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REVIEW

QTL Analysis of Leaf Photosynthesis in Rice

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Abstract

Recent progress in genomics has promoted understanding of genetic mechanisms controlling complex crop production traits. In rice, yield is one of the most complex traits and generally determined by relationship between sink and source ability. While several quantitative trait locus (QTL) genes have been identified for sink-size traits, there has been little progress in genetic analysis for source ability. One of the reasons is that the source ability such as photosynthesis is a dynamic trait, hampering efforts to make precise measurements due to the technical difficulty involved in handling so many plants simultaneously. To facilitate genetic studies for source ability, we developed rapid and precise methods to evaluate leaf photosynthesis, one of the important factors for source ability in rice. QTL analysis using such methods successfully detected several promising QTLs and verified the potential utility of the evaluation methods. Our work reflects the importance of steady observation of rice plants in paddy fields and includes the application of the latest technology used in various areas of science to develop improved criteria for each trait toward advanced genetic studies.

Discipline: Crop production
Additional key words: QTLs, sink and source, yield

Introduction

The global population exceeded 7 billion in 2011, and is still rising at the rate of 80 million per year[59]. Asian people, who comprise 60% of the world population, eat rice as a staple. In the 1960s, Asia had concerns during a food crisis because most of the prime agriculture land had been cultivated and/or arable land was diminishing due to urbanization and industrialization[27]. The average rice yields in most Asian countries excluding Japan were only between 1.5 and 2.0 t ha^{-1} in those days. To cope with the stagnant yield potential, the International Rice Research Institute (IRRI) was established in 1960 by the Rockefeller and Ford Foundations at Los Baños in the Philippines. Subsequently, the development of IR8, known as the first high-yield "miracle rice", with an intensive cultivation system, made it possible to virtually double Asia's rice production in the two decades from the mid-1960s to the mid-1980s and helped avert food shortages in Asia[26]. This success is well-known as the "Green Revolution" in rice[6]. Nevertheless, the demand for rice production is still rising due to the continuous population increase. According to the simulation model, the predicted population growth to 9 billion by 2050 will require a 60-70% increase in rice production without assuming no net expansion of the cultivated area[11,58]. To meet this requirement, rice production must be promoted by increasing the rice yield per unit land area.

Rice yield is expressed as total biomass × harvest index (HI, the ratio of grain weight to total aboveground weight). In the 1960s, IR8 attained a yield potential of 10 t ha^{-1} with a semi-dwarf plant type, which conferred lodging resistance at high N inputs, abundant tillers, erect leaves, and high HI[67]. However, targeted yield gain by further increasing HI in semi-dwarf rice varieties is not promising, because the HI of high-yield varieties released since IR8 is already consid-

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ered to have virtually peaked[18,34]. Therefore, further improvement in the yield potential in rice using a different strategy is required to boost biomass production[42,65].

Canopy photosynthesis, expressed as a function of the leaf area index (LAI), canopy architecture, and leaf photosynthesis[33], has been determined to account for most of the variation in biomass production and yield[3,41]. Improvement in any of the contributors to canopy photosynthesis therefore represents a potential increase in yield and biomass production. However, recent studies have suggested that the LAI potential has already peaked for most crops[18], and recent high-yield cultivars already possess optimal canopy architecture in maize, rice, and wheat[41,45]. Accordingly, leaf photosynthesis is the factor helping boost the yield of these crop species although there are controversies in a number of crop species over whether raising the photosynthesis rate can help improve the yield[3]. To reveal the causal association of photosynthesis with final yield, the best way is to identify genetic factors controlling photosynthesis and compare yield potential between recurrent varieties and near-isogenic lines (NILs) that differ only in terms of photosynthesis rate[20,32,68].

Over the last two decades, advances in molecular genetics technology with the entire rice genome sequence have facilitated quantitative trait locus (QTL) analysis for complex traits of agronomic importance[22,56,63]. To date, more than 1,000 QTLs have been detected for 21 trait categories in rice according to the Q-TARO database[66]. On rice yield, genetic analysis has advanced for sink-size traits; some genes of QTLs have been identified and cloned, including the grain number per panicle of *GN1a*, *APO1*, *DEP1* and *WFP*[2,19,35,57] and the grain size of *GW2*, *GS3*, *qSW5* and *GS5*[7,30,46,47]. Identification of these QTLs revealed the potential to enhance sink capacity in rice plants by pyramiding the QTLs[62]. However, recent yield trials using NILs pyramiding *GN1* and *APO1* showed only that enlarging sink size did not elicit any remarkable increase in yield potential, thus suggesting that enhancing source ability, such as photosynthesis, together with large sink size, would help boost the yield potential (Fig. 1)[38]. Natural variations in photo-

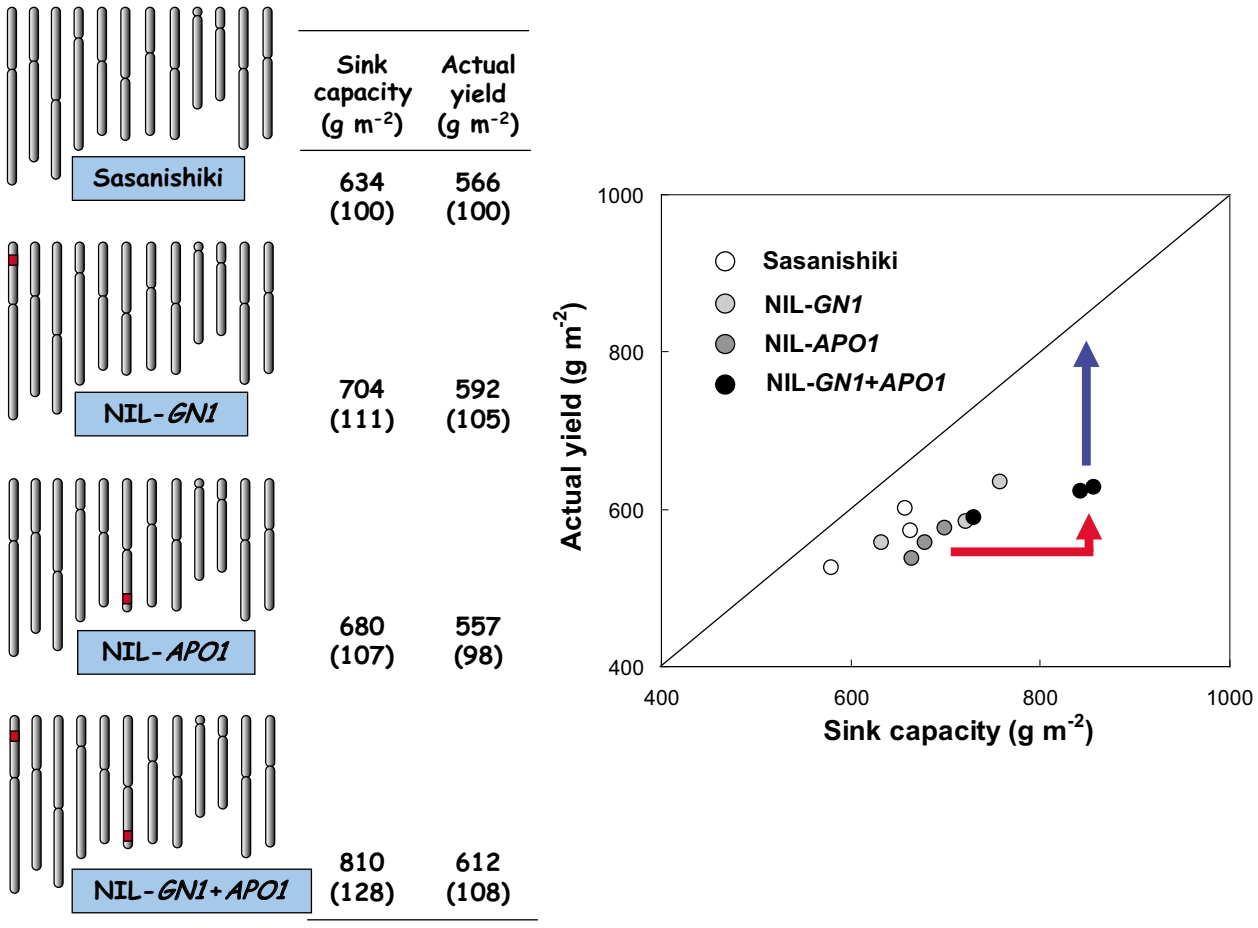

Fig. 1. Yield comparison among Sasanishiki and its sink size near-isogenic lines (NILs)
Graphical genotypes of each NIL with sink capacity and actual yield are shown on the left. Numbers in parentheses show percentages relative to Sasanishiki. Red and blue arrows indicate small actual yield gain and yield gain to achieve in combination with source ability, respectively.

synthesis rate have been reported in previous rice studies[20,24,37], meaning improvement of photosynthesis is surely recognized as an important target and expected to be possible, although the genetic analysis for photosynthesis lags behind sink size, the cause of which is discussed later.

In this review, we will start by briefly reviewing the photosynthesis process from the perspective of endogenous plant factors that cause variations in the rate of leaf photosynthesis. Second, we will introduce rapid and precise methods we have developed to evaluate endogenous factors toward genetic analysis of photosynthesis in rice. Subsequently, the progress of our genetic analysis will be described and finally, we will discuss the future perspective of physio-genetic studies on photosynthesis and its association with rice yield potential.

Photosynthesis process

The basic theory of photosynthesis can be traced back to great work by Prof. Graham Farquhar. Farquhar et al.[8] proposed a biochemical model of photosynthesis, which successfully integrated ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) kinetics and chain electron transport to the gas exchange of whole leaves. Their theoretical model effectively matched actual measurements of photosynthesis and gas exchange parameters under various environmental conditions[60], hence its widespread use and application in numerous subsequent photosynthetic studies.

The photosynthesis rate is determined by a balance between demand and supply functions for CO_2[9,29]. The demand function can be described by measuring the relationship between the photosynthesis rate (A) and intercellular CO_2 concentration (Ci), namely the A-Ci curve (Fig. 2). At low CO_2, the photosynthesis rate linearly increases with increasing CO_2 concentration. In this region, CO_2 limits the Rubisco activity but ribulose-1, 5-bisphosphate (RuBP) in the Calvin cycle exists in saturating quantities, meaning this region is called a RuBP-saturated or CO_2-limited region. Because the slope of this linear region is sharper depending on the amount of active Rubisco, the initial slope reflects carboxylation efficiency. Generally, C3 plants grown under natural ambient conditions demonstrate intercellular CO_2 concentration within this region. At high CO_2, the photosynthesis rate rises gently with increasing CO_2 concentration. In this region, CO_2 saturates the activity of Rubisco but the amount of RuBP in the Calvin cycle restricts the activity of Rubisco, which ultimately originates from limited electron transport in light reactions.

The supply function represents the diffusion of CO_2 from the atmosphere through stomata to the carboxylation sites in chloroplasts. Considering the diffusion of CO_2 into the leaf, the photosynthesis rate for the supply function can

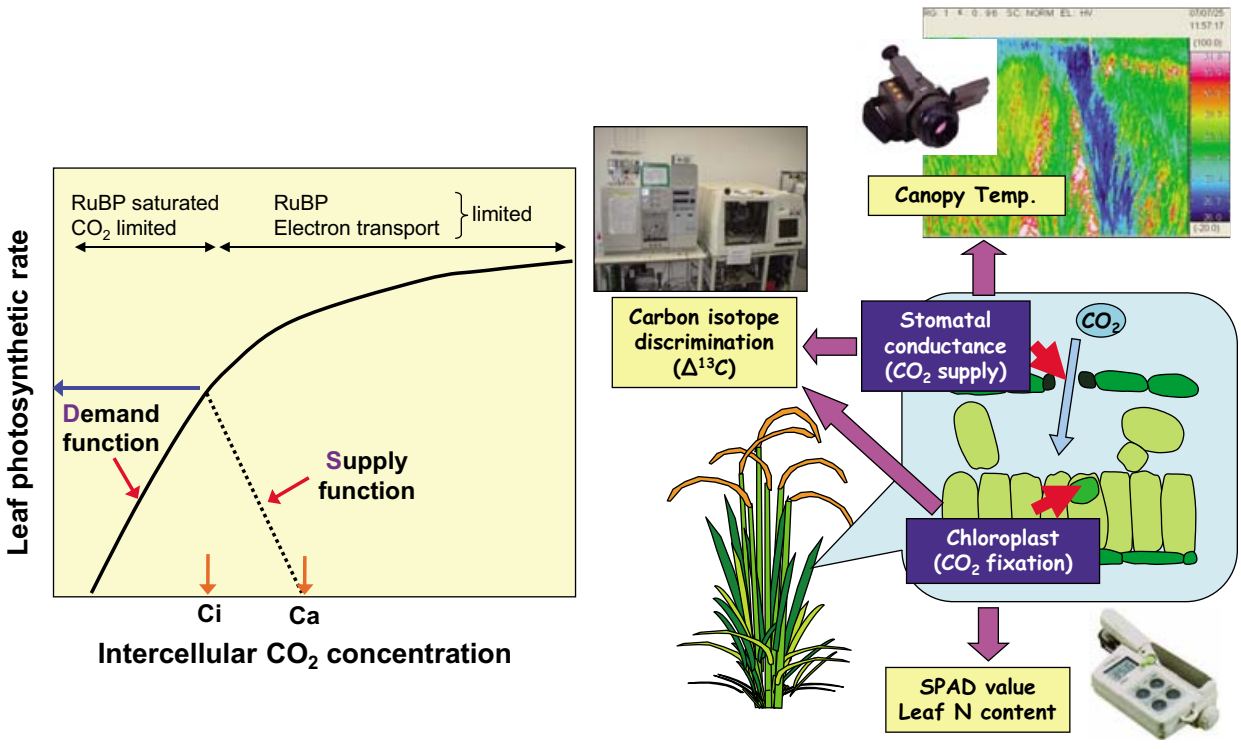

Fig. 2. Strategies to evaluate leaf photosynthesis
The left side shows the relationship between leaf photosynthesis rate and intercellular CO_2 concentration (Ci). Ca is the atmospheric CO_2 concentration. The right side shows our methods used to evaluate leaf photosynthesis. RuBP: Riburose-1, 5- bisphosphate.

be described by Fick's law as follows:

$$Photosynthesis\ rate = gs(Ca - Ci) \quad (1)$$

where *gs* is the stomatal conductance and Ca the CO_2 concentration at the leaf surface. In Fig. 2, the dotted linear line shows Equation 1 and *gs* is the slope of the line. The intersection of the supply and demand functions leads to the actual rate of photosynthesis and Ci when the photosynthesis rate is measured at Ca. The theory of photosynthesis suggests three strategies to improve photosynthesis in C3 species, namely to increase carboxylation efficiency for the demand function, *gs* for the supply function or both.

The photosynthesis rate is generally measured by a portable gas exchange system, which enables precise real-time measurements in the field[31]. Presumably therefore, direct measurements by portable monitors should be appropriate way when performing genetic analysis for photosynthesis. However, such genetic analysis requires evaluation of multiple genetic plants or lines. Because direct measurements of photosynthesis are time-consuming and laborious, it may be inappropriate for numerous plants evaluations. Besides, during real-time measurements, environmental conditions always vary, making it hard to precisely measure photosynthesis under constant conditions. These demerits may explain why genetic analysis for source ability or photosynthesis is behind that for sink size.

Development of evaluation methods

To advance genetic analysis for photosynthesis, the authors utilized the theory of photosynthesis described above, namely CO_2 demand and supply functions. As demand function, we initially tried to evaluate carboxylation efficiency via the Soil and Plant Analyzer Development (SPAD) value (Fig. 2). The SPAD value can be measured with a digital chlorophyll meter, which provides a non-destructive method for estimating leaf chlorophyll content by measuring the light absorption of specific spectral bands in living leaves[4,61]. Because chlorophyll is a component of chloroplast, higher chlorophyll content or a higher SPAD value can reflect a large number or volume of chloroplasts, namely large carboxylation sites. The methods used to measure SPAD values are simple and quick, and indeed close correlations between SPAD values and the photosynthesis rate have been observed in rice[25,28]. Accordingly, the SPAD measurement can be used as an appropriate method for genetic analysis for photosynthesis[54]. However, it should be noted that the SPAD meter was affected by temperature[55]. We noticed that the SPAD value varied between clear and cloudy days under field conditions and found that it decreased when the SPAD meter was heated by sunlight. To avoid such environmental effects, leaves were harvested and SPAD values were measured in the laboratory at constant temperature.

Infrared thermometers can be used to detect plant leaf or canopy temperatures rapidly and non-destructively (Fig. 2). Although leaf temperatures vary constantly, they are theoretically considered to reflect *gs* as a supply function of CO_2, hence we tried to establish the precise method to evaluate *gs* using the infrared thermometer as follows.

Energy balance models demonstrate how the leaf temperature varies when the heat of vaporization is extracted from leaves in the transpiration process. Because wide stomatal apertures cause higher transpiration, changes in leaf temperature reflect those in *gs* if all other conditions, including aerodynamic resistance, are constant[14,23,36]. These facts support the theory that leaf temperature measured with the infrared thermometer may be a promising criterion for *gs* as supply function of CO_2. However, handling leaf temperature directly is problematic, since it is prone to vary when exposed to changes in microclimates such as radiation, wind speed, air temperature and vapor pressure deficit (VPD). This issue can be solved if all multiple genetic lines (>100) could be measured simultaneously. Infrared thermometers, however, have limited resolution and field of vision, making such simultaneous measurements impractical. Therefore, the direct use of leaf temperature is unsuitable for genetic analysis[1].

To cope, Horie et al.[17] proposed a new remote-sensing technique to estimate canopy diffusive conductance, a reflection of *gs*, through an energy balance model, which considered all principal microclimate factors except wind speed. The new method responded positively to microclimate factors as well as canopy temperature and successfully detected varietal differences in canopy diffusive conductance. Accordingly, it can be an effective tool to evaluate *gs* under field conditions. Nevertheless, the method still has some downsides. First, the diffusive conductance can be evaluated not for line plots but canopy plots, which requires a large field area when we investigate multiple genetic lines. Second, it takes 2 to 3 min. to finish each measurement, which may still make it time-consuming to cover multiple genetic lines. Based on these, we established an alternative method of estimating rice varietal differences in *gs* via infrared thermography[53]. Our method arranged rice varieties with one row per variety and one rice variety set as a reference control every three varieties. Thermal images of four varieties, including the control, were simultaneously recorded in each image. The control variety provided a reference temperature for calculating the leaf temperature difference (CTd), which represents the temperature difference between the control and other varieties. Other microclimate data were recorded, not used to calculate CTd but used as background information. Each measurement required only 20 to 30 seconds and the calculated CTd was closely related

to *gs*[53]. Therefore, we concluded that our method had overcome the demerits of that proposed by Horie et al. and that CTd could be an effective criterion of *gs* as a supply function of CO_2 and applicable to genetic analysis.

We also focused on stable carbon isotope discrimination ($\Delta^{13}C$) for genetic analysis (Fig. 2). During photosynthesis, plants discriminate against the stable isotope of carbon (^{13}C) present in ambient CO_2. Farquhar et al.[10] defined the extent of this discrimination as $\Delta^{13}C$ and proposed that $\Delta^{13}C$ could reflect the mean ratio of intercellular to ambient CO_2 concentration (Ci/Ca);

$$\Delta^{13}C = a + (b - a)Ci/Ca \quad (2)$$

where *a* is the discrimination that occurs during diffusion of CO_2 into the intercellular airspaces (4.4 ‰), and *b* is the discrimination associated with carboxylation by Rubisco (~29 ‰). Because Ci is determined by the balance between the CO_2 demand and supply functions as described above, $\Delta^{13}C$ can also represent the results of the balance, which indicates that variation in $\Delta^{13}C$ should reflect that in carboxylation efficiency or *gs*. In rice, natural variations in $\Delta^{13}C$ have been observed in previous studies[16,24,40]. The major advantage of using $\Delta^{13}C$ is the fact that samples can be stored and used in automated measurements[5]. These characteristics of $\Delta^{13}C$ are suitable for evaluating multiple genetic lines, hence we decided to use $\Delta^{13}C$ in genetic analysis for photosynthesis.

In summary, we utilized the SPAD value as a criterion of carboxyl efficiency, CTd as that of stomatal aperture, and $\Delta^{13}C$ as that of both carboxyl efficiency and stomatal aperture during the photosynthesis process.

Genetic analysis

Based on the evaluation methods we developed, we conducted genetic analysis for leaf photosynthesis-related traits. For genetic analysis for SPAD value, we used backcross inbred lines (BILs) derived from a cross between a *japonica* variety, Sasanishiki and an *indica* high-yield variety, Habataki and detected a QTL on the long arm of the chromosome (chr.) 4[54] (Fig. 3). The QTL exhibited a significant effect (R^2 = 31.3%) and the Habataki allele increased the SPAD value. The effect of the QTL was confirmed by a chromosome segment substituted line (CSSL) and a higher photosynthesis rate was observed in the CSSL than

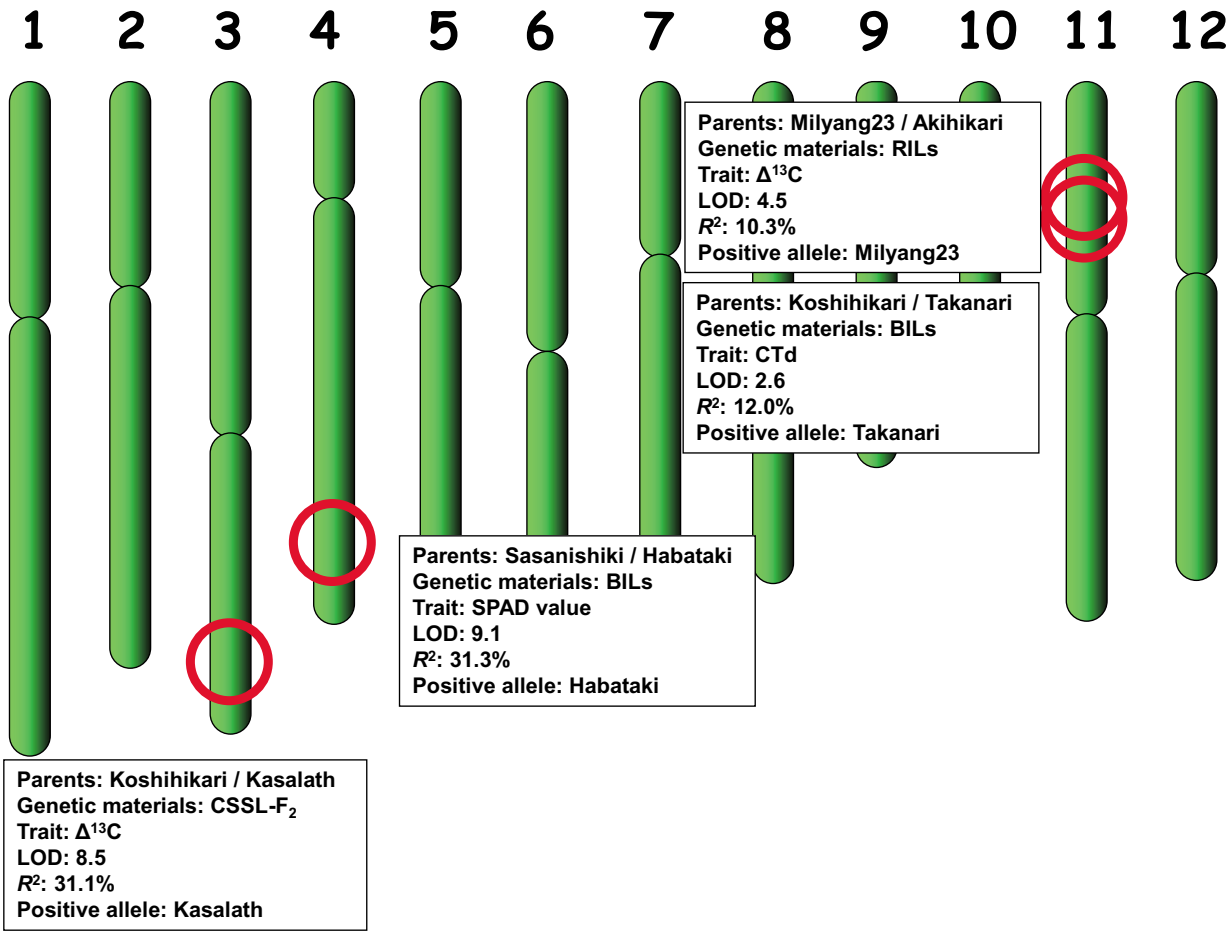

Fig. 3. Summary of promising QTLs for photosynthesis-related traits we detected using some mapping populations

Sasanishiki. These results indicated the potential utility of the SPAD value in genetic analysis for photosynthesis. To date, we have narrowed down the QTL region to 1798-kb intervals and are currently in the process of map-based cloning of the QTL.

On genetic analysis for CTd, we used BILs derived from a cross between a *japonica* variety, Koshihikari and an *indica* high-yield variety, Takanari and detected a QTL on the short arm of chr. 11[21]. The Takanari allele helped enhance CTd, namely decrease the canopy temperature compared with Koshihikari. The effect of the QTL is under confirmation using a CSSL with the corresponding segment from Takanari.

On genetic analysis for $\Delta^{13}C$, we detected two promising QTLs. We used CSSLs derived from a cross between Koshihikari and a traditional *indica* variety, Kasalath and detected a QTL increasing $\Delta^{13}C$ with the Kasalath allele on the long arm of chr. 3[52]. The QTL was confirmed by subsequent genetic analysis with F_2 progenies derived from a cross between the CSSL and Koshihikari. The association between $\Delta^{13}C$ and *gs* was also revealed by comparing the CSSL with Koshihikari. These results also represented successful genetic analysis for photosynthesis using $\Delta^{13}C$. Conversely, the study found negative effects based on a variation in heading date to $\Delta^{13}C$ and photosynthesis. Because agronomical traits are dynamic and vary as plants grow up, variation in heading date often masks indigenous variations in other traits[15,44]. To avoid effects caused by variation in heading date, we recommend analysis at the same sampling time or using genetic materials without segregation for heading date. Another QTL for $\Delta^{13}C$ was detected on the short arm of chr. 11 with recombinant inbred lines (RILs) derived from a cross between a high-yield *indica* variety, Milyang23 and a *japonica* variety, Akihikari[50]. The Milyang23 allele increased $\Delta^{13}C$. Interestingly, the QTL was co-located with that for CTd above described. The allele-decreasing canopy temperature was associated with that increasing $\Delta^{13}C$ and although the two QTLs were detected in different populations, the direction of the allele effects suggests that there may be a QTL gene controlling *gs* in this genomic region and that the QTLs may have resulted from pleiotropy of the gene. Fine mapping will reveal whether or not our hypothesis can apply.

As described above, we have successfully detected promising QTLs for each photosynthesis-related trait. One

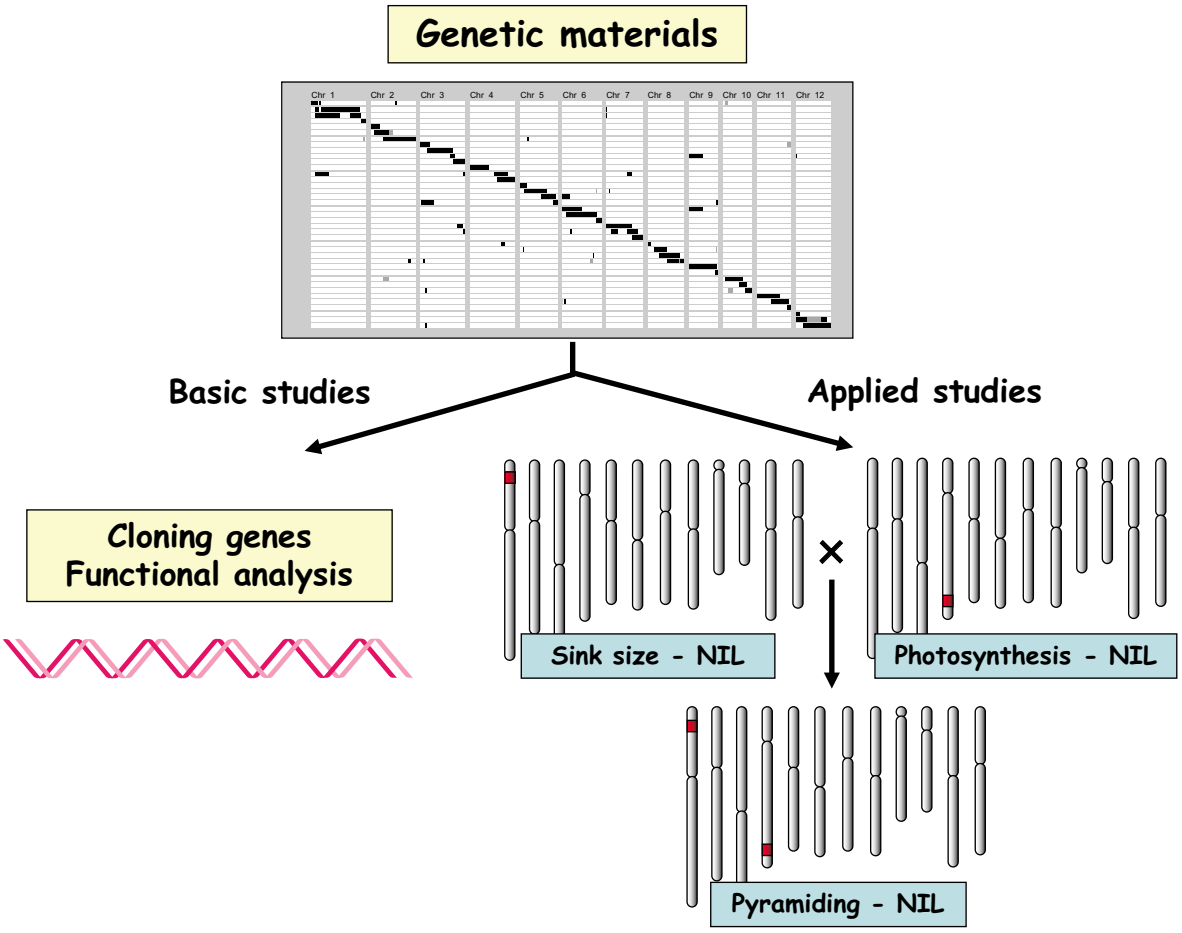

Fig. 4. Utility of genetic materials for future research

of the key points for subsequent studies is to confirm whether a combination of these QTLs would lead to enhancement of the demand and/or supply function, and result in a further increase in leaf photosynthesis. For example, we have an interest in combining the QTL for the SPAD value on the long arm of chr. 4 (demand function) with the QTL for Ctd or $\Delta^{13}C$ on the short arm of chr. 11 (supply function) in the same genetic background. These studies are important to genetically understand leaf photosynthesis, which is why we are currently conducting them.

Future perspectives

Recent advances in genomics enriched infrastructure such as genetic materials and DNA markers for genetic analysis. In particular, several CSSLs, which are useful plant materials for the detection and fine mapping of QTLs for target traits, have been successfully developed in rice[13,43,51]. The CSSLs also have the advantage of further basic studies such as map-based cloning of a QTL gene and its functional analysis (Fig. 4). Currently, we are developing reciprocal CSSLs derived from a cross between Koshihikari and Takanari. Takanari is one of the highest-yielding varieties in Japan and achieves nearly 10 t ha^{-1} of grain yield due to its large sink size and higher source ability[49]. Therefore, thorough investigation of the reciprocal Koshihikari/Takanari CSSLs, the evaluation methods introduced in this review could reveal genetic mechanisms underlying the leaf photosynthesis. However, the rice yield is a factor not only of the sink and source relationship but also depends on the translocation of assimilates between source and sink. Unfortunately, the translocation has not been eco-physiologically understood well, meaning information on the genetic mechanism is limited[48]. Further understanding of the translocation mechanism of assimilates is inevitable to improve yield potential. Besides, lodging resistance should be also considered to achieve high yield potential. Genetic analysis of lodging resistance is steadily advancing and recently a QTL associated with lodging resistance was successfully identified and cloned in rice[39]. As described in this review, the key point to promote genetic analysis is the precise evaluation of target traits for multiple genetic lines[12,64]. It is important for crop physiologists to create new precise scales for target traits. Steady and persistent observation of rice plants in paddy fields and the application of the latest technology used in various scientific fields would shed light on the development of improved criteria for each trait.

Finally, it should be noted that understanding the genetic mechanism of each yield component, such as sink size and photosynthesis, is not the ultimate goal of our studies. It will be necessary to determine the effects of modification of such component on the yield and ultimately find an appropriate combination of such component to increase the yield potential. Such applied studies are necessary to feedback information to breeding program and cultivation systems. Accordingly, we are conducting yield trials using a pyramiding line of sink size and photosynthesis QTLs (Fig. 4). To advance both basic and applied studies effectively, combining the phenotyping method with genetic materials would be important.

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References

1. Araus, J. L. et al. (2002) Plant breeding and drought in C_3 cereals: what should we breed for? *Ann. Bot. London*, **89**, 925–940.
2. Ashikari, M. et al. (2005) Cytokinin oxidase regulates rice grain production. *Science,* **309**, 741–745.
3. Evans, L. T. (1993) *Crop evolution, adaptation and yield.* Cambridge University Press, Cambridge, UK, pp. 516.
4. Chubachi, T. et al. (1986) The diagnosis of nitrogen nutrition of rice plants (Sasanishiki) using chlorophyll meter. *Nihon Dojo Hiryo Gaku Zasshi* (*Jpn. J. Soil Sci. Plant Nutr.*), **57**, 90–93 [In Japanese].
5. Condon, A. G. et al. (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Sci.*, **27**, 996–1001.
6. Evenson, R. E. & Gollin, D. (2003) Assessing the impact of the green revolution, 1960 to 2000. *Science*, **300**, 758–762.
7. Fan, C. et al. (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.,* **112**, 1164–1171.
8. Farquhar, G. D. et al. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, **149**, 78–90.
9. Farquhar, G. D. & Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.,* **33**, 317–345.
10. Farquhar, G. D. et al. (1982) On the relationship between carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Aust. J. Plant Physiol.*, **9**, 121–137.
11. Foley, J. A. et al. (2011) Solutions for a cultivated planet. *Nature*, **478**, 337–342.
12. Fukuoka, S. et al. (2010) Integration of genomics into breeding in rice. *Rice*, **3**, 131–137.
13. Fukuoka, S. et al. (2010) Germplasm enhancement by developing advanced plant materials from diverse rice accessions. *Breed. Sci.*, **60**, 509–517.
14. Hatfield, J. L. et al. (1987) Use of canopy temperatures to identify water conservation in cotton germplasm. *Crop Sci.*, **27**, 269–273.

15. Hori, K. et al. (2012) Variation in heading date conceals quantitative trait loci for other traits of importance in breeding selection of rice. *Breed. Sci*., (in press).
16. Horie, T. et al. (2003) Physiological traits associated with high yield potential in rice. *In* Rice science: innovations and impact for livelihood, eds. Mew, T.W. et al., IRRI, Los Baños, Philippines, 117–145.
17. Horie, T. et al. (2006) Genotypic difference in canopy diffusive conductance measured by a new remote-sensing method and its association with the difference in rice yield potential. *Plant Cell Environ.*, **29**, 653–660.
18. Horton, P. (2000) Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. *J. Exp. Bot.*, **51**, 475–485.
19. Huang, X. et al. (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat. Genet.,* **41**, 494–497.
20. Hubbart, S. et al. (2007) Trends in leaf photosynthesis in historical rice varieties developed in the Philippines since 1966. *J. Exp. Bot*., **58**, 3429–3438.
21. Ikka, T. et al. (2010) QTL analysis for differences in leaf temperature in rice using infrared thermometer. *Breed. Res.*, **12 (Extra 1)**, 43 [In Japanese].
22. International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. *Nature,* **436**, 793–800.
23. Jones, H. G. (1999) Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. *Plant Cell Environ.*, **22**, 1043–1055.
24. Kanemura, T. et al. (2007) Evaluation of genotypic variation in leaf photosynthetic rate and its associated factors using rice diversity research set of germplasm. *Photosynth. Res.*, **94**, 23–30.
25. Kato, M. et al. (2004) Photosynthesis and dry matter production during ripening stage in a female-sterile line of rice. *Plant Prod. Sci.*, **7**, 184–188.
26. Khush, G. S. (2001) Rice improvement at IRRI: An example of international collaboration. *Breeding Res*., **3**, 281–286.
27. Khush, G. S. (2001) Green revolution: the way forward. *Nature Rev. Genet.,* **2**, 815–822.
28. Kumagai, E. et al. (2009) Correlation of chlorophyll meter readings with gas exchange and chlorophyll fluorescence in flag leaves of rice (*Oryza sativa* L.) plants. *Plant Prod. Sci.*, **12**, 50–53.
29. Lambers, H. et al. (1998) *Plant physiological ecology.* Springer-Verlag, New York, USA, pp. 540.
30. Li, Y. et al. (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nat. Genet.,* **43**, 1266–1269.
31. Long, S. P. & Bernacchi, C. J. (2003) Gas exchange measurements, what can they tell us about underling limitations to photosynthesis? Procedures and source error. *J. Exp. Bot.,* **54**, 2393–2401.
32. Long, S. P. et al. (2006) Can improvement in photosynthesis increase crop yields? *Plant Cell Environ*., **29**, 315–30.
33. Loomis, R. S. & Connor, D. J. (1992) *Crop Ecology: Productivity and Management in Agricultural Systems.* Cambridge University Press, Cambridge, UK, pp. 556.
34. Mann, C. C. (1999) Crop scientists seek a new revolution. *Science,* **283**, 310–314.
35. Miura, K. et al. (2010) *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nat. Genet.,* **42**, 545–549.
36. Monteith, J. L. (1973) *Principles of Environmental Physics.* Edward Arnold, London, UK, pp. 241.
37. Ohsumi, A. et al. (2007) A model explaining genotypic and ontogenetic variation of leaf photosynthetic rate in rice (*Oryza sativa*) based on leaf nitrogen content and stomatal conductance. *Ann. Bot. London*, **99**, 265–273.
38. Ohsumi, A. et al. (2011) Evaluation of yield performance in rice near-isogenic lines with increased spikelet number. *Field Crop Res.,* **120**, 68–75.
39. Ookawa, T. et al. (2010) New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. *Nature Commun.*, **1**, 132.
40. Peng, S. et al. (1998) Transpiration efficiencies of *indica* and improved tropical *japonica* rice grown under irrigated conditions. *Euphytica*, **103**, 103–108.
41. Peng, S. (2000) Single-leaf and canopy photosynthesis of rice. *In* Redesigning rice photosynthesis to increase yield, eds. International Rice Research Institute, Philippines, 213–228.
42. Peng, S. et al. (2000) Grain yield of rice cultivars and lines developed in the Philippines since 1966. *Crop Sci*., **40**, 307–314.
43. Rice Genome Resource Center (2012) URL: http://www.rgrc.dna.affrc.go.jp/stock.html.
44. Rebetzke, G. J. et al. (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. *Theor. Appl. Genet.*, **118**, 123–137.
45. Richards, R.A. (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *J. Exp. Bot.,* **51**, 447–458.
46. Shomura, A. et al. (2008) Deletion in a gene associated with grain size increased yields during rice domestication. *Nat. Genet.,* **40**, 1023–1028.
47. Song, X. J. et al. (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat. Genet.,* **39**, 623–630.
48. Takai, T. et al. (2005) Time-related mapping of quantitative trait loci controlling grain-filliing in rice (*Oryza sativa* L.). *J. Exp. Bot.*, **56**, 2107–2118.
49. Takai, T. et al. (2006) Rice yield is closely related to crop growth rate during late reproductive period. *Field Crop Res.*, **96**, 328–335.
50. Takai, T. et al. (2006) Mapping of QTLs controlling carbon isotope discrimination in the photosynthetic system using recombinant inbred lines derived from a cross between two different rice (*Oryza sativa* L.) cultivars. *Plant Prod. Sci.*, **9**, 271–280.
51. Takai, T. et al. (2007) Development of chromosome segment substitution lines derived from backcross between indica donor rice cultivar 'Nona Bokra' and japonica recipient cultivar 'Koshihikari'. *Breed. Sci.*, **57**, 257–261.
52. Takai, T. et al. (2009) Detection of a quantitative trait locus controlling carbon isotope discrimination and its contribution to stomatal conductance in *japonica* rice. *Theor. Appl. Genet.*, **118**, 1401–1410.
53. Takai, T. et al. (2010) Canopy temperature on clear and cloudy days can be used to estimate varietal differences in stomatal conductance in rice. *Field Crop. Res.*, **115**, 165–170.
54. Takai, T. et al. (2010) A quantitative trait locus for chloro-

phyll content and its association with leaf photosynthesis in rice. *Rice*, **3**, 172–180.
55. Takai, T. (2012) Originality and ingenuity for genetic analysis on source ability in rice. *J. Crop Res*., **57**, 67–71.
56. Tanksley, S. D. (1993) Mapping polygenes. *Anuu. Rev. Genet.,* **27**, 205–233.
57. Terao, T. et al. (2010) A gene controlling the number of primary rachis branches also controls the vascular bundle formation and hence is responsible to increase the harvest index and grain yield in rice. *Theor. Appl. Genet.,* **120**, 875–893.
58. Tester, M. & Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science*, **327**, 818–822
59. UNFPA. (2011) *State of world population.* New York, USA, pp.124.
60. von Caemmerer, S. & Farquhar, G. D. (1981) Some relationship between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376–387.
61. Watanabe, S. Y. et al. (1980) Development of a digital chlorophyll meter: I. Structure and performance. *Nihon Sakumotsu Gakkai Kiji (Jpn. J. Crop Sci.*), **49**(Extra), 89 [In Japanese].
62. Yamamoto, T. et al. (2009) Towards the understanding of complex traits in rice: substantially or superficially? *DNA Res.*, **16**, 141–154.
63. Yano, M. & Sasaki, T. (1997) Genetic and molecular dissection of quantitative traits in rice. *Plant Mol. Biol.,* **35**, 145–153.
64. Yano, M. (2007) Genetic and molecular dissection of quantitative traits and its application in rice breeding. *Breed. Res.*, **9**, 135–140 [In Japanese].
65. Ying, J. et al. (1998) Comparison of high-yield rice in tropical and subtropical environments. I. Determinants of grain and dry matter yields. *Field Crop. Res.,* **57**, 71–84.
66. Yonemaru J.-i. et al. (2010) Q-TARO: QTL Annotation Rice Online Database. *Rice*, **3**, 194–203.
67. Yoshida, S. (1972) Physiological aspects of grain yield. *Annu. Rev. Plant Physiol.* **23**, 437–464.
68. Zelitch, I. (1982) The close relationship between net photosynthesis and crop yield. *Bioscience*, **32**, 796–802.