

Research Paper

A novel QTL associated with rice canopy temperature difference affects stomatal conductance and leaf photosynthesis

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Canopy temperature can be a good indicator of stomatal conductance. To understand the genetic basis of phenotypic differences in stomatal conductance between average and high-yielding rice (*Oryza sativa* L.) cultivars, we conducted a quantitative trait locus (QTL) analysis of canopy temperature. We developed reciprocal series of backcross inbred lines (BC₁F₆) derived from a cross between the average-yielding *japonica* cultivar ‘Koshihikari’ and the high-yielding *indica* cultivar ‘Takanari’. A stable QTL, *qCTd11* (QTL for canopy temperature difference on chromosome 11) on the short arm of chromosome 11, accounted for 10.4 and 19.8% of the total phenotypic variance in the two lines; the ‘Takanari’ allele decreased the canopy temperature difference value. A chromosome segment substitution line carrying the Takanari *qCTd11* showed a greater reduction in canopy temperature than ‘Koshihikari’, and had higher stomatal conductance and photosynthetic rate. These results suggest that *qCTd11* is not only involved in canopy temperature, but is also involved in both stomatal conductance and photosynthetic rate.

Key Words: canopy temperature difference, chromosome segment substitution lines, photosynthetic rate, quantitative trait loci, rice (*Oryza sativa*), stomatal conductance.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods in the world, and is eaten daily by over half of the world’s population (IRRI 2016; <http://irri.org/>). Increasing crop productivity is a global challenge, and improvements in grain yield are required on the existing available land area to meet the demand of future populations (Hubbart *et al.* 2007, Takai *et al.* 2013, Taylaran *et al.* 2011). As a result, many studies have been undertaken to identify potential approaches to increase rice yield per unit land area. Rice yield is determined by many factors, including sink size, source strength, and carbohydrate translocation. Many genetic studies on the identification and characterization of sink-related genes have been conducted, because sink-related characteristics, such as grain size and number, are obvious

traits for yield improvement (Sakamoto and Matsuoka 2008). In contrast, fewer genetic studies have been conducted on source strength and carbohydrate translocation.

Leaf photosynthesis is one of the main aspects of source strength. Both the carbon dioxide (CO₂) supply to the chloroplasts and the demand for CO₂ in the chloroplasts generally determine the photosynthetic rate, and the CO₂ supply is governed by the diffusion of CO₂ from the atmosphere through stomata. One of the factors involved in the CO₂ supply is stomatal conductance (g_s) (Farquhar and Sharkey 1982, Takai *et al.* 2013). In addition, g_s has increased in the last 50 to 80 years, and is crucial for obtaining high yields (Roche 2015). It is essential for leaf photosynthesis and high yield potential in modern crops, and can be used as an index of photosynthetic capacity and yield. In general, g_s is measured as the rate of gas exchange; however, this method is time-consuming and laborious, and is inappropriate for use in genetic analysis and breeding programs (Takai *et al.* 2010). Leaf temperature depends on stomatal opening, with the temperature decreasing as stomata open and as the evaporation rate increases; a major determinant of leaf temperature is

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the rate of evaporation or transpiration from the leaf (Furbank *et al.* 2009). Therefore, leaf temperature is a useful indicator of g_s and transpiration (Jones 2004). In addition to leaf temperature, canopy temperature depression (CTD), the difference between the air and canopy temperature, has been used as an indicator of g_s and an index of crop water status (Fischer *et al.* 1998, Hatfield *et al.* 1987).

Infrared thermometers based on infrared thermometry and thermography can be used to rapidly and non-destructively measure temperatures on the leaf or canopy (Jones 2004, Takai *et al.* 2010). O'Toole *et al.* (1984) reported that the Crop Water Stress Index, which is based on infrared thermometry of canopy temperatures, is neither destructive nor disruptive, and is an efficient approach to measuring water stress. Inoue (1990) showed a reduction in photosynthesis, transpiration, and g_s in the canopies of corn (*Zea mays*) and wheat (*Triticum* spp.) plants stressed by a root-reducing treatment, and infrared imagery revealed an inverse change in canopy surface temperature. Previous studies have made use of infrared thermometry to identify a quantitative trait locus (QTL) for leaf temperature in rice under drought stress (Babu *et al.* 2003, Yue *et al.* 2005). These findings suggest that infrared thermometers are useful for estimating canopy temperatures.

A wide range of plant and environmental factors affect leaf or canopy temperature; as the environment is constantly changing, it is necessary to consider the dynamic nature of leaf temperatures in a study on leaf temperature (Jones 2004). Jones (2004) suggested that resolution and sensitivity to environmental conditions, thermal dynamics, spectral properties, and the sun and view angle should be considered when optimizing measurements taken in the field. It may be possible to reduce the influence of environmental factors by recording leaf temperatures in conjunction with appropriate reference temperatures, such as control plants or wet-and-dry reference surfaces (Jones 1999, Leinonen *et al.* 2006). Takai *et al.* (2010) proposed that the canopy temperature difference (CTd), which is the difference in canopy temperature to that of a control plant, should be used for precise estimations of leaf or canopy temperature. Furthermore, as there is a strong correlation between CTd and g_s , then CTd could be used to estimate varietal differences in g_s , even under low irradiance (Takai *et al.* 2010).

In addition to being a useful index to estimate g_s , canopy temperature is also important for another reason. An increase in temperature reduces the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which consequently decreases the photosynthetic rates of C_3 plants (Long 1991, Vu *et al.* 1997). High temperatures can also lead to a high vapor pressure deficit (VPD), resulting in increased evaporative demand and greater transpiration rates (Way *et al.* 2013). Loss of water from epidermal and guard cells under a high transpiration rate directly causes the closure of stomata under low humidity, and leads to a decrease in photosynthesis (Dai *et al.* 1992, Mott *et al.* 1991). Prashar *et al.* (2013) reported a clear negative associ-

ation between canopy temperature and final tuber yield in a potato (*Solanum tuberosum*) mapping population grown with an ample water supply. These results suggest that a rise in canopy temperature reduces photosynthetic capacity, and that a low canopy temperature might be necessary for a high photosynthetic capacity and yield.

'Takanari' is an *indica* rice variety that is one of the most productive rice varieties in Japan, and consistently attains higher grain yields and dry matter production than commercial *japonica* varieties in Japan (Taylaran *et al.* 2009). It is a semi-dwarf variety, and has a large sink size because of a high spikelet number per panicle, strong source characteristics, and a high carbohydrate translocation capacity (Takai *et al.* 2014, Taylaran *et al.* 2011). 'Takanari' leaves contain high levels of nitrogen (N) and RuBisCO, and have greater g_s values than leaves of some *japonica* varieties (Taylaran *et al.* 2011). Therefore, it has a higher rate of leaf photosynthesis than common varieties in Japan, even at the same rate of N application. These characteristics make 'Takanari' a good model for investigating the genetic mechanisms underlying high yield potential in modern productive rice varieties to increase rice production.

In a previous study, we developed reciprocal chromosome segment substitution lines (CSSLs) derived from a cross between 'Takanari' and 'Koshihikari', a leading *japonica* cultivar, and demonstrated that CSSLs are useful for investigating the genetic mechanisms underlying yield potential in 'Takanari' (Takai *et al.* 2014). We detected many QTLs for yield using the CSSLs, and several QTLs exhibited a trade-off (Takai *et al.* 2014). For example, an allele that increased sink-size traits, such as spikelet number per panicle or per square meter, was associated with decreased ripening percentage and 1000-grain weight, suggesting that increased sink size does not increase yield in both 'Koshihikari' and 'Takanari' backgrounds (Takai *et al.* 2014). 'Takanari' exhibited a high rate of ripening despite a large sink size caused by a high photosynthetic rate and carbohydrate translocation capacity (Takai *et al.* 2014). Although major QTLs for sink-size traits (such as spikelet number per panicle) have been identified and cloned, including *GN1a* (Ashikari *et al.* 2005) and *APO1* (Ookawa *et al.* 2010), effective QTLs for high source strength and translocation have not been identified. Therefore, there is a need to find QTLs in 'Takanari' in order to increase yield in the 'Koshihikari' background.

In this study, we detected QTLs for leaf or canopy temperature using rice varieties that exhibit high and low canopy temperatures in the field ('Koshihikari' and 'Takanari', respectively). We previously used infrared thermography as a simple method of evaluating varietal differences in canopy temperature that could be used to measure CTd and to investigate the relationship between CTd and g_s and leaf photosynthetic rate (P_n) (Takai *et al.* 2010). We further validated this approach using the two varieties with different g_s and P_n , and the CSSL derived from a cross between them.

Materials and Methods

Plant materials and growth conditions

F₁ plants from a cross between ‘Koshihikari’ and ‘Takanari’ were backcrossed to ‘Koshihikari’ (for ‘Koshihikari’/‘Takanari’/‘Koshihikari’, BKT) or ‘Takanari’ (for ‘Koshihikari’/‘Takanari’/‘Takanari’, BTK) to produce BC₁F₁ plants. The two backcrosses produced 95 BKT and 99 BTK BC₁F₁ plants (Takai *et al.* 2013, 2014), which were then self-pollinated four times by the single-seed descent method. Eighty-seven progeny lines from each BKT and BTK (BC₁F₆) cross were designated as backcross inbred lines (BILs). In 2009, 15 plants per line from BKT and BTK were grown in a paddy field at the National Institute of Crop Science, Miraidaira, Ibaraki, Japan (36°0′N, 140°0′E). Germinated seeds were sown in mid-May, and the seedlings were transplanted to the field in early June at a spacing of 15 cm between rows and 30 cm between columns. ‘Koshihikari’ and ‘Takanari’ seedlings were planted as reference controls every three lines. Nitrogen (N, as a slow-release fertilizer), phosphorus (P, as P₂O₅), and potassium (K, as K₂O) were applied as basal fertilizers at 60, 120, and 90 kg ha⁻¹, respectively.

A CSSL (SL1) carrying the QTL for CTd from ‘Takanari’ was selected by marker assisted selection from progeny of a BC₄F₁ plant in which the target QTL was heterozygous on the ‘Koshihikari’ genetic background. SL1, ‘Koshihikari’, and ‘Takanari’ were used in the physiological experiments. The plants were grown in a paddy field and in pots standing in a water pool at the Institute of Crop Science Tsukuba, Japan (36°1′N, 140°6′E) in 2014–2016. Seeds for field and pots were sown in mid-April and mid-May, respectively, and seedlings were transplanted in mid-May and mid-June, respectively. Month-old seedlings of each variety were transplanted into three-rowed plots in the field or 1/5000 a Wagner pots filled with alluvial soil (2.5 kg per pot). For the field culture, each row contained 12 hills, with 15 cm between hills and 30 cm between rows. ‘Koshihikari’ seedlings were planted as a reference control every two varieties. The amounts of fertilizer applied as basal dressing were ~1.5 kg m⁻² compost, 8 g N m⁻², 8.9 g P m⁻², and 4.3 g K m⁻². Additional N was applied as a top-dressing (30 kg ha⁻¹) three weeks after transplanting. For the pot culture, the ten pots per each variety were randomly arranged in rows, and the arrangement was changed at two weeks and one month after transplanting. Plants in pots were grown outdoors at a density of five pots per row, with a 20-cm spacing between hills and one plant per pot, under water-logged conditions. Fertilizer was applied at rates of 0.5, 0.5, and 0.5 g per pot of N, P, and K, respectively, as a basal dressing, and 0.3 g of N per pot was applied as a top-dressing at the booting stage. Finally, the pots were arranged into two-rowed plots per each variety.

DNA extraction and genotyping

A fresh leaf blade from each seedling at 30 days after

transplanting (DAT) was collected for DNA extraction by the cetyl trimethylammonium bromide method (Murray and Thompson 1980). A total of 140 DNA markers, containing 138 simple sequence repeat (SSR) markers and two in/del markers distributed across the whole genome, were selected from the 141 PCR-based markers described by Takai *et al.* (2014), except for *APOI*. The PCR amplification conditions are described in Matsubara *et al.* (2008) for the *GN1a* and SSR markers; the PCR conditions using the *sd1* marker were slightly different: PCR amplification was performed for 35 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C, followed by 5 min at 72°C.

Measurements of canopy temperatures and plant characteristics

Canopy temperature was measured using the same methods as described previously (Takai *et al.* 2010). For the BILs, field measurements were taken from 12:00 to 15:00 on a clear day during the reproductive stage at 45 DAT. Thermal images of five varieties, including two ‘Koshihikari’ (in the BKT line) and ‘Takanari’ (in the BTK line) as reference varieties, were simultaneously recorded at least three times. Canopy temperatures of the varieties were calculated based on spot temperatures of the selected image-area using image analysis software (NS9200, NEC Avio, Tokyo, Japan).

For the CSSL, field measurements were taken from 09:00 to 14:00 on a clear day from the maximum tillering stage to the stage just prior to heading. The thermometers were set approximately 4.5 m or 2 m above the leaf surface, with the sun at the measurer’s front or back, respectively. The angle of depression was maintained at 25°. Thermal images of the varieties, including ‘Koshihikari’ as a reference variety, were simultaneously recorded at least four times. Canopy temperatures of the CSSLs were calculated using the original program developed for accurate estimation of canopy temperature from thermal images. Typical areas of the canopy in each plot were selected by drawing squares. Canopy temperature in each plot was estimated based on the mean temperature of enclosed leaf pixels that were extracted from background pixels in a visible image, using the program (**Fig. 1, Supplemental Fig. 1**). The program is available as free software (<http://phenotyping.image.coocan.jp/thermo/>).

The field air temperature and relative humidity were recorded every 1 min, using a dual-sensor instrument (2119A, Eto Denki, Tokyo, Japan) connected to a data logger (Thermic 2300A, Eto Denki) and a dual-sensor and data logger instrument (THM10-TH, Wako, Osaka, Japan) in a forced-ventilated radiation shield (Fukuoka *et al.* 2011) in 2014–2016. We calculated VPD from air temperature and relative humidity (Murray 1967). The amount of solar radiation was also recorded every 1 min, using a pyranometer (PCM-01(L), Prede, Tokyo, Japan) connected to a data logger (Thermic 2300A, Eto Denki). We calculated photosynthetically active radiation (PAR) from solar radiation (Stanhill and Fuchs 1977) and PPFD from the PAR (Thimijan and

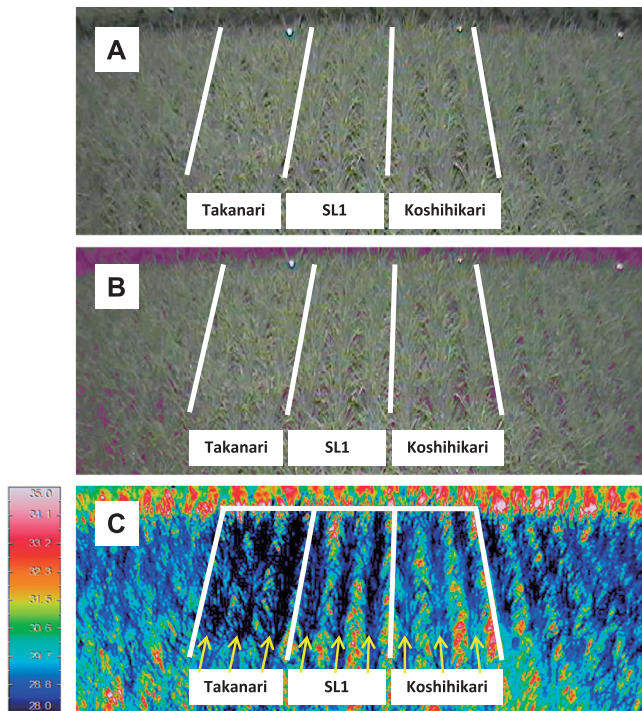


Fig. 1. Visible (A and B) and thermal (C) images of three rice varieties cultivated in a paddy field. The images were taken at 11:13 on 8 July 2014 (48 days after transplanting). The reference variety was ‘Koshihikari’. The purple areas in (B) were eliminated as background. The central column of each variety in (C) was analyzed to determine the canopy temperature.

Heins 1983). On the wind speed, it varies extremely around each plant, but it is difficult to monitor it correctly in the experimental blocks. Therefore, we estimated leaf temperature when the wind speed was weak and stable.

When analyzing the BILs, days to heading (DTH) was defined as the number of days until the appearance of the first panicle; after the mature stage, culm length, panicle length, and panicle number were also recorded. These agronomic traits were scored in 10 plants per line, and mean values were calculated for each line. When analyzing the CSSL, DTH was defined as the number of days until over 50% of the plants had panicles. DTH of ‘Koshihikari’, SL1, and ‘Takanari’ was 74, 71, and 75, respectively, grown in a paddy field in 2014, 59, 58, and 74, respectively, grown in pots in 2015, and 74, 74, and 75, respectively, grown in a paddy field in 2016. At the mature plant stage, the maximum widths and lengths of the topmost expanded leaves, and plant height, were also recorded.

Evaluation of CTds for QTL analyses

The CTds between the BILs or CSSL and ‘Koshihikari’ or ‘Takanari’ (as controls) were calculated from canopy temperature values of individual varieties using the following formula:

$$CTd = CT_{line} - CT_{ref}$$

where CT_{line} is the canopy temperature value of each BIL or CSSL, and CT_{ref} is the canopy temperature value of the reference variety, ‘Koshihikari’ for BKT and CSSL or ‘Takanari’ for BTK. The data were pooled and used for calculating the mean canopy temperature value.

QTL mapping for CTds

Genetic linkage maps of the BKT and BTK populations were constructed using MAPMAKER/EXP version 3.0b (Lander *et al.* 2009). One hundred and forty genetic markers (138 SSR and two in/del markers) were used for both BILs. QTL analyses were performed by composite interval mapping using the Zmapqtl program (model 6) in QTL Cartographer v. 2.5 software (Wang *et al.* 2005). Genome-wide threshold values ($\alpha = 0.05$) were used to detect putative QTLs based on the results of 1000 permutations.

Evaluation of P_n , g_s , and transpiration rate

P_n , g_s , and transpiration rate were measured in the morning on clear days, using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA) on the topmost expanded leaves that were attached to the main culms of plants grown in the paddy field. PPFD at the leaf surface was maintained at $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, the reference CO_2 concentration at $390 \mu\text{mol mol}^{-1}$, the leaf chamber temperature at 30°C , and the relative humidity at $75 \pm 5\%$. The chlorophyll content of the leaves used for estimating P_n and g_s was measured as soil-plant analysis development (SPAD) values using a chlorophyll meter (SPAD-502, Konica Minolta Japan, Inc., Tokyo, Japan).

Statistical analyses

All of the statistical analyses were performed using JMP Statistical Discovery software (SAS Institute Inc., Cary, NC, USA).

Results

Phenotypic variations in the BILs and their parental cultivars

In previous studies, leaf temperature and CTD have been used as indirect methods of estimating g_s (Fischer *et al.* 1998, Hatfield *et al.* 1987). However, leaf temperature and CTD are directly affected by changes in climate, including wind speed, air temperature, VPD, and radiation, while CTD is used for more precise estimations of leaf or canopy temperature (Takai *et al.* 2010). Therefore, we estimated changes in CTD and CTd in response to these factors using infrared thermal imaging. Although the CTds of ‘Koshihikari’ and ‘Takanari’, the parental cultivars of the BILs, varied with air temperature, VPD, and PPFD, we consistently observed low and stable CTds between the two cultivars under different climatic conditions (Fig. 2). In addition, stable CTds and variations in CTds were observed in different experimental blocks of the rice field (Fig. 2). Therefore, we focused on CTds between ‘Koshihikari’ as a reference and

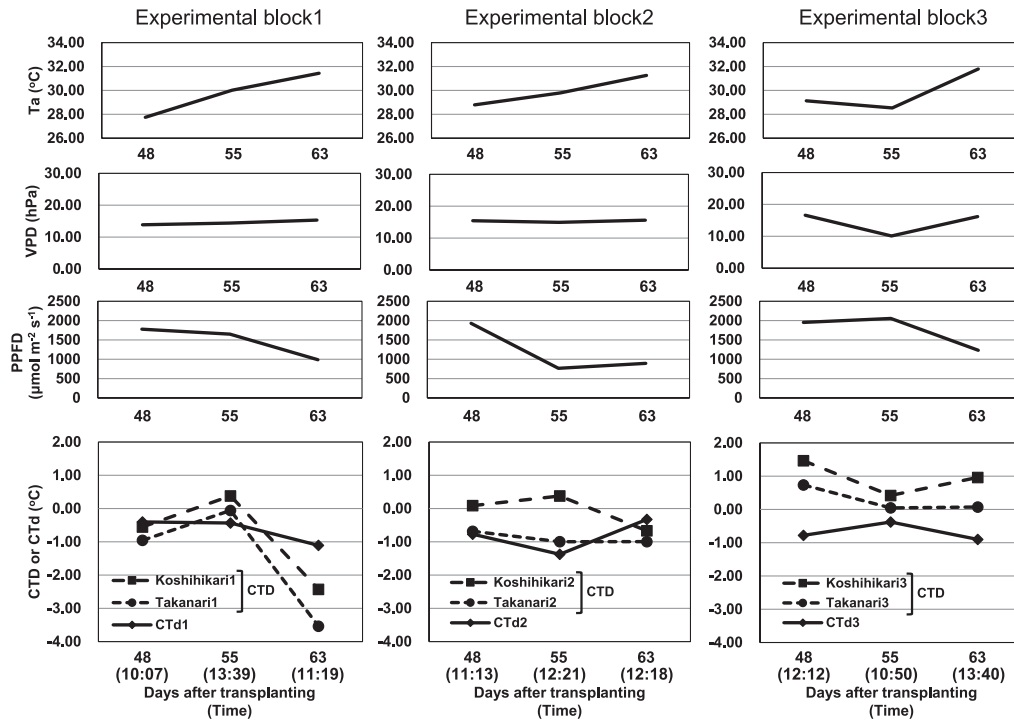


Fig. 2. Changes in air temperature (T_a), vapor pressure deficit (VPD), photosynthetic photon flux density (PPFD), canopy temperature depression (CTD) in ‘Koshihikari’ and ‘Takanari’, and canopy temperature difference (CTd) between ‘Koshihikari’ and ‘Takanari’ in three experimental blocks of a rice field in 2014.

the other lines.

‘Takanari’ had a lower leaf temperature than ‘Koshihikari’, and the mean CTd between ‘Koshihikari’ and ‘Takanari’ was minus 0.6°C . The CTds in both BILs (BKT and BTK) varied from -0.8 to 0.6°C and from -0.4 to 1.2°C , respectively (Fig. 3), indicating that this trait is controlled by several QTLs. The CTds in the BILs were mostly between ‘Koshihikari’ and ‘Takanari’, and revealed a transgressive segregation. CTd was weakly correlated with DTH ($r = 0.2219$ in BKT and $r = 0.3065$ in BTK) and panicle number ($r = 0.2645$ in BKT and $r = 0.2438$ in BTK); there was no significant correlation with culm length ($r = 0.1965$ in BKT and $r = 0.0854$ in BTK) or panicle length ($r = 0.0244$ in BKT and $r = 0.0681$ in BTK) (Fig. 4). These results indicated that decreases in leaf temperature were unlikely to be caused by pleiotropic effects related to morphological changes.

QTL mapping for CTd in the BILs

The genotypes of the two reciprocal BILs are presented in Supplemental Fig. 3. In BKT, five putative QTLs were detected on chromosomes 3, 9, 10, 11 and 12 (Table 1). Phenotypic variance and the additive effects of these QTLs varied from 0.10 to 0.16 and -0.11 to 0.15, respectively. In BTK, two putative QTLs were detected on chromosomes 9 and 11 (Table 2). Phenotypic variance and the additive effects of these two QTL were 0.09 and 0.20, and -0.09 and 0.15, respectively. In considering priority of target QTLs for further analysis, we focused on QTLs including the

‘Takanari’ allele that reduced CTds, without taking into consideration any pleiotropic effects for other aspects of the phenotype. In BKT, the largest QTL on chromosome 3 ($qCTd3$) was probably a pleiotropic effect of the heading date QTL ($qHD3$). The direction of effect of the second

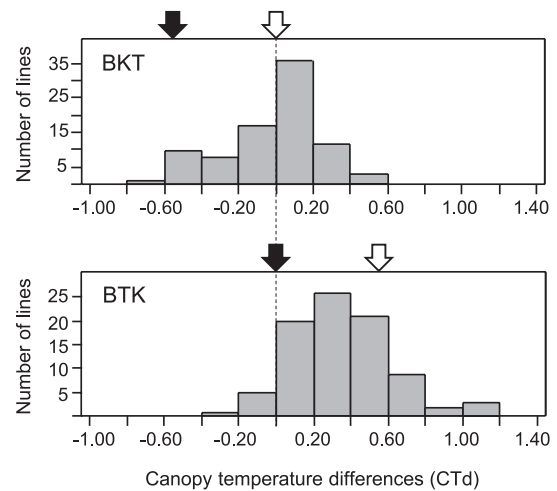


Fig. 3. Frequency distribution of canopy temperature differences (CTds) in two reciprocal populations crossed between ‘Koshihikari’ and ‘Takanari’. Black and white arrows indicate CTds in ‘Takanari’ and ‘Koshihikari’, respectively. The reference variety was ‘Koshihikari’ in BKT or ‘Takanari’ in BTK for the calculation of CTds as described in Materials and Methods. The weather conditions at the measurements were shown in Supplemental Fig. 2.

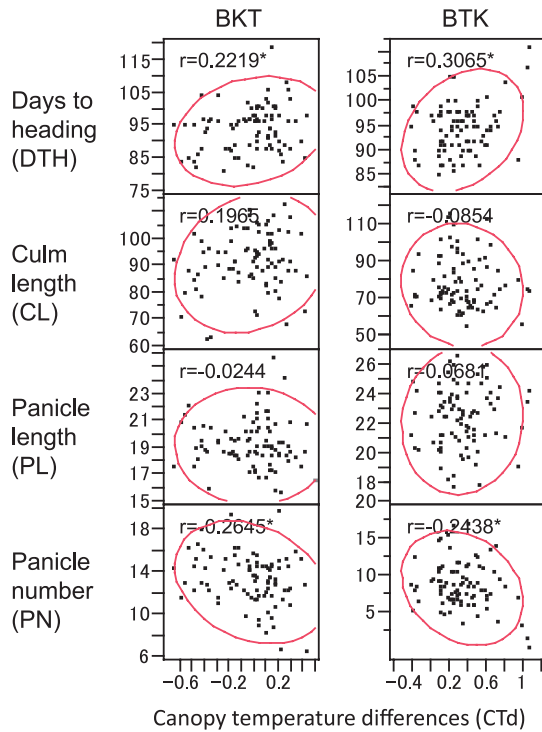


Fig. 4. Correlation between canopy temperature differences (CTDs) and four basic agronomic traits. The reference variety was ‘Koshihikari’ in BKT or ‘Takanari’ in BTK for the calculation of CTDs as described in Materials and Methods. Asterisks indicate significant differences in the correlation coefficient (* $P < 0.05$; Pearson’s product-moment correlation coefficient).

largest QTL (*qCTd9*) was opposite that of other QTLs: the ‘Koshihikari’ allele decreased CTds, possibly as a consequence of a wide tiller angle. The well-known gene, *TAC1*, is located in the same region as this QTL (Yu *et al.* 2007). Although a weak correlation was found between CTd and panicle number (Fig. 4), no significant QTL for panicle number was detected in the two populations. Of three other QTLs (*qCTd10*, *qCTd11* and *qCTd12*), only the region around *qCTd11* was detected in the reciprocal population, BTK (Table 2). Here, we chose *qCTd11* as the primary target for further analysis. This chromosome 11 QTL explained 10.4% and 19.8% of the phenotypic variance (R^2) in BKT and BTK, respectively (Table 1, 2).

Evaluation of a major QTL for CTd using the CSSL

To confirm the existence of a major QTL for CTd (*qCTd11*), we produced a CSSL (SL1) that carried the QTL from ‘Takanari’ on the ‘Koshihikari’ genetic background. SL1 was homozygous for the ‘Takanari’ allele between RM26123 and RM3701 (Fig. 5). Using infrared thermal imaging, we measured CTds between ‘Koshihikari’ and SL1 or ‘Takanari’ from the maximum tillering stage to the stage just prior to heading in two different growth conditions in 2014, i.e., paddy field and pots in a water pool (Fig. 6). This growth period included the stage when field measurements of BILs were taken. Takai *et al.* (2010) reported higher P_n and g_s values in ‘Takanari’ than in ‘Koshihikari’ in the growth period between maximum tillering and heading, and detected significant CTds between the two varieties in the stage just prior to heading. Therefore, we

Table 1. Putative QTLs controlling canopy temperature difference, heading date, culm length and panicle length in BKT population

Trait	QTL	Chromosome	Flanking marker interval (Mb)	LOD	R^2 ^a	AE ^b
Canopy temperature difference (°C)	<i>qCTd3</i>	3	RM6970 (32.444)–RM7389 (36.162)	5.00	0.16	0.15
	<i>qCTd9</i>	9	RM6235 (16.676)–RM6971 (21.893)	4.13	0.12	–0.11
	<i>qCTd10</i>	10	RM1859 (13.557)–RM6737 (18.784)	3.86	0.13	0.12
	<i>qCTd11</i>	11	RM5599-1 (3.829)–RM3701 (8.105)	3.39	0.10	0.09
	<i>qCTd12</i>	12	RM7344 (17.950)–RM3448 (19.948)	3.12	0.11	0.15
Heading date (days)	<i>qHD3</i>	3	RM3199 (30.422)–RM7389 (36.162)	3.69	0.09	–3.24
	<i>qHD6</i>	6	RM3408 (4.598)–RM3330 (11.065)	14.58	0.50	5.02
Culm length (cm)	<i>qCL1</i>	1	RM1297-1 (28.613)–RM6321 (42.924)	15.34	0.41	10.06
	<i>qCL3</i>	3	RM6970 (32.444)–RM7389 (36.162)	3.46	0.07	3.89
	<i>qCL6.1</i>	6	RM3408 (4.598)–RM3330 (11.065)	4.70	0.11	3.74
	<i>qCL6.2</i>	6	RM5957 (24.523)–RM1370 (28.534)	4.26	0.09	–3.59
Panicle length (cm)	<i>qPL5</i>	5	RM3838-1 (16.554)–RM3295 (22.333)	3.99	0.16	–1.10
	<i>qPL6</i>	6	RM5957 (24.523)–RM1370 (28.534)	4.90	0.21	–0.90

^a Phenotypic variance explained by each QTL.

^b Additive effect of the allele from Koshihikari compared with that of Takanari.

Table 2. Putative QTLs controlling canopy temperature difference in BTK population

Trait	QTL	Chromosome	Marker interval (Mb)	LOD	R^2 ^a	AE ^b
Canopy temperature difference (°C)	<i>qCTd9</i>	9	RM6235 (16.676)–RM3808 (20.548)	2.83	0.09	–0.09
	<i>qCTd11</i>	11	RM3701 (8.105)–RM5824 (14.528)	5.04	0.20	0.15

^a Phenotypic variance explained by each QTL.

^b Additive effect of the allele from Koshihikari compared with that of Takanari.

QTL for canopy temperature difference in rice

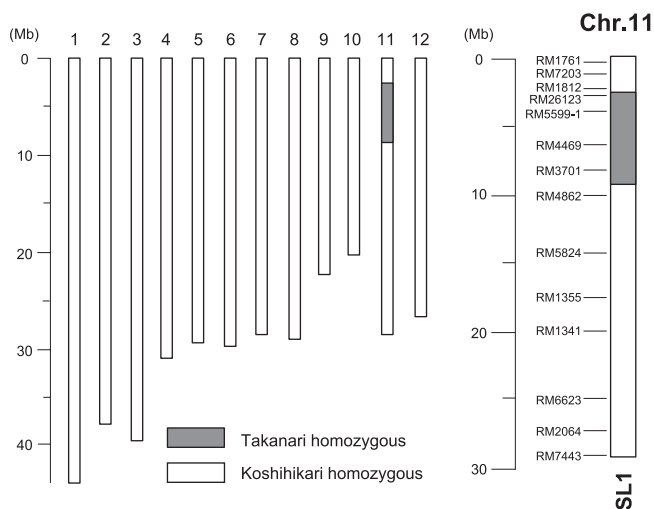


Fig. 5. Genotype of SL1, which was developed from a cross between ‘Koshihikari’ and ‘Takanari’.

decided that this growth period was suitable for measuring CTds. We replicated the experiment in a paddy field and pots to examine the effects of growth conditions on CTd. Similar results were obtained under the two growth conditions (**Fig. 6**). CTds of SL1 and ‘Takanari’ in the rice field ranged between -0.50 and -0.64°C (average, -0.57°C), and between -0.69 and -0.88°C (average, -0.76°C), respectively (**Fig. 6A**). Similar results were obtained in 2015 and 2016. Similarly, the CTds of SL1 and ‘Takanari’ in the pot experiments ranged between -0.43 and -1.04°C (average, -0.80°C), and between -0.60 and -1.37°C (average, -1.04°C), respectively (**Fig. 6B**). We also analyzed long-term and diurnal changes in CTds between ‘Koshihikari’ and SL1, or ‘Takanari’, in pot experiments in 2015. The CTds of SL1 and ‘Takanari’ varied from -0.33 to -0.64°C (average, -0.44°C), and from -0.48 to -1.10°C (average, -0.85°C), respectively, during the growth stage until heading (**Fig. 7A**). In addition, the CTds of SL1 and ‘Takanari’ varied from -0.33 to -0.39°C (average, -0.35°C), and from

-0.63 to -0.77°C (average, -0.69°C), respectively, from morning to afternoon in the stage just prior to heading (**Fig. 7B**). The canopy temperature of SL1 was consistently and significantly lower than that of ‘Koshihikari’, and slightly higher than that of ‘Takanari’, under the two growth conditions. Although the CTds, especially in ‘Takanari’, largely varied and the standard deviations were large in **Fig. 7A**, the order of the three lines was significantly same during most of the growing period. These results confirm that *qCTd11* reliably decreases canopy temperature in ‘Koshihikari’.

Morphological characteristics, such as maximum leaf width and length and plant height, in SL1 were similar to those in ‘Koshihikari’, while plant height in ‘Takanari’ was lower than that in the other two lines (**Supplemental Fig. 4**). In addition, DTH for SL1 was the same as that for ‘Koshihikari’, but shorter than that for ‘Takanari’. As with the BIL results, these results suggest that morphological changes may not have caused the low leaf temperature in SL1.

P_n, g_s, and transpiration rate of the CSSL

Transpiration strongly correlates with g_s , and leaf temperature is a useful indicator of g_s and transpiration (Jones 2004). In addition, CTd has previously been shown to correlate strongly with g_s and P_n (Takai *et al.* 2010). Here, we measured g_s , P_n , and the transpiration rate in SL1 in pot experiments in which the CTds were greater than those of SL1 plants in the paddy field (**Fig. 6**). SL1 had significantly higher g_s , P_n , and transpiration rates than ‘Koshihikari’, similar to those of ‘Takanari’ in 2015 (**Fig. 8B–8D**). The SL1 SPAD value was slightly higher than that for ‘Koshihikari’ (**Fig. 8E**). However, we also found that SL1 had significantly higher g_s in the rice field and higher g_s , P_n , and transpiration rates in the pot experiments than ‘Koshihikari’ in 2014, although SPAD values were similar in ‘Koshihikari’ and SL1 (**Supplemental Fig. 5**), suggesting that higher P_n might be resulted mainly from higher g_s . These results indicate that a low CTd is associated with high g_s ,

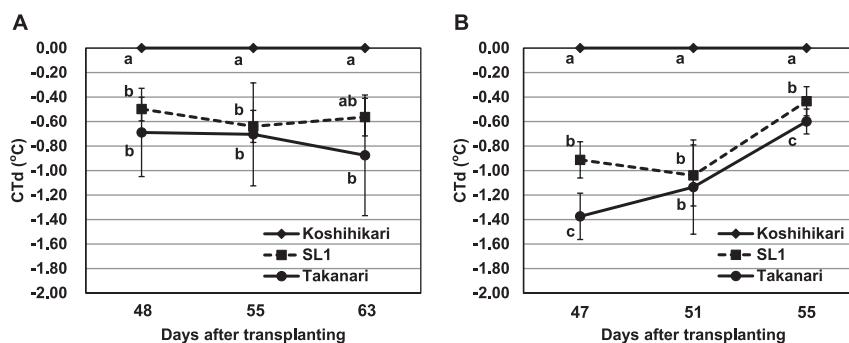


Fig. 6. Canopy temperature differences (CTds) between ‘Koshihikari’ and SL1, or ‘Takanari’, in plants grown in a paddy field (A) and pots (B) in 2014. All plants were evaluated before heading. The weather conditions at the measurements were shown in **Supplemental Table 1**. Vertical bars in (A) and (B) represent the S.D.s of four replicates of experimental blocks and eight replicates of measurements, respectively. Different letters indicate significant differences ($P < 0.05$; Tukey’s honest significant difference test).

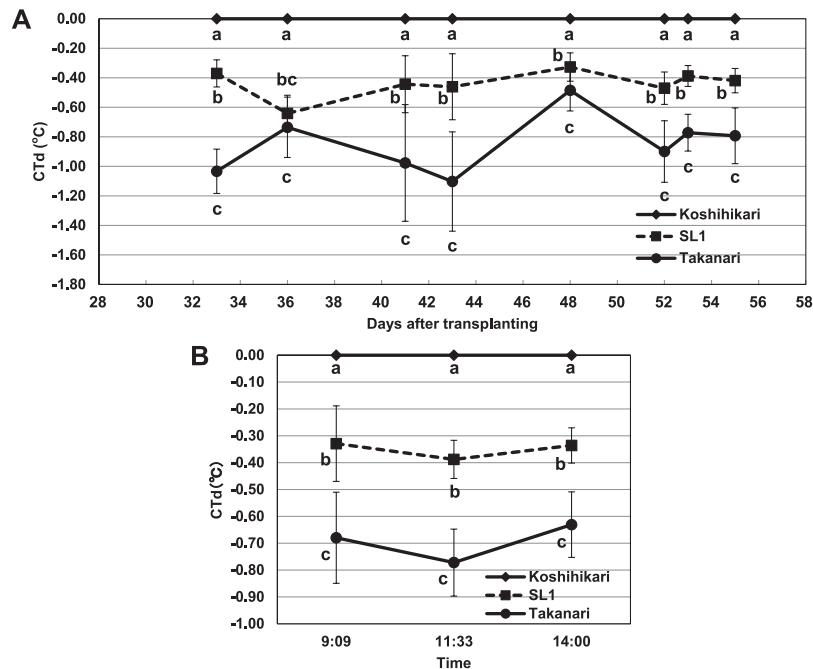


Fig. 7. Long-term and diurnal changes in canopy temperature differences (CTDs) between ‘Koshihikari’ and SL1, or ‘Takanari’, in plants grown in pots from the maximum tillering stage to the stage just prior to heading (A), and from morning to afternoon at 53 days after transplanting (B), in 2015. The weather conditions at the measurements were shown in [Supplemental Table 1](#). Vertical bars in (A) and (B) represent the S.D.s of 6–12 and eight or nine replicates of measurements, respectively. Different letters indicate significant differences ($P < 0.05$; Tukey’s honest significant difference test).

P_n , and transpiration rates in SL1, suggesting that the chromosome segment from ‘Takanari’ in SL1 might include genes that were related to leaf photosynthesis.

Discussion

QTL mapping for CTd

We mapped a major genetic factor for CTd on chromosome 11, between RM5599 and RM5824 ([Table 1, 2](#)). The QTL was the mutual one in both BILs (BKT and BTK). QTLs for canopy temperature have been identified in rice under drought stress (Babu *et al.* 2003, Yue *et al.* 2005). Babu *et al.* (2003) detected a major QTL for canopy temperature on chromosome 2 using a doubled-haploid population from a cross between an upland *japonica* ecotype, with a deep and thick root system and low osmotic adjustment, and an *indica* ecotype, with a shallow root system and high osmotic adjustment. However, the QTL did not match QTLs for drought resistance. Yue *et al.* (2005) also detected QTLs for canopy temperature on chromosome 2 in paddy soil conditions, and on chromosomes 1, 5, 6, 8, and 11 in sandy soil conditions, using a recombinant inbred population derived from a cross between an upland rice and an *indica* rice. The QTLs on chromosomes 2, 6, and 8, and one on chromosome 5, were co-located on the QTL for drought response index and leaf rolling score, respectively. ‘Takanari’ has a larger root surface area than ‘Koshihikari’, and it has been suggested that this characteristic supports high stomatal con-

ductance through the maintenance of high leaf water potential and high hydraulic conductance (Taylaran *et al.* 2011). Although it is unclear whether the QTL on chromosome 11 matches those detected in this study, the same gene(s) might play important roles in controlling canopy temperature and drought resistance because it is possible that high stomatal conductance caused by root characteristics is related to the two phenotypes.

Confirmation of qCTd11 using the CSSL

We showed that SL1, carrying *qCTd11* from ‘Takanari’ on the ‘Koshihikari’ genetic background, consistently and significantly had a lower canopy temperature than ‘Koshihikari’ ([Figs. 6, 7](#)), indicating that *qCTd11* is a stable and effective QTL for decreasing canopy temperature in ‘Koshihikari’. The canopy temperature of SL1 was slightly higher than that of ‘Takanari’ ([Figs. 6, 7](#)). [Table 1](#) shows that several putative QTLs (other than *qCTd11*) that decrease canopy temperature in the ‘Takanari’ genotype were detected on chromosomes 3, 10, and 12, although *qCTd11* was the only QTL detected in both genetic backgrounds. These QTLs (except *qCTd11*) might have some effect on canopy temperature, although they were unstable. Babu *et al.* (2003) detected a QTL for canopy temperature in rice under drought stress on chromosome 2, and Yue *et al.* (2005) identified six QTLs including one on chromosome 2. These findings suggest that, in addition to *qCTd11*, a minor QTL(s) for CTd might exist.

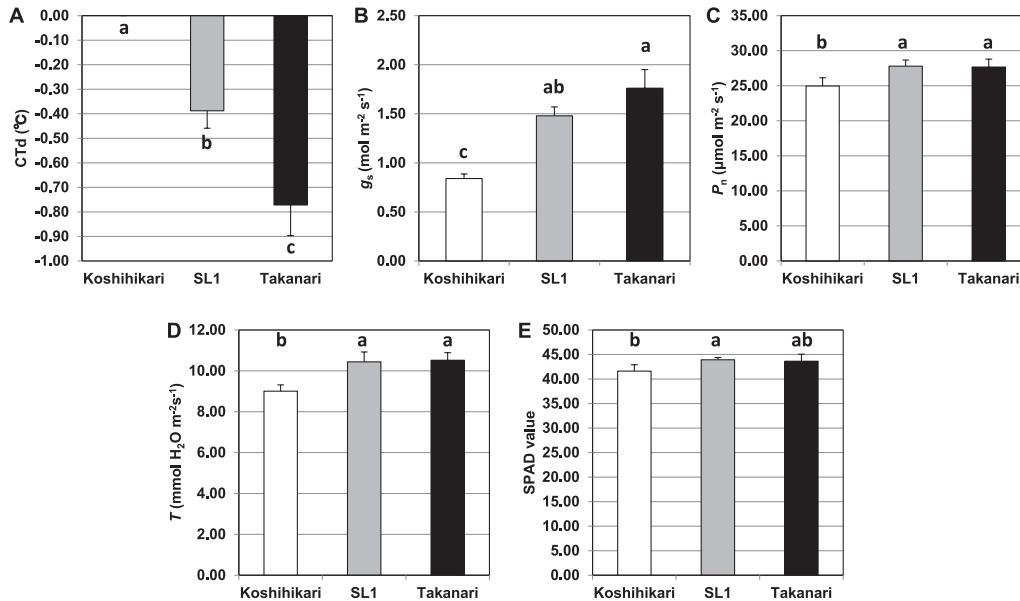


Fig. 8. Comparison of canopy temperature differences (CTDs) between ‘Koshihikari’ and SL1, or ‘Takanari’ and leaf photosynthesis in ‘Koshihikari’ and SL1, or ‘Takanari’. CTDs (A) were measured at 53 days after transplanting in 2015. Stomatal conductance (g_s) (B), leaf photosynthetic rate (P_n) (C), transpiration rate (T) (D), and soil-plant analysis development (SPAD) value (E) per leaf area in each topmost expanded leaf of the main culm of ‘Koshihikari’, SL1, and ‘Takanari’ grown in pots were determined on the same day as the CTD measurements. All plants were evaluated before heading. Values are means \pm S.D. (A, $n = 9$; B–E, $n = 3$ or 4). Different letters indicate significant differences ($P < 0.05$; Tukey’s honest significant difference test).

Relationship between $qCTd11$ and leaf photosynthesis and plant characteristics

‘Takanari’ has a lower leaf temperature and a higher g_s than other varieties, including ‘Koshihikari’ (Horie *et al.* 2006, Takai *et al.* 2014). In this study, we found a major QTL for CTd ($qCTd11$), which is an index of leaf temperature difference (Tables 1, 2). Takai *et al.* (2010) reported that CTd is significantly correlated with g_s , indicating that CTd reflects variation in g_s under high solar radiation, and that CTd is also closely correlated with P_n ; therefore, CTd reflects the effects of variations in CO₂ supply on leaf photosynthesis. Fig. 8 shows that SL1 had a lower CTd and higher P_n and g_s , than ‘Koshihikari’, supporting the hypothesis that a high g_s causes increases in P_n and a low leaf temperature; therefore, $qCTd11$ could be related to stomatal conductance and leaf photosynthesis.

The P_n and g_s values of ‘Takanari’ are higher than those of ‘Koshihikari’, even at low irradiance (500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and increase as the PPFD increases (Takai *et al.* 2010). In SL1, the P_n and g_s values were also higher than those in ‘Koshihikari’ at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and increased as the PPFD increased (Supplemental Fig. 6). These results indicate that $qCTd11$ is involved in stomatal conductance and leaf photosynthesis.

Negative associations between temperature and plant height have been observed in wheat and potato (Prashar *et al.* 2013, Rebetzke *et al.* 2012), although it is unclear what causes low temperatures in tall crops. In wheat, several QTLs for canopy temperature and leaf porosity are collocated with a known QTL for plant height and phenologi-

cal development (Rebetzke *et al.* 2012). We found that plant height and DTH in ‘Takanari’, which has a low canopy temperature, were shorter and longer, respectively, than in ‘Koshihikari’, a reference variety. In addition, two dwarf QTLs, $d-27$ (Yu *et al.* 1992) and $Tid1$ (Sunohara *et al.* 2004), are located between RM5599 and RM4469 (International Rice Genome Sequencing Project 2005) according to the Rice Annotation Project Database (RAP-DB) IRGSP-1.0 (Sakai *et al.* 2013) and the QTL Annotation Rice Online (Q-TARO) database (Yonemaru *et al.* 2010). However, CTd was not significantly correlated with culm length (Fig. 4), and plant height and DTH in SL1, which carried $qCTd11$ from ‘Takanari’ in the ‘Koshihikari’ genetic background, were similar to those in ‘Koshihikari’ (Supplemental Fig. 4). These results suggest that $qCTd11$ is not involved in morphological or phenological changes, and that these changes may not have caused the lower leaf temperature in SL1.

Takai *et al.* (2010) suggested that variations in leaf N content might affect the CTd, because leaf N content may be related to g_s (Hubbart *et al.* 2007, Yoshida and Coronel 1976). Supplemental Fig. 5 shows that the SPAD values, which are related to leaf N content, of SL1 were not significantly different to those of ‘Koshihikari’, although the SL1 SPAD values in pot experiments in 2015 were slightly higher than those in ‘Koshihikari’. In addition, we obtained similar SPAD values in SL1 to those in ‘Koshihikari’ in field experiments in 2016 (data not shown), suggesting that leaf N content was only weakly associated with the CTd between ‘Koshihikari’ and SL1.

In conclusion, we mapped *qCTd11* for the CTd between ‘Koshihikari’ and ‘Takanari’ on chromosome 11, and demonstrated that SL1, which carried *qCTd11* from ‘Takanari’ in the ‘Koshihikari’ genetic background, consistently and significantly had a lower canopy temperature and higher g_s and P_n than ‘Koshihikari’ over several years and in two experiments (field and pot). Our results suggest that *qCTd11* might be a significant factor for CTd, and is related to g_s and P_n . We confirmed the efficacy of measuring CTd using infrared thermography, and verified the relationship between the CTd and g_s and P_n in the field. The results of our study will be useful for cloning novel gene(s) related to canopy temperature, g_s , and P_n , and for breeding programs that aim for high g_s , P_n , and yield.

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