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THESE

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Cécile JULIA

CIRAD-Département BIOS, Umr AGAP (équipe PAM)

THERMAL STRESSES AND SPIKELET STERILITY IN RICE: SENSITIVE PHASES AND ROLE OF MICROCLIMATE

STRESS THERMIQUE ET STERILITE DES EPILLETS CHEZ LE RIZ : LES PHASES SENSIBLES ET LE ROLE DU MICROCLIMAT

Soutenance le 20 décembre 2012 devant le jury composé de:

Bruno ANDRIEU	Directeur de recherche INRA (Thiverval-Grignon)	Rapporteur
Folkard ASCH	Professeur à l'Université d'Hohenheim (Allemagne)	Rapporteur
Thierry SIMONNEAU	Directeur de recherche INRA (Montpellier)	Examinateur
Olivier TURC	Chargé de recherche INRA (Montpellier)	Examinateur
Vincent ALLARD	Chargé de recherche INRA (Clermont-Ferrand)	Examinateur
Michael DINGKUHN	Chercheur CIRAD (Montpellier) & IRRI (Philippines)	Directeur de thèse

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ABSTRACT :

Cultivated rice (*oryza sativa*) is a staple crop facing production uncertainty because of future climate changes. Predicting how the future climates will impact on rice is necessary to make appropriate decisions to cope with it.

In this context, thermal stress is one of the main abiotic stress to address. At the reproductive stage, rice spikelets are sensitive to cold and to heat which can lead to spikelet sterility. However, it is not the air temperature but the temperature of the sensitive organ itself during some specific sensitive stages that is involved.

Cold affects spikelet fertility through 1) meiosis disruption during the early microspore stage (sensitive organs enclosed by the leaf sheaths and exposed to the temperature of the floodwater and not the atmosphere), 2) incomplete panicle exertion (insufficient peduncle elongation). Heat induces sterility mostly by disrupting pollination and reducing pollen fertility during anthesis (sensitive organ located at the top of the canopy and exposed to air temperature)

Coping with thermal stress involves different strategies: physiological tolerance of a particular variety; temporal escape of the stress by phenology and time of day of anthesis (TOA) adjustments; and stress avoidance through microclimate generated by crop architecture and transpiration.

This Ph.D. aims to characterize the effect of environment on 1) phenoly, 2) TOA, and 3) temperature difference between air at 2m and panicle during flowering (canopy structure taken into account). To attain this goal, the same experiment was conducted with four rice varieties irrigated and grown in four different climatic environments (Philippines dry season, Senegal dry-hot and dry-cold seasons, France temperate summer) in 2009-2010. Standard meteorological (T, RH, wind speed, solar radiation at 2m from ground) and micrometeorological (Twater, T inside canopy, T and RH in the panicle layer) data were made in parallel of measurements of phenology, canopy architecture, and organ temperature with infra red (IR) thermotracer during flowering.

Even though few varietal differences were observed within a site, a great variability of TOA response to environment was shown and explained with Tair(min) and VPD (Vapour Pressure Deficit) observed before anthesis. However, anthesis duration is stable and limited to 2 hours per day.

Architecture and canopy transpiration rate generate a microclimate where sensitive organs temperature can sensibly differs from air temperature measured at 2 meters from the ground.

During anthesis, difference between panicle and air temperature (TD) observed varied between -9.5 and +2°C. TD was explained mostly by transpiration and modeled by VPD and Tair.

We then insist on the necessity to take into account TD in models predicting spikelet sterility, by showing the significant correlation of sterility observed at maturity (due to cold and heat) and the panicle temperature (Tp) during sensitive stages of the reproductive phase across sites and climates, what was previously not possible using only Tair. Those results showed that for irrigated rice, humid and moderately hot environments are more subject to heat stress sterility than very hot but dry environments because of panicle and canopy transpiration is favored by high VPD.

Ultimately, to answer the need in evaluating the impact of different climate change scenarios and the adaptation of crop response to those changes, RIDEV V.2 crop model (predicting phenology, TOA, Tp and spikelet sterility) was developed to integrate the previously presented results. This model is described and a simulation example is commented. RIDEV Tp submodel is presented and compared to another Tp model (IM2PACT) developed independently with a different approach in Japan. Those two models are robust and future collaboration will lead to complete model validations and maybe integration in a new modeling tool.

The results of this study will enable in a near future to 1) help breeders providing them new interest traits for thermal tolerance, and 2) define geographic zoning for high heat stress risk for irrigated rice, for present and future climate change scenarios. Complementary studies are needed to apply this approach to non irrigated system.

Keywords: *Oryza sativa* L., Thermal stress, Coping mechanisms, microclimate, Spikelet sterility, Modeling.

RESUME :

Le riz (O*ryza sativa* L.), culture céréalière la plus importante au monde, est menacé par les changements climatiques. Il est essentiel de déterminer de quelle manière et dans quelle mesure sa production sera affectée par ces changements afin de faire face en prenant les décisions stratégiques adéquates dans le futur.

Le stress thermique est un des facteurs de stress abiotique majeur à prendre en compte. Les inflorescences de riz sont sensibles au froid et à la chaleur ce qui se traduit par une stérilité des épillets à floraison. Ce n'est cependant pas directement la température de l'air qui est en cause mais la température des tissus à des stades de développement précis.

Le froid est source de stérilité *via* deux principaux processus qui sont (1) la perturbation de la méiose au début du stade microspore (organes sensibles localisés à la base et soumis à la température de l'eau), et (2) une exersion paniculaire incomplète (élongation des entrenœuds ralentie). La chaleur affecte principalement la pollinisation et la fertilité du pollen au moment de l'anthèse (organes sensibles localisés au sommet de la canopée et soumis à la température de l'air).

Pour faire face au stress thermique, la plante dispose de plusieurs stratégies : la tolérance physiologique d'une variété particulière; l'échappement temporel au stress par l'ajustement de la phénologie et de l'heure de l'anthèse; et l'évitement du stress par le microclimat généré par la culture grâce à son architecture et à sa transpiration.

Cette thèse a pour but de caractériser l'effet des composantes climatiques sur 1) La phénology, 2) l'heure de l'anthèse et 3) la différence de température entre l'air à 2m et la panicule à floraison (structure de canopée prise en compte).

Pour cela, le même essai a été mené au champ sur quatre variétés de riz irrigué cultivées dans quatre environnements climatiques contrastés (la saison sèche aux Philippines, les saisons sèches chaude et froide au Sénégal, et l'été tempéré du sud de la France) en 2009-2010. Des données météorologiques classiques (T, RH, vitesse du vent, radiation à 2m du sol) ainsi que des données micrometeorologiques (Teau, T dans la canopée, T et RH dans la couche paniculaire) ont été relevées en parallèle de mesures phénologiques, architecturales, et de température d'organe à l'aide de mesures Infrarouges prises à floraison.

Bien que peu de différence variétale ait été observée au sein de chaque site, une grande variabilité de l'heure de l'anthèse en réponse à l'environnement a été mise en évidence et expliquée par Tair(min) et VPD (Vapour Pressure Deficit) observés antérieurement à l'anthèse. La duree de l'anthese, par contre, est stable et limitée a environ 2 heures par jour.

L'architecture et le taux de transpiration de la canopée génèrent un microclimat dans lequel la température des organes reproducteurs peut considérablement différer de la température de l'air à deux mètres du sol. Au moment de l'anthèse, des différences de température (TD) entre -9.5 et +2°C ont été observées. Cette différence est principalement expliquée par la transpiration et modélisée par le VPD et Tair. Dans un second temps, on souligne la nécessité de prendre en compte TD dans les modèles de prédiction de stérilité paniculaire en montrant la corrélation significative entre la stérilité (chaud ou froid) observée à maturité et la température paniculaire aux stades sensibles au travers des différents sites et climats, ce qui était auparavant impossible en utilisant seulement Tair. Ces résultats montrent que pour le riz irrigué à floraison, un climat moyennement chaud et humide est plus dangereux qu'un climat très chaud mais sec en termes de risque de stérilité due à la chaleur, à cause de la transpiration de la panicule et de la canopée impulsées par un fort VPD.

Enfin, aux vues de la nécessité d'évaluer l'impact des différents scenarios climatiques et l'adaptation des variétés à ceux-ci, le modèle de culture RIDEV V.2 (qui prédit la phénologie, l'heure de l'anthèse, la température de la panicule et son taux de stérilité) a été développé afin d'intégrer les résultats présentés précédemment. Ce modèle est décrit de façon complète et un exemple de simulation est présenté. Les simulations de TD par RIDEV sont comparées à celles d'un autre modèle (IM2PACT) à l'approche différente et développé indépendamment au Japon. Ces deux modèles s'avèrent robustes et de futures collaborations mèneront à une validation complète de chaque modèle voire une intégration de ceux-ci à un nouvel outil.

Les résultats de cette étude permettront dans un futur proche 1) d'aider les sélectionneurs en apportant de nouveaux traits d'intérêts, et 2) de définir un zonage des territoires à haut risque de stress thermique pour le riz irrigué, pour des scénarios climatiques actuels et anticipés. Des études complémentaires seront nécessaires pour permettre l'application de cette approche aux systèmes non irrigués.

Mots clés: *Oryza sativa* L., Stress thermique, Mécanismes d'évitement, Microclimat, Stérilité des épillets, Modélisation.

CONTEXT, OBJECTIVES AND OVERVIEW OF THE THESIS

CONTEXT

Hosting project

This study was part of the RISOCAS project (developing RIce and SOrghum Crop Adaptation Strategies for climate change in vulnerable environments in Africa) (RISOCAS, 2011) funded by the BMZ (German Ministry of Economic Cooperation and Development) and coordinated by the Institute of Crop Production and Agroecology in the Tropics and Subtropics of Hohenheim University (Stuttgart, Germany). In a climate change context, this project aimed to develop strategies for coping with the increasing climate variability and weather extremes. To attain that goal, a broad range of varietal types of rice and sorghum were studied on existing climatic gradients that cover expected ranges of change, such as temporal/intra-annual gradients (irrigated rice in Senegal), latitudinal gradients (sorghum on a N-S transect in Mali) and altitudinal gradients (upland rice in Madagascar), in collaboration with AfricaRice, CIRAD (Agricultural Research for Development, Montpellier, France), IER (Institute for Rural Economy, Bamako, Mali), and FOFIFA (National Center for Applied Research for Rural Development, Antananarivo, Madagascar). Relevant meteorological data, site-specific soil characteristics and water balances, and parameters of growth and yield were monitored. These data are being used by the project consortium to identify valuable traits and ideotype concepts for varietal improvement and to develop/improve, calibrate and field validate crop models.

The points of departure for modeling were the existing models RIDEV in its original now technically and scientifically outdated version (Dingkuhn *et al*. 1995a, Wopereis *et al*. 2003), SARRAH (Dingkuhn *et al.* 2003, Heinemann *et al*. 2008) and SAMARA (Dingkuhn 2011 unpublished) crop models. The resulting tools will allow predictive applications in the context of climate change scenarios.

Many questions remain open on how the new abiotic stress combinations associated with climate change translate into phenology and yield and which varietal types are better adapted to the expected variation patterns of temperature, water availability, and atmospheric CO_2 concentration. The RISOCAS project aims at predicting the appropriateness of complex trait combinations by providing physiological models that are well validated for targeted environments.

The present multi-site study is one of four Ph.D. theses supported by RISOCAS and is focused on irrigated rice and thermal stresses at reproductive stage. Its role within RISOCAS is to:

- gather microclimate data along the soil-water-canopy-air gradient and organ temperatures across 4 contrasting environments;
- to link them to crop performance, in particular spikelet sterility; and
- to integrate the results to a crop model.

The thesis research was based at CIRAD (Phenotypic plasticity and Adaptation of perennial Monocots team of the AGAP unit, BIOS Department). The work was implemented at field research sites at AfricaRice in Senegal, the International Rice Research Institute (IRRI) in the Philippines and the Centre Francais du Riz (CFR) in France.

Rice in the world

Cultivated rice is an annual cereal with a C3 metabolism and belongs to the *Poaceae* botanical family. The two cultivated species *Oryza sativa* L. (Asian rice) and *Oryza glaberrina* Steud. (African rice), were respectivelely domesticated around 10 000 BC in China and 2500 BC in the Niger inland delta in Mali (MacNeish *et al*. 1995). *O. sativa* has two major genetic groups (sometimes classified as subspecies) called *japonica* (short-grained, tropical upland and temperate lowland rice) and *indica* (long-grained, tropical, mainly lowland rice). Around 90% of the rice produced in the world is cultivated in Asia at sometimes very high cropping intensity (3 growing seasons per year in some parts of South East Asia). Africa and Latin America provide around 7% of global production, at about 25 M tons each *per annum* (Seck *et al*. 2012). *O. sativa* cultivated under irrigated, flooded conditions represents around 50% of the world rice grown area (158 M hectares grown with rice in 2009) and provides 75% of the global rice supply (700 M tons produced in 2009) (IRRI, AfricaRice and CIAT 2010). During the last 50 years, world rice production has more than tripled and average yield more than doubled, to attain 4.3 tonnes per hectare in 2010 (China being the largest rice producer with 197.2 M tons and Australia the most productive with 10.8 t/ha) (FAOSTAT 2011).

Irrigated flooded systems are intensive in terms of management and fertilizer inputs and attain higher yields than other rice systems. Developed countries with highest yields, such as Korea, Japan, the USA or Australia, grow rice exclusively under irrigated conditions (Seck *et al*. 2012). This is also true for all temperate rice environments and (sub)tropical-arid environments such as the Sahel and Egypt. World population growth keeps increasing the demand for rice (Wild 2003) but arable land surface is limited or even diminishing in key areas such as China because of urbanization, or in marginal rainfed ecosystems due to unsustainable intensification and soil degradation (Daily *et al.* 1998). There is no doubt that the

much needed increases in global rice production must come from intensification of production (thus increasing attainable yields where they are currently low) and by reducing the yield gap (thus achieving the locally attainable yield). To attain food security, this must be achieved in a climate change context, which is a challenging goal to the global rice research and development community (Lal 2000).

The potential impact of research on yields can be expected to be greatest in environments and systems where (1) the biophysical potential for production is high, namely through the abundance of water high climatic potential for production; and (2) actual yields frequently fall short of this potential through yield reducing factors that can theoretically be overcome. One such group of situations is high-radiation, irrigated environments affected by thermal stresses that can be overcome with improved crop tolerance, avoidance or temporal escape strategies (Dingkuhn *et al*. 1995b, Dingkuhn and Miezan 1995, Dingkuhn 1995). The present thesis research addresses this problem.

Rice cycle and reproductive stage

The rice crop takes between 3 and 6 months from germination to maturity, depending on the variety and the environmental factors (Yoshida 1981). The cycle is divided in 3 main phases, namely, the vegetative phase, the reproductive phase and the maturation or grain filling phase. The vegetative phase starts at germination and is characterized by the development of the roots and the leaf area needed for resource acquisition, and tillering which sets the potential number of panicles at maturity (Yoshida 1981). The end of the vegetative phase is marked by a photoperiod-sensitive period (PSP) which can significantly delay panicle initiation under long-day conditions if the genotype is sensitive to them. The PSP can last between 2 and 200 days in extreme cases (Yoshida 1981). The PSP ends with panicle initiation: The apical meristel stops initiating leaves and instead initiates phytomers constituting the panicle.

The reproductive stage starts at the panicle initiation when the panicle begins to develop inside the sheath, and it ends with anthesis. During the reproductive phase, the panicle goes through differentiation (successive differentiation of neck node, rachis and branches, spikelets, and pollen) and stem elongation (internode elongation), followed by heading (panicle emergence from the sheath) and flowering or anthesis (opening of spikelets and mostly autogamous pollination). A panicle is composed of a base marked by a bulge (neck node), a primary axis (rachis) that supports several primary and secondary branches, holding pedicels at the end of which are the spikelets. The spikelets are composed of two envelopes (lemma and palea) enclosing stigma and pistil (Figure 1 a,b).

The initiation of the panicle primordium starts about 1 month before heading (depending on the environment), and the panicle differentiation ends when the pollen is fully matured (Yoshida 1981). At that time the panicle is still enclosed in the sheath and located at the bottom of the canopy. It is then pushed up to near the top of the canopy by internode elongation, particularly the uppermost internode

or peduncle (Figure 1a). For a given panicle, flowering starts between 0 and 3 days after heading and occurs on only a few hours every day for different successive spikelets, during a period of 4-10 days depending on the variety and the environment (Julia and Dingkuhn 2012). At anthesis, the lemma and palea open, and anthers dehisce and from then on remain outside the spikelet. Anther dehiscence usually occurs just before or when the lemma and palea open. Consequently, many pollen grains fall onto the stigma of the same spikelet, resulting if self-pollination or autogamy. After about 2h the spikelet closes again, leaving the anthers outside. (Yoshida 1981). Pollen grains deposited on the stigma germinate and rehydration to allow the pollen tube to elongate. Fertilization is normally achieved 5-6 hours after anthesis (Yoshida 1981).

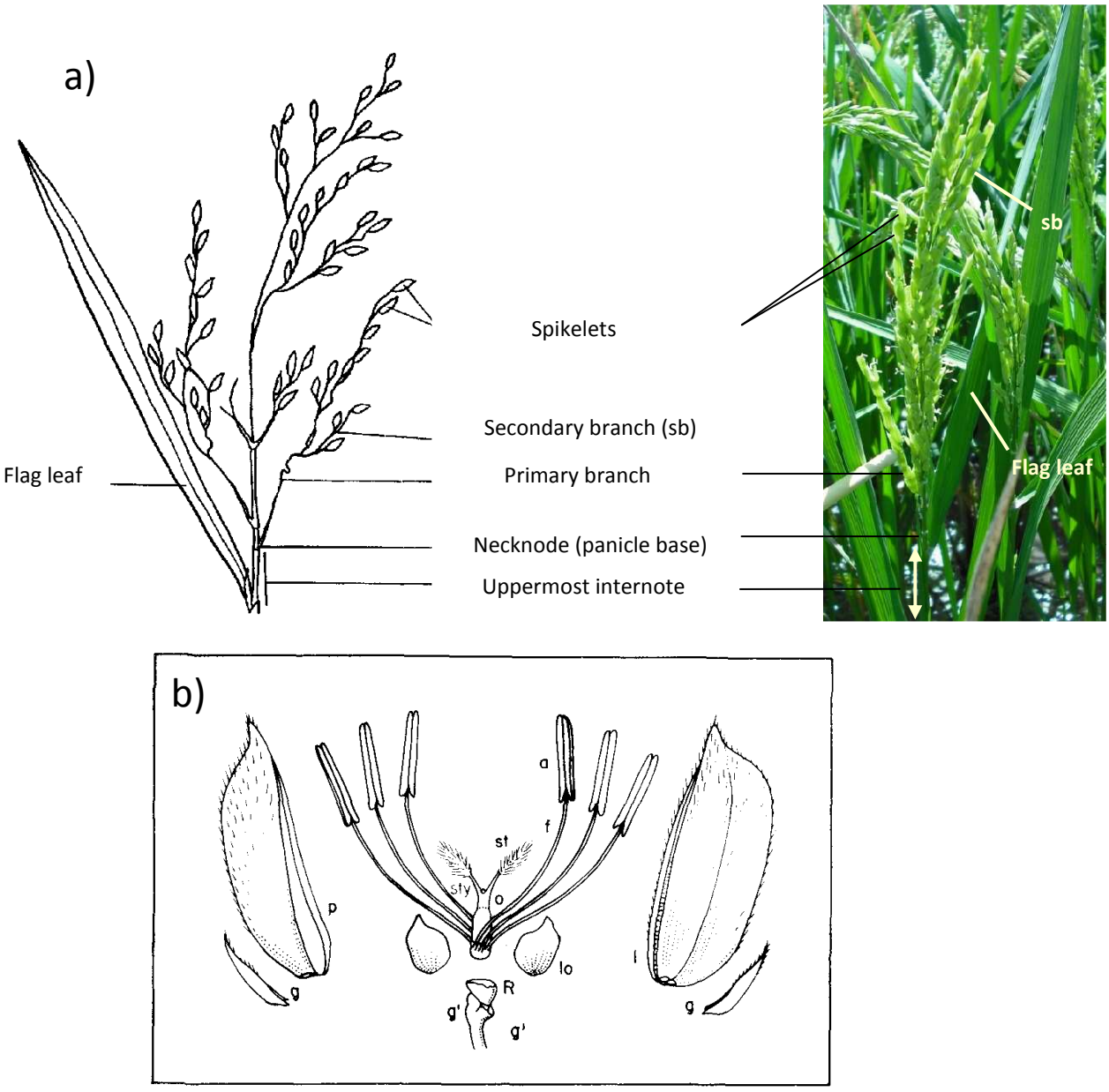

Figure 1 a,b: Detail of the rice panicle morphology, scheme and field photo (Julia field experiment in Camargue, France, 2010) (a); and rice spikelet morphology from Yoshida (1981) (b). l=lemma, p= palea, lo= lodicules, o=ovary, st=stigma, sty= style, a= anther, f= filament.

Rice crop performance under thermal stress

Rice is a tropical plant but has wide genetic diversity, allowing it to be cultivated between the 53°N parallel (in Russia) and the 40°S parallel (in Argentina) (Nguyen 2005), including diverse climates ranging from tropical, subtropical humid or arid to temperate conditions. Rice is also grown at more than 2000 m altitude (e.g., Nepal) and under arid conditions if irrigated (e.g., Sahel region and Egypt). Some of these climates (like temperate, subtropical or high-altitude) cause thermal stresses, particularly if cold or heat spells coincide with sensitive phases of rice phenology, causing injury and yield loss. Along with water-related constraints (drought or water scarcity for irrigation, and sometimes submergence; Bouman *et al.* 2007), thermal stresses is a foremost abiotic constraint and largely determines where rice can be cultivated and where not.

Thermal stress and climate change: actual situation and future implication for rice production

Chilling stress

Low temperature and cold damage are associated with rice grown under high latitude (temperate climate), the cool season in arid-tropical environments and tropical high-altitude regions. Mid-altitude mountainous zones are frequently cultivated with upland rainfed rice. This ecosystem is important for local food security but of minor global economic importance (less than 5% of the world rice production according to Seck *et al*., 2012). High-altitude rice is generally irrigated in terraced systems (e.g., Nepal, Madagascar). Rice-growing areas in the temperate environments (latitude higher than 23°27'N or 23°27'S) represent about 20% of the world's total rice area and are located in East Asia (China, Korea, Japan), Central Asia, North America (USA), South America (Uruguay), Australia, Mediterranean North Africa, and some parts of Europe (Italy, France, Spain). In these environments, mainly temperate-*japonica* rices are grown under flooded conditions.

In Asia, cold damage is regularly reported in several countries like China, South Korea, Japan and more recently in Vietnam and India. In the north-east provinces in China for example, cold damage due to low temperatures occurs frequently and can reduce grain production by 30%. The estimated yield loss due to low temperature is 3-5 million tons per year (Temperate Rice Research Consortium 2007).
In West Africa, cold is also a problem during the cold season for arid or semi arid areas like the Sahel (Dingkuhn *et al.* 1995a,b). Rice double cropping calendars need to be carefully managed to avoid cold-induced sterility (Dingkuhn, 1995), and changes in climatic patterns can increase the cold damage and may lead to changes in cropping calendars and cropping systems (De Vries *et al.* 2011).

Global warming and heat stress

During the last century, Asia, Africa and South America experienced a 0.7–1.0°C increase in average temperature and climate models predict an increase of global temperature due to climate change of 2°C to 4.5°C by the end of the 21st century (Salinger 2005; IPCC 2007). The temperature rises are likely to be higher locally (particularly in vulnerable areas like subtropical regions) and heat stress may become a major threat for staple crops like rice (IPCC 2007).

Higher temperatures can reduce rice yield and grain quality (Fitzgerald and Resurreccion 2009, Kim *et al.* 2011) by altering phenology (Mitchell *et al.* 1993; Peng *et al.* 2004; Craufurd *et al.* 2009) and inducing heat injury at during periods of extreme temperatures (Spiertz *et al*. 2006). Crop model applications to the current climate change scenarios predict a global mean rice yield reduction by 41% by the end of the century (Ceccarelli *et al*. 2010). In line with these simulations, Horie *et al.* (1996) predicted that the yield reduction of current rice varieties in southern Japan would be up to 40% for future climate change scenarios. In a preliminary study using NASA Prediction of Worldwide Energy Resource (POWER), meteorological station data, and air temperature thresholds found in the literature (Tair max=34°C for flowering), Laborte *et al.* (2012) showed that many areas cultivated with rice in Asia and western Africa have experienced frequent heat stress events during the sensitive reproductive stage between 1983 and 2011 (Figure 2). Indeed, high temperature and heat damage were regularly reported in many countries including major rice producers like India (Mohandrass *et al*. 1995) or China (Fan *et al*. 1979, Huang *et al*. 2004, Tian *et al*. 2009, Tao *et al*. 2012). Heat affects rice based cropping systems from irrigated Sahelian rice in West Africa (Dingkuhn and Sow 1997, Haefele *et al.* 2002) to irrigated systems in Japan (Hasegawa *et al*. 2009, 2011).

The increase of mean temperature is the result of both higher minimum and maximum temperatures. High temperatures at night or during the day act differently on the plant. Several studies showed that higher night-time temperatures reduced rice yields (Kukla and Karl 1993, Ziska and Manalo 1996, Peng *et al*. 2004, Sheehy *et al*. 2005) by 10% for every 1 ^{o}C increase in minimum temperature (Peng *et al*. 2004) and can also affect grain quality (Lanning *et al*. 2011). Kanno and Makino (2010) showed a positive effect of low (but not stressful) night temperatures on yield.

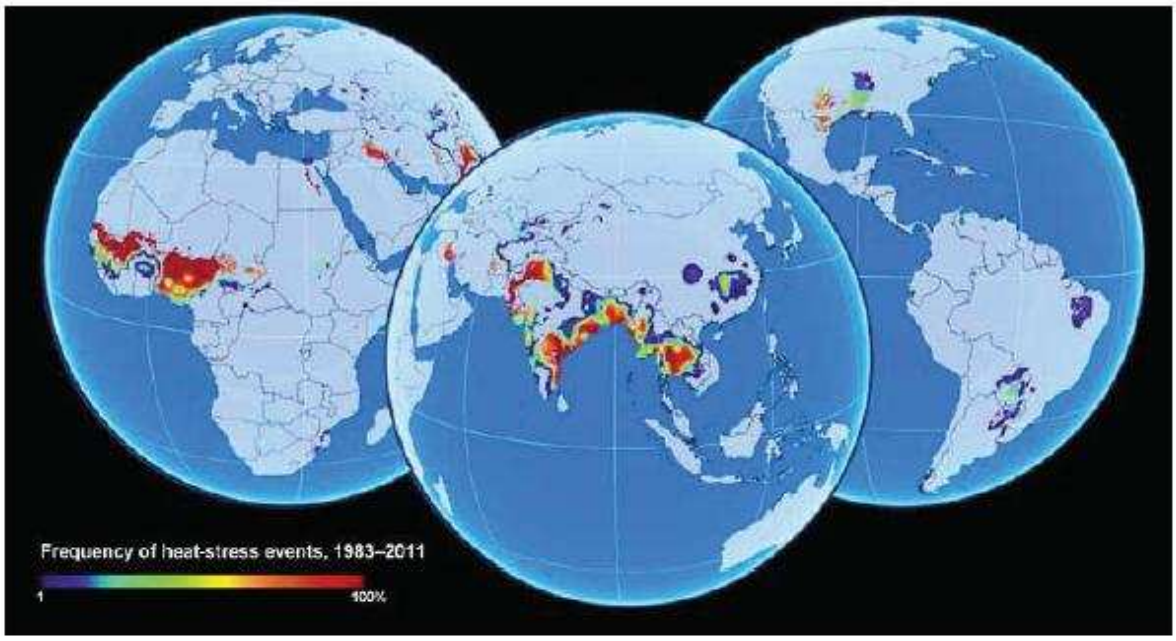

***Figure 2*: Frequency of heat event potentially damaging to rice crop across the world and calculated between 1983 and 2011 (from Laborte et al. 2012).**

Rice sensitive stages to thermal stress during reproductive phase

Extreme temperatures affect plant phenology, growth and reproduction, and physiological temperature thresholds define the optimal temperature window for the rice crop. The size of the window depends on the phenological phase and the critical physiological processes happening during the phase. The reproductive stage has the smallest temperature window of the cycle (Yoshida 1981). Krishnan *et al*. (2011) summarized maximum temperature thresholds for each stage of the rice cycle. The entire reproductive phase (from panicle initiation to flowering) is sensitive to thermal stress, but there are time windows that are particularly sensitive and directly impact on spikelet fertility and ultimately, on yield if it is sink limited.

Figure 3 summarizes the thermally sensitive stages and time windows for rice. Rice is very temperature sensitive from early pollen development to flowering, in particular the early microspore stage when microspores (young pollen grains) are being formed and released from the tetrads after meiosis (Satake and Hayase 1970). At this stage, cold floodwater can cause sterility because the reproductive organs are still located at the bottom of the culm prior to stem elongation, and heat stress is unlikely to occur in this micro-environment (Dingkuhn *et al*. 1995b). Particularly heat sensitive is the anthesis stage (Satake and Yoshida 1978, Nishiyama and Blanco 1980, Nishiyama and Satake 1981, Baker *et al*. 1992, Jagadish *et al*. 2007), when the reproductive organs are directly exposed to the atmosphere. The panicle is thus the most sensitive organ of the rice plant to extreme temperatures, and within the panicle, the male gametophyte is the most sensitive part (Saini 1997; Mamun *et al*. 2006). A similar

sensitivity of the female gametophyte has not been reported. The resulting sterility is thus male sterility. This male sterility can be caused by 1) failure to develop viable pollen, namely due to chilling at the microspore stage; 2) or failure of pollination or pollen tube outgrowth after pollination during anthesis because of cold but particularly heat. A third cause can be failure of anthesis itself, if spikelets cannot open when failing to emerge in time from the enclosing flag leaf sheath, which can also be caused by thermal stresses but mostly, by drought (Jagadish *et al*. 2011).

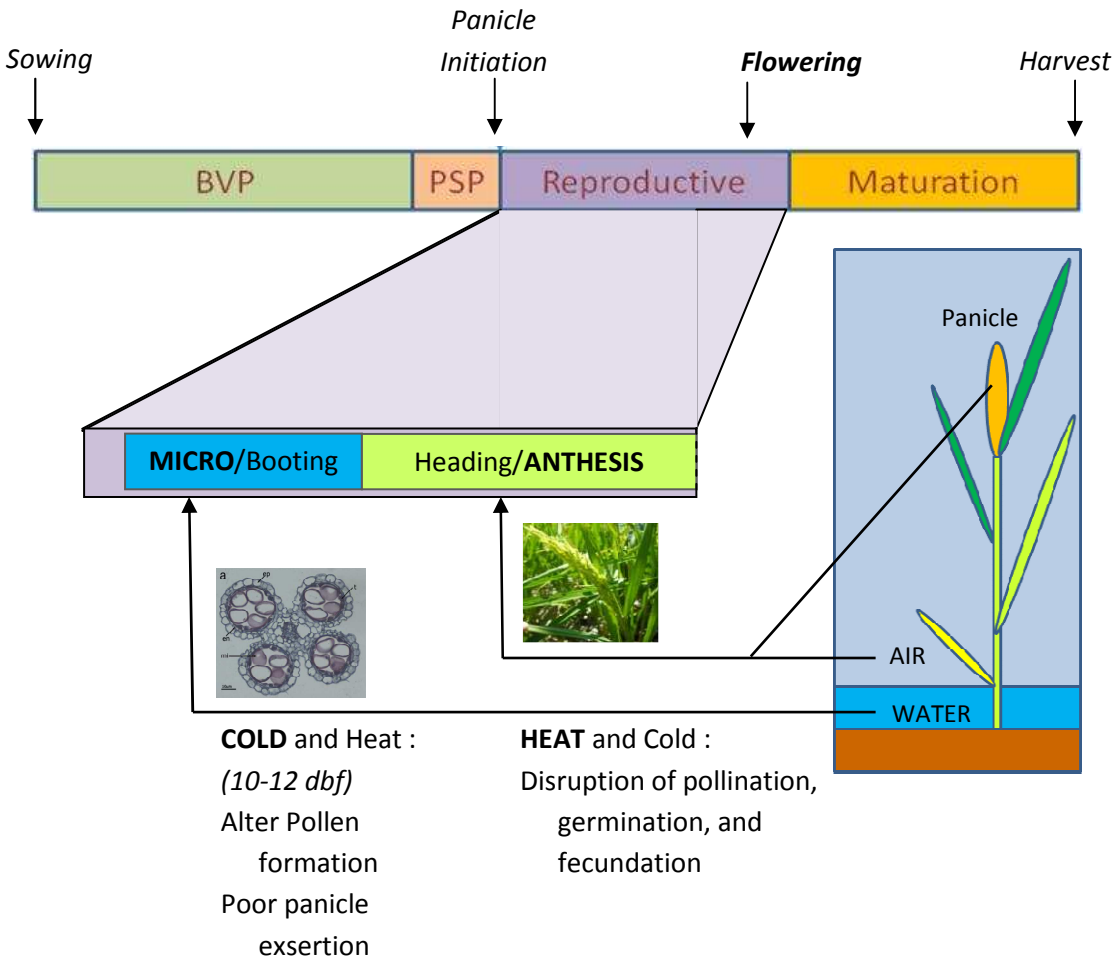

***Figure 3** : Summary of rice heat and cold sensitive stages replaced in the whole cycle and the reproductive phase. Capital and bold letter indicate the most important stress and corresponding stage. Arrows link the stage and the physical location of the thermal sensitive organ (panicle) within the plantation (rice plant drawing on the right). BVP =Basic Vegetative Phase ; PSP= PhotoSensitive Phase; MICRO= Microsporogenesis; dbf= day before flowering.*

Physiology behind cold injury

Early work reported that minimum air temperature ranging between 15-20°C at microspore stage can drastically decrease rice production (Satake 1976, Yoshida 1981). Mamun *et al.* (2006) presented a review of current knowledge on low temperature damage on microspore development, including cellular

scale observations. When exposed to cold at microspore stage, anther layers and microspores suffer from distortions, deformations and lesions, (*cf.* Light microscope images figure 4). The microspore cell walls are not well formed and show tapetal hypertrophy (cellular layer of anther envelope abnormally thick). Cold treatment also induces excessive starch accumulation in the anther endothecium (inside cellular layer of anther wall) and premature callose degradation around the developing microspores (Mamun *et al.* 2006). Consequently, there is a physiological breakdown of regulatory and possibly metabolic processes at the cellular level.

At the whole-plant scale, cold affects phenology (temporal organization of development including flowering time) (Dingkuhn and Miezan 1995; Dingkuhn 1995). This extends the duration of the cycle and in many situations increases the probability of chilling stress affecting the sensitive stages. Cold can also inhibit organ elongation processes in general (stunting) and peduncle elongation in particular (Chung *et al*. 1979, Hamdani *et al*. 1979) resulting in incomplete panicle exertion at heading. Spikelets remaining enclosed in the sheath are mostly infertile.

All these stresses effects affect yield through sink limitation (number of fertile spikelets developing into grains). Chilling effects on assimilate source capacity are generally less severe than those on the sink. The earliest and latest stages of the plant cycle are most sensitive, resulting in either failure of crop establishment or incomplete grain filling. These phenomena are not discussed here because they are outside the scope of this thesis research.

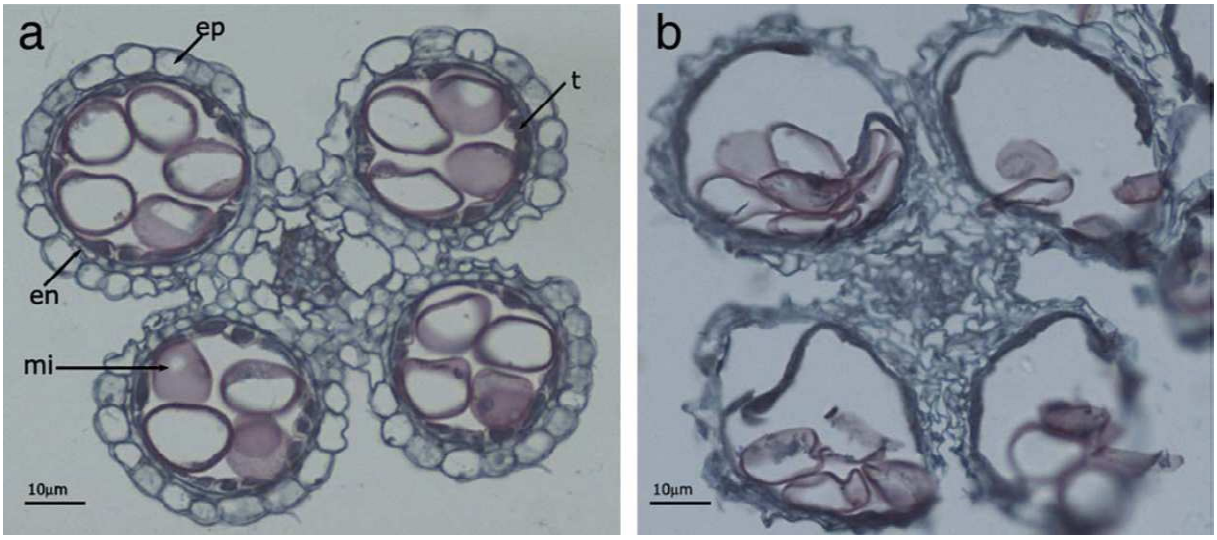

***Figure 4 a, b*: Light microscope images of transverse sections of microsporangia showing overview of anther at vacuolated stage from Mamun et al. (2006). (a) Anther growing in normal temperature comprise spherical microspores and well-shaped anther cell layers. (b) Chilling stress drastically altered the shape and development of the microspores and other cell layers. Ep= epidermis; en= endothecium; t= tapetum; mi= microspore.**

Genetics and breeding work on cold tolerance

At the molecular level, in response to cold, rice plants express a series of genes that may be related to adaptive responses. Transcription factors (TF) play important roles in enhancing plant cold tolerance and Wang *et al*. (2003) have identified a TF (OsbHLH1) involved in the cold signal-transduction pathway. A recent study conducted at CIRAD demonstrated that the zink-finger protein coded by the SAP network gene, acting as TF, may have a key role in abiotic stress tolerance including chilling (Ben Saad et al., 2011). The ortholog of this gene from *Aeluropus littoralis*, a halophyte, was over-expressed in Nippon Bare rice and rendered the plant highly tolerant to salinity, drought, chilling and oxidative stress, while expressing a large number of resident genes known to be related to abiotic stress tolerance. Many other genes are known to contribute to chilling tolerance in rice during vegetative growth, but little is still known on the genetic control of the specific processes affected by chilling during reporoductive development.

Cold is a threat at every stage of the cycle (most sensitive stage being microsporogenesis) and improving cold tolerance in rice is very important in term of rice breeding. Degree of panicle exertion is an important trait used for cold screening, tolerant varieties having a better panicle exertion than sensitive ones (Nanda and Seshu 1979, Pandey and Gupta 1993). Cold tolerance at plant reproductive stage is a complex trait controlled by many genes. Three regions of the rice genome have been identified to have a direct link to cold tolerance (Temperate Rice Research Consortium, 2007). QTLs and genes have been detected for cold tolerance traits. A common strategy in breeding for cold tolerance is to cross *japonica* and *indica* type varieties to obtain cold tolerant varieties which maintain high yield, the yield potential traits coming from the *indica* germplasm (Andaya *et al*. 2003, Ye *et al.* 2010, Saito *et al*. 2001, 2010).

In irrigated systems, water and crop management can also contribute to control cold injury. Maintaining deep floodwater in plots reduces day-night temperature differences (increased system inertia) and increases the minimum temperature, thus protecting the young panicle from cold stress at microsporogenesis, especially under high N fertilization (Williams *et al.* 1994). At this stage, the sensitive and developing panicle is located at the base of the plant (inside the sheath) and in the water.

Physiology of heat stress inducing spikelet sterility

At the biochemical level, abiotic stresses such as heat affect protein structure through unfolding and misfolding (which can lead to protein denaturation or aggregation), and reduce enzyme activity including Rubisco, the major enzyme involved in photosynthesis (Prasad *et al*., 2004). Heat also induces membranes dysfunction (higher fluidity of membrane lipids, increased leakage) (Savchenko *et al*., 2002, Falcone *et al*. 2004). Oxidative stress from the production of reactive oxygen species (ROS) in chloroplasts

and mitochondria (Mohammed *et al*. 2009*b*) is involved in most physiological stresses including heat, and is ultimately responsible for cell death following stress. In this respect, the study by Ben Saad (2011) previously mentioned is of interest because the over-expression of the TF gene AlSAP caused tissue tolerance to H_2O_2 in rice, along with increased general abiotic stress tolerance including chilling. Antioxidant and ROS quenching capacity may thus also be an important component of heat tolerance in rice.

Rice is relatively more tolerant to high temperatures during the vegetative phase (Yoshida *et al.* 1981, Prasad *et al.* 2006a) but highly susceptible during the reproductive phase, particularly at flowering stage and during anthesis (Matsui *et al*. 2001, Jagadish *et al.*2008). Many studies have been done in controlled environments and they have shown that maximal (or day-time) temperatures above 34ºC (Satake and Yoshida, 1978; Jagadish *et al*., 2007) or 35ºC (Matsui *et al*., 2001) during anthesis induce spikelet sterility. The stigma, however, is tolerant to heat (Yoshida *et al*. 1981), but high temperatures during rice booting and flowering cause spikelet sterility by reducing the number and the quality of pollen grains produced (Shimazaki *et al*. 1964, Takeoka *et al*. 1992, Sage *et al*. 2008, Endo *et al*. 2009) or released (Prasad *et al*. 2006a) by disrupting anther dehiscence (Matsui *et al*. 2000; Jagadish *et al*. 2010*a*), pollination (Prasad *et al*. 2006a, Jagadish *et al*. 2010*a*), and pollen germination (Endo *et al.* 2009, Jagadish *et al*. 2010*a*).

Heat stress at flowering induces a chain reaction by inhibiting the swelling of pollen grains (rehydration) and also limits anther dehiscence and grains interception by stigmas. The number of pollen grains intercepted by the stigma is correlated with grain fertility and Jagadish *et al*. (2010*b*) showed that 10 to 20 pollen grains need to be intercepted by the stigma to ensure successful pollen germination. Consequently, genotypes producing much pollen have greater fertility.

Strategies to cope with heat stress

Rice strategies to deal with thermal stress (cold or heat) can be divided into two types: Avoidance or escape processes which represent the adaptability to a genotype or cropping system through minimizing the stress vector itself; and real physiological tolerance under exposure to the stress vector, which is a genotypic characteristic and expressed at the tissue level.

Escaping or avoiding the stress

In terms of crop management, shifting planting dates to adapt the crop calendar to changing climate patterns is an obvious possibility, but may be sometimes limited by the length of growing season particularly in double or triple cropping systems (Dingkuhn, 1995; Nagarajan *et al*. 2010). Short duration

varieties are a partial solution of this category. A frequent problem, however, is that heat spells are unpredictable.

Work has been done to characterize the early heading and flowering traits (Jiang *et al*. 2007, Takahashi *et al*. 2009), short duration carries a yield penalty which may be unacceptable in high-input, intensified irrigated system where availability of water generates high yield potential (Jagadish *et al*. 2012). When shifting sowing dates and growing short cycle varieties are insufficient as solutions to heat, genetic improvement is called for. There may be some scope for reducing the yield penalty of earliness, for example. Probably more promising is advancing the time of day of anthesis (TOA). This trait was characterized in both controlled (Ishimaru *et al*. 2010) and field experiments (Kobayashi *et al*. 2010, Julia and Dingkuhn 2012) and early TOA varieties might in the future be developed by breeders.

Previous studies showed that canopy cooling can be an effective avoidance response to high temperature under low relative humidity. No breeding efforts have been made to our knowledge in this area. Panicle or spikelet temperature, and not air temperature, would be the reference in screening for adapted germplasm in this respect (Weerakoon *et al*. 2008, Matsui *et al*. 2007). More attention should be paid to crop architectural avoidance of heat stress affecting the panicle, and the possibility to combine good panicle exertion with hiding it within the shade of the canopy. We found no references in the literature on this issue, however.

Structural and physiological tolerance

Physiological tolerance is expressed under actual exposure of the plant or organ to heat stress that cannot be escaped from or avoided. Tolerance can be intrinsic to the genotype (constitutive) or acquired through a hardening process (inducible response). Among the structural traits involved in heat tolerance are a greater production of viable pollen grains to ensure pollen germination and fecundation (Prasad *et al*. 2005, Matsui *et al*. 2000; Jagadish *et al*. 2010a), and a specific structure of anther allowing a better dehiscence (Matsui *et al*. 2001, Matsui and Omasa 2002).

Physiological response to heat stress is linked to the production of diverse heat shock proteins (HSP) (Hsieh *et al*. 1992, Sung *et al*. 2001, Koo *et al.* 2003) which induce high temperature tolerance to the plant (Wahid *et al*. 2007) thru their molecular chaperone role in protein structure and degradation (Wang *et al*. 2004). (*cf*. summary in Figure 5 below).

In proteomic studies Jagadish *et al*. (2010b, 2011) identified 18 different HSP in spikelets and anthers of N22 (genetic group *aus)*, the international heat tolerant check variety for rice. A great variability in level of expression of HSP was observed in response to high temperature. However, HSP is not the only protection to heat damage. Growth hormones like ethylene, salicylic acid and abscisic acid seem to be involved, and act as antioxidants and provide for thermal membrane stability (Larkindale *et al*.

2005, Mohammed and Tarpley 2009), requiring further studies. Shah *et al*. (2011) gave a insightful review of the different mechanisms involved in physiological heat tolerance of rice, but little information is available on evaporative cooling allowing the sensitive organs to avoid the heat, a mechanism that is particularly effective with abundant water under irrigation.

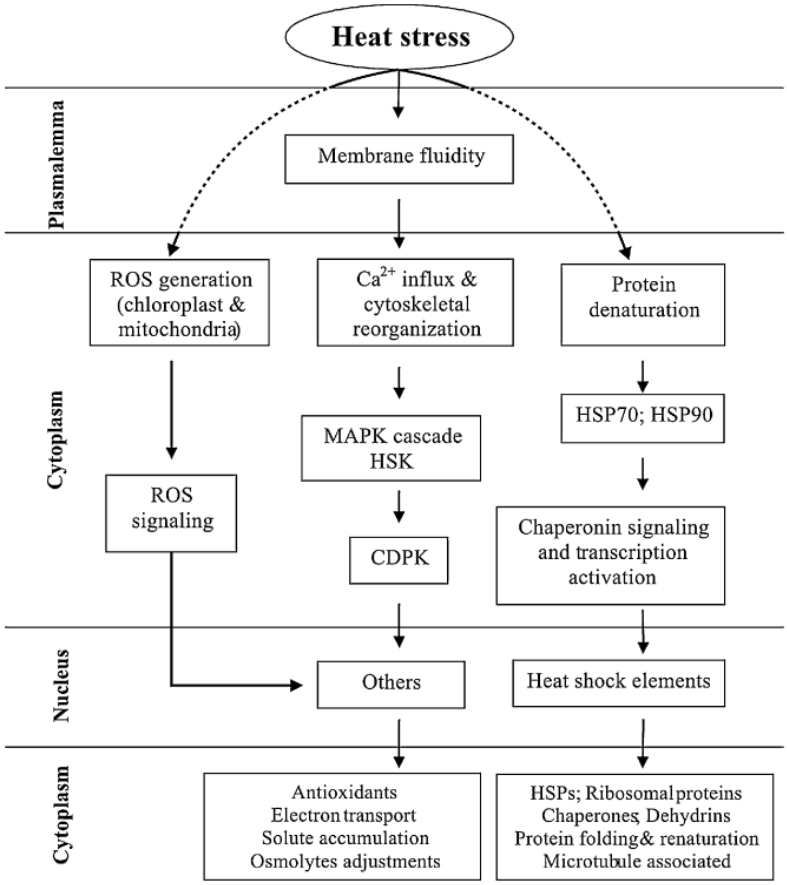

***Figure 5**: Proposed heat-stress tolerance mechanisms in plants. MAPK, nitrogen activated protein kinases; ROS, reactive oxygen species; HAMK, heat shock activated MAPK; HSE, heat shock element; HSPs, heat shock proteins; CDPK, calcium dependent protein kinase; HSK, histidine kinase. From Wahid et al. (2007) and partly adopted from Sung et al. (2003).*

Heat stress interaction with drought and CO_2

Global increase in temperature will be locally associated with other stress vectors such as drought or increasing CO_2 concentration, hence the stress interactions need to be adressed. Indeed, the variability of precipitation and occurrence of extreme weather increases, drought and heat are frequently associated (Easterling *et al*. 2000, Groisman *et al*. 2005, Sun *et al*. 2007, Wassmann *et al.* 2009b). For tobacco and arabidopsis, Rizhsky *et al*. (2002, 2004) showed that drought induces a closure of stomata to reduce water loss by transpiration. In case of simultaneous heat, the canopy cannot evacuate the extra

heat by transpiration cooling, which increases the risk of heat damage. Response to heat stress associated with drought has been recently addressed by Jagadish *et al.* (2011) using proteomics. Even if combined stress did not involve the same molecular responses, it is clear that combined stress decreases rice productivity more than individual stress.

Global warming is also characterized by increased greenhouse gases and particularly CO_2. The interaction between CO_2 and temperature on the crop is less clear than the synergistic negative effect of heat and drought. CO_2 reduces photorespiration particularly for C3 plants like rice which potentially enhances yield (Baker *et al*. 1990, 1992; Ziska and Teramura 1991). However, CO_2 combined with heat also induces a reduction in transpiration cooling which leads to an increase in canopy temperature (Matsui *et al*. 2007) and thus may have a global negative effect on rice crop (Moya *et al*. 1998; Wassmann *et al.* 2009a). From simulations based for north-west India, Lal *et al*. 1998 predicted that the positive effect of elevated CO_2 would be canceled for an increase in temperature of 2°C, and a 20% yield reduction in case of water shortage in the same thermal and CO_2 concentration conditions.

Combined stress effects are not simply the addition of two individual stresses and each couple of stress represents as a new stress. Gene pyramiding for different stresses is a promising approach using marker-assisted selection, for example in the case of submergence and salinity stress (Jagadish *et al.* 2012).

Breeding for heat tolerance

Ziska *et al*. (1996) demonstrated genetic diversity in the response of rice yield to high CO_2 concentration and high temperature for 17 cultivars in controlled environments. Heat stress response in rice is a quantitative trait controlled by multiple genes having interactive effects. Sequencing of the full rice genome (IRGSP 2005) and high resolution QTL mapping for several traits including heat (Ismail *et al*. 2007) helped the research. Rice genetic resources for tolerance to high temperature at flowering have been identified in both *indica* and *japonica* groups and are being used as genetic donors (Matsui *et al*. 1997, Matsui *et al*. 2001, Prasad *et al*. 2006a), and morphological and molecular markers were developed in order to accelerate breeding (Cao *et al.* 2003, Zhang *et al*. 2008, 2009, Xiao *et al*. 2010, Jagadish *et al*. 2010b). A recent study (Ye *et al.* 2011) includes N22 (international heat tolerant control) × IR64 population mapping. N22 is so far the most heat tolerant variety, but alternative donor materials need to be identified because of the poor combining ability of N22. Ishimaru *et al.* (2010) recently demonstrated the positive effect of introgressing the early morning flowering gene from *Oryza officinalis* into *O. sativa* to increase spikelet fertility under heat. Jagadish *et al.* (2012) proposed to combine different molecular techniques and to make use of the Global Rice Phenotyping Network of GRISP (Global Rice Science

Partnership) using standard methods to enhance of the probability of gene discoveries for heat tolerance and other stresses (*i.e*. drought and salinity).

As for most environmental stresses, a simple genetic or agronomic solution is unlikely to be satisfactory and only the combination of genetic tolerance and avoidance with appropriate crop calendars and water management will work for rice.

Identifying risk environments: importance of modeling organ temperature

Facing climate change: Modeling for identifying potentially risky weather patterns

At the end of the 1960's, an important phenomenon equivalent to the industrial revolution and called the green revolution completely changed the rice market until now. In the 50's and 60's, the demand for increasing rice production was urgent and crucial to face the threat of famine mostly due to the exponential population growth in some developing countries like India. Thanks to the availability of a great germplasm collection, conventional breeding allowed IRRI to release more than 40 semi-dwarf high-yielding varieties (some of them becoming globally adopted mega varieties), with high tillering ability, erect leaves and lodging resistance that produced more grain per plants, and responded favourably to fertilizers and irrigation (Hubbart *et al.* 2007, Paterson and Li 2011). Thanks to those mega rice varieties (from the original 'miracle rice' variety IR8 to more recent IR64 and IR72), yields improved consistently in Asia but less in Africa because of the greater diversity of cropping systems and other factors.

However, the positive impact of the green revolution is now declining as while population keeps increasing. New solutions need to be sought which, in addition, have to work in a challenging climate change context. To face the decline, different large international research programs have been initiated like the Green Super Rice (founded by Bill and Melinda Gates Foundation, Green Super Rice 2007), IRRI-Stress Tolerant Rice for Africa and South Asia (STRASA) and C4 Rice Project (Mitchell and Sheehy 2006, Hibberd *et al.* 2008) now under the umbrella of GRISP involving three CGIAR centers and many advanced research centers worldwide.

Temperature issues are prominent in this global collaborative context. However current modeling tools for rice do not consider many of the physiological mechanisms involved in heat tolerance and avoidance, thus requiring improved tools to accurately map out risk environments and the potential impact new varieties might generate, and where.

In this context, a certain number of features of thermal stress need to be understood better. Warmer temperatures are not always synonymous with yield loss, and spikelet sterility depends on other climatic parameters as well.

For instance, a recent study on rice reported serious heat stress damage under moderately hot (maximum Tair=35°C) and humid conditions in China (Tian *et al*., 2010) at flowering, whereas another

study did not find any serious damage under extremely hot (maximum air T=40°C) and dry conditions in Australia (Matsui *et al*., 2007). The role of humidity has been previously described for controlled environments (Weerakoon *et al.* 2008, Yan *et al.* 2010) and those studies confirm the negative effects of high humidity with increasing temperature. But there is a need to further characterize the link between climatic parameters and heat stress damage in the field. The key parameter involved in heat damage is not the air temperature itself, but the temperature of the sensitive organ (the panicle) at the precise time of anthesis, which can differ sensibly from air temperature because of panicle transpiration cooling (Yoshimoto *et al*. 2005, Prasad *et al.*2006a,b, Matsui *et al.* 2007, Jagadish *et al.* 2007, Weerakoon *et al*. 2008, Yan *et al*. 2008, 2010). On the basis of information on how climate translates into time of day of flowering (TOA) and the panicle temperature at TOA, there is a need to identify high-risk climate profiles, today and for future climate-change scenarios, to reduce the threat of rice yield loss due to heat. To attain that goal, we need 1) a better comprehension of the effect of the climatic parameters on the sensitive organ's temperature during sensitive stages in the field, 2) a modeling approach to give better prediction of potential yield loss in a given climatic environment. At the moment, no multi-site and field study involving contrasting climate patterns is available to analyze rice sterility response to climate. Such a study must imperatively take into account the microclimate generated by the crop itself.

Modeling of thermal stress and sterility: Current situation

Models can be process-based or statistical. Process-based models relate input to output variables using knowledge of physical and physiological mechanisms (in the case of biological models), based on previous research, and they generally require parameterization of many auxiliary parameters related to the biological entity and the processes formalized. Statistical models empirically relate relevant input and output variables using a sufficient number of observations to capture system response, but without descending to the elemental process level. Statistical models have limited domain of validity beyond the observed domain and thus tend to be inaccurate when used for extrapolation (Lobell *et al*. 2010), but they provide a rapid solution and can be robust and accurate if broad variation of all relevant input variables is available. There are three different methods used in statistical modeling: the time series method (time series data from a single experimental site), the panel (variation in both time and site), and the cross section (variation in site) model. All those method have strength and limits discussed by Lobell and Burke (2009). In a more recent study Lobell *et al* 2010 tested the prediction power of the three methods against a 'perfect crop model' (robust and well validated) on data from 200 different locations in Africa. The yield responses to temperature, precipitation, and solar radiation were investigated and the cross-section method model provided the most accurate predictions for temperature.

An alternative consist in combining a mechanistic and statistical approach. Many crop models were built in the past two decades but they are facing difficulties regarding the multiplicity of environments. The prediction of the potential effects of climate change on rice yields is a particular challenge because it involves new environments, in part not available yet for experimental study.
No biological model is entirely mechanistic because this would require descending to ever smaller scales. Simplifications are introduced, and the parameters for such simplified algorithms are calibrated empirically using experimental data and statistics – either on a reductionist basis for each individual process or systemically by statistical parameter optimization against integrative observed variables such as yield.

With regards to thermal response of rice spikelet sterility, the major available crop models follow a very simple and probably inaccurate approach (e.g., ORYZA2000 (Bouman 2001), CERES/DSSAT (Jones *et al*. 2003), SARRAH (Dingkuhn *et al.* 2003)). The panicle sink size on a per-area basis, corresponding to spikelet number X potential grain weight, is dimensioned during the phenological phase corresponding to panicle development (reproductive phase, from panicle initiation to flowering), as a function of growth and assimilate partitioning processes. The resulting sink capacity is then down-sized according to stress coefficients derived from environmental conditions during sensitive phases of panicle development. In the case of heat stress, the maximum daily air temperature during the flowering period is used and the sterility response calibrated according to estimated critical temperatures governing the response.
This simple approach has the weakness of not considering the microclimate generated by the crop, including organ temperature. Dingkuhn et al. (1995b) introduced the simulation of floodwater temperature for modeling of cold stress effects on spikelet sterility, which resulted in much improved simulation of thermal stress effects for irrigated rice in the Sahel (Dingkuhn and Miezan, 1995; Dingkuhn, 1995). This also improved the simulation of phenology, which is temperature dependent and governs the timing of temperature stress sensitive phases. Heat effects on spikelet sterility at flowering, however, was still simulated with air temperatures as stress vector. The present research will lay the basis for developing an improved version of RIDEV that fully considers microclimate and organ temperature.
More recently, the heat balance model for rice IM2PACT was developed at Tsukuba University in Japan (Yoshimoto *et al*. 2011). This model, although not (yet) a full crop simulator, simulates canopy and panicle temperature on the basis of climatic variables and some crop morphological variables. The research generating IM2PACT model coincided with the present thesis research. It was not possible to fully exchange data but a preliminary model comparison will be attempted further down.

OBJECTIVE AND OVERVIEW OF CHAPTERS

Within the RISOCAS project (RISOCAS 2011), extensive panel type data were collected from different environments in order to improve crop (rice, sorghum) models, particularly regarding thermal responses of the crop. The ultimate aim of the project, which involved four PhD theses and partners Hohenheim University, CIRAD, AfricaRice, IER of Mali and FOFIFA of Madagascar, was to 1) Identify valuable traits for better adapted cultivars; 2) Extrapolate varietal responses and adaptation potentials for different climate change scenarios; 3) Develop ideotype concepts for varietal selection; and 4) Develop a basis for tactical and strategic decision making to adapt African cereal cropping systems to climate change. The present PhD study furnished answers to specific questions, namely the microclimatic basis of thermal response of rice spikelet sterility and its modeling. The thesis focused on the reproductive phase and the flowering stage of rice, including the phenology that governs its timing and the thermal micro-environment the sensitive organs are exposed to. It also included observations of the relevant canopy properties during sensitive phases and the sterility observed on panicles at maturity.

This objective was realized by the generation and analysis of micro-meteorological and crop data based on similar field experiments implemented in four contrasting climatic environments and using four morphologically distinct rice varieties. At the core of the resulting data base are rice panicle temperatures observed by IR imagery in the field, backed by the necessary simultaneous environment observations to interpret them. The work concludes by integrating the results in a new version of the crop model RIDEV specifically simulating rice phenology and thermal sterility. The aim is to subsequently incorporate RIDEV into broadly used crop models such as ORYZA2000 (Bouman 2001) and the more detailed physiological models EcoMeristem (Luquet *et al.* 2006) and SAMARA.

In the 1st chapter, we studied the phenology and the in particular the timing of the reproductive phase and the flowering stage of irrigated rice, as well as the time of day of anthesis (TOA) in response to the various climatic environments. Anthesis happens during a distinct time of day and this decides on whether the panicle is exposed to heat stress or not. This chapter was therefore entitled "***Variation in time of day of anthesis in rice in different climatic environments***". The hypothesis of this sub-study was that TOA dependes on genotype and environment and can be predicted on this basis. Chapter 1 has been published in 2012 by the European Journal of Agronomy.

Chapter 2 focuses on the organ temperature (developing or exerted panicle) as a function of climatic environment during the main thermally sensitive stages *i.e*. miscroporogenesis (main cold sensitive stage) and anthesis (main heat sensitive stage), in order to predict organ temperature from crop and environment data. This information was then to be linked to observed spikelet sterility on the same plots at maturity. This second chapter named "***Predicting heat induced sterility of rice spikelets requires***

simulation of crop-generated microclimate", addresses the hypothesis that *i)* organ temperature can strongly differ from the air temperature (classically measured at 2m from the ground), *ii)* the temperature difference between the air and the organ can be explained and predicted from crop and climatic variables across different environments, and *iii)* spikelet sterility can be better predicted by taking into account the microclimate and the organ temperature, as compared to air temperature.

Chapter 3 presents the new RIDEV model developed on the basis of this thesis research, as a prototype. This model was commissioned by AfricaRice and a 9-month extension of the thesis research was funded for model development. A commented terminal report of this project is presented here, written by the author of this thesis and the project leader, Dr. Michael Dingkuhn. The full source code of the model is presented and commented.

The 4th chapter presents two modeling exercises, 1) a comparative simulation of observed panicle temperature in different environments using a statistical model developed here (similar to RIDEV) and IM2PACT, the heat balance model from Japan; and 2) an application of RIDEV to an independent set phenology and spikelet sterility data.

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CHAPTER 1:

Variation in time of day of anthesis in rice in different climatic environments

Cécile Julia[1], Michael Dingkuhn[1,2]*

[1]Centre International de Recherche Agronomique pour le Developpement (CIRAD), BIOS Department, UMR AGAP, F-34398 Montpellier, France

[2]International Rice Research Institute (IRRI), Crop and Environment Science Division (CESD), DAPO Box 7777, Metro Manila, Philippines

*Corresponding author

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ABSTRACT

Thermal stress and in particular heat during anthesis causes sterility in rice inflorescences. Rice spikelets open in the morning and close a few hours later. Genotypic variation in the time of day of anthesis is considered an escape mechanism from thermal stress, but little is known on its dependency on environmental conditions. One traditional, cold-tolerant rice cultivar and three improved tropical rice cultivars were grown in four different climatic environments under flooded conditions to study the environmental response of time of day of anthesis. The environments were the cold-dry season and the hot-dry season in Senegal, the temperate summer in Southern France and the dry season in the Philippines. The time of day when the first spikelets opened, a maximum of spikelets were open and the last spikelets closed was observed daily on a population basis (2 m2 plots replicated 3 times). The time of these observations was expressed as hours after sunrise (hasr) calculated from astronomic day length and the local time of solar noon. Aggregate flowering periods were 4–10 d at panicle scale and 14–32 d at population scale. Within a day, onset of anthesis was earliest in the Philippines (3.4 hasr) and latest in the cool season in Senegal (6.75 hasr), the other

environments being intermediate. The duration from onset to end of anthesis varied between 1.82 h in the Philippines and 2.77 h in France. Genotypic differences in time of anthesis and duration of anthesis were small. Across all genotypes and environments, 80% of the variation of the time of maximum anthesis could be explained with the mean minimum air temperature (Tmin) during the 7 d preceding any given anthesis event. Linear, multiple regression models determined for each cultivar using Tmin and vapor pressure deficit (VPD) observations from the three tropical environments explained 94% of variation of time of anthesis onset and end. Low Tmin thereby delayed and low VPD advanced anthesis processes. Under the assumption that panicle temperature during anthesis is indeed a major determinant of spikelet fertility in rice, it is concluded that the sensitivity of time of day of anthesis to air temperature and humidity is an effective eco-physiological adaptation of the rice crop. Prospects for improved modeling approaches for the prediction of thermal sterility in rice are discussed.

INTRODUCTION

Rising temperatures caused by climate change will likely depress rice yields in many parts of the world, according to pridictions using crop models (Baker et al., 1992; Sheehy et al., 2006a,b)). The most important mechanism of damage caused by heat is ineffective pollination during anthesis, which can be observed in the 35–40 ◦C temperature range. Exposure of panicles for a few hours during flowering can induce spikelet sterility and thus, grain set (Satake and Yoshida, 1978; Nishiyama and Blanco, 1980; Nishiyama and Satake, 1981; Baker et al., 1992).

Dingkuhn (1995) mapped irrigated rice environments in the Sahel for risks of thermal stresses causing sterility using the phonological model RIDEV (Dingkuhn, 1997; Wopereis et al., 2003). Sterility risks caused by either heat or chilling were a function of local climate, cultivar and cropping calendars. Yield simulations backed by field experiments (Dingkuhn and Sow, 1997) confirmed that thermal stresses are a dominant factor for potential rice yields in semi-arid tropical environments such as the Sahel. These studies, however, did not take into account environment effects on time of day of anthesis which were unknown.

Although flowering on a given rice panicle can be observed during 3–9 consecutive days, and during even longer periods at population scale depending on synchronization of flowering among tillers and plants, anthesis of a given spikelet happens only on one day during the morning, lasting about 2 h (Yoshida, 1981; Sheehy et al., 2007). Rice is a mainly autogamous plant but requires spikelets to open and anthers to dehiss for pollination. After the brief anthesis period, spikelets close again but dehisced anthers remain outside the spikelet.

The time of day of anthesis of rice is under genetic control. Observations in both field and controlled environments indicated some diversity within cultivated *Oryza sativa* rice (Sheehy et al., 2005; Jagadish et al., 2008) and much greater diversity among wild rices, which are a genetic source of traits for early flowering time (Ishimaru et al., 2010). The latter authors demonstrated that introgression of early-flowering traits is effective in reducing heat-induced spikelet sterility under experimental conditions.

Little is known on environmental effects on time of day of flowering in rice. A recent field study in Japan (Kobayasi et al., 2010) indicated that high air temperature (Ta) and solar radiation (Rs) advance anthesis, and high atmospheric vapor pressure deficit (VPD) in the early morning delays anthesis. Although these results were statistically highly significant, however, the three environmental factors explained only 40% of the observed variation in flowering time. Kobayasi et al. (2010) also reported differences in sensitivity among rice cultivars to Ta, Rs and VPD.

The present study aimed at quantifying climatic effects on the time and duration of anthesis in irrigated rice crops, in order to enable better predictions of sterility risks with models. Four cultivars were grown and observed in the field in four contrasting environments: the hot-dry season in Senegal, the cool-dry season in Senegal, the temperate summer season in Camargue in France and the dry season in the Philippines. The specific objective was to identify climatic predictor variables for time of day of flowering and to establish a model of anthesis time that is valid across diverse climatic environments.

MATERIALS AND METHODS

Experiment sites, design and management

Field experiments were conducted between January 2009 and July 2010 in 4 environments, named hereafter Senegal hot-dry season, Senegal cool-dry season, France temperate summer and Philippines dry season. In Senegal, the same field was used located on the Sahel research station of the Africa Rice Center (ARC) at Ndiaye in the Senegal river delta (16◦20_N, 16◦26_W, 5 m asl). The soil was a clay soil (Orthothionic Gleysol), containing 40–54% clay, 44–30% silt and 16% sand, with pH(H2O) 6.5. The field in Fance was located on the research station of the French Center for Rice (CFR) located in the Rhone delta (Camargue, 43◦70_N, 4◦56_E, 4 m asl). The soil was a sandy loam type consisting of 15.8% clay, 46.5% silt and 37.8% sand, with pH(H2O) 8.2, constituting a 40–50 cm layer of fluvial sediments covering deposited on a saline, acidsulphate Mangrove soil. In the Philippines, the field was located in the lowland part of the experiment farm of the International Rice

Research Institute (IRRI) (14◦16_N, 121◦25_E, 16 m asl). The soil was an Andaqueptic Haplaquoll composed of 58% clay, 33% silt and 9% sand, with pH(H2O) of 6.6.

Elemental plot size was 16 m2 (France and Senegal) or 20 m2 (Philippines). In all cases, the field was embedded in a large irrigated rice area. In all environments, the experiment was conducted under fully irrigated flooded conditions until surface drainage at about 14 d after end of flowering. Floodwater depth was maintained at 5–10 cm except during crop establishment, when floodwater depth was adapted to seedling size.

A randomized complete block design with 3 replications (2 in France) was used. Crop establishment differed among sites following standard practices for the respective stations: in the Philippines and France, seedlings were sown and raised in seedling trays in the greenhouse, then 15-d old plants (29 d in France) were manually transplanted into the wet field at a density of 25 hills m^{-2} (20 cm spacing), each hill having 2 plants. In Senegal, pre-germinated seed was directly, manually dibbled onto the wet field using the same density and geometry as in the Philippines and France. At all sites, inorganic fertilizer and crop protection inputs were used according to local standards for high-input, fully protected systems. Weed control was manual, however, and conducted as needed to keep plots weed free.

Sowing dates are provided in Table 1, along with flowering dates and flowering duration. The cold-adapted cultivar Chomrong, which flowers extremely early under tropical conditions, was sown later than the other cultivars in Senegal in order to reduce the time window of flowering observations.

Genetic materials

Four O. sativa L. cultivars were in the experiment at all sites and seasons. Among them, 3 were improved, medium-duration, indicatype, semidwarf varieties including IR64 (international check variety known to be well adapted to conditions in both Senegal and Philippines), IR72 (similar to IR64 but having slightly longer duration and smaller flag leaves), and Sahel 108 (local check in Senegal, characterized by tall erect flag leaves shading the panicle). The fourth cultivar was the traditional, lowland-adapted japonica type Chomrong from Nepal, a short-duration cultivar that is cold tolerant and adapted to the high altitudes. Chomrong builds a distinct canopy structure at flowering with a high panicle layer located above the leaves due to long peduncles and pronounced panicle exertion. The 4 cultivars were thus selected for their different crop architecture at flowering stage and for different thermal adaptation. The choice of Chomrong as a japonica type was also motivated by reports attributing earlier anthesis to this group (Imaki et al., 1987; Singh et al., 2006). Seed for IR64 and Sahel 108 was obtained from the rice germplasm collection of CIRAD; for

Chomrong from the CIRAD-FOFIFA rice breeding program in Antsirabe, Madagascar; and for IR72 from the IRRI germplasm bank in the Philippines.

Climate and micro-meteorological measurements

At each site and season, agro-meteorological measurements were continuously made at 2 m above ground at the center of the experimental field during the reproductive and ripening periods of the crops: March and April 2009 in the Philippines, August and September 2009 in France, and January–March 2010 and May–June 2010 in Senegal. They were backed by data from standard weather stations located on the same experimental station.

For each experiment the meteorological data including air temperature (T), relative humidity (RH) and solar radiation (Rs) was measured in the middle of the plot where an agro-meteorological station was set up for the entire duration of the experiments. The instruments were located at 2 m from the ground, or approximately 1–1.5 m above the canopy. We used Campbell Inc. equipment (UK): a MP100A Rotronic temperature and Relative Humidity sensor held in a naturally ventilated radiation shield for the air temperature and humidity, and a A100R cup anemometer for the wind speed. A CNR1 (Campbell Inc., UK) net radiometer was set up 30 cm above the canopy to measure solar radiation (Rs) and net radiation (not used in this study). We recorded the data with a Campbell CR1000 data logger every 5 s and average over 1 min for the entire periods of observations in the field.

Daily potential evapotranspiration (PET) was calculated according to the FAO Irrigation and Drainage Paper No 56 (Allen et al., 1998) and using the hourly average of Rg, T, RH and wind speed measured in the field. Average daily vapor pressure deficit (VPD) was calculated from daily Tmin, Tmax, RHmin, and RHmax using FAO standards (Allen et al., 1998) based on the following equation:

$$\mathrm{VPD} = \frac{1}{100} \times \frac{e°(Tmin)\ \mathrm{RHmax} + e°(Tmax)\ \mathrm{RHmin}}{2}$$

where $e^{\circ}(T) = 0.6108 \exp 17.27T/(T + 237.3)$ is the saturated vapour pressure at T (in kPa). (RH and T given in percent and in degree Celcius, repectively)

Time of day of anthesis observations

Measurements of time of day of anthesis (TOA) were done at the population scale on all panicles on a 1.4 m × 1.4 m (49 hills) called the observation square. Therefore, the results include observations made on panicles located at different positions in the panicle layer and confound main culms with tillers. Every subplot was observed daily during the entire flowering period every 30 min or less, from sunrise until anthesis was terminated on the last spikelets (about midday or early

afternoon). On a given day, only panicles in the observation square that had attained at least 50% heading (panicles halfway exerted or more) were considered. This was the stage at which anthesis would start at the earliest, as previously verified for all genotypes and environments. Within this sub-population of panicles, we defined as the onset of anthesis the time of day when at least 5 panicles in the observation square had started anthesis (at least one opened spikelet visible per panicle), the maximum of anthesis when all panicles of the sub-population had attained anthesis on at least one spikelet, and the end of anthesis when all the panicles had terminated anthesis as indicated by spikelet closure and droopiness and change of color of stamens. In addition, 10 panicles per plot were marked and observed for the number of days elapsing from first to last anthesis events (except Camargue).

Walking between plots can accelerate anthesis through physical stimulus. This was avoided by maintaining a 2-row border for the observation square, and entering into the plot was strictly avoided. All observations were therefore made from the inter-subplot space, with a maximal distance of 1.2 m between observer and plant.

The TOA was expressed as hours after sunrise (hasr). Solar time of sunrise was calculated on the basis of day length (DL), which was computed using the FAO method (Allen et al., 1998) as follows:

$$DL = \frac{24}{\pi} \arccos[-\tan(Decli).\tan(Lat)]$$

where Decli is the declination angle (in radians) between the rays of the Sun and the plane of the Earth's equator, and is a direct function of the date (in days after the spring equinox), Lat is the latitude (in radians).

Local legal time (LT), which can substantially deviate from solar time depending on location within a time zone, was converted into solar time.

Statistical analysis

Analyses of variance (ANOVA), correlation matrices and multiple, linear, stepwise regression analyses were computed with StatBox Pro (Grimmer Soft) software embedded in Excel (Microsoft).

Table 1. Dates of sowing, duration of flowering at the plant population and panicle scales, and duration from sowing to maximum flowering for four rice genotypes in four environments. Experiments in Senegal and Philippines had 3 replications, in France 2 replications. ANOVA was conducted within sites (lower-case letters in annotated in main Table) and across sites (appended Table). Means within columns having common group annotation are statistically not different (P>0.05) according to Tukey test. SEM indicated standard error of mean of 3 replications (2 in France).

Site	Genotype	Sowing date	Flowering duration (d)				Sowing to flowering (d)	
			Population	SEM	Panicle	SEM		SEM
Senegal	Chomrong	13/03/2010	19.7 b	-	6.2 bc	± 0.1	64.3 a	-
(hot-dry	S108	24/02/2010	13.7 a	± 0.7	4.2 a[(1)]	± 0.1	88.3 bc	± 2.7
season)	IR64	24/02/2010	13.0 a	± 0.0	6.4 bc	± 0.0	87.7 bc	± 2.0
	IR72	24/02/2010	12.0 a	± 0.0	6.0 b	± 0.1	95.0 cd	± 0.0
Senegal	Chomrong	10/11/2009	31.7 c	± 0.3	10.2 d	± 0.1	82.3 ef	± 0.9
(cool-dry	S108	30/10/2009	14.0 a	± 0.0	7.1 c	± 0.1	103.0 bc	± 1.0
season)	IR64	30/10/2009	14.0 a	± 0.0	6.8 bc	± 0.0	107.7 b	± 3.7
	IR72	30/10/2009	15.0 ab	± 1.0	7.1 c	± 0.1	120.0 a	± 0.9
Philippines	Chomrong	26/01/2009	14.0 a	± 0.0	-	-	52.0 a	± 0.0
(dry	S108	26/01/2009	13.0 a	± 0.0	4.5 a	± 0.1	68.0 b	± 0.0
season)	IR64	26/01/2009	14.0 a	± 0.0	4.2 a	± 0.0	72.0 c	± 0.0
	IR72	26/01/2009	14.0 a	± 0.0	4.0 a	± 0.0	76.0 d	± 0.0
France	Chomrong	20/04/2009	17.0 a	± 0.0	-	-	129.0 a	± 0.0
(temperate	S108	20/04/2009	15.0 b	± 0.0	-	-	126.0 a	± 0.0
summer)	IR64	20/04/2009	15.0 b	± 0.0	-	-	129.0 a	± 0.0
	IR72	20/04/2009	15.0 b	± 0.0	-	-	128.5 a	± 2.5

(1) The short flowering duration observed for this particular variety is explained by a short heading duration and an incomplete panicle exertion (spikelets stuck in the sheath) compared to other varieties which fully completed panicle exertion. For Senegal Cool-dry season, all varieties had a longer heading duration and poor panicle exertion (except for cold tolerant Chomrong) due to low temperatures at heading and at microspore stages.

ANOVA		Flowering duration (Population)		Sowing to flowering (d)	
		Mean	Group	Mean	Group
Site	France	15.5	A	128.5	A
	Senegal HDS	14.5	C	84.4	B
	Senegal CDS	18.7	B	103.2	C
	Philippines	13.8	C	67.0	D
Genotype	Chomrong	20.5	A	82.5	A
	IR72	14.0	B	104.9	B
	IR64	14.0	B	99.2	C
	Sahel 108	13.9	B	96.4	C

RESULTS

Time and duration of flowering at the population scale

Duration from sowing to onset of flowering (Table 1) was shortest in the Philippines, longest in France and intermediate in Senegal. Chomrong had the shortest duration to flowering in all environments except France, where its duration was similar to that of the other genotypes.

Onset of flowering was observed between 17 and 29 May in the hot-dry season in Senegal, between 29 January and 26 February in the cool-dry season in Senegal, between 18 March and 11 April in the Philippines and between 23 and 28 September in France. The duration of flowering at the population scale was about two weeks due to variation in flowering among plants, panicles and spikelet position within panicles. Only cv. Chomrong deviated from this observation in Senegal, with a flowering period of 19 d in the hot-dry season and 32 d in the cool-dry season (Table 1). At the scale of the individual panicle, the duration of flowering was much shorter, between 4 and 6 d in the hot-dry season and between 7 and 10 d in the cool-dry season in Senegal (panicle-scale observations were incomplete at the other sites). The difference between flowering duration at population and panicle scale was due to de-synchronization among tillers and plants, which was large for Chomrong, a traditional cold tolerant variety.

Environmental conditions during flowering

Assuming that time of day of anthesis is affected by environment at or shortly before anthesis, we calculated both the conditions on the day of anthesis and the mean conditions during the 7 d preceding anthesis. Since the 7-d means had a greater explanatory power for the observed timing of anthesis than environmental conditions on single days (data not presented), we use here only the 7-d means, based on calculations for each observation of anthesis and then averaged for the genotype and site or season (Table 2).

Solar radiation was high and very similar among environments, at 20.1–22.1 MJ d−1. Minimum air temperature was lowest in France and in the cool-dry season in Senegal (15.4–18.6 ◦C), with minima on individual days dropping to 9 ◦C in France. Temperature minima (Tmin) were highest and very constant in the Philippines (23.2–23.9 ◦C) and intermediate and also very constant in the Senegalese hot-dry season (21.4–21.5 ◦C). Maximal air temperature (Tmax) was lowest in Camargue (28.4–29.7 ◦C), highest in the Senegalese hot-dry season (31.6–35.6 ◦C), and intermediate in the other environments.

Three weather parameters were used to characterize air humidity and atmospheric demand for water vapor: mean daily relative humidity (RH), mean vapor pressure deficit (VPD) and potential

evapotranspiration (PET) which integrated Rg, T, RH and wind speed. The most humid environment was the Philippines where VPD was only 0.5 kPa, as compared to the hot and cool seasons in Senegal with about 2 kPa. France was intermediate with 1.4 kPa.

Time of day of anthesis

Time of day of anthesis was expressed as hours after sunrise (hasr) (Table 3) because day length and the time of solar noon varied among environments. Onset of anthesis was earliest in the Philippines (3.4 hasr) and latest in the cool season in Senegal (6.75 hasr). France and the hot-dry season in Senegal were intermediate. Differences among environments were highly significant ($P < 0.001$) and the SEM was extremely small because of high number of (daily) observations throughout the flowering period.

These differences in time of day of onset of anthesis were carried over to maximum anthesis (when all spikelets flowering on that day were open) and end of flowering (when all spikelets had closed again). The duration of anthesis thus varied to a lesser degree than the time of day of anthesis, between 1.82 h in the Philippines and 2.77 h in France (Table 3). Genotypic differences in time of anthesis and duration of anthesis were small and not consistent among environments.

Table 2. Mean environmental conditions during the 7d preceding anthesis events . Rg, global radiation;Tmin, minimum temperature; Tmax, maximum temperature; RH, mean relative humidity; VPD, mean varor pressure deficit; PET, potential evapo-transpiration; SEM, standard error of mean of 3 replications (2 in France).

Site	Genotype	Rg (MJ)	SEM	Tmin (°C)	SEM	Tmax (°C)	SEM	RH . (%)	SEM	VPD (kPa)	SEM	PET (mm)	SEM	Day length
Senegal	IR64	20.5	± 0.3	21.4	± 0.1	35.6	± 0.4	56.6	± 1.8	2.0	± 0.1	6.2	± 0.1	12.8
(hot-dry	IR72	22.1	± 0.2	21.4	± 0.2	31.6	± 0.4	72.5	± 1.5	1.0	± 0.1	5.0	± 0.1	12.9
season)	S108	20.5	± 0.3	21.5	± 0.1	35.6	± 0.4	56.9	± 1.8	2.0	± 0.1	6.2	± 0.1	12.8
	Chomrong	20.8	± 0.3	21.5	± 0.1	35.0	± 0.4	59.1	± 1.8	1.9	± 0.1	6.0	± 0.1	12.8
Senegal	IR64	21.1	± 0.3	15.4	± 0.2	30.5	± 1.0	68.0	± 4.0	1.6	± 0.2	4.6	± 0.2	12.9
(cool-dry	IR72	22.0	± 0.2	18.0	± 0.2	35.4	± 0.5	57.1	± 1.6	2.5	± 0.1	6.2	± 0.1	12.8
season)	S108	20.5	± 0.4	15.5	± 0.2	31.1	± 1.0	64.8	± 4.2	1.7	± 0.2	4.7	± 0.2	12.9
	Chomrong	20.4	± 0.3	16.4	± 0.3	32.8	± 0.6	60.4	± 2.1	2.1	± 0.1	5.1	± 0.2	12.9
Philippines	IR64	21.1	± 0.5	23.9	± 0.1	32.1	± 0.2	82.8	± 0.4	1.0	± 0.0	4.7	± 0.1	12.3
(dry	IR72	20.1	± 0.8	23.9	± 0.1	32.3	± 0.2	84.1	± 0.9	1.0	± 0.0	4.5	± 0.2	12.4
season)	S108	20.3	± 0.7	23.8	± 0.1	31.2	± 0.3	83.6	± 0.6	0.9	± 0.1	4.5	± 0.2	12.3
	Chomrong	21.5	± 0.8	23.2	± 0.1	31.5	± 0.1	85.6	± 0.2	0.7	± 0.0	4.4	± 0.1	12.1
France	IR64	20.9	± 0.3	17.7	± 0.5	28.9	± 0.4	69.1	± 0.8	1.4	± 0.0	4.8	± 0.1	13.0
(summer)	IR72	20.9	± 0.3	16.7	± 0.7	28.4	± 0.3	67.8	± 0.7	1.4	± 0.0	4.7	± 0.1	12.9
	S108	21.6	± 0.4	18.6	± 0.1	29.7	± 0.4	70.6	± 0.8	1.5	± 0.0	5.0	± 0.1	13.2
	Chomrong	21.1	± 0.3	17.0	± 0.6	28.7	± 0.4	68.5	± 0.8	1.4	± 00	4.8	± 0.1	13.0

***Table 3**. Time of anthesis (h after sunrise) and time from onset to end of anthesis (h) for 4 genotypes in 4 environments. Means of 3 replications (2 in France), each consisting of several consecutive daily observations , are presented with SEM (standard error of mean of replications) . ANOVA was conducted within sites (lower-case letters annotated in main Table) and across sites (appended Table). Means within columns having common group annotation are statistically not different (P>0.05) according to Tukey test.*

		Time of anthesis (h after sunrise)						Duration (h)	
Site	Variety	Mean onset	SEM	Mean max.	SEM	Mean end	SEM	Mean	SEM
Senegal	IR64	4.4 b	± 0.06	5.1 a	± 0.02	6.6 c	± 0.01	2.2 bc	± 0.06
(hot and dry	IR72	4.5 b	± 0.05	5.3 a	± 0.03	6.9 b	± 0.05	2.5 ab	± 0.02
season)	S108	4.6 b	± 0.03	5.2 a	± 0.03	6.7 bc	± 0.02	2.0 c	± 0.03
	Chomrong	3.9 c	± 0.08	5.1 a	± 0.10	6.6 c	± 0.01	2.8 a	± 0.07
Senegal	IR64	6.9 a	± 0.05	7.5 a	± 0.11	9.0 a	± 0.05	2.1 bc	± 0.09
(cold season)	IR72	6.5 a	± 0.14	7.4 a	± 0.19	8.7 a	± 0.11	2.2 bc	± 0.02
	S108	6.8 a	± 0.07	7.5 a	± 0.10	9.0 a	± 0.03	2.2 bc	± 0.05
	Chomrong	6.8 a	± 0.03	7.6 a	± 0.05	9.0 a	± 0.02	2.2 bc	± 0.03
Philippines	IR64	3.5 a	± 0.01	4.3 a	± 0.01	5.3 a	± 0.00	1.8 a	± 0.01
(hot and dry	IR72	3.5 a	± 0.01	4.3 a	± 0.01	5.3 a	± 0.00	1.8 a	± 0.01
season)	S108	3.5 a	± 0.00	4.2 a	± 0.01	5.2 a	± 0.01	1.8 a	± 0.00
	Chomrong	3.1 a	± 0.03	4.1 a	± 0.00	5.1 a	± 0.00	1.9 a	± 0.03
France	IR64	5.0 a	± 0.02	6.3 a	± 0.03	7.7 a	± 0.02	2.8 a	± 0.04
(temperate	IR72	4.9 a	± 0.06	6.3 a	± 0.02	7.7 a	± 0.02	2.8 a	± 0.06
summer)	S108	5.1 a	± 0.06	6.3 a	± 0.01	7.8 a	± 0.01	2.8 a	± 0.05
	Chomrong	4.9 a	± 0.05	6.4 a	± 0.02	7.7 a	± 0.01	2.8 a	± 0.06
	Mean	*5.0*		*5.9*		*7.3*		*2.3*	

Relationship between time of anthesis and environmental variables

Within a given site or season, we did not observe any significant effect of the environment on the time of anthesis, probably because of the small variability of weather conditions (data not presented). Across genotypes and environments, however, variables of anthesis time were significantly ($P < 0.05$) correlated with all environmental variables observed, except solar radiation (Table 4). The best single predictor variable was the daily minimum air temperature (negative), with R values between −0.82 and −0.89. The mean air temperature also highly predictive (R between −0.7 and −0.77) whereas Tmax was not. Consequently, low minimum air temperatures were associated with late onset and end of anthesis.

Table 4. Partial correlation matrix for anthesis variables (h after sunrise for onset, maximum and end) vs. environmental variables (definitions as in Table 2) averaged over the 7 days preceding any given daily observation.

	Rg	Tmean	Tmin	Tmax	RH	PET	VPD	Day length
Onset	0.01	**-0.70**	**-0.82**	-0.10	**-0.40**	**-0.40**	**0.51**	**0.54**
Maximum	0.00	**-0.77**	**-0.89**	**-0.19**	**-0.42**	**-0.45**	**0.53**	**0.61**
End	0.03	**-0.74**	**-0.88**	**-0.14**	**-0.50**	**-0.43**	**0.59**	**0.71**

Significant (P<0.05) R values by bi-lateral test in bold print

Variables related to atmospheric humidity (VPD, RH) or demand for water vapor (PET) were generally correlated with anthesis variables in a sense indicating that dry air conditions delay anthesis. No correlation was observed between anthesis variables and solar radiation, whereas there was a significant, positive correlation with day length. This was not an artifact of calculation because time of anthesis was expressed relative to the local time of sunrise (including civil twilight), taking into account local deviation of noon from solar noon, as well as date and latitude specific day length. It is possible, however, that apparent day length effects were caused by correlations among day length and climatic variables, which are not independent because linked to season.

Multiple, linear, stepwise regression analyses explaining time of onset and end of anthesis identified between 2 and 4 predictor variables, depending on genotype (Table 5). They were similar for Chromrong, Sahel 108 and IR64 where they included in all cases VPD and the minimum or average temperature, with Tmin always being the first variable to be taken into the model. Patterns were different for IR72, however, where temperature variables entered the model only on the 3rd iteration, because humidity and day length related variables had greater predictive power.

For better comparability among genotypic models, we chose Tmin and VPD as common predictors of the time of maximal anthesis (Table 6). Prediction coefficients (R2) were greater than 0.9 for all genotypes except Chromrong (0.76). T ratios (as absolute values) were generally much greater for Tmin than for VPD except for IR72, where VPD had greater predictive power than Tmin. In IR64, the contribution of VPD was not significant. All models had $P < 10E-8$.

Table 5. Multiple stepwise linear regression analyses explaining onset and end time of anthesis (h after sunrise) for four rice genotypes across data from Senegal, Philippines and France experiments. All predictor variables were retained at the end of the stepwise procedure according to Statbox Pro software (Grimmer Soft inc.). Predictor combinations in bold print gave the lowest P value. Rg, global radiation; Tavg, Tmin, Tmax; mean, minimum and maximum daily temperature; RH, mean air humidity; PET, potential evapo-transpiration; VPD, mean vapor pressure deficit; DL, day length.

	Rg	Tavg	Tmin	Tmax	RH	PET	VPD	DL	R^2	Rg	Tavg	Tmin	Tmax	RH	PET	VPD	DL	R^2
	Chomrong, onset									Chomrong, end								
1 var			X						0,59			X					0,63
2 var		X		X					0,72		X					X		0,80
3 var					X	X	X		0,77					X	X	X		0,86
4 var		**X**			**X**	**X**	**X**		**0,81**		**X**			**X**	**X**	**X**		**0,90**
5 var		X		X	X	X	X		0,84		X			X	X	X	X	0,91
All	X	X	X	X	X	X	X	X	0,84	X	X	X	X	X	X	X	X	0,92
	Sahel 108, onset									Sahel 108, end								
1 var			X						0,91			X						0,95
2 var			X	X					0,95			X				X		0,98
3 var			**X**	**X**				**X**	**0,96**			**X**				**X**	**X**	**0,99**
4 var		X	X	X				X	0,96	X	X	X					X	0,99
5 var		X	X	X	X			X	0,97	X	X	X				X	X	0,99
All	X	X	X	X	X	X	X	X	0,96	X	X	X	X	X	X	X	X	0,99
	IR64, onset									IR64, end								
1 var			X						0,77			X						0,87
2 var		X		X					0,82			X					X	0,93
3 var			X	X	X				0,84		**X**			**X**		**X**		**0,94**
4 var		**X**		**X**	**X**		**X**		**0,86**			X		X		X	X	0,95
5 var		X	X	X	X		X		0,86	X	X			X	X	X		0,95
All	X	X	X	X	X	X	X	X	0,85	X	X	X	X	X	X	X	X	0,95
	IR72, onset									IR72, end								
1 var							X		0,89					X				0,85
2 var							**X**	**X**	**0,91**							X	X	0,94
3 var		X	X				X		0,91		**X**					**X**	**X**	**0,97**
4 var	X	X	X				X		0,92		X				X	X	X	0,97
5 var		X	X			X	X	X	0,92		X	X			X	X	X	0,97
All	X	X	X	X	X	X	X	X	0,93	X	X	X	X	X	X	X	X	0,97

Table 6. Multiple regression analyses explaining time of maximal anthesis (h after sunrise) with minimum air temperature (means of 7d before anthesis for any given observation; °C) and daily average VPD (mean of 7d before anthesis; KPa), across Senegal, Philippines and France experiments.

Genotype		Chomrong	Sahel 108	IR64	IR72
Constant	Coeff.	11.58	13.05	12.27	6.68
Tmin	Coeff.	-0.340	-0.377	-0.337	-0.122
	Std.Coeff.	-0.779	-0.947	-0.939	-0.348
	T ratio	-12.6	-37.4	-21.7	-6.32
	P	<10E-8	<10E-8	<10E-8	<10E-8
VPD	Coeff.	0.614	0.174	0.100	1.189
	Std.Coeff.	0.318	0.105	0.0577	0.6997
	T ratio	5.15	4.16	1.334	12.72
	P	<0.000001	<0.0001	n.s.	<10E-8
Regression	N	63	48	50	48
	adjusted R^2	**0.764**	**0.972**	**0.915**	**0.928**
	F	102.8	848.2	269.8	273.2
	P	<10E-8	<10E-8	<10E-8	<10E-8

Thermal conditions at the time of onset of anthesis

Since low daily minimum temperatures delayed anthesis, it can be hypothesized that anthesis actually happened at different time of day but at the same ambient temperature. This was not the case (Table 7). All genotypes did begin anthesis at nearly the same temperature in any given environment, but this temperature was much lower in France than in the tropical environments. In the case of Sahel 108 and IR64, anthesis also began at significantly ($P < 0.05$) lower temperatures in the Senegal cool-dry season than in the hotdry season and in the Philippines.

Consequently, the delay in anthesis under low minimum temperatures caused anthesis to begin under warmer conditions, but this effect was not strong enough to cause isothermal patterns of anthesis onset across environments. A single critical temperature for onset of anthesis thus does not exist.

Table 6. Air temperature at onset of anthesis for four rice genotypes in four environments. SD, population standard deviation for all observations pooled.

	Chomrong		Sahel 108		IR64		IR72	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Senegal hot-dry season	25.2	0.7	26.2	0.8	26.0	0.8	24.7	0.8
Senegal cool-dry season	24.4	1.9	23.1	2.0	22.9	1.9	26.2	0.8
Philippines dry season	25.2	0.2	25.7	0.3	26.1	0.4	26.2	0.3
France summer season	21.1	2.0	22.6	0.5	22.0	1.6	20.8	2.0

Thermal response of anthesis within and across environments

The thermal response of time of anthesis as described above was generated by thermal variations between environments and to a much lesser degree within them (Fig. 1a: response of maximum anthesis). The four environments formed distinct thermal clusters was observed. Shorter integration intervals increased the scatter (and slightly reduced predictive power of environmental variables), whereas longer integration intervals reduced the scatter within environments but also reduced the goodness of correlations.

The France environment was the only temperate environment in this study, and had the greatest day-to-day variations in Tmin. When removing it from the analysis, linear correlations with very little scatter (mean R2 of 0.94) were observed for each genotype (Fig. 1b). Their slope was similar for the onset and the end of anthesis vs. Tmin, indicating that duration of anthesis was constant across thermal environments. Sahel 108 and IR64 had identical thermal response, whereas Chromrong and IR72 showed slightly but significantly ($P < 0.05$) different response.

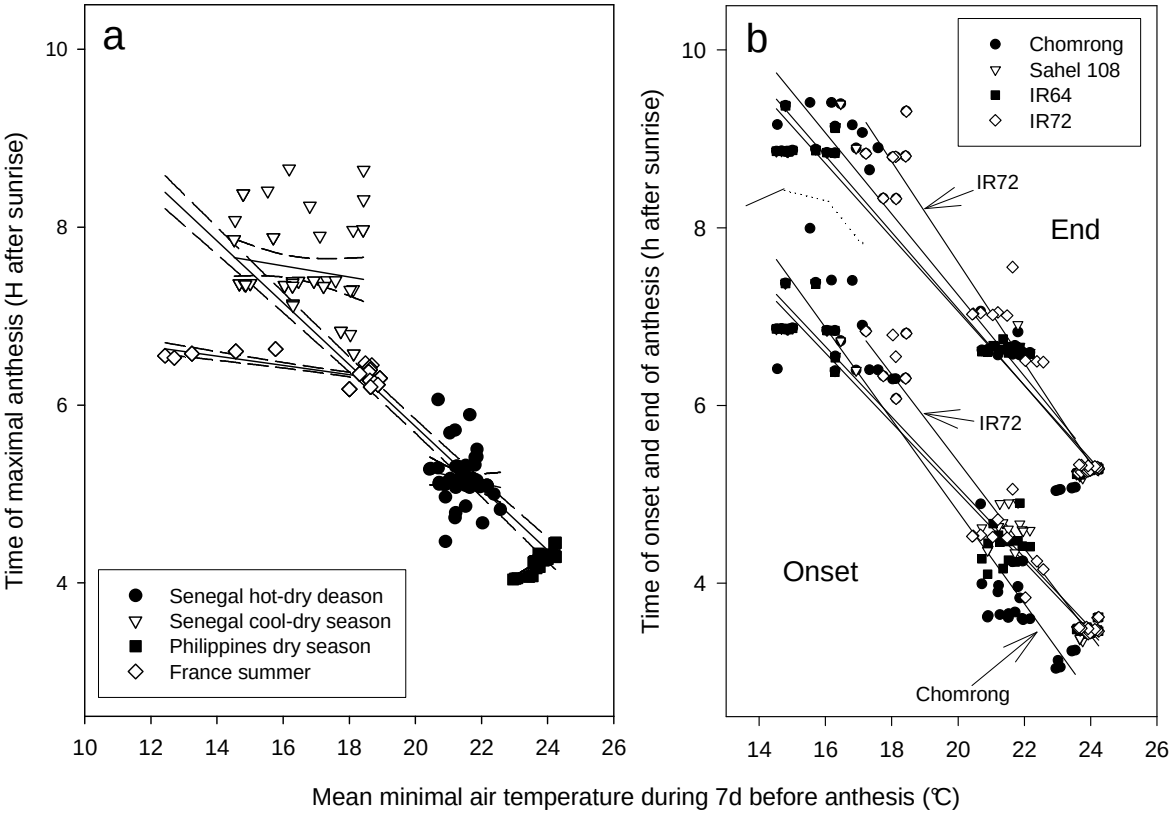

***Figure1**: Relationship of time of anthesis vs. minimum air temperature (Tmin). Fig. 1a: Maximal anthesis vs. Tmin. Correlation across 4 environments, Y=12.7-0.348X with R^2=0.80; within environments, not significant. Fig.1b: Onset and end of anthesis vs. Tmin, by genotype across 3 tropical environments (Senegal and Philippines). Correlations for onset of anthesis: Chomrong, Y=15.2-0.517X, R^2=0.90; Sahel 108, Y=13.0-0.396X, R^2=0.96; IR64, Y=12.9-0.392X, R^2=0.97; IR72, Y=15.2-0.489X, R^2=0.94. Correlations for end of anthesis: Chromrong, Y=16.4-0.456X, R^2=0.88; Sahel 108, Y=15.7-0.431X, R^2=0.95; IR64, Y=15.4-0.416X, R^2=0.97; IR72, Y=18.9-0.567X, R^2=0.95. Correlations marked by arrows are significantly (P<0.05) different from those of other genotypes.*

DISCUSSION

Atmospheric temperature and VPD strongly affect time of day of anthesis

This study found strong variations in the rice spikelets' time of onset and end of anthesis among climatic environments, whereas the duration of anthesis varied comparatively little. Across environments, atmospheric Tmin and VPD, averaged over the 7 d preceding any given anthesis event, explained almost all of the variation. As previously reported by Kobayasi et al. (2010), high temperatures and low VPD cause spikelets to open earlier in the morning. In contrast to that study, no effect of solar radiation was found here. The correlations reported here, however, were much stronger even for Tmin alone (R2 = 0.80 across all environments and R2 = 0.94 for the three tropical environments), as compared to R2 = 0.4 reported for the multiple regression model reported by Kobayasi et al. (2010). This difference was probably due to the larger range of thermal and humidity conditions encountered in our study, which led to stronger variability in time of day of onset and end of anthesis.

We did not find any effect of environment on anthesis time within a site and season, which was probably caused by insufficient variability, but leaves the remote possibly that site effects were caused by third, non-climatic factors. This, however, is extremely unlikely because (1) management including irrigation were optimal in all environments and (2) the cool and the hot season in Senegal, based on the same plots, gave very different times of anthesis that were consistent with the overall trends observed in this study. We thus conclude that the regression models obtained here reflect true effects of weather variables on time of anthesis. It is possible, however, that other environmental variables but Tmin and VPD that varied little in our study, such as Rg (20.1–22.1 MJ d−1), would affect time of day of flowering in rice if they varied more strongly. Kobayasi et al. (2010) reported such effects of Rg. Day length, which varied between 12.1 and 13.2 h in our study, was significantly ($P < 0.05$) correlated with time of day of flowering, particularly with the time at which anthesis ended (Table 4). Nishiyama and Blanco (1981) reported that decreasing day length by 1 h (or applying a dark treatment before anthesis) could delay (or advance) the onset of flowering. In contrast to these authors, however, we found a positive correlation of anthesis time of day with day length, suggesting the causes behind the effects were different in both studies. In field experiments, environmental variables that change seasonally are inter-correlated. But the effects of Tmin and VPD remained largely unchanged when day length was included in the multiple regression analyses, indicating that temperature and atmospheric drought have strong effects.

We assumed in this study that time of day of flowering is regulated at the level of the individual spikelet because anthesis events on a panicle are spread over an extended period,

probably due to different physiological age and topological position among spikelets. The fact that average environmental conditions during the 7 d preceding any anthesis event explained time of flowering better than those observed on the day of anthesis itself suggests the involvement of regulatory processes happening prior to the anthesis event itself. Sheehy et al. (2007) suggested that the the time of day of anthesis within a given panicle is uniform, which would imply that regulation occurs at the scale of the entire panicle before the first spikelet opens. We cannot test this hypothesis with the present data because this study was conducted at population and not panicle scale, but it would explain the several observations in Camargue that do not fit into the overall thermal response shown in Fig. 1a. In these cases, very low Tmin occurred during the end of the observation period but appeared not to delay anthesis further. If time of day of anthesis is set before the first spikelets on a panicle open, however, these late cold nights would indeed be ineffective, and even the apparent outliers in Fig. 1a would fall into the common relationship across all sites.

The genotypes studied here behaved very similarly. The thermal response of Chomrong, a cold-adapted cultivar from Nepal, and of IR72, a high-yielding tropical cultivar, differed slightly ($P < 0.05$; Fig. 1) from that of IR64 and Sahel 108, which are also highyielding tropical cultivars. IR72 also exhibited greater sensitivity to VPD and lower sensitivity to Tmin as compared to the other genotypes, as indicated by the different factor effects in multiple linear regressions (standardized coefficients and T ratios in Table 6). Similar observations were reported by Kobayasi et al. (2010) on other cultivars. Compared to the extremely large differences in time of anthesis observed by Sheehy et al. (2007) on various cultivated and wild *Oryza* species in the Philippines, however, the genotypic differences reported here were small. Anthesis onset varied among the species between pre-dawn and the afternoon, with 09:33 h noted for O. sativa. This was matched by our observations (3.4 h after sunrise in the Philippines. A similar duration of anthesis of about 2 h was observed in both studies.

Variation in time of day of anthesis has adaptive value

Thermal stresses affect rice spikelet sterility during two critical periods, first at microspore stage during which meiosis is impeded (Satake, 1976), and about two weeks later during anthesis when pollination happens (Satake et al., 1978; Matsui et al., 1997a,b; Farrell et al., 2006; Jagadish et al., 2007). The former sensitive phase is frequently affected by chilling (Hayase et al., 1969; Satake et al., 1988) but rarely by heat because at this stage, the inflorescence is still enclosed by the leaf sheaths near the bottom of the canopy (Yoshida, 1981). During anthesis, however, the panicle is fully exposed to the atmospheric conditions near the top of the canopy. Anthesis early in the day provides for escape from peak temperatures during the day (Nishiyama and Blanco, 1980; Jagadish et al.,

2007). Ishimaru et al. (2010) recently demonstrated that introgression of an early-anthesis trait effectively reduces sterility under hot conditions. The adaptive value of anthesis happening early in the day is thus an established fact.

We propose that the observed effects of air temperature and VPD on time of day of anthesis are both of adaptive value. Earlier anthesis under high temperatures at the beginning of the day thereby increases the probability of escape from the even higher temperatures later on during the day. The advantage conveyed by the VPD response is related to transpirational cooling of the panicle, or the absence of it when VPD is low. Rice panicles are known to achieve significant cooling through spikelets' epidermal pores that cannot be regulated, as opposed to stomata (Terai et al., 1990). Spikelet temperature can thus be by several degrees Celsius higher under humid than under dry conditions at any given ambient temperature (Matsui et al., 2007; Yan et al., 2008). Earlier anthesis under humid conditions provides for potential escape from rising ambient temperatures during the day.

Towards better models predicting impacts of climate on spikelet sterility

Most rice crop models that simulate spikelet sterility caused by thermal stresses, such as ORYZA 2000 (www.knowledgebank.irri.org/oryzabeta/) or DSSAT/CERES (Jones et al., 2003), take into account neither the time of day of anthesis, nor the temporal spread of the flowering process, nor the actual organ temperature which depends on the microclimate generated by the crop. The RIDEV model (Dingkuhn, 1997; Wopereis et al., 2003) went one step further in partially simulating microclimate including floodwater temperature, which is affected by ground cover and feeds back on phenology and chilling induced sterility at microspore stage. The present study provides crucial information necessary to improve such models. It will enable incorporating algorithms predicting the climate-dependent temporal spread of the heat sensitive pollination process, both within the day (time of onset and end of anthesis) and throughout the crop's flowering period (which depends on the level of de-synchronization of flowering at panicle, plant and population scales). The present study demonstrated that these escape mechanisms possess substantial adaptive phenotypic plasticity. Their efficacy can thus be predicted for different environments through modeling. But a major challenge will be to generate the necessary data to calibrate such models for different genotypes.

More research, however, is needed to incorporate into crop models all the important processes of the plant system determining risks of thermal sterility. The present study provided information on the temporal dimension of a heat sensitive process, anthesis, but not on the actual temperature the spikelet suffers during those periods. Matsui et al. (2007) reported that rice panicles

can attain temperatures by more than 6 ◦C lower than ambient und dry conditions, enabling avoidance of sterility. Conversely, Tian et al. (2010) reported panicle temperature exceeding air temperature by 4 ◦C under humid and wind free conditions. Recently, Yoshimoto et al. (2011) presented a model called IM2PACT that predicts panicle temperature under variable field conditions. As another factor related to climate change, rising atmospheric CO2 increases canopy temperature through partial stomatal closure (Yoshimoto et al., 2005). These processes need to be considered if model predictions of thermal sterility in rice are to be accurate.

CONCLUSION

This study provided evidence of strong effects of weather, notably Tmin and VPD, effects on the time of day of anthesis in rice. This response is of great adaptive value, particularly for scenarios of global warming, under which heat induced spikelet sterility is expected to be a constraint to yield. As another factor for escape from heat stress, the aggregate duration of flowering at the panicle and population were quantified, representing a risk spreading mechanism. Models predictive of thermal sterility in rice need to consider the temporal plasticity of the anthesis process, along with accurate simulation of spikelet temperature during the critical periods.

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CHAPTER 2:

Predicting heat induced sterility of rice spikelets requires simulation of crop-generated microclimate

Cécile Julia[1], Michael Dingkuhn[1,2]*

[1]Centre International de Recherche Agronomique pour le Developpement (CIRAD), BIOS Department, UMR AGAP, F-34398 Montpellier, France

[2]International Rice Research Institute (IRRI), Crop and Environment Science Division (CESD), DAPO Box 7777, Metro Manila, Philippines

*Corresponding author

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ABSTRACT

Extreme temperatures cause spikelet sterility in rice and thus yield losses. Predicting sterility is difficult because organ temperature may differ from air temperature. Four rice genotypes were planted under irrigated flooded conditions in a similar replicated design in four environments: the relatively humid dry season in the Philippines, the summer season in southern France and the cold-dry and hot-dry seasons in northern Senegal. Panicle temperature was measured by IR photography on ca. 4000 images, complemented with simultaneous monitoring of micro-climatic variables on the floodwater-canopy-air continuum. Spikelet sterility was observed at the population scale at grain maturity, and canopy morphology was also characterized (plant height, leaf area index, panicle position within the canopy and panicle exertion). The period and time of day of anthesis (TOA) was estimated using a model developed on the same experiments as described in a previous paper. Panicle temperature varied between 9.5 °C below and 2 °C above air temperature at 2m. During TOA it was on average slightly warmer than the air in the Philippines and significantly colder in Senegal. Spikelet sterility was disaggregated into three components caused by chilling at microspore stage,

incomplete panicle exertion at anthesis and high panicle temperature at anthesis. Chilling caused up to 100% and heat up to 40% sterility, the former mainly in the Senegal cool-dry season and the latter in the Philippines. All genotypes avoided heat sterility in the hot-dry season in Senegal despite air temperatures up to 40 ^{o}C, by a combination of escape (early TOA) and avoidance (transpiration cooling). Only one genotype had no chilling induced sterility due to physiological tolerance. It is concluded that heat stress causing sterility is more likely to occur in warm-humid than hot-arid environments due to humidity effects on transpiration cooling. Models predicting global warming effects on sterility losses need to consider microclimate and organ temperature, and research is now needed on the genetic control of panicle transpiration cooling.

INTRODUCTION

It is well established that high temperatures occurring at anthesis stage can cause spikelet sterility in rice and thus, yield losses (Satake and Yoshida 1978, Nishiyama and Blanco 1980, Nishiyama and Satake 1981a, Baker *et al*. 1992). Global warming is likely to aggravate this problem, and crop models have thus been used to predict rice yield losses associated with climate change (Baker *et al*. 1992, Sheehy *et al*. 2006). In a recent study, we suggested that such extrapolations are unreliable if they do not take into account the variability of time of day of anthesis (TOA; Julia and Dingkuhn 2012). Rice spikelets open only during about two hours to achieve pollination, a process that happens earlier in the day and thus helps escape from heat damage when nights are warm and/or days are humid.

Adaptive plasticity of TOA is not the only heat protection found in rice. Some cultivars show physiological tolerance of the reproductive processes, such as pollen quantity, morphology of the reproductive organs and up-regulation of heat shock proteins (Jagadish *et al*. 2010). Rice panicles can also avoid heat damage through evaporative cooling caused by transpiration. Matsui et al. (2007) reported that rice panicles can attain temperatures by more than 6 ^{0}C lower than ambient when atmospheric evaporative demand is high, whereas under humid conditions, panicle temperature can exceed air temperature by 4 ^{0}C (Tian *et al*. 2010). In fact, rice panicles do not possess functioning stomata (O'Toole and Garrity 1984, Ishihara *et al*. 1990, Ekanayake *et al*. 1993) and transpire through permanently open epidermal pores that can be more or less clogged with silica deposits (Tahahashi and Kurata 2007, Takahashi *et al*. 2008). On the basis of micro-climatic observations and measurements of panicle temperature, Yoshimoto *et al*. (2011) recently presented a model called IM^{2}PACT using a radiative heat balance to predict rice panicle temperature.

Multi-environment, field-scale studies linking rice panicle temperature to climatic conditions and to the resulting spikelet sterility are unavailable. As shown by Julia and Dingkuhn (2012), such

studies, to be conclusive, must also observe the variability of the temporal spread of flowering at plant and population scale, as well as the variability of TOA. Furthermore, yet another physiological source of spikelet sterility must be factored into such an analysis, namely, incomplete panicle exertion. Modern semi-dwarf rice cultivars have short peduncles and peduncle elongation is sensitive to stresses, resulting in lower portions of the panicle to remain stuck in the enclosing leaf sheath during drought (Muthurajan *et al*. 2011) or thermal stresses. Spikelets that do not participate in panicle exertion remain sterile but this sterility cannot be attributed directly to organ temperature at anthesis. Lastly, hot, semi-arid environments are prone to cold nights, resulting not only in heat stress during anthesis but also chilling stress during the early microspore stage (Satake 1970, Dingkuhn *et al*. 1995). Shimono *et al*. (2005) showed that spikelet sterility is correlated with the temperature of the panicle at this stage, enclosed by the leaf sheaths. During the early microspore stage, the panicle is exposed to the temperature of the floodwater and not the atmosphere. This type of chilling injury should thus be studied using the water temperature (Dingkuhn *et al.* 1995, Shimono *et al*. 2007).

Crop-generated microclimate, morphology, the timing of phenological processes and their sensitivity to organ temperature interact to determine the final incidence of spikelet sterility. The present study, which had the same experimental basis as the precursor study on time of day of anthesis (Julia and Dingkuhn 2012), sought to (1) relate observed panicle temperature during heading and flowering stages to climatic and microclimatic conditions, and (2) relate observed spikelet sterility observed at maturity at the population scale to the temperature experienced by panicles during the period and the time of day they underwent anthesis. Furthermore, we sought to dissociate the observed sterility incidence into fractions attributable to heat stress at anthesis, chilling stress and incomplete panicle exertion. For this purpose, four contrasting rice genotypes were grown under flooded conditions in near-identical experiments in four environments: the cold-dry and hot-dry seasons in Senegal, the summer season in southern France and the dry (but comparatively humid) season in the Philippines.

MATERIALS AND METHODS

Experiment sites, design and management

Field experiments were conducted between January 2009 and July 2010 in 4 environments, named hereafter Senegal hot-dry season, Senegal cool-dry season, France temperate summer and Philippines dry season. In Senegal, the same field was used located on the Sahel research station of the Africa Rice Center (ARC) at Ndiaye in the Senegal river delta (16°20’N, 16°26’W, 5m asl). The soil

was a clay soil (Orthothionic Gleysol), containing 40–54% clay, 44–30% silt and 16% sand, with pH(H_2O) 6.5. The field in Fance was located on the research station of the French Center for Rice (CFR) located in the Rhone delta (Camargue, 43°70'N, 4°56'E, 4m asl). The soil was a sandy loam type consisting of 15.8% clay, 46.5% silt and 37.8% sand, with pH(H_2O) 8.2, constituting a 40-50cm layer of fluvial sediments covering deposited on a saline, acid-sulphate Mangrove soil. In the Philippines, the field was located in the lowland part of the experiment farm of the International Rice Research Institute (IRRI) (14°16'N, 121°25'E, 16m asl). The soil was an Andaqueptic Haplaquoll composed of 58% clay, 33% silt and 9% sand, with pH(H2O) of 6.6.

Elemental plot size was 16m² (France and Senegal) or 20m² (Philippines). In all cases, the field was embedded in a large irrigated rice area. In all environments, the experiment was conducted under fully irrigated flooded conditions until surface drainage at about 14d after end of flowering. Floodwater depth was maintained at 5-10 cm except during crop establishment, when floodwater depth was adapted to seedling size.

A randomized complete block design with 3 replications (2 in France) was used. Crop establishment differed among sites following standard practices for the respective stations: In the Philippines and France, seedlings were sown and raised in seedling trays in the greenhouse, then 15-d old plants (29d in France) were manually transplanted into the wet field at a density of 25 hills.m^{-2} (20cm spacing), each hill having 2 plants. In Senegal, pre-germinated seed was directly, manually dibbled onto the wet field using the same population density and geometry as in the Philippines and France. At all sites, inorganic fertilizer and crop protection inputs were used according to local standards for high-input, fully protected systems. Weed control was manual, however, and conducted as needed to keep plots weed free.

The cold-adapted cultivar Chomrong, which flowers extremely early under tropical conditions, was sown later than the other cultivars in Senegal in order to reduce the time window of flowering observations.

For all the sampling and measurement procedures we excluded the 3 border lines of the subplot to avoid border effects. In all environments, experimental plots were located within a larger, planted (flooded) irrigation scheme, thus avoiding oasis effects at the field scale.

Genetic materials

Four *Oryza sativa* L. cultivars were in the experiment at all sites and seasons. Among them, 3 were improved, medium-duration, indica-type, semidwarf varieties including IR64 (international check variety known to be well adapted to conditions in both Senegal and Philippines), IR72 (similar to IR64 but having slightly longer duration and smaller flag leaves), and Sahel 108 (local check in

Senegal, characterized by tall erect flag leaves shading the panicle). The fourth cultivar was the traditional, lowland-adapted japonica type Chomrong from Nepal, a short-duration cultivar that is cold tolerant and adapted to the high altitudes. Chomrong builds a distinct canopy structure at flowering with a high panicle layer located above the leaves due to long peduncles and pronounced panicle exertion. The 4 cultivars were thus selected for their different crop architecture at flowering stage and for different thermal adaptation. Seed for IR64 and Sahel 108 was obtained from the rice germplasm collection of CIRAD; for Chomrong from the CIRAD-FOFIFA rice breeding program in Antsirabe, Madagascar; and for IR72 from the IRRI germplasm bank in the Philippines.

Agro-climatic measurements

At each site and season, agro-meteorological measurements were continuously made at 2m above ground at the center of the experimental field during the reproductive and ripening periods of the crops: March and April 2009 in the Philippines, August and September 2009 in France, and January-March 2010 and May-June 2010 in Senegal. They were backed by data from standard weather stations located on the same experimental station.

For each experiment the meteorological data including air temperature (T), wind speed (V), relative humidity (RH) and solar radiation (Rs) was measured in the middle of the plot where an agro-meteorological station was set up for the entire duration of the experiments. The instruments were located at 2m from the ground, or approximately 1 to 1.5m above the canopy. We used Campbell Inc. equipment (UK): a MP100A Rotronic temperature and Relative Humidity sensor held in a naturally ventilated radiation shield for the air temperature and humidity, and an A100R cup anemometer for V. K thermocouples were placed in the water of each subplot to measure water temperature. They were placed within the plant base to avoid any direct contact with incident solar radiation. We recorded the data with a Campbell CR1000 data logger every 5s and average over 1 min (4 minutes for the Philippines) for the entire periods of observations in the field. For each thermal image of plant leaves of panicles, corresponding, simultaneous environment data was thus available.

For every minute (or every 4 minutes for the Philippines) we calculated the average potential evapotranspiration (PET), the Vapor Pressure Deficit (VPD), and the sun height (H) according to the FAO Irrigation and Drainage Paper No 56 (Allen et al., 1998). VPD was calculated based on the following equation:

$VPD = e_s \times (1- RH/100)$

Where $e_s = e°(T) = 0.6108 \exp \frac{17.27\,T}{T+237.3}$ is the Saturation Vapor Pressure at T (in kPa)

(RH and T expressed in percent and in degree Celcius, repectively)

The solar altitude angle or sun height (H) is a function of the solar time and the date and is calculated from the following equation:

$$\sin H = \sin(\mathrm{Lat}) \sin(\mathrm{Decli}) + \cos(\mathrm{Lat}) \cos(\mathrm{Decli}) \cos\omega$$

where: Lat is the latitude (in radian), Decl the solar declination (in radian), ω the solar time angle at midpoint of the period of time considered (in radian).

Micro-meteorological measurement

For all the sites, a mobile micrometeorological station was set up in the field and moved from a genotype to another when the subplot reached the flowering stage. This station was left at least for two consecutive days on the same subplot.

Temperature sensors were placed at 3 different heights within the canopy: A MP100A Rotronic temperature and Relative Humidity sensor (Campbell Inc. equipment, UK) held in a naturally ventilated radiation shield was set up in the middle of the panicle layer; a T107 temperature sensor (Campbell Inc. equipment, UK) also held in a naturally ventilated radiation shield was positioned inside the canopy between the panicle layer and the water; and a T107 temperature sensor was placed in the water. A net radiometer CNR1 (Campbell Inc., UK) was set up 30cm above the canopy to measure the incident solar radiation (Rs) and net radiation (not used in this study).

We recorded the data with a Campbell CR1000 data logger every 5s and averaged them over 1 min (4 min for the Philippines) for the entire periods of observations in the field.

All equipment was clamped on a 3m long metal pole previously rammed into the soil. Each subplot had a pole set up before the crop reached flowering stage except in France and in the Philippines where a single pole was carefully moved with all equipment attached from a subplot to another.

Measurement of phenology for cold sensitive stage

To link the incidence of spikelet sterility observed at maturity with the environmental conditions measured during the sensitive stages, it is necessary to identify the periods of the sensitive stages. For heat stress, the main sensitive stage is flowering which is easy to observe directly in the field (for details of measurements of flowering periods at crop, panicle and spikelet scale (TOA) refer to Julia and Dingkuhn, 2012). Regarding the chilling stress, the most sensitive stage (microsporogenesis) is not directly visible on the plants. We used an indirect method based on the Auricle distance (AD) measurements which is the distance between the auricle of the flag leaf (last leaf) and the auricle of the penultimate leaf. It is a non-destructive measurement to obtain a fair approximation of the time

window when microsporogenesis occurs on the developing panicle enclosed in the same culm. Previous studies involving destructive sampling and microscopy of developing pollen reported the cold sensitive stage for AD values between -16cm and +2cm, with some variation among genotypes (Satake *et al*. 1970, Imin *et al*. 2006). Note that when the flag leaf is emerging and therefore still partially in the sheath, AD<0; when the flag leaf is totally exerted, AD≥0. To avoid destructive sampling, we assumed that the cold sensitive stage occurred within the time window corresponding to -16cm<AD<2cm for all genotypes and sites.

Daily AD measurements were made during both cold and hot season in Senegal on 6 plants (1 tiller/plant) per variety and per replication. The tillers were previously tagged when the flag leaf lamina was visible. The AD was measured with a simple ruler. When AD<0, the flag leaf auricle is not directly visible because located inside of the sheath, but the observer easily finds its position by gently pressing the sheath to feel the auricle bulge.

We also measured the panicle heading and anthesis duration on the same tagged tillers (cf. Julia and Dingkuhn 2012). On this basis, using weather data and for each individual tiller observed, we calculated the thermal time in degree-days between microsporogenesis and onset of anthesis. This was then used to evaluate the chilling sensitive time window for the whole subplot (tiller de-synchronization included: cf. Julia and Dingkuhn 2012). We also applied these results to the trials in the Philippines and France where no measurement of AD where done.

During microsporogenesis, the sensitive organ (young panicle) is located at the bottom of the tiller which is in the water. We therefore calculated the daily water average, minimum and maximum temperatures and we averaged them over the cold sensitive period for each subplot to relate it to the sterility observed at maturity.

IR measurements on plants at flowering

A total of approximately 4000 IR images of panicles and leaves were made in all environments except France. Images were taken on all days falling into the flowering period, between one h before onset of anthesis until one h after end of anthesis. The time of anthesis varied among environments (cf. Julia and Dingkuhn 2012). We took at least 10 images per subplot and per measurement session. Each shot contained between one and five panicles well visible.

All thermal images were taken with a thermo tracer TH7800 with microbolometer FPA system developed by NEC San-ei Instruments, Ltd (distributed by Impac, France). It operates in the wavebands 0.78–1.00 µm and has a thermal resolution of 0.1 °C within the measuring range of -20°C to 100°C. The images have a resolution of 320×320 pixels. All images were analysed with a dedicated software called Microspec Pro 4.0.10 (San-ei Instruments, Ltd). Emissivity of panicles and leaves was

set to 0.98 for the entire study. The camera provides a black and white image for visible light for the same frame taken for the IR image but its quality was not satisfying so a digital photo of the same scene was taken just before the IR shot with a commercial camera (Fujifilm, Finepix J10, 8.2Mpixel). To avoid any side interference with the measurement, the operator made sure to have the sun always in his back, and bright and hot backgrounds on the scene were avoided. The images were taken at a distance of approximately 50 cm from the plants at an angle of -30 to 0 degrees relative to horizontal. Shading of the scene by the operator was avoided. More than half of the measurements were made on panicles that were at least half exerted and located in the center of the panicle layer within the canopy, but measurements were also made on panicles having different position in the canopy or exertion, and this information was recorded. For each panicle shot we measured its position from the ground with a ruler, the flag leaf angle on the stem with a protractor, the approximate panicle exertion as percentage , as well as the flowering status (pre-, onset, mid-, end- or post-anthesis).

Images were taken irrespective of weather in order to capture a maximal range of environmental conditions.

The images where analysed manually on the computer afterwards. The Microspec software allows to manually trace object contours and extract the mean temperature within them. For every image we outlined panicles (at least 1 per shot) and leaves (at least 2 leaves per shot, data not presented here) and extracted their average temperatures. The recorded information was entered into a data base where it was combined with microclimatic, agro-climatic, agronomic and morphological information. This allowed multi-variate queries and extraction of information associated with the specific time of an IR scene and the crop concerned.

Plant and canopy measurement at flowering

At flowering, the height of the canopy (position of the highest leaf on the subplot) and of the panicle layer (lowest and highest panicle position on the subplot) were measured from the ground with a 1.50m ruler. Two measurements per subplot were taken and averaged daily.

Panicle exertion and Sterility at maturity

Panicle exertion is the extent of panicle emergence from the enclosing flag leaf sheath. It was used here to estimate the fraction of spikelet sterility attributable to incomplete exertion. At maturity, panicle exertion was measured with a ruler on 15 tillers of 15 plants chosen randomly per subplot (border plants excluded). We calculated the panicle exertion as the percentage of the exerted panicle length over total panicle length. Total panicle length is the distance from the top of

the panicle to the neck node. The first spikelet is located a few centimeters above the neck node position (≤ 3cm, data not shown). Therefore, for a panicle exertion superior or equal to 90%, no spikelet is stuck inside the sheath and 100% of the spikelets are exerted. The density of spikelets is not constant along the panicle but this was not measured. We thus assumed proportionality between the exertion the spikelet-bearing part of the panicle and the fraction of spikelets exerted.

For the estimation of total spikelet sterility, 10 individual plants per sub-plot were randomly sampled and used to measure yield components such as panicle number per plant, panicle weight, and number of spikelets and grains per panicle (data not presented). The 1000 filled grain weight and 1000 unfilled grain weight was also measured. The sterility was calculated as follows:

$$\text{Sterility}\ (\%) = 100 \times \frac{\text{number unfilled spikelets}}{\text{total spikelet number}}$$

To study the sterility due to thermal stress, we removed the fraction of sterility due to incomplete panicle exertion. The sterility corrected is:

$$\text{Sterility corrected}\ (\%) = 100 \times \frac{\text{Sterility}-\text{NE}}{100-\text{NE}}$$

Where NE is the non exerted fraction of the panicle in percent

Distinction of heat-induced sterility (at anthesis) and chilling-induced sterility (at microsporogenesis) was made by plotting the corrected sterility values against minimum floodwater temperature, which gave a distinct response at low temperatures. This enabled identifying cases affected by chilling, which were then removed from a similar plot for corrected sterility vs. panicle temperature at anthesis.

Data base construction and analysis

For each site, at a given time and for given variety we used Access (Microsoft Office 2007) to combine data on organ temperature with the corresponding agro-meteorolgical, micro-meteorogical, phenological and morphological measurements for the same time and sub-plot. Information obtained at maturity (sterility, panicle exertion) was also included We obtained a file of nearly 5000 complete data sets that was exported to Excel (Microsoft Office 2007). We used Excel Stat (embedded in Excel) to perform multiple linear correlations among variables for subsets or the entire file.

RESULTS

Climatic profiles of environments during flowering

The three sites provided four markedly different climatic environments during the period the 4 genotypes flowered (Fig1). The cool-dry and hot-dry seasons in Senegal had strong variations in Tmax (24-44 °C) combined with low relative humidity during the day (frequently below 20%, data not presented). This resulted in extremely variable and sometimes very high VPD (up to 4 kPa). Differences between Tmin and Tmax were greatest in the Sengal cool-dry season, with Tmin sometimes dropping below 12 °C , whereas, in the hot-dry season Tmin was generally above 20 °C . One of the short periods when Tmax reached about 40 °C in the cool-dry season was a Harmattan dust storm, with solar radiation reduced by about 10%.

The dry season in the Philippines was is a humid environment (average RH 85%). Tmin-Tmax differentials were small and temperatures were comparatively constant. Tmin was nearly constant at about 24°C. VPD was generally below 1.5 kPa and mostly below 1 kPa. France temperate summer was intermediate between Senegal and the Philippines in terms of Tmin-Tmax differences and VPD. Average VPD was 1.7 kPa ranging between 1 and 2.3 kPa. Both Tmin and Tmax decreased during the experiment, with Tmin dropping below 10°C (end of the summer).

Solar radiation (not presented) was similar for all sites (around 20 MJ/day) but in the Philippines we experienced many cloudy and rainy days compared to the long-term average. Within the 60 days of measurements, 24 days had rain. In France, 4 days of rain were recorded in September but no rain was recorded during the two experimental periods in Senegal.

Water temperature (Figure 1, solid symbols) measured under canopies having at least a leaf area index of 5 (data not presented) generally showed less diurnal variation than air temperature. It also had less day to day variation than air temperature. This was particularly striking in Senegal where maximum water temperature did not follow at all the strong oscillations of Tmax (air), whereas in France water temperature followed air temperature closely.

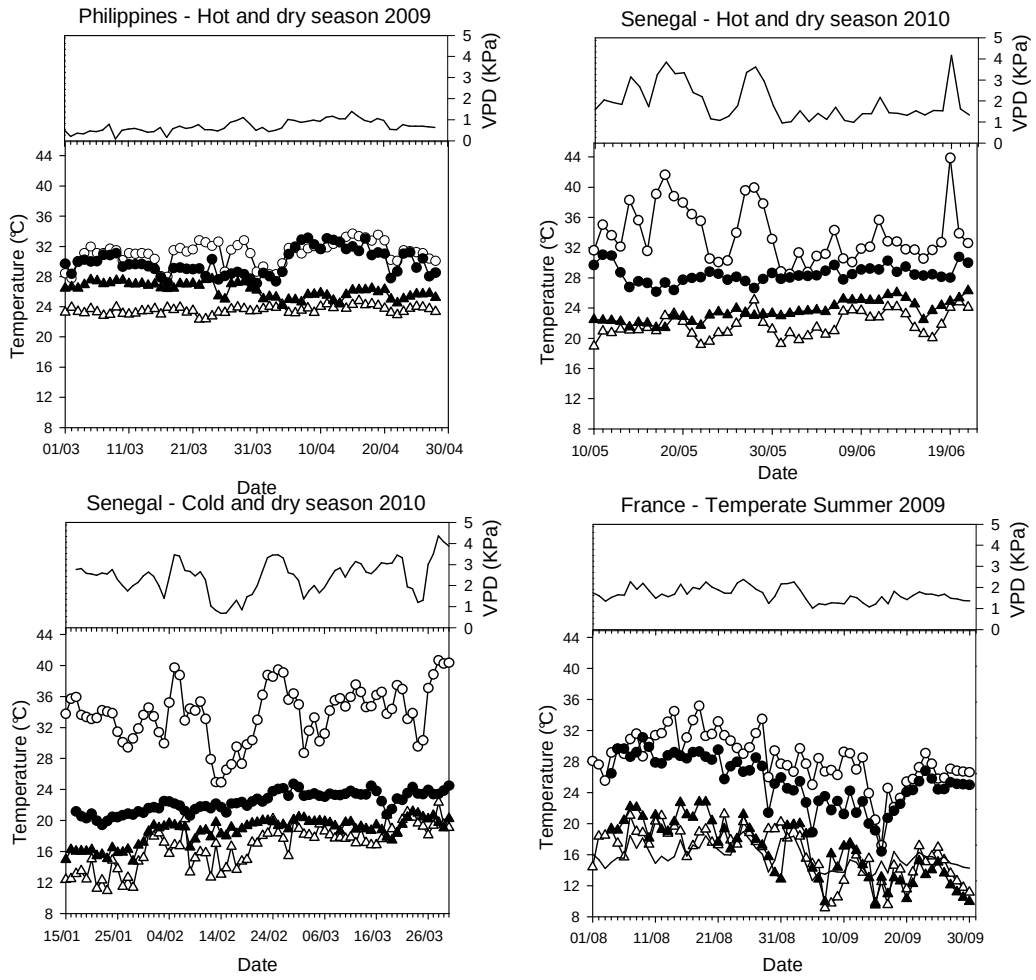


***Figure 1**: Climatic environment description for each site and season during the time window of flowering observation in the field (approximately 2 months). Are presented daily minimum and maximum temperatures of both air at 2 meters and water in the plot. The VPD is presented in the upper part of the graphs.*

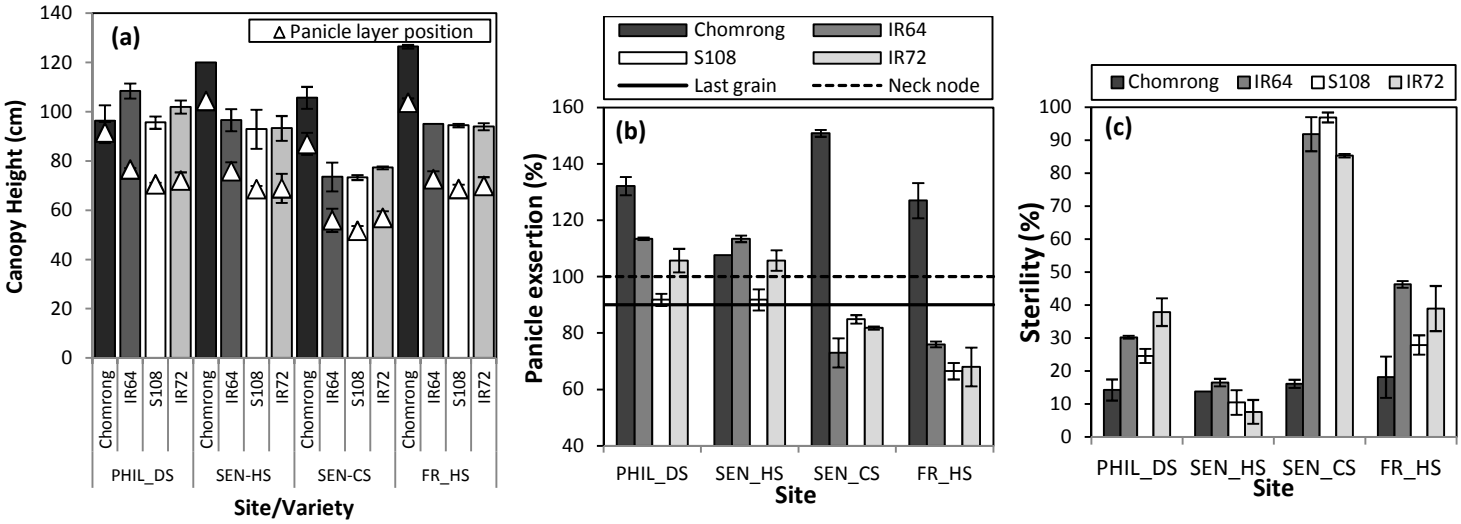
Variation in canopy height, panicle position, panicle exertion and spikelet sterility

Canopy height at flowering was similar for the three semidwarf rices IR64, Sahel 108 and IR72 within any of the four environments (Figure 2a, vertical bars). However, while it was between 95 and 110 cm in the Philippines, France and the Senegal hot-dry season, it was reduced to less than 80 cm in the Senegal cool-dry season, indicating stunting associated with the low night temperatures. IR64 differed slightly from the other semidwarfs by being significantly ($P < 0.05$) taller in the warm-humid environment of the Philippines. The cold tolerant traditional genotype Chomrong displayed a markedly different pattern from the semidwarfs by showing no height depression in the Senegal cool-dry season (as compared to the Philippines) but growing considerably taller in the Senegal hot-

dry season and in France, which was associated with an extended crop duration caused by photoperiod sensitivity (phenology was reported by Julia and Dingkuhn 2012).

Chomrong also differed from the semidwarfs in having a higher absolute and relative panicle position (relative to canopy height) at flowering (Figure 2a, white triangles). This was particularly the case in the Philippines, where Chomrong had smaller canopy height than in the other environments but its panicles were located at the top of the canopy due to long peduncles. By contrast, Sahel 108 had panicles located deep inside the canopy, a trait this cultivar had explicitly been selected for in Senegal.

When judging panicle exertion from the position of the panicle neck node relative to the top of the enclosing leaf sheath (broken horizontal line in Figure 2b), exertion was incomplete for the three semidwarfs in the two cooler environments, Senegal cool-dry season and France. Exertion was always complete in Chomrong due to long peduncles. Sahel 108 panicle exertion was incomplete in all environments. However, when judging panicle exertion from the position of the lowest spikelets on the panicle (solid horizontal line in Figure 2b), exertion was nearly complete for Sahel 108 in the Philippines and in the Senegal hot-dry season, but not in the cooler environments. Consequently, a large fraction of spikelets on panicles of the semidwarfs never emerged from the enclosing flag leaf sheath in the two cool environments and thus remained sterile.



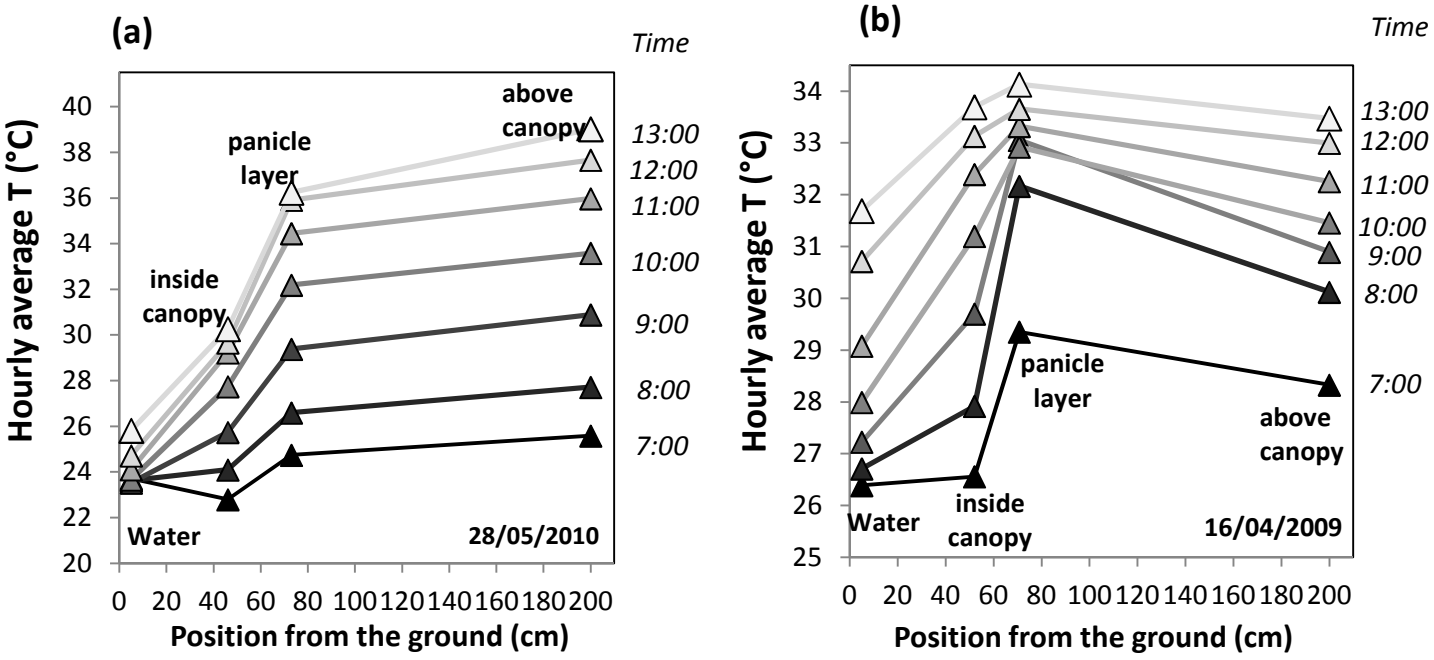
<u>Figure 2 a, b, c</u>: Canopy structure measurements and sterility for the 4 sites and varieties: (a) Canopy height in cm from the ground (position of the highest leaf on the subplot), and position of the center of the panicle layer in cm from the ground (white triangles); (b) Panicle exertion (in %) and (c) Sterility (in %) measured at maturity.

The bulk percentage of empty (sterile) spikelets at population scale varied markedly among environments and genotypes, except for Chomrong which had nearly constant sterility between about 13 and 20 % (Figure 2c). Sterility of the three semidwarfs, however, was near-total in the

Senegal cool-dry season whereas it was small in the Senegal hot-dry season. Sterility was greater than the level considered normal and agronomically acceptable (10-20%) for the semidwarfs in both the Philippines and France, ranging from 26 to 48%. This was in part due to poor panicle exertion in France but not in the Philippines.

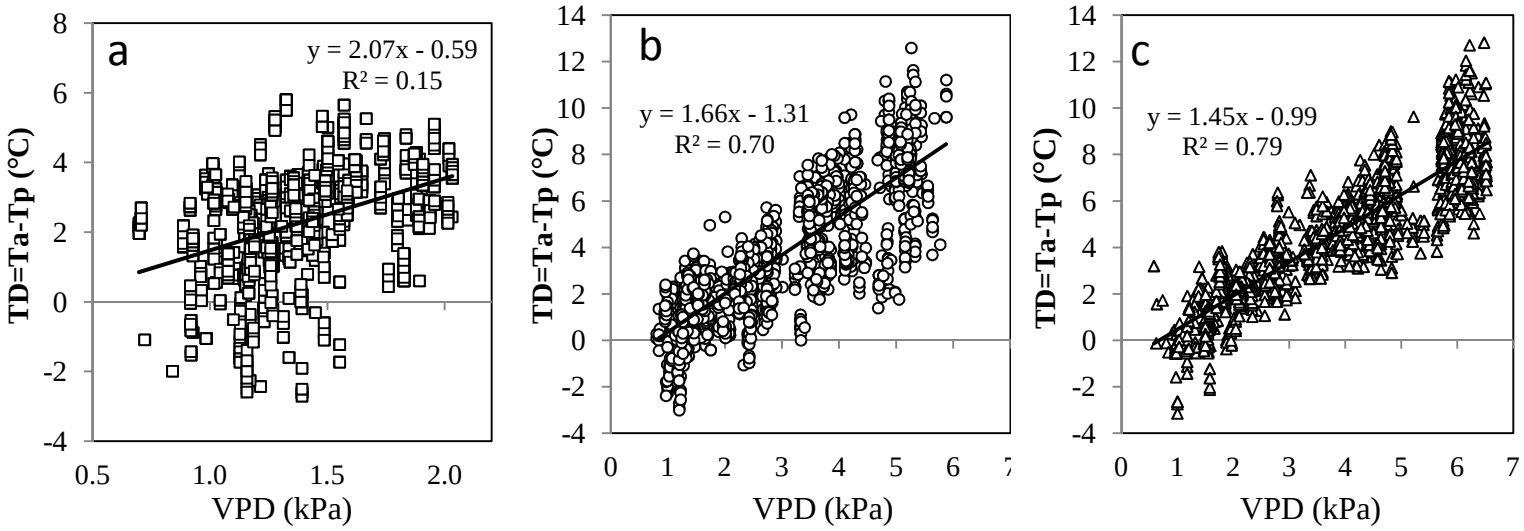
Relationships between air, water, panicle and leaf temperature

Temperature gradients between the floodwater and the atmosphere at 2m within the planted plots differed strongly among environments and changed during the day (Figure 3: example of IR64 on a clear day in the Senegal hot-dry season and in the Philippines). In Senegal (Figure 3a), the floodwater did not follow the strong diurnal fluctuations of the air temperature at 2m and remained close to the minimum air temperature even at midday. Air temperature within the canopy and near the top of the canopy (panicle layer) showed intermediate behavior. In the Philippines (Figure 3b), water temperature was slightly cooler than that of the air at 2m but showed similar oscillations. The air at the panicle layer, however, was warmer than that at 2m, a trend that was observed on a majority of days (data for other days not presented). The microclimate generated by the crop was thus markedly different from



<u>Figure 3 a, b</u>: Temperature gradients from water to 2 meters above the ground at different time of the day: hourly average temperatures from 7am to 1pm for the variety IR64 in Senegal during the hot and dry season (a) and in the Philippines during the dry season (b). The gradients presented were measured on hot days and the temperature sensors were placed at four levels: in the air (at 2m from ground), inside the panicle layer, inside the canopy (between the panicle layer and the water surface), and in the water.

The temperature difference (TD) between the air at 2m (weather station embedded in field) and the panicle (derived from IR images) was highly significantly correlated ($P<0.0001$, $R^2 >= 0.7$) with air VPD at 2m in the Senegal hot-dry and cool-dry seasons (Fig. 4bc). This correlation showed much greater scatter ($R^2 = 0.15$) in the Philippines (Fig. 4a) because VPD was lower and varied less. These correlations confounded effects of genotype and time of day (observations between 0900 and 1630 h). No other single environmental variable explained TD as much as did VPD at 2m.



<u>Figure 4 a, b, c</u>: Relationship of the difference between panicle temperature and air temperature at 2m (TD) and the air vapour pressure deficit (VPD) calculated at the same time. Correlations for TD vs VPD : Philippines dry season (a); Senegal hot and dry season (b); and Senegal cold and dry season (c).

A multiple linear regression model was constructed to explain TD, across all sites (except France where no IR images were taken), genotypes and time of day. As explanatory variables we considered weather variables at 2m, the available information on canopy structure and solar angle. Although the best combinations of predictive variables varied slightly among sites and genotypes (details not presented), the general model presented in Fig. 5 had almost the same predictive skills as site- or genotype-specific models. It included three weather variables (VPD, Tair and Rs measured at 2m), one crop variable (Top_P, indicating the height of the upper boundary of the panicle layer) and the sun angle as calculated from solar time of day and day length (H_deg). Inclusion of variables related to nebulosity such as actual over potential Rg did not further improve the correlation and were thus disregarded.

The general model explained 80.3 % of the variation of TD for 4914 cases (about 4000 IR images). Compared to the multiple regression model, VPD alone explained 65% of the variation ($Y = 1.29x + 0.02$; $R^2 = 0.65$), indicating that VPD was by far the most important predictor. Along with H and Ta_2m, VPD had a positive effect on TD, thus lowering panicle temperature relative to ambient (Table1). Solar radiation and (Rs) and Top_P increased panicle temperature.

Observed panicle temperature was up to 9.5 °C cooler and up to 2 °C warmer than Ta_2m (Figure 5). Calculated vs. observed values were linearly correlated and had roughly constant scatter on all regions of the relationship, indicating the absence of region specific bias. The entire range of variation occurred in the Sengal hot-dry and cool-dry seasons, whereas the range of variation of TD was only between 0 and 4 °C in the Philippines (note the distribution of symbols on Figure 5 indicating environments). The fit of the general model for TD was not significantly different for the four genotypes (data not presented).

***Table 1**: Results of the multiple regression of 4916 observations across the 2 seasons in Senegal and the Philippines to explain TD. The 5 Parameters of the models, equations and standardized coefficients, Analysis of variance.*

Model Equation :	TD1 = -1.578 + 1.093*__VPD__+0.189*__Tair__ - 3.367E-02*__TopP-__3.99E-03*__Rs__ + 3.31E-02*__H__					
Source	**Normalized coefficient**	**SD**	**t**	**Pr > |t|**	**Lower boundary (95%)**	**Upper boundary (95%)**
VPD_2m	0.685	0.013	53.738	< 0.0001	0.660	0.710
Ta_2m	0.249	0.012	20.079	< 0.0001	0.225	0.274
Top_P	-0.236	0.006	-36.652	< 0.0001	-0.249	-0.224
Rs	-0.336	0.008	-43.486	< 0.0001	-0.351	-0.321
H deg	0.181	0.008	24.048	< 0.0001	0.166	0.195
Model and variance analysis	**MCE**	**R^2**	**R^2 ajusted**	**Sum of squares**	**F**	**Pr>F**
5 parameters	**1.337**	**0.803**	**0.803**	**26802.5**	**4009.4**	**< 0.0001**

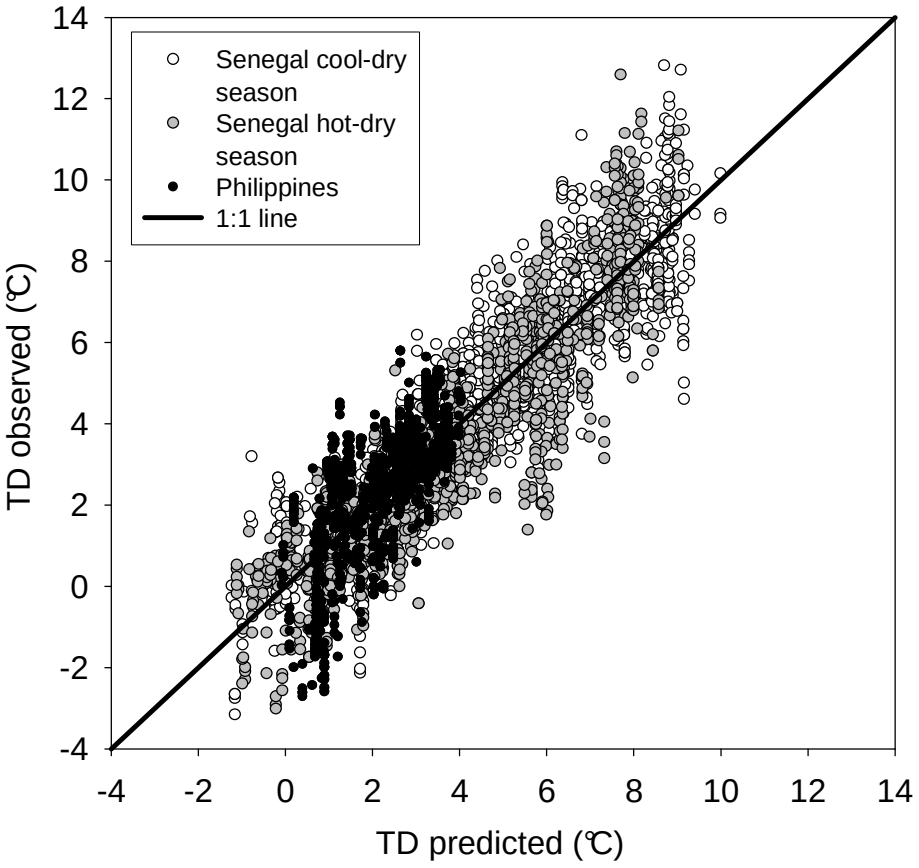


***Figure 5**: TD predicticted by the 5 parameters model vs. TD observed for the 3 sites (Senegal cool-dry season Senegal hot-dry season, and Philippines).*

Panicle temperature at anthesis

A model predicting the time of day at anthesis (TOA) described by Julia and Dingkuhn (2012) and based on the same experiments, in combination with the model of TD described above (Table 1), was used to predict the mean panicle and air temperature during anthesis for each trial, genotype and replication (Figure 6). Panicle temperature during anthesis was on average slightly higher than air temperature at 2m in the Philippines Figure 6a), whereas it was by about 4 ^{o}C lower than air in both Senegalese environments and about 1.2 ^{o}C lower in France. Consequently, although both environments in Senegal were hotter than the other environments during the roughly two hours during which anthesis took place, panicles were significantly hotter in the Philippines.

No significant differences in mean air or panicle temperature were observed among genotypes, all environments confounded (Figure 6b).

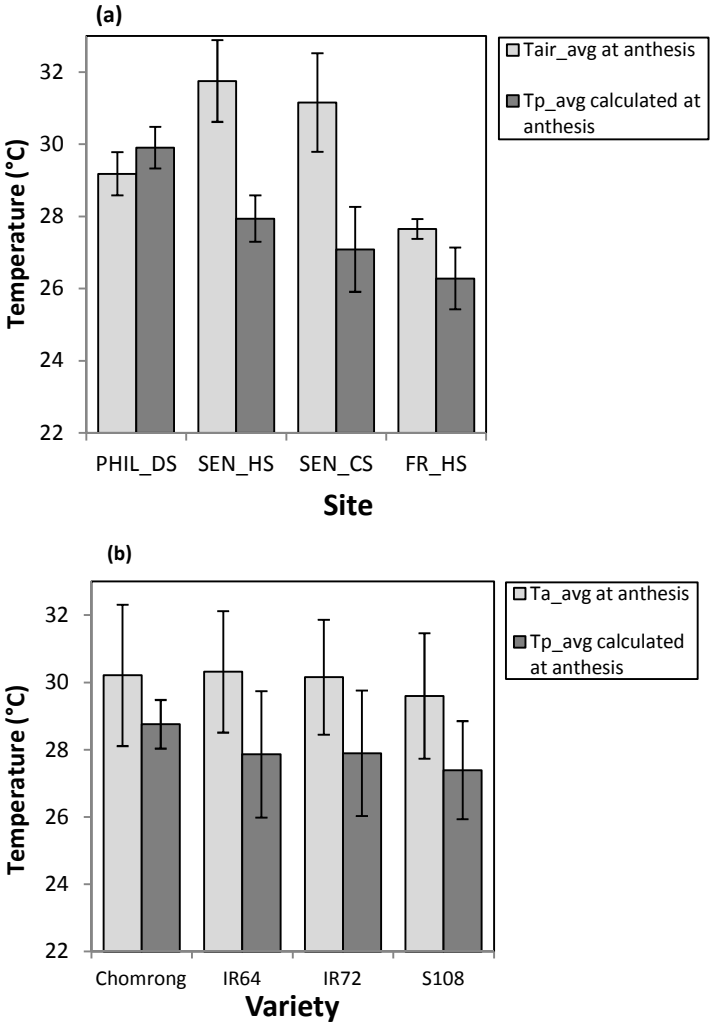


***Figure 6 a, b*: Calculated Tp at anthesis from the TD predictive model for each site (a) and variety (b).**

Chilling induced sterility

In order to estimate heat induced sterility, it was necessary to determine which combinations of genotype x environment were affected by chilling induced sterility; because these cases had to be eliminated from the analysis of heat induced sterility. Heat and chilling induced sterility are macroscopically indistinguishable because they are both chiefly male sterility.

Chilling induced sterility is mainly caused by low water temperatures during microsporogenesis stage (Dingkuhn *et al*. 1995). Observed spikelet sterility (corrected for estimated sterility caused by incomplete panicle exertion) was therefore plotted against the observed floodwater temperature at that stage (Figure 7). The three semidwarfs showed a strong and similar response and attained between 80 and 100% sterility as minimum water temperature dropped to 13-

15 °C. The cold-tolerant genotype Chomrong, however, showed no response within that temperature range.

On the basis of these observations, five environment x genotype combinations affected by chilling stress were eliminated from the subsequent analysis for heat-induced sterility (Senegal cool-dry season: IR64, Sahel 108 and IR72; France: IR64 and IR72).

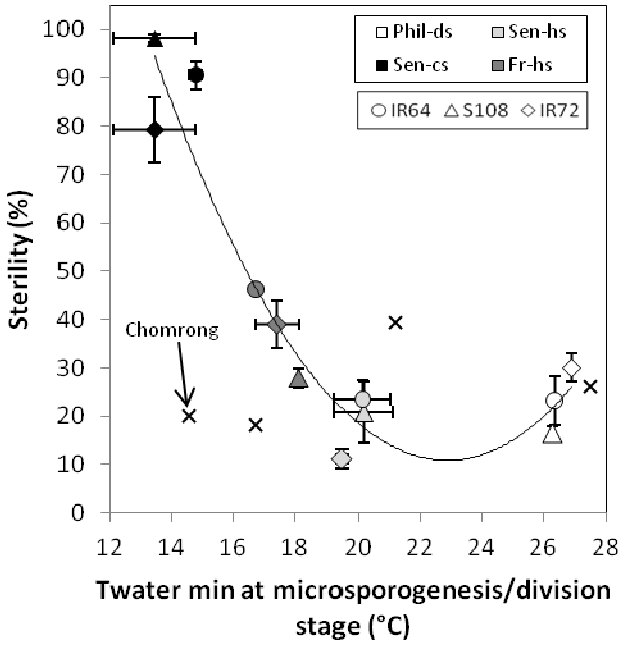
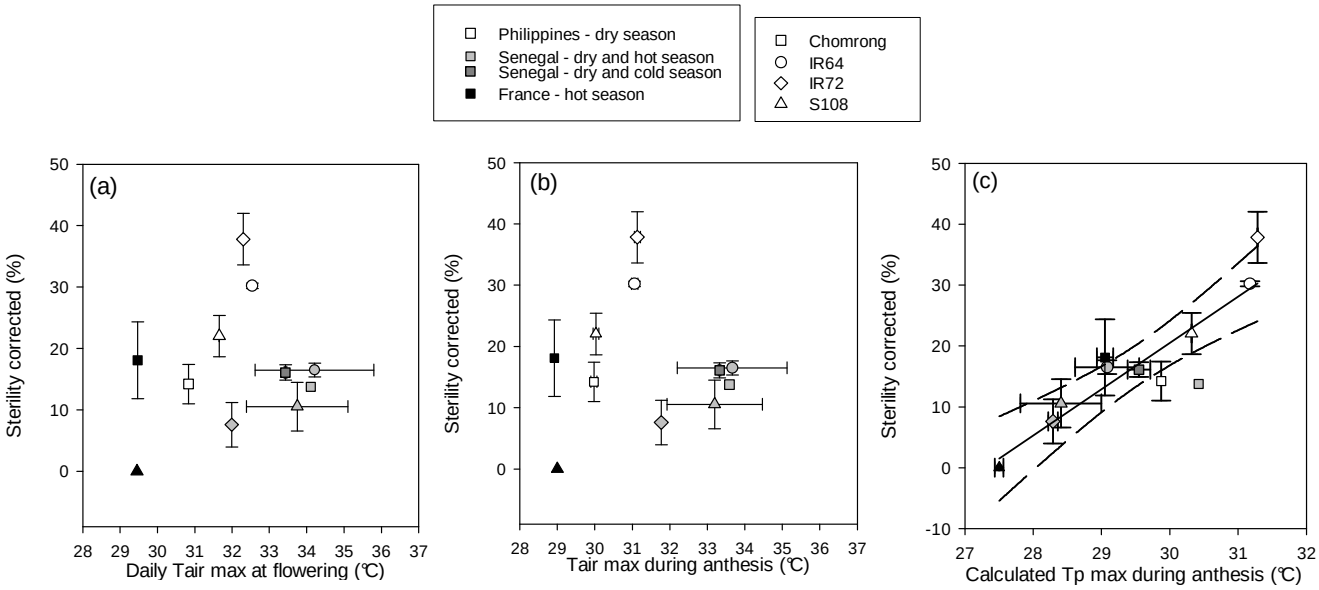


***Figure 7**: Correlation between the minimum temperature of water during the microporogenesis stage and the percentage of sterility observed at maturity for the 4 contrasted environments and 4 varieties. Chomrong was left out of the regression for being the control for cold tolerance. The other observations were strongly fitted with a polynomial equation: $Y=0.94x^2 - 43.10x + 503.90$ ($R^2 = 0.92$). The Twater min threshold to reach 50% sterility calculated is 16.4°C.*

Heat induced sterility

The remaining observations after elimination of cases affected by chilling stress were analyzed for heat effects at anthesis on spikelet sterility (Figure 8). Corrected spikelet sterility (with the estimated fraction attributed to incomplete panicle exertion subtracted) was plotted against temperature observed during the period of flowering. When the mean maximal daily air temperature during the days of anthesis was used, no relationship was observed between sterility and temperature (Figure 8a). This was also the case when the maximal air temperature occurring during TOA, averaged over the flowering period, was used (Figure 8b). A highly significant, positive correlation (R^2=0.79; P<0.01), however, was observed when the reference temperature was that of the panicle during TOA, averaged over the days the flowering period lasted (Figure 8c). By extrapolation, the correlation predicts a critical temperature generating 50% sterility (CT50; Dingkuhn *et al*. 1995) of 33-34 °C, all genotypes and environments confounded.



<u>Figure 8 a, b, c</u>: Relationship of maximum temperatures at flowering vs sterility corrected: (a) Daily maximum air temperature average over the flowering period vs Sterility; (b) maximum air temperature during anthesis average over the flowering period; (c) Correlation for maximum Tp at anthesis average over the flowering period vs Sterility. Correlation across 4 environments: y = 7.61x - 207.74, R^2 = 0.79.

DISCUSSION

The approach

This study was distinguished by the large geographic and climatic range of environments, to which the same genotypes were exposed in a similar experimental setup. All environments had high solar radiation but they differed in photoperiod (for detailed description of radiation environments refer to Julia and Dingkuhn 2012) as well as in VPD and temperature means and extrema. Our analytical approach was to extract patterns of plant responses, such as transpiration cooling, using queries from a data base that included nearly 4000 IR images of plants taken in various environments at different times of day, along with corresponding microclimatic, crop morphological and phenological observations. The precursor study (Julia and Dingkuhn 2012) provided us with models to estimate the timing of sensitive stages (e.g., period and time of day of anthesis), to which we then applied the empirical microclimatic models extracted from the database.

This approach was effective in (1) disaggregating the bulk spikelet sterility observed at crop maturity into its various causes, and (2) evaluating the escape, avoidance and tolerance mechanisms at work.

Thermal stress caused sterility by at least three distinct mechanisms

The three major causes of thermal sterility in rice considered in this study are well known. Chilling of spikelet primordia during microsporogenesis stage is most commonly associated with low minimum temperatures of the air and particularly the floodwater in irrigated rice. It was investigated in the field by Dingkuhn *et al*. (1995) and modeled in a systems context by Dingkuhn (1995). Although water management options give some scope for the management of this stress (Satake *et al*. 1988), temporal escape though genotypic phenology (Dingkuhn and Miezan 1995) and cropping calendars (Dingkuhn 1995, Shreshta *et al*. 2011) are probably more effective. In the present study, sterility of the traditional cold-tolerant cultivar Chomrong from Nepal proved insensitive to chilling within the range of conditions studied, whereas the three tropical semidwarfs had near-total sterility when daily minimum floodwater temperature dropped to ≤ 15 ^{o}C during the sensitive stage. Physiological cold tolerance at the reproductive stage, as observed in Chomrong, can thus be very effective and has been previously described as involving anti-oxidative enzymes protecting the tissues (Kuk *et al*. 2003, Sato *et al*. 2011). Tolerant genotypes also produce pollen in greater quantity compared to sensitive varieties (Satake 1970), thus increasing probability of successful pollination. The number of pollen grains is directly linked to spikelet fertility (Farrell *et al*. 2006).

The second cause of sterility observed here was incomplete panicle exertion from the enclosing flag leaf sheath (Griest 1986). Poor elongation of internodes and peduncle during heading can be caused by stresses such as drought (O'Toole and Namuco 1983, Muthurajan *et al.* 2011) and chilling (Chung 1979, Hamdani *et al*. 1979). Most spikelets trapped in the sheath remain sterile although the causes are not fully known (Jagadish *et al*. 2011). Breeding of short-strawed high yielding rices has increased the risk of incomplete panicle exertion because peduncles are shorter. The three semidwarf cultivars in this study were all affected by this problem in the Senegal cool-dry season and in France, the environments having low night temperatures, whereas the taller traditional cultivar Chomrong was not. Sahel 108, selected by breeders in Senegal aiming at avoiding bird damage and improving light use through panicles hidden deep in the canopy (Dingkuhn, pers. obs. 1995), had particularly poor panicle exertion even in the warmer environments.

We estimated the extent of sterility caused by incomplete panicle exertion from the fraction of spikelet-bearing panicle length failing to exert. This estimate may carry some systematic error due to heterogenous distribution of spikelets along the panicle. The potential effect of this error on the calculation of heat sterility (as a residual after subtraction of sterility caused by poor exertion) was small, however, and unlikely to significantly affect the results.

Heat induced sterility affected up to nearly 40% of the spikelets located on the exerted part of the panicle. The sterility vs. panicle temperature relationship during anthesis, for all genotypes

confounded because they appeared to respond similarly, indicated that sterility commences already at less than 30 ^{o}C and attains 50% at about 33-34 ^{o}C. Tropical rice has been reported to turn male-sterile at about 35 ^{o}C (Matsui et al, 1997*a*) but these measurements were based on air temperature which may not be the same as panicle temperature even under controlled conditions. Furthermore, there is considerable genetic diversity in physiological heat tolerance of rice spikelets (Shah et al., 2011).

Heat sterility occurred in the most humid and not in the hottest environment

We observed more heat sterility in the Philippines than in the considerably warmer environment of the Senegal hot-dry season. During the site and season specific time of day of anthesis (Julia and Dingkuhn 2012), panicle temperature was higher in the Philippines (and slightly higher than ambient temperature) than it was in Senegal (where panicles were much cooler than ambient). This result emphasizes the great importance of air humidity and other factors affecting transpiration cooling of the panicle. Any prediction of heat sterility from air temperature alone across different levels of VPD and solar radiation is thus prone to very large systematic errors. It is therefore essential that crop models used for such purposes consider the microclimate generated by the crop and organ temperature, which is generally not the case today, with one notable and recent exception (Yoshimoto *et al*. 2011). These authors developed a heat balance model simulating panicle temperature in rice canopies. This model is currently being incorporated into a rice crop growth model. Also the present results are being translated into a model predicting heat (and also cold) sterility of rice on the basis of the rice phenology model RIDEV that was conceived for similar purposes in the 19s in Senegal (Dingkuhn 1997).

Rice has highly effective escape, avoidance and tolerance mechanisms to thermal stresses

The present study demonstrated that rice panicles can cool their panicles very effectively under conditions of high VPD, involving high transpiration rates that were not measured here. Panicle temperature was by up to 9.5 ^{o}C below air temperature at 2m. This value is very high but is not far from 6.8 °C, the maximum temperature difference between air and panicle observed by Matsui *et al.* (2007) in Australia under hot and dry conditions based on IR measurements taken at TOA on a different rice cultivar. Rice canopies transpire through rigid epidermal pores located on that cannot be regulated like stomata (Tahahashi and Kurata 2007, Takahashi *et al.* 2008), enabling the panicle to maintain transpiration cooling even under drought conditions which are frequently associated with heat. This avoidance response, however, is not effective in warm-humid environments.

The precursor paper to this study (Julia and Dingkuhn 2012) described another adaptation of rice panicles to heat based on temporal escape. The short and singular period of TOA for each individual spikelet (ca. 2h) happens earlier in the day as nights are warmer and as air humidity is higher. In humid environments day-night temperature differences are small and consequently, nights relatively warm. This leads to earlier anthesis in the day and may help escaping peak temperatures at midday. The transpiration cooling and temporal escape processes together thus constitute complementary adaptations for coping with heat.

In our study, heat did not affect panicle exertion whereas chilling did. Incomplete panicle, although related to thermal stresses among other stresses, is a phenomenon restricted largely to modern semidwarf varieties and thus has been introduced by breeding. The long peduncles of traditional landraces of rice are, among other things, a morphological avoidance trait for physiological stress effects on sterility.

Consequences for research on adaptation to climate change

Our results have implications for the crop modeling approaches to be used for predictions of climate change impacts on rice production. Currently used crop models such as DSSAT (Jones *et al.* 2003), SARRAH (Dingkuhn *et al.* 2003) or ORYZA2000 (Bouman 2001) do not consider microclimate nor organ temperature when simulating thermal stresses. For example, it was predicted with such models that for every 1 °C increase in global temperature a yield loss of 7.2 % is to be expected (Krishnan *et al.* 2007). The general notion of increasing yield losses due to heat sterility under global warming conditions is probably true but its true magnitude and geographic distribution might be significantly different from current predictions. In particular, even small increases in air temperature might cause large effects in humid environments whereas rice crops in semi-arid, irrigated situations might be more resilient due to transpiration cooling.

There may be significant trade-offs between heat avoidance and crop water use. We can provide no quantitative information here on how water-saving management practices will affect the efficacy of transpiration cooling. Current or anticipated water scarcity in many semi-arid irrigated systems in the Sahel (de Vries 2010) and the Indo-Gangetic Plains (Abrol 1999) has triggered research on water-saving practices including alternate wetting and drying (AWD, Cabangon *et al*. 2001) and aerobic culture conditions (Tuong and Bouman 2003), along with complementary breeding efforts (Atlin and Lafitte 2002). Although rice panicles cannot regulate their transpiration (O'Toole and Garrity 1984, Ishihara *et al*. 1990, Ekanayake *et al.* 1993), water-saving practices are bound to reduce transpiration cooling and soil/water evaporative cooling at the canopy and systems level, necessarily resulting in warmer plants. This effect must imperatively be quantified and the associated sterility

risks predicted before water-saving technologies are implemented at a larger scale in hot-dry environments.

Lastly, rising atmospheric CO_2 levels are known to reduce stomatal conductance of rice, thus increasing canopy temperature by 1-2°C for anticipated conditions in 2050 (FACE experiments in Japan: Yoshimoto *et al.* 2005). Our study did not consider this long-term effect but research is under way elsewhere to quantify this effect for temperate environments using FACE experiments (Tokida *et al.* 2010). Extrapolation of such results to tropical conditions, however, may require similar experiments in the tropics, both in humid and semi-arid environments.

Efforts should also be made to study the genetic control and available genetic diversity in rice for the escape and avoidance responses described here. Some such information is available on TOA (Kobayasi *et al*. 2010), but not on its adaptive phenotypic plasticity as described by Julia and Dingkuhn (2012). Transpiration cooling capacity of rice panicles has not yet been studied in terms of genetic control and diversity.

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CHAPTER 3:

Development of RIDEV V.2, Rice Model of Phenology and Thermal Sterility of Spikelets

Michael DINGKUHN, Cecile JULIA and Jean-Christophe SOULIE

CIRAD/IRRI

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INTRODUCTION

The following report describes the development of RIDEV, a crop model simulating rice phenology and thermal-stress related spikelet sterility, developed in the context of this thesis. In fact, the CGIAR center AfricaRice commissioned the development of RIDEV in a 6-month project that also funded the extension of the stipend for this thesis after the initial 3-year period. The RIDEV report can thus be seen as a direct product of the thesis research, adding an engineering component to the scientific research described in chapters 1 and 2. The model was developed entirely on the basis of the data generated by this thesis.

RIDEV makes use of (1) the information on phenology and time of day of anthesis as described in chapter 1, and (2) panicle temperature and its relationship with observed spikelet sterility as described in chapter 2. Additional data analyses, not described in the two chapters of floodwater temperature (important for the simulation of chilling induced sterility) are also presented in this report.

There is one notable difference between the analysis of panicle temperature presented in chapter 2 and RIDEV. The multiple linear regression model developed in chapter 2 on the basis of observations in 3 environments (Senegal cool and hot dry seasons, Philippines) was not available yet at the time RIDEV was programmed in its current form:

Model 1

TD = 1.578 + 1.093 VPD_2m + 0.189 Ta_2m – 0.0337 Top_P – 0.00339 Rs + 0.0331 H

Where TD =Ta-Tp, difference between air and panicle temperature (in °C)

VPD_2m = Vapour pressure deficit at 2m from the ground (in kPa)

Ta_2m = Air temperature at 2m from the ground (in °C)

Top_P = the position of the highest panicle from the ground (in cm)

Rs = Solar radiation (in W/m²)

H = sun height or solar angle (in degree)

Instead, a different regression model based on the Senegal data only was used, as follows (details in the RIDEV report, below):

Model 2

TD = 0.782 + 0.422 Ta_2m -0.0443 RH_2m – 0.00287 Rs – 8.05 Z – 6.59 LTR

Where TD =Ta-Tp, difference between air and panicle temperature (in °C)

Ta_2m = Air temperature at 2m from the ground (in °C)

RH_2m = Relative humidity at 2m from the ground (in %)

Rs = Solar radiation (in W/m²)

LTR = Light transmission ratio

The two models yielded very similar results. RIDEV will be modified to implement Model 1 in its final version.

REPORT

1. Project synopsis

This 6-month project generated the prototype of RIDEV V.2, a much improved new edition of the phenology /sterility rice model RIDEV V.1 of the 1990s. As such, it involved far greater investment than the 6-month funding of the PhD student Cecile JULIA: It is the result of a nearly 4-year PhD thesis and several man-months of senior scientists Michael Dingkuhn and Jean-Christophe Soulie. On the scientific side, innovations include the simulation of panicle temperature and environment dependent time of day of anthesis (needed for accurate sterility simulations under heat), as well as the choice of 4 models of photoperiodism. On the informatics and development side, the model has been developed as a shared library using C++ and is coupled with with the statistical programming software R for graphical user interface and estimation tools, enabling multi-environment parameter

optimizations in a user-friendly way. This system now needs through validation before it begins its life (the software as a whole or only the model itself) in the hands of very diverse user communities. These will include decision aide at the producer or extension level, research on crop/system adaptation to climate change and variability, and phenotyping of crop panels for genetic studies. The model's source code, screens of the interface and templates for external files are provided with this report.

2. Scientific background and objectives

2.1 The old RIDEV

This project served to develop a new version of RIDEV, a cereal model of crop phenology and thermal stresses causing sterility of the spikelets on inflorescences, causing yield losses. The original RIDEV model, developed in 1995 for irrigated rice systems in the Sahel (Dingkuhn, 1997; Wopereis et al., 2003), was implemented in GW Basic language and had two versions, RIDEV_Sim (enabling predictive simulations for climatic risk analyses and farmers' guidance to chose appropriate crop calendars) and RIDEV_Cal (enabling parameterization against target files containing phenological observations, using a simple optimization procedure). The model was initially used for a regional study of thermal risks (Dingkuhn et al., 1995), varietal characterization (Dingkuhn and Miezan, 1995) and elaboration of cropping calendars (Dingkuhn, 1995), and thereafter as an agronomic decision tool by WARDA (now AfricaRice) and its NARS partners.

RIDEV differs from other crop models (1) in its simplicity, (2) focus on phenology and sterility, and (3) consideration of crop microclimate, namely simulation of organ temperature as a function of the apex' position in the soil-floodwater-atmosphere continuum.

The original RIDEV model increasingly became obsolete for a couple of reasons:

- The software basis (GW Basic on DOS) is outdated
- No investment in software maintenance and improvement
- Changes in germplasm, for which no calibration was available
- Apparently, changes in crop phenology at the Senegal study sites, either through genetic drift in the varieties (analogous to sorghum and millet during the same period? (…)) or climate change (CC), resulting in loss of model accuracy with the old parameter settings.

2.2 Need for a new RIDEV and projected users

A new version of RIDEV is needed, based on modern programming language, expanded domain of geographic validity and incorporation of new scientific knowledge on rice biology. The model is needed for the following applications:

- Simulator of the crop time frame of a new decision toll for farmers currently under development at AfricaRice and IRRI, the Rice Manager (predictive mode of model application)
- Applications in the context of climate risk analyses for crops, measurement of CC impacts and development of adaptation strategies for CC, as pursued by CCAFS, GRiSP and other CGIAR Research Programs (predictive mode)
- Model-assisted phenotyping of photothermal response traits in rice and cereals in general, through parameter optimization against observed datasets, as required by the GRiSP Global Rice Phenotyping Network (…) and the ORYTAGE project (reverse or heuristic mode)

The first two applications require a modernized equivalent of RIDEV_Sim, but will also require an equivalent of RIDEV_Cal as parameterization tool. The last application (phenotyping) mainly requires a new equivalent of RIDEV_Cal equipped with a powerful parameter optimization tool suited to multi-environment fitting for hundreds of genotypes (context of genetic QTL and association studies).

The main potential users are AfricaRice, IRRI and CCAFS, as well as their local, regional and global collaborators.

2.3 Skills expected from the new RIDEV (V.2)

The following skills are expected of RIDEV V.2:

- Simulation of the date of the main phenological events panicle initiation (PI), flowering (F) and maturity (M) of the crop as a function of genotype, sowing date, cultural practice (flooded-irrigated or upland; transplanting or direct seeding), thermal conditions of the apical growing point and day length dynamics (function of calendar date and geographical latitude)
- Simulation of fraction of sterile spikelets resulting from adverse temperatures (heat or cold) of the affected organs during their sensitive periods (before and at flowering)
- Achievement of such simulations with minimal data requirements to enable applications at sites where environmental data are scarce: daily minimum and maximum air temperature (Tmin, Tmax), geo-reference, and if necessary, some commonly available atmospheric variables from weather stations (but not solar radiation, which is frequently unavailable)
- Achievement of model calibration for specific genotypes using easily measureable phenological variables and spikelet sterility fraction (but this for a variety of sowing dates and/or latitudes as source of thermal and day-length variation)

- An in-built tool for parameter optimization using automated sensitivity analysis, suited for multi-environment fitting of parameters and serial parameterization of large genotype numbers as needed for model-assisted phenotyping

The specific skill of the original RIDEV, namely to simulate key components of microclimate (e.g., floodwater temperature) are to be conserved and improved in RIDEV V.2. In addition, heat-induced spikelet sterility is to be simulated on the basis of two innovations, (1) time of day of anthesis and (2) panicle temperature at that time. These innovative improvements benefit from the results of Cecile Julia's thesis on exactly those issues (Chapters 1 and 2).

Lastly, RIDEV V.2 will also optionally simulate phenology under dryland conditions (but without considering drought), in a mode that simply bypasses the calculation of the microclimate specific to flooded irrigated rice (use of air temperature at 2m instead of simulated microclimate).

2.4 Product package delivered upon completion of project

Along with this terminal report (due date 31 March 2012), or not more than 1 month after its submission, we will provide the following products to AfricaRice:

- An operational prototype of RIDEV V.2 including a user interface permitting individual simulations, external-file driven series of simulations and automatic parameterization (multi-environment fitting using optimization tool)
- Test parameter optimizations and simulation outputs for selected Risocas field results: (1) 3 rice varieties x 11-13 sowing dates under full irrigation at Ndiaye, Senegal (thesis Stürz) and (2) 9 sorghum genotypes x 3 sowing dates x 3 sites in Mali
- Source code of the model: RIDEV model and user interface written in Tcl/Tk, parameter optimization tool written in R, the two linked through user interface
- Preliminary, "minimalist" instruction manual for user

3. Description of RIDEV V.2 model

3.1 Simulation of thermal time

Daily heat units (degree-days) are calculated from Tmin and Tmax of the appropriate entity (air, floodwater, a weighted average of both, or panicle, depending on developmental stage) using a linear broken-stick model. A physiological temperature Tphys is calculated as state variable. Temperatures below Tbase are disregarded (Tphys=0), temperatures between Tbase and Topt are considered effective (Tphys=Tact-Tbase), and temperatures above Topt are also considered ineffective (Tphys=Topt).

To account for diurnal patterns of T, which potentially incur Tact<Tbase or Tact>topt during part of the day), an empirical standard distribution of T during the 24-h cycle is implemented with hourly integration. This relative diurnal pattern is dimensioned on a daily basis using T extrema (Tmin and Tmax of the entity concerned) and calculated day length including civil twilight (the night and day portions of the pattern are "stretched" or "compressed" according to photoperiod). A partial Tphys is calculated for each hour and accrued for 24 hours.

Thermal time of a day is equal to Tphys of the same day.

3.2 Simulation of phenological phases

RIDEV V.2 considers sequentially a Basic Vegetative Phase (BVP), a Photoperiod-Sensitive Phase (PSP), a Reproductive phase (RPR) and a maturation phase (MAT). BVP, RPR and MAT are considered to be of constant thermal duration for a given genotype, unless transplanting shock as parameterized by the user extends the BVP. PSP thermal duration is considered to depend on both temperature and day length on the basis of 4 alternative models the user can choose from.

Thermal time (or a day-length dependent term) is accrued until a critical value is attained, followed by the onset of the subsequent phonological phase.

3.3 Simulation of photoperiod sensitivity

Because considerable uncertainty persists on the way how day length affects PSP duration, and because different cereal species behave differently, the user is given the choice of 4 models of photoperiod sensitivity.

PP Model 1: Linear "quantitative" model

This model follows the classical concept of Major and also resembles the model implemented in the original RIDEV. It accrues [dPsp = PPsens * Tphys * max(PP-PPcrit, 0)] using [Psp = Psp + dPsp] until [PSP >= 1], which is when panicle initiation (PI) happens and the RPR starts. PPsens sets the sensitivity to day length (0…1) and PPcrit (10…12) sets the day length below which no response to it occurs. The model assumes a gradual (quantitative) response, as opposed to a threshold-type (qualitative) response.

PP Model 2: Curvi-linear model able to approximate both qualitative and quantitative responses

This model is identical to Model 1 except for the introduction of parameter PPexp (1…10), an exponent that renders the effect of day length progressive. This is found in many traditional cereals,

notably in sorghum and millet. If [PPexp=1] then Model 2 gives the same results as Model 1.

PP Model 3: Effect of day-to-day change of day length

This model was considered by Clerget et al. (2004) for sorghum and supposes that short-day plants are actually decreasing-day plants. The model calculates at day-to-day change in day length [dPP = PP-PPold] and accrues the term [dPsp = PPsens * Tphys * max(dPP-PPcrit, 0)]. PPsens can assume values [0…1] and PPcrit [0…0.2], the latter usually with very small values (0.003 or less). This model does not always give convincing results but was considered here because it makes a fundamentally different assumption on photoperiodism.

PP Model 4 : Impatience

This model was specifically developed for sorghum where it is the only one able to accurately predict the crop's phenology in the off-season (Dingkuhn et al., 2008). Its broader applicability for crops remains to be evaluated but there are indications that it captures a generic phenomenon. The principle is that PI occurs when the plant is exposed to a critical day length (shortness of day), but that this threshold moves as the plant's wait state (PSP) gets longer. This is the "impatience" principle, analogous to animal ethology (threshold lowering under prolonged wait states for an external signal). PPsens=0…1.5, PPexp=0.1…1, SeuilPP=12.5…15. (PPcrit, a parameter also present in this model, was permanently set to 11.5 because it seems not to vary.)

3.4 Simulation of floodwater temperature

Floodwater temperature can be very different from air temperature through evaporative cooling and shading by the canopy. We used data generated by the thesis of Cecile Julia from Senegal (cold and hot seasons), Camargue (summer) and IRRI (dry season) to construct an empirical model. An initial attempt to use a multiple regression model was abandoned because it gave unrealistic predictions for extremely humid situations (characterized by small daily Tmax-Tmin differentials) that were not represented in the data but do occur in the monsoonal wet season. We thus constructed a logical model that forced T(water)-T(air) differentials to be equal to zero when air Tmax-Tmin equals zero (theoretical humid situation with minimal sunlight).

The resulting model is presented in the box below. Its parameters were optimized against observations using the solver utility of Microsoft Excel.

Empirical, optimized models to calculate water T from Tmax and Tmax-Tmin of the air as well as the crop light transmission ratio (LTR) as calculated from LAI:

Model TWmax:

TWmax-Tmax = [a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR] * (Tmax+c)/20
therefore:
TWmax = Tmax + [a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR] * (Tmax+c)/20
A=-0,728; b=0,275; c=-18,46

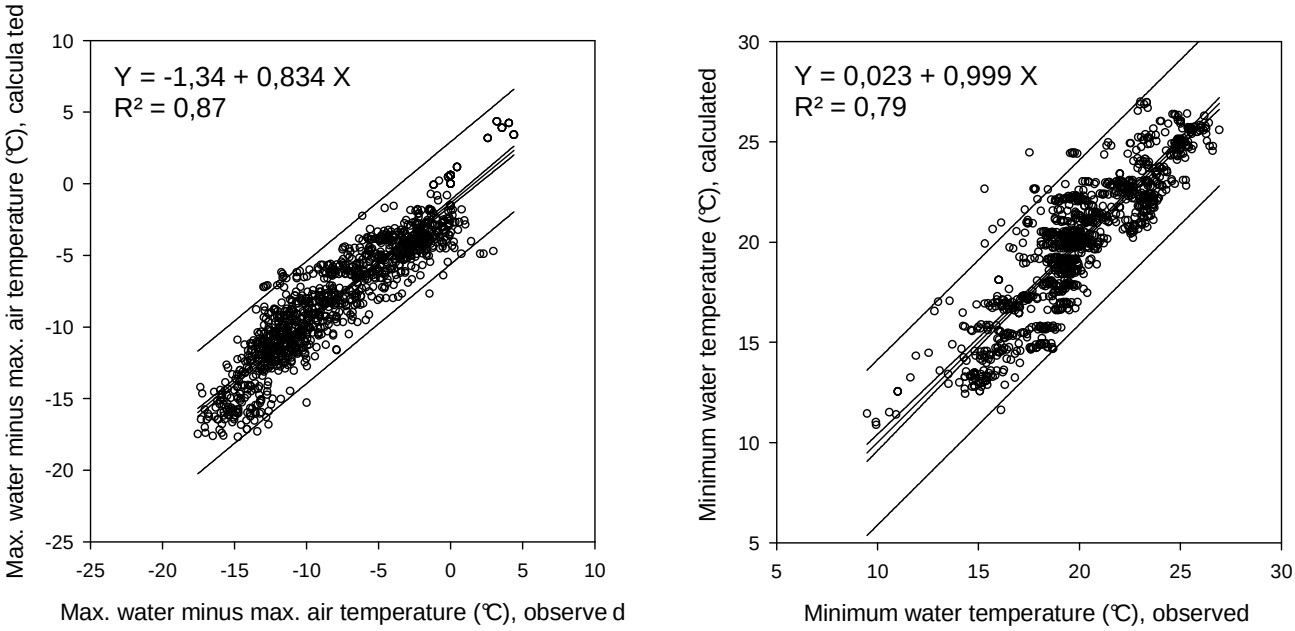
Model TWmin:

TWmin = Tmin + a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR + c((Tmax-Tmin)^1.2)
A=0,658; 0,425; c=-0,303

These models gave the simulated vs observed correlations shown in the Figure 1 below.

The models require validation with independent data but they are likely to be robust because (1) they are based on a large range of climatic situations and more than 1000 observations, and (2) they force T(water)-T(air) differentials towards zero for humid and low light situations, which is true for theoretical considerations.

TWmax varied between 18 ^{o} below Tmax (on hot, dry days under a dense crop cover) and 5 ^{o} above Tmax (on hot, dry days in the absence of a crop cover). TWmin was on average similar to Tmin of the air because it varied by a few degrees in both directions (not presented).



Model:
TWmax-Tmax = [a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR] * (Tmax+c)/20
TWmax = Tmax + [a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR] * (Tmax+c)/20
a =-0.728, b = 0,275, c = -18,46

Model:
TWmin = Tmin + a(Tmax-Tmin)*(1-LTR) + b(Tmax-Tmin)*LTR + c((Tmax-Tmin)^1.2)
a = 0,658, b = 0,425, c = 0,303

<u>Figure 1</u>. Correlations between calculated and observed water temperatures. Left : daily max and min differential ; right : minimum temperature.

3.5 Simulation of apex temperature of the plant

Under irrigated conditions, it was assumed that Tapex = Twater until PI. Thereafter, the apex rises above the floodwater due to stem elongation and gradually approximates the air temperature. This was simulated by using weighted averages between Twater and Tair, evolving from PI (Tapex=Twater) to flowering (Tapex=Tair).

For upland, non-irrigated condition, we assumed Tapex=Tair. This is a simplification and probably erroneous, but it may serve as such as a reference for the approach used by other crop models, which do not consider microclimate.

3.6 Simulation of panicle temperature

For model development for panicle temperature, data was available for the cold and hot seasons in Senegal, based on thermal images (thesis Cecile Julia). Corresponding data for IRRI (Philippines) were not yet available and will be used for model validation.

The following model predicting the panicle-air temperature differential (TD) was determined by multiple linear regression analysis:

Table 1: Description of the model explaining the air and panicle temperature difference TD: Results of the Multiple linear regression of TD against 5 parameters (air temperature, relative humidity and solar radiation at 2m, the panicle relative position in the canopy and the light transmission ratio); and results of the analysis of variance. The equation was found using data from Senegal only (cold and hot dry seasons).

TD = 0,782 + 0,422 Ta_2m - 0,0443 RH_2m - 0,00287 Rs - 8,05 Z - 6,59 LTR
With :
Ta_2m = T(air) at 2m; RH_2m = rel. air humidity at 2m; Rs = solar radiation (W/sqm);
Z = mean panicle position relative to canopy height (fraction, 0.7…1);
LTR= canopy light transmission ratio (0…1 theoretically, but mostly 1)

Multiple linear regression :

	Coeff	Stdev	Std Coef	t-ratio	P
Constant	7,82E-01	5,56E-01	0,00E+00	1,40E+00	8,01E-02
Ta_2m	4,22E-01	1,04E-02	6,29E-01	4,04E+01	0,00E+00
RH_2m	-4,43E-02	2,50E-03	-2,99E-01	-1,77E+01	0,00E+00
Rs	-2,87E-03	1,77E-04	-1,37E-01	-1,62E+01	0,00E+00
Z	-8,05E+00	3,38E-01	-1,94E-01	-2,38E+01	0,00E+00
LTR	-6,59E+00	4,49E-01	-1,31E-01	-1,47E+01	0,00E+00

R2 = 0,819 R2 ajusted = 0,818

Analysis of variance

	DF	SS	MS	F	P
Regression	5,00	19493,33	3898,67	2613,61	0,00^{E}+00
Residual error	2896,00	4319,90	1,49		
Total	2901,00	23813,23			

The following Figure presents graphically the fit of this model:

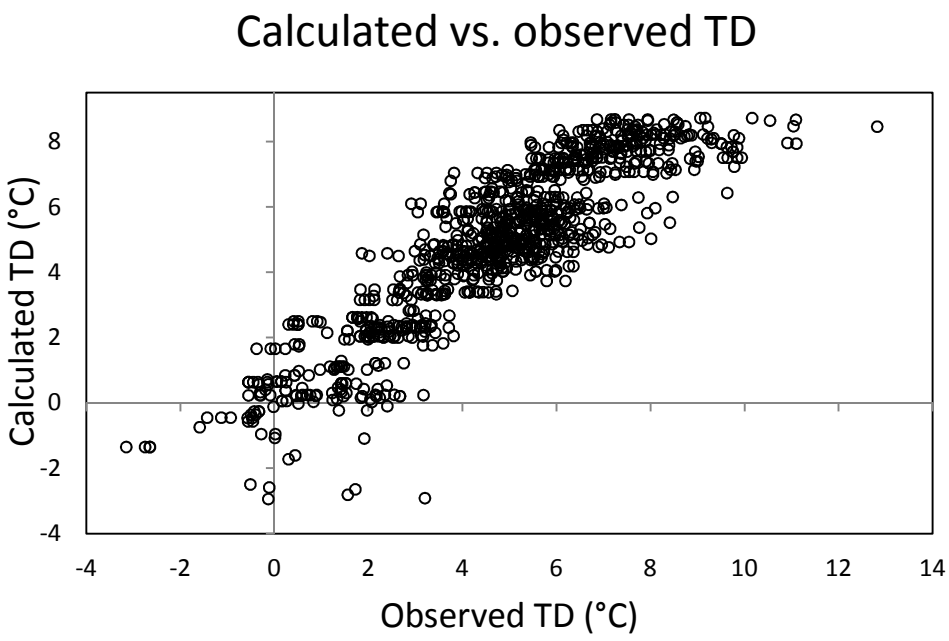


Figure. 2. Correlation between simulated and observed panicle temperature

We used this relationship in the RIDEV V.2 model to calculate the daily maximal panicle temperature TPANmax) from canopy properties and weather conditions at the hottest time of the day. For the minimum panicle temperature we assumed equilibrium with the air (TPanMin = Tmin) because transpiration can assumed to be negligible at night and Rs is nil.

The following model was thus implemented, which includes an estimate of incident global radiation at midday (W/sqm) from Rs (MJ):

RsMax = 22 // at 12h day length

WattPerSqm = 1000 * (Rs * 12 / daylength) / RsMax

TPanMax = Tmax – (0.78 + 0.422 * Tmax – 0.0443 * RHmin – 0.00287 * WattPerSqm – 8.05 * Z – 6.59 * LTR)

TPanMin = Tmin

Panicle temperature at any given time of day was estimated by applying TPANmax and TPANmin to a standard diurnal, relative temperature pattern as described further below.

3.7 Simulation of time of day of anthesis and panicle temperature at anthesis

Combined results from Senegal, France and the Philippines showed that environmental effects on time of day of anthesis are large whereas varietal differences were small in the study (Fig. 3). High night temperatures (Tmin) advanced anthesis, providing potentially for escape from heat stress. High RH also advanced anthesis (not shown), potentially providing for escape from heat stress associated with poor transpirational cooling under humid conditions. An article was submitted to Eur. J. Agronomy on these results and accepted pending minor revision.

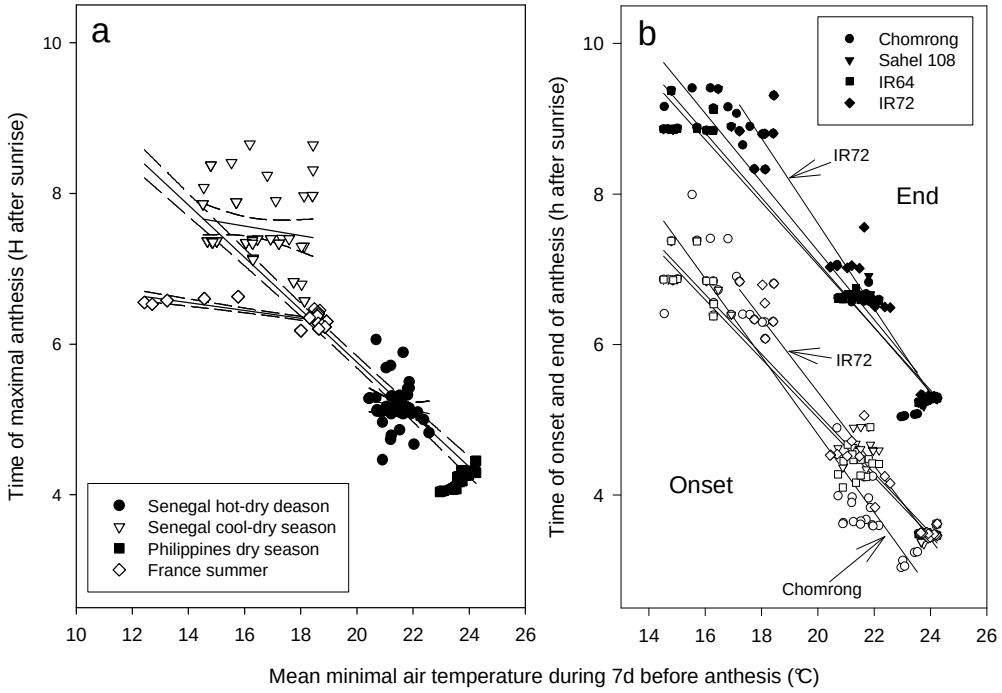


Figure. 3 a, b. Relationship of time of anthesis vs. minimum air temperature (Tmin). Maximal anthesis vs. Tmin. Correlation across 4 environments, Y=12.7-0.348X with R^2=0.80; within environments, not significant (a). Onset and end of anthesis vs. Tmin, by genotype across 3 tropical environments (Senegal and Philippines) (b). Correlations for onset of anthesis: Chomrong, Y=15.2-0.517X, R^2=0.90; Sahel 108, Y=13.0-0.396X, R^2=0.96; IR64, Y=12.9-0.392X, R^2=0.97; IR72, Y=15.2-0.489X, R^2=0.94. Correlations for end of anthesis: Chromrong, Y=16.4-0.456X, R^2=0.88; Sahel 108, Y=15.7-0.431X, R^2=0.95; IR64, Y=15.4-0.416X, R^2=0.97; IR72, Y=18.9-0.567X, R^2=0.95. Correlations marked by arrows are significantly (P<0.05) different from those of other genotypes.

We only considered the thermal effect on anthesis time in RIDEV V.2. Since duration of anthesis was constant at about 2h, we simulate only the time of mid anthesis using the results shown in Fig. 3b, and express it as the number of hours after sunrise (hasr). A crop parameter sets the time of anthesis under conditions of [Tmin=20^{o}C] expressed in hours before noon (HrAnthBefNoon20C). The following equation is implemented in RIDEV V.2:

AnthesisTime = (14 – 0.4 * Tmin) + (24-PP)/2 – HrAnthBefNoon20C

Consequently, if the genotypic parameter [HrAnthBefNoon20C=0h] and [Tmin=20°C] then anthesis centers at midday (11h-13h). If [Tmin = 25°C] then anthesis centers at 10h, etc.

For the time of anthesis, the panicle temperature is estimated on the basis of a diurnal simulation of panicle temperature, using a relative diurnal template (explained further below) and the simulated TPANmax and TPANmin of the day.

3.8 Simulation of diurnal patterns of temperature

The diurnal template used for generation of hourly temperatures from Tmin and Tmax is shown in Figure. 4.

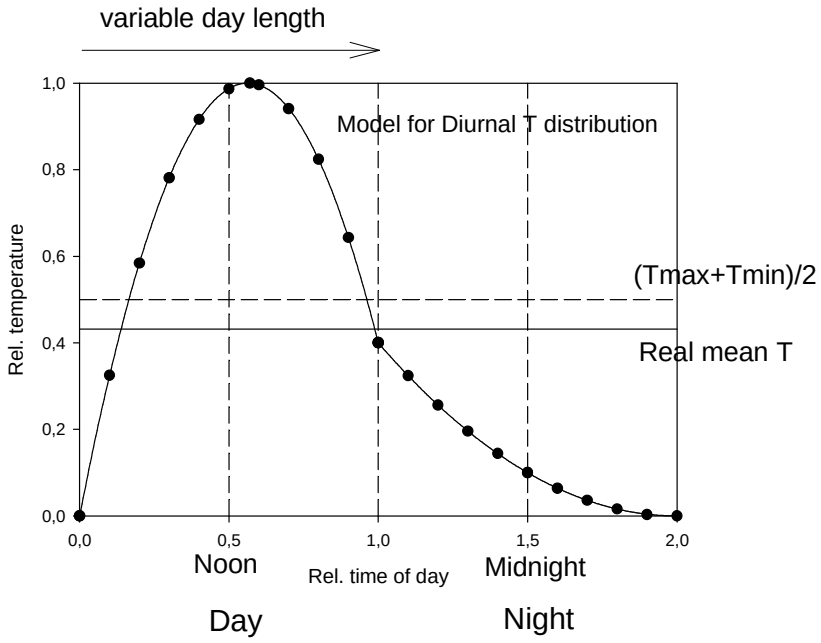


Figure. 4. Relative diurnal pattern (template) for temperature distribution used to calculate hourly temperatures from daily min and max temperatures of the entities concerned (air, water or panicle). Calculated day length is used to « stretch » or compress the relative day or night periods. Empirical equations used are [$Y=3.55\ X - 3.15\ X^2$] (day) and [$0.4 - 0.8\ X + 0.4\ X^2$] (night).

3.9 Simulation of LAI and LTR

LAI simulation is needed because the calculation of floodwater and panicle temperature requires information on ground cover. In the absence of biomass simulation in RIDEV, we chose to drive LAI with the following information:

- Thermal time expressed as fraction of potential daily thermal time, generated by the model [Tphys / (Topt – Tbase)], range 0…1
- Initial LAI as a function of population POP and individual initial plant leaf area PLAini: [LAI = POP * PLAini / 10000]. POP and PLAini are parameters to be set by users. POP range is typically 50000…500000 plants/ha and PLAini is in the order of 0.0001 m^2.

- Maximal leaf relative growth rate (LRGRmax), range 1.1…1.5 (resulting in daily multiplication of LAI by 1.1 to 1.5 during exponential growth). Thus, [LAI = LAI * LRGRmax * Tphys/(Topt-Tbase)]. LRGRmax is a crop parameter.
- Once LAI exceeds 1, linear growth is implemented using [LAI = LAI + (LRGRmax – 1) * 1 * Tphys / (Topt – Tbase)]
- LAI is capped by the parameter LAImax
- If the transplanting option is chosen by user, a transplanting shock can be implemented that delays both LAI and phenological development. The user chooses the duration of the shock (parameter DDtransplantingShock, in degree-days, range 0…100), during which development stops and leaf growth is halved.

This simple model can easily be calibrated to approximate common LAI patterns (post-flowering dynamic are of no importance to RIDEV). No particular accuracy is needed because LAI simulation only serves to estimate ground cover for water and panicle temperature calculation.

Light transmission ratio (LTR) is calculated with Lambert-Beer's law, using the value of 0.6 for the extinction coefficient:

LTR = exp (-0.6 * LAI)

LTR ranges from 0 (dense crop) to 1 (no crop).

3.10 Simulation of spikelet sterility

Thermal sterility is simulated on a range from 0 (none) to 1 (total). It is simulated for 3 different, successive sensitive phases, each having a specific sensitivity set by a crop parameter:

1. Microspore stage: From 280 to 140 dd before flowering (sensitivity to cold based on minimum water temperature; parameter CritSterCold1, range ca. 20 °C…10 °C)
2. Panicle exertion process: From 140 dd before flowering to flowering (sensitivity to cold based on minimum air temperature; parameter CritSterCold2, range ca. 20 °C…10 °C)
3. Flowering stage: From 50 dd before flowering until 50dd after flowering (sensitivity to heat based on temperature of the panicle at the hour of mid-anthesis; parameter CritSterHeat, range ca. 30 °C…40 °C)

The mean thermal conditions during the sensitive phases are used to calculate the sterility fractions. The 3 crop parameters set the temperature (°C) at which the sterility effect sets on, based on a

constant progression of 0.2 per ^{o}C (resulting in 100 % sterility as the temperature progresses by 5 ^{o}C beyond the critical value (parameter).

A 4th, constant source of sterility is simulated which stands for a genotypic baseline sterility (parameter SterBase, range 0…1). Typical values are between 0.1 and 0.2. A value of 1.0 would indicate genetic sterility as in male-sterile breeding lines.

The calculation of total sterility (base, SterCold1, SterCold2, SterHeat) is not additive because it cannot exceed the value 1. It is calculated as follows:

SterTot = SterBase + (1 – SterBase) * (1-[(1-SterCold1) * (1-SterCold2) * (1-SterHeat)])

3.11 Model parameters, input variables and output variables

3.11.1 Model parameters

There are 10 model parameters setting the general conditions of the simulation, 8 setting conditions for crop phenology (of which not all are always required, depending on choice of PP model), and 4 setting conditions for sterility.

<u>General conditions:</u>

Latitude	XX.XX, decimal
SowingDate	dd/mm/yyyy
Flooding	0 or 1 binary
Transplanting	0 or 1 binary
DDTransplantingShock	0…100 (dd)
POP	50000…500000 (plants per ha)
PLAini	ca. 0.0001 (m^2)
LRGRmax	1.1…1.5 (adjusted visually to obtain appropriate LAI dynamics)
LAImax	2…12
Z	Fraction of panicle height over canopy height (0.6…1)

<u>Phenology:</u>

BVPsum	duration of BVP (200…800 dd)	can be optimized
RPRsum	duration of RPR (200…500 dd0	never optimize together with BVP; set to 350
MATsum	duration of MAT (200…500 dd)	can be optimized
PPsens	)	
PPcrit	)	occur in different combinations in the 4 photoperiodism models
PPexp	)	to choose from. Value ranges as described further up. Can be optimized
SeuilPP	)	

<u>Sterility:</u>

SterBase	0…1, typically 0.1 to 0.2	)	
SterCold1	20…10 ^{o}C	)	Can be optimized (rice)
SterCold2	20…10 ^{o}C	)	

SterHeat 30…40 °C)

Only phenology and sterility parameters can be optimized and can be selected on the optimization menu (Annexe 2). The other parameters must then be fixed manually beforehand. Optimization can be performed as multi-fitting across different environments, cultural practices, population densities etc., but always for the same genotype (variety). A minimum diversity of sowing dates and/or latitudes (thus, day length and thermal conditions must be in the sample to allow meaningful optimizations of phenology.

Optimization scenarios are read from an external spreadsheet.

3.11.2 Input variables from external source file (Annexe 3)

Tmin	daily minimum air T (°C)
Tmax	daily maximum air T (°C)
RHmin	daily minimum relative humidity (%)
Rs	daily cumulative solar global radiation (MJ)

(Optimizations also require observed crop data: duration from sowing to flowering (days), duration from sowing to maturity (days), and sterility (fraction).

3.11.3 Output variables

DSowEndBVP	Days from sowing to end of BVP
DSowPi	Days from sowing to PI
DSowFlowering	Days from sowing to flowering
DSowMaturity	Days from sowing to maturity
SterCold1Out	Cold sterility phase 1 (microspore)
SterCold2Out	Cold sterility phase 2 (panicle exertion process)
SterHeatOut	Heat sterility (flowering period)
SterTotOut	Total sterility

4. Next steps

The model and software delivered with this end-of-project report is **a prototype**. It needs validation on several points, namely the calculation of water and panicle temperature. Given the **diverse applications** of the optimization/simulation system, or at least its heartpiece the model itself, by **numerous partners** (AfricaRice and its regional partners, users of the Rice Manager, projects of CCAFS, projects of the GRiSP phenotyping network), **initial validation should be through and durable**.

This report will be amended in late 2012 as some validation work and additional publications become available.

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CHAPTER 4:

Preliminary modeling exercises and perspectives for future applications

INTRODUCTION

This chapter describes some preliminary modeling exercises using data generated by this thesis on plant organ temperature and spikelet sterility, as follows:

1. Comparison of the empirical regression model of panicle temperature described in chapter 2 (similar to the one used in the RIDEV phenology and sterility model, chapter 3), with the energy balance model IM2PACT of canopy and panicle temperature recently developed in Japan (Yoshimoto *et al.* 2011), using the crop data generated by this thesis research.
2. Optimization of RIDEV parameters on a set of Sahel 108 rice crop data obtained from two other sources (ORYTAGE project of CIRAD, thesis of Sabine Stuerz of Hohenheim University) for the Ndiaye site in Senegal, which also figured as an experimental site in this thesis.

The empirical regression model (chapter 2) and RIDEV (chapter 3) were already described in detail. The model IM2PACT (Integrated Micrometeorology Model for Panicle And Canopy Temperature) was developed independently by a Japanese team from NIAES (National Institute for Agro-Environmental Sciences) in Tsukuba, Japan, under the leadership of Dr. Toshi Hasegawa. IM2PACT (Yoshimoto *et al.*, 2011) is a simple micrometeorology model focusing on canopy and panicle temperatures, based on classical heat balance equations. It was calibrated with leaf conductance and panicle temperature measurements made on a temperate *japonica* rice variety ('Akita-Komachi') grown in the field in Northern Japan in 2000 and in Eastern China in 2003 (Yoshimoto *et al*., 2005a,b), and validated with independent data from a transpiration suppression experiment on two *japonica* rice varieties ('Akita-Komachi' and 'Hatsu-Boshi') at NIAES in 2005 (Yoshimoto *et al.*, 2011), involving the spraying of a wax-type water dispersible chemical on leaves to reduce leaf transpiration.

IM2PACT is composed of two sub-models, 1) a canopy micro-meteorology sub-model estimating the air temperature and humidity at the panicle's position inside the canopy, and calculating the heat balance between the whole canopy and the air above the canopy; and 2) a

panicle temperature sub-model which is a heat balance model between panicle and the air in the vicinity of panicle obtained through 1).

The model comparison (first part of this chapter) is only preliminary because at the time of this writing, no independent field data with sufficient detail to satisfy the requirements of both models are available. The model comparison is thus limited to the data generated by this thesis. Second part B will apply RIDEV in its current form (prototype) to independent field data from third sources, aiming at calibrating the model with its in-built parameter optimization tool and evaluating the quality of the calibration.

MODEL COMPARISON ACROSS THE SITE USING THE PHD DATA

In chapter 2, we underlined the necessity to predict sensitive organ temperature, in particular panicle temperature, to explain and predict the spikelet sterility caused by heat stress. The objective of this section is to compare the output of two models developed independently (regression model described in chapter 2 and IM2PACT) in terms of simulated vs. observed panicle temperature.

Materials and methods

Using the crop and meteorology datasets with available panicle temperature measurements (Senegal cool and hot dry seasons, Philippines), we calculated the temperature difference between air (2m) and panicle (TD) with our empirical model (Model 1) and IM2PACT. Model 1 was as follows:

Model 1

$$TD = 1.578 + 1.093\ VPD\_2m + 0.189\ Ta\_2m - 0.0337\ Top\_P - 0.00339\ Rs + 0.0331\ H$$

Where TD =Ta-Tp, difference between air and panicle temperature (in °C)
VPD_2m = Vapour pressure deficit at 2m from the ground (in kPa)
Ta_2m = Air temperature at 2m from the ground (in °C)
Top_P = the position of the highest panicle from the ground (in cm)
Rs = Solar radiation (in W/m²)
H = sun height or solar angle (in degree)

The input and output variables of the two models are summarized in the Table 1. Model 1 is a statistical model using 5 input variables including three standard meteorological inputs (VPD, Ta, and Rs), one canopy-dependent input (ground height of panicle layer) and the solar angle which is the sun position from the horizon (*cf.* Chapter 2). IM2PACT is more complex because it is calculating the heat balance within the canopy. Therefore it needs the location of the site (latitude), four standard

meteorological inputs (Ta, RH, Rs, and Wind Speed), three crop-dependent inputs (LAI, canopy and panicle heights), and calendar date and the time.

For IM2PACT, constant values for leaf stomatal and panicle conductance were used for all sites and all varieties to calculate the sensible and latent heat flux involved in the Tp calculation. These values were taken from measurements made in Japan on a single rice variety ('Akita-Komachi').

***Table 1**: Summary of the inputs and outputs of the two panicle temperature models, Model 1 and IM2PACT.*

Model	*INPUTS*	*OUTPUTS*
Model 1	Vapour pressure deficit (kPa) Air temperature at 2m Ta (°C) Panicle up-layer position (cm) Solar Radiation (W/m²) Solar angle (°)	***Temperature difference TD Ta-Tp** (°C)*
IM2PACT	Latitude Day of year Time of day (h) Air temperature at 2m Ta (°C) RH at 2m (%) Wind Speed at 2m (m/s) Solar Radiation (W/m²) Leaf Area Index Canopy height (cm) Panicle height (cm)	Sensible and latent heat flux (W/m²) Soil heat flux (W/m²) Radiation temperature of canopy surface (°C) Soil surface temperature (°C) Sensible and Latent heat flux at panicle surface (W/m²) Air temperature inside canopy (°C) Relative humidity inside canopy (g/m3) ⇩ ***Panicle temperature Tp** (°C)*

Results and discussion

Model 1 gave a good approximation of TD as plotted against RH_2m (Figure 1, left graphs, black open circles), for each of the genotypes and across all sites. The scatter of calculated values was less pronounced than the scatter for the observed values, indicating that some sources of variation were not simulated. This was probably because Model 1 uses the average ground height of the panicles at population scale, whereas the observed TD was based on individual ground height of given panicles. As a result, simulations gave an accurate but less scattered TD-RH relationship.

Therefore, Model 1 is not sensitive to individual panicle position and all the panicles of the same plot have the same parameter value for panicle ground height, unlike IM2PACT which uses

individual panicle position as input (cf. Table 1). Accordingly, IM2PACT simulations gave a similar scatter as that observed for the calculated values of TD (Figure 1, right).

Despite the fact that IM2PACT calibration was made on independent datasets that had a lesser range of TD (-2°C <TD< 3°C) as compared to our data (-12.7°C <TD< 4°C), IM2PACT simulations predicted the observed TD vs. RH relationship remarkably well over this larger range. IM2PACT therefore is a robust model. The robustness of Model 1 cannot be evaluated here because no independent data was available for its validation. However, Model 1 was is based on an extremely broad range of climatic situations, probably covering much of the variation that is possible in irrigated rice systems.

While Model 1 simulated TD for the 4 genotypes equally well, this was not the case for IM2PACT. While simulations for the 3 semidward rice (IR64, IR72 and Sahel 108) were overall accurate, TD was under-estimated with IM2PACT and the scatter was larger for the tall-traditional variety Chomrong. Chomrong had greater variation in panicle ground height and position relative to canopy height than the semidwarfs. It is also possible that panicle conductance differs among genotypes. Such differences would be absorbed by the statistical model 1 whereas IM2PACT explicitly factors in panicle conductance, which was then falsely considered as constant in this exercise.

A very recent study (Fukuoka *et al.* 2012) confirmed this hypothesis by showing differences in panicle conductance among 21 rice varieties grown in the field. The conductance measured covered a wide range of values (between 0.15 and 0.67 cm s-1) leading TD variation between 2.1 and 3.5°C. In order to consider such varietal differences in panicle conductance, simulations with IM2PACT would require empirical information of genotypic differences, whereas Model 1 (and thus, the RIDEV model) would be insensitive to them.

The correlations between calculated and observed TD were better with Model 1 than with IM2PACT (Figure 2). This is not surprising because Model 1 was calibrated using the same dataset whereas IM2PACT was calibrated with an independent dataset. Nevertheless, The simulated-observed fit was remarkably good for IR64 and Sahel 108 (both in terms of R^2 and slope), and poor only for Chomrong (inaccurate slope, indicating a systematic error related to unknown genotype characteristics).

Perspective for further model comparisons and improvements

An immediate next step towards the implementation of Model 1 in RIDEV for extrapolative purposes (beyond the present scope of environments and/or genotypes) will be its validation with independent field data, such as those generated by NIAES for IM2PACT calibration and validation. On this basis a full comparison of model accuracy and robustness between the two models will be possible. However, at this point we can already conclude that the two models converge remarkably well, despite the different approaches and experimental bases.

Model development should not stop with this achievement, however. An important new objective will be the simulation of TD in water limited situations, such as water-saving crop management where trade-offs between plot-level water consumption and transpiration cooling (to avoid heat injury) needs to be considered. For such extended objectives, the choice between statistical approaches (Model 1) or mechanistic heat balance approaches (IM2PACT) needs to be carefully considered.

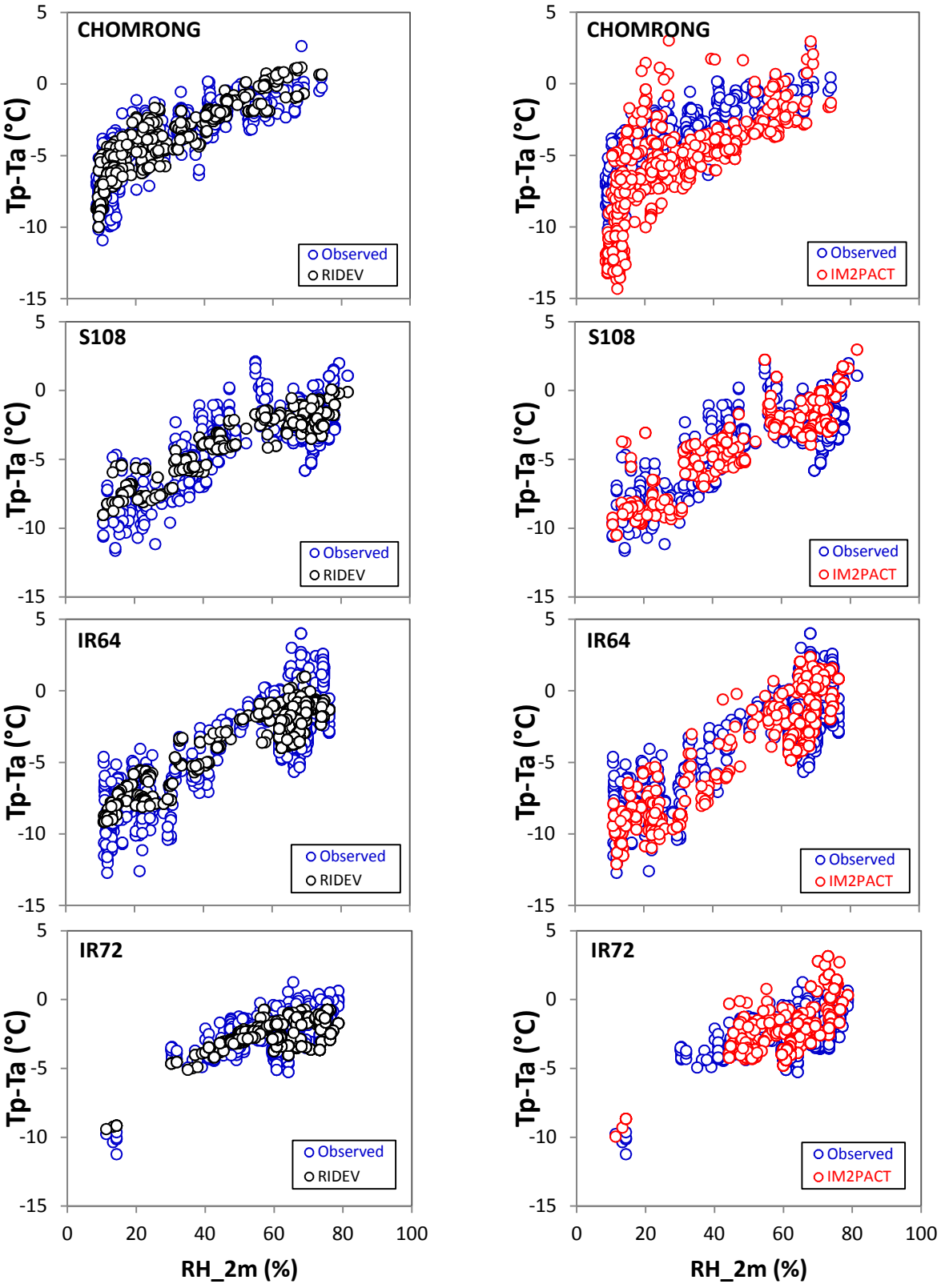

***Figure 1**: Comparison of observed Tp-Ta (blue open circles) and calculated Tp-Ta, temperature difference between panicle and air at 2m, for the four rice varieties across 3 environments (Philippines hot and dry Season, Senegal hot and dry season, and Senegal cold and dry season) in function of the relative humidity measured at 2m (RH_2m). Tp-Ta was calculted using both RIDEV panicle temperature submodel (black open circles) and IM2PACT (red open circles).*

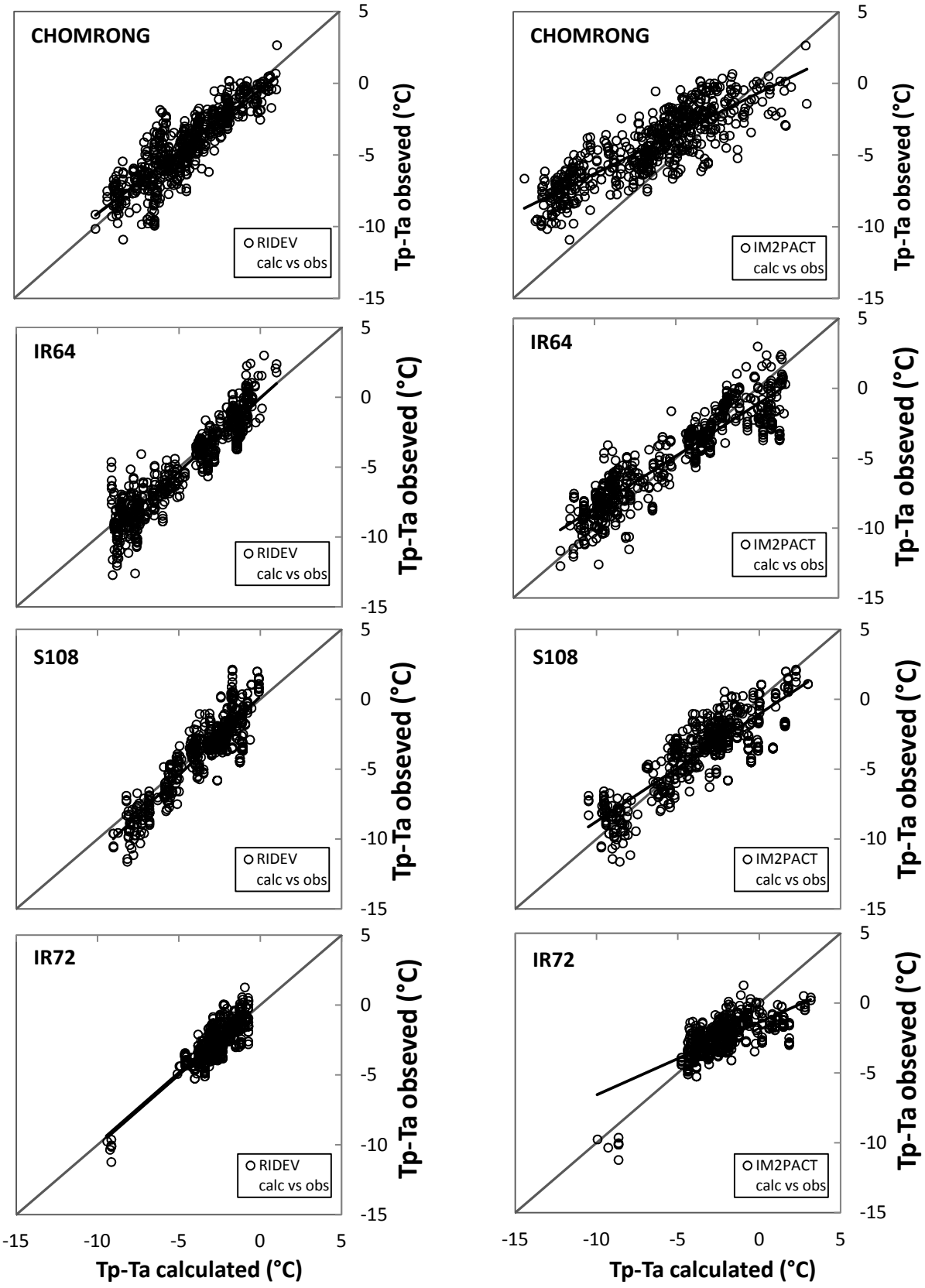

***Figure 2**: Calculated vs. observed TD for the four rice varieties across 3 environments (Philippines hot and dry Season, Senegal hot and dry season, and Senegal cold and dry season). Correlation for calculated vs. observed TD for RIDEV Model 1: Chomrong, Y= 0.86X-0.56, R^2 = 0.76; IR64, Y= 1.05X-0.06, R^2 = 0.87; Sahel 108, Y = 1.13X+0.20, R^2 = 0.81; IR72, Y= 1.02X+0.17, R^2 = 0.69. For IM2PACT : Chomrong, Y= 0.56X-0.70, R^2 = 0.72; IR64, Y= 0.74X-1.14, R^2 = 0.83; Sahel 108, Y = 0.77X-1.07, R^2 = 0.74; IR72, Y= 0.52X-1.38, R^2 = 0.50. (P<0.05 for all regressions).*

RIDEV APPLICATION : AN EXAMPLE OF SIMULATION

The RIDEV model as described in chapter 3 was applied to an independent data set obtained from two different sources for the Ndiaye site in Senegal, using the cultivar Sahel 108. This aggregate data set covered year-round plantings, thus exposing the crop to different thermal regimes at different developmental stages.

The objective of this modeling exercise was to test the model's parameter optimization routine and to evaluate the model's ability to simulate intra-annual patterns of variation of crop duration and spikelet sterility.

Materials and Methods

Crop data (days from sowing to flowering and maturity, fraction spikelet sterility) was obtained from the ORYTAGE project (CIRAD in collaboration with AfricaRice ca. : 200 indica rice genotypes exposed to 6 sowing dates in 2009 ; Sahel 108 figuring as 4x replicated check) and the Ph.D. thesis of Ms. Sabine Stuerz (unpublished; University of Hohenheim ; 12 sowing dates in 2008-2010 ; 4 replications, but only means available). Daily weather data were obtained from AfricaRice.

The RIDEV model was calibrated by parameter optimization using R-Genoud (Mebane *et al.* 2011) in two steps : (1) optimization of phase durations for SumBvp and SumMatu with SumRepr set to 400 ^{o}C.d , and of the photoperiodic parameters PPexp and PPsens ; and (2) optimization of the sterility parameters CritSterCold1 (mid-reproductive phase), CritSterCold2 (late reproductive phase), Crit SterHeat (period of anthesis) and SterBase (baseline sterility fraction). The exponential sub-model for photoperiod response was chosen.

Each optimization procedure, essentially a sensititivity analysis by genetic algorithm aiming at minimizing the simulation error (cost function based on least sum of squares), involved several 1000 simulations. The rapidity of the model allowed 10000 simulations to be done in about 30 min.

Simulation outputs from the optimized (parameterized) model were days to flowering, days to maturity, and sterility fractions attributed to chilling stress at two pre-floral stages and to heat stress at anthesis stage. Other simulation outputs such as duration of BVP, PSP, RPR and MAT phases, time of day of anthesis, and panicle T at anthesis were not used in this study.

Results and Discussion

The optimized model parameter values and the simulation results as compared with observation are shown in Figure 3. The cardinal temperatures were 10.6 ^{o}C (Tbase) and 31.7 ^{o}C (Topt), and thus fell into the normal range for rice (Dingkuhn and Miezan, 1995). Sterility was not sensitive to chilling at mid-ripening (CritSterCold1 = 10.6 ^{o}C) but highly sensitive to chilling at late ripening stage (about last 10d before anthesis ; CritSterCold2 = 18.7 ^{o}C). It was also quite sensitive to heat at anthesis (CritSterHeat = 29.7 ^{o}C). Note that the latter is based on panicle temperature, which mostly corresponded to much higher air temperatures (*cf*. chapter 2).

Phenology in terms of days to flowering and maturity (Figure 3, top) was well simulated, with the exception of one out of 18 sowing dates (under-estimation for October 2009 sowing). The extraordinarily late flowering observed in the field for that date was probably caused by severe chilling, as indicated by extreme stunting and high leaf senescence levels (visual observations by the author of the data, Ms. Sabine Stuerz, Hohenheim University). The annual oscillation of crop duration was caused in larger part by thermal effects and smaller part by photoperiod, to which the genotype was mildly sensitive.

Total sterility simulations (Figure 3, center) followed the observed seasonal patterns, with near-total sterility for sowing in late September / early October. But simulated total sterility varied strongly with sowing date in a very « nervous » manner. This was due to the narrow temporal windows of sensitivity of sterility of temperature, between 10 and 14 days. Widening these windows of sensitivity would make the response less « nervous » and thus reduce noise, but would not be faithful to existing physiological knowledge. A conceptual problem thus arises: How can a sterility model be calibrated and validated with field data if sterility by nature varies extremely within the same environment, caused by small differences in sowing date and/or phenology ?

The model explained some of the observed sterility with heat effects (Figure 3, bottom). Simulated heat effects on sterility behaved even more « nervously » than that caused by chilling, and showed less clear seasonal peaks.

We also tested model calibrations / optimizations using the Linear and Impatience options for photoperiod response. The Impatience model gave the best fit for phenology and the linear model the worst, the exponential model being intermediate. Optimized cardinal temperatures were similar for the three options but the models differed significantly in the parameterization of the BVP. The three alternative phenology models also gave quite different parameter values for sterility simulation, notably the ponderation between effects of CritSterCold1, CritSterCold2, CritSterHeat and SterBase (data not presented). This constitutes a problem in model parameterization because it

is difficult to assess which one of the phenological options is most appropriate from a biological perspective, and the choice will subsequently affect the simulation results.

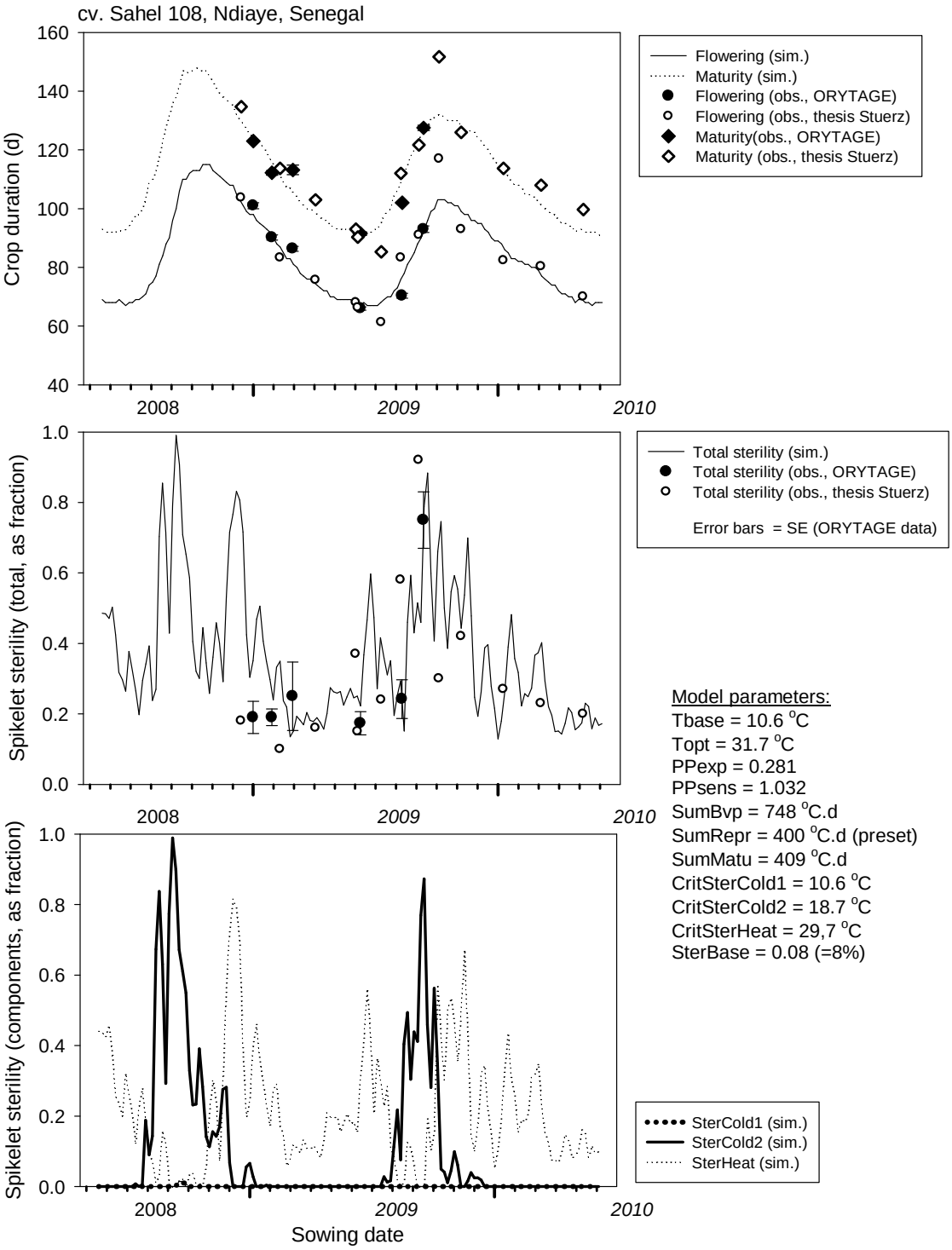

***Figure. 3**. Comparison of simulations with the calibrated RIDEV model with observations on crop duration (top) and spikelet sterility (center), and simulation of chilling and heat effects on sterility ; for Sahel 108 planted at different dates at Ndiaye, Senegal. Field data courtesy of ORYTAGE project and the Ph.D. project of Ms. Sabine Stuerz (RISOCAS project, Hohenheim University).*

Next steps

We will study more genotypes (from available ORYTAGE project data) in order to make a better-founded choice of phenological model options within the RIDEV framework for rice. We will also enlarge the existing datasets on phenology and sterility using supplemental data from different environments, notably different altitudes in Madagascar (ORYTAGE database). This may also help further refine the algorithms of RIDEV. AfricaRice is in the process of generating additional data sets in the field aimed at validating RIDEV.

OUTLOOK FOR MODELING OF CROP RESPONSE TO THERMAL CONTRAINTS

The validated model will be used to parameterize ca. 200 indica rice genotypes studied in Senegal and Madagascar within ORYTAGE. This model-assisted phenotyping exercise will feed into a genome-wide association study (GWAS) for the porpose of discovering genes and alleles affecting the cardinal temperatures, phenological phase duration, photoperiod response and temperature induced sterility.

The validated model will also be used for predictive purposes such as (1) mapping rice environments for thermal constraints and genotypic adaptation to them, for past and anticipated climate scenarios; and (2) decision support in devising locally adapted cropping calendars (incorporation of RIDEV in the rice manager software under development at AfricaRice and IRRI).

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SYNTHESIS, DISCUSSIONS AND PERSPECTIVES

SYNTHESIS

The aim of this thesis was to characterize poorly known traits and mechanisms involved in heat and cold tolerance of rice during sensitive stages of the reproductive phase, and to lay the basis for their incorporation into crop models to predict the risk of yield losses due to thermal stresses in a global climate change context. This work was made on irrigated rice, a high-potential staple crop grown in highly diverse climatic environments many of which are prone to extreme temperatures. To attain this goal, the coping mechanisms of rice were divided into

(1) temporal escape through phenology,

(2) temporal escape through time of day of flowering (TOA),

(3) avoidance through transpirational cooling of the canopy,

(4) avoidance through morphology enabling good panicle exertion, and finally

(5) physiological tolerance.

Emphasis was on (2) and (3) because these mechanisms are the least well known at present, but an effort was made to observe and integrate all five mechanisms to enable an inclusive modeling approach to thermal stress.

A set of four *O. sativa japonica* and *indica* varieties including a traditional one (Chomrong) and 3 improved ones (IR64, IR72, S108) with different morphologies and thermal tolerances were studied in four replicated field experiments in contrasting environments (humid-tropical in the Philippines, temperate summer in France, and semi-arid tropical (Sahelian) climate during the cool-dry and hot dry seasons in Senegal). For all combinations of genotype and environment, detailed phenological (Microspore, Flowering, TOA) and morphological (canopy structure) measurements were coupled with a characterization of the micro-climate along the soil-water-canopy-atmosphere gradient and IR imagery of leaves and panicles. This enabled estimation of the temperature of the stress-sensitive organ during its sensitive phases and at its location during those phases (submerged in the floodwater at microspore stage, near top of canopy at flowering).

The analysis of the data brought out two new and agro-ecologically important findings:

(1) TOA was confirmed to last about 2 hours and happened chiefly in the late morning, but it was found to vary strongly among environments. This plasticity of TOA could be predicted with environment temperature and humidity and was found to be adaptive (escape from high midday temperatures). Although no varietal differences were observed, the adaptive plasticity of TOA is an escape trait of major significance. If not taken into account, model predictions of heat induced sterility are bound to be flawed.
(2) Panicle temperature was up to 10°C below ambient under dry conditions but slightly higher than ambient under humid conditions. Consequently, heat injury was not observed in the hottest but in moderately warm but humid environments. This is necessarily a game-changer for crop-model based predictions of heat sterility because the risk prone environments are not the same as predicted with present crop models.

In addition, this thesis also provided quantitative information on chilling induced sterility caused by cold floodwater at microspore stage and poor panicle exertion due to insufficient peduncle elongation. No genotypic differences in physiological temperature stress tolerance were found except for pronounced chilling tolerance of Chomrong, a variety from Nepal.

The escape and avoidance mechanisms were described in chapters 1 and 2. In chapter 3 we introduced the crop model RIDEV and described its construction and improvement to take into account the results of chapter 1 and 2 (TOA and panicle temperature). We then compared our regression model of panicle temperature with another model of panicle temperature developed independently in Japan (IM2PACT). Finally, we presented some simulations made with the new RIDEV modeling tool in chapter 4. Despite the different modeling approaches and data used by the present study and the colleagues in Japan, the two models of panicle temperature agreed very well. RIDEV calibration and simulations based on independent data were also conclusive.

DISCUSSION

TOA plasticity and temporal escape of thermal stress

Although duration of anthesis varied little, its hour of onset during the day (TOA, corrected for solar noon and day length) varied by several hours among the different climatic environments. TOA responded in an adaptive sense (thus avoiding high spikelet temperature during anthesis) as a function of Tair and VPD experienced before anthesis. Plasticity of TOA is thus an escape mechanism

to avoid unfavorable thermal conditions at flowering. Under cold conditions anthesis is delayed and under hot conditions it is advanced to earlier hours of the morning. The earlier anthesis under humid conditions provides a complementary protection to heat at flowering, because panicle cooling is less effective under low VPD. We did not observe a significant variability of TOA among the four rice varieties we studied in the field. However, previous studied have shown diversity in TOA (Sheehy *et al.* 2005, Jagadish *et al*. 2008) particularly in wild rice varieties (Ishimaru *et al.* 2010), and this diversity should be used in breeding as trait for heat escape in the future, particularly in hot and humid climate where the avoidance mechanism of transpiration cooling is ineffective (*cf.* next paragraph). Ishimaru *et al.* (2010) already demonstrated the effectiveness of introgressing the early TOA trait to reduce spikelet sterility under hot conditions. Research efforts should continue in this domain, but should also include our finding of TOA having significant adaptive plasticity. Screening of genetic materials in only one environment might cause bias by not considering this plasticity, which may even vary among genotypes.

Transpirational panicle cooling and climatic profiles causing heat stress

Panicle temperature during the precise period of TOA was confirmed to be the best criterion to explain heat-induced spikelet sterility, better than Tair(max) or Tair(TOA). This phenomenon necessarily escapes study if a narrow range of climatic environments is considered, but it manifests itself in multi-climatic studies. This work is the first to investigate such a wide range of transpiration cooling effects of panicles on the same genetic materials across different environments. The importance of this heat avoidance mechanism mostly depends on the evaporative demand of the air above the canopy (VPD), but is also sensitive to canopy architecture. The results emphasize the risk of heat damage for rice under warm and humid conditions (low VPD), and explains the high and relatively stable yields frequently observed under very hot but dry conditions (high VPD) in Senegal (Dingkuhn and Sow, 1997) or Australia (Matsui *et al*. 2007). Consequently, the notion of absolute heat is of limited pertinence if not backed up with information on air humidity.

In our study, among the four climatic environments, the hottest environment (Senegal hot and dry season) was not the most prone to heat stress at flowering but the Philippines climate. Indeed, in the Philippines, the high relative humidity (RH) does not allow an efficient panicle cooling leading to higher Tp and a frequent occurrence of Tp>Tair at anthesis compared with the Senegal environment (Table 1). In Senegal, the low RH counterbalances the high temperature effect by favoring panicle and canopy transpiration, a heat avoidance mechanism. In France, even though RH was higher than in Senegal, Tair was always inferior to 30°C during anthesis. But many environments exist in the monsoonal humid tropics where conditions are even more humid and warmer than

observed in the Philippines in this study, probably leading to even higher Tp. Such sites were unavailable here and future studies should be extended to them.

***Table 1* : Climatic determinants of heat stress.**

This table presents the occurrence (in number of day) of Tp > Tair at anthesis for the whole flowering period of IR72 in four different environments characterized by air temperature (Tair) and relative humidity (RH) measured at 2m, and averaged over the anthesis duration. The table also summarizes the panicle temperature (Tp) calculated with RIDEV and average over the anthesis duration as well. The percentage of spikelet sterility corrected (sterility due to poor panicle exertion subtracted) is also presented. Sterility mainly due to cold was not specified. The red color underlines the risk of heat stress: hot (Tair>30°C) and humid (RH>60%) conditions at anthesis limiting panicle transpirational cooling and leading to Tp>Tair.

	METEO DURING ANTHESIS		Tp CALCULATED DURING ANTHESIS			STERILITY CORRECTED
SITES AND SEASONS	*RH (%)*	*Tair (°C)*	*Tp (°C)*	*Nb of day with Tp < Tair*	*Nb of day with Tp ≥ Tair*	*(%)*
Senegal cold and dry season	32.5 ±11.4	33.3 ±2.9	27.0±1.1	15	0	-
Senegal hot and dry season	49.5 ±9.8	30.8 ±2.9	27.7±1.3	12	0	7.6
Philippines dry season	75.3 ±4.5	30.5 ±1.0	29.8±0.9	4	8	37.8
France temperate summer	60.3 ±11.2	27.3 ±2.2	25.3 ±1.5	14	1	-

No significant varietal difference of Tp was observed in our study and the same Tp model was thus used for the four rice cultivars. In the RIDEV model, the panicle layer position from the ground is the only morphological parameter considered. Under a given climatic condition, a higher panicle layer will result in higher Tp. More research, however, is needed on the crop architectural effects on Tp. Theoretically, coolest panicles should be observed under maximal shading of panicles by flag leave and large green leaf area having a high transpiration rate, but this architecture might be associated with poor panicle exertion and thus needs more study.

Very recent results showed genetic diversity of panicle conductance among 21 cultivars (Fukuoka *et al.* 2012), suggesting that there might be variability of Tp response to the environment among varieties. Phenotypic diversity of Tp traits should therefore be more investigated in the future.

To evaluate the potential risk of diverse climatic environments on rice crop, the concept of heat stress must be revised to take into account the air evaporative demand. In the future, crop model predictions of heat stress using weather and architecture dependent panicle temperature will help to improve the agro-ecological zoning, the predictions of climate change impacts and strategies of varietal improvement (an example of heat stress maps based on air temperature alone is

presented in Figure 2 of the Introductive chapter of this thesis, *cf*. p.16). This may change both the geographic foci and the varietal selection strategies for heat stress research for rice.

General consequences for applied research

So far no crop model simulating rice spikelet sterility takes into account TOA and Tp, except the recently released IM2PACT heat balance which is currently being extended to achieve full crop model skills (Yoshimoto *et al.* 2011), and RIDEV generated with the help of the present study.

Rice crop models accurately simulating thermal stresses are urgently needed to (1) establish global and regional agro-ecological maps of stress impacts now and in the future, (2) to provide decision tools allowing producers to better cope with climatic risks, and (3) to guide breeders and geneticists to adjust breeding criteria, selection environments and phenotyping approaches. So far, no genetic information is available on the adaptive plasticity of TOA and the heat avoidance achieved with transpiration cooling, and the available genetic diversity has not yet been characterized.

Integrated approaches are needed to consider the interactions among the numerous plant traits and responses with regards to thermal stresses, to be implemented in crop models having agronomic simulation skills (cultural practices and cropping systems). These integrated approaches should be sensitive to both heat and chilling stresses because they can occur in the same environments. Furthermore, climate change will likely change the geographic domains of crops (migration), involving stresses at both ends of the thermal spectrum.

New research dynamics being triggered by the present results

RIDEV will be at the center of a new research dynamics. At IRRI, an initiative was taken to develop new global maps of thermal constraints for rice (Improvement of Figure 2 in Introduction chapter). In the context of the Climate Change Agriculture and Food Security (CCAFS) program of the CGIAR, such maps will also be extended to future climate scenarios. In parallel, AfricaRice and IRRI are collaborating to develop a new decision tool for farmers called Rice Manager that will make use of RIDEV.

Collaboration between Tsukuba University (NIAES) and IRRI, involving also CIRAD, is under discussion to validate and possibly integrate both IM2PACT and RIDEV models, validate them on a broader basis and extend their skills to variable water resources and CO_2 levels.

Lastly, RIDEV is now being used for genomic studies in the context of the GRiSP Global Rice Phenotyping Network (GRiSP 2011). It serves to extract, in reverse mode application, genotypic photothermal constants from multi-environment phenomics data. These genotypic photothermal constants such as cardinal temperatures, photoperiodic parameters, phenological phase durations

and critical temperatures for thermal sterility will be defined at traits and mapped genetically by Genome-Wide Association Studies (GWAS).

New research questions arising from this thesis

The need to characterize the genetic diversity for the escape and avoidance mechanisms described here was already mentioned.

Our study was conducted under irrigated systems and was focused on thermal stress only. Other abiotic stresses combined with heat stress and potentially induced by global warming need to be address as well. In particular, our results suggest that there should be major trade-offs between water-saving and heat avoidance, because any reduction of transpiration or evaporation from paddy fields through modified cultural practices (e.g., alternate wetting and drying, AWD 2009) is bound to increase canopy temperature. The existing recommendations or technology options for water saving in dry-hot areas must thus be reviewed on the basis of such research because they may be risky.
Furthermore, it is unknown whether rice panicles are able to sustain their transpiration under water deficit: Although they are not able to regulate their conductance as leaves do through stomata, water deficit might reduce panicle transpiration through xylem cavitation (Stiller *et al*. 2003) or other mechanisms and needs further study.

Another issue requiring new research efforts is the probable trade-offs between CO_2 fertilization from rising atmospheric concentrations and the reduction in transpiration cooling of canopies caused by CO_2 (stomatal closure in leaves). Hasegawa research team based at NIAS in Tsukuba (Japan) demonstrated in Free-Air CO_2 Enrichment (FACE) field experiments that this effect can be substantial (Namakura *et al.* 2010). This effect needs to be built into future scenarios of heat stress.

Lastly, rice is probably not the only crop species avoiding heat injury by transpiration cooling and plasticity of TOA. In wheat, the temperature difference between the air and spikes was reported to be up to 5 °C (Ayeneh *et al*. 2002), suggesting that a similar heat avoidance mechanism as in rice is also present in other crops.

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GENERAL CONCLUSION

This multi-environment, agro-ecological study provided evidence for the adaptive value and the variability of two poorly understood traits involved in rice thermal stress tolerance during the reproductive phase: the environment driven plasticity of time of day of anthesis (TOA; an escape mechanism) and panicle transpiration cooling (avoidance). The environmental controls of the two traits were characterized mostly by VPD and air temperature. Environmental and phenological determinants of chilling induced spikelet sterility were also studied and largely confirmed previous findings. The results were incorporated into RIDEV V.2, a new crop model predicting spikelet sterility from simulated phenology and microclimate inspired by a simpler model developed in the 1990s.

This study demonstrates the need to distinguish between the general climatic environment as measured by agro-meteorological stations and the environment the critical physiological processes are exposed to. The commonly used crop models generally do not make this distinction, which may be one of their crop parameters' lack of robustness across different physical environments. In the case of thermal constraints to rice performance, the resulting errors are very large: Depending on environment, rice panicles can be by 10 degrees C cooler or 2 degrees C warmer than the air at 2m, and the heat-sensitive processes of pollination can happen early in the morning or after noon. Plant and canopy structure further modify exposure of the inflorescence to heat. Given these variations, air minimum or maximum temperatures alone have no predictive value of the thermal stress across different climatic environments.

Although the present findings are specific to irrigated rice and the fertility of its inflorescence, we suggest that the importance of microclimate generated by the crop and its organs is more general because it affects phenology and metabolism throughout the crop cycle. Crop models should therefore be equipped with modules calculating organ temperature for all physiological processes that are critically dependent on temperature, for rice and other crop species. This is particularly important for predictions of the impact of climate variability and change, including effects of rising atmospheric CO_2 concentrations.

The present study gave rise to the development of a modeling tool that will stand at the center of numerous applied research projects with agronomic and genetic objectives. Further research, however, is necessary to extend the present approach to water-limited environments, rising CO_2 concentrations and different plant and canopy architectures. This will probably require a more mechanistic approach to crop thermodynamics, as well as more explicit simulation of plant and canopy structure.

***Résumé**:*

Les inflorescences de riz sont sensibles au froid et à la chaleur ce qui se traduit par une stérilité des épillets à floraison. Ce n'est cependant pas directement la température de l'air qui est en cause mais la température des tissus à des stades de développement précis. Les stratégies pour faire face au stress thermique sont: 1) la tolérance physiologique d'une variété particulière; 2) l'échappement temporel au stress par l'ajustement de la phénologie et de l'heure de l'anthèse (TOA); 3) l'évitement du stress par le microclimat généré par la culture. Cette thèse a pour but de caractériser l'effet des composantes climatiques sur 2) et 3), et pour ce, le même essai a été mené au champ sur quatre variétés de riz irrigué cultivées dans quatre environnements climatiques contrastés (Philippines, 2 saisons aux Sénégal, France).

Bien que peu de différences variétales aient été observées au sein de chaque site, il existe une grande variabilité de l'heure de l'anthèse et de la différence de température (TD) entre panicule (Tp) et air (Tair) en réponse à l'environnement. La durée de l'anthèse est stable et limitée à environ 2 heures par jour, alors que l'heure de l'anthèse varie de 3.4 à 6.75 heure solaire. Au moment de l'anthèse, TD observée varie entre-9.5 et +2 °C. TOA et TD sont principalement caractérisés par Tair et VPD (Vapour Pressure Deficit) observés antérieurement (TOA) ou pendant (TD) l'anthèse. De plus, il existe une corrélation significative entre la stérilité (chaud ou froid) observée à maturité et Tp aux stades sensibles. Ces résultats montrent qu'en termes de risque de stérilité paniculaire pour le riz irrigué à floraison, un climat moyennement chaud et humide est plus dangereux qu'un climat très chaud mais sec car un fort VPD favorise la transpiration de la canopée et des panicules. TOA et TD ont ensuite été intégrés au modèle de culture RIDEV V.2 (qui prédit la stérilité) et les simulations de TD ont été comparées aux résultats d'un autre modèle de Tp (IM2PACT) développé indépendamment au Japon. Ces deux modèles s'avèrent robustes, et de futures collaborations mèneront à une validation complète de chaque modèle voire une intégration de ceux-ci à un nouvel outil en vues d'étudier l'impact des changements climatiques sur les cultures.

Les résultats de cette étude permettront dans un futur proche 1) d'aider les sélectionneurs en apportant de nouveaux traits d'intérêts, et 2) de définir un zonage des territoires à haut risque de stress thermique pour le riz irrigué, pour des scénarios climatiques actuels et anticipés. Des études complémentaires seront nécessaires pour permettre l'application de cette approche aux systèmes non irrigués.

***Abstract**:*

At the reproductive stage, rice spikelets are sensitive to cold and to heat which can lead to spikelet sterility. However, it is not the air temperature but the temperature of the sensitive organ itself during some specific sensitive stages that is involved. There are three different strategies to cope with thermal stress: 1) physiological tolerance of a particular variety; 2) temporal escape of the stress thanks to phenology and time of day of anthesis (TOA) adjustments; 3) stress avoidance through microclimate generated by crop architecture and transpiration. This PhD aims to characterize the effect of environment on 2) and 3) and to attain this goal, the same experiment was conducted with four rice varieties irrigated and grown in four different climatic environments (Philippines, Senegal two seasons, France).

Even though few varietal differences were observed within a site, a great variability of TOA and difference of temperature (TD) between panicle (Tp) and air (Tair) exists in response to the environment. Anthesis duration is stable and limited to 2 hours per day, whereas time of onset of anthesis varied between 3.4 to 6.75 hours after sunrise. During anthesis, observed TD varied between -9.5 and +2.5 °C. TOA and TD are mostly explained with Tair and VPD (Vapour Pressure Deficit) observed before (TOA) or during (TD) anthesis. A significant correlation between spikelet sterility (due to cold or heat) and Tp at sensitive stages was established across sites and varieties. Those results showed that for irrigated rice, humid and moderately hot environments are more subject to heat stress sterility than very hot but dry environments, because panicle and canopy transpiration are favored by high VPD. Ultimately, RIDEV V.2 crop model (predicting spikelet sterility) was developed to integrate the previous results and Tp simulations were compared to another Tp model (IM2PACT) simulations, developed independently with a different approach in Japan. Those two models are robust and future collaborations will lead to complete model validations and maybe integration in a new modeling tool to answer the need in evaluating the impact of different climate change scenarios and the adaptation of crop response to those changes.

In a short term, the results of this study will enable to 1) help breeders providing them new interest traits for thermal tolerance, and 2) define geographic zoning for high heat stress risk for irrigated rice, for present and future climate change scenarios. Complementary studies are needed to apply this approach to non irrigated system.