



Field phenomics for response of a rice diversity panel to ten environments in Senegal and Madagascar. 1. Plant phenological traits

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ABSTRACT

The GRiSP Global Rice Phenotyping Network seeks to assemble a multi-environment, multi-trait phenomics resource for rice (*Oryza sativa* L.) diversity panels in order to understand existing genetic diversity and identify genes/alleles conveying adaptation and yield potential. Phenology is central to plant adaptation and productivity in different agro-ecologies. We studied a panel of 209 accessions, including 189 indica types and 20 acc. representing other genetic groups. The panel was field-phenotyped under flooded conditions for duration to flowering (F) for 6 sowing dates in Senegal (capturing different thermal and day length regimes) and during two years at two altitudes (mid, 857 m asl; high, 1497 m asl; with similar latitude) in Madagascar. The experiments had an augmented design in Senegal (6 blocks × 6 dates as subplots and 209 acc. as sub-subplot) with 4 replicated check varieties; and a complete block design with 3 replications at 2 sites × 2 years in Madagascar. To dissociate different traits contributing to variation of F, five genotypic Index Variables were calculated. Among them, PCA identified three as describing 98% of diversity: Estimated basic vegetative phase (BVP), photoperiod (PP) sensitivity, and altitude response. Three clusters were identified within the indica group and related to genotype origin, agronomic type and ecosystem of adaptation. Cluster C1 was PP-sensitive, C2 had short BVP and C3 had long BVP. Within C1, a sub-cluster was highly altitude sensitive, and within C3 two sub-clusters were comparatively altitude insensitive. Harvest index (HI), aboveground dw and stem height observed in the most favorable environment (Senegal wet season) showed distinct patterns among clusters, with C2 having high HI and C1 being tall. The phenomics resources will be further analyzed using a crop model and genome-wide association (GWA) studies.

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1. Introduction

Phenology in terms of the timing of plant developmental processes, governing organogenesis and notably flowering time, is of foremost importance to adaptation to the biophysical environment, both in agronomic and ecological terms (Dingkuhn, 1995; Fukai, 1999; Shrestha et al., 2012, 2013). Phenology determines temporal escape from abiotic stresses and prevalence of pests and diseases that are linked to seasonal climatic patterns (Kouressy

et al., 2008), and sets the amount of time available to deploy organs that acquire resources such as radiation, water and nutrients. Rice (*Oryza sativa* L.) possesses considerable genetic diversity in phenological responses because as a species, it extends from temperate to tropical environments, from sea level to altitudes above 2000 m asl, from arid to humid monsoonal climates, and from permanently flooded to dryland habitats (GRiSP, 2013).

Foremost environmental factors for phenology of rice are temperature and photoperiod (Dingkuhn et al., 1995), sometimes modulated by physiological stresses (Fukai, 1999; Suji et al., 2012) and to a lesser extent, resources such as phosphorus (Sahrawat et al., 1995). In irrigated flooded systems, temperature is the main driver of rice phenology because stress factors and nutri-

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ent resources are usually controlled, but photoperiod can strongly impact on the flowering date of the more sensitive traditional cultivars (Craufurd et al., 2003). Since early anthropogenic selection and breeding implicitly selected for these response traits by selecting for integrative traits such as yield and crop duration, we can expect substantial genetic diversity in the species for physiological component traits of phenology. These component traits may include cardinal temperatures (base, optimal and maximal temperatures for development), the thermal duration of the basic vegetative phase (BVP) and subsequent reproductive phases, as well as component traits for photoperiod responses such as critical day length parameters and the strength of the day length effect itself (Dingkuhn et al., 1995). Furthermore, the rate of organogenetic rhythms governing phytomer succession contribute to overall phenology and interact with the photoperiod response of phase duration (Gutjahr et al., 2012).

Temperature perception of the plant system is also complex because it depends on the developing organ and its position in the soil–water–canopy–atmosphere continuum, in which considerable thermal gradients can exist (Yoshimoto et al., 2011; Julia and Dingkuhn, 2013). In irrigated, shallowly flooded rice as studied here, the apical shoot meristem driving phenology is located most of the time under the water line but emerges in the course of internode elongation during the reproductive phase. Phenomics research aiming at investigating trait diversity and ultimately, trait genetic control, can pursue a reductionist approach for specific component traits in controlled environments or alternatively expose the germplasm to diverse field environments in a more integrative approach capturing whole-plant adaptation. The latter is frequently useful if the results are to be directly relevant to breeding, where the traits' breeding value depends on its adaptive value in the field. The present study focuses on diverse field environments while attempting component trait dissection *a posteriori* using information on environmental variation.

This study used a diversity panel of 209 rice accessions composed for Cirad's ORYTAGE phenotyping project which is part of the GRiSP Global Rice Phenotyping Network (<http://ricephenonetwork.irri.org>). The panel consisted of a sub-population of indica types (189 accessions) and 20 accessions representing other genetic groups within the species for comparative purposes. Indica types, generally considered as a sub-species because of significant genetic barriers to hybridization with japonica types (Oka, 1958, 1964), have many agronomic advantages in terms of yield potential and grain quality but are largely secluded from cool habitats because of thermal adaptation (Tai, 2012). The detection of tolerance traits to diverse thermal habitats within the indica group is thus of great economic and eco-physiological interest.

The panel was composed to (1) characterize genetic diversity in climatic adaptation traits and (2) enable genome-wide association studies (GWAS) aiming at gene/allele discovery (Courtois et al., 2013). Particular choices were made with respect to geographic origin, with an over-representation of accessions originating from West Africa and Madagascar that have harsh climates in terms of atmospheric aridity (Sahel irrigated environments) or low temperatures (Madagascar highlands). These two regions have seen an influx of Asian rice germplasm during the past 500 years or more (Radanielina et al., 2013) on different routes. The indica materials grown in the Madagascar highlands came from the Malaysian peninsula and India but have since then been subjected to selection under the cool climate at 850–1750 m asl. In West Africa, high altitude environments are absent but low night temperatures can cause significant stresses in irrigated or seasonally flooded Sahel environments (Dingkuhn, 1995).

The objective of this study was to expose the rice diversity panel to diverse climatic environments under irrigated flooded conditions resulting from six different sowing dates in Senegal and

two altitudes and years in Madagascar; and to extract from the observed variation of duration to flowering (F) phenological component traits related to the effect of genotypic BVP and sensitivity to photoperiod and low temperatures. The companion paper to this study (Dingkuhn et al., submitted) carries the analysis to spikelet sterility caused by chilling.

We use the resulting data resource on phenology to analyze the structure of genotypic diversity within the indica group, its relationship with genetic groups, and its relationship with the accessions' geographic origin, the hydrological ecosystem of cultivation (rainfed-upland or flooded-lowland) and the cultivar type (traditional or improved). This also permits identifying contrasting genotypes for future in-depth studies on the functional ecology, physiology and application in breeding. The data generated by this study will be further used for genetic analysis using GWAS.

2. Materials and methods

A rice diversity panel was phenotyped for days to flowering in the field in diverse environments under irrigated flooded conditions in Senegal in 2009/2010 (seasonal environments: 6 sowing dates) and in Madagascar in 2009 and 2010 (two altitudes). These environments were selected to cover climatic diversity including cool and hot environments.

2.1. Genetic materials

The population taken from the ORYTAGE species-wide (*O. sativa* L.) diversity panel of Cirad (<http://ricephenonetwork.irri.org/diversity-panels/orytage-diversity-panels>) which is also a resource for the GRiSP Global Rice Phenotyping Network (<http://ricephenonetwork.irri.org>). For the present study, a sub-sample of 189 indica accessions was grown, augmented for comparative purposes with 20 genotypes representing other genetic groups (3 aus, 3 temperate japonica, 12 tropical japonica, and 2 aromatic accessions). The indica population covered improved and traditional varieties from all tropical regions but had large sub-populations from Madagascar (34) and W-Africa (44, thereof 30 improved lines from AfricaRice bred in Senegal) to capture adaptations to the climatic constraints at the experimental sites. Twenty improved varieties and lines were from IRRI (Philippines). A complete list of accessions' geographic origin and seed sources is presented in Table S1.

2.2. Experiment in Senegal

The experiment was conducted at the AfricaRice Sahel Station at Ndiaye, Senegal (16°12'N, 16°16'W) at 8 m asl altitude. The experiment design was an augmented design with 6 blocks and 2 factors (main plot = sowing date, sub-plot = genotype). Each block had 6 sowing dates and 38 genotypes plus 4 replicated check varieties. Check varieties were IR64 as international high yielding variety (HYV) check, Sahel 108 as local HYV check, N22 from India (aus) as heat tolerant check and Chomrong from Nepal (temperate japonica) as cold tolerant check. Sub-plot size was 1 m × 1 m and pre-germinated seed was hill-wise dibbled onto wet puddled soil at 20 cm × 20 cm spacing and a rate of 3–4 seeds per hill, thinned after emergence to 2 seedlings per hill.

Fields were flooded 5–10 cm deep following stand establishment until maturity. Fertilizer inputs were 120–60–60 (N–P–K, kg ha⁻¹) as triple-split application following local practice, using urea, diammonium phosphate (DAP) and potassium chloride (KCl). Weed control was by hand and birds were controlled with nylon fishnets from heading to maturity. The soil was a heavy, slightly acid vertisol clay (percolation <1 mm d⁻¹) with 47% clay, 40% silt and 13% sand.

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