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Temporal chlorophyll fluorescence signals to track changes in optical properties of maturing rice panicles exposed to high night temperature

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ABSTRACT

High night temperature (HNT) significantly influences rice grain filling dynamics. A novel phenotyping approach using chlorophyll fluorescence was employed to track changes in the optical properties of maturing rice panicles exposed to control and HNT. Two contrasting rice genotypes, Gharib (HNT sensitive) and N22 (highly tolerant), were exposed to control (23 °C) and HNT (29 °C), from panicle initiation till maturity. Changes in the optical properties of rice panicles throughout maturity were evaluated under field conditions by measuring (i) effective quantum yield of photosystem II efficiency (Φ_{II}), (ii) steady-state chlorophyll fluorescence (F_S) and (iii) ratio of emitted chlorophyll fluorescence at 690 and 735 nm, under excitation at 435 nm (F_{690}/F_{735}). Numerous vegetative indices (Vis) were correlated with fluorescence measurements to prove the accuracy of the phenotyping method. Φ_{II} was selected as the most potent fluorescence parameter (i) to track changing optical properties of maturing rice panicles under both control and HNT and (ii) to estimate the elusive change point initiating rice panicle senescence. Detection of Φ_{II} change point allows for larger genetic diversity scans under field conditions and for identifying novel donors for increasing rice yields and incorporating resilient strategies to reduce impact of HNT stress on grain-filling.

1. Introduction

Flowering in rice is a critical developmental stage, when seed numbers are determined. Flowering, along with the seed-filling phase, is considered highly sensitive to temperatures above critical thresholds (Shah et al., 2011). One major component accompanying climate change is the rapid increase in minimum night temperature compared with maximum day temperature at the global (Vose et al., 2005), country (Zhou et al., 2004; Welch et al., 2010; Rao et al., 2014) and farm level (Peng et al., 2004). A significant negative impact of high night temperature (HNT) stress (Jagadish et al., 2014) on rice yield has been confirmed by controlled-environment studies (Kanno and Makino, 2010; Mohammed and Tarpley, 2009, 2010, 2011; Mohammed et al., 2013) and recently from field experiments (Shi et al., 2013). Interestingly, under field conditions, the major cause of reduced yield under HNT was due to the overall reduction in biomass and non-structural carbohydrate (NSC) content, particularly in the panicles (Shi et al., 2013). In addition, the authors documented reduced grain width and 1000 grain weight, which could potentially be a result of reduced active grain filling duration (Kim et al., 2011), that is not captured by the routine protocols established to determine grain maturity.

Anthesis or flowering of rice (reviewed by Yoshida and Nagato, 2011) generally begins upon panicle emergence or on the following day and is consequently considered synonymous with heading, depending on the genotype under investigation. Rice follows a top-down flowering pattern, with the entire panicle flowering lasting for about 4 to 7 days (Moldenhauer and Slaton, 2005). Each spikelet on a rice panicle initiates its grain filling process 4 to 5 days past flowering, with the initiation of starch granule formation (Fitzgerald and Resurreccion, 2009). Superior spikelets are defined as those that are generally located on the apical primary branches

Abbreviations: Cc, total carotenoids; Chl-*a*, chlorophyll *a*; Chl-*b*, chlorophyll *b*; Chl-F, chlorophyll fluorescence; CP, fitted change point; DAF, days after flowering; DS, dry season; Φ_{II}, effective quantum yield of photosystem II efficiency-QY; F_S, steady-state chlorophyll fluorescence level; HNT, high night temperature; NSC, non-structural carbohydrate; PRI, photochemical reflectance index; PSII, photosystem II; Vis, vegetative indices; WS, wet season.

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of a rice panicle, they flower first and produce larger and heavier seeds grains, while inferior spikelets are located on the proximal secondary branches, flower late and are either sterile or produce poorly filled grains (Yang and Zhang, 2010). The grain filling and maturation stage, occurring after anthesis, are characterized by grain growth and increases in weight, and are concomitant with the translocation of stored assimilates in the culms or from current photosynthesis (Fu et al., 2011; Yang and Zhang, 2010). The translocated sugars, which is sucrose in the case of rice, are converted to starch in the grain with the help of invertases, starch synthase, sucrose synthase, adenosine diphosphate-glucose pyrophosphorylase and starch branching enzyme and other enzymes involved in sugar metabolism (Yang and Zhang, 2006, 2010). All these changes are reflected in the progressive change in grain color as grain filling continued. A feature of rice panicle ripening is the change in color as a consequence of chlorophyll disappearance, when the yellowish coloration, due to other unknown pigments, becomes perceptible.

The presence of functional chlorophyll, a major photosynthetic pigment, showed that the photochemical efficiency of photosystem II (PS II) in fruit is similar to that in leaves (Carrara et al., 2001), since fruits from numerous species develop as a green photosynthetic tissue (Gillaspy et al., 1993). Rice panicles contribute to photosynthesis as well (Imaizumi et al., 1997). On the basis of chlorophyll, the photosynthetic capacity of a spikelet was found to be similar, while the estimated gross amount of photosynthetically assimilated carbon in the panicle is 30% of that in a flag leaf (Imaizumi et al., 1990). In addition, it was highlighted that the transpiration rate of the rice panicle reached maximum at heading, when the panicle color is similar to the leaf and decreased thereafter with age as grain filling progressed (Ishihara et al., 1990), providing evidence of carbon entry facilitating assimilation in the rice floral tissue. In addition, it has been highlighted, that rice genotypes characterised with rapid leaf senescence also exhibit rapid panicle senescence (Seo et al., 1981). In principle, photosynthesis suffers a substantial decrease during the period of color change, in which chlorophyll or chloroplast function is lost (Bean et al., 1963). These color changes can be followed by measurements of different optical signals. Chlorophyll fluorescence (Chl-F) provides a fast and non-destructive assessment of the loss of chloroplast function, and has been effectively used to follow senescence and/or ripening in a range of harvested plant tissues and organs (Adams et al., 1990; Armstrong et al., 1997). Since Chl-F is induced by direct excitation of chlorophyll molecules of PSII by light and their immediate relaxation, its characteristic is altered by functionally poor or rich PSII. During ripening, the level of Chl-F emission can be affected by two major changes—(i) a decrease in chlorophyll content or (ii) a loss of photosynthetic competence per unit chlorophyll. A decline in Chl-F has been reported to follow a decrease in chlorophyll content in papaya fruit (Bron et al., 2004), and was reflected in the loss of chloroplast function with advancing maturation, for example, in apples (Song et al., 1997).

Extending rice grain filling period has been a target of rice breeding programs during the past couple of decades. The underlying benefit is to delay panicle senescence, which would allow sufficient time for assimilates to fill the reproductive units efficiently, a novel route to increase stagnating rice yields. The grain filling duration in rice under temperate conditions such as Japan is much longer, whereas a significantly shorter duration under tropical conditions such as the Philippines is considered as a key bottleneck for further yield enhancement. Progress has not been made in this direction due to the lack of a standardized phenotyping protocol to establish the right approach to ascertain and track progress during grain filling. To ensure that a larger diversity of rice accessions is explored to address this highlighted bottleneck, a high-throughput phenotyping approach is needed. To date, there are no reports related to the use of Chl-F for the detection of rice panicle maturity, even though previous findings showed that Chl-F measurements in conjunction with other conventional methods appear to be a useful tool to follow ripening in chlorophyll-containing fruits, and thus potentially rice panicles. Hence, the objectives of our studies were (i) to investigate temporal changes in Chl-F and supportive reflectance signals of rice panicles exposed to control and HNT during ripening, (ii) to unravel the association between known vegetative tissue reflectance indices (Vis) and those obtained from panicle fluorescence and reflectance measurements, and (iii) to identify the best Chl-F parameter that can detect the elusive point trigerring panicle senescence and its rate of advancement under HNT.

2. Materials and methods

Field and greenhouse experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, Philippines (14°11′N, 121°15′E). Three independent experiments were carried out during the wet season (WS) of 2013 and dry season (DS) of 2014.

2.1. Plant material

Based on phenotypic data obtained from a larger genotypically diverse set of 36 rice genotypes in response to HNT stress (Zhang et al., 2013), followed by a comprehensive physiological and molecular characterization (Shi et al., 2013), N22 (HNT tolerant) and Gharib (a susceptible genotype), were selected for this study. Seed dormancy was broken by exposure to 50 °C for 3 days, followed by pre-germination and sowing in seeding trays. Fourteen-day-old seedlings were transplanted on July 11 during the 2013 WS and on January 5 during the 2014 DS, at a spacing of 0.2 × 0.2 m, with four seedlings per hill. During the 2013 WS, phosphorus (15 kg P ha^{-1} as single superphosphate), potassium (20 kg K ha^{-1} as KCl) and zinc (2.5 kg Zn ha^{-1} as zinc sulfate) were incorporated one day before transplanting. Nitrogen fertilizer in the form of urea was applied in four splits (30 kg ha^{-1} as basal, 20 kg ha^{-1} at mid-tillering, 20 kg ha^{-1} at panicle initiation and 30 kg ha^{-1} just before heading). The amount of P, K and Zn fertilizers was doubled in the 2014 dry season as per the standard recommendations, while the 150 kg N was applied in four splits with a proportional increase in each of the splits. Manual weeding was employed to maintain weed-free plots. Need-based chemical spraying was undertaken to control whorl maggots (*Hyddrelia philippina* Ferino) during the early vegetative stage and yellow stem borers (*Scirpophaga incertulas*) at the flowering stage.

2.2. HNT tents and stress treatment

Six field-based temperature-controlled tents were used to study the impact of HNT on the optical properties of rice panicles during the grain filling and ripening stages. For details on the facility and the operation of the tents, readers are directed to Shi et al. (2013). A photographic view and details of the facility are also provided as Supplementary data Fig. S1. In brief, the tents were designed to impose HNT with high accuracy—by ensuring they were leak-proof and provided with sufficient air flow to avoid buildup of CO_2 and relative humidity. Plants were exposed to HNT (29 °C [actual = 28.6 ± 0.1 °C during 2013 WS and 28.6 ± 0.5 °C during 2014 DS]) and 23 °C [23 ± 0.1 °C during 2013 WS and 22.9 ± 0.1 °C during 2014 DS] as control temperature. Each tent of size 18 m^2 was considered as an independent replicate, two (in 2013) and three (in 2014) each for control and HNT treatments. The tents were covered during nights (1800–0600) to impose HNT stress and completely opened during the day (0600–1800) to avoid other confounding factors, such as reduced light, etc.; and to obtain results that would