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Genotypic Responses of Upland Rice to an Altitudinal Gradient

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Preface

This thesis is based on multi-locational field trials conducted in Madagascar and the greenhouse trials carried out in the University of Hohenheim, Germany in the Crop Water Stress Management Section of the Department of Plant Production and Agroecology in the Tropics and Subtropics. The thesis is submitted together with the enclosed three peer reviewed manuscripts in a partial fulfilment of the requirement for Ph.D. degree at the Faculty of Agricultural Sciences.

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List of Abbreviations

AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of variance
BVP	Basic vegetative phase
C:N	Carbon nitrogen ratio
CF	Chlorophyll fluorescence
CI	Chlorophyll index
DAO	Days after onset of treatments
Er	Early sowing
ET_o	Potential evapotranspiration
FAO	Food and Agriculture Organization
FL	Flowering
F_m	Maximal fluorescence in dark adapted state
F_m'	Maximal fluorescence in light-adapted state
F_m^r	Maximal fluorescence at far-red light
F_o	Minimal fluorescence at a modulated light
F_o'	Minimal fluorescence intensity in light-adapted state
F_o'/F_m'	Efficiency of excitation energy capture
F_s	Transient fluorescence at steady state
FYM	Farm yard manure
GLM	General linear model
GM	Germination
GnYd	Grain yield (t ha^{-1})
HA	High altitude
IPCA	Interaction principle component analysis
IPCC	Intergovernmental Panel on Climate Change
IRRI	International Rice Research Institute
LA	Low altitude
LSD	Least significant difference of means
Lt	late sowing
LUE	Light use efficiency

MA	Mid altitude
MP	Maturity phase
MS	Mean sum of square
N	Nitrogen
NA	Data not available
NPQ	Non-photochemical quenching related to excess energy re-emitted as heat
NPQ_F	Fast relaxing non-photochemical quenching
NPQ_S	Slow relaxing non-photochemical quenching
NPR	Net photosynthetic rate
N-supply	Nitrogen supply
NUE	Nitrogen use efficiency
PCA	Principle Component Analysis
PFS	Percentage of filled spikelet (%)
PI	Panicle initiation
PM	Physiological maturity
PP	Photoperiod
PPFD	Photosynthetic photon flux density
PPT	Percentage of productive tillers (%)
PRI	Photochemical reflectance index
PSI	Photoperiod sensitivity index
PSII	Photosynthesis II
PSP	Photoperiod sensitive phase
q_N	Non-photochemical quenching related to excess energy re-emitted as light
q_P	Photochemical quenching related to derived photosynthesis
r^2	Coefficient of determination
RCTV	Relative contribution to variance
REG	Regression
RF	Rainfall
RP	Reproductive phase
RUE	Radiation use efficiency
SD	Standard deviation
SE	Standard error

SLP	Saturated light pulse
SLW	Specific leaf area
SPAD	Soil-Plant Analysis Development
SPP	Spikelets per panicle (n)
SR	Solar radiation
SRES	Special Report on Emission Scenarios
SS	Sum of square
SSP	Percentage of spikelet sterility
T_{base}	Critical lower temperature for development (base temperature)
TGW	Thousand grain weight (gm)
T_{max}	Maximum air temperature
T_{mean}	Mean air temperature
T_{min}	Minimum air temperature
TPH	Tillers per hill (n)
T_{sum}	Accrued number of heat units required for flowering
VP	Vegetative phase
VPD	Vapour pressure deficit
Yr	Year
ΦNO	Non-regulated heat dissipation
ΦNPQ	Non-photochemical quenching
ΦPSII	Maximum quantum yield of Photosynthesis II

Summary

Adaptation strategies are required for crops to cope with changing climate. The impact of climate change on crop production is not straight forward to predict as extreme events comprise multiple combination of abiotic stresses and their impact differs in crop physiological growth stages. The mechanism on how new abiotic stress combinations translate into phenology and yield, and which cultivars are better adapted is yet unclear. Crop growth models are available that have been parameterized and validated for some aspects of possible climate change scenarios but in view of complex interactions crop responses to climate change are difficult to predict. On the other hand, prediction of the complex ideotype trait combinations may be interesting for breeders but physiological models are required that are well validated for the target environments. In upland rice grown under rainfed conditions without surface water accumulation methane emission is negligible and therefore greenhouse gas emission much lower compared to irrigated paddy rice systems. In addition, growing demand for rice and the increasing pressure on irrigated land leads to development of upland rice areas to supplement irrigated rice. Therefore, this study investigates genetically diverse upland rice genotypes from a wide range of origins across altitudinal gradient locations. The main objective of this study is to investigate genotypic responses of upland rice to different environments in order to calibrate crop growth models, which allow the evaluation of effects of climate change on upland rice systems.

Multi-locational field (three locations: 1625, 965 and 25 m asl) trials comprising non-replicated phenological plots with five sowing dates (monthly staggered) in two consecutive years creating thirty different environments, and replicated physiological yield trials with two sowing dates (monthly staggered; early and late sowing) in two consecutive years creating twelve different environments were established in Madagascar. Ten contrasting upland rice genotypes were included in both field trials. Meteorological data were recorded on a daily basis during trial periods. Developmental stages were observed in the phenological plots; in the physiological plots yield and yield components were recorded. In addition, greenhouse trials were conducted with one upland rice genotype subjected to seven N-supply levels in a hydroponic system at the University of Hohenheim in order to understand the relationship

between chlorophyll index, photochemical reflectance index and chlorophyll fluorescence parameters. Various statistical tools were applied to analyse field and greenhouse data sets.

The phenological trial showed that duration to flowering was 117, 81 and 67 d in high (HA), mid (MA) and low (LA) altitudinal locations respectively. 90% of the total variance was explained by location when pooled over genotype, location, sowing dates and year. In HA, factors such as genotype, sowing date and year equally contributed to the observed variability whereas in MA year was the most determining factor and genotype had no significant contribution. Similarly, in LA sowing date was the main influencing factor and year had no significant effect. Aggregated data over locations, sowing dates and years indicated that each degree Celsius rise in mean air temperature decreased crop duration by 5 to 9 days depending upon genotype. Basic genotypic thermal constants T_{base} ranged from 9.8 to 13.9 °C and T_{sum} from 816 to 1220 °C d within the selected genotypes. Cold tolerant genotypes were less affected by lower T_{min} (14 °C) at booting to heading stage regarding spikelet sterility in HA, whereas others were highly affected at 15 °C (cold stress). Similarly, both cold sensitive and tolerant genotypes were affected by T_{max} (above 30 °C) at flowering in MA and LA locations (heat stress).

Grain yield and yield components were highly affected by location, year, sowing date, and genotypes and the interactions between these yield-determining factors were obvious. In HA, early sown cold tolerant genotypes had more than 5 t ha^{-1} grain yield and one month delay in sowing led to highly reduced yield whereas other genotypes had very poor yield on both sowing dates due to cold stress. In MA, yield difference between sowing date and genotypes was small (4.3 - 4.9 t ha^{-1}). Grain yield in LA was vulnerable due to frequent tropical storms. Yield stability analysis showed that cold tolerant genotypes had above average stability. AMMI model for grain yield showed that environment and genotype by environment interactions were highly significant. Yield components determined during specific development stages of the genotype such as tillers per hill and percentage of filled spikelets were mainly influenced by environment, spikelets per panicle and thousand grain weight were influenced by genotype, and percentage of productive tillers was equally influenced by both genotype and environment. PCA biplots showed that all HA environments were equally influenced by all weather parameters with minimum air temperature having the strongest positive influence on genotypic performance. In all MA environments genotypic performance

in all phenophases was strongly and positively influenced by rainfall, and strongly and negatively influenced by vapour pressure deficit, solar radiation and potential evapotranspiration. In the LA environments, main weather parameters influencing genotypic performance were maximum temperature and high rainfall accompanied by strong winds.

The field measured SPAD values of the upper canopy leaves reflected the location specific N-remobilization and leaf senescence levels after flowering. Similarly, PRI values showed the abiotic stress responses among development stages and locations along the altitudinal gradient. These readings showed that genotypes were efficient in radiation use and N-remobilization after flowering in MA. The unsynchronized relationship between source (leaf) and sink (grain) explained the yield penalty. Emphasis on identification of morpho-physiological traits contributing to cold tolerance should be placed for further breeding.

We conclude that genotypic responses of upland rice cultivars differed across altitudinal gradients. Genotypes that are well adapted in HA can easily be adapted in MA without yield decrease. But genotypes well adapted in MA may show a huge yield penalty in HA due to lower temperature during reproductive phase and consequently reduced sink formation. Frequent tropical storms and high temperature reduced yield potential in LA. Therefore, HA has a large potential for the future food security considering climate change scenarios. At present, MA is favorable for upland rice production systems, whereas LA is highly vulnerable and is expected to be even more vulnerable in future. Those results on genotype-specific responses to environmental conditions allow further improvement of crop models such as RIDEV and SAMARA (synthesis of SARRAH and EcoMeristem), which can be used to test a number of phenotypic traits x environments combinations to define ideotypes of upland rice varieties adapted to changing climate and cropping calendars. Genotypic responses of phyllochron, biomass production and crop growth rate, and radiation use efficiency across altitudinal gradients will be included to parameterize these models. In this regard, collaborations with AfricaRice, CIRAD and IRRI are ongoing.

Zusammenfassung

Um mit veränderten Klimabedingungen zurechtzukommen, sind Kenntnisse über die Anpassungsstrategien von Nutzpflanzen notwendig. Die Auswirkungen des Klimawandels sind komplex, da Extremereignisse gemeinsam mit verschiedenen Kombinationen abiotischer Stresse auftreten und die Auswirkungen je nach physiologischer Entwicklungsphase unterschiedlichen Ausprägungen unterliegen. In wie fern sich verschiedene Sorten hinsichtlich ihrer Anpassungsfähigkeit unterscheiden, ist größtenteils noch unbekannt. Zwar existieren Wachstumsmodelle, die für einige mögliche Klimaszenarien parametrisiert und überprüft wurden, aber die komplexen Wechselwirkungen, die der Reaktion auf klimatische Veränderungen zugrunde liegen, sind noch nicht zufriedenstellend geklärt. Zusätzlich besteht großes Interesse seitens der Pflanzenzüchtung, die Eignung verschiedener Idiotypkombinationen für verschiedene Zielumwelten vorherzusagen. Aufgrund der zu vernachlässigenden Methanemission von regenbewässertem und nicht überstauten Trockereis, führt dessen Anbau zu einer geringeren Produktion von klimaschädlichen Treibhausgasen Gleichfalls führt die wachsende Nachfrage nach Reis und der damit wachsende Druck auf bewässertes Land zu einem Ausbau der Trockenreisproduktion, um die Nassreisproduktion zu ergänzen. Diesbezüglich wurde in dieser Studie der Anbau verschiedener, weltweit verbreiteter Trockenreisgenotypen in Anbauregionen verschiedener geographischer Höhe untersucht. Das Hauptziel dieser Studie ist es, die genotypischen Reaktionen von Trockenreis auf verschiedene Umwelten zu untersuchen und die Ergebnisse in ein Wachstumsmodell einfließen zu lassen, welches die Abschätzung von Auswirkungen des Klimawandels auf Trockenreisproduktionssysteme erlaubt.

Es wurden multilokale Feldexperimente in Madagaskar (drei Standorte: 1625, 965 und 25 m NN) durchgeführt. Zur Untersuchung phänologischer Eigenschaften wurden in nicht wiederholten Blöcken mit 5 verschiedenen Aussaatterminen und zwei aufeinanderfolgenden Jahren 30 Umwelten geschaffen. Physiologische Ertragsversuche wurden in wiederholten Blöcken angelegt und zu zwei verschiedenen Aussaatterminen (frühe und späte Aussaat, monatlich versetzt) jeweils in zwei aufeinanderfolgenden Jahren durchgeführt, so dass insgesamt 12 verschiedene Prüfumwelten abbildet wurden. In beiden Feldexperimenten wurden jeweils zehn kontrastierende Trockenreisgenotypen untersucht. Meteorologische

Daten wurden täglich in beiden Versuchen aufgezeichnet. In Phänologie-Blöcken wurden die Entwicklungsstadien beobachtet und in Physiologie-Blöcken wurden Ertrag und Ertragskomponenten gemessen. Um die Zusammenhänge zwischen Chlorophyll-Index, photochemischer Reflektion und Chlorophyllfluoreszenz zu verstehen, wurden mit einem Trockenreisgenotyp zusätzlich Gewächshausversuche zur N-Versorgung an der Universität Hohenheim in einem hydroponischen System durchgeführt. Zur Analyse der Datensätze aus Gewächshaus- und Feldversuchen wurden verschiedene statistische Werkzeuge eingesetzt.

Der Versuch zur Phänologie zeigte, dass die Zeit bis zur Blüte 117, 81 und 67 d in der Hoch- (HA), Mittel- (MA) und Tieflage (LA) betrug. Bei Mittelwerten aus Genotypen, Höhenlage, Aussaatdatum und Jahr konnte 90% der Gesamtvarianz durch die Höhenlage erklärt werden. In HA trugen die Faktoren Genotyp, Aussaatdatum und Versuchsjahr gleich stark zur beobachteten Variabilität bei, während in MA das Versuchsjahr den größten Einfluss und Genotyp keinen signifikanten Einfluss hatte. In LA war das Aussaatdatum ebenfalls der Faktor mit dem größten Effekt. Über Höhenlage, Aussaatdatum und Jahr zusammengefasste Werte weisen darauf hin, dass jedes Grad Celsius Temperaturerhöhung die Zeit von Keimung bis Blüte je nach Genotyp um 5 bis 9 Tage verkürzte. Die sortenspezifischen Kardinaltemperaturen T_{base} der untersuchten Genotypen lag zwischen 9.8 und 13.9 °C und T_{sum} zwischen 816 und 1220 °C d. Eine geringere T_{min} (14 °C) zwischen Rispenschwellen und Rispenschieben hatte auf kältetolerante Genotypen bezüglich der Ährchensterilität in HA einen kleineren Effekt, während andere Genotypen diesbezüglich bei 15 °C (Kältestress) stark beeinflusst wurden. T_{max} (über 30 °C) hatte sowohl auf kältetolerante als auch auf kälteempfindliche Genotypen in MA und LA einen Effekt (Hitzestress).

Höhenlage, Jahr, Aussaatdatum und Genotyp hatten starken Einfluss auf Kornertrag und Ertragskomponenten und die Wechselwirkungen zwischen diesen ertragsbestimmenden Faktoren waren offensichtlich. In HA lag der Ertrag bei früh ausgesäten kältetoleranten Genotypen bei über 5 t ha^{-1} und eine um einen Monat später erfolgte Aussaat hatte negative Effekte auf den Ertrag, während der Ertrag bei den übrigen Genotypen zu beiden Aussaatterminen sehr gering war. In MA waren die Ertragsunterschiede zwischen den Aussaatterminen und Genotypen gering (4.3 - 4.9 t ha^{-1}). Für Trockenreis muss in LA durch häufig auftretende tropische Stürme mit Ertragseinbußen gerechnet werden. Eine Analyse der Ertragsstabilität zeigte, dass kältetolerante Genotypen eine überdurchschnittliche

Ertragsstabilität aufwiesen. Eine Modellierung des Kornertrages mit AMMI zeigte, dass der Effekt der Umwelt und die Genotyp/Umwelt Interaktion hochsignifikant waren. Ertragskomponenten, die in spezifischen Entwicklungsstadien gebildet wurden, wie Bestockungstriebe pro Pflanze und der Anteil gefüllter Ährchen, wurden hauptsächlich durch den Faktor Umwelt beeinflusst. Die Anzahl an Ährchen pro Rispe und das Tausendkorngewicht wurden durch den Genotyp bestimmt und der Anteil an produktiven Bestockungstrieben war gleichermaßen vom Genotyp und Umwelt beeinflusst. Die PCA Biplots zeigten, dass alle HA Umwelten gleich stark durch die Wetterparameter beeinflusst waren, wobei die minimale Lufttemperatur den stärksten Einfluss auf die genotypische Leistungsfähigkeit hatte. Die genotypische Leistungsfähigkeit in allen phänologischen Stadien war in allen MA Umwelten positiv durch Niederschlag, und stark negativ durch Dampfdruckdefizit, Einstrahlung und potentielle Evapotranspiration beeinflusst. In den LA Umwelten waren die Wetterparameter, die den größten Einfluss auf die genotypische Leistungsfähigkeit hatten, die maximale Temperatur und eine durch Starkwind begleitete hohe Niederschlagsmenge.

Die im Feld gemessenen SPAD-Werte von Blättern der oberen Bestandesebene zeigten standortbedingte Remobilisierung von N und Blattalterungsprozesse nach der Blüte. PRI-Werte verwiesen auf abiotische Stresse, die je nach Anbaugebiet und Entwicklungsphase variierten. Diese Werte zeigten, dass Reis in MA effizienter in Bezug auf Strahlungsnutzung und N-Remobilisierung nach der Blüte war. Die nicht aufeinander abgestimmte Beziehung zwischen Source (Blatt) und Sink (Korn), die zu Ertragseinbußen führt wurde durch die Identifizierung von morpho-physiologischen Merkmalen erklärt und hervorgehoben, was in Zukunft zur Entwicklung Kältetoleranter Genotypen in der Pflanzenzüchtung beitragen kann.

Wir schlussfolgern, dass sich die sortenspezifischen Reaktionen von Trockenreis innerhalb des Höhengradienten unterscheiden. An HA angepasste Genotypen können ohne Ertagseinbußen an MA angepasst werden. Jedoch können an MA angepasste Sorten aufgrund geringerer Temperaturen während der reproduktiven Phase und daher reduzierter Sink-Bildung große Ertragseinbußen in HA zeigen. Häufige tropische Stürme und hohe Temperaturen reduzieren das Ertragspotential in LA. In Anbetracht von Klimawandelszenarien haben daher HA ein großes Potential für die zukünftige Ernährungssicherung. Gegenwärtig bieten MA günstige Bedingungen für die

Trockenreisproduktion, während die Produktion in LA hochgradig anfällig ist und zukünftig wahrscheinlich noch anfälliger werden wird. Diese Ergebnisse bezüglich der genotypspezifischen Reaktionen auf Umweltbedingungen erlauben eine weitere Optimierung von Wachstumsmodellen wie RIDEV und SAMARA (enstanden aus SARRAH und EcoMeristem), die für die Überpüfung von Interaktionen von phänotypischen Merkmalen und Umweltbedingungen genutzt werden können, um Ideotypen von an den Klimawandel und an Anbaukalender angepasste Trockenreissorten zu beschreiben. Für die Parameterisierung dieser Modelle werden genotypische Reaktionen von Phyllochron, Biomasseproduktion und Wachstumsraten und Strahlungsnutzungseffizienz entlang eines Höhengradienten einbezogen. Diesbezüglich werden derzeit Kollaborationen mit AfricaRice, CIRAD und IRRI unterhalten.

1. General introduction

1.1. Background

Rapid growth in food demand and slower growth in food production is imbalance in the world food market (Trostle, 2008). According to FAO (2010), 13.6% people of the estimated world population are undernourished and the number of hungry people is increasing due to neglect of agriculture relevant to very poor people by governments. On the other hand, the significant inflation of food price is creating increased disparity between food sufficient and deficient people, and the number of starving people will drastically increase in the future (Parry et al., 2004; Parry et al., 2005). However, although the socio-economic system and political conflicts may cause poverty and hunger, climate change cannot be excluded as the main cause (Bohle et al., 1994). Climate change threatens agricultural production systems through higher and more variable temperatures, changes in precipitation patterns, and increased occurrences of extreme events such as droughts and floods (Nelson, 2009). The rise in temperature driven by increasing concentrations of greenhouse gases (CO_2, CH_4 and N_2O) leads to a decrease in cereal productivity in low latitudes and a potential increase for cereal productivity in mid to high latitudes (IPCC, 2007). The Special Report on Emission Scenarios (SRES) for the 21st century projected increased yields in colder environments and decreased yields in warmer environments due to an increase frequency of warmer spells, and crops failure due to increased drought spells, intense tropical cyclone activity, and salinisation of irrigation water. Rainfed ecosystems, where an increasing share of the poorest and the most vulnerable population resides have inherent problems in the agricultural sector and the production level. Such areas will be more affected by changes in agro-climatic conditions than in most other parts of the world (Wassmann and Dobermann, 2007). Climate change is increasingly a reality, and these changes are already being observed (Wassmann et al., 2010a). Crop adaptation strategies such as adjustment of planting dates and crop varieties to fit an adapted cropping calendar, crop relocation and improved management practices are required to cope with changing climate. But significant gaps in knowledge still exist on the complex interactions of how specific crops are affected by shifting planting dates and how varieties respond in terms of crop duration and grain yield under various climatic conditions over time and to adapted management practices.

1.2. Impact of climate change on crop production

The impact of climate change on crop production is not straight forward to predict because the increased frequency of extreme weather events subject the crop periodical to multiple combinations of abiotic stresses and the intensity of impact varies between crop physiological growth stages. For example, the potentially beneficial effects on crop biomass production due to increase in the atmosphere CO_2 are offset by following increase in temperature and as a result reduces grain yield associated with decrease in sink formation, shortening of growth duration and increase in maintenance respiration (Mathews and Wassmann, 2003). High temperature during reproductive stage increases spikelet sterility due to reduced pollen viability causing yield loss (Matsui et al., 1997b) and enhanced CO_2 levels further aggravate this problem due to reduced transpiration cooling in the rice crop (Matsui et al., 1997a). Wassmann and Dobermann (2007) reported on interactive stress between high temperature and humidity on spikelet sterility. Peng et al. (2004) and Sheehy et al. (2006) showed negative correlations between increasing night temperature (minimum temperature) and grain yield due to variation in solar radiation, differential effects of night and day temperature on tillering, leaf expansion, stem elongation, grain filling, and crop phenology. Crop responses to elevated CO_2 also depend on nitrogen supply. Ziska et al. (1996) stated that photosynthetic and growth responses are limited due to a lack in sink size for excess carbon when additional CO_2 was supplied in N-limited conditions. Kim et. al. (2003), found strong positive interactions between N-supply and carbon gain under CO_2 enriched condition due to an increased leaf are index with increasing N availability, which enhanced the positive effect of higher quantum yield under CO_2 elevated conditions. The mechanism on how the new abiotic stress combinations translate into phenology and yield, and which cultivars are better adapted to the expected variation in patterns of temperature and water availability still remains unclear. In field conditions, there is lack of clear understanding of the complex interactions between maintenance respiration and development stage, crop water and N status, temperature, and CO_2. However, crop growth models are available that have been parameterized and validated for some aspects of possible climate change scenarios, but the complex interactions are not captured well in these models that seek to predict crop response to climate and climate change (Wassmann and Dobermann, 2007).

1.3. Crop adaptation strategies to climate change

Phenology, growth, and attainable yield of any crops are subject to seasonal climatic patterns. Regional adapted genotypes are available but an unpredictably changing climate requires identification of new genotypes adaptable to changes, along with appropriate management to fit in the cropping calendar. In order to develop coping strategies in terms of varietal development and crop management to avoid negative impacts due to increasing climate variability and weather extremes, a broad range of cultivars need to be studied on existing climatic gradients that cover expected ranges of change (e.g., altitudinal temperature gradients). While in previous activities genotype by environment interactions have been studied, neither the full range of adaptation mechanisms nor the full range of expected environmental changes induced by climate change have been addressed yet. Adoption of new or modified ideotype concepts combining several specific adaptations based on reliable information on existing genetic resource materials might be interesting for breeders to cope with changing agro-climatic conditions. But, to predict the appropriateness of complex trait combinations, in turn, requires physiological models that are well validated for targeted environments.

1.4. Rice (*Oryza sativa L.*)

Rice is a staple food for most of the countries in Asia, Africa, and Latin America providing 21% of world human per capita energy and 15% per capita protein (Maclean et al., 2002), and it will continue to be the main staple food (Sombilla et al., 2002) for most of the poor people in the future. Therefore, rice production must increase dramatically in spite of climate change impacts to fight poverty and provide food security (Wassmann et al., 2009). According to FAOSTAT (2009), rice was harvested from 158.3 million ha with the total production of 685.2 million tons in worldwide of which 90% of the world's rice production was from Asia (89% of the harvest area), 6% from Latin America (5%) and 4% from Africa (6%). Rice is cultivated in a wide range of environments. Irrigated lowland rice is cultivated in 79 million ha, rainfed lowland rice in 54 million ha, rainfed upland rice in 14 million ha (Figure 1) and about 11 million ha rice are grown under flood-prone environments (Maclean et al., 2002). However, although upland rice represents a small proportion of the rice production area, it is the most dominant rice in Latin America (75%) and West Africa (50%) (Maclean et al.,

2002). Upland rice production systems are highly heterogeneous, with climates ranging from humid to subhumid, soils from relatively fertile to highly infertile, and topography from flat to steeply sloping. It is often intercropped or relay cropped with maize, sorghum, soybean, cowpea, cassava, sugarcane, coconut and spices which makes it complicated to quantify upland rice production area and often they are not counted (Gupta and O'Toole, 1986).

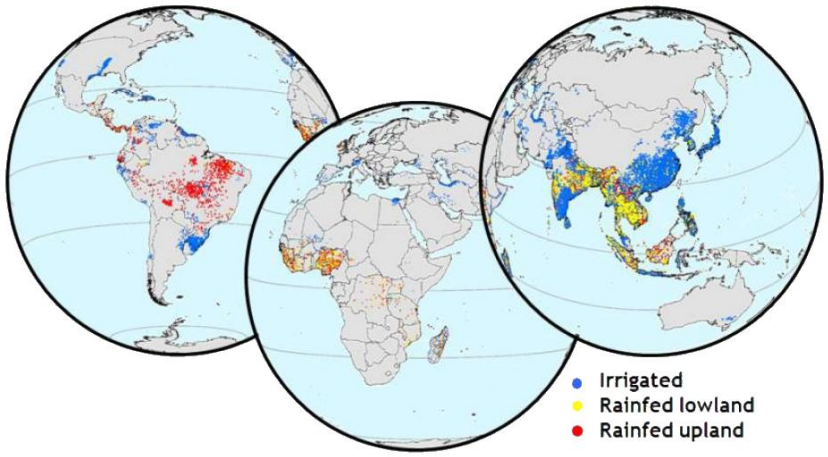

Figure 1 Three major rice growing environments around the world. Each dot represents 5000 ha of rice cultivated area. (Source: http://irri.org/about-rice/rice-facts/rice-production-and-processing, accessed on 5/2/2012)

Upland rice is also known as dryland rice and is normally grown under rainfed conditions without surface water accumulation for any significant period of time and regarded as more favourable in climate change scenarios induced by rise in temperature as methane (CH_4) emmisions are fairly negligible in this cropping system (Wassmann et al., 2000). On the other hand, limited use of inorganic and/or organic fertilizer and pesticides by poor farmers emits lower nitrous oxide (N_2O) (Bouwman et al., 2002). Rise in temperature definitely favours upland rice system in the higher altitudes of the Tropics and Subtropics (where low temperature is the main constraint) in terms of reduced crop duration and increased sink formation leading to higher grain yield. In addition, the growing demand for rice and the increasing pressure on irrigated land is leading to the vital development of upland rice to supplements irrigated and rainfed lowland rice. Upland rice production systems where most of the poorest people are concentrated on their marginal land (vulnerable to climate change), are still short of information available, and research has concentrated less on upland rice systems as compared to more intense rice production systems.

2. Hypothesis and objectives

2.1. Research hypothesis

Availability of water and nutrients, spatial and temporal climatic conditions, and the length of the cropping season are the most important factors that affect upland rice yield. Rice grown in temperate climate has longer crop duration as compared to when grown in tropical climate. Provided that water is not a limiting factor, arid areas tend to result in higher yields because of more intense solar radiation due to a lack of cloud cover and thus enhanced photosynthesis. These factors can explain much of the variation in crop duration and grain yield.

The basic hypothesis of this study is that the phenology of upland rice genotypes responses to an altitudinal gradient and monthly staggered sowing dates, because the weather experienced by a genotype differs in phenological phases in different environmental conditions. Yield components are determined during specific phenological phases and the climatic environment has a strong influence on yield components. Grain yield is directly and/or indirectly affected by yield components. Locations with high precipitation are prone to N-depletion due to leaching losses affecting crop N-supply. Low N-supplied leaves of upland rice have a lower chlorophyll index (SPAD) and reduced leaf-N content leading to decreased photochemical quenching and increased non-photochemical quenching, which is correlated with changes in photochemical reflectance index (PRI) as a stress indicator. PRI correlates with net CO_2 uptake and radiation use efficiency measured by gas exchange at different phenological phases and can thus be employed as a tool to estimate the efficiency of genotypic assimilation under N limited conditions.

2.2. Research objectives

The main objective of this study is to investigate genotypic responses of upland rice in different environments aiming to combine crop responses with a model that allows evaluation of climate change scenarios in view of genotypic adaptation mechanisms. Thus, making it possible to propose crop ideotypes for adaptation to specific environmental changes and thus to develop a basis for tactical and strategic decision making tools to adapt agriculture to a changing climate.

Field trials on phenology, yield and yield component of upland rice assessment across altitudinal gradient have not been reported so far. Therefore, this study was initiated with the following specific objectives:

- to identify phenological responses at different altitudes so that basic genotypic thermal constants are estimated and assessed genotypic thermal responses in spikelet sterility to improve phenological parameters of crop growth models for rainfed upland rice production systems,
- to analyze genotype by environment interactions that enhance characterization of genotypic specific traits (yield components) that significantly contribute to stabilize grain yield across altitudinal gradient locations, and
- to facilitate non-destructive monitoring methods for upland rice leaf-N status with the help of chlorophyll index, photochemical reflectance index and chlorophyll fluorescence parameters under variable N-supply as stress indicator.

3. Literature review

Climate change scenarios indicate that temperature rise potentially affects rice grain yields through changes in metabolism and phenology (Wassmann et. al., 2010b), reduced tiller number during vegetative phase (Yoshida, 1981), increased spikelet sterility due to reduced pollen viability at flowering stage (Matsui et al., 2000), and shortens grain filling phase which in turn effects on grain quality (Counce et al., 2005). In high altitudes where low temperature is the main constraint, rice production will benefit from climate change induced temperature increase in terms of crop duration, grain yield, broader varietal choice and a widened window for introducing new crops into the cropping calendar (Shrestha et. al., 2011).

3.1. Phenology of upland rice

Characterization of the existing variability of upland rice germplasms in terms of phenological responses to variable temperature, day length, and water availability is required for assessing the potential of strategically adapting rice production systems to changing climate. Rice genotypes are short-day plants and crop duration is strongly influenced by their sensitivity to photoperiod (PP) and temperature (Dingkuhn and Miezan, 1995). Under optimal conditions (temperature between 20 and 30 °C and photoperiods of less than 12 hours), crop duration mainly depends upon the genotype-specific duration of the basic vegetative phase (BVP). The BVP is followed within a few days by panicle initiation (PI) under inductive conditions (Sié et al., 1998b; Sié et al., 1998a). The vegetative phase (VP) extends longer in photoperiod sensitive rice genotypes due to a longer photoperiod sensitive phase (PSP) (Figure 2). The PP-insensitive genotypes have the shortest PSP. Drought during germination and flowering delays developmental phases (Wopereis et al., 1996), but accelerates ripening (Dingkuhn and Le Gal, 1996).

Japonica cultivars are more sensitive to temperature and less to photoperiod than Indica cultivars (Fukai, 1999). Considering these effects of abiotic factors on developmental phases, lower temperatures increase crop duration from germination to flowering (Shrestha et al., 2011). Temperature is the main driving force for development in photoperiod insensitive genotypes and heat unit accumulation and thus crop duration depend on the genotypic cardinal temperatures such as temperature sum, and base and optimum temperatures. Flowering of photoperiod insensitive rice cultivars can be simply predicted with two

genotypic constants, critical lower temperature for development (T_{base}) and accrued number of heat units required for flowering (T_{sum}) within the range of linear response of plant development (Dingkuhn et al., 1995; Shrestha et al., 2011).

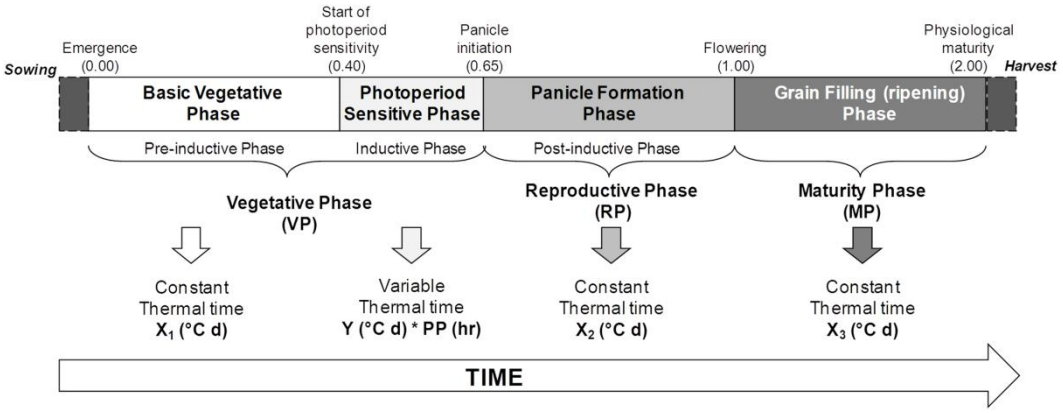

Figure 2 Demonstration of phenophases and stages of a typical rice cultivar. (Source: Dingkuhn and Asch, 1999)

3.2. Grain yield and yield components

Grain yield of any genotype in any given environment is determined by yield components (Yoshida, 1981) developed at different phenophases and growth stages (Figure 3). The yield potential is determined by the number of tillers formed during the vegetative growth phase, the number of panicles induced at the end of the vegetative stage, the number of spikelets formed in each panicle during the panicle development stage, the number of fertile spikelets determined between booting and flowering stage, and the final individual grain weight determined at the grain filling phase (Dingkuhn and Kropff, 1996). All yield components are strongly influenced by the environmental conditions the plant experiences during the respective phases the components are developed. The final yield of a given cultivar depends on the interactions between the genotype, its responses to environmental conditions, and management practices (Messina et al., 2009). Under the same management, the interaction between the genotype and the environmental characteristics is the sole determinant of varietal performance (Dingkuhn et al., 2006). Additive Main effect and Multiplicative Interaction (AMMI) model is widely used to test the effects of such interactions (Yan et al., 2007; Gauch Jr et al., 2008; Sanni et al., 2009). Pb Samonte et al. (1998) used path coefficient analyses to understand direct and/or indirect effects of yield components on grain yield and Nassir and Ariyo (2006) showed that the environment has a strong influence on yield components in

upland rice. In 2007, de Hann et al. applied principal component analysis (PCA) tools to interpret genotype by environment interactions including high variance of data, which is capable to interpolate visually in biplots (Gabriel, 1971). Statistical method developed by Finlay and Wilkinson (1963) to analyze genotypic yield stability across different environments is widely used in breeding activities.

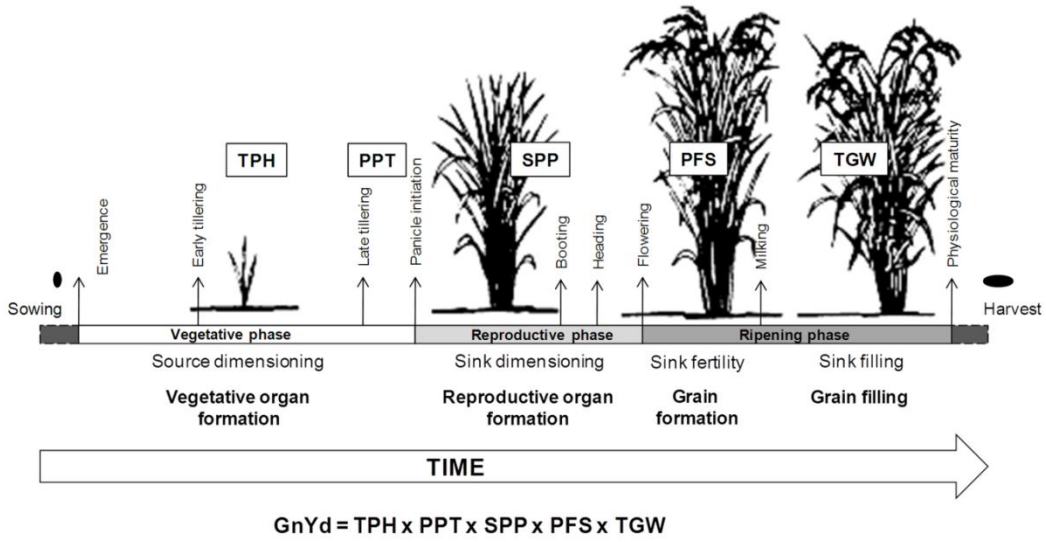

Figure 3 Demonstration of yield components determined at different phenophases and stages. Abbreviations: GnYd, grain yield (t ha^{-1}); TPH, tillers per hill (n) expression of tillers per unit area; PPT, percentage of productive tillers (%) expression of panicle number per unit area; SPP, spikelets per panicle (n) expression of sink size; PFS, percentage of filled spikelet (%) expression of grain number per unit area; TGW, thousand grain weight (gm) expression of physical mass.

3.3. Indices indicating crop N status at leaf scale

Nitrogen is the most limiting plant nutrient element on global scale and particularly in agricultural production systems where N fertilizer application is the main driver of plant growth and yield (Tilman, 1999; Becker et al., 2003). There is widespread lack of understanding about the role of N as the main yield-limiting factor and the importance of adequate applications and optimal timing of N fertilizer (Wopereis et. al., 1999). Dynamics of soil N mineralization, nitrate (NO_3^-) leaching, nitrous oxide (N_2O) emissions and crop N uptake were studied in the field at two sites in the lowland and the upper mid-hills of Nepal with contrasting temperature regimes and durations of the dry-to-wet season transition period to establish partial N balances of the cropping system (Becker et al., 2007). This study found that N loss associated with NO_3^- leaching and N_2O emission was higher in high altitude (high

precipitation areas) from the paddy rice production system. In Madagascar, the low altitudes (coastal areas) have high precipitation during wet season (Appendix I and II). Rapid and non-destructive diagnosis of plant N status is necessary to optimise N fertilizer application and use-efficiency in such areas. In agriculture, the main focus generally is on yield. The driving forces in crop yield are a source (leaves) and a sink (spikelets) for carbohydrates. The dry matter stored in the grains comes from reserves produced in the vegetative phase and assimilates produced during grain filling and are largely determined by climate and N-supply (Dingkuhn and Kropff, 1996). Leaf photosynthetic characteristics of rice are affected by leaf-N content rather than by genotype or species (Keulen and Seligman, 1987). Peng et al. (1993) estimated rice leaf-N content using SPAD and emphasised it as a powerful and rapid research tool to relate rate of leaf senescence due to leaf-N absorbing ability of SPAD reading. The leaf of rice plant is a major storage organ for N. The major source of N for new developing leaves of mature rice plants is the N mobilization from older senescing leaves (Ladha et al., 1998a). Ladha et al. (1998a) mentioned that a key aspect of N-use efficiency (NUE) in relation to yield is the mechanism that regulates degradation of photosynthetic proteins (leaf-N content) in upper canopy leaves (the youngest fully developed leaves or the flag leaves after flowering).

Chlorophyll index (SPAD) estimate leaf chlorophyll density (Markwell et al., 1995) and is widely used to monitor the N status of plants (Samborski et al., 2009) and optimize N fertilizer management in rice (Ladha et al., 1998b; Varinderpal et al., 2010). Current interest is high in applying suitable indices with a physiological background at plant or leaf scale (Guo and Trotter, 2004) which will allow for spatially explicit N fertilizer application. The photochemical reflectance index (PRI) is one of such parameters indicating changes of the epoxidation state of xanthophyll cycle pigments (Gamon et al. 1992, 2001). The xanthophyll cycle protects the functionality of photosystem II (PSII) (Demmig et al., 1987; Müller et al., 2001) under conditions when either light intensity is high (photoinhibitory conditions) or photochemical quenching by carboxylation is reduced (e.g., drought-induced stomatal closure or N-deficiency-induced low enzyme concentrations). Xanthophyll cycle pigments are indicative of photosynthetic light use efficiency (LUE) or the rate of carbon dioxide uptake by foliage per unit energy absorbed (Gamon et al., 1997). Gamon et al. (1997) also found positive correlation between PRI and photosynthetic radiation use efficiency (RUE). It is used in studies of vegetation productivity prior to senescence and plant responses to stress. In a similar context, chlorophyll fluorescence parameters help to assess the relative partitioning of

absorbed light energy into photochemical (energy used for photosynthesis) and non-photochemical (excess energy dissipation) quenching and numerous examples illustrate effects of N-supply on this energy partitioning (Figure 4). Kumagi et al. (2007; 2009; 2010) observed decreases of photochemical quenching (q_p) and increases of non-photochemical quenching in *Oryza sativa* L. leaves under low compared to high N-supply.

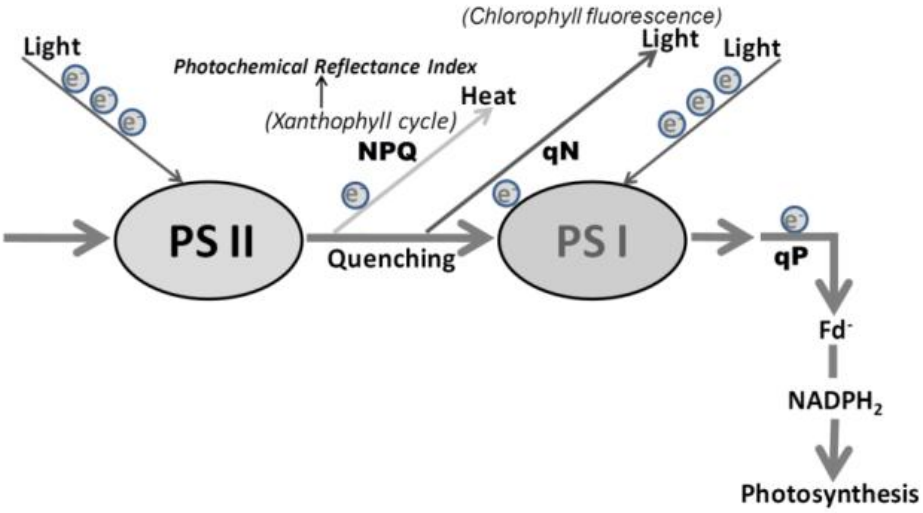

Figure 4 Demonstration of photochemical quenching (qP) related to derived photosynthesis, non-photochemical quenching (qN) related to excess energy re-emitted as light and non-photochemical quenching (NPQ) related to excess energy re-emitted as heat.

4. Materials and methods

This study comprises field based multi-locational trials and greenhouse trials. Multi-location field trials consist of non replicated phenological plots also known as Mini Rice Garden (Dingkuhn et al., 1995 and Shrestha et al., 2011) and replicated yield based physiological plots along three altitudinal locations with monthly staggered sowing dates in Madagascar for two years. The greenhouse trials were conducted in the University of Hohenheim under the Water-stress Management Section of the Department of Plant Production and Agroecology in the Tropics and Subtropics.

4.1. Multi-locational field trial

4.1.1. Locational characteristics

Three locations differing in altitude along a temperature gradient in Madagascar (Andranomanelatra, 1625 m asl; Ivory, 965 m asl and Ankepaka, 25 m asl) were selected for field trials of ten upland rice genotypes in two consecutive years (2008/09 and 2009/10). Experimental fields were located in the high altitude (HA) at 19°46'45.3" S and 47°06'24.5" E, mid altitude (MA) at 19°33'16.8" S and 46°25'29.1" E and low altitude (LA) at 22°11'31.6" S and 47°52'32.7" E. HA and LA were on the east aspect facing towards Indian Ocean whereas MA was on the west aspect facing towards the Mozambique Channel (Figure 5).

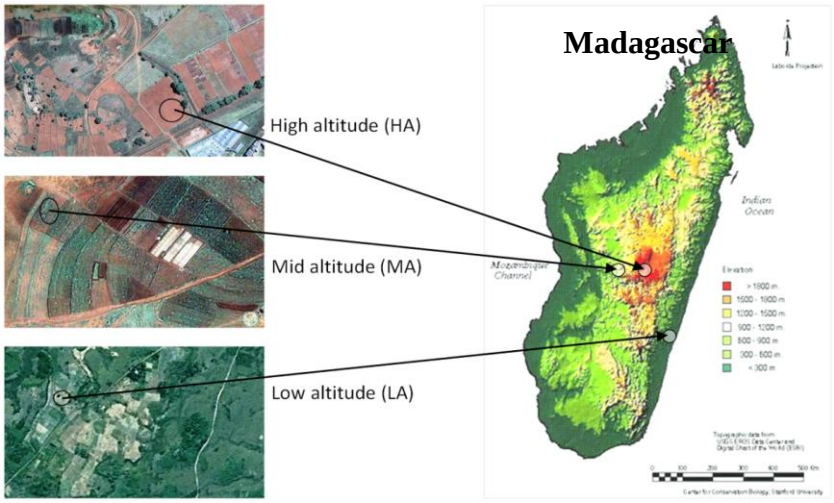

Figure 5 Altitudinal gradient multi-locational field trial conducted areas display in the map of Madagascar. (Source: Google map and http://roland.ratsimiseta.free.fr/madasite/presentation/html/Site-carte%20relief.htm, accessed date: 5/2/2012)

4.1.2. Climatic patterns

Climatic data were recorded from an automatic meteorology station, ENERCO 404 Series, (CIMEL Electronique, Paris, France) in the HA and MA locations, and HOBO U30 Series, (Onset HOBO Data Loggers, Pocasset, Massachusetts, USA) in LA location which were set up close to the experimental plots.

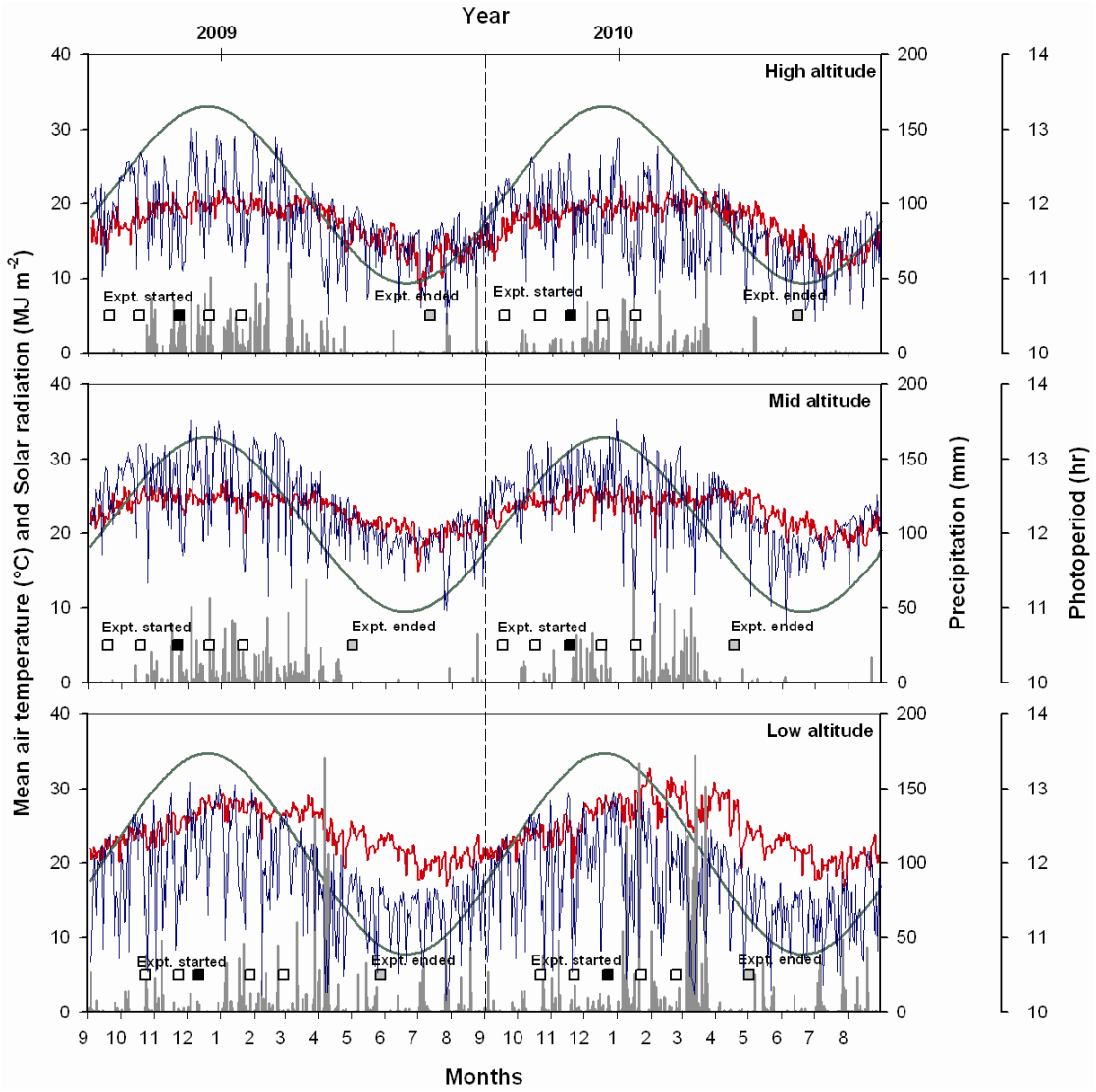

Figure 6 Daily weather patterns of two experimented years of three different altitudinal locations in Madagascar. The red lines are 24 hours mean air temperature (°C), blue lines are solar radiation (MJ m^{-2} d^{-1}), dark green lines are photoperiod (h) and vertical dark gray bars depicts total daily precipitation (mm). The white square boxes indicate sowing dates, black square boxes indicate locally practiced sowing date and the dark gray square boxes indicate end of the experiment period of the year. (Source: Appendix I and Appendix II)

HA and MA had similar photoperiod whereas LA had 10 minutes more photoperiod during January and 10 minutes less during July compared to HA and MA. Average solar radiation was higher in MA as compared to HA and LA locations (Figure 6). In the HA location, daily mean air temperature (T_{mean}) was 7 – 22 °C in the first growing season and slightly higher with 10 – 23 °C in the second year during the experimental periods. In MA location, T_{mean} was similar in both years with 19 -27 °C. In the LA location T_{mean} was 17 – 29 °C in the first year and more variable with 15 – 33 °C in the second year. Precipitation amount during the experimental period varied between locations and years. The HA location had 1545 and 1044 mm of precipitation in the first and second season, respectively. Precipitation in the MA location was 1317 mm in the first and 1069 mm in the second season. The coastal LA location received 1411 mm in the first and 2435 mm in the second season. Several tropical cyclones occurred during the experimental periods. Cyclone Eric (east coast, 19 Jan 2009), cyclone Fanele (west coast, 21 Jan 2009 with winds of 210 km hr^{-1} and heavy rains), Category 1 cyclone Jade (east coast, 6 April 2009 with winds of 93 km hr^{-1}), cyclone Edzani (east coast, 11 Jan 2010 with winds of 185 km hr^{-1}), cyclone Hubert (300 km southeast of Antananarivo, 10 March 2010 with maximum sustained winds of 65 km hr^{-1} and heavy showers) were strong tropical cyclones that occurred between Jan 2009 and March 2010. This list of cyclones is reported here, as such whether events affected the extent of sterility of certain sowing dates and genotypes.

4.1.3. Soil properties

Bench mark soil samples (before the start of experiment in the first year) were collected within 30 cm depth from all three locations and were analysed in the University of Hohenheim. HA had clay soil (sand 12%, silt 41% and clay 17%) of pH 4.5 and carbon nitrogen ratio (C:N) 13, MA had clay loam soil (sand 36%, silt 18% and clay 65%) of pH 4.5 and C:N 13, and LA had silt loam soil (sand 51%, silt 41% and clay 18%) of pH 4.0 and C:N 15 which were dominant in upland rice ecosystem in Madagascar. Soil total N is comparatively low in MA. Some of the physical and chemical properties of these locations are tabulated below (Table 1).

Table 1 Physical and chemical properties of the soils in high altitude location (HA), mid altitude location (MA) and low altitude location (LA) within 30 cm depth.

Soil properties	Location		
	HA	MA	LA
Chemical			
Total C (weight%)	4.8	1.1	2.2
Total N (weight%)	0.38	0.09	0.14
P Bray I (mg kg^{-1})	5.0	4.4	12.6
K Bray I (mg kg^{-1})	46	28	25
Mg sol. $CaCl_2$ (mg $100g^{-1}$)	5	4	6
Effective CEC ($mmol_c$ kg^{-1})	43	23	40
Al exch. (rel.%)	26	13	58
Ca exch. (rel.%)	59	67	25
K exch. (rel.%)	5	4	3
Mg exch. (rel.%)	10	16	12
Na exch. (rel.%)	1.1	< 0.5	3
Cu DTPA (mg kg^{-1})	0.3	4.6	0.4
Fe DTPA (mg kg^{-1})	37	82	405
Mn DTPA (mg kg^{-1})	30	737	7
Zn DTPA (mg kg^{-1})	0.9	2.6	5.2
Physical			
Bulk density (g cm^{-3})	1.2	1.3	1.4
Saturated hydraulic conductivity (cm hr^{-1})	0.23	0.17	1.41
Saturation (cm^3 water cm^{-3} soil)	0.54	0.51	0.48
Field capacity (cm^3 water cm^{-3} soil)	0.45	0.34	0.30
Wilting point (cm^3 water cm^{-3} soil)	0.30	0.23	0.12
Plant available water (cm^3 water cm^{-3} soil)	0.15	0.12	0.18

4.1.4. Genotype characteristics

Ten contrasting genotypes including seven tropical japonica, one temperate japonica and two interspecific crosses (Table 2) were selected for this study. Botramaintso and Chhomrong are traditional landraces adapted to the middle and higher altitudes of Madagascar and Nepal respectively. Botramaintso was selected due to its growth vigour. Chhomrong is a high tillering, cold tolerant genotype rapidly diffused after it's released in 2006 in the HA of Madagascar. B22 and Primavera are improved varieties from Brazil grown at MA and LA. Nerica 4 (WAB 450-I-B-P-91-HB), WAB 878 (WAB 878-6-12-1-1-P1-HB), and IRAT 112 are selected genotypes for MA in Madagascar. Nerica 4 was selected for its morphological characteristics (stay-green syndrome, erect leaves, and low plant height). WAB 878 was

selected for its growth. FOFIFA 161, FOFIA 167 and FOFIFA 172 are improved varieties, adapted to HA of Madagascar and cold tolerant.

Table 2 Characteristics of the *Oryza sativa* upland rice cultivars used in the study. Abbreviations: G1 to G10, genotypes; trop, tropical; temp, temperate; isc, interspecific crosses; imp, improved; trad, traditional. (Source: Appendix I and Appendix II).

Genotype	Variety name	Sub-species	Type	Cross (Parents)	Growing altitude	Country of origin
G1	B22	trop japonia	imp	CNA 095-BM30-BM27_P35-2	mid-low	Brazil
G2	Botramaintso	trop japonica	trad	Local upland variety	mid	Madagascar
G3	Chhomrong	temp japonica	trad	Local lowland/upland variety	high	Nepal
G4	FOFIFA 161	trop japonica	imp	IRAT 114 / FOFIFA 133	high	Madagascar
G5	FOFIFA 167	trop japonica	imp	CA 148 /SHINEI	high	Madagascar
G6	FOFIFA 172	trop japonica	imp	IRAT 265 57-2 / Jumli Marshi	high	Madagascar
G7	IRAT 112	trop japonica	imp	IRAT 13 / Dourado Precoce	mid	Ivory Coast
G8	NERICA 4 (WAB 450-I-B-P-91-HB)	isc	imp	WAB 56-104 / CG 14//2*WAB 56-104	mid	Benin
G9	Primavera	trop japonica	imp	IRAT 10 / LS85-158	mid-low	Brazil
G10	WAB 878 (WAB 878-6-12-1-1-P1-HB)	isc	imp	CG14/IRAT 144	mid	Ivory Coast

4.1.5. Environmental characteristics

The locally practiced sowing date in HA location was between mid October and mid November, in MA location was between mid November and mid December and in the LA location was between mid December and mid January. The phenology trials comprised of five sowing dates (monthly staggered) and the physiology trials comprised of two sowing dates (early and late) in three locations (HA, MA and HA) in two consecutive years (2008/09 and 2009/10), thus creating thirty different growing environments in phenology trials (Appendix I) and twelve in physiology trials (Appendix II).

4.1.6. Experimental design and crop management

The phenology trial comprised five blocks of sowing dates in each location and year. Ten genotypes were randomized within each block without replication. Each genotype plot was 1 m x 1 m in size, plant sown with 0.2 m x 0.2 m spacing (25 hills m^{-2}). Similarly, the physiology trial was designed as split plot with sowing date as main plot and genotypes as sub-plot arranged in a randomized complete block design and were replicated three times. Each plot size was 18.24 m^2 (4.8 m X 3.8 m) in HA and 11.52 m^2 (3.2 m X 3.6 m) in MA and

LA. Hill to hill spacing was 0.2 m x 0.2 m spacing (25 hills m^{-2}) as in phenology trials. Local practice was followed in both trials. Seven to eight seeds per hill were direct seeded and adjusted to five plants per hill at seedling stage.

Plots in MA and LA locations were mulched with *Stylosanthes* to avoid soil moisture loss through evaporation. In all locations, early-sown plots were manually irrigated to avoid drought stress during vegetative growth phases. Complex fertilizer (11:22:16 N-P-K) at a rate of 300 kg ha^{-1}, dolomite 500 kg ha^{-1} and FYM 5 t ha^{-1} was applied as basal dose at the time of sowing. Top dressing was done with urea (46% N) at the rate of 35 kg ha^{-1} and 30 kg ha^{-1} at first and second weeding, respectively. Manual weeding was done as required. Systematic fungicide (Carbenstor-500 SC) was applied at the rate of 1 L ha^{-1} to control leaf blast (*pyriculariase*) when symptoms appeared.

4.1.7. Observation and data analysis

Genotypic specific phenological stages were carefully observed from each plot of the Mini Rice Garden in all three locations at each planting date during crop cycles, and sterility percentage was determined at harvest. Similarly, biomass, grain yield and yield components were determined at harvest from replicated physiological plots. Yield components were measured from 8 hills (2 hills from 4 corners of the plot) excluding 2 border lines. Bulk grain yield was obtained from the central area of 3.8 m^2 in MA and LA and 5.7 m^2 in HA. SPAD and PRI reading were taken at different intervals (phenophases) during cropping season. The average of three replicated measurements from a youngest fully developed leaf represented the SPAD and PRI values of a plant. Statistical analyses were performed in GenStat 13th Edition (VSN International Ltd, UK) and SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). Statistical tools such as Proc Univariate analysis, Proc GLM and Proc MIXED models for analysis of variance (ANOVA), PROC REG for linear regression, Additive Main Effects and Multiplicative Interaction (AMMI) model and Principal component analysis (PCA) were used to analyse field data. Relative contribution to variance (RCTV) of factors were estimated from respective mean sum of square (MS) to the total MS. Genotypic variance were calculated as the ratio of genotype MS to total MS (sum of genotype MS, environment MS, genotype and environment interaction MS and error MS) and similar procedure was followed for environmental variance. The effects of environments on grain yield and yield components for each genotype were calculated as the percentage deviation from genotype mean. Positive

values represent losses and negative values gains in yield and yield components as compared to the genotype mean. SigmaPlot Version 10.0 (Systat Software, Inc., Washington St., Chicago, USA) was used for graphical representation (box plots, scattered plots, bar plots and biplots) of the results. Refer appendix I and II for further details.

4.2. Greenhouse trial

4.2.1. Hydroculture

Cold-tolerant rice (*Oryza sativa L*. spp. *temperate japonica*) cultivar Chhomrong was grown in a greenhouse from August 2009 to October 2009 in a hydroponic system using Yoshida nutrient solution with the following nutrient element composition (mM): 1.43 N as NH_4NO_3, 0.32 P as $NaH_2PO_4.2H_2O$, 0.51 K as K_2SO_4, 1.00 Ca as $CaCl_2$, 1.65 Mg as $MgSO_4.7H_2O$; (µM): 9.10 Mn as $MnCl_2.4H_2O$, 0.07 Mo as $(NH_4)_6.Mo_7O_{24}.4H_2O$, 18.50 B as H_3BO_3, 0.15 Zn as $ZnSO_4.7H_2O$, 0.16 Cu as $CuSO_4.5H_2O$, and 35.81 Fe as $FeCl_3.6H_2O$. N treatments started on August 28 by supplying the plants with nutrient solution with seven different N concentrations (0.18, 0.36, 0.71, 1.43, 2.86, 4.28, 5.71 mM N L^{-1}). N treatments were randomized within a block and replicated three times (randomized complete block design). The pH of the nutrient solutions was adjusted to 5.0-5.5. The greenhouse had average air temperatures of 35 °C / 20 °C day/night and 30% / 75% day/night rH. Extra light was supplied with Philips SON-T Agro 400W bulbs during the 12 hr photoperiod (8:00 a.m. to 8:00 p.m.) keeping the light intensity 400 µmol m^{-2} s^{-1} photosynthetic active photon-flux density (PPFD) at the leaf level.

4.2.2. Measurements and observations

Measurements were done on the youngest fully expanded leaf (leaves 8 or 9). The measurements were taken on 12 and 20 days after onset of treatments (DAO) for seven N levels and, additionally, of three N levels (0.36, 1.43, 4.28, mM N) on 28 DAO. Chlorophyll index (CI) was measured by SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) which calculates the SPAD value (nontrivial ratios) based on the intensity of light transmitted around 650 nm (red band) where absorption by chlorophyll is high and a reference wavelength around 940 nm (infra red band) where absorption by chlorophyll is low as shown in the equation below.

$$CI = \log_{10}[(\frac{T_{940}}{I_{940}})/(\frac{T_{650}}{I_{650}})]$$

Where, T_{940} is the transmittance of infra red wave length (940 nm), I_{940} is the irradiance of infra red, T_{650} is the transmittance of red light wave length (650 nm) and I_{650} is irradiance of red light. Photochemical reflectance index (PRI) was measure by PlantPen PRI 200 (Photon Systems Instruments Ltd., Brno, Czech Republic) which estimates the PRI values based on the intensity of light reflected at 531 nm which is sensitive to xanthophyll cycle pigments and 570 nm as a reference wavelength as shown in the equation below.

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$$

Where, R_{531} indicates reflectance centered close to 531 nm, a wavelength sensitive to changes in leaf pigments of xanthophyll cycle epoxidation state (plays role in light absorption) and R_{570} indicates reflectance centered near 570 nm, a reference wavelength unaffected by xanthophylls activity (reduce the effect of chloroplast movement). Chlorophyll fluorescence (CF) parameters (F_o, minimal fluorescence in the dark-adapted state; F_m, maximal fluorescence in the dark-adapted state; F_m', maximal fluorescence in the light-adapted state; F_o', minimal fluorescence in the light-adapted state; F_s, transient fluorescence at steady state F_m^r, maximal fluorescence at far-red light) were measured with the GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) after a dark-adaptation period of 30 minutes (appendix III). Measurements of these CF parameters allow for the calculation of standard parameters such as:

non-photochemical quenching (NPQ) = $(F_m - F_m') / F_m'$,
fast relaxing non-photochemical quenching (NPQ_F) = $(F_m / F_m') - (F_m / F_m^r)$ and
slow relaxing non-photochemical quenching (NPQ_S) = $(F_m - F_m^r) / F_m^r$.

4.2.3. Data analysis

The experiment was laid out as a completely randomized design with three replications. Statistical analyses were performed with SAS – Version 9.00 (SAS Institute Inc., Cary, NC, USA). One-way ANOVA was used to evaluate the significance of N-supply on measured parameters. LSD with α=0.05 was used to compare N levels. Standard error (SE) at each N level was calculated from standard deviation (SD) and number of replicates (n) as SE = $(SD / n^{0.5})$.

5. Results

5.1. Crop duration varies in altitudinal location

Crop duration was longest in the HA location and decreased in MA and LA locations. Location explained more than 90% of the total variance (pooled over genotype, location, sowing dates and year) in crop duration at different phenological stages (Figure 7). Days from germination to panicle initiation, flowering and physiological maturity were 72 d (± 2.0), 117 d (± 1.4) and 145 d (± 1.5) in the HA location; 45 d (± 0.9), 81 d (± 1.1) and 102 d (± 1.5) in the MA location; and 32 d (± 0.7), 67 d (± 1.1) and 83 d (± 1.4) in the LA location.

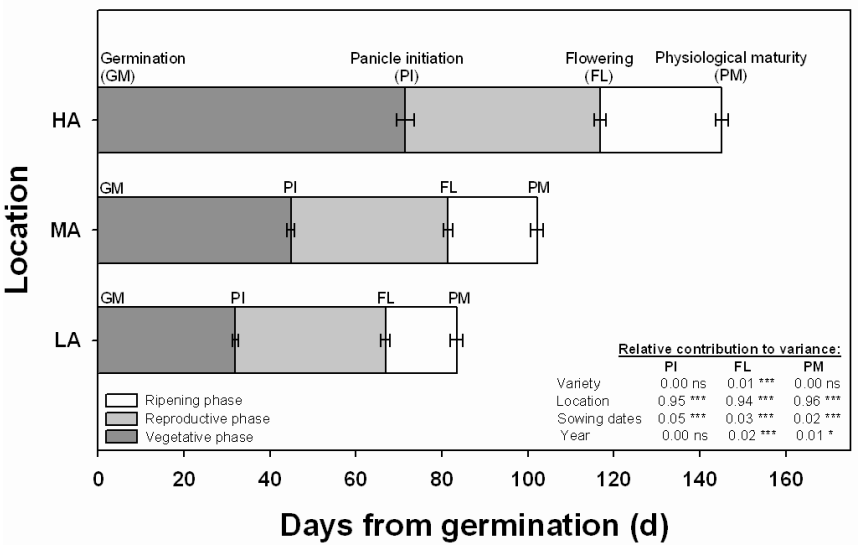

Figure 7 Different phenological phases of upland rice in three different locations. The horizontal bars represent the standard error of mean (n=100 for data sets without missing information) aggregated over genotypes, sowing dates and year. ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. (Source: Appendix I).

5.1.1. Factors determining crop duration in a given location

In the HA location, year explained 40% of the total variance (pooled over genotype, sowing dates and year) as compared to genotype (35%) and sowing dates (25%), indicating that genotype, sowing dates and year were all contributing to the observed variability (Figure 8). Similarly, in the MA location, year explained 65% and sowing dates 31% of the total variance, while genotype did not contribute significantly to total variance. And in the LA

location, sowing date explained 84% of the total variance, while genotype explained only 15% and year had no effect.

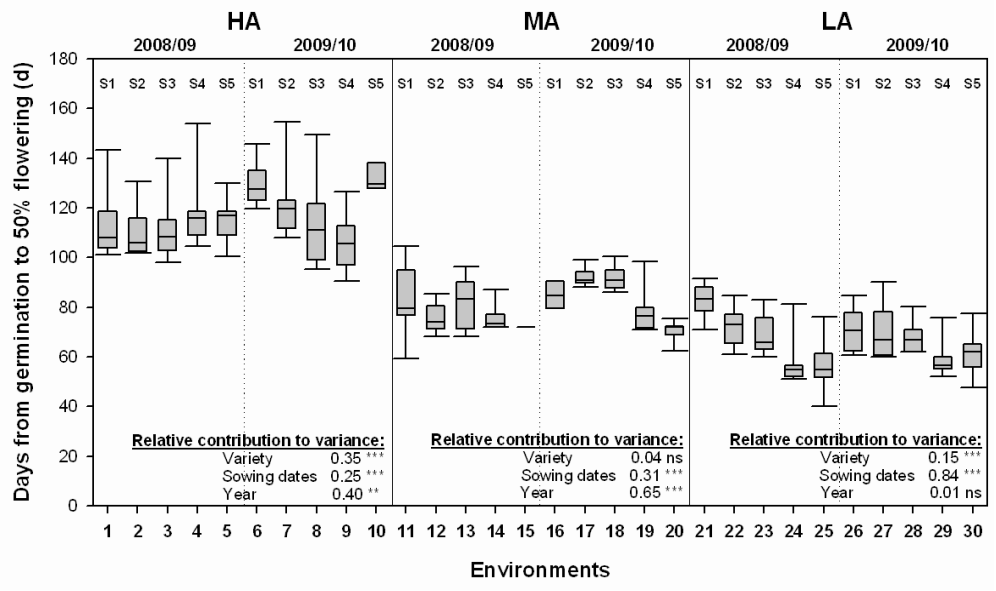

Figure 8 Quartile box plots (between 5% and 95%) showing crop duration from germination to 50% flowering across thirty different environments (E1 to E30). ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. (Source: Appendix I).

5.1.2. Crop duration as a function of mean air temperature

The selected genotypes were photoperiod insensitive as the photoperiod sensitivity index (PSI) was less than 0.3 (Appendix I). Therefore, day-length had no significant effects on crop duration of the selected genotypes. Air temperature experienced at different developmental phases by a given upland rice cultivar had substantial effect on crop duration depending upon location, sowing dates and year. Aggregated data over locations, sowing dates and years in the linear regression analyses of varietal responses indicated that each 1 °C rise in mean air temperature (T_{mean}) decreased crop duration by 5 to 9 days to flowering (Figure 9, see also Figure 5 of Appendix I for other genotypes) depending upon genotype. Duration to flowering of landrace cultivar Botramaintso (G2) decreased by 9 days and cold-tolerant cultivar FOFIFA 172 (G6) by 5 d. The selected genotypes tended to show similar relationships within one location (Figure 9, see also Figure 5 of Appendix I for other genotypes), while the relationship differed between locations indicating that there were location-specific constraints that affected crop duration. In the HA location, five staggered sowing dates over two years experienced T_{mean} of 18 - 20 °C while the corresponding genotypic-specific days to flowering

varied from 90 d to more than 158 d. Similarly, T_{mean} in the MA location did not vary much (24 - 25 °C) but days to flowering ranged from 57 to105 d. In the LA location, T_{mean} varied 24 - 29 °C and the corresponding days to flowering ranged from 39 to 92 d.

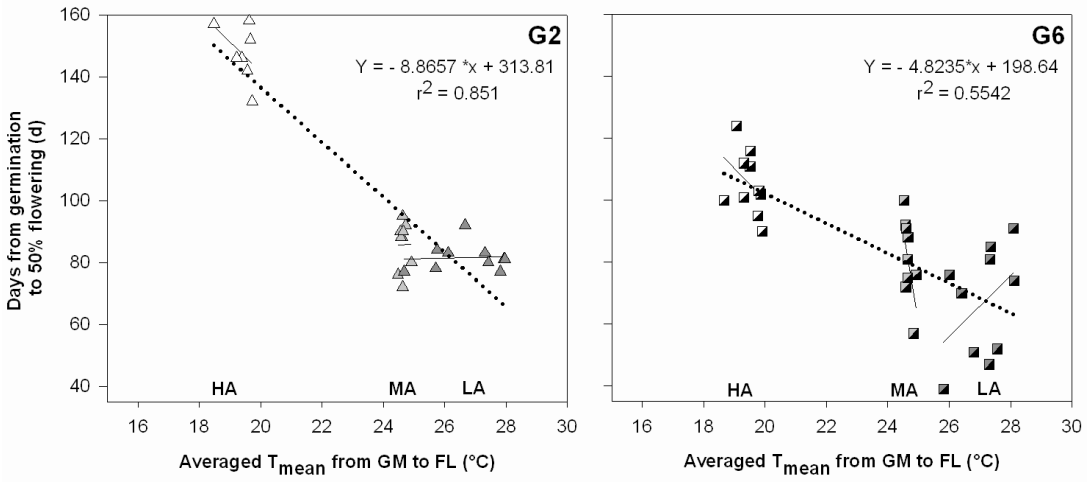

Figure 9 Relationship between crop duration in days (germination to 50% flowering) and the corresponding averaged mean air temperature (T_{mean}) in degree Celsius experienced (germination to 50% flowering) by two contrasting upland rice genotypes Botramaintso (G2) and FOFIFA 172 (G6). Symbols with white, light gray and dark gray color represent high altitude (HA), mid altitude (MA) and low altitude (LA) respectively. The dotted lines represent linear regression line pooled over location, sowing dates and year. The solid lines represent linear regression line pooled over sowing dates and year. (Source: Appendix I).

5.1.3. Estimation of genotypic thermal constants

Pooled over locations, sowing dates and years in the linear regression of varietal responses showed that FOFIFA 172 (G6) had the highest T_{base} (13.9 °C) and the lowest T_{sum} (816 °C d) whereas Botramaintso (G2) had the highest T_{sum} (1220 °C d) and 11.4 °C T_{base} (Figure 10). FOFIFA 161 (G4) had the lowest T_{base} (9.8 °C) and 1157 °C d T_{sum} (Figure 10, see also Figure 6 of Appendix I for other genotypes). Slopes (T_{base}) of a genotype differed between locations when aggregated data sets of sowing dates and year were regressed for individual location (e.g., the MA location had a steeper slope compared to HA location).

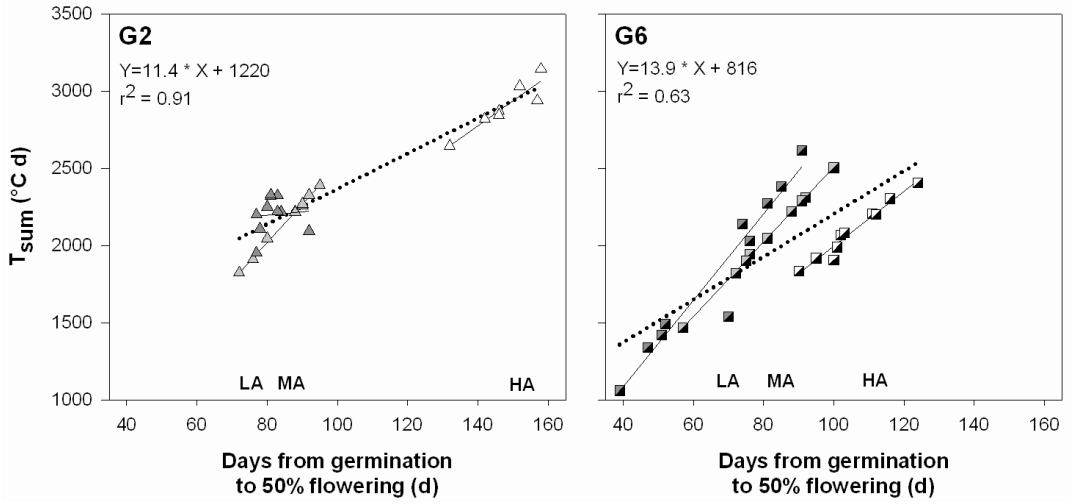

Figure 10 Linear regression of accrued thermal duration to flowering (to the basis of zero) known as T_{sum} (°C d) against the accrued number of days to 50% flowering from germination (d) of by two contrasting upland rice genotypes Botramaintso (G2) and FOFIFA 172 (G6). Symbols with white, light gray and dark gray color represent high altitude (HA), mid altitude (MA) and low altitude (LA) respectively. The dotted lines represent linear regression line pooled over location, sowing dates and year. The solid lines represent linear regression pooled over sowing dates and year. (Source: Appendix I).

5.2. Yield and Yield components at different altitudes

Grain yield and yield components were significantly affected by year, location, sowing date, and genotypes and interactions between these three treatment factors were obvious (Appendix II, Table 3). Pooled over genotypes and sowing dates, grain yield was about 1.7 times higher in the MA than in the LA, and 2.0 times higher than in HA locations (Table 3). In the HA location, Chhomrong (G3) and FOFIFA 172 (G6) had more than 2 t ha^{-1} of grain yield even when lately sown and attained more than 5 t ha^{-1} when sown early. Contrary, Botramaintso (G2) and Primavera (G9) had low grain yield in the HA location for both early and late sowing. In the MA location, average grain yield of genotypes varied from 4.3 to 4.9 t ha^{-1} and differences between sowing dates and varieties were small. However, Botramaintso (G2) attained more than 4 t ha^{-1} when sown early and less than 2.5 t ha^{-1} when sown late. FOFIFA 161 (G4) and Nerica 4 (G8) performed better when sown late. Chhomrong (G3) and IRAT 112 (G7) consistently yielded about 4.1 and 5.2 t ha^{-1} respectively, in MA irrespective of sowing date and year. Grain yields of Chhomrong (G3) and FOFIFA 172 (G6) were lower in LA than in HA location while the opposite was observed for Botramaintso (G2) and Primavera (G9) with the latter realizing the highest grain yield at LA.

Table 3 Varietal performance on grain yield (t ha^{-1}) of selected upland rice cultivars across twelve environments. Least significant difference (LSD) at $P \leq 0.05$. HA, high altitude; MA, mid altitude; LA, low altitude; Er, early sowing; Lt, late sowing; Yr, year; E1 to E12, environments. (Source: Appendix II).

Genotype	HA Er Yr 1 E1	HA Er Yr 2 E2	HA Lt Yr 1 E3	HA Lt Yr 2 E4	MA Er Yr 1 E5	MA Er Yr 2 E6	MA Lt Yr 1 E7	MA Lt Yr 2 E8	LA Er Yr 1 E9	LA Er Yr 2 E10	LA Lt Yr 1 E11	LA Lt Yr 2 E12
B22	0.5	2.1	0.0	2.5	5.1	4.5	6.3	5.6	3.3	3.2	2.5	2.4
Botramaintso	0.2	0.7	0.0	0.0	4.0	5.3	2.4	1.9	1.5	3.0	0.6	1.3
Chhomrong	5.2	7.0	2.4	4.3	4.0	4.1	4.1	4.3	1.1	3.5	2.1	1.9
FOFIFA 161	3.6	3.6	2.9	4.1	3.2	3.8	5.6	5.4	4.0	2.7	1.0	2.0
FOFIFA 167	3.9	5.0	1.8	4.0	5.0	3.8	4.3	3.6	0.8	3.5	1.3	3.3
FOFIFA 172	4.2	5.7	3.4	4.2	5.2	3.9	4.5	3.4	1.3	3.7	2.7	2.4
IRAT 112	0.9	2.1	0.3	2.5	5.3	5.0	5.7	4.9	5.7	2.6	2.5	3.2
Nerica 4	2.2	3.1	0.3	3.1	3.3	4.0	5.8	5.3	4.9	2.8	2.0	3.2
Primavera	0.2	0.5	0.0	0.8	5.1	4.3	4.8	4.7	3.3	3.1	3.3	4.1
WAB 878	0.2	1.6	0.0	1.7	4.9	3.9	5.0	5.2	2.4	2.8	2.1	3.1
Mean	2.10	3.10	1.10	2.70	4.50	4.30	4.90	4.40	2.80	3.10	2.00	2.70
LSD	1.04	1.10	0.85	0.71	1.30	1.28	1.14	1.09	0.81	1.11	0.69	1.11

5.2.1. Yield stability and G by E interaction

5.2.1.1. Yield stability

Based on linear regression between genotype and environment mean yields (Figure 11a), regression coefficients of each variety were plotted against varietal mean grain yield to visualize yield stability (Figure 11b). B22 (G1) and IRAT 112 (G7) had the highest regression coefficients due to the highest yields in high yielding environments but comparably low yields in low yielding environments (Figure 11a) and accordingly were classified as responsive to environmental conditions with an average yield stability (Figure 11b). Yield stability was measured in terms of slope and position. Chhomrong (G3) and FOFIFA 172 (G6) were the highest yielding varieties in low yielding environments and had low to medium grain yields in the most productive environments resulting in the lowest regression coefficients. All cold tolerant genotypes, namely Chhomrong (G3), FOFIFA 161 (G4), FOFIFA 167 (G5), and FOFIFA 172 (G6), cluster in the high yielding group in both low and high yielding environments as they have low regression coefficients. These genotypes had above average yield stability and were well adapted to all environments without significant yield penalty. WAB 878 (G10) and Primavera (G9) had average yield stability but were less responsive to more productive environments. The local landrace Botramaintso (G2) had low yields across all environments and consequently a regression coefficient close to one and below average yield stability. Nerica 4 (G8) had a regression coefficient similar to

Botramaintso (G2), indicating below average yield stability (Figure 11b) but yielded consistently higher than Botramaintso (G2) in productive environments (Table 3).

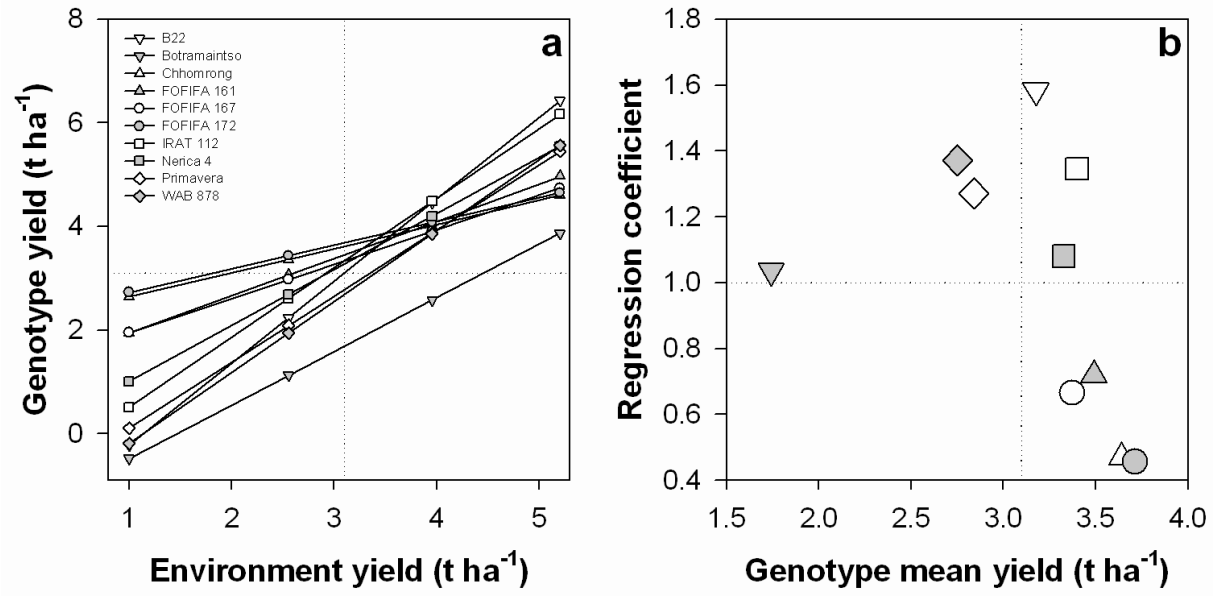

Figure 11 Yield stability of selected upland rice genotypes across twelve environments (E1 to E12). (a) Fitted linear regression lines of each genotype yield (t ha^{-1}) across environment yield (t ha^{-1}). Horizontal and vertical dotted lines are population mean yield (3.1 t ha^{-1}) of ten genotypes across twelve environments. (b) Scattered plot of regression coefficient versus genotype mean yield (t ha^{-1}). Vertical dotted line depicts population mean yield (t ha^{-1}) and the horizontal dotted line depicts the line representing regression coefficient equals to 1. (Source: Appendix II).

5.2.1.2. Genotype by Environment interaction

The ANOVA table for the AMMI model (Table 4) showed that the interaction between genotypes and environments were highly significant and thus with IPCA-1 and IPCA-2. The AMMI-1 biplot (Figure 12a) indicates similar environmental adaptation for Chhomrong (G3), FOFIFA 167 (G5) and FOFIFA 172 (G6); and for B22 (G1), IRAT 112 (G7), Primavera (G9), and WAB 878 (G10), whereas Botramaintso (G2), Nerica 4 (G8), FOFIFA 161 (G4) seem to be less clearly adapted to certain environments. The environments in MA (E5-E8, see also Appendix II) cluster closely to each other whereas the environments in HA (E1-E4, see also Appendix II) and LA (E9-E12, see also Appendix II) are widely scattered within clusters in the lower and upper part of the biplot, respectively, indicating that sowing date and years resulted in comparatively more variable environments in HA and LA than in MA. The

AMMI-2 biplot (Figure 12b) revealed significant differences in sensitivity to and variability in environmental interactions among the genotypes. Chhomrong (G3), FOFIFA 167 (G5), and FOFIFA 172 (G6) clustered far from the origin and closely associated with their HA high yielding environments (E1, E2, and E3) indicating their adaptation to high altitude environments and their sensitivity to unfavourable environments.

Table 4 Analysis of variance from AMMI for grain yield in the twelve environments and the proportion of the total variance attributable to the source of variation. ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. Abbreviation: df, degree of freedom; SS, sum of square; MS, mean square; and F pr., F probability. (Source: Appendix II).

Source	df	SS	MS	SS (%)
Total	359.0	1086.3	3.0	
Treatments	119.0	997.5	8.4***	
Block	24.0	15.9	0.7 **	
Genotypes	9.0	114.4	12.7***	11.5
Environments	11.0	451.0	41.0***	45.2
Interactions (G x E)	98.0	432.1	4.4***	43.3
IPCA1	19.0	293.0	15.4***	67.8
IPCA2	17.0	73.0	4.3***	16.9
Residuals	62.0	66.1	1.1***	
Error	214.0	73.0	0.3	

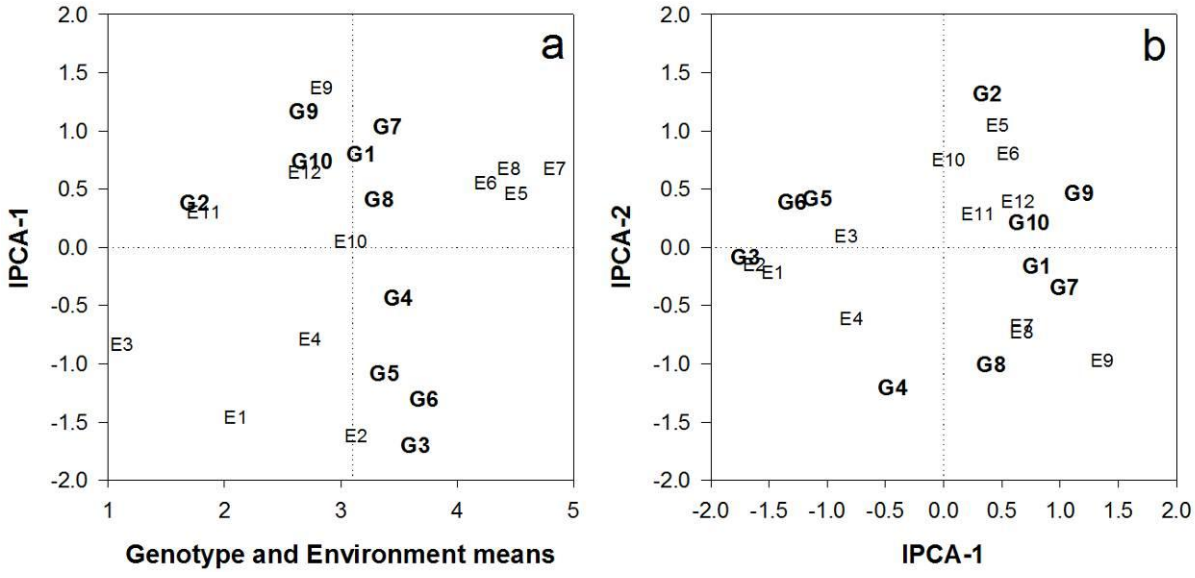

Figure 12 AMMI biplots of ten upland rice cultivars across twelve environments. (a) AMMI-1 biplot where ordinate is Interaction Principal Component Axes 1 (IPCA-1) scores and abscissa is Genotype and Environment mean grain yield (t ha^{-1}). (b) AMMI-2 biplot where ordinate is IPCA-2 and abscissa is IPCA-1. (Source: Appendix II).

However, FOFIFA 167 (G5) and FOFIFA 172 (G6) also showed a good yield performance in E5 and E7 (Table 3), not reflected in the AMMI-2 biplot. Botramaintso (G2) was singled out in the upper right corner of the biplot clustering together with E5, E6 and E10, which is in line with its duration requirements (long duration) favoured by early sowing. Primavera (G9) and WAB 878 (G10) are located closer to the origin, indicating a broader adaptation to environmental variation and clustered in between their favourable MA environments E5, E6 and E7, E8. In their environmental responses they are similar to B22 (G1) and IRAT 112 (G7) which in contrast showed a good yield performance across a slightly larger environmental range as they perform well also in E9 and E10 (Table 3), respectively. Opposite of Botramaintso (G2) in the lower right, Nerica 4 (G8) is located relatively far away from the origin, indicating a strong sensitivity to environment also reflected in being clustered together with its most favourable environments E7, E8 and E9. FOFIFA 161 (G4), which clustered together with the other cold tolerant varieties (G3, G5 and G6) in the yield stability analysis (Figure 11b) is singled out in the AMMI-2 biplot in the lower left, clearly distinguished from G3, G5 and G6. Despite its great distance from the origin, FOFIFA 161 (G4) shares favourable environments (E7-E9) with a larger number of varieties (G8, G7, G1) but also performed well in E4 indicating responses to specific environmental conditions affecting the yield building process. According to Figure 12b, the most contrasting environments were E2 (early sowing, year 2, HA), E5 (early sowing, year 1, MA), and E9 (early sowing, year 1, LA) being located far from the origin and in opposite corners of the plot. Consequently, genotypes most closely associated with these environments reflect earlier selection processes aiming at specific environmental adaptation. Similarly, the environments E3 (late sowing, year 1, HA) and E11 (late sowing, year 1, LA) were not closely related to any of the varieties, indicating general environmental problems affecting yield that were not related to specific environmental adaptation and consequently resulted in low yield performance for most genotypes.

5.2.2. Environmental effects on yield and yield components

5.2.2.1. Phenotypic traits influenced by genotype and environment

Genotype and environment variance was computed for different phenotypic traits to estimate genotypic and environmental influence (Table 5). Grain yield was mainly influenced by environment. Yield components such as TPH and PFS were highly influenced by

environment whereas SPP and TGW were more genotypic and less influenced by environment. PPT was equally influenced by both genotype and environment.

Table 5 Environmental and genotypic variance estimated from phenotypic variance. GnYd, grain yield; TPH, tillers per hill; PPT, percentage of productive tillers; SPP, spikelets per panicle; PFS, percentage of filled spikelets; TGW, thousand grain weight. (Source: Appendix II).

Variable	Variance	
	Genotypic	Environmental
GnYd	0.22	0.70
TPH	0.31	0.67
PPT	0.45	0.34
SPP	0.62	0.34
PFS	0.34	0.60
TGW	0.63	0.33

5.2.2.2. Yield and yield components across different environments

Yield performance of individual genotypes in a given environment reflects the cumulative environmental effects on the different processes involved in building the final yield. Thus, the changes in yield as compared to the mean genotypic yield across environments (environmental yield gains or penalty) will be a result of the environmental effects on yield components developed during specific phenological phases in this environment. The percentage change in yield and yield components was calculated from genotype mean across different environments for each genotype (Table 6) with negative numbers indicating gains and positive numbers indicating losses. Cooler climate in the HA was reflected to the longer vegetative phase (Figure 7) leading to a higher number of tillers per hill (TPH) in almost all genotypes for all years and sowing dates (Table 6). For all but the cold tolerant genotypes (Chhomrong, FOFIFA 161, FOFIFA 167, and FOFIFA 172) this gain in yield potential was off-set by a strong decrease in the percentage of filled spikelets (PFS) leading to yield penalties of up to 100% particularly in E3 (late sowing, year 1, HA). Compared to HA, all genotypes in MA showed yield gains, on an average in the range of 12 – 30 % for the cold tolerant varieties and between 40 and 95% in the cold sensitive varieties. The main effect for these yield gains was observed for the late reproductive phase, particularly in PFS and grain weight (TGW). Due to warmer condition during the vegetative phase the duration was shorter in MA and consequently yield potential was reduced by reduced TPH.

Table 6 Percentage change on grain yield and yield components from genotype mean. Positive values are losses (%) and negative values are gain (%) from genotype mean. Er, early sowing; Lt, late sowing; Yr, year; E1 to E12 are environments; NA, data not available. (Source: Appendix II).

Genotype		HA Er Yr 1 E1	HA Er Yr 2 E2	HA Lt Yr 1 E3	HA Lt Yr 2 E4	MA Er Yr 1 E5	MA Er Yr 2 E6	MA Lt Yr 1 E7	MA Lt Yr 2 E8	LA Er Yr 1 E9	LA Er Yr 2 E10	LA Lt Yr 1 E11	LA Lt Yr 2 E12
B22	GnYd	84	34	99	21	-61	-42	-99	-77	-4	1	21	24
	TPH	-54	-13	-24	-13	16	3	-23	10	-2	39	30	30
	PPT	18	-5	0	5	-7	-4	-3	8	-4	-4	1	-4
	SPP	11	1	19	-3	-15	18	-2	-4	16	-12	-55	26
	PFS	83	46	98	29	-56	-49	-53	-45	-28	-13	-5	-8
	TGW	18	-4	27	4	-8	-6	-8	-3	-9	-12	1	0
Botramaintso	GnYd	88	59	100	100	-130	-201	-36	-9	12	-70	63	25
	TPH	-17	-14	-10	-19	5	-2	-15	2	-5	34	21	21
	PPT	-5	-9	28	-4	-6	-7	-7	4	-1	7	9	-9
	SPP	43	-31	46	100	-8	19	16	-19	-18	-54	-3	7
	PFS	75	85	100	100	-50	-65	-11	-4	-30	4	23	-29
	TGW	3	9	NA	NA	-1	-13	0	15	8	-20	12	-13
Chhomrong	GnYd	-42	-91	34	-17	-9	-12	-11	-18	71	5	43	49
	TPH	-30	-23	-37	-52	-5	30	-5	15	4	28	5	69
	PPT	-5	-5	-1	-5	-4	1	0	-3	18	-3	-2	10
	SPP	6	-33	5	-23	-10	-1	-2	-18	-2	33	2	43
	PFS	-24	-22	29	-5	-5	-22	-2	-7	23	-2	16	21
	TGW	12	0	18	20	-5	-8	5	-5	13	-27	-6	-16
FOFIFA 161	GnYd	-3	-4	17	-17	9	-10	-60	-56	-14	24	71	44
	TPH	-29	19	-35	-27	21	-7	-55	8	-2	29	40	36
	PPT	-1	-8	-3	-2	-1	3	0	5	-5	0	18	-6
	SPP	22	-29	19	5	-2	19	12	-44	5	-31	0	24
	PFS	-12	-22	15	-22	-20	-15	0	1	-8	47	19	16
	TGW	15	2	20	6	-3	-1	-1	-11	-7	-16	6	-10
FOFIFA 167	GnYd	-17	-47	45	-20	-49	-12	-27	-8	75	-4	61	3
	TPH	-33	2	-24	-26	12	9	-25	3	-7	30	36	23
	PPT	-2	-9	4	-4	-8	4	10	17	5	-6	-7	-3
	SPP	26	-16	15	9	-23	12	2	-27	-10	-7	-2	22
	PFS	-17	-38	54	-12	-26	-24	-5	-4	43	10	27	-8
	TGW	12	9	-2	9	-6	-6	-2	3	10	-18	11	-20
FOFIFA 172	GnYd	-12	-52	10	-14	-40	-4	-21	7	64	-1	28	35
	TPH	-37	-16	-32	-29	19	14	-21	22	-6	27	28	32
	PPT	-3	-6	15	-5	-4	-4	-2	-5	26	-3	-2	-8
	SPP	28	-6	19	13	-24	9	27	2	-40	-20	-30	20
	PFS	-10	-17	-11	-17	-19	-12	-13	-7	35	26	34	11
	TGW	10	-1	10	0	-5	-5	-7	-11	16	-15	5	1
IRAT 112	GnYd	74	37	91	25	-56	-48	-67	-45	-67	23	27	5
	TPH	-52	-11	-17	-7	10	6	-19	6	-16	27	26	47
	PPT	10	-1	1	-1	-2	-2	-1	-3	-2	9	-5	-3
	SPP	22	-9	13	13	-3	10	1	2	-30	-15	-15	12
	PFS	73	47	89	18	-55	-50	-44	-40	-16	-4	12	-31
	TGW	26	9	24	5	-5	-4	-2	-8	-12	-11	0	-21
Nerica 4	GnYd	35	8	90	6	0	-19	-73	-59	-47	16	39	4
	TPH	-31	-4	-13	-42	28	-1	-25	-6	6	43	18	28
	PPT	-1	1	0	1	-2	2	0	2	1	0	-4	-1
	SPP	11	-11	-4	-14	1	23	8	-9	-10	-20	-3	28
	PFS	39	26	91	32	-45	-51	-43	-33	-24	9	28	-28
	TGW	20	10	-6	3	-5	-5	-4	-9	2	0	13	-18
Primavera	GnYd	95	84	100	70	-80	-52	-71	-66	-18	-10	-16	-44
	TPH	-40	-6	-23	-23	6	25	7	-2	6	21	NA	28
	PPT	5	-2	2	-8	4	1	-5	15	1	-9	NA	-4
	SPP	19	-18	0	-3	-15	9	-2	-13	2	-4	NA	26
	PFS	95	95	100	97	-69	-74	-57	-57	-45	-26	NA	-58
	TGW	5	16	NA	-27	4	2	2	8	7	-4	NA	-12
WAB 878	GnYd	92	40	100	39	-77	-40	-83	-90	13	-3	23	-13
	TPH	-78	13	-19	-11	27	19	-15	7	-13	33	8	31
	PPT	18	-3	4	-1	-3	2	-1	1	-4	-5	-4	-3
	SPP	28	-8	13	18	-9	6	1	-26	10	-20	-28	15
	PFS	89	45	100	43	-78	-64	-51	-51	-10	1	16	-40
	TGW	13	-14	25	-2	-12	-4	-5	-1	9	-15	12	-7

Large variation among the genotypes was observed for sink size formation, as the environmental effects on total number of panicles per tiller or percentage of productive tillers (PPT) and number of spikelets per panicle (SPP) varied widely among sowing dates and genotypes in MA. In LA, generally genotypes responded to environmental conditions with a penalty in yield ranging on average between 32-42% in the cold tolerant varieties and between 3-10% for the others. Primavera was the only genotype responding relatively favourable to the LA environment with yield gains of about 22% on an average. No clear pattern emerged from the analysis of the environmental responses of the yield components in relation to yield responses in LA. The environmental effects on yield components and their contribution to final yield varied widely among sowing dates within specific genotypes as well as within sowing dates across genotypes.

5.2.2.3. Principal component analysis of yield components and environments

The principal component analysis (PCA) of yield components and environments revealed the genotypic relationships between the environmental influences on the yield components during the phenophases they were established and the importance of the effects on the yield component for the final yield in the respective environment (Figure 13). The principal component axis PCA-1 and PCA-2 explained more than 90% of the variation observed among the genotypes, with the exception of Chhomrong and the three FOFIFA varieties where the PCA-1 and PCA-2 accounted only for 79 – 90% of the variation. In the Figure 13, the closer the projection of environment scores of the genotype to its yield components (latent vector), the higher the percentage reduction of the yield component of that genotype in that environment. In other words, the farther the environment scores of the genotype deviate from its yield components; the lower is the percentage reduction of the yield component. The selected genotypes included in this study responded differently and strongly to the different environments. In cold sensitive cultivars B22, Botramaintso, IRAT 112, Nerica 4, Primavera and WAB 878 the HA environment induced severe spikelet sterility (high percentage reduction of PFS) that strongly reduced the potential yield. In cold tolerant cultivars Chhomrong, FOFIFA 161, FOFIFA 167, and FOFIFA 172 the HA environment induced reductions in TGW often associated with reductions in SPP indicating an environmental influence on sink size and problems during the grain filling phase. In the MA environments,

final yield was not affected by a specific environmental influence on specific yield components in B22, Botramaintso, FOFIFA 172, IRAT 112, Primavera, and WAB 878.

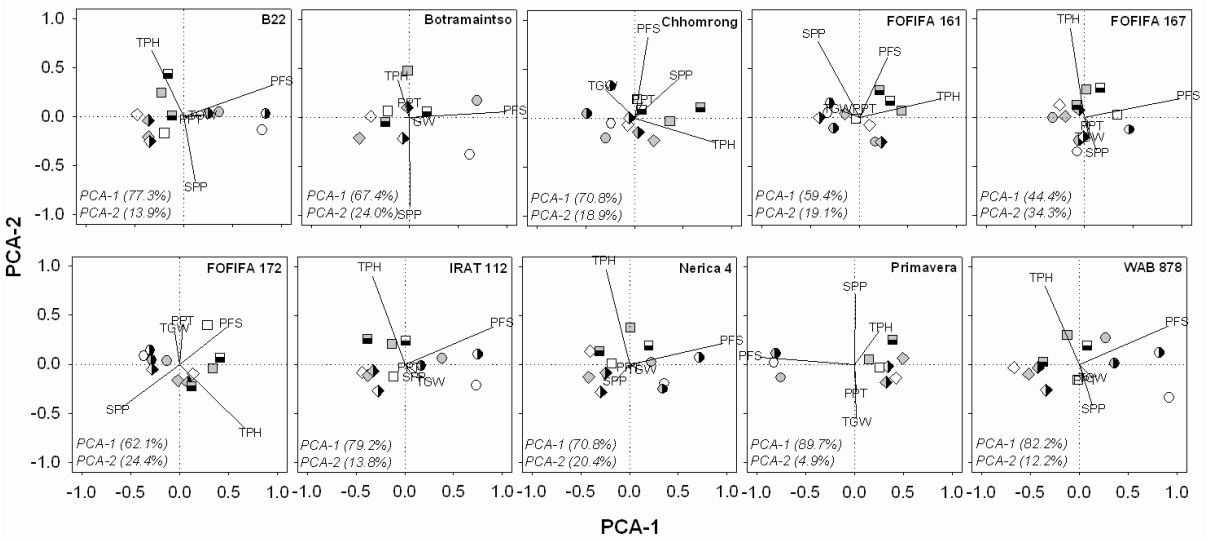

Figure 13 Percentage reduction of yield components from overall mean interacting with different environments. Yield components (TPH, PPT, SPP, PFS and TGW) as the latent vector loadings and environments as the scores are shown in the PCA biplots with principal component (PC) Axis-2 against PC Axis-1 of ten upland rice genotypes. The symbols used in biplots represent environments. The circles are high altitude, diamond shapes are mid altitude and the square boxes are low altitude environments. Symbols with white colors are early sowing in the first year, gray colors are early sowing in the second year, half white and half black colors are late sowing in the first year and half gray and half black colors are late sowing in the second year. The values in the parenthesis (brackets) are the variation explained by the respective PC Axis. (Source: Appendix II).

The same environment influenced the sink size in Chhomrong, FOFIFA 161, and FOFIFA 167 through reductions in PPT indicating rather an environmental influence during the tiller formation phase, whereas in Nerica 4 MA environments strongly reduced the sink size through reductions in SPP indicating adverse environmental influence during the booting phase and panicle development. The LA environments strongly shortened the duration to flowering in all genotypes (Figure 13) which is strongly reflected in the influence of the LA environments on TPH in all genotypes (Figure 13). In Chhomrong, LA environments additionally reduced SPP indicating problems in balancing sink-source dimensions, whereas in the three FOFIFA genotypes LA environments had strong effects on PFS which either reflects heat sterility or additional biotic stresses during panicle formation such as mold.

5.2.3. Weather parameters exerting major influence in specific environments

As shown in Figure 13 environments strongly influence genotypic yield via the individual yield components formed during specific development stages of the genotype. These influences are directly related to the weather experienced by each genotype during its phenophases (vegetative, reproductive, and ripening phases). The PCA of mean weather conditions each genotype experienced during its phenophases explained between 85 and 90% of the genotypic variation for the respective phenophases by PCA-1 (abscissa) and PCA-2 (ordinate) (Figure 14). The figure shows that all HA environments were equally influenced by all weather parameters with minimum air temperature (T_{min}) having the strongest positive influence on genotypic performance which is reflected in the duration to flowering (Figure 7 and Figure 8), TPH and PFS (Table 6). In all MA environments genotypic performance in all phenophases was strongly positively influenced by rainfall (RF) and strongly negatively influenced by vapour pressure deficit (VPD), solar radiation (SR), and potential evapotranspiration (ET_o). These factors affect mainly water use, water use efficiency, and photosynthesis and, thus, sink build-up and sink filling. This is reflected in the influence of the yield components PPT, SPP and TGW on yield performance in MA environments (Figure 13 and Table 6). In the LA environments the main weather parameters influencing genotypic performance were temperature and rainfall. Particularly higher temperatures during the early development exert a strong influence on the duration to flowering, shortening the vegetative development and thus influence the source build-up, as reflected in the negative effect of TPH on yield in these environments. However, a larger variation in the influence of the specific weather parameters was observed related to the different planting dates. Genotypic performance was strongly negatively influenced by maximum air temperature (T_{max}) in the early sowing of the first year negatively affecting PFS in the cold tolerant varieties, whereas the late sowing date in the first year and both sowing dates in the second year were strongly influenced by RF. In these cases, high rainfall was accompanied by strong winds (tropical cyclone) increasing lodging and by low VPD increasing mold infections both strongly affecting the yield performance of sensitive genotypes.

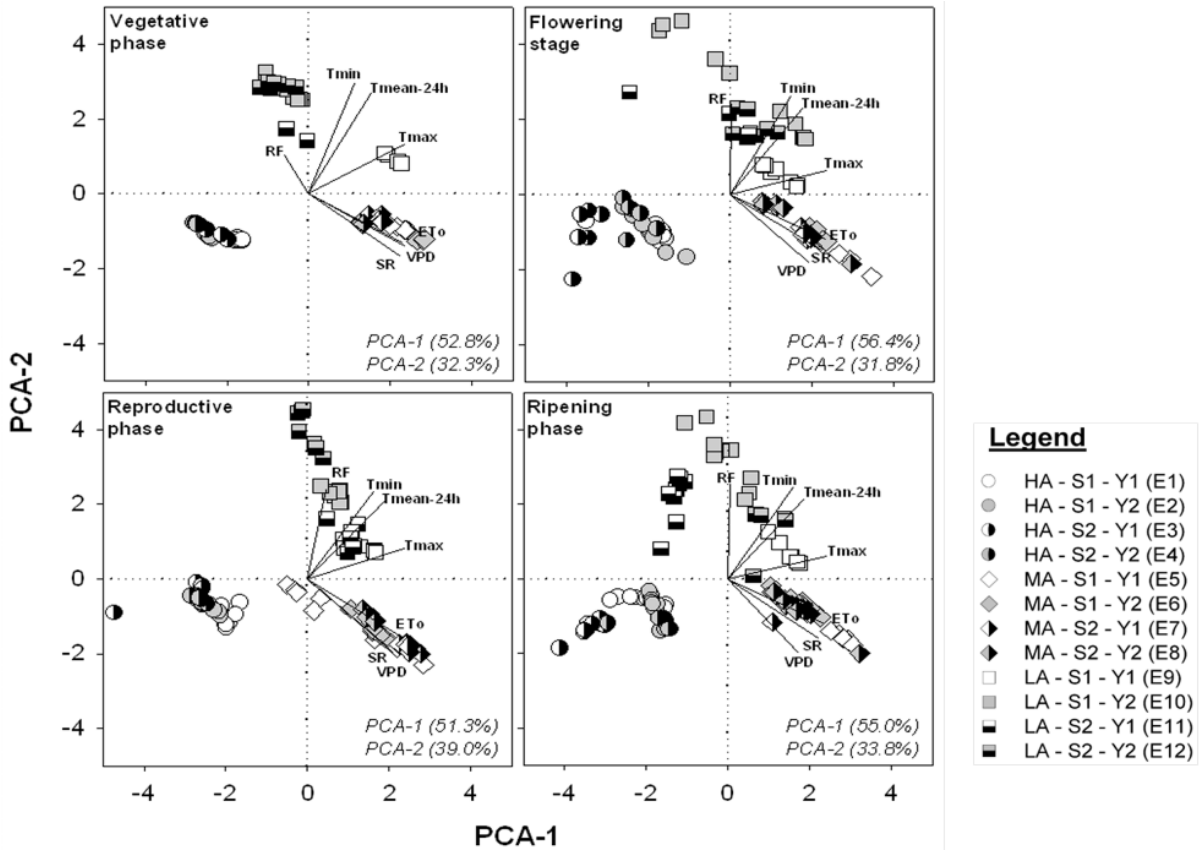

Figure 14 PCA biplots of averaged weather experienced by each genotype during its phenological stages across twelve environments. Weather parameters minimum air temperature (T_{min}), maximum air temperature (T_{max}), 24 hours mean air temperature ($T_{mean24h}$), precipitation (RF), solar radiation (SR), vapor pressure deficit (VPD) and potential evapotranspiration (ET_o) are the latent vector loadings; and weather experienced by genotypes during its phenological stages across twelve environments are the scores of PCA. The symbols used in biplots represent corresponding environments as shown in the legend. The values in the parenthesis are the variation explained by the respective PC-axis. (Source: Appendix II).

5.3. Thermal stress effects on spikelet sterility

The univariate analysis for the main effects of location, genotype, sowing date and year showed that the percentage of spikelet sterility (SSP) varied between locations, genotypes and sowing dates. In the HA location, variation was mainly due to genotype and sowing dates, but in MA and LA locations variation was more due to sowing date (more than 74%) and less by genotype (less than 18%) (Table 7).

Table 7 Source of variance and its relative contribution to variance on percentage of spikelet sterility (SPP) in three locations and pooled over location. ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. HA, high altitude; MA, mid altitude and LA, low altitude. (Source: Appendix I).

Source of variance	Pooled		HA		MA		LA	
Location	0.88	***						
Genotype	0.05	***	0.50	***	0.16	**	0.18	ns
Sowing date	0.07	***	0.49	***	0.82	***	0.74	***
Year	0.00	ns	0.01	ns	0.02	ns	0.08	ns

5.3.1. Spikelet sterility due to cold and heat stresses

Spikelet sterility was affected by low temperature (cold stress) between booting and heading stages (averaged T_{min} < 18 °C) in the HA location (Figure 15a). However, cold tolerant genotypes were less affected in this location when early sown. MA and LA locations were affected by heat stress at flowering stage (average T_{max} > 30 °C) in environment E11, E16, E20, E21, E22, E23, E26 and E28 (see also Appendix I) depending on genotypic crop duration (Figure 15b). Cold-tolerant cultivar Chhomrong (G3), and cold-sensitive cultivar IRAT 112 (G7) were selected as reference genotypes to quantify spikelet sterility due to thermal stress. Spikelet sterility regressed across averaged T_{min} exposed between booting and heading stages to determine cold stress (Figure 15a), and averaged T_{max} exposed during flowering stage for heat stress (Figure 15b). Chomrong (G3) had less than 40% spikelet sterility when averaged T_{min} was around 13 to 14 °C (Figure 15a) and 100% sterility when T_{min} was below 12 °C. A similar relationship was found to genotypes FOFIFA 161 (G4), FOFIFA 167 (G5) and FOFIFA 172 (G6). Cold sensitive genotypes IRAT 112 (G7) had less spikelet sterility at 19 °C and the sterility was close to 80% at 15 °C and 100% at 13 °C (Figure 15a). Similar behavior was observed for other genotypes B22 (G1), Botramaintso (G2), Nerica 4 (G8), Primavera (G9) and WAB 878 (G10), but the sterility was 100% when the averaged T_{min} was close to 15 °C. Data for spikelet sterility was not available between 15 °C and 18 °C as the crop did not experience these range of temperatures across location, sowing dates and year. Chhomrong (G3) and IRAT 112 (G7) had heat stress when the averaged T_{max} at flowering was above 30 °C (Figure 15b). Averaged T_{max} close to 30 °C had less than 20% sterility and 100% sterility was extrapolated above 34 °C. However, other genotypes had similar trend, 100% sterility was below 34 °C.

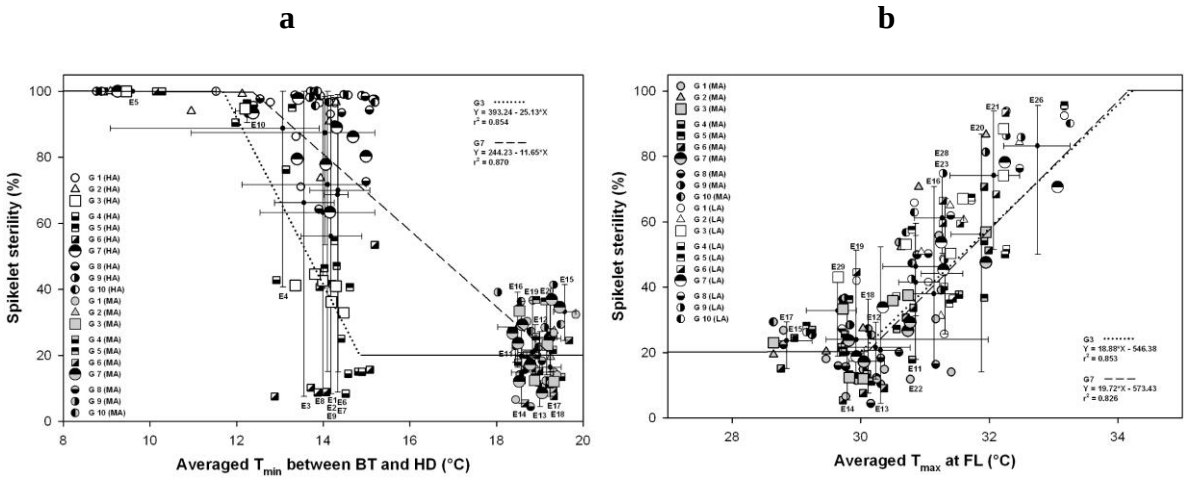

Figure 15 (a) Relationship between spikelet sterility and the averaged T_{min} actually observed between booting and heading stages, individually determined for each genotype, location, sowing dates and year. **(b)** Relationship between spikelet sterility and the averaged T_{max} actually observed during flowering stage (± 7 days), individually determined for each genotype, location, sowing dates and year. Chhomrong (G3) and IRAT 112 (G7) were taken as reference genotypes to represent cold tolerant and sensitive genotypes respectively in the linear regression due to its spikelet sterility variation from less than 30% to 100% within the range of 14 and 20 °C averaged T_{min} from booting to heading in HA and MA locations, and less than 30% to more than 80% within the range between 30 and 32 °C averaged T_{max} during flowering stage in MA and LA locations. (Source: Appendix I).

5.4. Crop N-status and its effects on final yield

5.4.1. N-supply effects on SPAD

SPAD values or chlorophyll index (CI) of a rice leaf increased significantly with increasing N supply (Figure 16a) and levelled off when N supply was higher than 2.86 mM N on both 12 and 20 days after onset of treatments (DAO). In agreement with data collected at 12 and 20 DAO, SPAD values increased with increasing N supply when measured 28 DAO at N supply levels of 0.36, 1.43 and 4.28 mM N (Figure 16a). Comparing three measurement dates, SPAD values decreased with increasing leaf age at low N supply. Positive correlation between SPAD values and leaf-N content (g kg^{-1}) was observed (Figure 16b) where the second order polynomial (quadratic) equation had coefficient of determination (r^2) of 0.95. The threshold SPAD value 40 corresponds to 32 g kg^{-1} leaf-N (Figure 16b). A similar relationship between

SPAD and chlorophyll$_{(a+b)}$ content (g m^{-2}) was observed (Figure 16c) with the coefficient of determination of 0.67.

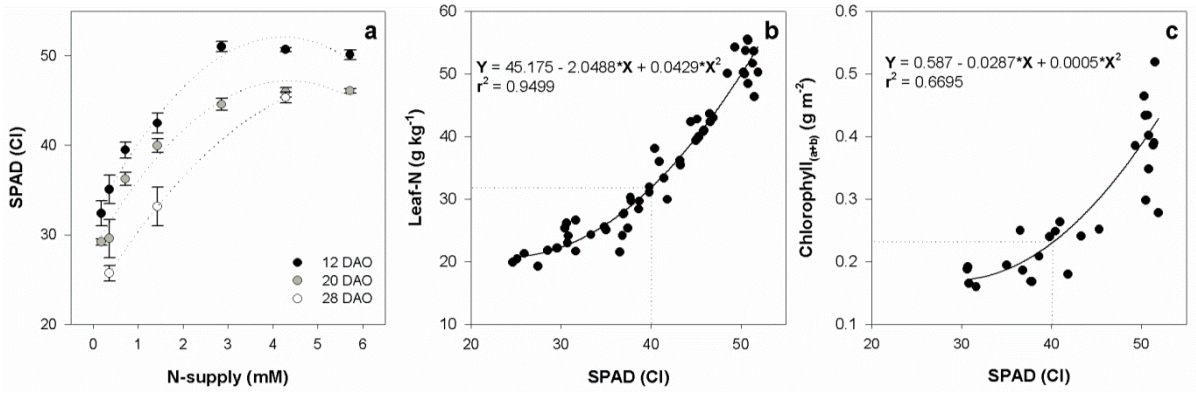

Figure 16 Effects of N-supply on SPAD and leaf-N content (g kg^{-1}) of the youngest fully expanded rice leaf measured at 12, 20 and 28 DAO of 0.037 kg m^{-2} average specific leaf weight (SLW).

5.4.2. Relationship between SPAD, PRI and NPQ

Dark-adapted and light-adapted PRI values had positive correlation with SPAD values on both 20 and 28 DAO (Figure 17). In agreement with data collected at 20 DAO, light-adapted PRI increased with increasing SPAD values when measured at 28 DAO (Figure 17b), which was not observed in dark-adapted PRI (Figure 17a). The threshold SPAD value 40 correspond to less than 0.10 light adapted PRI value (Figure 17b).

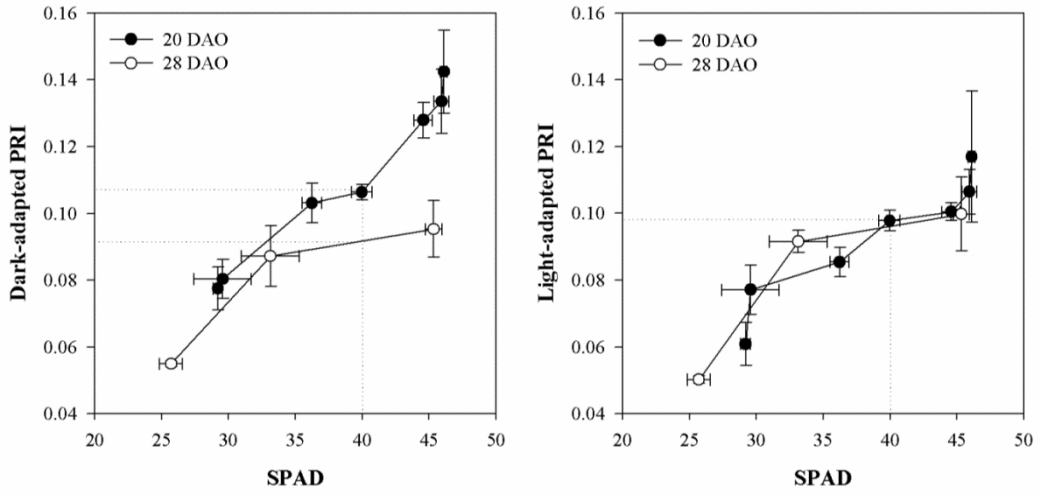

Figure 17 Relationship between SPAD and dark- and light adapted PRI on 20 (filled symbols) and 28 (open symbols) DAO. Vertical and horizontal bars indicate standard error (n=3 leaves).

NPQ and NPQ_F correlated negatively with SPAD, dark- and light-adapted PRI values at 20 DAO (Figure 18). NPQ and NPQ_F and SPAD values measured at 28 DAO fitted well to that at 20 DAO while the agreement was not as good for dark-adapted PRI readings at N level 4.28 mM N at 28 DAO. NPQ_S did not correlate with SPAD, dark- and light- adapted PRI values at 20 and 28 DAO. The relationship between fluorescence parameters and SPAD and PRI values were not linear over the whole range of data. E.g., when SPAD values were higher than 45, chlorophyll fluorescence varied while SPAD values did not differ significantly any more.

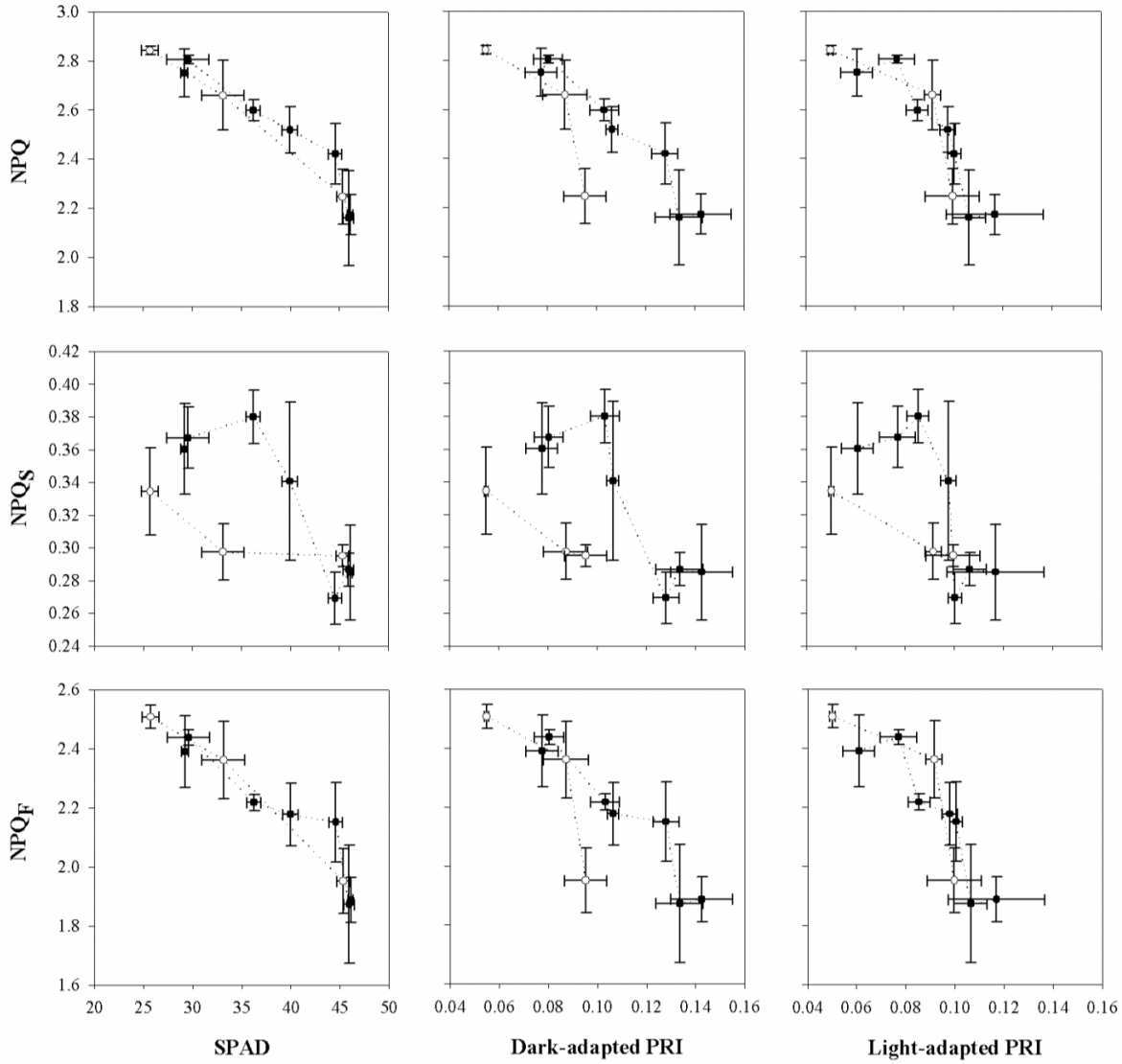

Figure 18 Relationship between NPQ, NPQ_S, and NPQ_F and SPAD, and dark- and light-adapted PRI values at 20 (filled symbols) and 28 (open symbols) DAO. Vertical and horizontal bars indicate standard error (n=3 leaves). (Source: Appendix III).

5.4.3. Field measurement of SPAD and PRI values at different phenophases

SPAD and PRI values showed distinct trend in HA, MA and LA locations at different phenophases. In the HA location, SPAD values of the fully developed youngest leaves are above threshold value 40 at reproductive and grain filling phases (Figure 19). However, PRI values are comparatively always lower compared to SPAD values at both reproductive and grain filling phases. In the early sowing date, PRI is far below SPAD at flowering stage. Similarly, in the MA, SPAD value is lower than threshold during panicle initiation stage and close to threshold before flowering, further decreased from flowering to physiological maturity stages (Figure 19). PRI is comparatively higher than SPAD during vegetative phase and decreased gradually. Similar patterns were observed in LA on both early and late sowing dates, but the differences with SPAD and PRI are much more pronounced than in MA location. SPAD values are always above threshold all over development stages after panicle initiation, except at panicle initiation stage in late sowing date.

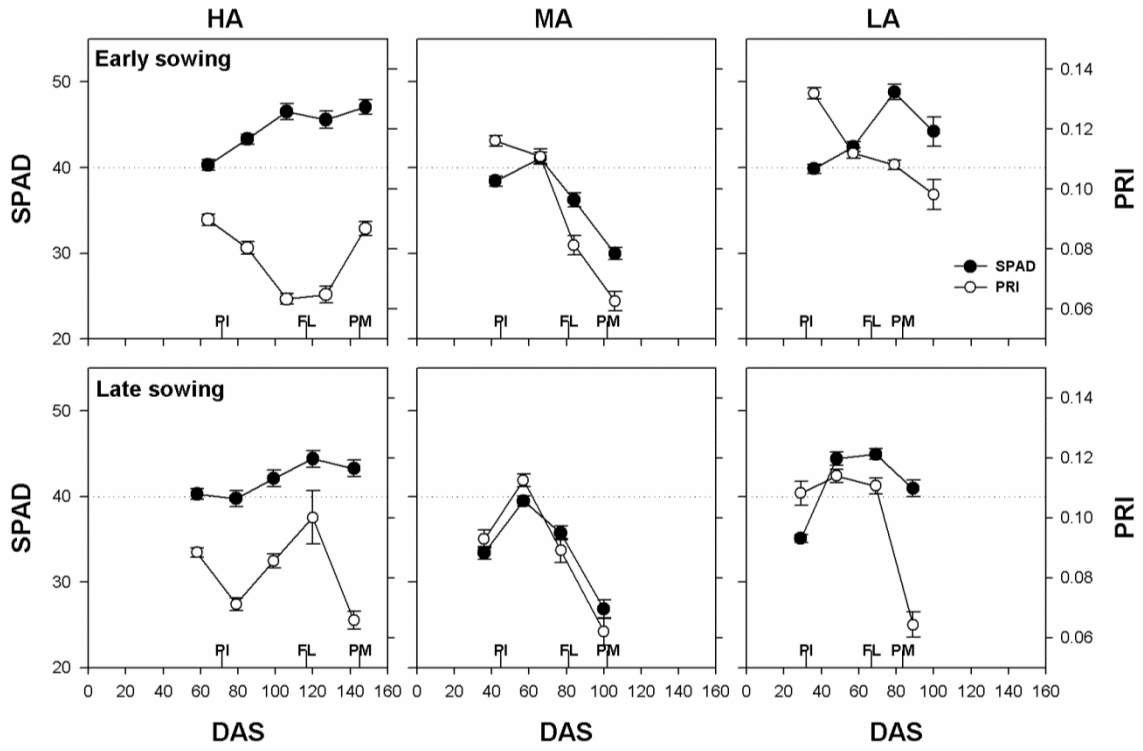

Figure 19 SPAD and PRI readings (pooled over genotypes) during cropping period in high (HA), mid (MA) and low (LA) altitudinal locations in the second year. The vertical bars indicates standard errors (n=30). DAS, days after sowing; PI, panicle initiation stage; FL, flowering stage; and PM, physiological maturity.

Leaf-N content was estimated from the SPAD values measured in the field condition based on greenhouse trials (Figure 16). Leaf-N content of the fully developed youngest leaf is higher (above 32 g kg^{-1} which corresponds to the threshold SPAD value 40, see also Figure 16b) in HA and LA locations compared to MA location (Figure 20). In the HA altitude, leaf-N gradually increased during reproductive phase. The leaf-N content of the flag leaf (fully developed youngest leaf after flowering) did not decreased much. A similar trend was observed in LA location but the leaf-N decreased significantly during grain filling phase. In the MA location, leaf-N increased to a maximum level before flowering and decreased drastically until physiological maturity on both sowing dates. Leaf-N content was always lower in late sowing than early sowing date.

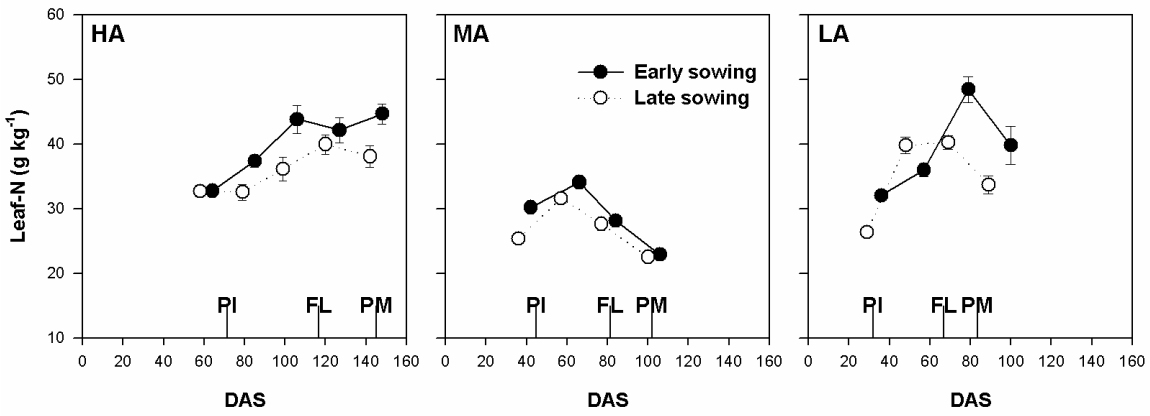

Figure 20 Leaf-N content (g kg^{-1}) (pooled over genotypes) during cropping period in high (HA), mid (MA) and low (LA) altitudinal locations in the second year. The vertical bars indicates standard errors (n=30). DAS, days after sowing.

6. Discussion

1.1. Thermal effects on crop duration

Crop duration is influenced by both genotype and environment and is a major determinant of source and sink potential (Dingkuhn and Kropff, 1996). Crop duration of photoperiod insensitive rice cultivars is influenced by accrued heat units during its development stages (Dingkuhn et al., 1995) while photoperiod sensitive genotypes are influenced by day length during the photoperiod sensitive phase (PSP) between basic vegetative phase (BVP) and reproductive phase (RP). In our field trial, all selected genotypes had a photoperiod sensitivity index (PSI) of less than 0.3, and were classified as photoperiod insensitive; thus the PSP had no effect on crop duration within the five sowing dates. Consequently, crop duration varied due to temperature accrued over time which was mainly affected by altitudinal temperature gradients between locations. The warmer the location (MA and LA locations) the faster is the phenological development and the shorter the crop duration (Figure 7). Within locations, variation was due to early or late sowing date (season specific) which is also influenced by temperature accrued over time and genotypic characteristics that differs from genotype to genotype in the course of accumulating the number of thermal units required to complete phenological phases. In HA and MA locations, but not in the LA location, crop duration varied between years with shorter crop duration in the first year. This could be explained by inter-annual variation in temperature. The relationship between crop duration and average mean air temperature (from germination to flowering) differed between locations. However, the relationship tended to remain similar within locations for all selected genotypes (Figure 9). This may be due to spatial variability in seasonal annual climate (Wassmann et al., 2009) such as daily amplitude of minimum and maximum and/or day and night temperatures (in HA location); rainfall amount, frequency and distribution; soil properties in terms of nitrogen status (Dingkuhn et al., 1991), and soil moisture condition (Wopereis et al., 1996) such as temporal drought (in MA location) or flooding (in LA location).

Consideration of a wide range of environments in the linear regression gave better results for T_{base} and T_{sum} rather than estimating within narrow and limited environments (e.g., five sowing dates within one location (Figure 10). Dingkuhn et al. (1995) claimed that establishing thermal constants from field experiments can lead to difficulties predicting the exact crop duration with the RIDEV model if the thermal conditions (micro climate) are not closely monitored and are insufficiently variable among planting dates. Field-based studies would

either result in wrong genotypic constants, wrong predictions of crop duration, or both. However, these statements were based on horizontally scattered locational field trials. Our locations were vertically (altitudinal) arranged and daily temperatures varied substantially.

1.2. Environmental effects on yield and yield components

Yield stability across environments is commonly accompanied by a yield penalty in favourable, high yielding environments (Peng et al., 2006; Acuña et al., 2008), i.e. Chhomrong and FOFIFA 172 in this study (Figure 11). In the current study, environments were not only defined by different locations but also by sowing dates early and late in the season for two different years. A cluster analyses showed that the 12 environments differed significantly in their average combination of abiotic factors (data not shown). When combined with the environmental characteristics, associations between genotypes and environments emerged that were only partly reflecting the original environments the genotypes were selected for (Figure 12). Since crop duration is strongly influenced by temperature and altitudes vary in seasonal mean temperatures due to the altitudinal temperature gradient of 7 °C per km at 60% air humidity (Houghton and Cramer, 1951), variations in yield observed for the different altitudes can be explained with differences in genotypic adaptation and with temperature effects on duration shifting the different phenological phases responsible for the formation of the different yield components to more or less favourable conditions depending on altitude (Lu et al., 2008; Bajracharya et al., 2010). Tillers per hill, the percentage of filled spikelets followed by number of spikelets per panicle were the yield components most influential on yield at different altitudes (Table 6 and Figure 13). Temperature effects on spikelet sterility (both cold and heat sterility) and on sink-source relationships have been well described for rice (e.g. Dingkuhn et al., 1995; Shrestha et al., 2011; Dingkuhn and Kropff, 1996). Variation in grain yield among planting dates within altitudes was not mainly due to temperature but rather due to the combinations of abiotic factors the genotypes experienced during the different phenological stages during which the different yield components were formed. These combinations strongly differed among altitudes (Figure 14). The combinations of abiotic factors during specific development stages in concert with the genetic predisposition of the genotype determine the level of penalty the respective yield component will inflict on final grain yield.

1.3. Thermal stress on spikelet sterility

Among yield components, percentage of spikelet sterility is highly sensitive to thermal stress and a major component explaining variability of grain yield at a given environment. Sterility determines sink dimensioning (Yoshida, 1981) and is sensitive to environmental stresses (Dingkuhn et al., 1995). Spikelet sterility is caused by indehiscence of the anthers which reduces effective pollination due to either poorly germinating pollen or high wind speed and heavy rain (tropical cyclone) during flowering time. In cold-sensitive genotypes spikelet sterility exceeded 80% across five sowing dates in the HA location. Both cold-sensitive and tolerant genotypes had less than 49% sterility in the MA location and more than 53% sterility in the LA location across five sowing dates (Figure 15). Similarly, sterility varied between sowing dates. The variation in percentage of spikelet sterility was mainly due to cold stress between booting and heading stages when panicles were developing (disturbed meiosis in male floral organs), and heat stress at flowering stage when matured pollens are ready to be intercepted by stigma (poor pollen shedding and germination). Complete sterility was observed below 15 °C (averaged T_{min} between booting to heading) due to cold stress in HA location. However, some genotypes had complete sterility below 12 °C. Similarly, most of the genotypes had 100% spikelet sterility below 34 °C (averaged T_{max} at flowering stage) due to heat stress in MA and LA locations (Figure 15b). This is contrary to a previous study on irrigated rice. De Vries et al. (2011) observed spikelet sterility below 20 °C due to cold and heat stress above 35 °C. Dingkuhn et al. (1995) found cold sterility below 18 °C T_{min} at booting. In this study the deviation of temperature regime may be due to specific genotypes (cold tolerant) included in this experiment and other factors causing sterility beside temperature such as physiological stress associated with soil type (Takeoka et al., 1992) and drought at anthesis stage (Ekanayake et al., 1989). Moreover, low solar radiation on cloudy days (Vergara, 1976; Welch et al., 2010), high day and night temperatures (Welch et al., 2010), drought in combination with high temperature (Rang et al., 2011), wind speed (Matsui et al., 1997b) and a combination of high humidity and temperature (Weerakoon et al., 2008) affect sterility and the interaction between these factors is naturally strong under field conditions.

1.4. Field measurement of SPAD and PRI

N-supply effects on SPAD values of the fully developed youngest rice leaves, and at lower N-supply levels the SPAD values also depend on leaf age (Figure 16a). SPAD is useful tool to estimate leaf-N content (Figure 16b). Peng et al. (1993) and Esfahani et al. (2008) found better linear relationship between SPAD values and leaf-N content when SPAD values are corrected with specific leaf weight (SLW). But in our hydroponic study in the greenhouse, leaf-N had better fitted in quadratic function to SPAD value without correction. Similar function was found between chlorophyll$_{(a+b)}$ and SPAD which is in agreement with Markwell et al. (1995). The threshold SPAD value of 40 (Huang et al., 2008) corresponds to 32 g kg^{-1} leaf-N and 0.24 g m^{-2} chlorophyll$_{(a+b)}$ content in our study (Figure 16b). PRI values were affected by N-supply. NPQ was significantly affected by N-supply and correlated with SPAD and PRI values (Figure 18). Higher NPQ values indicate an increased thermal dissipation of absorbed energy and this regulated heat dissipation is closely linked to xanthophyll cycle activity protecting PSII against photoinhibition under a combination of N deficiency and high light (Verhoeven et al., 1997; Kumagai et al., 2007, 2009a, 2009c, 2010). Non-photochemical quenching can be analysed by following the relaxation after actinic light is switched off (Walters and Horton, 1991; Horton et al., 1996). Relaxation studies identified fast (NPQ$_F$) and slow (NPQ$_S$) relaxation quenching. In this study, NPQ$_F$ was affected by N supply, and this relaxation parameter is considered to reflect the extent for zeaxanthin formation. This finding which has not been reported for N-supply effects so far is indirect evidence that low N supply induced xanthophyll cycle activity and that dark-adapted PRI values are able to indicate this at least in the low-N range (Appendix III). An increased activity of the xanthophyll cycle is indirectly indicated by the change of PRI values from high to low N supply. As both SPAD and dark-adapted PRI values indicated insufficient N supply when the N concentration of the nutrient solution was below 1.43 mM N, both non-destructive measurements can be used to assess the N status of rice leaves in terms of N deficiency. However, as xanthophyll cycle activity is responsive to all stressors which affect lumen pH, PRI values should not be used for N diagnosis as a stand-alone tool. PRI can be a tool for rapid stress assessment, particularly in cropping systems where not only the N fertilizer demand needs to be estimated but stress responses to water or temperature to be considered as well (Appendix III).

MA location had N limiting soil condition (Table 1) when compared with HA and LA locations which was reflected in the SPAD reading (Figure 19). Despite N limiting condition,

MA location had higher yield (Table 3) depicting favourable environmental condition for upland rice. This explains that genotype selection and environmental conditions were more important yield-limiting factors than N-application in our study. However, Huang et al. (2008) claimed that site-specific and time-specific improved N management practice ameliorates NUE in paddy rice. Gradual decline in SPAD and PRI values (Figure 19), and leaf-N content (Figure 20) after flowering in the MA location may explain efficient translocation from the fully developed youngest leaf (source) to panicles (sink) which resulted to higher yield. SPAD values and leaf-N content in the HA location did not decline after flowering and tend to decline in LA location, PRI values at different phenological stages (Figure 19) showed that these locations are influenced by abiotic stresses and as a result sever yield penalty. Kumagai et al. (2009b) suggested that the SPAD reading of the flag leaves of rice cultivars during the ripening stage has the potential to estimate the photosynthetic capacity and is affected by various environmental factors such as irradiance, temperature, humidity, and N conditions, and can be used as a stress indicator. Leaf net photosynthetic rate (NPR) is correlated with nitrogen content (Yoshida and Coronel, 1976; Peng et al., 1995) and the decline in NPR is correlated with decline in of chlorophyll content during leaf senescence (Kura-Hotta et al., 1987; Makino et al., 1983; Ladha et al., 1998a). The content of leaf-N and chlorophyll has been used to quantify leaf senescence during reproductive and ripening stages (Ray et al., 1983; Makino et al., 1983; Kura-Hotta et al., 1987). The gradual decrease of leaf-N content is directly related to biomass production and grain yield of rice crop (Ray et al., 1983). Changes in nitrogen and chlorophyll contents of 4^{th} (counting from the top) and flag leaves as a transition in the source-sink relationship at the onset of leaf senescence have been studied (Mae et al., 1983; Makino et al., 1984; Ladha et al., 1998a). Yoshida (1981) and Ray et al. (1983) claimed that the top three leaves contribute most to grain yield. Mae (1997) further added that the top three leaves assimilate majority of carbon for grain filling during ripening phase and provide large proportion of remobilized-nitrogen for grain development during their senescence. Delaying leaf senescence in order to increase the time for producing and transferring nutrients to the grain has been a source for a stay green genotype (Fu and Lee, 2008; Liu et al., 2010). In the HA, cold tolerant genotype had the similar behaviour but without yield loss. These genotypes under increased temperature (e.g., in MA) matured rapidly along with accelerated leaf senescence without yield penalty.

7. Conclusions

This study was able to show how crop duration of upland rice cultivars varies at different altitudinal gradient locations. Variation in crop duration is location specific. Genotype, year and sowing dates are equally contributing to observed variability in HA whereas genotype in MA and year in LA was not significantly contributing to variability. Unit increment in mean air temperature decreases crop duration by 5 to 9 days depending upon genotype. The predicted rise in air temperature is favourable for upland rice cultivation at high altitudes in terms of crop duration and grain yield. Genotypic characteristics are more important with regard to spikelet sterility in HA, whereas in MA and LA environmental parameters have a greater importance. Unsynchronized relationship between source and sink due to unfavourable environment results poor grain yield. Morpho-physiological traits contributing to cold tolerance need to be identified for further breeding. This study for the first time attempted to relate yield stability across environments with the environmental effects on the different yield components determinant for final yield of upland rice in order to be able to select or breed genotypes suited for newly emerging rice growing environments along an altitude gradient location-specific. The contribution of individual yield components to final yield changes with the environmental conditions the rice experiences during the development stages and that this effect may have a stronger influence on final yield than the genetic control of the individual yield components is shown. The varieties chosen for this study represented a cross section of the upland rice genetic diversity. The multitude of growing environments allowed showing, that the original environments the genotypes were selected for favoured certain combinations of traits that were in most cases not ideally combined for environments facing changes due to changing climate. Therefore, new combinations of traits are required to better exploit the environmental potential which may only be possible via advanced crop models simulating the environmental effects on yield components and their interdependencies to develop ideotype for the target environments thus guiding breeding and selection efforts. The phenological responses determining crop duration, the reported basic genotypic thermal constants, and the analyses of genotypic thermal responses with regard to spikelet sterility reported here provide valuable information for the improvement of rice phenological and growth models urgently needed to develop new genotypes and better adapted cropping calendars.

8. Perspectives

Crop duration, foliage N concentration, dilution of foliar N after vegetative-growth phase, leaf senescence and grain yield have been studied on paddy rice (Dingkuhn et al., 1991; Kura-Hotta et al., 1987; Makino et al., 1984). However, such information is not available for upland rice studied along an altitude gradient. Yield stability of different rice genotypes across environments including climatic and edaphic factors have been studied (e.g., Anyanwu, 2009; Wade et al., 1999) but never considered adverse environmental conditions across altidutinal gradient. Direct and indirect effect of yield components and their relative contribution on final yield have been studied (e.g., Pb Samonte et al., 1998; Nassir and Ariyo, 2006). The contribution of individual yield components to final yield changes with the environmental conditions the rice experiences during the development stages, and the stronger influence of this effect on final yield than the genetic control of the individual yield components is the main focus of this study. Wassmann and Dobermann (2007) clearly stated that crop growth models are available that have been parameterized and validated for some aspects of possible climate change scenarios but the complex interactions are not captured well in these models that seek to predict crop response to climate and climate change. This study tried to capture genuine focus on this aspect. The results mentioned here is a part of the study area. Further plant physiology based results on biomass production and crop growth rate, net assimilation rate, radiation and water use efficiencies, and phyllochron studies are under data processing stage and will be ready for calibration and validation of crop growth models at the final stage. The results obtained on the differentiated responses of various genotypes to environmental conditions in the current study allow to further develop crop models based on physiological responses such as IMPATIENCE (Dingkuhn et al., 2008), RIDEV (Dingkuhn, 1997; Wopereis et al., 2003), SARRAH (Kouressy et al., 2008), EcoMeristem (Luquet et al., 2006; Dingkuhn et al., 2006) and SAMARA (synthesis of SARRAH and EcoMeristem) or in order to test a large number of traits x environments combinations to define ideotypes of upland rice varieties adapted to changing climate and adapted cropping calendars. In this way, emerging high altitude rice cropping environments can contribute substantially to future food security through the urgently needed identification or breeding of suited genetic material. Collaborations with AfricaRice, CIRAD and IRRI to this effect are ongoing.

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Appendix I

Phenological responses of Upland Rice Grown Along an Altitudinal Gradient

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Abstract: High altitude upland rice production systems are expected to benefit from climate change induced increase in temperatures. The potential yield of rice genotypes is governed by the thermal environment experienced during crop development phases when yield components are determined. Thus, knowledge on genotypic variability in phenotypic responses to variable temperature is required for assessing the adaptability of rice production to changing climate. Although, several crop models are available for this task, genotypic thermal constants used to simulate crop phenology vary strongly among the models and are under debate. Therefore, we conducted field trials with ten contrasting upland rice genotypes on three locations along an altitudinal gradient with five monthly staggered sowing dates for two years in Madagascar with the aim to study phenological responses at different temperature regimes. We found that, crop duration is equally influenced by genotype selection, sowing date and year in the high altitude. In contrast, in mid altitudes genotype has no effect on crop duration but year and sowing date strongly affect crop duration. At low altitudes crop duration is more affected by sowing date and less by genotype and year. Every 1°C increment in mean air temperature decreases crop duration (germination to flowering) by 5 to 9 days depending on genotype. Using a wide range of environments for estimating thermal constants (Tbase and Tsum) allowed for more accurate results under field conditions. Whereas the mid altitudes represent favorable conditions for upland rice, grain yield is strongly affected by low temperatures at high altitudes and severly influenced by frequent tropical cyclones at low altitudes. In high altitude, genotype explained 68% of variation in spikelet sterility, whereas in mid and low altitudes environment explained more than 70% of the variation. The phenological responses determining crop duration and yield, the reported basic genotypic thermal constants, and the analyses of genotypic thermal responses with regard to spikelet sterility reported here, provide valuable information for the improvement of rice phenological and growth models urgently needed to develop new genotypes and better adapted cropping calendars.

Research Highlights:

- The knowledge on genotypic variability in phenotypic responses to variable temperature is required for assessing the adaptability of rice production to changing climate.
- Every 1 ℃ increment in mean air temperature decreases crop duration by 5 to 9 days depending on genotype.
- The phenological responses determining crop duration and yield, and the genotypic thermal responses with regard to spikelet sterility provide valuable information for the improvement of rice growth models to develop new genotypes and better adapted cropping calendars to climate change.

1 **Title of the paper**

2 Phenological responses of Upland Rice Grown along an Altitudinal Gradient

3

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Abstract

High altitude upland rice production systems are expected to benefit from climate change induced increase in temperatures. The potential yield of rice genotypes is governed by the thermal environment experienced during crop development phases when yield components are determined. Thus, knowledge on genotypic variability in phenotypic responses to variable temperature is required for assessing the adaptability of rice production to changing climate. Although, several crop models are available for this task,, genotypic thermal constants used to simulate crop phenology vary strongly among the models and are under debate. Therefore, we conducted field trials with ten contrasting upland rice genotypes on three locations along an altitudinal gradient with five monthly staggered sowing dates for two years in Madagascar with the aim to study phenological responses at different temperature regimes. We found that, crop duration is equally influenced by genotype selection, sowing date and year in the high altitude. In contrast, in mid altitudes genotype has no effect on crop duration but year and sowing date strongly affect crop duration. At low altitudes crop duration is more affected by sowing date and less by genotype and year. Every 1°C increment in mean air temperature decreases crop duration (germination to flowering) by 5 to 9 days depending on genotype. Using a wide range of environments for estimating thermal constants (T_{base} and T_{sum}) allowed for more accurate results under field conditions. Whereas the mid altitudes represent favorable conditions for upland rice, grain yield is strongly affected by low temperatures at high altitudes and severly influenced by frequent tropical cyclones at low altitudes. In high altitude, genotype explained 68% of variation in spikelet sterility, whereas in mid and low altitudes environment explained more than 70% of the variation. The phenological responses determining crop duration and yield, the reported basic genotypic thermal constants, and the analyses of genotypic thermal responses with regard to spikelet sterility reported here, provide valuable information for the improvement of rice phenological and growth models urgently needed to develop new genotypes and better adapted cropping calendars.

Key words: Crop duration; Sowing date; Spikelet sterility; Temperature; Thermal stress

Introduction

On a global scale rice is the most important crop in terms of daily carbohydrate supply in human diet. Rice cultivated mainly in tropical and subtropical environments are increasingly exposed to climate-change induced adverse abiotic conditions which of heat stress and flooding are major threats in the tropical areas. In subtropical environments a combination of drought and unfavorable rainfall distribution are considered major growth constraints. Additionally, freshwater in subtropical environments becomes less available in some paddy rice systems on a regional scale. These conditions and particularly the forecasts of future climate may potentially de-stabilize the rice-dominated food markets (Wassmann et al., 2010). Upland rice production systems are expected to increasingly contribute to the world-wide rice market especially in mountainous areas and where freshwater resources are overexploited. Upland rice cultivation differs from paddy production systems in many regards. Water availability is more likely lower during at least some of the developmental stages, thereby reducing the plants' potential yield. Soil temperature is subject to greater variability as compared to the standing water body of lowland paddy rice, exposing the growth meristems to higher fluctuation in the thermal regime. These environmental conditions in combination with frequently inadequate nutrient supply result in substantially lower yields of upland than of paddy systems and usually increase inter-annual yield variability. However, climate-change induced increases in temperature may allow for the extension of upland rice systems into areas with cooler climatic conditions in the high altitude tropics (David, 1994; Shrestha et al., 2011).

In this context, characterization of existing variability in the germplasm of rice in terms of phenotypic responses to variable temperature and day length is required for assessing the potential of strategically adapting rice production systems to changing climate. Crop growth models such as RIDEV, OryzaS, Oryza2000 and CERES are available as decision support tools to systematically evaluate genotypic variability in phenology. However, most of the parameterization of these models is based on lowland rice germplasms and data acquired in tropical environments, resulting in poor predictive power of these models in rainfed upland environments particularly in terms of phenological development (Shrestha et al., 2011; van Oort et al., 2011).

Generally, rice genotypes are short-day plants and crop duration is strongly influenced by their sensitivity to photoperiod and temperature (Dingkuhn and Meizan, 1995). Under optimal conditions (temperature between 20 and 30 °C and photoperiods of less than 12 hours), crop

duration mainly depends upon genotype-specific duration of the basic vegetative phase (BVP). The BVP is followed within a few days by panicle initiation (PI) under inductive conditions (Sié et al., 1998a, b). Photoperiod insensitive rice genotypes have the shortest photoperiod sensitive phase (PSP). In rainfed rice, drought during germination and flowering delays developmental phases (Wopereis et al., 1996), but accelerates ripening (Dingkuhn and Le Gal, 1996). Flowering time is commonly used to determine final crop duration as reproductive and ripening phases are assumed to be fairly constant in general for any genotype in a given environment (Yoshida, 1981).

Japonica cultivars are more sensitive to temperature and less to photoperiod than indica cultivars (Fukai, 1999). Considering these effects of abiotic factors on developmental phases, lower temperatures increase crop duration from germination to flowering. Flowering of photoperiod insensitive rice cultivars can be predicted with two genotypic constants, critical lower temperature for development (T_{base}) and accrued number of heat units required for flowering (T_{sum}) within the range of linear response of plant development (Dingkuhn et al., 1995; Shrestha et al., 2011).

Crop models such as RIDEV and OryzaS are able to estimate spikelet sterility of rice cultivars if the genotypic-specific threshold temperatures for cold and heat stresses are defined. The default critical temperatures for cold (during booting to heading) and heat (during flowering) stress are 18 °C and 37 °C in both models, yielding 100% spikelet sterility for temperatures below and above, respectively. However, these models were parameterized for low-altitude rice production systems and only for few genotypes. Potential of introducing cold-tolerant genotypes is indicated by the study of Shrestha et al. (2011), who reported a threshold temperature for cold stress distinctly below 18 °C for genotypes such as Chhomrong and Machhapuchre-3.

Field trials on phenology and spikelet sterility of upland rice assessment across altitudinal gradients have not been reported so far. Considering the basic assumption that phenology of photoperiod insensitive rice genotypes responds to altitudinal temperature gradient, this study intended to identify phenological responses of crop duration at different altitudes, estimate basic genotypic thermal constants and assess genotypic thermal responses in spikelet sterility. Results are intended to guide future breeding efforts for high altitude rice cropping systems and to improve phenological parameters of crop growth models for upland and rainfed rice production systems.

Materials and methods

Locational characteristics of Mini Rice Garden experiment

Three different altitude locations in Madagascar (Andranomanelatra, 1625 m asl; Ivory, 965 m asl and Ankepaka, 25 m asl) were selected for non-replicated phenological 'mini rice garden' studies following the concepts used by Dingkuhn et al. (1995) and Shrestha et al. (2011). A field-plot trial with five sowing dates (monthly staggered) and ten upland rice genotypes in two consecutive years (2008/09 and 2009/10) was established, thus creating thirty different rice growing environments (Table 1). Ten selected upland rice genotypes were randomized within a block (sowing date). Experimental fields were located in the high altitude (HA) at 19°46'45.3" S and 47°06'24.5" E, mid altitude (MA) at 19°33'16.8" S and 46°25'29.1" E, and low altitude (LA) at 22°11'31.6" S and 47°52'32.7" E. Climatic data were recorded from an Automatic Meteorology Station, ENERCO 404 Series, (CIMEL Electronique, Rue de Charonne, Paris, France) in the HA and MA locations, and Onset Hobo Weather Station, HOBO U30 Series, (MacArthur Blvd, Pocasset, Massachusetts, USA) in LA location which were set up close to the experimental plots. The HA and MA locations had a similar photoperiod, while the LA location had a 10 minutes longer and shorter photoperiod in January and July, respectively. In the HA location, daily mean air temperature (T_{mean}) was 7 – 22 °C in the first growing season and slightly higher with 10 – 23 °C in the second year during the experimental periods (Fig. 1). In MA location, T_{mean} was similar in both years with 19 -27 °C. In the LA location T_{mean} was 17 – 29 °C in the first year and more variable with 15 – 33 °C in the second year. Air temperature and relative humidity in the LA location were unavailable from 12 Jan 2010 due to technical problems of the Onset Hobo Weather Station's temperature and humidity sensor. Therefore, daily mean air temperature and relative humidity for rest of the period were taken from TinyTag Plus 2 data loggers (Gemini Data Loggers Ltd, Chichester, West Sussex, United Kingdom) placed in the experimental plots to measure air temperature above canopy level (Fig. 1). Precipitation amount during the experimental period varied between locations and years. The HA location had 1545 and 1044 mm of precipitation in the first and second season, respectively. Rainfall in the MA location was 1317 mm in the first and 1069 mm in the second season. The coastal LA location received 1411 mm in the first and 2435 mm in the second season. Several tropical cyclones occurred during the experimental periods: Cyclone Eric (east coast, 19 Jan 2009), cyclone Fanele (west coast, 21 Jan 2009 with winds of 210 km hr^{-1} and heavy rains); Category 1 cyclone Jade (east coast, 6 April 2009 with winds of 93 km hr^{-1}); cyclone Edzani (east coast, 11 Jan 2010 with

winds of 185 km hr^{-1}); cyclone Hubert (300 km southeast of Antananarivo, 10 March 2010 with maximum sustained winds of 65 km hr^{-1} and heavy showers). This list of cyclones is reported here, as such whether events affected the extent of sterility of certain sowing dates and genotypes.

Genotypes and crop management

Ten contrasting genotypes including seven tropical japonica, one temperate japonica and two interspecific crosses (Table 2) were selected for this study. Botramaintso and Chhomrong are traditional landraces grown at the middle and higher altitudes of Madagascar and Nepal, respectively. Botramaintso was selected due to its vigour growth. Chhomrong is a high-tillering, cold-tolerant genotype rapidly diffused after it's released in 2006 in the HA of Madagascar. B22 and Primavera are improved varieties from Brazil grown at mid- and low altitudes. Nerica 4 (WAB 450-I-B-P-91-HB), and WAB 878 (WAB 878-6-12-1-1-P1-HB), and IRAT 112 are selected genotypes for mid-altitude locations in Madagascar. Nerica 4 has stay-green characteristic and was selected due to its erect leaves and low plant height compared to other selected genotypes. WAB 878 was selected due to its vigour growth. Improved genotypes FOFIFA 161, FOFIFA 167 and FOFIFA 172 were introduced for high altitude locations of Madagascar due to cold tolerance.

The mini rice garden trial comprised five blocks of sowing dates in each location and year. Ten genotypes were randomized within each block. Each genotype plot was 1 m x 1 m in size, plant sown with 0.2 m x 0.2 m spacing and adjusted to 5 plants per hill at the seedling stage with the sowing dates as summarized in Table 1. Textures of soils were: 11.6% sand, 34.0% silt and 54.3% clay; pH 4.5 (HA); 40.2% sand, 20.2% silt and 39.7% clay; pH 4.8 (MA), and 16.3% sand, 63.3% silt and 20.4% clay; pH 3.9 (LA). Plots in MA and LA locations were mulched with *Stylosanthes* to avoid soil moisture loss through evaporation. In all locations, early-sown plots were manually irrigated to avoid drought stress during vegetative growth phases. Complex fertilizer (11:22:16 N-P-K) at a rate of 300 kg ha^{-1}, dolomite 500 kg ha^{-1} and FYM 5 t ha^{-1} was applied as basal dose at the time of sowing. Top dressing was done with urea (46 % N) at the rate of 35 kg ha^{-1} and 30 kg ha^{-1} at first and second weeding, respectively. Manual weeding was done as required. Systematic fungicide (Carbenstor-500 SC) was applied at the rate of 1 L ha^{-1} to control leaf blast (*pyriculariase*) when symptoms appeared.

Observation and data analysis

Development phases and growth stages were carefully monitored during crop cycles. Biomass, grain yield and yield components including sterility percentage were determined at harvest. Statistical analyses were done in GenStat 13th Edition (VSN International Ltd, UK) and SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). Crop duration of phenological phases, grain yield and percentage of spikelet sterility were analyzed with PROC Univariate. Main effect of genotypes, location, year and sowing dates were tested using general linear model (GLM) for analysis of variance (ANOVA) and relative contribution to variance (RCVE) of these factors were estimated. Box plots with 5% and 95% quantiles were produced with SigmaPlot Version 10.0 (Systat Software, Inc., Washington St., Chicago, USA) for all thirty environments. Photoperiod sensitivity Index (PSI) as the slope (absolute value) of the linear regression of days to flowering against 5 sowing dates for 3 locations and 2 year of each genotype was calculated according to Fukai (1999) and were accordingly classified. The relation between crop duration and average daily mean temperature between germination and 50% flowering was analysed by linear regression (PROC REG). The negative slope of the regression provided an estimation of decrement in crop duration to flowering due to increment in mean air temperature. Thermal constants T_{base} and T_{sum} were estimated from the linear regression of thermal duration to flowering (in terms of accrued °C to the basis of zero) against the accrued number of days with the intercept yielding T_{sum} and the slope T_{base}. Spikelet sterility due to cold stress was analysed in a scattered plot diagram where percentages of spikelet sterility was plotted against corresponding averaged minimum air temperature during booting and heading stages. Similarly, spikelet sterility due to heat stress was also analysed in a scattered plot diagram where percentages of spikelet sterility was plotted against corresponding averaged maximum air temperature during flowering stage (50% flowering time ± 7 days). Crops that were damaged by tropical cyclones in the LA locations were excluded from heat stress analysis.

Results

Crop duration

Location explained more than 90% of variance (Fig. 2) in crop duration at different phenological stages. Crop duration was longest in the HA location and decreased in MA and LA locations (Fig. 2). Pooled over genotypes, sowing dates and years showed that days from germination to panicle initiation, 50% flowering and physiological maturity were 72 d (± 2.0), 117 d (± 1.4) and

145 d (± 1.5) in the HA location, 45 d (± 0.9), 81 d (± 1.1) and 102 d (± 1.5) in the MA location, and 32 d (± 0.7), 67 d (± 1.1) and 83 d (± 1.4) in the LA location.

Variation in days from germination to 50% flowering within one location was explained by genotypic characteristics (long or short duration) and/or sowing dates (early or late) and/or year (climatic conditions). In the HA location, year explained 40% of the total variance as compared to varieties (35%) and sowing dates (25%) (Fig. 3), indicating that variety, sowing dates and year were all contributing to observed variability. Crop duration to flowering was shorter (112 d) in the year 2008/09 and longer (117 d) in the year 2009/10. Genotype Botramaintso (G2) had the longest duration (145 d) and Primavera (G9) the shortest (106 d) in the HA location. Sowing between mid-November and mid-December resulted in the shortest duration to flowering (109 – 110 d). In the MA location, year explained 65% and sowing dates 31% of the total variance, while variety did not contribute significantly to total variance. Duration to flowering was shorter (79 d) in the year 2008/09 and longer (84 d) in the year 2009/10. Early sowing (mid-September to mid-November) resulted in more than 80 d to flowering and late sowing (mid-January) in the shortest duration (70 d). In the LA location, sowing date explained 84% of the total variance, while variety explained only 15% and year had no effect. Early sowing (mid-October) resulted in the longest duration to flowering (77 d) and late sowing (mid-February) in the shortest (57 d). Genotype Botramaintso (G2) had the longest duration to flowering (83 d), whereas, all other genotypes had shorter duration (62 - 68 d).

Crop duration as a function of mean air temperature

All selected genotypes had a photoperiod sensitivity index (PSI) of less than 0.3 (Fig. 4), and were classified as photoperiod insensitive cultivars. Crops experienced different average mean air temperatures during their developmental phases depending upon location, sowing dates and year. Pooled data over locations, sowing dates and years in the regression analyses of varietal responses indicated that each 1 °C rise in mean air temperature decreased crop duration by 6 to 7 days to flowering (Fig. 5). However, crop duration of landrace Botramaintso (G2) decreased by 9 days and that of cold-tolerant cultivar FOFIFA 172 (G6) by 5 d. Genotypes tended to show similar relationships within one location (Fig. 5), while the relationship differed between locations indicating that there were location-specific constraints that affected crop duration. In the HA location, five staggered sowing dates over two years experienced mean air temperatures of 18 - 20 °C while the corresponding genotypic-specific days to flowering varied from 90 d to more

than 158 d. Similarly, mean air temperature in the MA location did not vary much (24 - 25 °C) but days to flowering ranged from 57 to105 d. In the LA location, mean air temperature varied 24 - 29 °C and the corresponding days to flowering ranged from 39 to 92 d.

Genotypic thermal constants

Pooled data over locations, sowing dates and years in the regression analyses of varietal responses showed that T_{base} of the ten genotypes ranged from 9.8 to 13.9 °C and T_{sum} from 816 to 1220 °C d (Fig. 6). Inverse correlation between T_{base} and T_{sum} (i.e., lower T_{base} resulted in proportionally higher T_{sum}) was observed (Fig. 7) with 0.644 coefficient of determination (r^2). FOFIFA 172 had the highest T_{base} (13.9 °C) and the lowest T_{sum} (816 °C d) whereas FOFIFA 161 had the lowest T_{base} (9.8 °C) and T_{sum} 1157 °C d. Botramaintso (G2) alone had the highest T_{sum} (1220 °C d) and T_{base} 11.4 °C. Slopes differed between locations when aggregated data sets of sowing dates and year were regressed for individual location (e.g., the MA location had a steeper slope compared to HA location).

Grain yield and spikelet sterility

Genotype, location and sowing dates were the main driving factors for variation in grain yield. In the HA location, variation in grain yield was mainly due to genotype and sowing date as both equally explained 49% to the total variance (Table 3). Genotypes such as Chhomrong (G3), FOFIFA 161 (G4), FOFIFA 167 (G5), and FOFIFA 172 (G6) had more than 2 t ha^{-1}, whereas, genotypes B22 (G1), Botramaintso (G2), IRAT 112 (G7), Nerica 4 (G8), Primavera (G9) and WAB 878 (G10) had less than 1 t ha^{-1} across five sowing dates in both years. These cold tolerant genotypes (G3, G4, G5 and G6) had higher yield when early sown and had higher yield penalty when sown later. Variation in grain yield in MA and LA locations were mainly due to sowing dates as it explained more than 74% of the total variance and less by year (less than 17%) (Table 3). In the MA location, sowing between mid-October and mid-December resulted in 3.3 - 4.2 t ha^{-1} of grain yield. Early sowing (mid-September) resulted in lower yield (1.2 t ha^{-1}) than late sowing (mid-January). Similarly, in the LA location sowing between mid-November and mid-January resulted in 1.2 - 1.7 t ha^{-1}, whereas early sowing (mid-October) gave 0.6 t ha^{-1} and late sowing (mid-February) 0.4 t ha^{-1} grain yields.

Percentage of spikelet sterility (SSP) also varied between genotypes, locations, and sowing dates. In the HA location, variation was mainly due to genotype and sowing dates, but in MA and LA

locations variation was more due to sowing date (more than 74%) and less by genotype (less than 18%) (Table 3). In the HA location, genotypes G3, G4 and G6 had 40 - 58% SSP, and G5 had 70% SSP. All other genotypes had more than 80% SSP. Early sowing dates (mid-September to mid-October) had less than 65% SSP and late sowing dates (mid-December to mid-January) had more than 86% SSP. In the MA location, SSP was between 19 and 49% depending on genotypes. Early sowing (mid-September) had 41% SSP and late sowing (mid-January) had 48% SSP. Sowing between mid-October and mid-December had 19 - 25% SSP. In the LA location, SSP ranged from 53 to 79% depending upon genotypes. Early sowing (mid-October to mid-November) had SSP between 69% and 79% where as late sowing (mid-February) 67% SSP. Sowing between mid-December and mid-January had SSP between 53% and 56%.

Spikelet sterility caused by thermal stress

Percentage of spikelet sterility (SSP) was highly influenced by environmental factors such as location, year and sowing dates (66%) and less by genotypic characteristics (34%). In the high HA location, genotype explained 68% and environment 32% of the total variance, whereas, in MA location genotype 29% and environment 71%, and in LA location genotype 14% and environment 86%. Spikelet sterility was affected by low temperature (cold stress) between booting and heading stages (averaged T_{min} < 18 °C) in the HA location (Fig. 8). However, cold tolerant genotypes were less affected in this location when early sown. In the MA location, early or late sowing dates had increased spikelet sterility. MA and LA locations were affected by heat stress at flowering stage (average T_{max} > 30 °C) in environment E11, E16, E20, E21, E22, E23, E26 and E28 depending on genotypic crop duration (Fig. 9). Cold-tolerant Chhomrong (G3), and cold-sensitive IRAT 112 (G7) were selected as reference genotypes to quantify spikelet sterility across averaged T_{min} exposed between booting and heading stages, and averaged T_{max} exposed during flowering stage. Chomrong had less than 40% spikelet sterility when averaged T_{min} was around 13 to 14 °C (Fig. 8) and 100% sterility when T_{min} was below 12 °C. A similar relationship was found to genotypes FOFIFA 161 (G4), FOFIFA 167 (G5) and FOFIFA 172 (G6). Cold sensitive genotypes IRAT 112 (G7) had less spikelet sterility at 19 °C and the sterility was close to 80% at 15 °C and 100% at 13 °C. Similar behavior was observed for other genotypes B22 (G1), Botramaintso (G2), Nerica 4 (G8), Primavera (G9) and WAB 878 (G10), but the sterility was 100% when the averaged T_{min} was close to 15 °C. Data for spikelet sterility was not available between 15 °C and 18 °C as the crop did not experience these range of temperatures across

location, sowing dates and year. G3 and G7 genotypes had heat stress when the averaged T_{max} at flowering was above 30 °C (Fig. 9). Averaged T_{max} close to 30 °C had less than 20% sterility and 100% sterility was extrapolated above 34 °C. However, other genotypes had similar trend, 100% sterility was below 34 °C.

Discussion

Phenological traits such as crop duration are the key attributes of rice cultivars determining potential yield, fit to the local cropping calendar, and ability to escape from thermal stress and drought or flooding (Dingkuhn and Asch, 1999) during different sensitive growth stages. Crop duration is influenced by both genotype and environment and is a major determinant of source and sink potential (Dingkuhn and Kropff, 1996). Crop duration of photoperiod insensitive rice cultivars is influenced by accrued heat units during its development stages (Dingkuhn et al., 1995) while photoperiod sensitive genotypes are influenced by day length during the photoperiod sensitive phase (PSP) between basic vegetative phase (BVP) and reproductive phase (RP). In our field trial, all selected genotypes had a photoperiod sensitivity index (PSI) of less than 0.3, and were classified as photoperiod insensitive; thus the PSP had no effect on crop duration within the five sowing dates. Consequently, crop duration varied due to temperature accrued over time which was mainly affected by altitudinal temperature gradients between locations. The warmer the location (MA and LA locations) the faster the phenological development and the shorter the growth duration (Fig. 2). Within locations, variation was due to early or late sowing date (season specific) which is also influenced by temperature accrued over time and genotypic characteristics that differs from genotype to genotype in the course of accumulating the number of thermal units required to complete phenological phases. In HA and MA locations, but not in the LA location, crop duration varied between years with shorter crop duration in the first year. This could be explained by inter-annual variation in temperature.

Genotype, sowing date and years equally influenced crop duration in the HA location. Shifting sowing dates within the location altered crop duration as different sowing dates exposed crops to different thermal environments during its development stages in this cool-temperature environment. Similarly, in the MA location, year and sowing dates were main factors determining crop duration, while, except for long-duration landrace Botramaintso (G2), sowing dates solely determined crop duration in the LA location (Fig. 3). This illustrates that temperature

alone was the determining factor for crop duration in photoperiod insensitive japonica rice cultivars.

A unit rise in temperature shortened crop duration by 5 to 9 days depending on genotype. In our field trial, the relationship between crop duration and average mean air temperature (from germination to flowering) differed between locations. However, the relationship tended to remain similar within locations for all selected genotypes (Fig. 5). This may be due to spatial variability in seasonal annual climate (Wassmann et al., 2009) such as daily amplitude of minimum and maximum and/or day and night temperatures (in HA location); rainfall amount, frequency and distribution; soil properties in terms of nitrogen status (Dingkuhn et al., 1991), and soil moisture condition (Wopereis et al., 1996) such as temporal drought (in MA location) or flooding (in LA location).

The developmental rate (germination to flowering) is often approximated by a linear function of mean temperature alone (Dingkuhn et al., 1995; Fukai, 1999). Consequently, estimates of genotypic-specific thermal constants are needed for model-assisted prediction of varietal performance (development stages) in contrasting environments. Therefore, estimation of accurate thermal constants plays a vital role to predict precise development stages. Craufurd et al. (2003) stated that derivation of accurate thermal constants requires many successive planting dates for better regression output. In this study, the range of T_{base} and T_{sum} values are similar to Dingkuhn et al. (1995) working under Sahelian conditions and Shrestha et al. (2011) in a high-altitude study with 8 staggered planting dates of 15 days interval. Our field trial showed that considering a wide range of environments in the linear regression gave better results for T_{base} and T_{sum} rather than estimating within narrow and limited environments (e.g., five sowing dates within one location – (Fig. 6). Dingkuhn et al. (1995) claimed that establishing thermal constants from field experiments can lead to difficulties predicting the exact crop duration with the RIDEV model if the thermal conditions (micro climate) are not closely monitored and are insufficiently variable among planting dates. Field-based studies would either result in wrong genotypic constants, wrong predictions of crop duration, or both. However, these statements were based on horizontally scattered locational field trials. Our locations were vertically (altitudinal) arranged and daily temperatures varied substantially.

Sowing dates generated location-specific differences in climatic conditions during different phenological phases and affected grain yield. The extent of sowing date effects on yield obviously depended on the genetic yield potential (genotypic specific sink capacity) under

adverse environmental conditions (source capacity). Dingkuhn and Kropff (1996) stated that grain yield is either sink or source limited, and both are usually balanced as they depend on the environment at all stages (initiation and development). Imbalances occur when the environment changes greatly during phases that are crucial to sink and source formation. In our field trial, cold tolerant cultivars expressed a high yield potential in the HA location where temperature was a clear constraint of yield. These cultivars when sown early had higher yields in the HA location than in other locations. As opposed to the HA location, the MA location is favourable in terms of climatic condition and the LA location vulnerable as affected by frequent tropical cyclone causing increased number of cloudy days and low solar radiation, and high humidity with high air temperature during cropping season.

Among yield components, percentage of spikelet sterility is highly sensitive to thermal stress and a major component explaining variability of grain yield at a given environment. Sterility determines sink dimensioning (Yoshida, 1981) and is sensitive to environmental stresses (Dingkuhn et al., 1995). Spikelet sterility is caused by indehiscence of the anthers which reduces effective pollination due to either poorly germinating pollen or high wind speed and heavy rain (tropical cyclone) during flowering time. In cold-sensitive genotypes spikelet sterility exceeded 80% across five sowing dates in the HA location. Both cold-sensitive and tolerant genotypes had less than 49% sterility in the MA location and more than 53% sterility in the LA location across five sowing dates (Fig. 8 and 9). Similarly, sterility varied between sowing dates. The variation in percentage of spikelet sterility was mainly due to cold stress between booting and heading stages when panicles were developing (disturbed meiosis in male floral organs), and heat stress at flowering stage when matured pollens was ready to be intercepted by stigma (poor pollen shedding and germination). Complete sterility was observed below 15 °C (averaged T_{min} between booting to heading) due to cold stress in HA location. However, some genotypes had complete sterility below 12 °C. Similarly, most of the genotypes had 100% spikelet sterility below 34°C (averaged T_{max} at flowering stage) due to heat stress in MA and LA locations (Fig. 9). This is contrary to a previous study on irrigated rice. De Vries et al. (2011) observed spikelet sterility below 20 °C due to cold and heat stress above 35 °C. Dingkuhn et al. (1995) found cold sterility below 18 °C T_{min} at booting. In this study the deviation of temperature regime may be due to specific genotypes (cold tolerant) included in this experiment and other factors causing sterility beside temperature such as physiological stress associated with soil type (Takeoka et al., 1992) and drought at anthesis stage (Ekanayake et al., 1989). Moreover, low solar radiation on cloudy

401 days (Vergara, 1976; Welch et al., 2010), high day and night temperatures (Welch et al., 2010),
402 drought in combination with high temperature (Rang et al., 2011), wind speed (Matsui et al.,
403 1997) and a combination of high humidity and temperature (Weerakoon et al., 2008) affect
404 sterility and the interaction between these factors is naturally strong under field conditions.
405
406 **Conclusion**
407 In this study we showed how crop duration of upland rice cultivars varies at different altitudinal
408 gradient locations when altering sowing dates and showed locational constraints for spikelet
409 sterility due to thermal stress. Variation in crop duration is location specific. Genotype, year and
410 sowing dates are equally contributing to observed variability in HA whereas genotype in MA and
411 year in LA was not significantly contributing to variability. Unit increment in mean air
412 temperature decreases crop duration by 5 to 9 days depending upon genotype. The predicted rise
413 in air temperature is favorable for upland rice cultivation at high altitudes in terms of crop
414 duration and grain yield. Genotypic characteristics are more important with regard to spikelet
415 sterility in HA, whereas in MA and LA environmental parameters have a greater importance.
416 Chhomrong and three selected FOFIFA genotypes are tolerance to cold induced sterility (T_{min}
417 less than 18 °C) and perform better when temperature improve (T_{max} less than 31 °C). Morpho-
418 physiological traits contributing to cold tolerance need to be identified for further breeding. The
419 phenological responses determining crop duration, the reported basic genotypic thermal
420 constants, and the analyses of genotypic thermal responses with regard to spikelet sterility
421 reported here provide valuable information for the improvement of rice phenological and growth
422 models urgently needed to develop new genotypes and better adapted cropping calendars.
423
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431 conducting field experiments.
432

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508

509 **Caption for Figures**:

510 Figure 1. Daily weather patterns of two years of field experiments in three different
511 altitudinal locations in Madagascar. The upper, middle and lower zigzag solid
512 lines in each locational plots are daily minimum, mean and maximum air
513 temperature (°C) respectively. The smooth bimodal solid lines are daily
514 photoperiod (h) and vertical grey bars depict total daily precipitation (mm). White
515 square boxes indicate early and late sowing dates, black square boxes indicate
516 recommended sowing dates and the gray square boxes indicate end of the
517 experiment.

518 Figure 2. Different phenological phases of upland rice in three different locations. The
519 horizontal bars represent the standard error of mean (n=100 for data sets without
520 missing information) aggregated over genotypes, sowing dates and year. ns, ***,
521 **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05,
522 respectively. Abbreviation: HA, high altitude; MA, mid altitude and LA, low
523 altitude.

524 Figure 3. Quartile box plots (between 5% and 95%) showing crop duration from
525 germination to 50% flowering across thirty different environments (E1 to E30,
526 also see Table 1). ns, ***, **, *: not significant or significant at P-value ≤ 0.001,
527 ≤ 0.01 and ≤ 0.05, respectively. Abbreviation: HA, high altitude; MA, mid
528 altitude; LA, low altitude and S, sowing dates.

529 Figure 4. The effect of sowing dates on duration to flowering from germination of ten
530 genotypes. The lines are produced from fitted values in the linear regression, and
531 the symbols represent genotypes. The linear regression for each genotype is pooled
532 over 3 locations and 2 years. The slope of each line estimates photoperiod
533 sensitivity index (PSI) of the genotype.

534 Figure 5. Relationship between crop duration (germination to 50% flowering) and the
535 averaged mean air temperature experienced (germination to 50% flowering).
536 Symbols with white, light gray and dark gray color represent high altitude (HA),

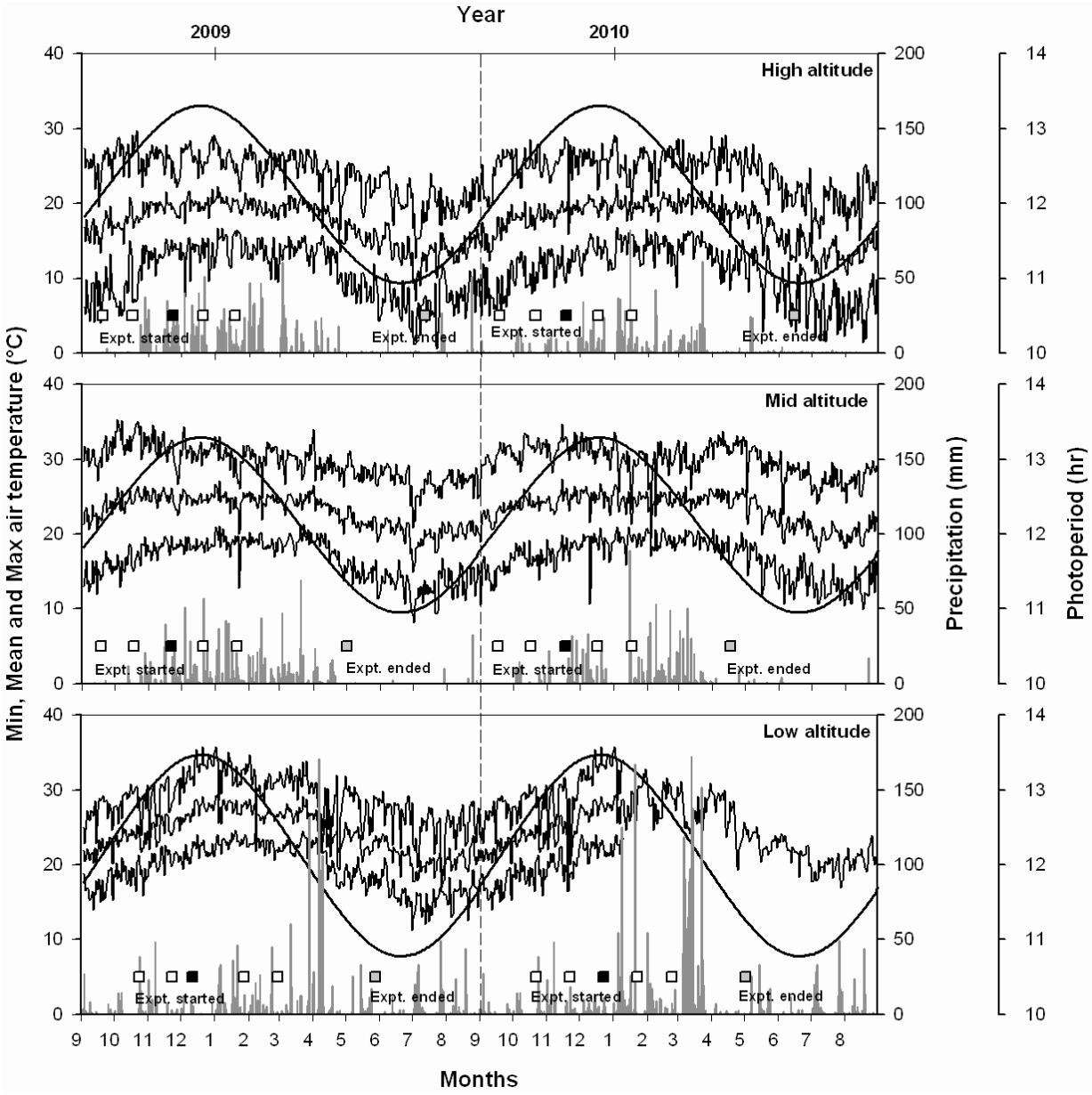
mid altitude (MA) and low altitude (LA). The dotted lines represent linear regression line over location, sowing dates and year. The solid lines represent linear regression over sowing dates and year. G1 to G10 represent genotypes (also see Table 2).

Figure 6. Linear regression of accrued thermal duration to flowering (to the basis of zero) known as T_{sum} (°C d) against the accrued number of days to flowering (d). Symbols with white, light gray and dark gray color represent high altitude (HA), mid altitude (MA) and low altitude (LA). The dotted lines represent linear regression pooled over location, sowing dates and year. The solid lines represent locational linear regression pooled over sowing dates and year. G1 to G10 represent genotypes (also see Table 2).

Figure 7. Relationship between thermal times required to progress from germination to flowering (T_{sum}) and critical lower temperature for development (T_{base}).

Figure 8. Relationship between spikelet sterility and the averaged T_{min} actually observed between booting and heading stages, individually determined for each genotype, location, sowing dates and year. Chhomrong (G3) and IRAT 112 (G7) were taken as reference genotypes to represent cold tolerant and sensitive genotypes respectively for linear regression due to its spikelet sterility variation from less than 30% to 100% (in HA and MA locations) within the range of less than 14 to 20°C averaged T_{min} from booting to heading.

Figure 9. Relationship between spikelet sterility and the averaged T_{max} actually observed during flowering stage (± 7 days), individually determined for each genotype, location, sowing dates and year. Chhomrong (G3) and IRAT 112 (G7) were taken as reference genotypes to represent cold tolerant and sensitive genotypes respectively for linear regression due to its spikelet sterility variation from less than 30% to more than 80% (in MA and LA locations) within the narrow range of more than 30 to 32°C averaged T_{max} during flowering stage.



565

566 Figure 1.

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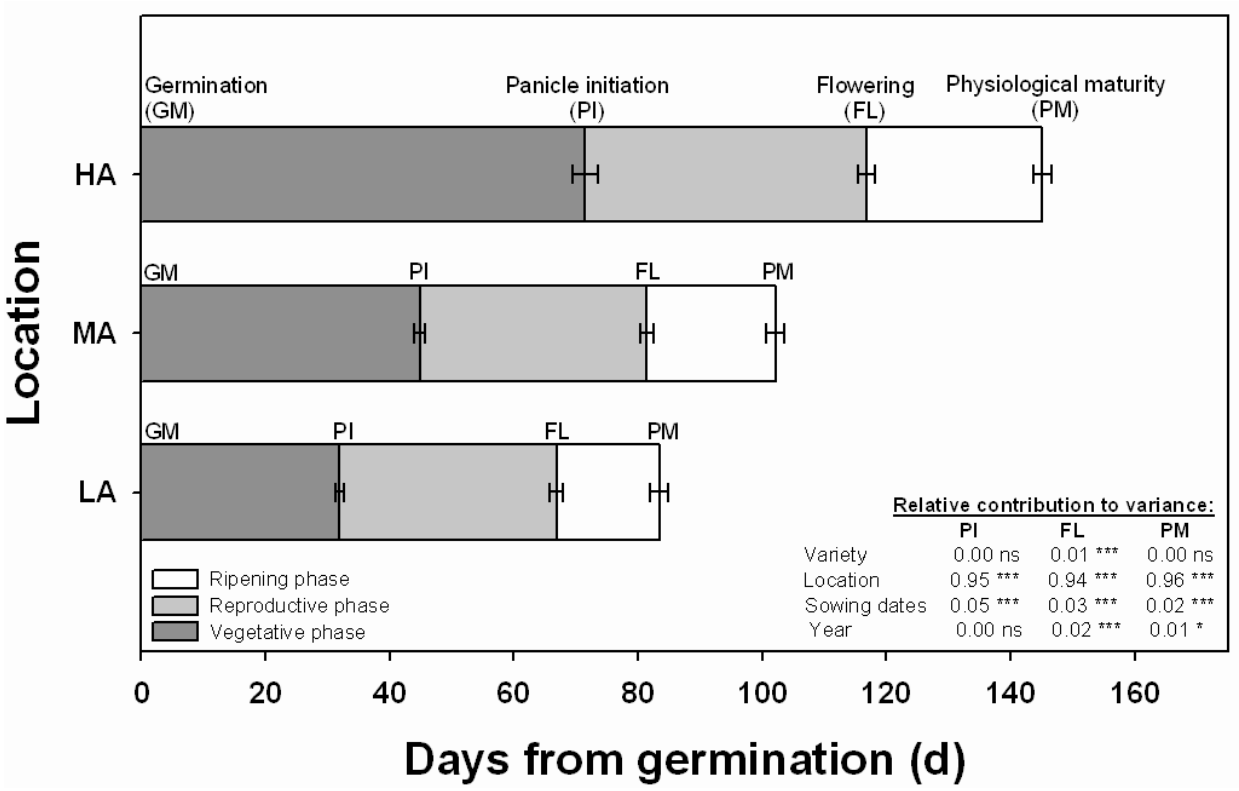

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569 Figure 2.

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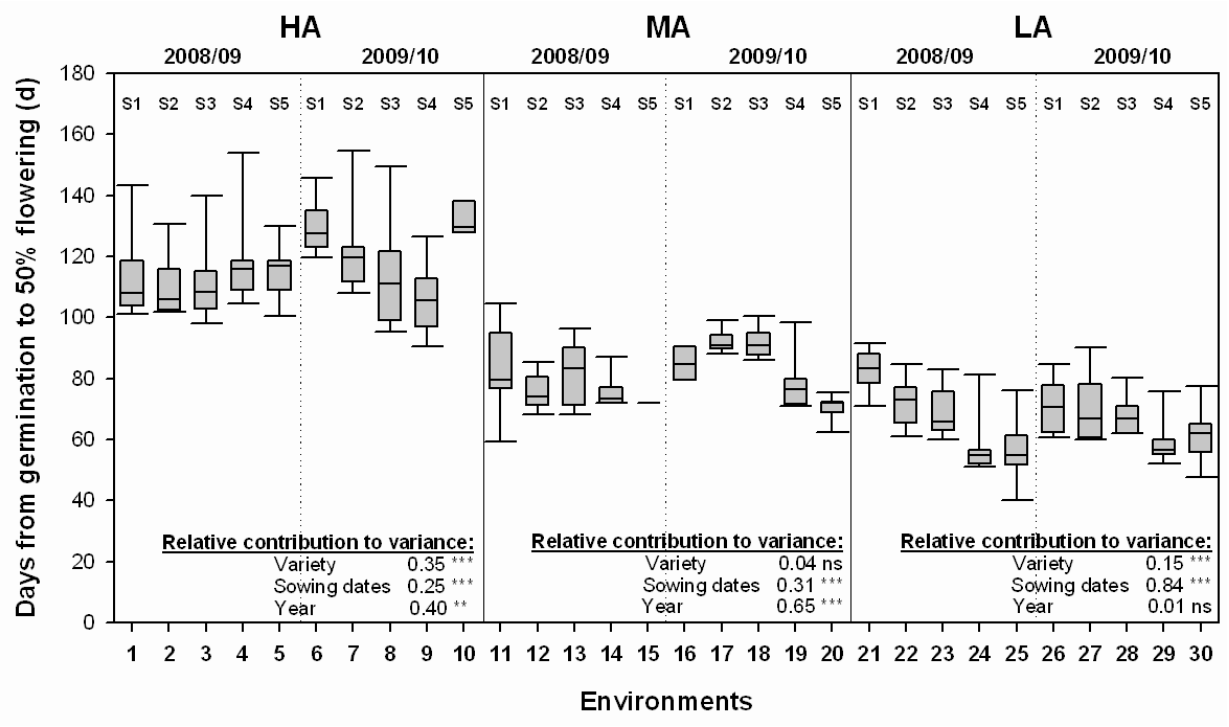

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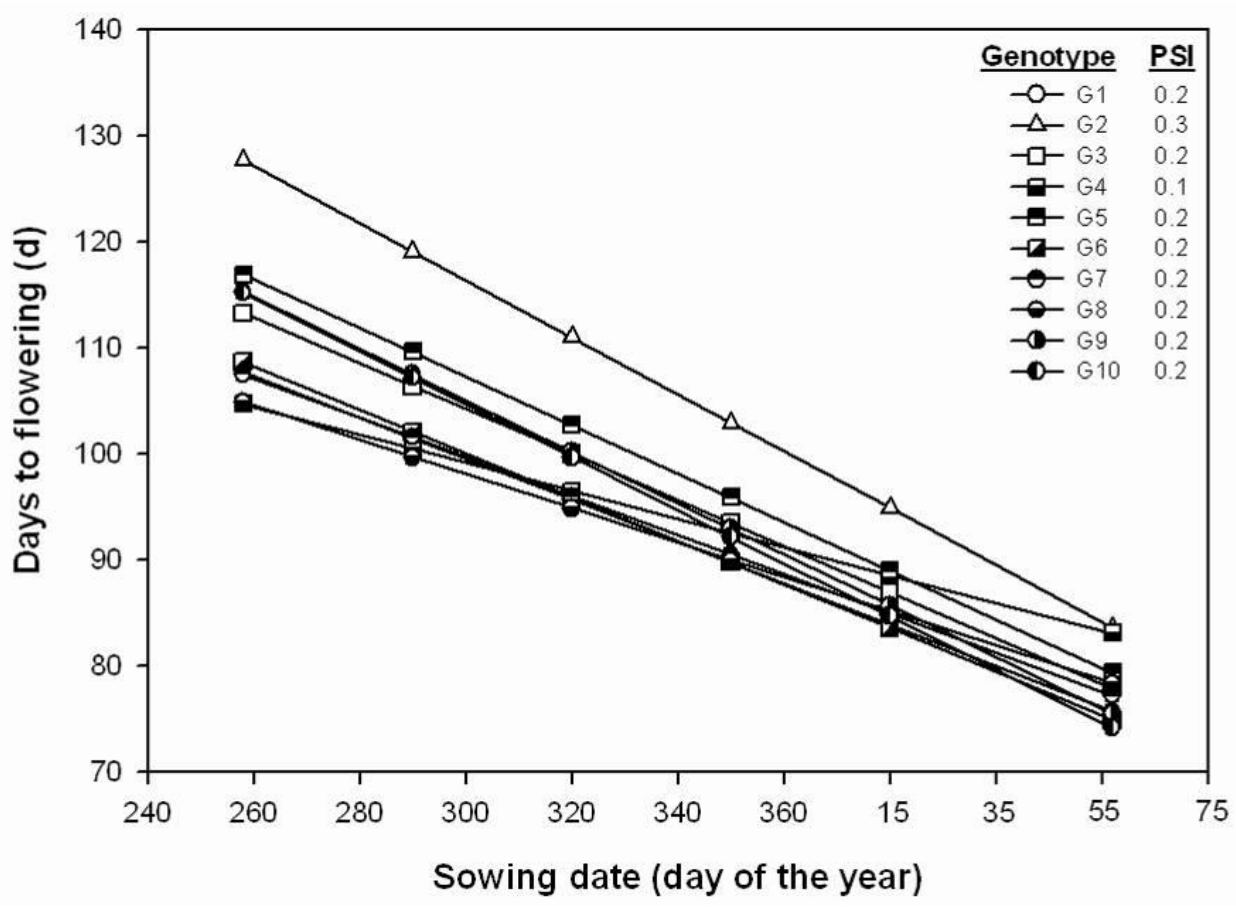

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575 Figure 4.

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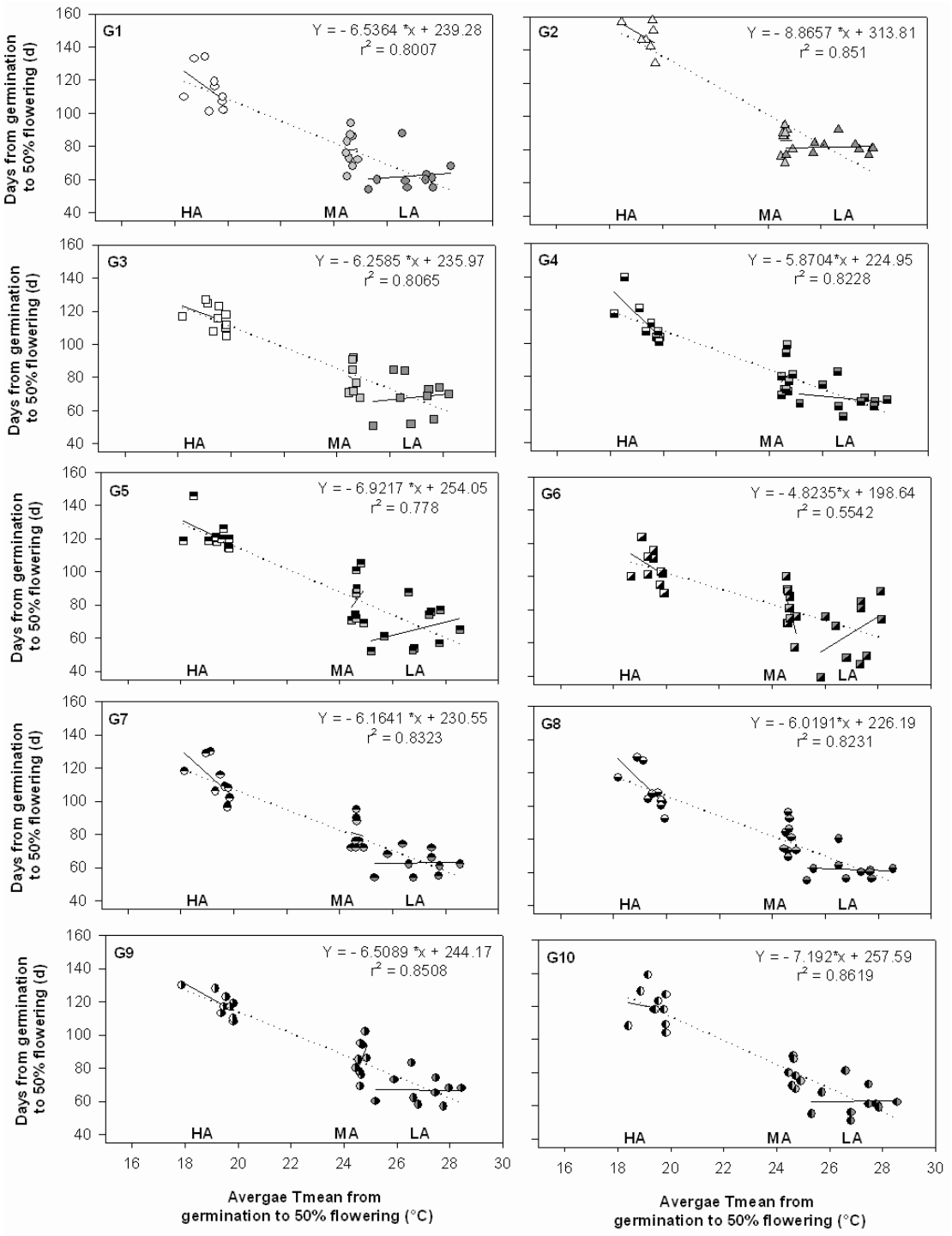

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578 Figure 5.

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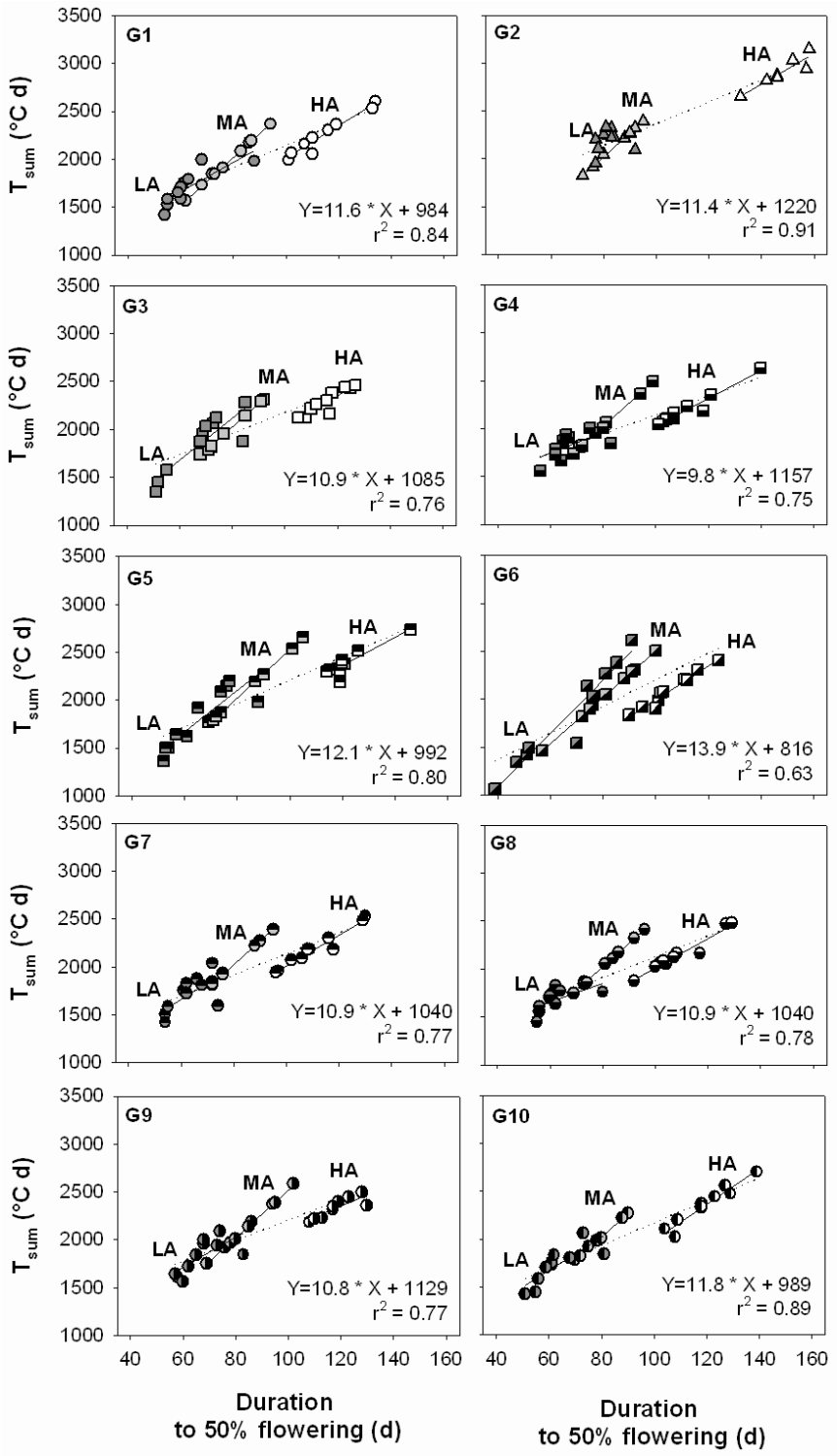

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581 Figure 6.

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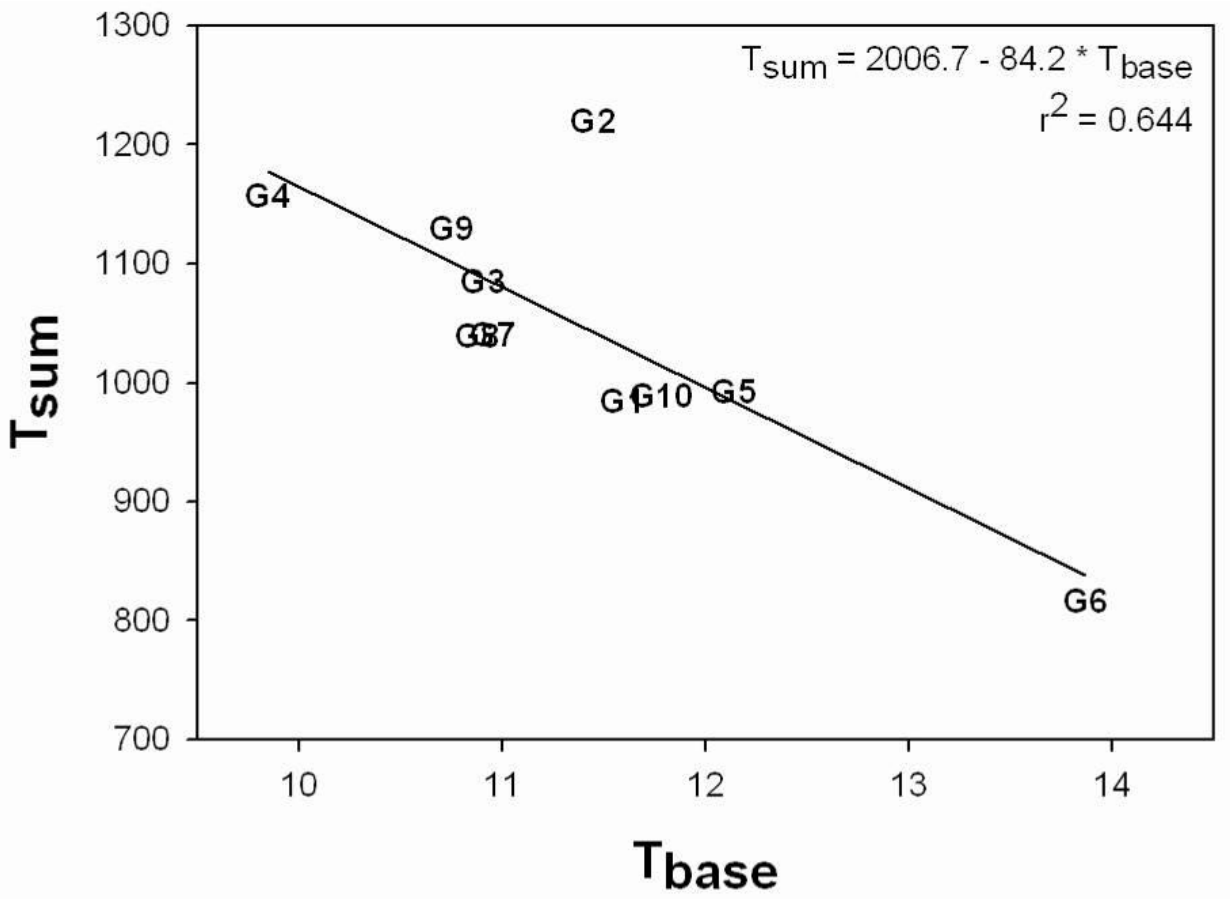

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584 Figure 7.

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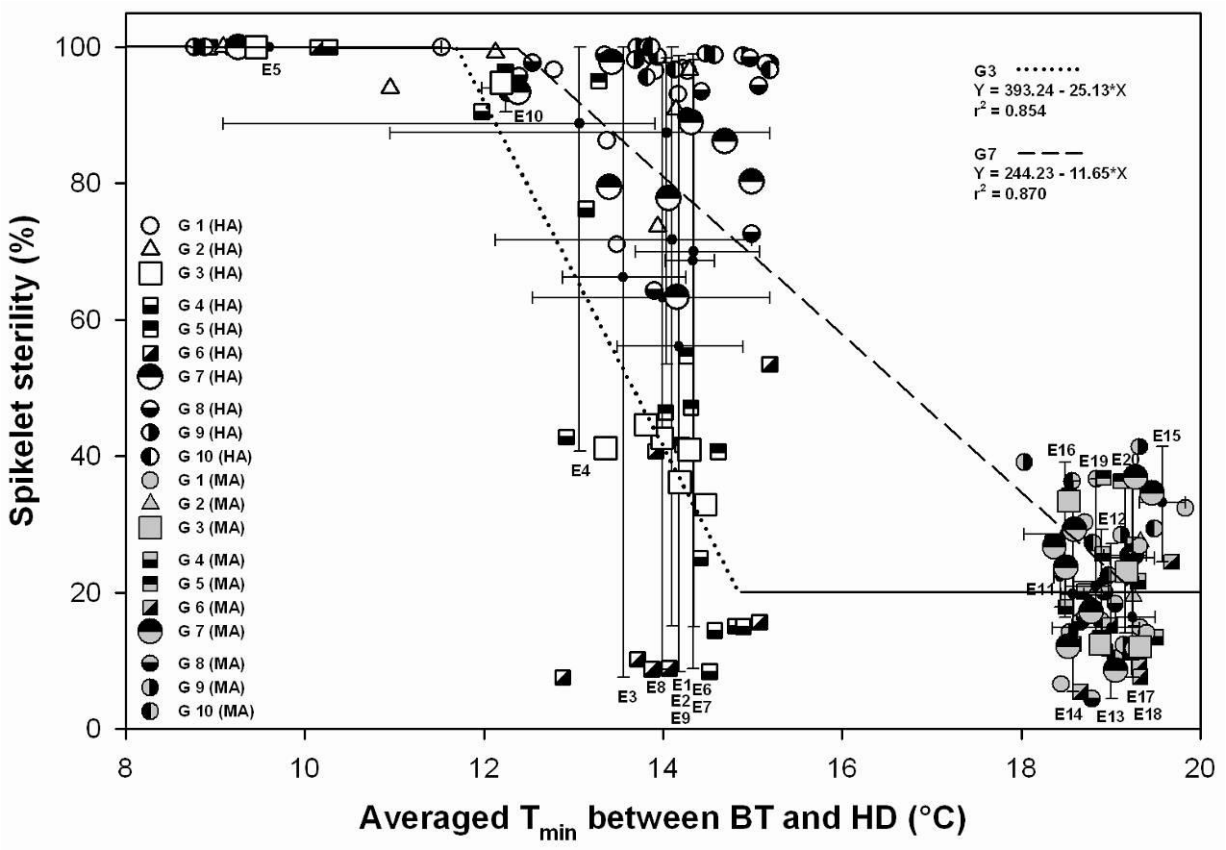

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587 Figure 8.

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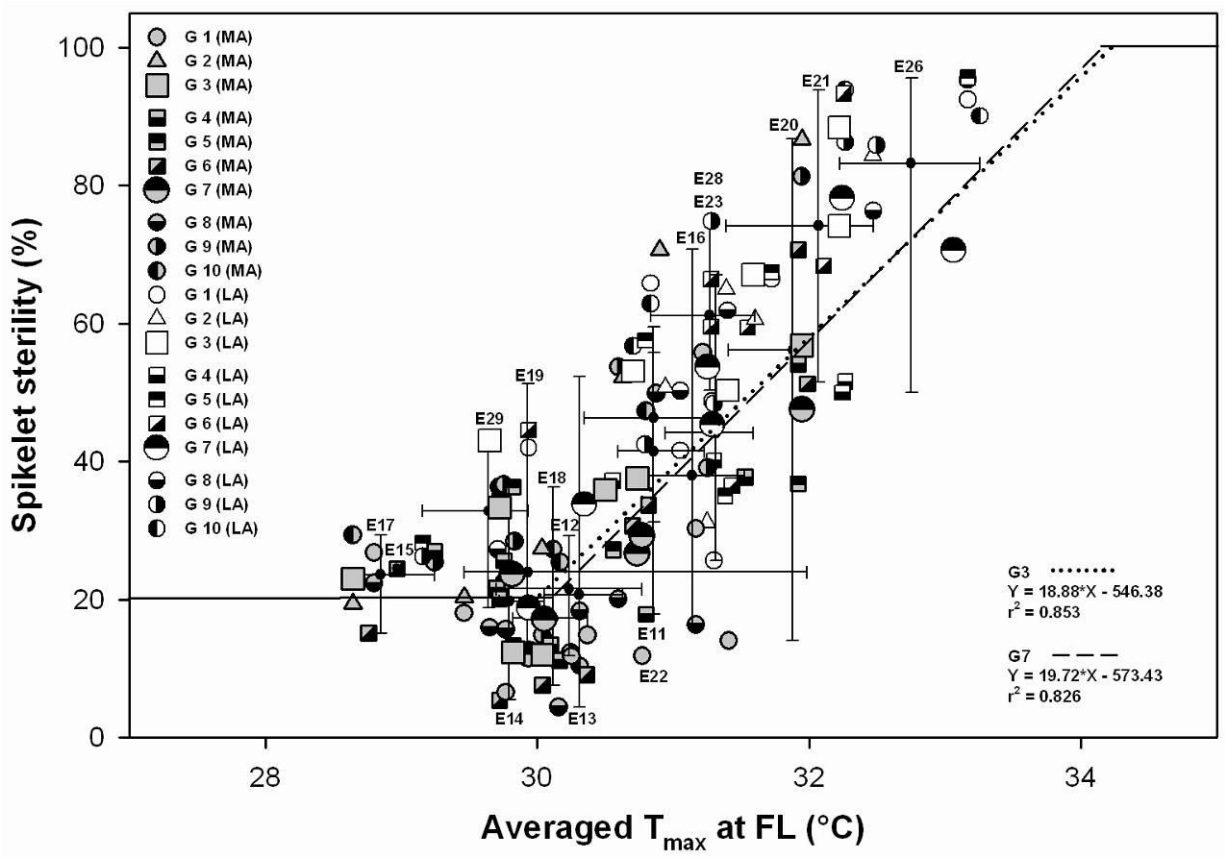

589

590 Figure 9.

591

Captions for Tables:

Table 1. Categorization of the environments (E1 to E30) based on location, sowing dates and year.

Table 2. Characteristics of the upland rice (*Oryza sativa L.)* genotypes (G1 to G10) selected for the study. Abbreviations: trop, tropical; temp, temperate; isc, interspecific crosses; imp, improved; trad, traditional.

Table 3. Source of variance and its relative contribution to variance on grain yield (GnYd) and percentage of spikelet sterility (SPP) in three locations and pooled over location. ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. Abbreviation:HA, high altitude; MA, mid altitude and LA, low altitude.

605 Table 1.

Location	High altitude (HA)		Mid altitude (MA)		Low altitude (LA)	
Year	Sowing date	Environment	Sowing date	Environment	Sowing date	Environment
2008/09 (1)	18-Sep-08	E1	17-Sep-08	E11	22-Oct-08	E21
	15-Oct-08	E2	17-Oct-08	E12	21-Nov-08	E22
	21-Nov-08	E3	20-Nov-08	E13	10-Dec-08	E23
	19-Dec-08	E4	19-Dec-08	E14	26-Jan-08	E24
	19-Jan-09	E5	19-Jan-09	E15	26-Feb-08	E25
2009/10 (2)	16-Sep-09	E6	15-Sep-08	E16	20-Oct-08	E26
	19-Oct-09	E7	15-Oct-09	E17	20-Nov-08	E27
	16-Nov-09	E8	16-Nov-09	E18	20-Dec-08	E28
	15-Dec-09	E9	16-Dec-09	E19	21-Jan-10	E29
	15-Jan-10	E10	16-Jan-10	E20	22-Feb-10	E30

606

607

608 Table 2.

Genotype	Cultivar name	Sub-species	Type	Cross (Parents)	Growing altitude	Country of origin
G1	B22	trop japonia	imp	CNA 095-BM30-BM27_P35-2	mid-low	Brazil
G2	Botramaintso	trop japonica	trad	Local upland variety	mid	Madagascar
G3	Chhomrong	temp japonica	trad	Local lowland/upland variety	high	Nepal
G4	FOFIFA 161	trop japonica	imp	IRAT 114 / FOFIFA 133	high	Madagascar
G5	FOFIFA 167	trop japonica	imp	CA 148 /SHINEI	high	Madagascar
G6	FOFIFA 172	trop japonica	imp	IRAT 265 57-2 / Jumli Marshi	high	Madagascar
G7	IRAT 112	trop japonica	imp	IRAT 13 / Dourado Precoce	mid	Ivory Coast
G8	NERICA 4	isc	imp	WAB 56-104 / CG 14//2*WAB 56-104	mid	Benin
G9	Primavera	trop japonica	imp	IRAT 10 / LS85-158	mid-low	Brazil
G10	WAB 878	isc	imp	CG14/IRAT 144	mid	Ivory Coast

609

610 Table 3.

Source of variance	HA		MA		LA		Pooled	
GnYd (t ha^{-1})								
Location							0.71	***
Genotype	0.49	***	0.04	ns	0.08	ns	0.05	***
Sowing date	0.49	***	0.82	***	0.74	***	0.23	***
Year	0.02	ns	0.14	*	0.17	ns	0.01	ns
SSP (%)								
Location							0.88	***
Genotype	0.50	***	0.16	**	0.18	ns	0.05	***
Sowing date	0.49	***	0.82	***	0.74	***	0.07	***
Year	0.01	ns	0.02	ns	0.08	ns	0.00	ns

611

Appendix II

Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes

Suchit Shrestha, Folkard Asch, Julie Dusserre, Alain Ramanantsoanirina and Holger Brueck

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Title: Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes

Article Type: Regular Article

Keywords: cold stress; crop duration; high altitude cropping systems; ideotype development; phenology; principal component analysis; sterility; yield stability

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Abstract: Grain yield in any given environment is determined by yield components developed at different phenophases. Yield components are influenced by the environmental conditions the plant experiences during the respective phases. The final yield of a given cultivar depends on the interaction between genotype and its responses to environmental conditions. Hence, it is necessary to evaluate the plasticity in yield components formation while selecting genotype for a given environment. For this, we conducted field trials comprising ten upland rice genotypes representing a large share of genetic variation, with two sowing dates in two consecutive years in three altitudinal locations creating twelve environments in Madagascar. Crop duration, grain yield and yield components (tillers per hill, panicles per tiller, grains per panicle, sterility, grain weight) were strongly affected by sowing dates, location, year and genotypes. Sowing date and years resulted in comparatively more variable environments in high and low altitude than in mid altitude. Yield stability across environments reflected the target environments the genotypes were originally selected for. Variation in grain yield among planting dates within altitudes was not mainly due to temperature but rather to the combinations of abiotic factors the genotypes experienced during the different phenological stages during which the different yield components were formed. Yield components and their contribution to environmentally induced yield penalties were analyzed in detail. The contribution of individual yield components to final yield changed with the environmental conditions the rice experienced during the development stages. This effect may have a stronger influence on final yield than the genetic control of the individual yield components. New combinations of traits are required to better exploit the environmental potential which may only be possible via advanced crop models simulating the environmental effects on yield components and their interdependencies to develop ideotypes for the target environments thus guiding breeding and selection efforts.

Research Highlights:

- Grain yield of a cultivar is determined by yield components developed at different phenophases and the environmental conditions during the respective phenophases influences yield components.
- The final yield of a given cultivar depends on the interaction between genotype and its responses to environmental conditions.
- New combinations of traits are required to develop ideotypes for the target environments.
- Advanced crop models simulating the environmental effects on yield components is only possible to better exploit the environmental potential.

Title of the paper

Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes

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Abstract

Grain yield in any given environment is determined by yield components developed at different phenophases. Yield components are influenced by the environmental conditions the plant experiences during the respective phases. The final yield of a given cultivar depends on the interaction between genotype and its responses to environmental conditions. Hence, it is necessary to evaluate the plasticity in yield components formation while selecting genotype for a given environment. For this, we conducted field trials comprising ten upland rice genotypes representing a large share of genetic variation, with two sowing dates in two consecutive years in three altitudinal locations creating twelve environments in Madagascar. Crop duration, grain yield and yield components (tillers per hill, panicles per tiller, grains per panicle, sterility, grain weight) were strongly affected by sowing dates, location, year and genotypes. Sowing date and years resulted in comparatively more variable environments in high and low altitude than in mid altitude. Yield stability across environments reflected the target environments the genotypes were originally selected for. Variation in grain yield among planting dates within altitudes was not mainly due to temperature but rather to the combinations of abiotic factors the genotypes experienced during the different phenological stages during which the different yield components were formed. Yield components and their contribution to environmentally induced yield penalties were analyzed in detail. The contribution of individual yield components to final yield changed with the environmental conditions the rice experienced during the development stages. This effect may have a stronger influence on final yield than the genetic control of the individual yield components. New combinations of traits are required to better exploit the environmental potential which may only be possible via advanced crop models simulating the environmental effects on yield components and their interdependencies to develop ideotypes for the target environments thus guiding breeding and selection efforts.

Key words: cold stress; crop duration; high altitude cropping systems; ideotype development; phenology; principal component analysis; sterility; yield stability

Introduction

Upland rice is cultivated in about 14 million ha (9% of the total rice cultivated area worldwide) of which 64% in Asia, 22% in Latin America and 13% in Africa accounting for 4% of the world's rice production (Rice Almanac, 2002). With the projected increase in mean temperatures of 0.4 – 0.6 °C per decade due to global warming, accompanied with a projected positive shift in annual mean precipitation for many of the high altitude cropping systems (IPCC, 2007), high altitude systems (above 1600 m asl) currently constrained by temperature stresses and short vegetation periods (Shrestha et al, 2011), may become suitable for rice production within the next decade. This could improve the productivity of high altitude cropping systems and help satisfying the increasing demand for rice. To date, few of the existing rice varieties may be suitable for the newly emerging, rainfed environments in high altitudes. In order to fully exploit the systems potential, crop adaptation strategies will be required in terms of varietal development and crop management. Rice is a thermophilic crop but sensitive to temperature extremes during specific developmental stages (Dingkuhn et al., 1995). Temperature is the main driving force for development in photoperiod insensitive genotypes and heat unit accumulation and thus crop duration depend on the genotypic cardinal temperatures such as temperature sum, and base and optimum temperatures. Yield in any given environment is the result of yield components developed in different development phases and growth stages. Yield potential is determined by the number of tillers formed during the vegetative growth phase, the number of panicles induced at the end of the vegetative stage, the number of spikelets formed in each panicle during panicle development, the number of fertile spikelets determined during the booting and flowering stage, and the final individual grain weight determined during the grain filling phase (Dingkuhn and Kropff, 1996). All yield components are strongly influenced by the climatic conditions the plant experiences during the respective phases the components are developed in. The final yield of a given cultivar depends on the interactions between the genotype, its responses to environmental conditions, and management practices (Messina et al., 2009). Under the same management, the interaction between the genotype and environmental characteristics is the sole determinant of varietal performance (Dingkuhn et al., 2006). To develop newly emerging rice cropping systems in high-altitude environments, it is important to select or breed cultivars that are adapted to the specific climatic conditions and that are able to realize as much of their potential as possible

under the given environmental constraints. Therefore, it is not only important to select genotypes that respond positively to favorable environmental conditions, but it is also necessary to be able to evaluate the plasticity in the yield components formation and their respective contribution to the final yield in responses to environmental conditions.

For this study, we took advantage of the large diversity of rice growing environments in different altitudes in Madagascar to investigate responses of yield components to changes in environmental conditions in a set of rice genotypes representing a large share of the global genetic variation of upland rice varieties. To date, there is little information on the responses of individual yield components to the climatic environment during which they are formed and the effect on the final yield in upland rice and if this could be exploited for breeding purposes. Pb Samonte et al. (1998) used path coefficient analyses to understand direct and/or indirect effects of yield components on grain yield and Nassir Adesola and Ariyo Omolayo (2006) showed that the environment has a strong influence on yield components in upland rice. However, neither study was performed along gradients in altitude. We employed a variety of statistical methods, such as genotype by environment interaction using genotype main effect plus genotype by environment interaction (GGE) biplot analysis or Additive Main effect and Multiplicative Interaction (AMMI) analysis (Gauch Jr, 2006; Yan et al., 2007; Acuña et al., 2008; Gauch Jr et al., 2008; Sanni et al., 2009) that are widely used to test the effects of genotype or environment, respectively. The objective of the study was to characterize genotypic specific traits (yield components) that significantly contribute to stabilize grain yield across different environments and that are particularly suited for selection or breeding of cultivars adapted to high-altitude cropping systems with variable climatic environment.

Materials and methods

Locations and environmental design

Three locations differing in altitude along a temperature gradient in Madagascar (Andranomanelatra, 1625 m asl; Ivory, 965 m asl and Ankepaka, 25 m asl) were selected for field trials with two sowing dates (early and late season sowing, one month apart) of ten upland rice genotypes in two consecutive years (2008/09 and 2009/10), thus creating twelve different rice growing environments. The experiment was designed as split plot with sowing date as main plot and genotypes as sub-plot arranged in a randomized complete block design with three

115 replication. Experimental fields were located in the high altitude (HA) at 19°46'45.3" S and
116 47°06'24.5" E, mid altitude (MA) at 19°33'16.8" S and 46°25'29.1" E and low altitude (LA) at
117 22°11'31.6" S and 47°52'32.7" E. HA and LA were on the east aspect facing towards Indian
118 Ocean whereas MA was on the west aspect facing towards the Mozambique Channel. Climatic
119 data were recorded from an automatic meteorology station, ENERCO 404 Series, (CIMEL
120 Electronique, Paris, France) in the HA and MA locations, and HOBO U30 Series, (Onset
121 HOBO Data Loggers, Pocasset, Massachusetts, USA) in LA location which were set up close to
122 the experimental plots. During the cropping season, average minimum air temperature (T_{min})
123 and maximum air temperature (T_{max}) were 13°C and 19°C, respectively, with 1300 mm of total
124 rainfall in HA. In MA, average T_{min} was 19°C and average T_{max} was 24°C with 1200 mm of
125 total rainfall during cropping season. LA had the highest total rainfall (2100 mm) with average
126 T_{min} 19°C and T_{max} 27°C during cropping season. HA and MA had similar photoperiod whereas
127 LA had 10 minutes more photoperiod during January and 10 minutes less during July compared
128 to HA and MA. Average solar radiation was higher in MA (Fig. 1). The recommended sowing
129 date in HA is between mid October and mid November, early sowing was done on 21/11/2008
130 and the late sowing on 19/12/1008 in the first year, and in the second year, early sowing was
131 done on 19/10/2009 and late sowing on 16/11/2009 (Fig. 1). Similarly, the recommended
132 sowing date is between mid November and mid December in MA, the early sowing was done
133 on 20/11/2008 and late sowing on 19/12/2008 in the first year, and in the second year, early
134 sowing was done on 16/11/2009 and late sowing on 15/12/2009. In the LA, the recommended
135 sowing date is between mid December and mid January, and early sowing was done on
136 10/12/2008 and late sowing 26/01/2009 in the first year, and in the second year, early sowing
137 was done on 21/12/2009 and late sowing was done on 21/01/2010. HA had clay soil of pH 4.5,
138 MA had clay loam soil of pH 4.5 and LA had silt loam soil of pH 4.0 which were dominant in
139 upland rice ecosystem in Madagascar. Each plot size was 18.24 m² (4.8 m X 3.8 m) in HA and
140 11.52 m² (3.2 m X 3.6 m) in MA and LA. Hill to hill spacing was 20 cm x 20 cm in all
141 locations.
142
143 *Genotypes and crop management*
144 Ten contrasting genotypes including seven tropical japonica, one temperate japonica and two
145 interspecific crosses (Table 1) were selected for this study. Botramaintso and Chhomrong are

traditional landraces adapted to the middle and higher altitudes of Madagascar and Nepal. Botramaintso was selected due to its growth vigour. Chhomrong is a high tillering, cold tolerant genotype rapidly diffused after it's released in 2006 in the HA of Madagascar. B22 and Primavera are improved varieties from Brazil grown at MA and LA. Nerica 4 (WAB 450-I-B-P-91-HB), WAB 878 (WAB 878-6-12-1-1-P1-HB), and IRAT 112 are selected genotypes for MA in Madagascar. Nerica 4 was selected for its morphological characteristics: stay-green syndrome, erect leaves, and low plant height. WAB 878-6-12-1-1-P1-HB was selected for its growth. FOFIFA 161, FOFIA 167 and FOFIFA 172 are improved varieties, adapted to HA of Madagascar and cold tolerant. In accordance with local practice, seven to eight seeds per hill were direct seeded and thinned to five plants per hill at seedling stage. In the MA and LA additional mulching was done with *Stylosanthes* to avoid soil moisture losses due to evaporation. Fertilizer (11:22:16 N-P-K) was applied as basal dose at a rate of 300 kg ha^{-1}, dolomite 500 kg ha^{-1} and FYM 5 t ha^{-1} at the time of sowing and top dressing was done with urea (46 % N) at 35 kg ha^{-1} at first weeding and 30 kg ha^{-1} at second weeding. Manual weeding was done as required. Systematic fungicide (Carbenstor-500 SC) was applied at the rate of 1 L ha^{-1} to control leaf blast (*Pyriculariase*) when symptoms appeared.

Measurements, observations, and data analysis

Genotype specific duration of phenological stages was recorded from each plot in all three locations at each planting date. Yield components were measured from 8 hills (2 hills from 4 corners of the plot) excluding 2 border lines. Bulk grain yield was obtained from the central area of 3.8 m^2 in MA and LA and 5.7 m^2 in HA. Three locations, two sowing dates, and two years were considered as twelve environments as source of variation. GenStat 13^{th} Edition (VSN International Ltd, UK) and SAS – Version 9.2 (SAS Institute Inc., Cary, NC, USA) were used for statistical analyses. Analysis of variance (ANOVA) was performed to test split plot (sowing date as main plot and genotypes as sub-plot) arranged in a randomized block design combined over location and year where locations and years are considered as fixed effect as explained in McIntosh (1983). Heterogeneity of genotype regressions on environment means accounting G x E interaction (Finlay and Wilkinson, 1963) was used for yield stability analysis. The main effect of genotype and environment and their interaction was tested using the Additive Main Effects and Multiplicative Interaction (AMMI) model (Yan et al., 2007; Gauch

Jr et al., 2008) which resulted in AMMI-1 and AMMI-2 biplots. AMMI-1 biplot consist of genotype and environment means on abscissa, and Interaction Principal Component Axes (IPCA) -1 on ordinate for genotype and environment scores. AMMI-2 biplot consist IPCA-1 on abscissa and IPCA-2 on ordinate. The effects of environmental changes on grain yield and yield components for each genotype were calculated as percentage deviation from genotype mean. Positive values represent losses and negative values gains in yield and yield components as compared to the genotype mean.

Principal component analysis (PCA) is a tool to interpret interactions including high variance of data (de Haan et al., 2007) commonly presented graphically as biplots (Gabriel, 1971). Environments plotted as scores and yield components as latent vectors in the biplot allow visual interpretation of interactions of yield components across different environments. PCA based on a variance-covariance matrix was performed for the percentage deviation of yield components from genotype means as latent vector loadings (size of contribution) and respective environments as principal component scores (projection) for two major variation axes -1 and -2. A similar PCA was performed for average rainfall (RF), average minimum temperature (T_{min}) and average maximum temperature (T_{max}), daily mean air temperature $T_{mean-24h}$), solar radiation (SR), vapour pressure deficit (VPD), and potential evapotranspiration (ET_o) as latent vector loading and environments as principal component scores at the vegetative phase, reproductive phase, flowering stage, and ripening phase for the ten genotypes. Genotypic variance was calculated as the ratio of genotype mean sum of square (ms) to total ms (sum of genotype ms, environment ms, genotype and environment interaction ms and error ms). Genotypic variance was calculated to estimate broad sense heritability (Nyquist and Baker, 1991). Similarly, environment variance was calculated as the ratio of environment ms to total ms to estimate environmental influence on the phenotype.

Results

Sowing date and location effects on grain yield and crop duration

Sowing date and location strongly influenced crop duration and grain yield of the selected genotypes (Table 2). In general duration to flowering increased by factor 1.8 from LA to HA across planting dates and genotypes from 68 to 125 days. However, the variation in duration to flowering among genotypes within the different environments was similar, although with an

increasing mean variation. Duration to flowering varied among planting dates for any specific genotype in LA between 5 and 31 with an average of 12 days, in MA between 7 and 26 with an average of 16 days, and in HA between 16 and 34 with an average of 24 days. Duration to flowering was longer than 100 days for all genotypes and planting dates in HA never shorter than 75 days in MA and required a minimum of about 60 days in LA, reflecting the thermal requirements of the genotypes. Among all selected genotypes, Botramaintso had the longest duration to flowering.

Yield stability and G X E

Grain yield, yield components, harvest index, and duration to 50% flowering were significantly affected by year, location, sowing date, and genotypes (Table 3) and interactions between these three treatment factors were obvious. Pooled over genotypes and sowing dates, grain yield was about 1.7 times higher in the MA than in the HA and LA locations (Table 2). In the HA location, Chhomrong and FOFIFA 172 had more than 2 t ha^{-1} of grain yield even when lately sown and attained more than 5 t ha^{-1} when sown early. Contrary, Botramaintso and Primavera had low grain yield in the HA location for both early and late sowing. In the MA location, average grain yield of genotypes varied from 4.3 to 4.9 t ha^{-1} and differences between sowing dates and varieties were small. However, Botramaintso attained more than 4 t ha^{-1} when sown early and less than 2.5 t ha^{-1} when sown late. FOFIFA 161 and Nerica 4 performed better when sown late. Chhomrong and IRAT 112 consistently yielded about 4.1 and 5.2 t ha^{-1} respectively, in MA irrespective of sowing date and year. Grain yields of Chhomrong and FOFIFA 172 were lower in LA than in HA location while the opposite was observed for Botramaintso and Primavera with the latter realizing the highest grain yield at LA. Based on the linear regression between varietal and environmental mean yields (Fig. 2a), regression coefficients of each variety were plotted against varietal mean grain yield to visualize yield stability (Fig. 2b). B22 and IRAT 112 had the highest regression coefficients due to the highest yields in high yielding environments but comparably low yields in low yielding environments (Fig. 2a) and accordingly were classified as responsive to environmental conditions with an average yield stability (Fig. 2b). Chhomrong and FOFIFA 172 were the highest yielding varieties in low yielding environments and had low to medium grain yields in the most productive environments resulting in the lowest regression coefficients. All cold tolerant genotypes, namely Chhomrong,

FOFIFA 161, FOFIFA 167, and FOFIFA 172, cluster in the high yielding group in both low and high yielding environments as they have low regression coefficients. These genotypes had above average yield stability and were well adapted to all environments without significant yield penalty. WAB 878 and Primavera had average yield stability but were less responsive to more productive environments. The local landrace Botramaintso had low yields across all environments and consequently a regression coefficient close to one and below average yield stability. Nerica 4 had a regression coefficient similar to Botramaintso, indicating below average yield stability (Fig. 2b) but yielded consistently higher than Botramaintso in productive environments (Table 2). The ANOVA table for the AMMI model (Table 4) shows that the interactions between genotypes and environments are highly significant and thus with IPCA-1 and IPCA-2. The AMMI-1 biplot (Fig. 3a) indicates similar environmental adaptation for Chhomrong (G3), FOFIFA 167 (G5), and FOFIFA 172 (G6), and for B22 (G1), IRAT 112 (G7), Primavera (G9), and WAB 878 (G10), whereas Botramaintso (G2), Nerica 4 (G8), FOFIFA 161 (G4) seem to be less clearly adapted to certain environments. The environments in MA (E5-E8, see also Table 2) cluster closely to each other whereas the environments in HA (E1-E4) and LA (E9-E12) are widely scattered within clusters in the lower and upper part of the biplot, respectively, indicating that sowing date and years resulted in comparatively more variable environments in HA and LA than in MA. The AMMI-2 biplot (Fig. 3b) revealed significant differences in sensitivity to and variability in environmental interactions among the genotypes. Chhomrong (G3), FOFIFA 167 (G5), and FOFIFA 172 (G6) clustered far from the origin and closely associated with their HA high yielding environments (E1, E2, and E3) indicating their adaptation to high altitude environments and their sensitivity to unfavourable environments. However, FOFIFA 167 (G5) and FOFIFA 172 (G6) also showed a good yield performance in E5 and E7 (Table 2), not reflected in the AMMI-2 biplot. Botramaintso (G2) was singled out in the upper right corner of the biplot clustering together with E5, E6 and E10, which is in line with its duration requirements (long duration) favoured by early sowing. Primavera (G9) and WAB 878 (G10) are located closer to the origin, indicating a broader adaptation to environmental variation and clustered in between their favourable MA environments E5, E6 and E7, E8. In their environmental responses they are similar to B22 (G1) and IRAT 112 (G7) which in contrast showed a good yield performance across a slightly larger environmental range as they perform well also in E9 and E10 (Table 2), respectively. Opposite

of Botramaintso (G2) in the lower right, Nerica 4 (G8) is located relatively far away from the origin, indicating a strong sensitivity to environment also reflected in being clustered together with its most favourable environments E7, E8 and E9. FOFIFA 161 (G4), which clustered together with the other cold tolerant varieties (G3, G5 and G6) in the yield stability analysis (Fig. 2b) is singled out in the AMMI-2 biplot in the lower left, clearly distinguished from G3, G5 and G6. Despite its great distance from the origin, FOFIFA 161 (G4) shares favourable environments (E7-E9) with a larger number of varieties (G8, G7, G1) but also performed well in E4 indicating responses to specific environmental conditions affecting the yield building process. According to Fig. 3b, the most contrasting environments were E2 (early sowing, year 2, HA), E5 (early sowing, year 1, MA), and E9 (early sowing, year 1, LA) being located far from the origin and in opposite corners of the plot. Consequently, genotypes most closely associated with these environments reflect earlier selection processes aiming at specific environmental adaptation. Similarly, the environments E3 (late sowing, year 1, HA) and E11 (late sowing, year 1, LA) were not closely related to any of the varieties, indicating general environmental problems affecting yield that were not related to specific environmental adaptation and consequently resulted in low yield performance for most genotypes.

Environmental effects on genotypic yield and yield components

Yield performance of individual genotypes in a given environment reflects the cumulative environmental effects on the different processes involved in building the final yield. Thus, the changes in yield as compared to the mean genotypic yield across environments (environmental yield gains or penalty) will be a result of the environmental effects on yield components developed during specific phenological phases in this environment. The percentage change in yield and yield components was calculated from genotype mean across different environments for each genotype (Table 5) with negative numbers indicating gains and positive numbers indicating losses. The longer duration in HA (Table 2) led to a higher number of tillers per hill (TPH) in almost all genotypes for all years and sowing dates (Table 5). For all but the cold tolerant genotypes (Chhomrong, FOFIFA 161, FOFIFA 167, and FOFIFA 172) this gain in yield potential was off-set by an strong decrease in the percentage of filled grains (PFS) leading to yield penalties of up to 100% particularly in E3 (late sowing, year 1, HA). Compared to HA, all genotypes in MA showed yield gains, on average in the range of 12 – 30 % for the cold

tolerant varieties and between 40 and 95% in the cold sensitive varieties. The main effect for these yield gains was observed for the late reproductive phase, particularly in filled spikelets (PFS) and grain weight (TGW). Due to the generally shorter duration in MA yield potential was reduced by reduced TPH. Large variation among the genotypes was observed for sink size formation, as the environmental effects on panicles per tiller (PPT) and spikelets per panicle (SPP) varied widely among sowing dates and genotypes in MA. In LA generally genotypes responded to environmental conditions with a penalty in yield ranging on average between 32-42% in the cold tolerant varieties and between 3-10% for the others. Primavera was the only genotype responding relatively favourable to the LA environment with yield gains of about 22% on average. No clear pattern emerged from the analysis of the environmental responses of the yield components in relation to yield responses in LA. The environmental effects on yield components and their contribution to final yield varied widely among sowing dates within specific genotypes as well as within sowing dates across genotypes.

Genotype and environment variance was computed for different phenotypic traits to estimate genotypic and environmental influence (Table 6). Days to 50% flowering and grain yield were mainly influenced by environment. Yield components such as TPH and PFS were highly influenced by environment whereas SPP and TGW were more genotypic and less influenced by environment. PPT was equally influenced by both genotype and environment.

The principal component analysis of yield components and environments revealed the genotypic relationships between the environmental influences on the yield components during the phenophases they were established and the importance of the effects on the yield component for the final yield in the respective environment (Fig. 4). The principal component axis PCA-1 and PCA-2 explained more than 90% of the variation observed among the genotypes, with the exception of Chhomrong and the three FOFIFA varieties where the PCA-1 and PCA-2 accounted only for 79 – 90% of the variation. In the figure, the closer the projection of environment scores of the genotype to its yield components (latent vector), the higher the percentage reduction of the yield component of that genotype in that environment. In other words, the farther the environment scores of the genotype deviate from its yield components; the lower is the percentage reduction of the yield component. The ten genotypes included in this study responded differently and strongly to the different environments. In B22, Botramaintso, IRAT 112, Nerica 4, Primavera and WAB 878 the HA environment induced severe spikelet

sterility that strongly reduced the potential yield. In Chhomrong, FOFIFA 161, FOFIFA 167, and FOFIFA 172 the HA environment induced reductions in TGW often associated with reductions in SPP indicating an environmental influence on sink size and problems during the grain filling phase. In the MA environments, final yield was not influenced by a specific environmental influence on specific yield components in B22, Botramaintso, FOFIFA 172, IRAT 112, Primavera, and WAB 878. The same environment influenced the sink size in Chhomrong, FOFIFA 161, and FOFIFA 167 through reductions in the total number of panicles (PPT) indicating rather an environmental influence during the tiller formation phase, whereas in Nerica 4 MA environments strongly reduced the sink size through reductions in number of spikelets per panicle (SPP) indicating adverse environmental influence during the booting phase and panicle development. The LA environments strongly shortened the duration to flowering in all genotypes (Table 2) which is strongly reflected in the influence of the LA environments on number of tillers per hill (TPH) in all genotypes (Fig. 4). In Chhomrong, LA environments additionally reduced SPP indicating problems in balancing sink-source dimensions, whereas in the three FOFIFA genotypes LA environments had strong effects on filled spikelet (PFS) which either reflects heat sterility or additional biotic stresses during panicle formation such as mold.

Weather parameters exerting major influence in specific environments

As shown in Figures 3 and 4 environments strongly influence genotypic yield via the individual yield components formed during specific development stages of the genotype. These influences are directly related to the weather experienced by each genotype during its phenophases (vegetative, reproductive, and ripening phases). The PCA of mean weather conditions each genotype experienced during its phenophases explained between 85 and 90% of the genotypic variation for the respective phenophases by PCA-1 and PCA-2 (Fig. 5).

Fig. 5 shows that all HA environments were equally influenced by all weather parameters with minimum air temperature (T_{min}) having the strongest positive influence on genotypic performance which is reflected in the duration to flowering (Table 2), tiller number per hill and spikelet sterility (Table 5). In all MA environments genotypic performance in all phenophases was strongly positively influenced by rainfall (RF) and strongly negatively influenced by vapour pressure deficit (VPD), solar radiation (SR), and potential evapotranspiration (ETo). These factors affect mainly water use, water use efficiency, and photosynthesis and, thus, sink

363 build-up and sink filling. This is reflected in the influence of the yield components PPT, SPP
364 and TGW on yield performance in MA environments (Fig. 4 and Table 5). In the LA
365 environments the main weather parameters influencing genotypic performance were
366 temperature and rainfall. Particularly higher temperatures during the early development exert a
367 strong influence on the duration to flowering, shortening the vegetative development and thus
368 influence the source build-up, as reflected in the negative effect of TPH on yield in these
369 environments. However, a larger variation in the influence of the specific weather parameters
370 was observed related to the different planting dates. Genotypic performance was strongly
371 negatively influenced by maximum air temperature (T_{max}) in the early sowing of the first year
372 negatively affecting PFS in the cold tolerant varieties, whereas the late sowing date in the first
373 year and both sowing dates in the second year were strongly influenced by RF. In these cases,
374 high rainfall was accompanied by strong winds increasing lodging (tropical cyclone) and by low
375 VPD increasing mold infections both strongly affecting the yield performance of sensitive
376 genotypes.
377
378 **Discussion**
379 Rice production systems along an altitude gradient, such as in Madagascar, have been
380 traditionally stratified into low altitude, mid-altitude and high-altitude environments. Varieties
381 have been specifically selected and bred for those environments adapted to local cropping
382 calendars aiming at maximal yields. Climate change renders the close relationship between
383 genotypic adaptation/specialization and growing environment dangerous, since environmental
384 factors will vary significantly more (temperature extremes, frequency and amount of rainfall,
385 intensity of solar radiation and VPD) (Meehl et al., 2007, Wassmann et al., 2009) and new
386 combinations of environmental factors may occur (e.g. higher temperatures combined with high
387 VPD or emergence of new pests in higher altitudes combined with changes in water
388 availability) (Weerakoon et al., 2008; Rang et al., 2011; Laštůvka, 2009; Kocmánková, 2009).
389 This may force changes in crop management i.e. shifts in planting dates which lead to
390 significant changes in crop duration particularly across altitude levels (Fig. 1 and Table 2)
391 which had been observed before for Nepal high altitude systems (Shrestha et al., 2011).
392 Exposing a given variety to environmental conditions different from the ones it was adapted to
393 increases the risk of yield loss or crop failure (Fig. 2a). Yield stability across environments is

commonly accompanied by a yield penalty in favorable, high yielding environments (Peng et al., 2006; Acuña et al., 2008), i.e. Chhomrong and FOFIFA 172 in this study (Fig. 2). In the current study, environments were not only defined by different locations but also by sowing dates early and late in the season for two different years. A cluster analyses showed that the 12 environments differed significantly in their average combination of abiotic factors (data not shown). This was reflected in the relatively large genotypic variation in duration and grain yield across environments (Table 2). When combined with the environmental characteristics, associations between genotypes and environments emerged that were only partly reflecting the original environments the genotypes were selected for (Fig. 3). Since crop duration is strongly influenced by temperature and altitudes vary in seasonal mean temperatures due to the altitudinal temperature gradient of 7 °C per km at 60% air humidity (Houghton and Cramer, 1951), variations in yield observed for the different altitudes can be explained with differences in genotypic adaptation and with temperature effects on duration shifting the different phenological phases responsible for the formation of the different yield components to more or less favorable conditions depending on altitude (Lu et al., 2008; Bajracharya et al., 2010). Tillers per hill, the percentage of filled spikelets followed by number of spikelets per panicle were the yield components most influential on yield at different altitudes (Fig. 4 and Table 5). Temperature effects on spikelet sterility (both cold and heat sterility) and on sink-source relationships have been well described for rice (e.g. Dingkuhn et al., 1995; Shrestha et al., 2011; Dingkuhn and Kropff 1996). Variation in grain yield among planting dates within altitudes was not mainly due to temperature but rather due to the combinations of abiotic factors the genotypes experienced during the different phenological stages during which the different yield components were formed. These combinations strongly differed among altitudes (Fig. 5). The combinations of abiotic factors during specific development stages in concert with the genetic predisposition of the genotype determine the level of penalty the respective yield component will inflict on final grain yield. We analyzed the influence of the different environments on individual yield components by linearly regressing the genotypic responses to environment against the environmental mean for the individual yield components. This is exemplarily shown for 4 genotypes in Fig. 6. A number of studies have been conducted in rice to differentiate the effects of the genetic make-up of the plant (genotype) and the effects of abiotic factors, such as temperature (environment). Ao et al. (2010) investigated the effect of increasing the relative

number of productive tillers per hill (PPT). They showed, that reducing the number of unproductive tillers did not positively influence yield, which is in line with finding from Moradpour et al. (2011) who determined tiller number to be the most important yield component for final yield across different planting dates and from Mishra and Salokhe (2010) for final yield across different water regimes in rice. In the present study all genotypes responded strongly with an increase in tiller number when the environmental conditions became more favorable, however, the importance of the overall tiller number for yield depended on the general adaptation of the genotype to the different altitude shown in Fig. 6 by the slope of tiller number relative to the slope of yield. When the slope TPH crossed over the slope of yield (e.g. Fig. 6b, FOFIFA 172), TPH had a strong influence on yield in favorable environments, whereas when the slope of TPH was flatter than the slope of yield (e.g. Fig. 6a, Botramaintso), TPH had only a minor influence on final yield. These results suggest that in environments favoring source built-up TPH should be a selection criterion for high potential yield. Panicle number per tiller (PPT) was found earlier to be a highly environment independent trait (Akinwale et al., 2011; Liu et al., 2008; Zhu et al., 2011), in our study PPT affected yield similarly across all environments (Fig. 4), however genotypic stability in this trait across environments varied strongly (Fig. 6), indicating a certain degree of genotypic plasticity in this trait to be considered in varietal selection for newly emerging rice cropping environments. The ultimate sink potential is defined by the number of spikelets per panicle (SPP). It has been shown before, that this trait is strongly genetically controlled (Akinwale et al., 2011; Kovi et al., 2011) and indirectly strongly influenced by temperature effects on PPT and on panicle length (Kovi et al., 2011). In the present study, SPP strongly affected yield, however, the effects varied strongly with altitude (Fig. 4) and the environment depending effect of SPP varied from strong in Primavera (Fig. 6d) via medium in Nerica 4 (Fig. 6c), weak in FOFIFA 172 (Fig. 6b) to negligible in Botramaintso (Fig. 6a). As in PPT, in SPP we also observed a degree of genotypic plasticity that can be exploited when selecting genotypes for specific environmental conditions. The final yield component to be considered, apart from PFS which was clearly temperature influenced and can only be managed through avoiding detrimental environmental conditions, is TGW which reflects the effectiveness of source mobilization at the end of the reproductive stage. TGW directly depends on cumulative mean temperature and cumulative solar radiation during the grain filling phase and the duration of the grain filling phase in combination with optimal

456 temperature and radiation conditions determines to a large extend the final grain yield of rice
457 (Yang et al., 2008). In addition, the maximal weight of a grain is determined by the size of the
458 hull which is supposedly genetically controlled (Yoshida, 1981). This is reflected in the effects
459 of TGW on grain yield in HA environments (Fig. 4 and Table 5) and in the slopes shown in Fig.
460 6. In unfavorable environments genotypes often suffered from source limitations for different
461 reasons, thus the influence of TGW on final yield was high. TGW increased with environments
462 becoming more favorable and the relative effect on yield was reduced at the same time (Fig. 6).
463 The analysis above has shown, that despite varying degrees of genetic control, most of the yield
464 components respond to environmental conditions and thus influence final yield. Thus,
465 theoretically, maximizing each component in the cascade should increase yield significantly.
466 Table 7 exemplarily shows the grain yield and yield components for 4 genotypes for the
467 respective highest yielding environment and the grain yield obtained when combining the
468 maximal values for the individual yield components observed for each genotype. The values
469 show, that in almost no case the highest possible value for a given yield component was
470 achieved in the combination of yield components resulting in the highest genotypic yield. In
471 addition, the environments in which highest values for individual yield components were
472 observed varied strongly among the genotypes, indicating that no optimal environment for
473 maximizing a specific yield component can be defined. Table 7 shows that in addition to the
474 genetic make-up of a genotype the interdependency of the individual yield components as well
475 as their environmental responses need to be taken into account when defining an ideotype for a
476 newly emerging rice growing environment. Equally, it would require fine tuning of cropping
477 calendar and management to the requirements of the genotype and the requirements of the
478 individual phases during which the yield components are developed (Poussin et al., 2003).
479 Therefore, models simulating the environmental effects on yield components and their
480 interdependencies will be needed to tackle this complex relationship to guide breeding and
481 selection for future environments.
482
483 **Conclusion**
484 In this study we attempted for the first time to relate yield stability across environments with the
485 environmental effects on the different yield components determinant for final yield of upland
486 rice in order to be able to select or breed genotypes suited for newly emerging rice growing

environments along an altitude gradient. We have shown that the contribution of individual yield components to final yield changes with the environmental conditions the rice experiences during the development stages and that this effect may have a stronger influence on final yield than the genetic control of the individual yield components. The varieties chosen for this study represented a cross section of the upland rice genetic diversity. The multitude of growing environments allowed showing, that the original environments the genotypes were selected for favoured certain combinations of traits that were in most cases not ideally combined for environments facing changes due to changing climate. Therefore, new combinations of traits are required to better exploit the environmental potential which may only be possible via advanced crop models simulating the environmental effects on yield components and their interdependencies to develop ideotype for the target environments thus guiding breeding and selection efforts.

Acknowledgements

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616 mapping of a major QTL controlling panicle number in rice. Molecular Breeding 27,
617 171-180.
618

620

621 **Caption for Figures**:

622 Figure 1.

623 Daily weather patterns of two experimented years (from Sept 2008 to Aug 2010) in sequence in
624 three different altitudinal locations in Madagascar. Solid zigzag lines are 24 hours mean air
625 temperature (°C), smooth solid lines are solar radiation (MJ m^{-2} d^{-1}), dotted lines are
626 photoperiod (h) and vertical grey bars depicts total daily precipitation (mm). White square
627 boxes indicate sowing and end of the early sowing and the gray square boxes indicate sowing
628 and end of the late sowing. Gray horizontal lines indicate crop durations. Abbreviation: SD,
629 short duration (IRAT 112); LD, long duration (Botramaintso); SW, sowing date; PI, panicle
630 initiation; FL, flowering; PM, physiological maturity.

631

632 Figure 2

633 Yield stability of ten upland rice genotypes across twelve environments (3 locations, 2 sowing
634 dates and 2 years). (a) Linear regression lines of genotype yield (t ha^{-1}) versus environment
635 yield (t ha^{-1}). Symbols used are the fitted values for each genotype. Horizontal and vertical
636 dotted lines are population mean yield (3.1 t ha^{-1}) of ten genotypes across twelve environments.
637 (b) Scattered plot of regression coefficient versus genotype mean yield (t ha^{-1}). Vertical dotted
638 line is population mean yield (t ha^{-1}) and the horizontal dotted line is the line representing
639 regression coefficient equals to 1.

640

641 Figure 3

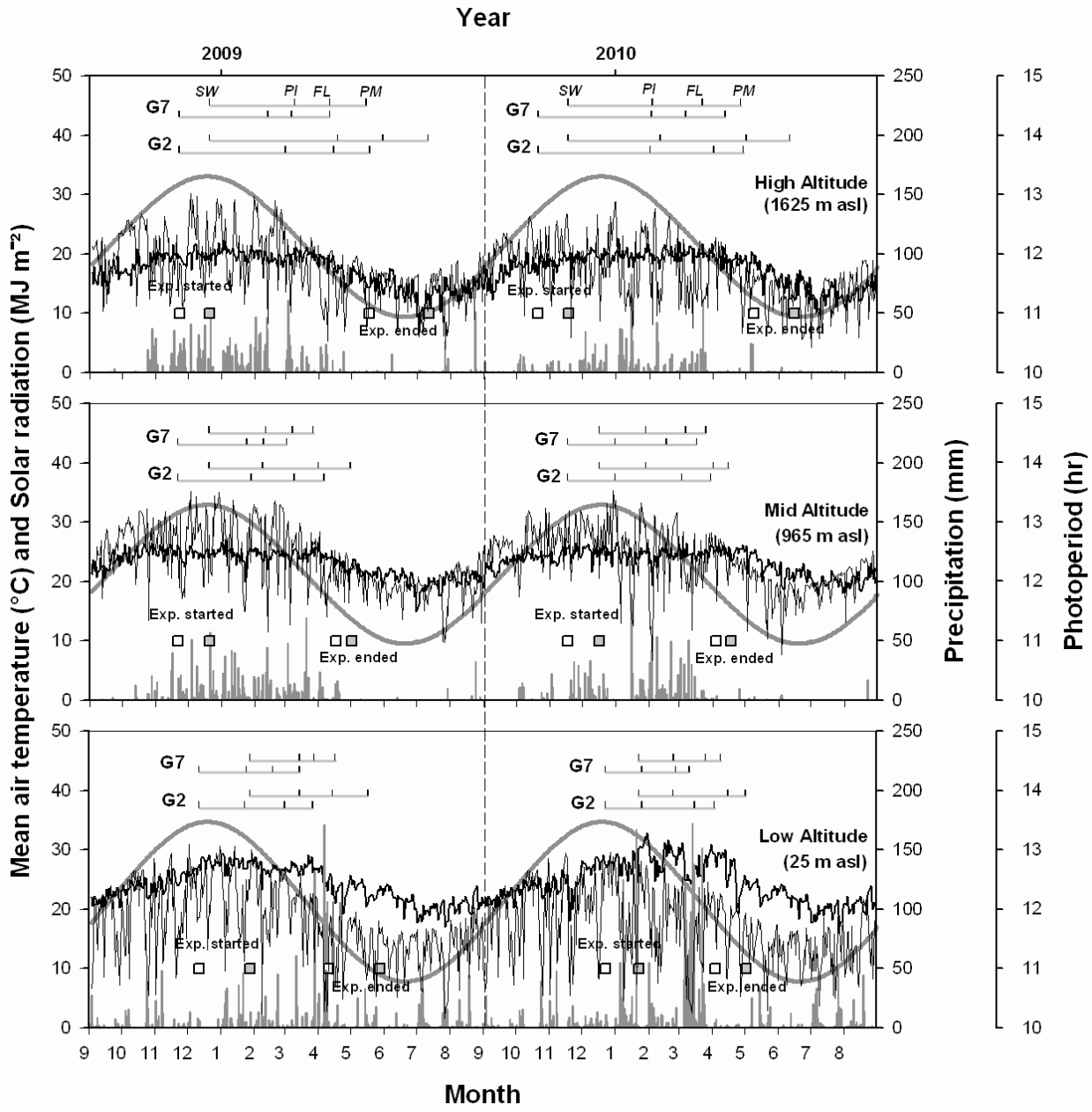
642 Biplot of Additive Main Effects and Multiplicative Interaction (AMMI) analysis for Genotypes
643 and environments. (a) AMMI-1 biplot where ordinate is Interaction Principal Component Axes
644 1 (IPCA-1) scores and abscissa is Genotype and Environment mean grain yield (t ha^{-1}). (b)
645 AMMI-2 biplot where ordinate is IPCA-2 and abscissa is IPCA-1.

646

647 Figure 4

648 Percentage reduction of yield components from overall mean interacting with different
649 environments. Yield components (TPH, PPT, SPP, PFS and TGW) as the latent vector loadings

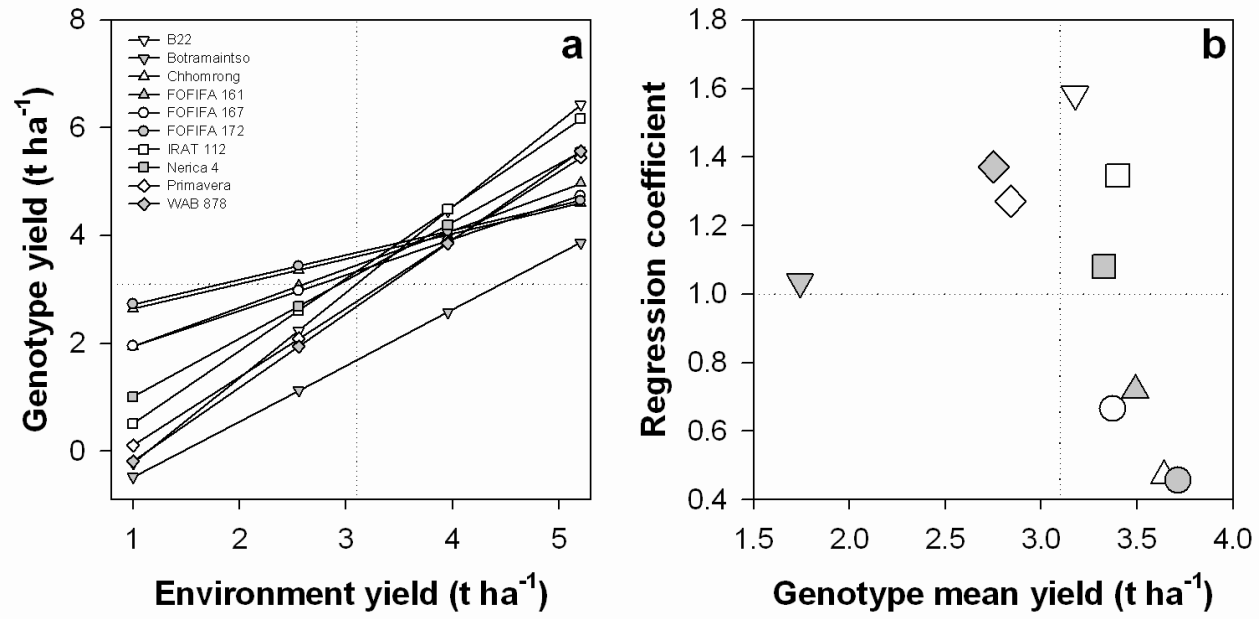
650 and environments as the scores are shown in the PCA biplots with principal component (PC)
651 Axis-2 against PC Axis-1 of ten upland rice genotypes. The symbols used in biplots represent
652 environments. The circles are high altitude, diamond shapes are mid altitude and the square
653 boxes are low altitude environments. Symbols with white colors are early sowing in the first
654 year, gray colors are early sowing in the second year, half white and half black colors are late
655 sowing in the first year and half gray and half black colors are late sowing in the second year.
656 The values in the parenthesis (brackets) are the variation explained by the respective PC Axis.
657
658 Figure 5
659 Biplots of averaged weather experienced by each genotype during its phenological stages across
660 twelve environments plotted PC axis-2 against PC axis-1.Weather parameters minimum air
661 temperature (T_{min}), maximum air temperature (T_{max}), 24 hours mean air temperature ($T_{mean24h}$),
662 precipitation (RF), solar radiation (SR), vapor pressure deficit (VPD) and potential
663 evapotranspiration (ET_o) are the latent vector loadings and weather experienced by genotypes
664 during its phenological stages across twelve environments are the scores of principal component
665 analysis. The symbols used in biplots represent environments. The circles are high altitude,
666 diamond shapes are mid altitude and the square boxes are low altitude environments. White
667 colors are early sowing in the first year, gray colors are early sowing in the second year, half
668 white and half black colors are late sowing in the first year and half gray and half black colors
669 are late sowing in the second year. The values in the parenthesis (brackets) are the variation
670 explained by the respective PC-axis.
671
672 Figure 6
673 Yield and yield components of four genotypes across 12 environments (linearly fitted values)
674 scaled from zero to hundred (zero represents minimum value and 100 represents maximum
675 value).
676



677

678 Figure 1.

679



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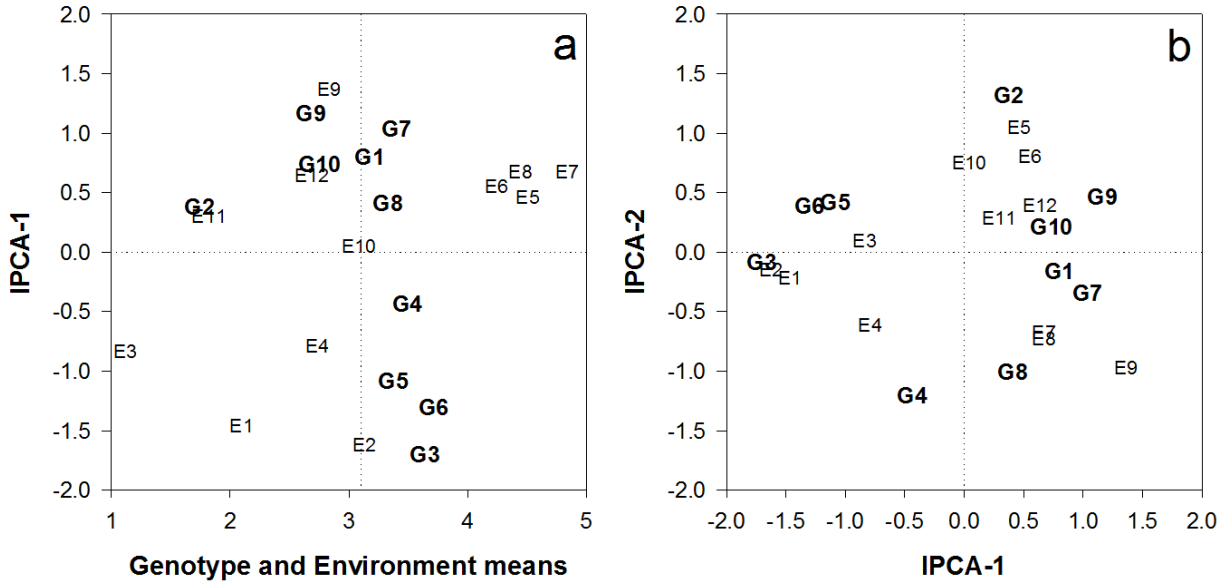
681 Figure 2

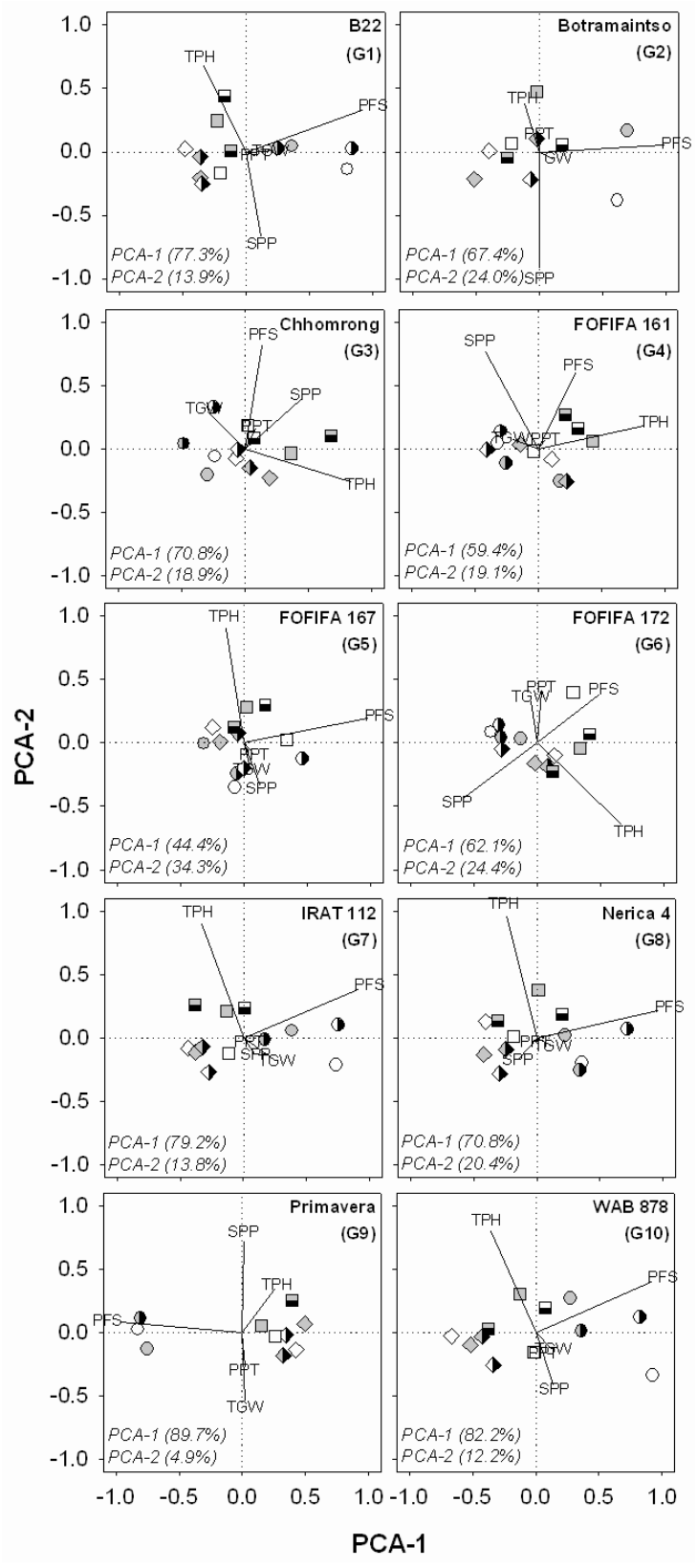
682



683

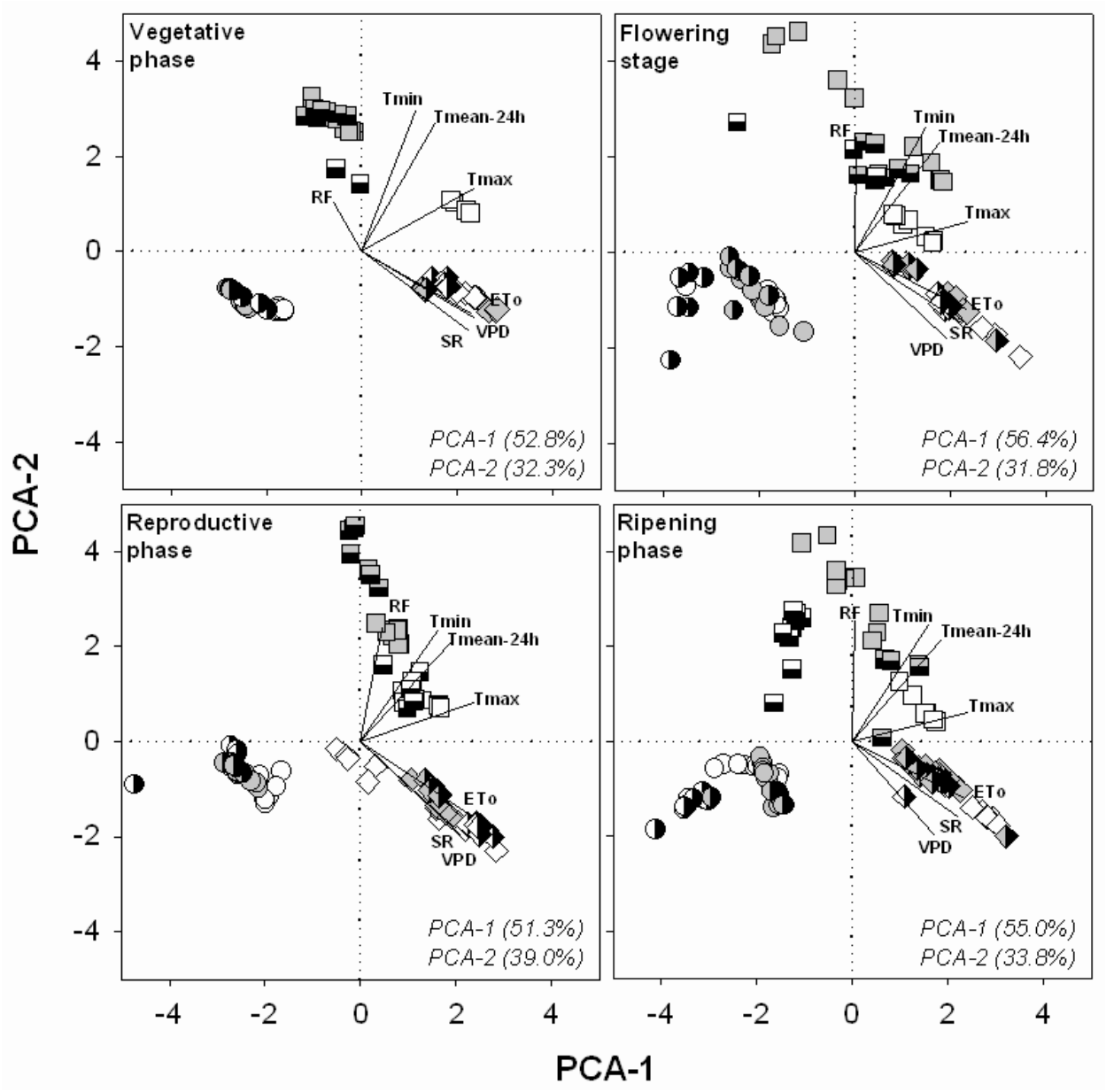
684 Figure 3.

685



686

687 Figure 4



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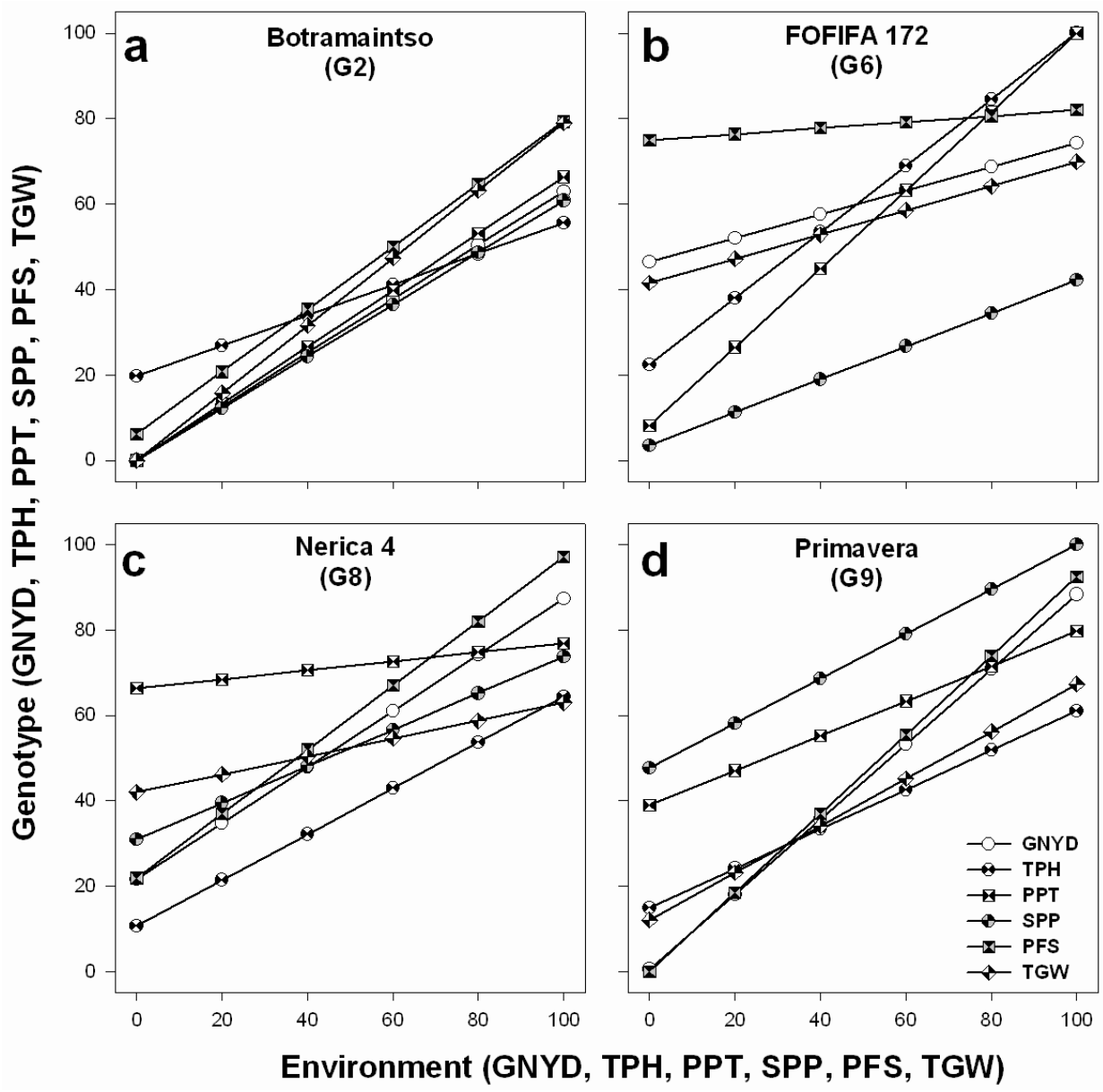
689 Figure 5

690



691

692 Figure 6

693

Captions for Tables:

Table 1. Characteristics of the *Oryza sativa* genotypes used in the study. Abbreviations: G1 to G10, genotypes; trop, tropical; temp, temperate; isc, interspecific crosses; imp, improved; trad, traditional.

Table 2. Varietal performance on grain yield (t ha^{-1}) and days to 50% flowering from sowing of ten upland rice cultivars across twelve environments. Least significant difference (LSD) at $P \leq 0.05$. Abbreviations: HA, high altitude; MA, mid altitude; LA, low altitude; Er, early sowing; Lt, late sowing; Yr, year; E1 to E12, environments.

Table 3. ANOVA table of split plot design combined over year and location where year and location effects are fixed. Abbreviation: GNYD, grain yield; THP, tillers per hill; PPT, percentage of productive tillers; SPP, spikelets per panicle; PFS, percentage of filled spikelets; TGW, thousand grain weight; HI, harvest index; df, degree of freedom; m.s., mean square; and F pr., F probability.

Table 4. Analysis of variance of AMMI model for grain yield in the twelve environments and the proportion of the total variance attributable to the source of variation. . ns, ***, **, *: not significant or significant at P-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively. Abbreviation: df, degree of freedom; m.s., mean square; and F pr., F probability.

Table 5. Percentage change on grain yield and yield components from genotype mean. Positive values are losses (%) and negative values are gain (%) from genotype mean. Abbreviation: GnYd, grain yield; THP, tillers per hill; PPT, percentage of productive tillers; SPP, spikelets per panicle; PFS, percentage of filled spikelets; TGW, thousand grain weight; HA, high altitude; MA, mid altitude; LA, low altitude; Er, early sowing; Lt, late sowing; Yr, year; E1 to E12 are environments; NA, data not available.

Table 6. Environmental and genotypic variance (heritability) in broad sense estimated from phenotypic variance (total variance + error). Abbreviations: FL, flowering; GnYd,

724 grain yield; TPH, tillers per hill; PPT, percentage of productive tillers; SPP, spikelets
725 per panicle; PFS, percentage of filled spikelets; TGW, thousand grain weight.
726
727 Table 7. Highest grain yield obtained within twelve environments and its corresponding yield
728 components of four genotypes compared to the yield that can be obtained from same
729 genotype with the highest yield components obtained within twelve environments.
730

731 **Table(s)**

732

733 Table 1.

Genotype	Variety name	Sub-species	Type	Growing altitude	Country of origin
G1	B22	trop japonia	imp	mid-low	Brazil
G2	Botramaintso	trop japonica	trad	mid	Madagascar
G3	Chhomrong	temp japonica	trad	high	Nepal
G4	FOFIFA 161	trop japonica	imp	high	Madagascar (FOFIFA)
G5	FOFIFA 167	trop japonica	imp	high	Madagascar (FOFIFA)
G6	FOFIFA 172	trop japonica	imp	high	Madagascar (FOFIFA)
G7	IRAT 112	trop japonica	imp	mid	Ivory Coast
G8	NERICA 4 (WAB 450-I-B-P-91-HB)	isc	imp	mid	Benin (WARDA)
G9	Primavera	trop japonica	imp	mid-low	Brazil
G10	WAB 878 (WAB 878-6-12-1-1-P1-HB)	isc	imp	mid	Ivory Coast

734

735 Table 2.

Genotype	HA Er Yr 1 E1	HA Er Yr 2 E2	HA Lt Yr 1 E3	HA Lt Yr 2 E4	MA Er Yr 1 E5	MA Er Yr 2 E6	MA Lt Yr 1 E7	MA Lt Yr 2 E8	LA Er Yr 1 E9	LA Er Yr 2 E10	LA Lt Yr 1 E11	LA Lt Yr 2 E12
Grain yield (t ha^{-1})												
B22	0.5	2.1	0.0	2.5	5.1	4.5	6.3	5.6	3.3	3.2	2.5	2.4
Botramaintso	0.2	0.7	0.0	0.0	4.0	5.3	2.4	1.9	1.5	3.0	0.6	1.3
Chhomrong	5.2	7.0	2.4	4.3	4.0	4.1	4.1	4.3	1.1	3.5	2.1	1.9
FOFIFA 161	3.6	3.6	2.9	4.1	3.2	3.8	5.6	5.4	4.0	2.7	1.0	2.0
FOFIFA 167	3.9	5.0	1.8	4.0	5.0	3.8	4.3	3.6	0.8	3.5	1.3	3.3
FOFIFA 172	4.2	5.7	3.4	4.2	5.2	3.9	4.5	3.4	1.3	3.7	2.7	2.4
IRAT 112	0.9	2.1	0.3	2.5	5.3	5.0	5.7	4.9	5.7	2.6	2.5	3.2
Nerica 4	2.2	3.1	0.3	3.1	3.3	4.0	5.8	5.3	4.9	2.8	2.0	3.2
Primavera	0.2	0.5	0.0	0.8	5.1	4.3	4.8	4.7	3.3	3.1	3.3	4.1
WAB 878	0.2	1.6	0.0	1.7	4.9	3.9	5.0	5.2	2.4	2.8	2.1	3.1
Mean	2.10	3.10	1.10	2.70	4.50	4.30	4.90	4.40	2.80	3.10	2.00	2.70
LSD	1.04	1.10	0.85	0.71	1.30	1.28	1.14	1.09	0.81	1.11	0.69	1.11
Days to 50% flowering												
B22	109	143	118	124	80	92	78	80	68	64	60	62
Botramaintso	143	163	160	165	108	106	101	106	79	83	77	82
Chhomrong	116	133	111	127	86	95	78	85	74	73	60	63
FOFIFA 161	111	134	111	124	81	92	79	83	70	73	63	69
FOFIFA 167	122	139	119	126	93	105	85	84	78	79	62	68
FOFIFA 172	101	127	108	120	90	100	76	82	90	84	59	62
IRAT 112	104	137	111	124	79	91	77	80	68	65	60	62
Nerica 4	105	133	112	124	79	91	76	79	70	68	61	63
Primavera	113	135	119	127	87	93	81	86	68	68	63	68
WAB 878	115	143	120	130	81	92	78	79	69	67	59	62
Mean	114	139	119	129	86	96	81	84	73	72	62	66
LSD	4	8	6	8	8	9	4	5	3	3	2	1

736

737 Table 3.

		GNYD		TPH		PPT		SPP		PFS		TGW		HI	
Source of variation	df	ms	F pr	ms	F pr	ms	F pr	ms	F pr	ms	F pr	ms	F pr	ms	F pr
Year	1	19.4	<.001	462.8	<.001	486.0	0.006	671.0	0.147	4022.5	<.001	387.6	<.001	0.041	<.001
Loc	2	181.5	<.001	1216.4	<.001	9.0	0.815	1118.2	0.047	44929.7	<.001	338.5	<.001	1.047	<.001
Year.Loc	2	19.7	<.001	13.1	0.188	870.6	<.001	5494.5	<.001	2488.9	<.001	132.6	<.001	0.408	<.001
Residual	12	0.7		6.8		43.3		279.4		71.4		7.6		0.001	
Sow	1	14.2	<.001	0.3	0.793	47.1	0.337	898.9	0.096	3164.2	<.001	4.7	0.4	0.008	0.004
Year.Sow	1	1.4	0.174	42.6	0.007	47.3	0.336	673.9	0.144	3226.7	<.001	12.3	0.183	0.007	0.008
Loc.Sow	2	10.6	<.001	166.7	<.001	227.0	0.029	1722.5	0.014	1063.9	<.001	11.3	0.201	0.043	<.001
Year.Loc.Sow	2	0.9	0.312	163.7	<.001	1.4	0.971	7970.7	<.001	1156.2	<.001	8.2	0.298	0.027	<.001
Residual	12	0.7		4.0		47.1		276.4		57.2		6.1		0.001	
Var	9	16.3	<.001	149.4	<.001	332.3	<.001	5615.1	<.001	5752.5	<.001	272.0	<.001	0.158	<.001
Year.Var	9	1.1	<.001	11.0	<.001	80.2	0.068	475.7	<.001	264.6	<.001	14.6	<.001	0.006	<.001
Loc.Var	18	16.3	<.001	14.6	<.001	152.3	<.001	523.4	<.001	4682.1	<.001	35.6	<.001	0.097	<.001
Sow.Var	9	4.8	<.001	9.4	0.003	94.8	0.028	176.4	0.054	325.0	<.001	34.6	<.001	0.006	<.001
Year.Loc.Var	18	2.1	<.001	10.7	<.001	63.4	0.12	95.1	0.438	252.7	<.001	23.6	<.001	0.029	<.001
Year.Sow.Var	9	1.9	<.001	6.4	0.047	95.8	0.026	105.6	0.341	177.3	<.001	10.9	0.006	0.018	<.001
Loc.Sow.Var	18	3.1	<.001	6.9	0.007	107.4	0.001	194.7	0.007	196.2	<.001	18.2	<.001	0.018	<.001
Year.Loc.Sow.Var	18	1.7	<.001	12.3	<.001	181.0	<.001	278.4	<.001	198.3	<.001	9.1	0.007	0.015	<.001
Residual	216	0.4		3.3		44.4		93.2		40.6		4.1		0.001	

738

739 Table 4.

Source	df	SS	MS	SS (%)
Total	359.0	1086.3	3.0	
Treatments	119.0	997.5	8.4***	
Block	24.0	15.9	0.7 **	
Genotypes	9.0	114.4	12.7***	11.5
Environments	11.0	451.0	41.0***	45.2
Interactions (G x E)	98.0	432.1	4.4***	43.3
IPCA1	19.0	293.0	15.4***	67.8
IPCA2	17.0	73.0	4.3***	16.9
Residuals	62.0	66.1	1.1***	
Error	214.0	73.0	0.3	

740

741 Table 5.

Genotype		HA Er Yr 1 E1	HA Er Yr 2 E2	HA Lt Yr 1 E3	HA Lt Yr 2 E4	MA Er Yr 1 E5	MA Er Yr 2 E6	MA Lt Yr 1 E7	MA Lt Yr 2 E8	LA Er Yr 1 E9	LA Er Yr 2 E10	LA Lt Yr 1 E11	LA Lt Yr 2 E12
B22	GnYd	84	34	99	21	-61	-42	-99	-77	-4	1	21	24
	TPH	-54	-13	-24	-13	16	3	-23	10	-2	39	30	30
	PPT	18	-5	0	5	-7	-4	-3	8	-4	-4	1	-4
	SPP	11	1	19	-3	-15	18	-2	-4	16	-12	-55	26
	PFS	83	46	98	29	-56	-49	-53	-45	-28	-13	-5	-8
	TGW	18	-4	27	4	-8	-6	-8	-3	-9	-12	1	0
Botramaintso	GnYd	88	59	100	100	-130	-201	-36	-9	12	-70	63	25
	TPH	-17	-14	-10	-19	5	-2	-15	2	-5	34	21	21
	PPT	-5	-9	28	-4	-6	-7	-7	4	-1	7	9	-9
	SPP	43	-31	46	100	-8	19	16	-19	-18	-54	-3	7
	PFS	75	85	100	100	-50	-65	-11	-4	-30	4	23	-29
	TGW	3	9	NA	NA	-1	-13	0	15	8	-20	12	-13
Chhomrong	GnYd	-42	-91	34	-17	-9	-12	-11	-18	71	5	43	49
	TPH	-30	-23	-37	-52	-5	30	-5	15	4	28	5	69
	PPT	-5	-5	-1	-5	-4	1	0	-3	18	-3	-2	10
	SPP	6	-33	5	-23	-10	-1	-2	-18	-2	33	2	43
	PFS	-24	-22	29	-5	-5	-22	-2	-7	23	-2	16	21
	TGW	12	0	18	20	-5	-8	5	-5	13	-27	-6	-16
FOFIFA 161	GnYd	-3	-4	17	-17	9	-10	-60	-56	-14	24	71	44
	TPH	-29	19	-35	-27	21	-7	-55	8	-2	29	40	36
	PPT	-1	-8	-3	-2	-1	3	0	5	-5	0	18	-6
	SPP	22	-29	19	5	-2	19	12	-44	5	-31	0	24
	PFS	-12	-22	15	-22	-20	-15	0	1	-8	47	19	16
	TGW	15	2	20	6	-3	-1	-1	-11	-7	-16	6	-10
FOFIFA 167	GnYd	-17	-47	45	-20	-49	-12	-27	-8	75	-4	61	3
	TPH	-33	2	-24	-26	12	9	-25	3	-7	30	36	23
	PPT	-2	-9	4	-4	-8	4	10	17	5	-6	-7	-3
	SPP	26	-16	15	9	-23	12	2	-27	-10	-7	-2	22
	PFS	-17	-38	54	-12	-26	-24	-5	-4	43	10	27	-8
	TGW	12	9	-2	9	-6	-6	-2	3	10	-18	11	-20
FOFIFA 172	GnYd	-12	-52	10	-14	-40	-4	-21	7	64	-1	28	35
	TPH	-37	-16	-32	-29	19	14	-21	22	-6	27	28	32
	PPT	-3	-6	15	-5	-4	-4	-2	-5	26	-3	-2	-8
	SPP	28	-6	19	13	-24	9	27	2	-40	-20	-30	20
	PFS	-10	-17	-11	-17	-19	-12	-13	-7	35	26	34	11
	TGW	10	-1	10	0	-5	-5	-7	-11	16	-15	5	1
IRAT 112	GnYd	74	37	91	25	-56	-48	-67	-45	-67	23	27	5
	TPH	-52	-11	-17	-7	10	6	-19	6	-16	27	26	47
	PPT	10	-1	1	-1	-2	-2	-1	-3	-2	9	-5	-3
	SPP	22	-9	13	13	-3	10	1	2	-30	-15	-15	12
	PFS	73	47	89	18	-55	-50	-44	-40	-16	-4	12	-31
	TGW	26	9	24	5	-5	-4	-2	-8	-12	-11	0	-21
Nerica 4	GnYd	35	8	90	6	0	-19	-73	-59	-47	16	39	4
	TPH	-31	-4	-13	-42	28	-1	-25	-6	6	43	18	28
	PPT	-1	1	0	1	-2	2	0	2	1	0	-4	-1
	SPP	11	-11	-4	-14	1	23	8	-9	-10	-20	-3	28
	PFS	39	26	91	32	-45	-51	-43	-33	-24	9	28	-28
	TGW	20	10	-6	3	-5	-5	-4	-9	2	0	13	-18
Primavera	GnYd	95	84	100	70	-80	-52	-71	-66	-18	-10	-16	-44
	TPH	-40	-6	-23	-23	6	25	7	-2	6	21	NA	28
	PPT	5	-2	2	-8	4	1	-5	15	1	-9	NA	-4
	SPP	19	-18	0	-3	-15	9	-2	-13	2	-4	NA	26
	PFS	95	95	100	97	-69	-74	-57	-57	-45	-26	NA	-58
	TGW	5	16	NA	-27	4	2	2	8	7	-4	NA	-12
WAB 878	GnYd	92	40	100	39	-77	-40	-83	-90	13	-3	23	-13
	TPH	-78	13	-19	-11	27	19	-15	7	-13	33	8	31
	PPT	18	-3	4	-1	-3	2	-1	1	-4	-5	-4	-3
	SPP	28	-8	13	18	-9	6	1	-26	10	-20	-28	15
	PFS	89	45	100	43	-78	-64	-51	-51	-10	1	16	-40
	TGW	13	-14	25	-2	-12	-4	-5	-1	9	-15	12	-7

742

743 Table 6.

Variable	Genotypic variance	Environmental variance
Days to 50% FL	0.10	0.90
GnYd	0.22	0.70
TPH	0.31	0.67
PPT	0.45	0.34
SPP	0.62	0.34
PFS	0.34	0.60
TGW	0.63	0.33

744

745 Table 7.

Genotype	Environment	GNYD	TPH	PPT	SPP	PFS	TGW
Botramaintso	E6	3.4	13	89	53	81	27
		8.3	15	91	101	81	29
			E4	E2	E10	E6	E10
FOFIFA 172	E2	6.2	20	95	56	91	25
		11.4	24	97	75	92	29
			E1	E12	E9	E5	E10
Nerica 4	E7	5.8	16	93	71	86	25
		10.9	18	98	94	91	29
			E4	E11	E10	E6	E12
Primavera	E5	5.2	12	87	109	81	22
		12.2	19	99	112	83	29
			E1	E10	E2	E6	E4

746

Appendix III

Chlorophyll Index, Photochemical Reflectance Index and Chlorophyll Fluorescence Measurements of Rice Leaf Supplied with Different N Levels

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Chlorophyll index, photochemical reflectance index and chlorophyll fluorescence measurements of rice leaves supplied with different N levels

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ABSTRACT

Rapid and non-destructive diagnosis of plant N status is highly required in order to optimise N fertilizer management and use-efficiency. Additionally to handheld devices for measurements of chlorophyll indices (e.g., SPAD meter) parameters of canopy reflectance via remote sensing approaches are intensively investigated and the photochemical reflectance index (PRI) appears to be a reliable indicator for changes of the epoxidation state of xanthophyll cycle pigments. In order to assess the suitability of a handheld PRI as an additional tool for N diagnosis, rice plants were grown in a nutrient solution experiment with seven N-supply levels (0.18–5.71 mM) and CI (SPAD) and PRI values and chlorophyll fluorescence parameters measured 20 and 28 days after onset of treatments. N-supply had effects on both CI (SPAD) and PRI values with a more reliable differentiation between levels. Maximum quantum yield of PSII (F_v/F_m), actual efficiency of PSII photochemistry (Φ_{PSII}) and regulated non-photochemical quenching (Φ_{NPQ}) did not differ significantly between N levels. Non-photochemical quenching (*NPQ*) and fast- relaxing *NPQ* (NPQ_F) were significantly affected by N-supply. *NPQ* and NPQ_F, but not the slow-relaxing component (NPQ_S), were correlated with CI (SPAD) and PRI values. This finding which has not been reported for N-supply effects so far is indirect evidence that low N-supply induced xanthophyll cycle activity and that PRI values are able to indicate this at least in plants subject to severe N deficiency.

1. Introduction

Nitrogen is the most limiting plant nutrient element on global scale and particularly in agricultural production systems where N fertilizer application is the main driver of plant growth and yield [41]. Due to interrelationships between nitrogen, carbon, and water cycles and the complex environmental impacts induced [44], tools are required for rapid and non-destructive diagnosis of plant N status in order to optimise N fertilizer application and use-efficiency. The SPAD-502 chlorophyll meter provides a rapid and non-destructive estimation of leaf chlorophyll density [31] and is widely used to monitor the N status of plants [39] and optimise N fertilizer management in rice [27,48,10,20,42].

Additionally to this handheld device, tractor-mounted sensors are used for diagnosis of N status [39]. Current interest is high in identifying suitable parameters of canopy reflectance which will allow for spatially explicit N fertilizer application. The photochemical reflectance index (PRI) is one of such parameters. PRI indicates changes of the epoxidation state of xanthophyll cycle pigments [13] and is used in both remote sensing approaches monitoring the light-use efficiency of plant canopies under environmental stressors [11,35,12,21,4] and in physiological studies at the leaf level [36,17,21]. The use of PRI as a screening tool for varietal yield differences was less successful [2,3].

The xanthophyll cycle protects the functionality of photosystem II (PSII) [9,33] under conditions when either light intensity is high (photoinhibitory conditions) or photochemical quenching by carboxylation is reduced (e.g., drought-induced stomatal closure or N-deficiency-induced low enzyme concentrations). Chlorophyll fluorescence measurements are the state-of-art approach to assess the relative partitioning of absorbed light energy into photochemical and non-photochemical quenching and numerous examples illustrate effects of N-supply on this partitioning. Decreases of relative quantum yield of PSII photochemistry (Φ_{PSII}), efficiency of excitation energy capture (F'_o/F'_m) and photochemical quenching (q_p) in *Triticum aestivum* L. flag leaves were observed under low compared to high N-supply by Cabrera-Bosquet [5]. Similar observations in *Oryza sativa* L. were presented in several papers by Kumagai et al. [24–26]. Under conditions of limited N-supply and high photon flux density, photochemical quenching was reduced and non-photochemical quenching increased by enhanced employment of the xanthophylls cycle in *Spinacia oleracea* L. [43] and *Chenopodium album* L. [22]. Contrarily, variation in N-supply had no effect on Φ_{PSII} and chloroplast pigment composition in a 21-year old *Pinus radiata* L. stand while leaf position in the canopy

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and thus exposure to light affected fluorescence parameters [38]. Genotypic differences are indicated in a study on N effects on fluorescence parameters in two *Triticum aestivum* L. cultivars with a decrease of Φ_{PSII} at three of five post-anthesis measurements in the low protein cultivar but the opposite trend in the high protein cultivar [29].

Gamon et al. [12] showed that PRI values correlated with xanthophyll cycle pigment epoxidation state, indicating that increased energy dissipation can be monitored by changes in PRI. The above mentioned effects of N deficiency on fluorescence parameters with the relative increases in non-photochemical quenching suggest that PRI measurements may be used as an indicator of plant N-supply. Indeed, Φ_{PSII} and PRI of different plant species were affected by N-supply and correlated [14]. Since the xanthophyll cycle is a general mechanism of photoprotection, any abiotic stressor is expected to affect Φ_{PSII} and PRI. Sarlikioti et al. [40] combined chlorophyll fluorescence measurements with PRI readings and showed that PRI could be used to monitor early water deficit stress in *Solanum lycopersicum* L. A correlation between Φ_{PSII} and PRI was also documented for *Magnifera indica* L. in a chilling experiment [46].

PRI measurements are mostly based on the use of spectroradiometers but a cheap handheld device (PlantPen PRI 200, Photon Systems Instruments Ltd., Brno, Czech Republic) recently has become available allowing for the assessment of PRI at a single leaf scale. In this study we compare the PlantPen PRI with chlorophyll meter (SPAD) readings and chlorophyll fluorescence parameters in rice under variable N-supply. We hypothesise that low N-supply increases non-photochemical quenching and that this is correlated with changes in PRI readings.

2. Materials and methods

2.1. Plant cultivation

Cold-tolerant rice (*Oryza sativa* L. spp. *temperate japonica*) cultivar Chomrong was grown in a greenhouse at the University of Hohenheim, Germany, from August 2009 to October 2009 in a hydroponic system using Yoshida nutrient solution [49] with the following nutrient element composition (mM): 1.43 N as NH_4NO_3, 0.32 P as $NaH_2PO_4{\cdot}2H_2O$, 0.51 K as K_2SO_4, 1.00 Ca as $CaCl_2$, 1.65 Mg as $MgSO_4{\cdot}7H_2O$; (μM): 9.10 Mn as $MnCl_2{\cdot}4H_2O$, 0.07 Mo as $(NH_4)_6{\cdot}Mo_7O_{24}{\cdot}4H_2O$, 18.50 B as H_3BO_3, 0.15 Zn as $ZnSO_4{\cdot}7H_2O$, 0.16 Cu as $CuSO_4{\cdot}5H_2O$, 35.81 Fe as $FeCl_3{\cdot}6H_2O$. After germination of seeds in moist sand, two rice plants were transferred into pots of 1 L volume and supplied for seven days each with 25%, 50% and 100% Yoshida solution. N treatments started on August 28 by supplying the plants with nutrient solution with seven different N concentrations (0.18, 0.36, 0.71, 1.43, 2.86, 4.28, 5.71 mM N). Nutrient solutions were renewed at the one day interval and the pH was adjusted to 5.0–5.5. Air temperatures and relative humidity (rH) were logged hourly with TGP-4500 Tinytag Plus 2 (Gemini Data Loggers Ltd., Chichester, United Kingdom) during the experiment. The greenhouse had average air temperatures of 35°/20 °C day/night and 30%/75% day/night rH. Extra light was supplied with Philips SON-T Agro 400W bulbs during the 12-h photoperiod (8 a.m.–8 p.m.) keeping the light intensity 400 μmol m^{-2} s^{-1} photosynthetic active photon-flux density (PPFD) at the leaf level.

2.2. Chlorophyll index (SPAD) and photochemical reflectance index (PRI)

The SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) calculates the SPAD value based on the intensity of light transmitted around 650 nm (red band) where absorption by chlorophyll is high and a reference wavelength around 940 nm [31]. Measurement of PRI values with the PlantPen PRI 200 (Photon Systems Instruments Ltd., Brno, Czech Republic) is based on the intensity of light reflected at 531 nm which is sensitive to xanthophyll cycle pigments and 570 nm as a reference wavelength. Both SPAD and PRI were measured from three points (upper, middle and lower parts of a leaf) and were averaged to represent individual measurement of a leaf. PRI values were measured on light-adapted leaves. Additionally, PRI values of dark-adapted leaves were recorded. In line with the protocol of chlorophyll fluorescence measurements (see below), plants were kept in the dark for 30 min and dark-adapted PRI values measured. SPAD values were measured on light adapted leaves only. Measurements were done on the youngest fully expanded leaf (leaves 8 or 9). SPAD and PRI measurements of plants of the seven N treatments were taken 20 days after onset of treatments (DAO) and, additionally, of three N levels (0.36, 1.43, 4.28 mM N) 28 DAO.

2.3. Chlorophyll fluorescence parameters

Leaf chlorophyll fluorescence was measured with the GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) after a dark-adaptation period of 30 min. Minimal fluorescence (F_o) was measured at a modulated light intensity of 1.2 μmol m^{-2} s^{-1} PPFD. Maximal fluorescence in the dark adapted state (F_m) was measured by imposing a saturated light pulse (SLP) of 4500 μmol m^{-2} s^{-1} PPFD for 0.8 s (Fig. 1). Then the leaf was continuously irradiated with actinic light of 1200 μmol m^{-2} s^{-1} PPFD for the next 10 min and a 0.8 s SLP was given at 60 s intervals to determine the maximal fluorescence (F'_m) in the light-adapted state. Minimal fluorescence intensity (F'_o) with all PSII reaction centres opened in light-adapted state was measured after switching off the actinic light. The transient fluorescence F_s is the steady state value of fluorescence immediately prior to the SLP. Leaves were kept under modulated light for 5 min and afterwards far-red light (740 nm) of 17 μmol m^{-2} s^{-1} PPFD was applied for 1 min and a SLP was given to measure the maximal fluorescence parameter F^r_m.

Measurements of chlorophyll fluorescence allow for the calculation of many derived parameters of which some are widely used [15,5,32]. Standard parameters are maximum quantum yield of

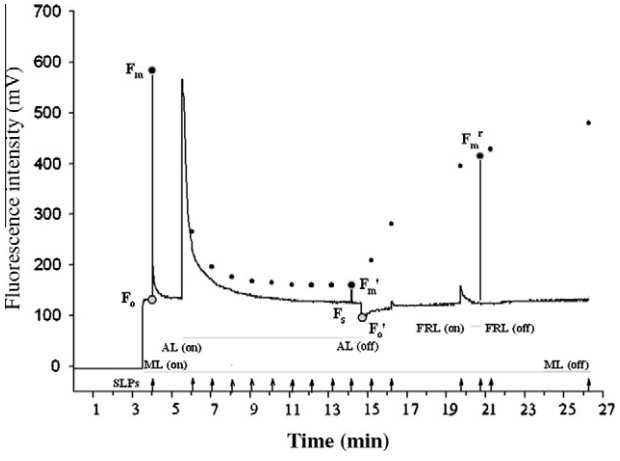

Fig. 1. Sequence of a chlorophyll fluorescence measurement. Modulated light (ML) switched-onto measure minimal fluorescence (F_o) and a saturated light pulse (SLP) applied to measure maximal fluorescence (F_m) of dark-adapted leaves, actinic light (AL) switched on and off as indicated, SLPs were applied every 60 s to measure maximal fluorescence in light (F'_m) at steady state condition, minimal fluorescence in the light (F'_o) after switching off AL. F_s steady-state fluorescence. Far-red light (FRL) switched on and off as indicated and SLPs applied to measure maximal fluorescence in the relaxation phase (F^r_m).

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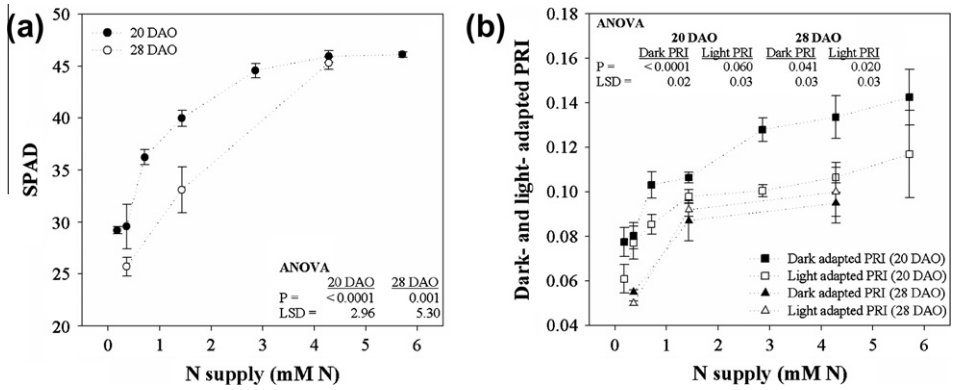

Fig. 2. Effect of N supply on SPAD and PRI of dark- and light-adapted fully expanded youngest rice leaves (leaf 8 or 9) at 20 and 28 DAO. Black and white circles indicate SPAD readings at 20 and 28 DAO respectively (a). Black and white square boxes indicate dark- and light-adapted PRI values at 20 DAO respectively and similar readings at 28 DAO are represented in black and white triangle boxes (b). Vertical bars indicates standard error ($n = 3$ leaves).

PSII, $(F_v/F_m) = (F_m - F_o)/F_m$, and $\Phi_{PSII} = (F'_m - F_s)/F'_m$, the actual efficiency of PSII photochemistry, indicating the proportion of absorbed light (energy) that is used in photochemistry. Non-photochemical quenching was calculated in two ways: $NPQ = (F_m - F'_m)/F'_m$ with values ranging from 0 to infinity and $\Phi_{NPQ} = (F_s/F'_m) - (F_s/F_m)$ [16,18,23], with values from 0 to 1, quantifying the proportion of regulated dissipation by heat (fraction of light absorbed by the PSII antennae that is dissipated thermally via ΔpH, trans-thylakoid pH gradient; and/or xanthophyll-regulated process). Primarily constitutive loss of non-regulated heat dissipation Φ_{NO} (the sum of fraction of light absorbed by PSII antennae that is lost by either constitutive thermal dissipation or via fluorescence) was calculated as F_s/F_m. With $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$ [18], N effects on the relative quenching by photochemistry and non-photochemical heat dissipation can be calculated. Fast and slow relaxing non-photochemical quenching (NPQ_F and NPQ_S) were calculated as $NPQ_F = (F_m/F'_m) - (F_m/F^r_m)$ and $NPQ_S = (F_m - F^r_m)/F^r_m$ [32]. After measurements of fluorescence parameters of plants at 28 DAO, leaves were supplied with 700 μmol PPFD m^{-2} s^{-1} and 380 μbar bar^{-1} of CO_2 in the leaf chamber (air temperature 25 °C, vapour pressure 15 Pa/kPa) for 15 min in order to investigate N-supply effects on steady-state CO_2 assimilation rate and stomatal conductance.

2.4. Leaf pigment composition and plant dry mass

Immediately after gas exchange measurements, leaves of plants were detached from the shoot, transferred into liquid nitrogen, and afterwards stored at −25 °C. Leaf chlorophyll and carotenoid absorptions were measured in 80% (v/v) acetone extracts with a Beckman DU-640 UV–VIS spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA) and converted to concentrations according to Lichtenthaler and Wellburn [28]. Leaf N concentrations of freeze-dried and ball-milled samples were measured with an EA3000 series CHNS-O Elemental Analyser (EuroVector, HEKAtech, Wegberg, Germany). Plants of the three N-supply levels (see above) were harvested 28 DAO, separated into root, stem and leaves, dried at 65 °C to constant weight and dry mass determined.

2.5. Data analysis

The experiment was laid out as a completely randomised design with three replications. Statistical analyses were performed with SAS – Version 9.00 (SAS Institute Inc., Cary, NC, USA). One-way ANOVA was used to evaluate the significance of N-supply on measured parameters. LSD with $\alpha = 0.05$ was used to compare N levels. Standard error (SE) of replications at each N level was calculated from standard deviation (SD) and number of replicates (n) as $SE = (SD/n^{0.5})$.

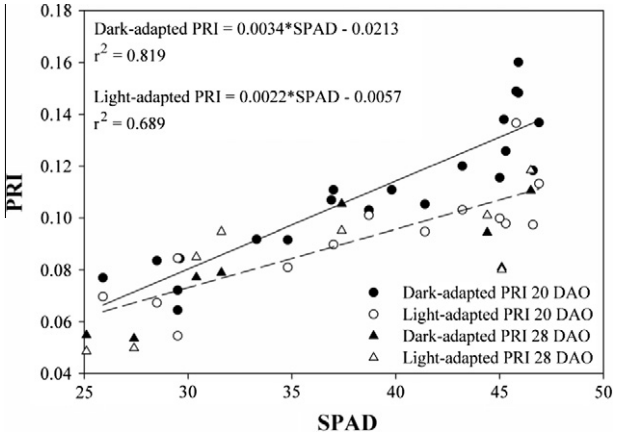

Fig. 3. Linear regression between dark- and light-adapted PRI and SPAD values measured at 20 (solid line) and 28 (dashed line) DAO.

3. Results

SPAD and PRI values increased with increasing N-supply (Fig. 2a and b). PRI values of dark-adapted leaves and SPAD values remained unaffected when N-supply increased from 0.18 to 0.36 mM, increased significantly with further increase in N-supply and levelled off when N-supply was higher than 2.86 mM N. Light-adapted PRI values were not significantly different when N-supply was higher than 1.43 mM N. In agreement with data collected at 20 DAO, SPAD values and dark- and light-adapted PRI increased with increasing N-supply when measured 28 DAO at N-supply levels of 0.36, 1.43 and 4.28 mM N (Fig. 2a and b). SPAD values were significantly different between the three N levels, while light- and dark-adapted PRI values indicated significant differences only between N levels 0.36 and 1.43 mM N. Comparing both measurement dates, SPAD values were similar for the high N treatment of 4.28 mM N whereas SPAD values decreased with increasing leaf age at low N-supply. PRI values of the three N treatments were consistently lower at 28 DAO as compared to 20 DAO. Dark- and light-adapted PRI values were positively correlated with SPAD values (Fig. 3). Light-adapted PRI values were lower than dark-adapted PRI values and these differences increased with increasing N-supply.

Variation in N-supply for 28 days resulted in significant differences between the three N levels in leaf N and carotenoid

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Table 1
Effects of N-supply (mM N) on leaf nitrogen content (%), relative biomass allocation to root dry mass (%), carotenoid$_{(x+c)}$ (μg cm^{-2} leaf), chlorophyll$_{(a)}$ and chlorophyll$_{(b)}$ (g m^{-2} leaf), stomatal conductance (mol m^{-2} s^{-1}) and assimilation (μmol m^{-2} s^{-1}) of the youngest fully expanded rice leaf (leaf 8 or 9) 28 DAO. *P*, significance of overall *F* test effects; LSD, least significant differences.

N-supply (mM N)	Leaf N (%)	Relative allocation (%)	Carotenoid$_{(x+c)}$ (μg cm^{-2} leaf)	Chlorophyll$_{(a)}$ (g m^{-2} leaf)	Chlorophyll$_{(b)}$ (g m^{-2} leaf)	Stomatal conductance (mol m^{-2} s^{-1})	Assimilation (μmol m^{-2} s^{-1})
0.36	1.98	28.8	2.63	0.16	0.06	0.12	10.17
1.43	2.57	29.4	3.60	0.19	0.05	0.12	12.87
4.28	4.29	22.9	5.85	0.34	0.08	0.30	19.60
P:	<0.0001	0.0499	<0.0001	0.0020	0.0014	0.0520	0.0410
LSD:	0.13	5.37	0.20	0.06	0.01	0.16	6.80

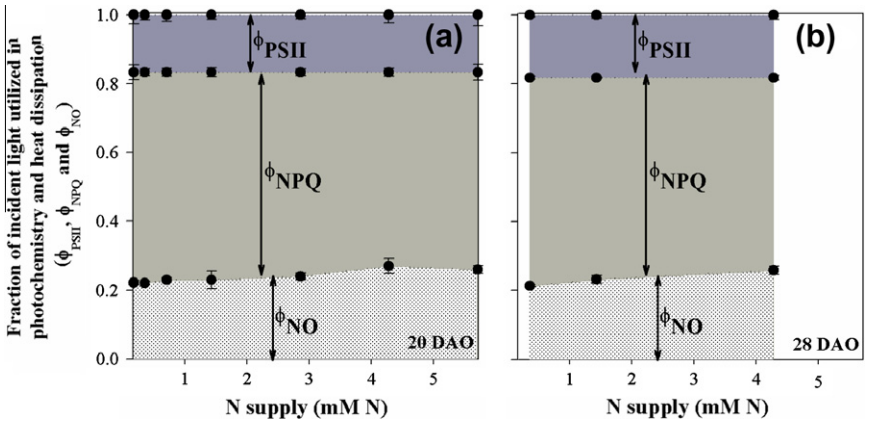

Fig. 4. Fraction of incident light utilised in photochemistry and heat dissipation (Φ_{PSII}, Φ_{NPQ} and Φ_{NO}) at different N supply levels measured (a) at 20 DAO and (b) at 28 DAO. Vertical bars indicates standard error (n = 3 leaves).

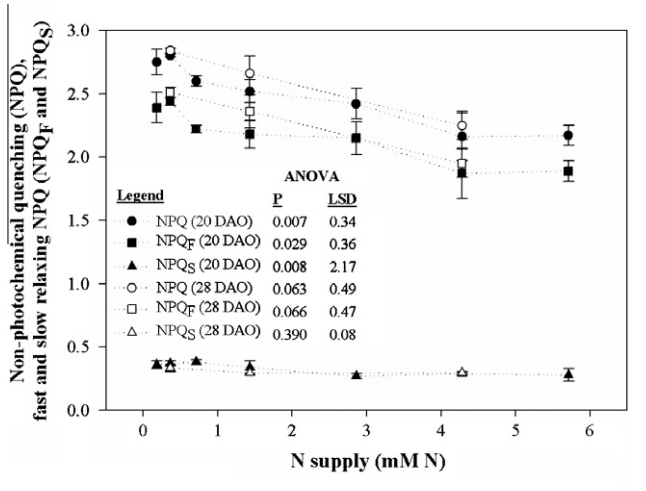

Fig. 5. Non-photochemical quenching (*NPQ*), fast and slow relaxing *NPQ* (*NPQ*$_F$ and *NPQ*$_S$) at different N supply levels measured at 20 and 28 DAO. Vertical bars indicates standard error (n = 3 leaves).

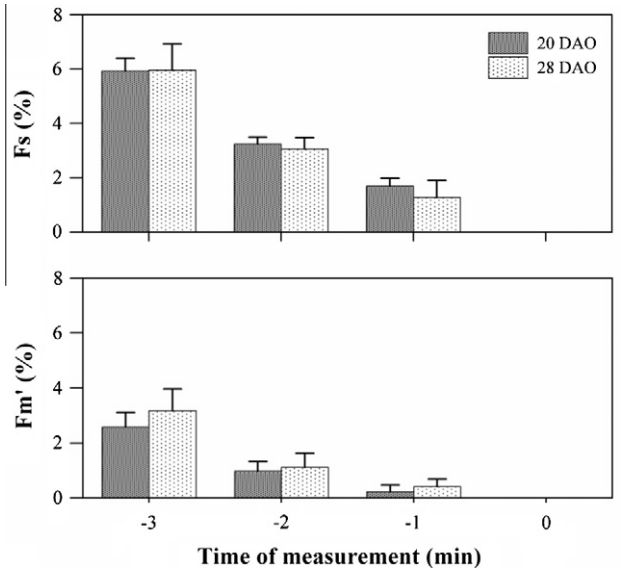

Fig. 6. Stability of records of F_s and F'_m 1–3 min before the final saturated light pulse were applied. Values are expressed as the change of F_s and F'_m relative to the final values of F_s and F'_m at time zero. Vertical bars indicates standard error (n = 3).

concentrations whereas chlorophyll concentrations and stomatal conductance were similar between the two lower N-supply levels (Table 1). At 28 DAO, shoot dry mass differed significantly between N levels and increased from 2.4 to 5.1 and 5.6 g per plant. Relative biomass allocation to root dry mass decreased significantly from the two lower (28.8% and 29.4%) to the highest (22.9%) N-supply level (Table 1).

Maximum quantum yield of PSII (F_v/F_m) and Φ_{PSII} did not differ significantly between the seven N levels. Mean values of F_v/F_m and Φ_{PSII} were 0.772 and 0.173, respectively at 20 DAO and 0.772 and 0.167 when measured at 28 DAO (data not shown). N-supply had no significant effect on regulated and non-regulated non-photochemical quenching (Fig. 4a) at 20 DAO and N-supply tended to increase Φ_{NO}·Φ_{NPQ} of high-N plants was significantly lower at 28 DAO whereas Φ_{NO} tended to increase with N-supply (Fig. 4b). Less than 20% of the incident light was utilised in photochemistry. About 60% was dissipated as Φ_{NPQ} and more than 20% as Φ_{NO} (Fig. 4a and b). The fraction of light utilised in photochemistry (Φ_{PSII}) and heat

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dissipation (Φ_{NPQ}) did not vary across seven N levels at 20 DAO and three N levels at 28 DAO.

Non-photochemical quenching (*NPQ*), and fast- and slow-relaxing *NPQ* (NPQ_F and NPQ_S) were significantly affected by N-supply at 20 DAO (Fig. 5). A significant decrease of *NPQ* was observed when comparing N supply levels of 0.36 mM with 4.28 and 5.71 mM. A similar effect of N-supply was observed for NPQ_F. N effects on NPQ_S were as well obvious with higher values at the three low N-supply levels and lower values of the three high N-supply levels. However, due to high variability of NPQ_S at N level 1.43 mM, the LSD test did not indicate significant differences between means. Similar effects on *NPQ*, NPQ_F and NPQ_S were observed at 28 DAO and, again, high variation within replicates constrained the detection of differences between means (Fig. 5). F_s and F'_m exhibited fairly stable values (only 2 and 1% higher values 1 min before readings were taken, Fig. 6) indicating that the measurement protocol allowed for the assessment of fluorescence parameters in nearly steady-state conditions.

NPQ and NPQ_F correlated negatively with SPAD, dark- and light-adapted PRI values at 20 DAO (Fig. 7). *NPQ* and NPQ_F and SPAD values measured at 28 DAO fitted well to that at 20 DAO while the agreement was not as good for dark-adapted PRI readings at N supply levels of 4.28 mM at 28 DAO. NPQ_S did not correlate with either SPAD or dark- and light- adapted PRI values at 20 and 28 DAO. The relationship between fluorescence parameters and SPAD and PRI values were not linear over the whole range of data. E.g., when SPAD values were higher than 45, chlorophyll fluorescence varied while SPAD values did not differ significantly any more.

4. Discussion

The hypothesis that low N-supply increases non-photochemical quenching and that this is correlated with changes in PRI readings is partially confirmed by this study although N deficiency was severe and resulted in a substantial decrease of dry mass and standard gas exchange parameters compared to high-N plants.

Low N-supply can increase the excitation pressure on PSII beyond the capacity of photochemical and non-photochemical quenching, thereby inducing photoinhibitory damage of PSII and

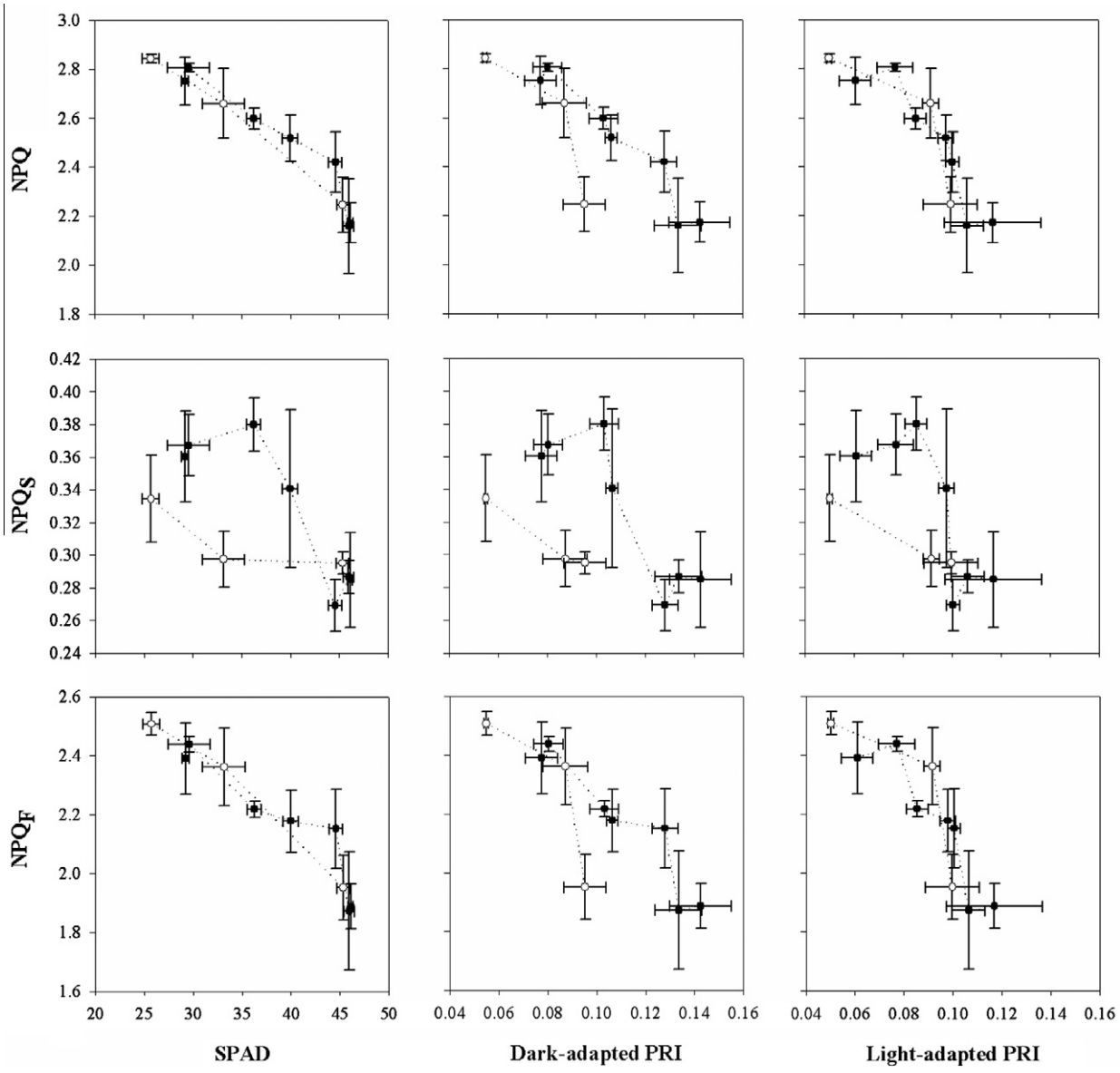

Fig. 7. Relationship between *NPQ*, NPQ_S, and NPQ_F and SPAD, and dark- and light-adapted PRI values at 20 (filled symbols) and 28 (open symbols) DAO. Vertical and horizontal bars indicate standard error ($n = 3$ leaves).

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a decrease of the maximal quantum yield of PSII, F_v/F_m, as illustrated by results of Verhoeven et al. [43], Netto et al. [34], Wu et al. [47], Kumagi et al. [25] and Pompelli et al. [37]. However, N-supply had no effect on F_v/F_m in this study and others [7,30,8,5]. Photoinhibitory damage is more pronounced in leaf tissue exposed to high light [43] or additional stressors such as water deficit or if leaves are already in the senescence phase. We assume that F_v/F_m was not affected by N-supply in this study as we measured the youngest fully expanded leaves and plants were cultivated in nutrient solution under comparably low light intensity.

The actual efficiency of PSII photochemistry, Φ_{PSII}, was not affected by N-supply and rather low compared to other studies which reported both values mostly in the range of 0.2–0.6 and pronounced N-supply effects on Φ_{PSII} [43,6,37,26]. Our findings that N-supply had no effects on regulated and non-regulated non-photochemical quenching at 20 DAO and the decrease of Φ_{NPQ} of high-N plant at the expense of Φ_{NO} instead of Φ_{PSII} at 28 DAO are against the initial hypothesis and not corroborated by other studies. We see no reason to question the protocol of fluorescence measurements which is comparable to that of many other studies. Analyses of F_s and F'_m indicate that both were recorded under nearly steady-state conditions. Furthermore, standard gas exchange parameters measured at 28 DAO were typical for rice leaves under varying N-supply and N effects on dry mass were pronounced, indicating, together with the range of SPAD meter values measured, that N deficiency was the exclusive factor limiting plant performance.

In contrast to Φ_{NPQ}, *NPQ* was significantly affected by N-supply and correlated with SPAD and PRI values. A comparison of *NPQ* values can be ambiguous if F_v/F_m values differ between treatments [32] but F_v/F_m values were not affected by N-supply in this study. Higher *NPQ* values indicate an increased thermal dissipation of absorbed energy and this regulated heat dissipation is closely linked to xanthophyll cycle activity protecting PSII against photoinhibition under a combination of N deficiency and high light [43,24–26].

Non-photochemical quenching can be analysed by following the relaxation after actinic light is switched off [45,19]. Relaxation studies identified fast (NPQ_F) and slow (NPQ_S) relaxation quenching. In this study, NPQ_F was affected by N-supply, and this relaxation parameter is considered to reflect the extent for zeaxanthin formation. This finding which has not been reported for N-supply effects so far is indirect evidence that low N-supply induced xanthophyll cycle activity and that dark-adapted PRI values are able to indicate this at least in the low-N range. The measurement protocol did not allow for a more detailed measurement of dark relaxation kinetics and may have underestimated NPQ_F to a certain extent [32]. In agreement with our finding that N-supply had no effect on F_v/F_m, NPQ_S, which is indicative of photoinhibition, was not affected by N-supply.

An increased activity of the xanthophyll cycle is indirectly indicated by the change of PRI values from high to low N-supply. As both SPAD and dark-adapted PRI values indicated insufficient N-supply when the N concentration of the nutrient solution was below 1.43 mM N, both non-destructive measurements can be used to assess the N status of rice leaves in terms of N deficiency. However, as xanthophyll cycle activity is responsive to all stressors which affect lumen pH, PRI values should not be used for N diagnosis as a stand-alone tool. The Plant Pen PRI 200 was only recently released and used by Sarlikioti et al. [40] to monitor early water deficit stress in *Solanum lycopersicum* L. Additionally to the measurement of PRI during the light phase we recorded dark-adapted PRI values which were higher than light-adapted PRI values and better correlated with fluorescence parameters. This finding is surprising as the conversion from zeaxanthin to violaxanthin after stress relaxation is usually fast and the xanthophyll cycle follows diurnals of light intensity [1]. We assume that carotenoids and xanthophyll cycle pigments persisted during the 30 min dark period imposed on plants but cannot explain why dark-adapted PRI values were better predicting leaf status parameters of fluorescence.

5. Conclusions

Evidently, N deficiency is not always affecting the efficiency of PSII photochemistry (Φ_{PSII}) and the relative contribution of quenching components. N-supply affected *NPQ* and NPQ_F both indicating increased xanthophyll cycle activity. This was partially reflected in PRI readings but further studies are required to evaluate if the Plant Pen PRI 200 can be used to monitor this central component of stress adaptation reliably. Particularly the poor ability of the Plant Pen PRI 200 to differentiate between medium to high N-supply levels indicate that further technical improvements may be helpful. Having proven this eventually positive, we feel that the PRI 200 can be a tool for rapid general stress assessment, particularly in cropping systems where not only the N fertilizer demand needs to be estimated but stress responses to water or temperature to be considered as well.

6. Abbreviations

CI	chlorophyll index
DAO	days after onset of treatments
F_m	maximal fluorescence in the dark adapted state
F'_m	maximal fluorescence in the light adapted state
F^r_m	maximal fluorescence in the far red light state
F_o	minimal fluorescence in the dark adapted state
F'_o	minimal fluorescence in the light adapted state
F_s	transient fluorescence at the steady state
F_v	variable fluorescence
mM	milimole
N	nitrogen
NPQ	non-photochemical quenching
NPQ_F	fast-relaxing non-photochemical quenching
NPQ_S	slow-relaxing non-photochemical quenching
PPFD	photosynthetic active photon-flux density
PRI	photochemical reflectance index
PSII	photosystem II
q_p	photochemical quenching
SLP	saturated light pulse
SPAD	soil plant analysis development
Φ_{NO}	primarily constitutive loss of non-regulated heat dissipation
Φ_{NPQ}	regulated non-photochemical quenching
Φ_{PSII}	actual efficiency of PSII photochemistry

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Statement of authors contribution

The thesis titled "**Genotypic responses of Upland Rice to an Altitudinal Gradient**" is submitted together with the enclosed peer reviewed three articles.

Paper I: *Title*: **Phenological responses of Upland Rice Grown Along an Altitudinal Gradient**.
Authors: Suchit Shrestha, Folkard Asch, Holger Brueck, Julie Dusserre and Alain Ramanantsoanirina
Journal: Environmental and Experimental Botany
Ref. No.: EEB-D-12-00032
Status: under review

Paper II: *Title*: **Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes**.
Authors: Suchit Shrestha, Folkard Asch, Julie Dusserre, Alain Ramanantsoanirina and Holger Brueck
Journal: Field Crops Research
Ref. No.: FIELD-D-12-00014
Status: under review

Paper III: *Title*: **Chlorophyll Index, Photochemical Reflectance Index and Chlorophyll Fluorescence Measurements of Rice Leaf Supplied with Different N levels**.
Authors: Suchit Shrestha, Holger Brueck and Folkard Asch
Journal: Journal of Photochemistry and Photobiology B: Biology
Ref. No.: JPHOTOBIOL-D-12-00027
Status: under review

Authors contributions:

Mr. Suchit Pd. Shrestha planned and conducted all field trials in Madagascar and the greenhouse trials in Hohenheim. He was responsible for the data analysis and writing of the manuscripts.

Prof. Dr. Folkard Asch was instrumental in setting the work up and was involved in the final stages of writing the manuscripts.

PD Dr. Holger Brueck guided on designing the trials, contributed to the data analysis and was involved in the final stage of manuscripts preparation.

Dr. Julie Dusserre provided administrative and technical support to conduct multi-locational field trials in Madagascar.

Dr. Alain Ramanantsoanirina provided administrative and logistic support to conduct multi-locational field trials in Madagascar.

Date: 7/2/2012, Hohenheim

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Declaration of Originality

Hereby I declare that this doctoral thesis is independently written by myself. In addition, I confirm that no other sources than those specified in the thesis have been used. I assure that this thesis, in the current or similar format, has not been submitted to any other institution in order to obtain a Ph.D. or any other academic degree.

Ich erkläre hiermit, dass ich diese Dissertation selbständig angefertigt habe. Es wurden nur die im Literaturverzeichnis aufgeführten Hilfsmittel benutzt und fremdes Gedankengut als solches kenntlich gemacht. Ich versichere, dass ich diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Institution zur Prüfung vorgelegt habe.

Date: 7/2/2012, Hohenheim **Suchit Prasad Shrestha**

Curriculum Vitae

Personal information

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Nationality	Nepali
Date of birth	20th Jan, 1972 in Kathmandu, Nepal
Gender	Male
Marital status Children	Married with Bipana Shrestha (Pradhan) on 16th Dec, 2004 One daughter (Subreena Shrestha) born on 23rd Sept, 2006

Personal skills and competences

Mother tongue(s): **Newari**

Other language(s): **Nepali, Hindi**

Self-assessment	**Reading**	**Listening**	**Speaking**	**Understanding**	**Writing**
European level					
English	Fluent	Fluent	Fluent	Fluent	Fluent
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Desired employment / Occupational field: **Agricultural Research**

Work experience

Dates	From August 1997 to October 2008
Occupation or position held	**Technical Officer** (Soil Science)
Main activities and responsibilities	Conducting experiments, analysing experiment data, writing proposals and reports
Name and address of employer	Nepal Agricultural Research Council (NARC), Singha Darbar Plaza, Nepal

Type of business or sector	Regional Agricultural Research Station (RARS), Lumle Agricultural Environment Unit (AEU), Khumaltar Soil Science Division (SSD), Khumaltar
Education and training	
Dates	2008-12
Title of qualification	**PhD**
Principal subjects/occupational skills covered	Genotypic responses of Upland Rice to an Altitudinal Gradient
Name and type of organisation providing education and training	University of Hohenheim, Department of Plant Production and Agroecology in the Tropics and Sub-tropics, Section of Water Stress Management, Stuttgart, Germany
Dates	2005-07
Title of qualification awarded	**Master of Science in Agriculture**
Principal subjects/occupational skills covered	Phenological and agronomical responses of rice genotypes in high altitude rice production system
Name and type of organisation providing education and training	University of Bonn, Agricultural Science and Resource Management in the Tropics and Sub-tropics (ARTS), Bonn, Germany
Dates	1996-97
Title of qualification	**Master's Degree in Economics**
Principal subjects/occupational skills covered	Agricultural Economics
Name and type of organisation providing education and training	Tribhuvan University, Institute of Humanities and Social Sciences, Patan Mulitple Campus (PMC), Patan, Nepal
Dates	1992-95
Title of qualification awarded	**Bachelor's Degree in Agriculture**
Principal subjects/occupational skills covered	Soil Science
Name and type of organisation providing education and training	Tribhuvan University, Institute of Agriculture and Animal Sciences (IAAS), Rampur Campus, Chitwan, Nepal
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Title of qualification	**Bachelor's Degree in Physics**
Principal subjects/occupational skills covered	Physics and mathematics

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Title of qualification awarded	**Proficiency Certificate in Physics**
Principal subjects/occupational skills covered	Physics and mathematics
Name and type of organisation providing education and training	Tribhuvan University, Institute of Science and Technology, Kathmandu Valley Campus (KVC), Lalitpur, Nepal
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Title of qualification awarded	**School Leaving Certificate**
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Name and type of organisation providing education and training	HMG Board, Lalitpur Secondary Boarding School (LSBS), Lalitpur, Nepal

Publications

Scientific Journal

(peer reviewed journals)

1. **Shrestha, S.**, Asch F., Brueck, H., Dusserre J., Ramanantsoanirina A., 2012, Phenological responses of Upland Rice Grown Along an Altitudinal Gradient. (under review in Environmental and Experimental Botany)
2. **Shrestha, S.**, Asch F., Dusserre J., Ramanantsoanirina A., Brueck, H., 2012, Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes. (Accepted in Field Crops Research)
3. **Shrestha, S.**, Brueck, H., Asch, F., 2012. Chlorophyll Index, Photochemical Index and Chlorophyll Fluorescence Measurements of Rice leaf Supplied with Different N levels. (Accepted in Journal of Photochemistry and Photobiology B: Biology) in press, available online
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Conference oral presentations	1. **Shrestha S.**, Asch F., Dusserre J., Ramanantsoanirina A., 2011, Yield Stability and Genotype x Environment Interactions of Upland Rice in Altitude Gradient in Madagascar. Tropentag 2011, October 5-7, Bonn, Germany. 2. **Shrestha S.**, Asch F., Bruck H., Ramanantsoanirina A., Dusserre J. 2011, Phenological Responses of Upland Rice grown Along an Altitude gradient. International conference on crop improvement, ideotyping and modeling for African cropping systems under climate change (CIMAC), 7-9 February, Hohenheim, Stuttgart, Germany. 3. Ramanantsoanirina A.,Dusserre J., Raboin L. M., **Shrestha S.**, Brueck H., Asch F. 2011, Temperature constraint in Upland rice improvement in the High Plateau of Madagascar. International conference on crop improvement, ideotyping and modeling for African cropping systems under climate change (CIMAC), 7-9 February, Hohenheim, Stuttgart, Germany.
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1. **Shrestha, S. P.**; Tripathi, B. P.; and Acharya, G. P. (2000). Long-term Soil Fertility Management in Maize/Finger Millet System in the Marginal Bari Lands of Western Hills of Nepal (1997-1999). Lumle Seminar Paper 2000. Kaski, Nepal: Agricultural Research Station, Lumle
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Books and booklets	1.	Soil Erosion and Balance of Plant Nutrient Loss from the Bari Land of Middle Hills of Nepal (in Nepali) Dr. BP Tripathi and **SP Shrestha**
	2.	Soil Fertility Management and Productivity in Rice-Wheat, Upland rice-Blackgram, Maize-Finger millet cropping system in the Hills (in Nepali) Dr. BP Tripathi, **SP Shrestha** and GP Acharya
	3.	Strategies for Soil Fertility Research in the Hills of Nepal (in English) RK Shrestha, SL Maskey, B Shrestha, BP Tripathi, RC Munankarmy, YG Khadka, EM Bhattarai, **SP Shrestha**

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