het korrelaantal (het product van aantal pluimen per m^2 en aantal korrels per pluim). In zout-water milieus hing deze positieve koppeling samen met eigenschappen die verband houden met de korrelvulling (product van korrelgewicht en percentage aartjesfertiliteit). Als meer droge stof naar de bovengrondse delen werd getransporteerd in plaats van naar de wortels dan bleek de korrelopbrengst in zoet-water milieus lager te zijn, maar in zilte milieus juist hoger te zijn. Dit gegeven suggereert dat het voor zoet-water milieus goed is rijstrassen te selecteren met grote wortelsystemen. Het positive effect daarvan is groter bij een lage dan bij een hoge stikstofbemesting. In zilte milieus dienen de zouten die zich in het bovengrondse plantendeel ophopen, zo veel mogelijk te worden verdund teneinde de zoutschade in de weefsels zo veel mogelijk te beperken. Dit geldt vooral voor het blad waar zich de grootste hoeveelheden zouten ophopen. Het is daarom gewenst om voor zilte milieus rassen te selecteren met een hoge spruit/wortel verhouding.

Een hoger stengelgewicht leidde tot minder pluimen per m^2 en tot een lagere korrelopbrengst. Dit effect hing samen met de sterke invloed van het aantal pluimen per m^2 op de opbrengst in zoet-water milieus. In zilte milieus had het stengelgewicht echter een positieve invloed op de korrelopbrengst, vooral bij een hoge stikstofbemesting. Hoge bladgewichten, een hoge bebladeringsindex en late bloei hadden alle een positief effect op korrelopbrengst bij zoet water. Dit hing samen met de positieve correlatie van deze eigenschappen met de belangrijkste opbrengst-componenten. In zilte milieus had een hoog bladgewicht evenwel een negatief effect op de opbrengst indien geen kunstmeststikstof werd gegeven en een positief effect op de opbrengst indien 100 kg N per ha werd toegediend. De rijstgenotypen die in zilte milieus worden geselecteerd zouden tevens vroeg tot middelvroeg in bloei moeten komen, omdat een uitstel van de bloei een negatief effect heeft op eigenschappen die verband houden met korrelvulling. Deze associaties tussen fysiologische eigenschappen en opbrengstcomponenten en opbrengst dienen te worden onderzocht in verschillende groeistadia

van het rijstgewas teneinde een beter begrip te ontwikkelen van de fysiologische mechanismen die bij opbrengstvorming onder gestresste en stressvrije condities een rol spelen.

In Hoofstuk 5 werden relevante fysiologische eigenschappen van laagopbrengende en hoogopbrengende rassen in verschillende groeistadia en onder vier verschillende milieus vergeleken. Het betrof de eigenschappen bebladeringsindex, het stikstofgehalte in het blad, gewicht van blad, stengel, pluim en van de totale bovengrondse massa, en de drogestofverdelingscoëfficiënten. Het doel van deze studie was na te gaan welke fysiologische eigenschappen kunnen leiden tot een hoge opbrengst in de verschillende milieus. Verschillen in fysiologische eigenschappen tussen hoogopbrengende en laagopbrengende rassen bleken groter te zijn in de zilte milieus dan in de zoetwater milieus. Bij zowel zoet water als zilt water bleken de niveaus van korrelopbrengst sterk beïnvloed te worden door de productie van totale biomassa en door het aandeel droge stof dat in de pluimen werd geïnvesteerd. In de zoetwatermilieus produceerden de rijstgenotypen die een groter bladoppervlak dan wel een hogere stikstofconcentratie in het blad hadden meer biomassa dan de genotypen die lagere waarden voor deze eigenschappen vertoonden. In zilte milieus bleek een hoge biomassa sterker gecorrelleerd met een groot bladoppervlak dan met een hoge stikstofconcentratie in het blad. Zoutstress gaf een verlaging van de bebladeringsindex, de bovengrondse biomassa en de korrelopbrengst maar deed de stikstofconcentratie in the blad en de groeiduur van de meeste rijstgenotypen toenemen. De bebladeringsindex, biomassa en korrelopbrengst erden echter onder zilte condities meer verlaagd bij zoutgevoelige dan bij zouttolerante rijstgenotypen. De verschillen in bebladeringsindex en biomassa tussen zouttolerante en zoutgevoelige typen waren groter gedurende de periode voor de bloei dan gedurende de periode na de bloei.

In Hoofstuk 6 werd nagegaan in hoeverre de biomassaproductie en de korrel-opbrengst van uiteenlopende rijstgenotypen konden worden gesimuleerd met behulp van het ecofysiologische gewasgroeimodel ORYZA1. Dit model werd tevens gebruikt om na te gaan welke fysiologische opties er zijn om de opbrengst van elk genotype in verschillende milieus te verhogen. ORYZA1 gaf betere schattingen van de korrel-opbrengst dan van de biomassaproductie en de voorspellingen waren beter voor zoet-water milieus dan voor zilte milieus. Over het algemeen was het model voor alle milieus in staat om onderscheid te maken tussen hoog-opbrengende en laag-opbrengende genotypen van rijst. Voor alle testmilieus werd tevens waargenomen dat sommige rijstgenotypen grote hoeveelheden biomassa produceerden ondanks het feit dat hun bebladeringsindex niet zo hoog was en ondanks dat ze ook lage waarden vertoonden voor de specifieke hoeveelheid stikstof in het blad en de groeiduur. Dit zou kunnen betekenen dat de onderzochte rassen ook verschilden in hun stikstof-

benuttingsefficiëntie. Andere onderzoekers hadden ook reeds gevonden dat er in rijst voor deze eigenschap genetische variatie bestaat. Voor zilte milieus is de verwachting dat de modeluitkomsten kunnen worden verbeterd door in de daarvoor geëigende subroutines rekening te houden met de zouteffecten op bladstikstofconcentratie en de fertiliteit van de aartjes.

In Hoofdstuk 7 worden de totale resultaten van de studie geïntegreerd en bediscussieerd. De resultaten laten zien dat het genetische opbrengstpotentieel van rijst kan worden verhoogd, zowel in milieus met stress als in stressvrije milieus, door gebruik te maken van een beter inzicht in de processen die een rol spelen bij opbrengstvorming op basis van opbrengstcomponenten en fysiologische eigenschappen. Tevens wordt uitgelegd hoe een QTL analyse kan worden benut om de vaak gememoreerde compensatie-mechanismen tussen opbrengstcomponenten van rijst op te helderen. Bovendien kunnen QTL analyses gebruikt worden om de variabele relaties tussen korrelopbrengst en opbrengstcomponenten over verschillende milieus op te helderen. Inzicht in de genetische achtergrond van dergelijke mechanismen kunnen veredelaars helpen bij het bepalen welke kruisingen nuttig zijn. Vervolgens kan gepoogd worden deze nuttige allelen te stapelen om aldus in de verschillende milieus de opbrengsten te verhogen. Tenslotte worden er suggesties gedaan om nieuwe wetenschappelijke inzichten uit andere vakgebieden, zoals biotechnologie, statistiek en systeemmodellering, beter te benutten in de plantenveredeling.

Curriculum vitae

Baboucarr Manneh was born in Sukuta, Kombo North district, Western Division, The Gambia, on March 3, 1969. He attended Sukuta Primary School from 1975 to 1981. He pursued his secondary education first at Gambia Muslim High School between 1981 and 1986, obtained his 'O' Level certificate in 1986, proceeded on to Gambia High School from 1986 to 1988 and graduated in 1988 with an 'A' Level certificate. He enrolled at Njala, University College, University of Sierra Leone from 1989 to 1993 and graduated in 1993 with a Bachelor of Science degree in Agriculture General. In March 1994, the National Agricultural Research Institute (NARI) of The Gambia employed him as an Assistant Research Officer first in the Agricultural Engineering Unit and then in the Socio-economics Unit. In August 1996, he followed an MSc programme in Crop Science at Wageningen University and graduated in January 1998 in the specialization Crop Breeding. His thesis research topic was 'The influence of level of nitrogen fertilization on the expression of yield QTLs in spring barley (*Hordeum vulgare* L.)'. On his return to NARI in February 1998, he was appointed head of the Cereals Research Programme and then in April 1998, Station Manager for the Sapu Research Station. He was awarded a Wageningen University sandwich fellowship in 1998 and began his PhD studies in September 1998 that led to the production of the thesis described herein. This work was part of a collaborative research project between the National Agricultural Research Institute (NARI) of The Gambia and the groups Laboratory of Plant Breeding and Crop and Weed Ecology of the Department of Plant Sciences, Wageningen University, Netherlands.

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