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Variations between the photosynthetic properties of elite and landrace Chinese rice cultivars revealed by simultaneous measurements of 820 nm transmission signal and chlorophyll *a* fluorescence induction

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ABSTRACT

The difference between the photosynthetic properties of elite and landrace Chinese rice cultivars was studied, using chlorophyll *a* fluorescence induction (mostly a monitor of Photosystem II activity) and I_{820} transmission signal (mostly a monitor of Photosystem I activity) to identify potential photosynthetic features differentiating these two groups, which show different degrees of artificial selection and grain yields. A higher fluorescence (related to PSII) IP rise phase and a lower P700$^+$ (related to PSI) accumulation were observed in the elite cultivars as compared to the landraces. Using these data, together with simulation data from a kinetic model of fluorescence induction, we show that the high IP rise phase and the low P700$^+$ accumulation can be a result of transient block on electron transfer and traffic jam on the electron acceptor side of PSI under a high [NADPH]/[NADP$^+$] ratio. Considering that the ferredoxin NADP$^+$ reductase (FNR) transcript levels of XS134 (a representative elite cultivars) remains unaffected during the first few minutes of light/dark transition compared to Q4145 (a representative landrace cultivars), which shows a strong decline during the same time range, we propose that the FNR of elite cultivars may take more time to be inactivated in darkness. During this time the FNR enzyme can continue to reduce NADP$^+$ molecules, leading to initially high [NADPH]/[NADP$^+$] ratio during OJIP transient. These data suggested a potential artificial selection of FNR during the breeding process of these examined elite rice cultivars.

Introduction

Photosynthesis is one of the most powerful and life-giving biological processes occurring on our Earth (Blankenship, 2014). Oxygenic photosynthesis is carried out on a large scale by plants, algae and cyanobacteria, where light energy is converted into stable-rich chemical compounds, with concomitant uptake of CO_2 and release of O_2 (Eaton-Rye et al., 2012). This process, as well as anoxygenic photosynthesis (Hunter et al., 2009) carried out by photosynthetic bacteria, is responsible for all life on Earth. The photosynthetic apparatus of higher plants includes, in addition to the enzymes involved in CO_2 assimilation, four membrane bound protein complexes (Ke, 2001; Wydrzynski et al., 2005; Golbeck, 2006; Shevela et al., 2013): (1) photosystem II (PSII), water-plastoquinone

Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzo-quinone; DCMU, 3,4-dichlorophenyl-1,1-dimethylurea; F_o, basal (initial, minimal) level of chlorophyll *a* fluorescence; F_m, maximal level of chlorophyll *a* fluorescence; FNR, ferredoxin NADP$^+$ reductase; F_v, variable chlorophyll *a* fluorescence (=F_m minus F_o); I_{820} nm, transmission signal at 820 nm; LED, light emitting diode; OJIP transient, chlorophyll *a* fluorescence induction where O is for F_o, P is for peak (equivalent to F_m in saturating light) and J and I are inflections between O and P; PC, plastcyanin; PQ, plastoquinone; PQH_2, plastoquinol; PS, photosystem; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RC, reaction centre.

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oxido-reductase, which catalyzes light driven oxidation of water and reduction of plastoquinone (PQ); (2) photosystem I (PSI), plastocyanin- ferredoxin oxido-reductase, which catalyzes the final stage of light reactions, i.e., reduction of ferredoxin and oxidation of plastcyanin (PC); ferredoxin is reduced via ferredoxin $NADP^+$ reductase (FNR), and by NADPH, formed from $NADP^+$, which, in turn, is reduced by PSI; (3) Cytochrome (cyt) b_6f, which catalyzes the transfer of electrons from plastoquinol (PQH_2) to PC.; and (4) ATP Synthase that uses proton motive force (pmf), which is made up of proton gradient ΔpH and $\Delta\psi$, to produce ATP from ADP and inorganic phosphate. Photochemical events of photosynthesis are initiated by the capture of incident photons by pigments in antenna complexes. Then, absorbed energy is efficiently transferred to photochemical reaction centres (RC), leading, ultimately, to the transfer of electrons through an electron transport chain from water to $NADP^+$, and the production of pmf.

Improvement of photosynthetic capacity of food crops has been considered to be a real challenge for plant scientists and crop breeders (Long et al., 2006; Murchie et al., 2008; Zhu et al., 2008; Evans, 2013), in order to cope with the enormous demand for food in the world. Several biotechnological approaches have been applied, using many targets affecting photosynthetic activity (Reynolds et al., 2000; Leegood, 2002; Long et al., 2006; Feng et al., 2007; Zhu et al., 2010; Ort et al., 2011; Gowik and Westhoff, 2011; Raines, 2011; Parry et al., 2013). Many current efforts to identify options to increase photosynthesis follow a rationale design approach, i.e., first identifying potential targets to engineer the plant, based on the current knowledge of photosynthesis, and then applying targeted engineering of these options, and finally examination of their consequences. Besides this approach, mining natural variations of photosynthesis is also regarded as a promising approach to identify a new genes or alleles for crop improvement. Mining variations can also be used to identify potential factors under natural or artificial selection. Here, we report a case study of variations and correlations of some photosynthetic parameters in two different groups of Chinese rice (*Oryza sativa*) cultivars, which show different degrees of artificial selection and also grain yield. The first group represents the elite cultivars, which exhibits high crop yield (Bao et al., 2006; Deng et al., 2007; Zhou and Yao, 2012), which has been under a strong artificial selection by plant breeders for high performance and is commercially used in China (For further details, see e.g., http://www.ricedata.cn/variety/.) Moreover, several cultivars of elite rice, such as: 9311, Minghui 63 (MH63) and Zhonghua 11 (ZH11) have been used as recurrent parent to produce super hybrid rice (Jiang et al., 2004; Zhang et al., 2012). On the other hand, the second group represents traditional Chinese rice varieties, developed between 1928 and 1996 (called here landraces), which mostly shows low crop yields, and is relatively less used these days.

In this study, Chl *a* fluorescence induction (OJIP, where O is for minimum fluorescence, P is for peak, and J and I are inflections), which reflects a progressive reduction of the PQ pool located on the acceptor side of PSII, and transmission changes at 820 nm (I_{820}), representing the redox level of the RC of PSI, were used to characterize these two groups. The OJIP fluorescence induction curve has been used extensively in photosynthesis physiology research (Govindjee, 1995), mainly due to the ease of measuring the OJIP signal. However, OJIP signal is influenced by a large number of biophysical processes. Hence, it has remained a major challenge to fully interpret the physical mechanism underlying each of individual phases of the OJIP curve, even though a number of theoretical studies are available that have explored this issue (Zhu et al., 2005; Lazar, 2009; Xin et al., 2013). In this study, we first explored the relationship between these parameters between landraces and elite cultivars of rice, and showed that there is a distinct increase in the IP phase in the OJIP curve of the chosen elite cultivars. Then, with a kinetic model of fluorescence induction, we evaluated the potential mechanisms underlying simultaneous changes in the IP phase rise and $P700^+$ accumulation and propose that variations in FNR can be a potential mechanism underlying these changes. Finally, we provided evidence showing that the expression level of *FNR* differed between elite and landraces, suggesting that *FNR* might be a gene under artificial selection during the breeding process.

Materials and methods

Plant material

For our measurements, two groups of Chinese rice (*Oryza sativa*) were used. The first group (elite group) was composed of ten accessions, namely, HE2219, KY131, WCC2, MH63, XS134, DHX-Z, DHX-W, ZH11, WY-4 and 9311. The second group (landraces) was also composed of ten accessions, namely, Ao Chiu 2 Hao (A4010), Chun 118-33 (E4050), Kin Shan Zim (H4080), Pan Ju (J4088), Shui Ya Jien (M4112), 4484 (Q4144), 4595 (Q4145), You-I B (Q4146), Chunjiangzao No. 1 (Q4147), and Wong Chim (X4203). (For further details, see e.g., http://www.ricedata.cn/variety/.)

Growth conditions

Rice seeds from the elite and landrace groups were grown for ~30 days in a soil seed bed located in Beijing (China) (39°55′N, 116°25′E). Then, from early June 2013, seedlings were transplanted into plastic pots (12 L volume) containing commercial peat soil (Pindstrup Substrate no. 4), and then grown under outdoor conditions. Measurements were started from early July 2013. During this period, the average lower temperature range was ~24–26 °C, and the average higher temperature range was ~30–31 °C, whereas the humidity range was ~61–75%.

Chlorophyll (Chl) a fluorescence induction (OJIP) and transmission changes at 820 nm (I_{820})

For a background on the use of Chl *a* fluorescence, see chapters in Papagergiou and Govindjee (2004), and for a basic background on detailed experimental technique, see Kalaji et al. (2014). OJIP and I_{820} measurements were recorded simultaneously using the Multifunctional Plant Efficiency Analyzer (M-PEA) (Hansatech, King Lynn, Norfolk, UK). In this instrument, wavelengths of light (from Light Emitting Diodes, LEDs) are: 625 ± 10 nm for the actinic light; 820 ± 25 nm for the modulated light; and 735 ± 15 nm for the far-red light. Plants were kept overnight at 24 °C in darkness. Then, after a 10-min dark adaptation, the attached uppermost fully expanded leaves were exposed for 0.5 s to saturating orange–red (625 nm) actinic light (5000 μmol photons m^{-2} s^{-1}) and modulated light (820 nm) provided by the LED. Measurements were repeated three to four times for each accession. The ratio of variable fluorescence F_v (the difference between the maximal fluorescence, F_m, fluorescence at the P level, and F_0, fluorescence at the O level) to F_m, i.e., F_v/F_m, was used to evaluate the maximum quantum yield of PSII. For the measurements shown later in Fig. 7, plants were kept overnight at 24 °C in darkness. Then, the attached leaves were light-adapted for 10 min in white light (600 μmol photons m^{-2} s^{-1}) provided by an external lamp projector. After that, Chl *a* fluorescence induction was recorded, as described above, first without, and then after 1, 3, 7, and 10 min dark-adaptation.

RNA isolation and real-time RT-PCR analysis

Total RNA was extracted from mature rice leaves at different dark-time point (0, 1, 3, and 10 min) using Purelink RNA Mini Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Concentration of each RNA sample was measured