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Research article

Construction of chromosome segment substitution lines of Dongxiang common wild rice (*Oryza rufipogon* Griff.) in the background of the *japonica* rice cultivar Nipponbare (*Oryza sativa* L.)

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ABSTRACT

Dongxiang common wild rice (*Oryza rufipogon* Griff.) is believed to have the northernmost distribution of any wild rice species. Many favorable genes were lost during rice domestication, and Dongxiang common wild rice is a potential source of many genes related to biological and abiotic stress resistance and high grain yield. Despite its importance, *japonica* rice has not yet been used as a background material for the construction of introgression lines carrying Dongxiang common wild rice chromosome segments. In this study, we used Dongxiang common wild rice as the donor parent and Nipponbare (*Oryza sativa* L), a reference-sequence *japonica* cultivar, as the recurrent parent to develop a set of 104 chromosome segment substitution lines (CSSLs) through crossing and backcrossing with marker-assisted selection based on 203 polymorphic molecular markers evenly distributed across 12 chromosomes. The 104 CSSLs covered 87.94% of the genome of Dongxiang common wild rice. Each CSSL contained an average of four introgressed segments, and the average segment length was 3.3 Mb. Quantitative trait locus (QTL) analysis of the panicle-related traits of the CSSLs at two sites (Beijing and Nanchang, Jiangxi Province) revealed 18 QTLs for eight traits. Among these 18 QTLs were two known grain length- and width-related genes and four novel QTLs. In addition, two QTLs were verified, and two novel QTLs were identified, for panicle neck length, a domestication-related trait. The developed CSSLs potentially represent a valuable population for detecting favorable genes in common wild rice and creating germplasm resources for *japonica* rice breeding.

1. Introduction

Rice breeding in China has undergone two major changes: from dwarf breeding to heterosis utilization in the last century and then to super rice cultivation, which is the approach used today. Every change in breeding practices has resulted from the exploration and utilization of important rice germplasm resources, but the homogenization of rice varieties is becoming increasingly severe. For example, the gene pool used for the breeding of parent materials of *japonica* rice varieties in northern China has narrowed, and the genetic diversity has declined sharply. Rice production is an eternal theme in the development of the rice industry. By 2050, the production of rice will need to increase by 60–70% to meet the needs of population growth in Asia (Tester and Langridge, 2010). In addition, in some countries, such as China, the improvement of living standards over the past decade has resulted in an increased demand for high-quality rice. Panicle-related traits, such as panicle length, grain number, grain length and width, are related to rice yield and play important roles in rice grain quality. However, the identification of new resources of existing varieties to further improve both rice yield and quality has proven difficult. Wild rice species are wild relatives of cultivated rice and contain large numbers of genes related to traits such as disease resistance, stress resistance, rice yield and grain quality. The use of wild rice species to expand the gene pool of cultivated rice and increase the genetic diversity of varieties is an effective approach for improving rice breeding and production (Huang et al., 2012).

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The genus *Oryza* includes cultivated rice and wild rice species. Cultivated rice comprises two species (*O. sativa* and *O. glaberrima)* with the AA genome type (2n = 24), whereas wild *Oryza* comprises 22 species (*O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*, *O. nivara*, *O. rufipogon*, *O. punctata*, *O. officinalis*, *O. rhizomatis*, *O. minuta*, *O. eichingeri*, *O. alta*, *O. grandiglumis*, *O. latifolia*, *O. australiensis*, *O. brachyantha*, *O. longiglumis*, *O. ridleyi*, *O. schlechteri*, *O. granulata*, *O. meyeriana*, and *O. coarctata*) with the AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK genome types (2n = 24 or 48) (Vaughan, 1994; Ge et al., 1999; Sanchez et al., 2013). Cultivated rice was domesticated from wild *Oryza* species, and during this domestication process, the diversity of morphological traits, including plant height, tillering number, flowering behavior, and panicle, leaf and seed characteristics, in cultivated rice was reduced by 40% relative to that in wild *Oryza* species. In addition, the domestication process of rice resulted in the loss of several biotic and abiotic stress-related genes (Sun et al., 2001; Xie et al., 2008). Due to a research emphasis on the identification of wild rice resources, many rice disease-resistance and stress-tolerance genes have been discovered in recent years. For example, many bacterial blight-resistance genes have been identified in wild rice, including *Xa21* from *O. longistaminata* (Ronald et al., 1992; Song et al., 1995), *Xa23* from *O. rufipogon* (Wang et al., 2015), *Xa33* and *Xa38* from *O. nivara* (Hemal et al., 2012; Kumar et al., 2012), *Xa27* from the tetraploid wild rice species *O. minuta* (Gu et al., 2004), and *Xa32(t)* from *O. australiensis* (Zheng et al., 2009). Furthermore, genes related to insect resistance, cold tolerance and male fertility have been found in wild rice (He et al., 2012; Huang et al., 2013; Xiao et al., 2015; Hu et al., 2016).

Three species of wild rice are distributed in China: *O. rufipogon*, *O. officinalis* and *O. meyeriana*. *O. rufipogon* and *O. sativa* share the AA genome type and are very closely related, and *O. rufipogon* is an ancestor of modern rice. To date, most varieties bred using conventional methods, hybridization and high-yield- and high-grain-quality-targeted breeding have *O. rufipogon* as one parent. China is rich in wild rice resources, which are widely distributed in six onshore provinces (Guangdong, Guangxi, Yunnan, Hunan, Fujian and Jiangxi) and two island provinces (Hainan and Taiwan) (Gao et al., 2000). One of these resources, *O. rufipogon* is believed to have the northernmost distribution of any species of wild rice (N 28°14') (Luo et al., 2012). Because this species was discovered in Dongxiang County, Jiangxi Province, in 1978, it is called Dongxiang common wild rice. This rice species exhibits abundant genetic diversity and is a potential source of many genes related to cold and drought tolerance, disease and insect resistance, wide cross-compatibility, fertility restoration, cytoplasmic male sterility, and high grain yield. Dongxiang wild rice plays important roles in basic rice research and industrial development and is known as "the panda of wild plants" (Tian et al., 2006; Xie et al., 2010).

Although common wild rice and cultivated rice have the same genome type, they exhibit substantial differences in their genome sequences. A stable and reliable genetic population is important for utilizing or studying common wild rice. The construction of temporary genetic populations, such as F_2 or BC_1 populations, and permanent primary genetic populations, such as doubled-haploid and recombinant inbred lines, is easy. However, due to interference from genetic background noise, such populations exhibit not only a low QTL detection efficiency but also poor stability, and QTL detection with fine mapping requires the construction of near-isogenic lines and secondary segregating populations (Yano, 2001). Chromosome segment substitution lines (CSSLs, also called introgression lines (ILs)) are not subject to the limitations of the abovementioned genetic populations. A CSSL population is generally developed through crossing, advanced backcrossing with marker-assisted selection (MAS) and self-crossing. Each final CSSL carries a single or a few chromosomal segments from the donor parent in the background of the recurrent parent and can thus be regarded as a near-isogenic line of the recurrent parent. Using *O. rufipogon*, *O. glumaepatula* and some improved cultivars as the donor parent, many sets of CSSLs and ILs have been constructed in rice (Tian et al., 2006; Tan et al., 2007; Hirabayashi et al., 2010; Qiao et al., 2016; Takai et al., 2014; Nagata et al., 2015; Uga et al., 2015). The CSSL strategy is particularly suitable for the construction of a genetic population with wild rice as the donor parent. CSSLs can solve the problem of the extreme distance between the early-generation populations of wild rice plants and their cultivated rice offspring (Kubo et al., 2002; Furuta et al., 2014). In addition, CSSLs can also minimize linkage drag, facilitate the map-based cloning of QTLs and produce new resources for CSSL-based rice breeding (Yamamoto et al., 2000).

In this study, we used Dongxiang wild rice as the donor and the *japonica* rice cultivar Nipponbare as the recurrent parent to construct a set of CSSLs comprising 104 families. The CSSL genotypes were analyzed using 203 molecular markers that uniformly covered the 12 chromosomes, and the set of constructed CSSLs covered 87.94% of the genome of Dongxiang wild rice. The subsequent characterization of CSSLs associated with the transmission of panicle-related traits from common wild rice to cultivated rice revealed several novel QTLs.

2. Materials and methods

2.1. Plant materials and construction of CSSLs

A Chinese common wild rice (*O. rufipogon* Griff.) accession (C35) collected from Dongxiang, Jiangxi Province, was used as the donor parent. Nipponbare, a typical *japonica* rice cultivar (*O. sativa* L.) used in the International Rice Genome Sequencing Project (IRGSP), was used as the recurrent parent. Extensive genomic information is available for Nipponbare, and this cultivar is used worldwide in rice functional genomics research. All materials, including plants of different generations during the construction of the ILs, were planted during two seasons each year: summer in Beijing (N39°36′, E116°32′) and winter in Sanya (N18°15′, E109°30′), Hainan Province, China.

2.2. Construction of CSSLs

A screening of the existing collection of 1017 simple sequence repeat (SSR) and insertion/deletion (InDel) markers and 217 InDel markers designed using C35 genome resequencing information identified a set of 203 markers that detected polymorphisms between Nipponbare and C35, and these markers were then used for CSSL analysis. These markers map to loci that are approximately uniformly distributed across the 12 rice chromosomes, with an average interlocus distance of 1.9 megabytes (Mb) (Ma et al., 2019).

The F_1 plant derived from a cross between Nipponbare and C35 was successively backcrossed three times with Nipponbare to produce BC_3F_1 plants (Fig. 1), and the genotypes of the BC_3F_1 lines were surveyed for further backcrossing. From BC_3 to BC_5, the backcrossing plant for each generation was selected by MAS, and self-crossing was performed five times to obtain the BC_5F_6 population. From BC_5F_2 to BC_5F_5, we selected an individual from each line by phenotype which represented the line. Randomly selected plants from the BC_5F_6 population were genotyped for construction of the CSSLs.

2.3. Extraction of DNA, PCR amplification, and product detection

Young leaves from C35 and Nipponbare plants were harvested, and genomic DNA was extracted using a conventional cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980).

Molecular marker primers were synthesized based on the sequences as previously reported (Ma et al., 2019). Each PCR amplification was performed in a volume of 10 μL, which contained 50 ng of DNA, 1.0 μL of 10 × PCR buffer, 0.3 μL of each dNTP (10 mmol L^{-1}), 0.5 U of Taq DNA polymerase, 0.5 μL of each forward and reverse primer (10 $\mu\text{mol L}^{-1}$), and a sufficient amount of double-distilled H_2O

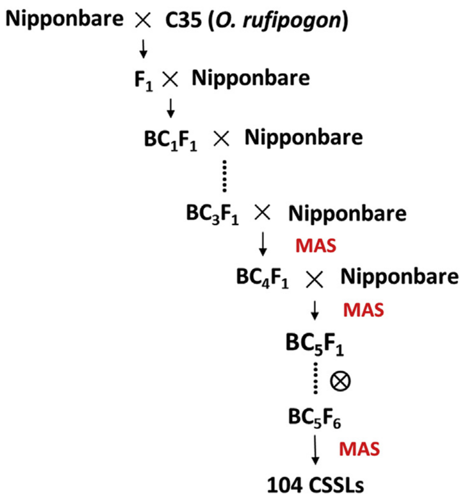

Fig. 1. Schematic of the process used for the construction of the 104 CSSLs in this study.

(ddH_2O). The PCR program consisted of a predenaturation step of 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s and a final extension of 72 °C for 5 min. The PCR products were visualized by electrophoresis on 8% polyacrylamide gels that were stained with silver nitrate (Bassam et al., 1991).

2.4. Evaluation of agronomic traits

The CSSLs and the recurrent parent Nipponbare were planted at the experimental station of the Institute of Crop Sciences (Chinese Academy of Agricultural Sciences) in Beijing and at the Rice Research Institute (Jiangxi Academy of Agricultural Sciences) in Nanchang, Jiangxi Province, during the summer. All the lines were planted in a complete randomized design with three replications. Each line was planted in four rows, with distances of 10 cm between plants within a row and 30 cm between rows. The field management strategy involved the conventional field planting methods for rice. At the time of harvest, 12 plants in the middle of each plot were selected for the evaluation of nine panicle-related traits: panicles per plant, panicle length, grains per panicle, seed setting rate, numbers of primary and second branches, panicle neck length (the length from the neck node to the flag leaf pulvinus), grain length and grain width. The grain length and width were detected using an automatic seed investigation machine (Wanshen, China), and the other traits were measured using the methods described by Han and Wei (2006). The maximal and minimal values of each agronomic trait were excluded, and the average of each trait was calculated for evaluation.

2.5. Data analysis

The physical map constructed by Ma et al. (2019) using IRGSP 2005 (ftp://ftp.ensemblgenomes.org/pub/plants/release-28/fasta/oryza_sativa/dna/) data was used to estimate the marker distances, lengths of the chromosomes and substituted chromosome segments and overall genome size, and these values were used for genome ratio calculations. GGT software (van Berloo, 1999) was used to construct the graphical genotypes and to calculate the percentage of the total genome in each CSSL based on a previously reported method (Tian et al., 2006; Qiao et al., 2016; Xu et al., 2010).

A QTL analysis of the nine panicle-related traits was conducted using the likelihood ratio test based on the stepwise regression for additive QTL (RSTEP-LRT-ADD) mapping method in QTL IciMapping software (Version 4.1), which allows the QTL mapping of CSSLs. The threshold logarithm of odds (LOD) score indicating a significant additive QTL was set to 2.5, and the other parameters were set to default values according to the software's user manual (Wang et al., 2016).

3. Results

3.1. Development of CSSLs

To construct the CSSLs, the F_1 plant derived from a cross between Nipponbare and Dongxiang wild rice was successively backcrossed three times with Nipponbare, and 187 BC_3F_1 lines were obtained. The genotypes of the BC_3F_1 lines were surveyed using 203 markers, and 101 plants were selected for backcrossing. MAS resulted in the identification of 87 and 79 CSSLs from BC_4F_1 and BC_5F_1, respectively, and the BC_5F_1 plants were self-crossed five times to obtain the BC_5F_6 population. The genotypes of 368 plants belonging to the BC_5F_6 population (each plant represented one line) were analyzed, and the genotyping results showed that nine of the 368 individuals did not contain an inserted *O. rufipogon* chromosome fragment and that 29 individuals contained too many inserted *O. rufipogon* chromosome fragments (more than 30). To ensure the highest possible coverage of the *O. rufipogon* genome, the lines with repetitive insertions or too many fragments were removed, which resulted in a total of 104 unique CSSLs.

3.2. Distribution of substituted segments in the CSSLs

The graphical genotypes of the 104 CSSLs were constructed according to the physical locations and genotypes of the 203 molecular markers in the CSSLs (Fig. 2). The 104 CSSLs contain 340 homozygous and 73 heterozygous introgressed chromosome segments (Table 1), and on average, each line carried 3.27 homozygous and 0.70 heterozygous segments. Heterozygous loci are problematic for evaluation because they segregate in the next generation and affect the phenotype. However, none of the tested plants were homozygous for all 73 regions, so we could not select purely homozygous lines for those heterozygous regions. The number of introgressed segments varied among the chromosomes. For example, the largest number of introgressed segments (57 homozygous + heterozygous segments) was found on Chr. 1, and the smallest number (13) was found on Chr. 6 (Fig. 2). Furthermore, the coverage of the *O. rufipogon* genome in the CSSLs varied among the chromosomes. Among the 12 chromosomes, Chr. 3 and Chr. 5 had the highest and lowest coverage rates (99.51% and 67.05%, respectively) (Table 1).

A total of 413 introgressed segments were detected in all 104 CSSLs. The size of the segments ranged from 0.19 Mb (on Chr. 12 of Line 101) to 17.33 Mb (on Chr. 6 of Line 62), with an average of 3.26 Mb. Approximately 62.7% of the introgressed segments were less than 3 Mb in size, 28% were 3–6 Mb in size, and only 9.1% of the segments were greater than 8 Mb in size (Fig. 3).

3.3. Phenotypic variation

Descriptive statistics of the panicle-related traits of the 104 CSSLs and the recurrent parent planted in Beijing and Nanchang are listed in Table 2 and Fig. 4. Because Dongxiang common wild rice cannot head in Beijing, we show the values for only Nipponbare in Beijing and Nanchang in Table 2. Phenotypic transgressive variation was observed for all panicle traits at the two sites (Fig. 4). Among the nine investigated traits, the greatest coefficients of variation were obtained for the panicle neck length (35.3% at Beijing and 39.4% at Nanchang), whereas the lowest coefficients of variation were found for the grain width (4.0% at Beijing and 3.8% at Nanchang). Overall, the variation at the Nanchang site was greater than that at the Beijing site.

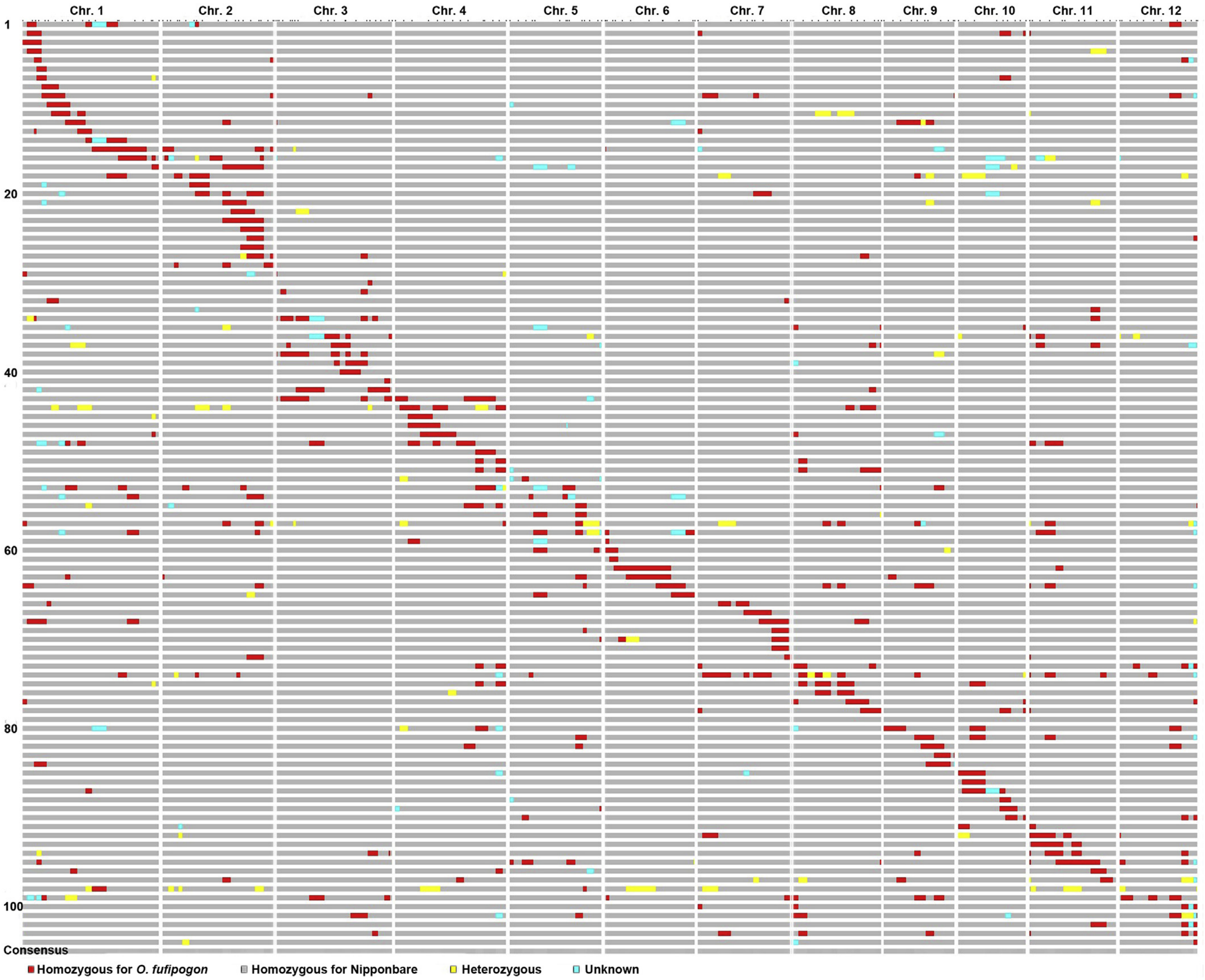

Fig. 2. Physical map of the 104 CSSLs. Each row represents a CSSL, and each column represents a marker locus. The genotype classes of the 203 DNA markers in each CSSL are shown in Fig. S1.

Table 1
Distribution of substituted chromosome segments of *O. rufipogon* in the CSSLs and its cumulative proportion represented by homozygous and heterozygous segments.

Chromosome	Homozygous segments		Heterozygous segments		Genome coverage length (Homo + Het, Mb)	Percent genome coverage (Homo + Het, %)[a]
	No. of segments	Average segment length (Mb)	No. of segments	Average segment length (Mb)		
1	46	3.91	11	24.32	41.46	95.81
2	44	3.36	12	22.30	35.35	98.36
3	39	3.10	5	8.56	36.23	99.51
4	28	3.79	10	18.49	35.27	99.35
5	27	2.22	3	11.88	20.09	67.05
6	11	5.52	2	12.52	27.88	89.22
7	22	3.36	4	9.39	24.02	80.87
8	36	2.48	5	16.64	25.16	88.47
9	20	3.45	5	8.80	21.38	92.92
10	17	3.68	4	12.46	16.12	69.45
11	23	3.28	5	14.88	26.42	91.04
12	27	2.27	7	14.74	18.84	68.42
Average	3.27	3.26	0.70	2.39	328.21 (Total)	87.94 (Total)

[a] Based on the proportion of each chromosome's physical length (in Mb) represented by at least one CSSL.

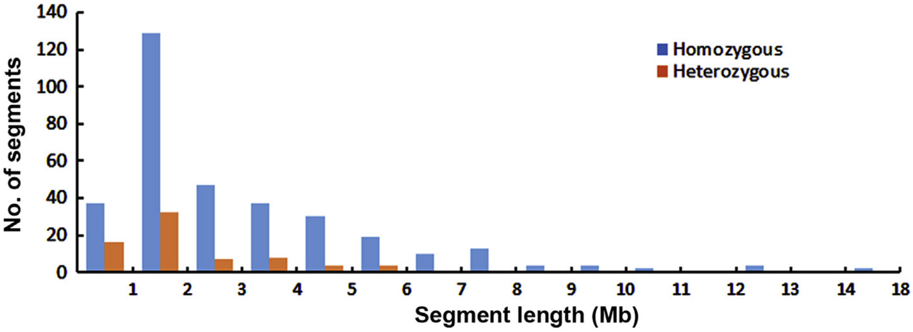

Fig. 3. Distribution of the length of substituted chromosome segments in the CSSLs.

3.4. QTL analysis of panicle-related traits using the CSSLs

QTL analyses of the nine panicle-related traits were performed separately at the sites in Beijing and Nanchang. QTLs were detected for all the traits with the exception of panicles per plant. In addition, 41 QTLs were detected in Beijing, 39 QTLs were detected in Nanchang (Table S1), and 18 significant QTLs were identified at both sites (Table 3 and Fig. 5). Here, the 18 QTLs are introduced.

3.4.1. Panicle length

One QTL (*qPL5-2*) for panicle length was detected near marker DX-C5-10 on Chr. 5, and the allele from common wild rice conferred an increase in panicle length.

3.4.2. Primary branches per panicle

One QTL (*qPBP8*) for primary branches per panicle was detected near marker DX-S8-14 on Chr. 8, and the allele from common wild rice conferred an increase in the number of primary branches per panicle.

3.4.3. Secondary branches per panicle

One QTL (*qSBP4-2*) for secondary branches per panicle was detected near marker DX-C4-12 on Chr. 4. The allele from common wild rice exerted a negative effect on the number of secondary branches per panicle.

3.4.4. Panicle neck length

Four QTLs (*qPNL1-2, qPNL1-3, qPNL8-1,* and *qPNL8-2*) for panicle neck length were detected on Chr. 1 and 8, and their effects had similar directions at both sites. The phenotypic variance explained by the individual QTLs at both sites varied from 3.2 to 22.1%. Two QTLs (*qPNL8-1* and *qPNL8-2*) derived from *O. rufipogon*, which were located near markers DX-C8-3 and DX-C8-12 on Chr. 8, respectively, exerted positive effects on panicle neck length, whereas the two other QTLs (*qPNL1-2* and *qPNL1-3*) had negative effects.

3.4.5. Grains per panicle

Two QTLs (*qGP3* and *qGP12*) for grains per panicle were detected: one near marker DX-S3-4 on Chr. 3 and one near DX-C12-10 on Chr. 12. Both QTLs contributed to a decrease in the number of grains per panicle. The phenotypic variance explained by these two QTLs ranged from 8.9 to 12.4%.

3.4.6. Seed setting rate

Three QTLs (*qSS2*, *qSS9*, and *qSS11*) for the seed setting rate were detected: one near marker DX-C2-1 on Chr. 2, one near marker DX-C9-5 on Chr. 9 and one near DX-C11-11 on Chr. 11. The three QTLs showed consistently negative effects on the seed setting rate at both sites, and the phenotypic variance explained by these three QTLs ranged from 9.4 to 14.2%.

3.4.7. Grain length

Four QTLs (*qGL1-3*, *qGL3-2*, *qGL8-1*, and *qGL9-1*) associated with grain length were detected. Three of these QTLs, which were located near marker DX-C1-18 on Chr. 1, indel-c3-12 on Chr. 3 and DX-C9-4 on Chr. 9, had positive effects on the grain length, whereas *qGL8*, which was detected near DX-C8-3 on Chr. 8, exerted a negative effect. The phenotypic variation explained by these four QTLs ranged from 8.1 to 14.4%.

3.4.8. Grain width

Two QTLs (*qGW8* and *qGW10*) associated with grain width were detected: one near marker DX-C8-15 on Chr. 8 and one near marker DX-C10-10 on Chr. 10. Both led to decreases in grain width, and the phenotypic variation explained by these QTLs ranged from 9.8 to 18.5%.

4. Discussion

In this study, we used an accession of *O. rufipogon* as a donor parent with MAS to screen 104 individuals from a 368-line BC_5F_6 population and constructed a set of CSSLs. The average coverage of the Dongxiang

Table 2
Statistics of panicle-related traits of Nipponbare and the 104-CSSL population at the Beijing and Nanchang locations.

Trait	Nipponbare (Beijing)	CSSLs (Beijing)			Nipponbare (Nanchang)	CSSLs (Nanchang)		
		Mean	CV (%)	Range		Mean	CV (%)	Range
Panicles per plant	11.7	10.5 ± 2.0	19.2	5.4–15.2	9.1	9.8 ± 3.0	23.5	5.4–18.7
Panicle length (cm)	20.4	21.2 ± 1.6	7.6	17.7–26.6	17.5	18.4 ± 2.1	8.3	14.5–27.9
Primary branches per panicle	11.3	11.5 ± 1.6	13.7	7.8–17.4	10.2	10.4 ± 2.3	14.5	6.3–18.6
Secondary branches per panicle	18.5	15.5 ± 5.0	32.1	3.4–30.0	16.4	14.2 ± 6.0	35.1	2.8–32.4
Panicle neck length (cm)	8.2	8.4 ± 3.0	35.3	0.3–21.4	7.2	7.8 ± 3.4	39.4	0.4–25.1
Grains per panicle	117.5	110.8 ± 21.3	19.2	56.2–162.6	103.1	106.1 ± 28.3	23.1	36.2–158.1
Seed setting rate (%)	96.4	89.7 ± 9.1	10.1	46.3–98.9	84.1	82.1 ± 10.5	13.2	36.3–93.3
Grain length (mm)	7.7	7.7 ± 0.3	4.1	6.7–8.8	7.6	7.5 ± 0.5	5.1	6.1–9.3
Grain width (mm)	3.4	3.3 ± 0.1	4.0	2.9–3.7	3.2	3.4 ± 0.3	3.8	2.1–3.9

CV, coefficient of variation.

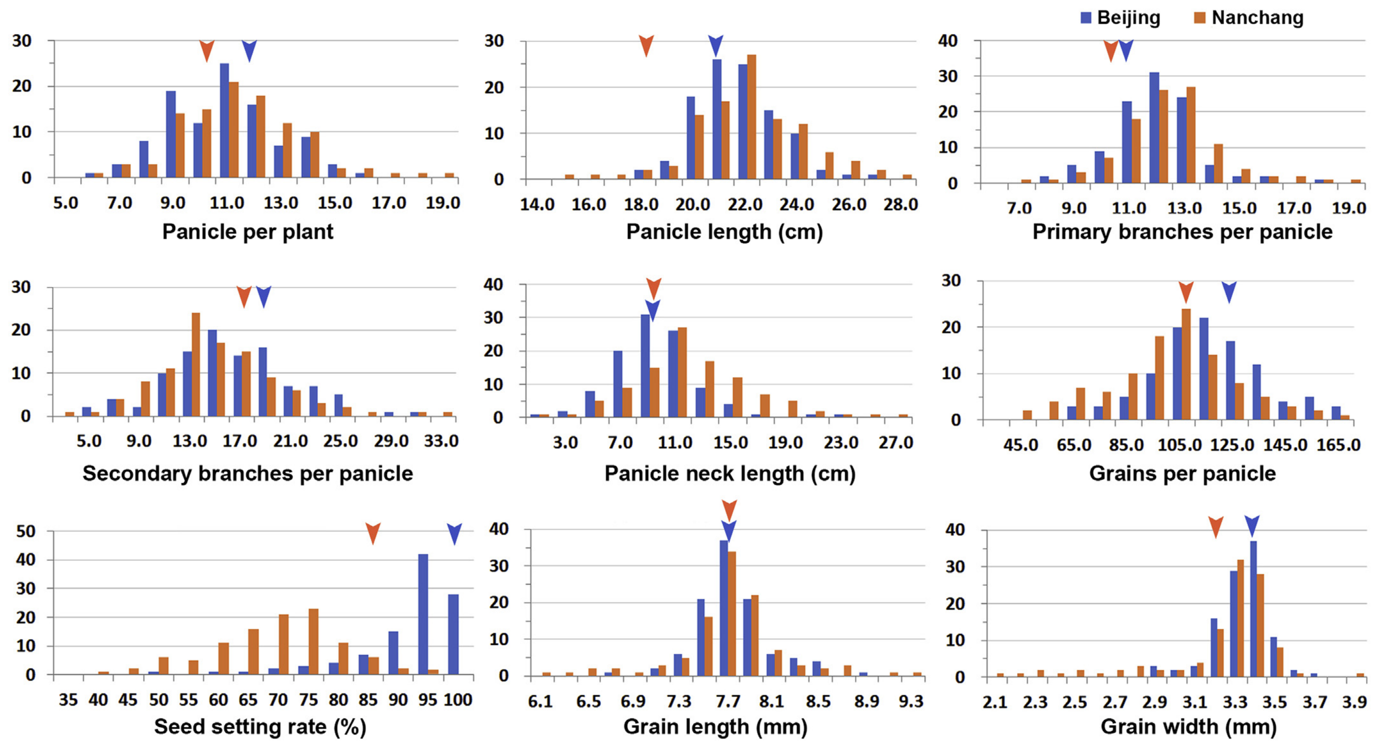

Fig. 4. Frequency distributions of nine panicle-related traits in the CSSLs. The vertical axis of each figure represents the number of CSSL individuals. The blue and orange rectangles represent the distribution of nine panicle-related traits in the CSSLs at the Beijing and Nanchang locations, respectively. The blue and orange triangles represent the positions of the means of Nipponbare in Beijing and Nanchang, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
QTLs for panicle-related traits detected in the CSSLs at both sites.

Trait	Locus	Marker	Chromo-some	Beijing			Nanchang		
				LOD	PVE (%)	Add	LOD	PVE (%)	Add
Panicle length	*qPL5-2*	DX-C5-10	5	4.6	17.9	1.1	3.9	14.3	2.2
Primary branches per panicle	*qPBP8*	DX-S8-14	8	7.1	18.1	1.3	5.8	16.1	1.9
Secondary branches per panicle	*qSBP4-2*	DX-C4-12	4	3.2	11.6	−2.1	2.9	8.6	−1.9
Panicle neck length	*qPNL1-2*	01–046	1	6.2	16.1	−1.7	4.3	9.9	−2.2
	qPNL1-3	Indel-c1-13	1	2.8	6.5	−1.4	2.5	3.2	−1.5
	qPNL8-1	DX-C8-3	8	6.9	17.8	6.4	8.7	22.1	5.1
	qPNL8-2	DX-C8-12	8	4.1	9.9	1.5	3.2	4.9	1.7
Grains per panicle	*qGP3*	DX-S3-4	3	3.8	11.5	−14.2	3.7	10.9	−16.3
	qGP12	DX-C12-10	12	2.9	8.9	−10.1	3.1	12.4	−13.6
Seed setting rate	*qSS2*	DX-C2-1	2	4.9	14.2	−12.8	4.1	10.2	−9.4
	qSS9	DX-C9-5	9	4.6	12.1	−4.9	3.9	9.4	−7.3
	qSS11	DX-C11-11	11	3.5	9.6	−10.6	3.6	10.5	−12.8
Grain length	*qGL1-3*	DX-C1-18	1	4.7	13.8	0.39	4.3	10.9	0.37
	qGL3-2	Indel-c3-12	3	3.3	9.4	0.12	3.4	9.5	0.18
	qGL8-1	DX-C8-3	8	3.5	10.1	−0.47	3.0	8.1	−0.16
	qGL9-1	DX-C9-4	9	4.9	14.4	0.29	5.0	14.0	0.27
Grain width	*qGW8*	DX-C8-15	8	2.6	9.8	−0.064	2.9	10.1	−0.069
	qGW10	DX-C10-10	10	4.8	18.5	−0.092	3.9	12.3	−0.12

wild rice genome in the CSSLs was 87.94%. A few chromosomal regions (nine molecular markers) were not covered by the CSSLs, consistent with results obtained in previous studies (Tian et al., 2006; Qiao et al., 2016; Kubo et al., 2002). Using the same Dongxiang wild rice accession as the donor parent, Tian et al. (2006) found that the genome coverage was only 67.5%, which is significantly lower than the 87.94% coverage obtained in this study. In another study, the coverage of Chr. 3 was only 27.4%, which the authors assumed was associated with a pollen-fertility QTL located on Chr. 3 (Cai and Morishima, 2002). In the present study, the coverage of Chr. 3 was 99.51%. We believe that the differences between these studies could be explained by two factors. First, we used more primers on Chr. 3 for MAS (30) than the authors of the previous study (10). Second, and more importantly, we used a different recurrent parent. The recurrent parent selected by Tian et al. (2006) was an *indica* rice variety, Guichao 2, whereas we used a *japonica* variety, Nipponbare. However, in this study, we did not investigate the genotype at BC_5F_2-BC_5F_5. At the time, we believed that we had a sufficiently large BC_5F_1 population to obtain a set of ideal CSSLs. However, a few chromosomal regions (nine molecular markers) were not covered by the CSSLs. If we had performed genotype analysis at BC_5F_2-BC_5F_5, we would have improved the quality of the CSSLs. This experience will help us design more comprehensive experiments for CSSL construction in the future.

Although the average genomic coverage reached 87.94%, the

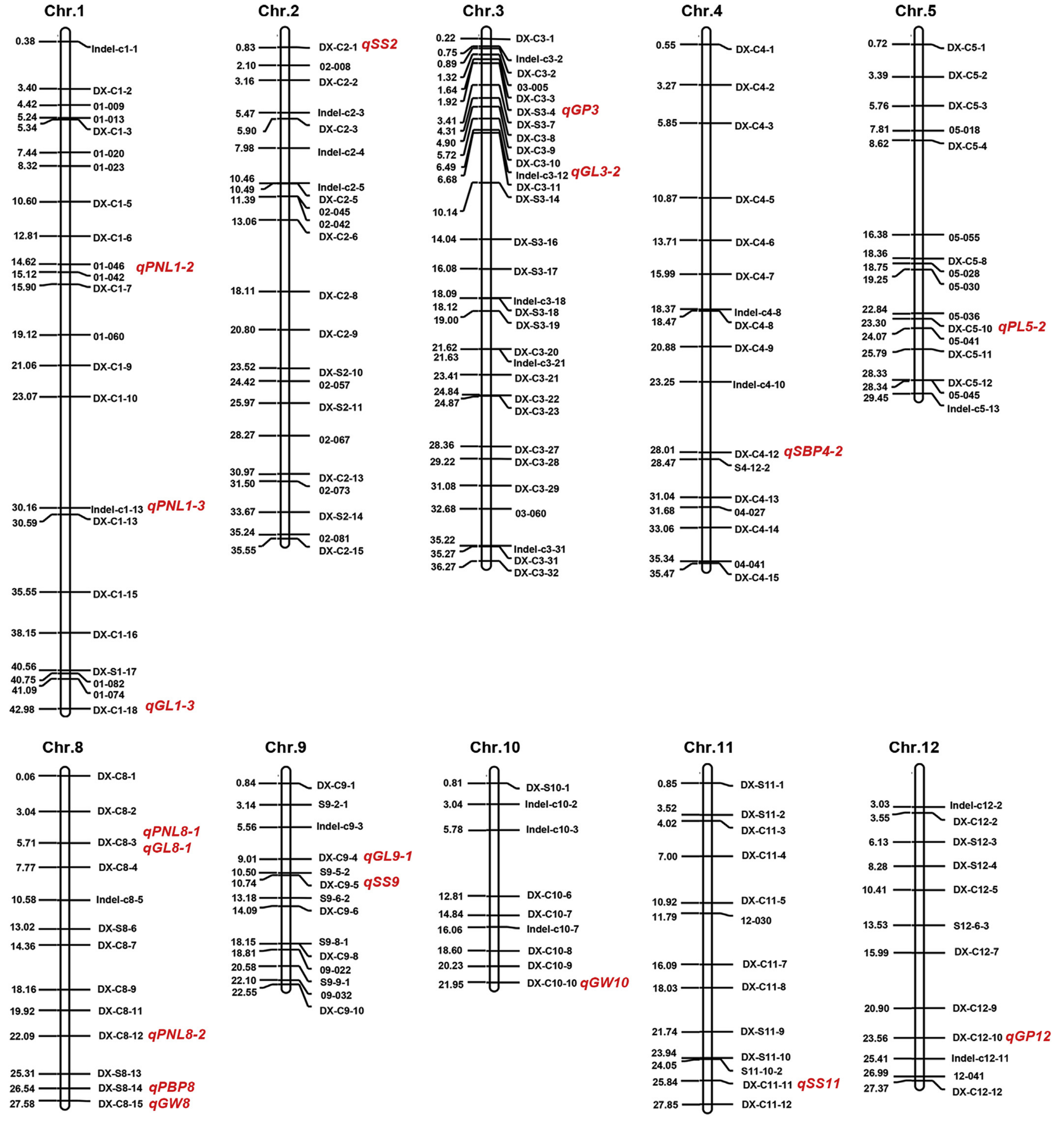

Fig. 5. Map locations of the QTLs associated with panicle-related traits detected at both sites. Only the chromosomes containing QTLs are displayed. The definitions of the abbreviations for the symbols representing the QTLs are listed in Table 3. The position of each marker is based on the physical distance shown to the left of each chromosome, and the molecular marker is shown on the right. The red characters represent the names of the QTLs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

coverage of Chr. 5, 10, and 12 was only 67–69%, which might have been due to the small number and uneven distribution of markers on these chromosomes. The presence of regions without coverage in this set of CSSLs might be because MAS was not performed until the BC_3 generation. The initiation of MAS at an earlier generation, such as BC_2, should make it possible to fill the gaps in this CSSL set and cover the entire wild rice genome. In addition, 29 of the 368 lines screened had an average number of introgressed segments from the Dongxiang wild rice genome that was greater than 30. This finding might have been obtained due to the lack of a successful cross in the BC_1-BC_3 generations, which would have resulted in these lines remaining in the BC_3 or an earlier generation. Therefore, we did not include these 29 lines in the CSSLs.

In contrast to many previous studies, in which an *indica* rice variety was generally selected as the parent (Qiao et al., 2016; Brondani et al., 2002), we selected a *japonica* variety as the recurrent parent. An important reason for the selection of *indica* rice varieties in previous studies is that sterility genes can be discovered from wild rice and used to produce sterile lines. The world-famous three-line hybrid rice, which has resulted in the production of more rice for China and the global population than ever before, is cultivated using this approach. *Japonica* rice exhibits substantial differences in plant morphology, ecological adaptability and genome composition compared with *indica* rice. Traditionally, *japonica* rice has been grown in northern China, and *indica* rice has been planted in southern China. In recent years, *japonica* rice has been planted in more southern areas in China than before due to its improved taste compared with *indica* rice. All common wild rice resources are grown in the south, where it is wet and warm, and the phenotypes and genomes of these resources are more similar to those of *indica* rice than to those of *japonica* rice. Among the common wild rice resources, Dongxiang wild rice grows in the most northern areas, and Dongxiang wild rice might be more genetically similar to *japonica* rice than other wild rice species are. The use of Dongxiang wild rice as the donor parent and the construction of CSSLs to improve the existing *japonica* rice varieties by enabling them to adapt to the southern planting region would be of great significance for expanding the ecologically suitable planting area of *japonica* rice and meeting the needs of industrial development.

Nipponbare is a *japonica* variety that was bred in the 1960s. Because Nipponbare is a reference *japonica* rice variety, there is abundant genomic sequence and annotation information available. In this study, Nipponbare was selected as the recurrent parent due to the comprehensiveness of its genomic sequence information, which facilitates the design of molecular markers and genotyping analyses for the construction of CSSLs. Although the agronomic traits of this variety are not as desirable as those of the varieties currently used for breeding, it is a good parent material. The BC_5F_6 generation in this study included some lines with performance-associated agronomic traits that were superior to those of other lines, including thicker stems, strong lodging resistance, improved yield traits, and high temperature resistance (data not shown). This finding confirms reports that wild rice with poor agronomic traits contains many excellent genes (Xiao et al., 1996, 1998), and this finding warrants further in-depth research.

In this study, we used Nipponbare as the recipient to construct Dongxiang wild rice ILs. These CSSLs can serve as a powerful research population for the discovery of genes that control important traits in wild rice and, particularly, for the cloning of genes underlying quantitative traits. Although the construction of CSSLs takes a long time, once the population is constructed, the genetic data can be reused many times for different phenotypes and environments and can be used to discover many genes derived from the superior traits of the donor parent.

Here, 18 QTLs controlling eight traits were detected at two sites. Among these QTLs, some contained genes that have been cloned, and some loci have been reported but contain genes that have not been cloned. For grain shapes, due to their high heritability, easy phenotypic determination, and the importance of their traits, many related genes, such as *GS3*, *GW5,* and *GW8* (*OsSPL16*), have been cloned (Mao et al., 2010; Wang et al., 2012; Liu et al., 2017). In this study, we detected four grain length-related QTLs and two grain width-related QTLs. Among these QTLs, the genes located at the *qGL3-2* and *qGW8* positions, which are *OsGL3b* and *GW8*, respectively, have been cloned (Wang et al., 2012; Yu et al., 2018), and the other four QTLs for grain shape traits have not been reported. The panicle neck length, which is an important panicle trait in the breeding of rice panicles (Xu et al., 2005), is a domestication-related trait (Xiong et al., 1999). The panicle of wild rice and rice landraces has a long neck (> 15 cm in length), whereas that of modern rice cultivars is generally short (< 4 cm), which can lower the panicle's center of gravity and thereby increase the risks of panicle breakage and plant lodging (Pan et al., 2004). In this study, we detected four QTLs for panicle neck length; among these, the loci of *qPNL1-2* and *qPNL1-3* have been reported, but the genes have not been cloned (Xiong et al., 1999; Qiao et al., 2008).

5. Conclusions

In conclusion, we constructed 104 CSSLs of *O. rufipogon* in a *japonica* rice background. The CSSL population covered approximately 87.94% of the wild rice genome, and the average introgressed segment length was 3.3 Mb. Each CSSL contained an average of four introgressed segments. In addition, we identified 18 panicle trait-related QTLs from the *O. rufipogon* donor. The genes of some of these QTLs have been cloned (*qGL3-2* and *qGW8*), and some of the QTLs were previously reported (*qPNL1-2* and *qPNL1-3*). However, the majority of the QTLs are novel. The CSSLs described herein could provide a powerful population for detecting favorable genes from common wild rice and for creating germplasm resources for *japonica* rice breeding.

Contributions

XM, LH and ML designed the study. BH and JT analyzed the phenotypic data. BH, JT, JZ, DC and LG contributed to genotype analysis. ML and HZ made crossing and backcrossing. XM, BH and JT performed QTL analysis and wrote the manuscript. All authors read and approved the final manuscript.

Declaration of competing interestCOI

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.041.

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