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Characterization of a genomic region that maintains chlorophyll and nitrogen contents during ripening in a high-yielding stay-green rice cultivar

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ABSTRACT

The high-yielding *indica* × *japonica* rice (*Oryza sativa* L.) cultivar Akenohoshi tends to maintain a higher photosynthetic rate during ripening owing to its higher nitrogen accumulation and nitrogen partitioning to leaves than the commercial *japonica* cultivar Koshihikari. By using recombinant inbred lines derived from a cross between Akenohoshi and Koshihikari, we detected at least 6 quantitative trait loci (QTLs) for maintaining higher leaf chlorophyll content, 4 QTLs for nitrogen content, and 5 QTLs for the rate of nitrogen transport to shoots during ripening in the paddy field. Then we developed two chromosome segment substitution lines carrying Akenohoshi segments on the short arm of chromosome 3, where the QTLs for chlorophyll content reduction were clustered, in the Koshihikari genetic background. The lines showed higher rate of nitrogen transport to shoots, leaf chlorophyll and nitrogen contents, and therefore a higher rate of leaf photosynthesis, than Koshihikari. We concluded that a 7.7-Mb region present in both two lines, named *qCHR1*, is important for maintaining chlorophyll and nitrogen contents during senescence. The Akenohoshi allele at *qCHR1* increased nitrogen accumulation and nitrogen partitioning to leaves during ripening, but did not change yield.

1. Introduction

Dry matter production of crops is determined by both the light-intercepting characteristics and leaf photosynthesis (Long et al., 2006). Leaf photosynthetic rate is determined by the photosynthesis capacity and is reduced by abiotic stress and with senescence. The photosynthesis capacity (a measure of maximum photosynthetic rate of leaves) is determined at full leaf expansion under saturating light, the ambient atmospheric concentration of CO_2, an optimum temperature, and a low vapor pressure deficit (Murata, 1961; Hirasawa et al., 2010). The photosynthetic rate of rice (*Oryza sativa* L.) is reduced in the afternoon owing to water stress even under submerged conditions (Hirasawa et al., 1992; Ishihara, 1995; Hirasawa and Hsiao, 1999). In rice, no new leaves are formed after heading, and the photosynthetic rate decreases with senescence in all leaves during the ripening stage (Makino et al., 1984; Jiang et al., 1988, 1999). Since dry matter produced after heading contributes approximately 60% to 80% of grain yield in rice (Kumura, 1995), maintaining the high rate of leaf photosynthesis after heading is vital for grain production.

The decrease in the photosynthetic rate during senescence is closely related to the decrease in the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary CO_2 fixation enzyme, in leaves (Makino et al., 1985; Imai et al., 2005). There is also a close correlation between leaf nitrogen content and Rubisco content, because Rubisco accounts for approximately

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30% of total leaf nitrogen (Mae et al., 1983; Makino et al., 1985; Evans, 1993). Thus, maintaining high leaf nitrogen content during senescence is important for maintaining the high photosynthetic rate. Nitrogen topdressing before and after heading is effective for maintaining a high rate of leaf photosynthesis during ripening and ensuring high yield in rice (Matsushima, 1995).

The delayed leaf senescence phenotypes are called "stay-green" (Thomas and Howarth, 2000; Kusaba et al., 2013). These phenotypes are classified into functional and non-functional. In functional stay-green plants, both leaf greenness and photosynthetic rate are maintained during senescence, whereas in non-functional ones, only leaf greenness is maintained, by delaying chlorophyll breakdown during senescence. The functional stay-green trait is beneficial for extending the assimilatory capacity of the canopy and improving crop yield potential; it is classified into type A (a delay in the onset of leaf senescence) and type B (a slower decrease in the rate of photosynthesis) (Thomas and Howarth, 2000). Several studies have focused on natural variation of leaf senescence in rice (Ishimaru et al., 2001; Jiang et al., 2004; Teng et al., 2004; Abdelkhalik et al., 2005; Yue et al., 2006; Yoo et al., 2007; Fu et al., 2011). Among them, Jiang et al. (2004), Yoo et al. (2007) and Fu et al. (2011) used stay-green rice cultivars as parents in QTL analysis, and detected some QTLs involved in chlorophyll degradation, which was evaluated from chlorophyll meter values. However, to confirm the effects of these QTLs on chlorophyll degradation, further characterization in a uniform genetic background is needed.

Akenohoshi is a high-yielding *indica* × *japonica* rice cultivar developed in 1984 by a 15-year government project aimed at the development of high-yielding cultivars in Japan (Hirota and Nomoto, 1990). A typical component that determines high yield in Akenohoshi is considered to be a long maturity period due to long leaf greenness after heading. During ripening, the leaf nitrogen content is maintained at a higher level in Akenohoshi than in the standard *japonica* cultivar, Nipponbare (Ookawa et al., 2003). This makes the rate of leaf photosynthesis higher in Akenohoshi than in Nipponbare throughout the ripening stage. Akenohoshi accumulates and partitions a larger amount of nitrogen to leaves after the panicle formation stage than Nipponbare does, resulting in a higher leaf nitrogen content during ripening (Ookawa et al., 2003).

To focus on QTLs in Akenohoshi that affect the photosynthetic rate during leaf senescence in the ripening stage, we have previously developed recombinant inbred lines (RILs) from a cross between Akenohoshi and the commercial *japonica* cultivar Koshihikari. By using the RILs, we have already detected a QTL for exudation rate, which may increase hydraulic conductance during the ripening stage (Yamamoto et al., 2016). In this study, using a similar genetic approach, we tried to detect QTLs for maintaining high chlorophyll and leaf nitrogen contents and a high rate of nitrogen transport from roots to shoots during ripening. We then confirmed and characterized the effect of one of the detected QTLs by using chromosome segment substitution lines (CSSLs).

2. Materials and methods

2.1. Plant materials and QTL analysis

We used 94 lines of an F_7 RIL population derived from a cross between Akenohoshi and Koshihikari developed by Yamamoto et al. (2016). DNA marker analysis and QTL analysis were performed as described previously (Yamamoto et al., 2016). On the basis of the results of QTL analysis, the F_1 plants were backcrossed to Koshihikari 4 times with marker-assisted selection. Finally, two CSSLs (SL3-1 and SL3-2), each carrying a different chromosome segment of Akenohoshi on the short arm of chromosome 3 in the Koshihikari genetic background, were developed. Using the RIL population and the CSSLs, we conducted 6 experiments (Exp 1–6; Table 1).

2.2. Cultivation of rice plants

The RILs in 2009 (Exp 1) and 2010 (Exp 2) and the CSSLs in 2013 (Exp 3), 2014 (Exp 5), and 2015 (Exp 6) were grown at the National Institute of Agrobiological Sciences (NIAS) in Tsukuba, Japan. Thirty days after sowing, 12 plants per line with three replications in Exp 1–3 and 72 plants (24 plants × 3 rows) per line with three replications in Exp 5 and 6 were transplanted into the paddy field (18.5 hills m^{-2}, one plant per hill). Compost was applied as a basal dressing (~1.5 kg m^{-2}) and inorganic fertilizer was applied at 8 g N m^{-2}, 8.9 g P m^{-2}, and 4.3 g K m^{-2}. No topdressing was applied. Heading date (HD) data of the RILs were reported previously (Yamamoto et al., 2016).

The CSSLs were also grown outdoors at NIAS in 2013 (Exp 4) in 12-L pots filled with a mixture of upland and paddy field soils (3 hills per pot; one plant per hill). Basal fertilizer was applied at 1.0 g N, 0.44 g P, and 0.83 g K per pot, and 0.2 g of N per pot 14 days before heading. Heading date and panicle number were recorded. Plant height was measured from soil surface to the top of the panicle of the main culm.

2.3. Determination of leaf chlorophyll content, photosynthetic rate, and stomatal conductance (Exp 1–3)

In Exp 1 and 2, leaf chlorophyll content was measured with a chlorophyll meter (SPAD-502; Konica Minolta, Osaka, Japan) in the flag leaves (CHF) and the third leaves below the flag leaves (CHT) of the RILs. CHF and CHT were measured at heading stage (CHF0 and CHT0) and 4 weeks after heading as ripening stage (CHF4 and CHT4). The rates of chlorophyll content reduction in the flag leaves (CHRF) and third leaves below the flag leaves (CHRT) were calculated as the ratios of CHF0 to CHF4 and of CHT0 to CHT4, respectively.

In Exp 3, leaf chlorophyll content in the flag leaves of the CSSLs was measured every week after heading as in Exp 1 and 2. The photosynthetic rate (P_n) and the stomatal conductance (g_s) of a flag leaf on a main stem were measured with a portable gas-exchange system (LI-6400; LI-COR, Lincoln, NE, USA) at 390 μL L^{-1} CO_2 (ambient concentration) in the leaf chamber, a photosynthetic photon flux density of 2000 μmol m^{-2} s^{-1}, a leaf-to-air vapor pressure difference of 1.5–1.8 kPa, and a leaf temperature of 30 °C. After P_n and g_s measurements, leaves were frozen and stored at −80 °C.

2.4. Determination of the nitrogen content (Exp 1 and 2)

The nitrogen concentration in frozen leaves was determined with a CN analyzer (MT700 Mark II; Yanako, Kyoto, Japan). The leaf nitrogen content was calculated on the basis of the leaf area measured with an area meter (AAM-9; Hayashi Denko, Tokyo, Japan) at both heading stage (NC0) and ripening stage (3 weeks after heading, NC3). The rate of nitrogen content reduction (NCR) was calculated as the ratio of NC0 to NC3.

At heading and maturity, 3 plants with an average number of ears were harvested per replication, separated into leaves, ears, and culms plus leaf sheaths, and dried in an oven at 80 °C for >72 h. Samples were powdered in a mill (WB-1; Osaka Chemical Ltd, Osaka, Japan), and the nitrogen concentration was determined as above. The nitrogen content of each organ was calculated as the product of dry weight and nitrogen concentration, and the sum of nitrogen contents of leaves, ears, and culms plus leaf sheaths was regarded as the nitrogen content of the above-ground parts.