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Analysis of quantitative trait loci affecting chlorophyll content of rice leaves in a double haploid population and two backcross populations

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ABSTRACT

Chlorophyll content, one of the most important physiological parameters related to plant photosynthesis, is usually used to predict yield potential. To map the quantitative trait loci (QTLs) underlying the chlorophyll content of rice leaves, a double haploid (DH) population was developed from an *indica/japonica* (Zhenshan 97/Wuyujing 2) crossing and two backcross populations were established subsequently by backcrossing DH lines with each of their parents. The contents of chlorophyll a and chlorophyll b were determined by using a spectrophotometer to directly measure the leaf chlorophyll extracts. To determine the leaf chlorophyll retention along with maturation, all measurements were performed on the day of heading and were repeated 30 days later. A total of 60 QTLs were resolved for all the traits using these three populations. These QTLs were distributed on 10 rice chromosomes, except chromosomes 5 and 10; the closer the traits, the more clustering of the QTLs residing on common rice chromosomal regions. In general, the majority of QTLs that specify chlorophyll a content also play a role in determining chlorophyll b content. Strangely, chlorophyll content in this study was found mostly to be lacking or to have a negative correlation with yield. In both backcross F_1 populations, overdominant (or underdominant) loci were more important than complete or partially dominant loci for main-effect QTLs and epistatic QTLs, thereby supporting previous findings that overdominant effects are the primary genetic basis for depression in inbreeding and heterosis in rice.

1. Introduction

Chlorophyll is a photosynthetic pigment and an essential component of the plant photosystem. Leaf chlorophyll content affects photosynthetic ability and thus is one of the most important physiological traits affecting yield in rice (Czyczyło-Mysza et al., 2013; Teng et al., 2004; Wang et al., 2008). Chlorophyll has been studied in many plants, such as sorghum (Kassahun et al., 2010), wheat (Czyczyło-Mysza et al., 2013), maize (Cai et al., 2012), and others, and can be divided into two groups of distinct biochemical properties, namely chlorophyll a and chlorophyll b. The former is favorable for absorbing long wavelengths, whereas the latter is favorable for absorbing short wavelengths. Recently, there have been a few reports regarding genetic dissection of chlorophyll content in rice leaves (Dong et al., 2007; Jiang et al., 2012; Takai et al., 2010; Yang et al., 2008; Zuo et al., 2007). For example, Dong et al. (2007) found seven quantitative trait loci (QTLs) for leaf chlorophyll content at tillering and heading stages. Zuo et al. (2007) detected 10 QTLs responsive for chlorophyll content before heading.

Another yield-related index in chlorophyll content is about chlorophyll retention along with rice maturation. Many physiological and biochemical changes occur during leaf senescence, such as the decline in photosynthetic capability, the loss of proteins, the death of organelles and cells, and others (Buchanan-Wollaston, 1997; Nooden et al., 1997). However, the most obvious characteristic accompanying leaf senescence is the yellowing of the leaves, which is caused by the reduction of chlorophyll content. Therefore, much attention has been given to the speed of reduction of the greenness of leaves as a key indication of the leaf senescence process (Abdelkhalik et al., 2005; Jiang et al., 2004; Peng et al., 1995; Toojinda et al., 2003). A few studies have revealed that leaf stay-green traits are genetically controlled and some correlative

Abbreviations: a, additive effect; BC, backcross; BCF_1, backcross F1; d, dominance; DH, double haploid; E-QTLs, epistatic quantitative trait loci; GF, grain-filling degree; HMP, mid-parental heterosis; ICa, leaf chlorophyll a content at developmental stage I; ICb, leaf chlorophyll b content at developmental stage I; IICa, leaf chlorophyll a content at developmental stage II; IICb, leaf chlorophyll b content at developmental stage II; II/ICa, ratio of IICa to ICa; II/ICb, ratio of IICb to ICb; IISF, SPAD values of the flag leaves measured at 30 days after heading; IISS, SPAD values of the second leaves measured at 30 days after heading; II/ISF, the ratio of IISF to ISF; II/ISS, the ratio of IISS to ISS; ISF, SPAD readings of the flag leaves measured on the day of heading; ISS, SPAD readings of the second leaves measured on the day of heading; KGW, grain weight per 1000 grains; LR, likelihood ratio; LOD, log likelihood value; M-QTLs, main-effect quantitative trait loci; PL, panicle length; QTLs, quantitative trait loci; R^2, relative contribution of a genetic component; RIL, recombinant inbred line; SDEN, setting grain density per panicle; SP, spikelet per panicle; SS, seed-setting rate; SSR, simple sequence repeat; TC, testcross; TP, number of tillers per plant; WY, Wuyujing; WYF1, DH × WY; WYHMP, mid-parental heterosis in WYF1; YD, grain yield per plant; ZS, Zhenshan; ZSF1, DH × ZS; ZSHMP, mid-parental heterosis in ZSF1 population.

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QTLs have been detected (Abdelkhalik et al., 2005; Fu et al., 2009, 2011; Jiang et al., 2004; Kassahun et al., 2010; Kumar et al., 2010; Lin et al., 2010; Liu et al., 2008; Subudhi et al., 2000; Xu et al., 2000a,b; Yoo et al., 2007). In rice, for example, Toojinda et al. (2003) detected some main-effect QTLs (M-QTLs) for leaf senescence on the short arm of chromosome 9. Jiang et al. (2004) identified a total of 46 M-QTLs in 25 chromosomal regions for stay-green traits using a double haploid (DH) population from an *indica*/*japonica* crossing, and 50 epistatic QTLs (E-QTLs) were detected on 66 loci distributed on all 12 chromosomes. Abdelkhalik et al. (2005) identified 11 QTLs in two backcross (BC) populations, of which the majority of QTLs for leaf senescence were detected on the short arm of chromosome 6 and on the long arm of chromosome 9. Based on a comparison of the effects of heterozygotes and homozygotes on the phenotypic values of each QTL genotype, it was found that partial dominance rather than overdominance was the reason for differential senescence.

Repeat experiments and utilization of more than one population allowed accuracy of QTL detection. A population possessing heterozygotes was helpful for the comprehensive analysis of gene actions, including additive and nonadditive genetic effects. According to the criteria, the populations composed of permanent recombinant inbred lines (RILs) or DHs or of temporary lines, such as F_2, F_{2-3}, and the BC_1F_2 lines, were not ideal for QTL analysis. The RILs and DHs have a shortage of heterozygotes and nonadditive genetic effects cannot be analyzed. However, in the F_2, F_{2-3}, and BC_1F_2 lines, although they carry heterozygotes, it is not possible to make repeated observations at the individual level or at the block level, or to perform multiple trials. Creating permanent populations possessing heterozygotes by testcrosses (TCs) or BCs from a recombinant inbred population (Li et al., 2001; Luo et al., 2001; Mei et al., 2003, 2005) or developing an "immortalized F_2" population generated by intermating between the RILs (Hua et al., 2002, 2003) are suitable strategies for resolving these problems. In this study three mapping populations were adopted, namely, a reported DH population (Jiang et al., 2004) and two BC populations. The DH population was developed from an *indica*/*japonica* (Zhenshan [ZS] 97/Wuyujing [WY] 2) crossing, and two BC populations were derived by backcrossing DH lines to each parent. The previous report (Jiang et al., 2004) used only one DH population to identify QTLs affecting chlorophyll; however, this study aimed to achieve three distinct points. One was to dissect the whole chlorophyll into two concrete components, chlorophyll a and chlorophyll b, and to analyze their personal genetic controls. Another was to use more than one population to achieve more convincing QTL findings. The third was to create immortalized BC populations possessing heterozygotes that allowed analysis of more gene actions to determine the relative importance of overdominant, additive, completely dominant, and partially dominant effects.

2. Materials and methods

2.1. Materials and field planting

The rice mapping populations used in this study included a set of 190 DHs derived from anther culture of the F_1 from a cross between *indica* rice ZS 97 that showed early senescence and *japonica* rice WY 2 with delayed senescence (Jiang et al., 2004). Subsequently, two BC populations were developed by crossing all 190 DH lines to each parent. According to the crosses with sufficient seeds for further repeat experiments, one BC population was successfully created with 149 BC F_1 (BCF_1) hybrids (hereafter simply called ZSF1) from crosses between the DH lines (used as the female parent) and one parent ZS 97. The second BCF_1 (hereafter simply called WYF1) population consisted of 143 hybrids from crosses between the DH lines and the other parent WY 2. The parents of the DHs (ZS and WY), the F_1 hybrid (ZS × WY), were used as controls.

The phenotypic performance was evaluated at the experimental field of Huazhong Agricultural University, Wuhan, China. All materials for the three populations were sown in the seedling nursery, and 27-day-old seedlings were transplanted into two-row plots, 10 plants with 16.5 cm of space between plants within a row and 26.4 cm of space between the rows. The plots were arranged in a randomized complete block design with two replications.

2.2. Trait measurements

The contents of chlorophyll a and chlorophyll b were separately determined by directly measuring the crude chlorophyll extracts from flag leaves on heading day (stage I) and also 30 days afterward (stage II). Instead of using the chlorophyll meter SPAD-502 (Minolta Co., Osaka, Japan) to determine the total chlorophyll content, as performed by Jiang et al. (2004), we used measurements that were similar to those of Inskeep and Bloom (1985). Five random plants from the middle of a row in each plot were tested. For each observed plant, three pieces of round leaves with a semi-diameter of 3 mm were punched from a flag leaf by a plunger, and then the 15 sampled pieces of leaves from 5 plants were immediately placed into a 50-ml tube filled with 5 ml N,N-dimethylformamide; 200 μl of thorough extracted solution (over the course of 24-h extraction dark on a shaker) was measured on the spectrophotometer DU-640 using visible light with wavelengths of 664.5 nm and 647 nm, respectively. The extinction coefficient could be transformed into content of leaf chlorophyll a and chlorophyll b according to the formula used by Inskeep and Bloom (1985) as follows: chlorophyll a $= 20.7A_{647} - 4.62A_{664.5}$ and chlorophyll b $= 12.7A_{664.5} - 2.79A_{647}$. $A_{664.5}$ and A_{647} in the formula represent the maximum extinction coefficients of chlorophyll a and chlorophyll b at 664.5 nm and 647 nm, respectively. Thus, for the flag leaves at developmental stages I and II, six chlorophyll-related traits resulted. The calculated values of chlorophyll a and chlorophyll b contents were designated as ICa ($\mu g \cdot cm^{-2}$) and ICb for the flag leaves at stages I and IICa and IICb for the flag leaves at stage II, respectively. The ratios of IICa to ICa and of IICb to ICb were used as indices for relative retention of greenness, designated as II/ICa and II/ICb, respectively.

The degrees of greenness of the flag and second leaves from five plants in the middle of a row were also measured for the three populations, on the day of heading and also 30 days after, using a Minolta Chlorophyll Meter SPAD-502 (Minolta Co., Osaka, Japan). The measurement was essentially as described by Jiang et al. (2004). The SPAD readings of the flag and second leaves measured on the day of heading were designated as ISF and ISS for the flag and second leaves, and the SPAD values at 30 days after heading were used as a measurement for retention degrees of greenness, designated as IISF and IISS, respectively. The ratios of IISF to ISF and of IISS to ISS were used as indices for relative retention of greenness, designated as II/ISF and II/ISS, respectively.

Yield and its component traits examined included grain yield per plant (YD; in grams), number of tillers per plant (TP), number of grains per panicle (GP), grain weight per 1000 grains (KGW; in grams), spikelet per panicle (SP), panicle length (PL; in millimeters), grain density per panicle (SDEN), grain-filling degree (GF; %), and seed-setting rate (SS; %) from eight randomly selected plants in the middle of the rows of all the three populations. The former four traits, YD, TP, GP, and KGW, were essentially as described previously by Yu et al. (1997). The SP was scored as the total number of spikelets divided by the number of reproductive tillers of a plant. The PL was measured as the average length from the bottom neck of three main panicles to their tips for each plant. The SDEN was scored as the number of grains divided by the PL, with average grain number per centimeter for three main panicles representative of each plant. The GF was scored as the percentage of the average weight of a single fertilized grain compared to the weight of single grain with mass density >1 in each plant, essentially as previously described by Niu et al. (2004) and Zhu et al. (1995). SS was scored as the number of grains divided by the total number of spikelets from the reproductive tillers of a plant.