Identification of Rice Chromosome Segment Substitution Line Z322-1-10 and Mapping QTLs for Agronomic Traits from the F_3 Population

F.M. Zhao[1,2], Y. Tan[1], L.Y. Zheng[1], K. Zhou[1], G.H. He[1], Y.H. Ling[1],
L.H. Zhang[2] and S.Z. Xu[2]*

[1]Laboratory of Application and Safety Control of Genetically Modified Crops, Rice Research Institute,
Southwest University, Chongqing, China, 400716
[2]Department of Botany and Plant Sciences, University of California, Riverside, CA, 92521, USA

Chromosome segment substitution lines (CSSLs) are powerful tools to combine naturally occurring genetic variants with favorable alleles in the same genetic backgrounds of elite cultivars. An elite CSSL Z322-1-10 was identified from advanced backcrosses between a *japonica* cultivar Nipponbare and an elite *indica* restorer Xihui 18 by SSR marker-assisted selection (MAS). The Z322-1-10 line carries five substitution segments distributed on chromosomes 1, 2, 5, 6 and 10 with an average length of 4.80 Mb. Spikelets per panicle, 1000-grain weight, grain length in the Z322-1-10 line are significantly higher than those in *Nipponbare*. Quantitative trait loci (QTLs) were identified and mapped for nine agronomic traits in an F_3 population derived from the cross between *Nipponbare* and Z322-1-10 using the restricted maximum likelihood (REML) method in the HPMIXED procedure of SAS. We detected 13 QTLs whose effect ranging from 2.45% to 44.17% in terms of phenotypic variance explained. Of the 13 loci detected, three are major QTL (*qGL1*, *qGW5-1* and *qRLW5-1*) and they explain 34.68%, 44.17% and 33.05% of the phenotypic variance. The *qGL1* locus controls grain length with a typical Mendelian dominance inheritance of 3:1 ratio for long grain to short grain. The already cloned QTL *qGW5-1* is linked with a minor QTL for grain width *qGW5-2* (13.01%) in the same substitution segment. Similarly, the previously reported *qRLW5-1* is also linked with a minor QTL *qRLW5-2*. Not only the study is important for fine mapping and cloning of the gene q*GL1*, but also has a great potential for molecular breeding.

Keywords: chromosome segment substitution line, QTL mapping, rice, yield, yield component trait

Introduction

Rice (*Oryza sativa* L.) serves as a main staple food for half of the world population. High yield has always been the target of rice breeding. However, yield-related traits are governed by many quantitative trait loci (QTLs) and easily influenced by genetic backgrounds

*Corresponding author; E-mail: shizhong.xu@ucr.edu

and environments (Liao et al. 2001). To date, large numbers of QTLs for yield and its components have been reported (Xing and Zhang 2010) and some major QTLs have been cloned, such as *GS3* (Fan et al. 2006), *GL3. 1* (Peng et al. 2012), *GW2* (Song et al. 2007), *qGW8* (Wang et al. 2012), *GW5* (Weng et al. 2008) and *Gn1a* (Ashikari et al. 2005). These studies facilitated a better understanding of the regulation mechanism of grain yield in rice. However, insufficient numbers of effective favorable alleles may impede the application of these QTLs in rice breeding programs (Zheng et al. 2011).

Chromosome segment substitution lines (CSSLs) can be used both for QTL mapping and for new cultivar development as female lines due to the unique feature of uniform genetic background (Furuta et al. 2014). Therefore, development of CSSLs is a useful step to improve breeding programs (Zamir 2001). Some CSSLs have been developed and used in isolation of alleles of rice target QTL (Ebitani et al. 2005; Wan et al. 2006; Yang et al. 2015). However, the QTLs were derived from naturally occurring variation, and favorite alleles are often separated in different germplasms. The use of a wider range of genetic variation is important (Takai et al. 2007) but the limited number of crossovers do not allow us to explore all naturally occurring genetic variants in rice. Therefore, novel CSSLs need to be developed.

In this study, we report a novel elite CSSL, named Z322-1-10, which was developed through advanced backcrosses between the recipient cultivar Nipponbare (a *japonica* rice cultivar) and the donor Xihui 18 (an elite *indica* rice restorer) by SSR markers MAS. We will describe how the CSSL Z322-1-10 was developed, how to perform QTL mapping using an F_3 population derived from this line and the statistical method used to perform data analysis.

Materials and Methods

Materials

An elite CSSL named Z322-1-10 was used as the material in the study. Z322-1-10 was derived from advanced backcrosses between Nipponbare as the recipient parent and Xihui 18 as the donor by SSR markers MAS. The Z322-1-10 line in BC_3F_4 is stable in inheritance. Nipponbare was an elite *japonica* rice cultivar which has been fully sequenced. The Xihui 18 is an elite *indica* rice restorer bred by our laboratory.

The material used for QTL analysis was the F_3 population derived from this cross with 150 individuals. The F_3 seeds were collected from 5 F_2 individual plants with long grains derived from the cross of Nipponbare and Z322-1-10 in Hainan Province of China in 2013. All the F_3 seeds and its parents (Nipponbare and Z322-1-10) were first pre-germinated by soaking in water at 25 °C for 48 h, and raised in the greenhouse at Southwest University, Chongqing, China, in March of 2014. Thirty seedlings of Z322-1-10 and 30 seedlings of Nipponbare along with 150 seedlings of the F_3 population were transplanted in each plot of the experiment field 40 days after sowing with a spacing of 16.67 cm between hills and 26.67 cm between rows, with conventional field cultivation management.

Development of Z322-1-10

The Z322-1-10 line was developed by crossing Nipponbare with Xihui18. The resulting F_1 hybrid was backcrossed with Nipponbare to produce 7 BC_1F_1 followed by successive backcrossing to Nipponbare to obtain 53 BC_3F_1 progenies. A total of 530 plants from the 53 lines were genotyped with 182 polymorphism SSR markers evenly distributed among all 12 chromosomes of the rice genome. A line named Z205-1-2 with nine substitution segments carrying 21 markers, including 10 heterozygous markers and 11 homozygous ones was found possessing excellent panicle phenotype. Eventually, 20 plants from each advanced generations of Z205-1-2 were used to select target segments with both marker and phenotype selection. Finally, a stable CSSL named Z322-1-10 in BC_3F_4 was identified. The line carried five substitution segments. The estimated length of the substitution segments was calculated using the method proposed by Paterson et al. (1991). The chromosome map was generated using the Mapchart 2.2 software package.

Measurement of agronomic traits

Heading date, plant height and leaf morphology between Z322-1-10 and Nipponbare are very similar and these traits are not of interest in this study. We measured the yield and yield related trait along with grain shape traits in the study. At maturity, 10 plants at the 3rd–7th hills of the middle two rows in Z322-1-10 and Nipponbare plots were individually harvested and measured for grain length, grain width, ratio of length to width, 1000-grain weight, panicle number per plant, spikelets per panicle, grain number per panicle, and seed-setting ratio. *t*-tests were conducted between Z322-1-10 and Nipponbare for these traits. The same traits mentioned above were also measured for the 150 F_3 individuals for QTL mapping. Simple statistical analysis such as *t*-test for the traits between Nipponbare and Z322-1-10 and the skewness and kurtosis in the F_3 population were conducted with intrinsic statistical functions within Excel 2003.

Extraction of rice DNA

Leaves of 150 F_3 individuals and their plants were sampled to extract DNA at 20 d after transplanting. DNA extraction was conducted using the CTAB method of McCouch et al (1988) with a slight modification.

PCR amplification and QTL mapping

The total reaction volume of PCR was 12.5 μL, including 1.25 μL of 10×PCR buffer, 0.65 μL of 25 mmol L^{-1} MgC1$_2$, 0.5 μL of 2.5 mmol L^{-1} dNTPs, 8.0 μL of ddH$_2$O, 1.0 μL of 10 μmol L^{-1} primers, 1.0 μL of template DNA, and 0.1 μL of 5 U μL^{-1} *Taq* DNA polymerase. After heating the PCR reaction to 94 °C and holding it for 3 min to complete the process of initial denaturation, the cycle at 94 °C was repeated for 20 s, 56 °C for 20 s, and 72 °C for 40 s, 35 times. Finally, the extension step at 72 °C was undertaken for 7

min. The PCR products were observed after the treatments of 10% native-PAGE and rapid silver dying (Panaud et al. 1996). The lanes of Nipponbare were marked by "–1", those of the heterozygotes were marked by "0", and the lanes of Xihui18 were marked by "1". Missing values were replaced by "." Lanes of each marker located on the substitution segments, together with the phenotypic values of each individual of the F_3 population were used to identify the putative QTLs using the restricted maximum likelihood (REML) method implemented in the HPMIXED procedure in SAS (SAS Institute Inc. 2009). The p-value of 0.05 was used as the threshold to decide whether a QTL is linked with these markers in the substitution segments. Detected QTLs were named by a letter q followed by abbreviation of the trait-chromosome-serial number.

Results

Identification of the substitution segments

Based on the development of the Z322-1-10 line, three plants of Z322-1-10 were used to validate the target segments with all 11 markers located on the substitution segments and detect the residual segments with 36 other markers on the 12 chromosomes. The results showed that Z322-1-10 is homozygous with no residual segments being detected. The Z322-1-10 carries five chromosome substitution segments with a total length of 24.01 Mb and an average length of 4.80 Mb. The five segments are distributed on chromosomes 1, 2, 5, 6 and 10. The substitution segments on chromosomes 1 and 2 are short arm-RM3426 -RM1167 and RM6834-RM1920-RM112, respectively, whose estimated lengths were 2.40 Mb and 4.80 Mb. The substitution intervals on chromosomes 5, 6 and 10 are nS-SR505-RM289, RM7412-RM494 and RM7020-RM6673, respectively, and corresponding lengths are 6.97 Mb, 3.50 Mb and 6.34 Mb (Fig. 1).

Phenotypic analysis of Z322-1-10

Compared to Nipponbare, panicle length, number of spikelets per panicle and 1000-grain weight in Z322-1-10 are significantly increased by 1.96 cm, 42.7 and 2.44 g, respectively, for the three traits. The seed setting ratio of Z322-1-10 (78.79%) is significantly lower than that of Nipponbare (89.97). No differences were observed for panicle number per plant and grain number per panicle between CSSL Z322-1-10 and Nipponbare (Table 1). Grain length and ratio of grain length to grain width in Z322-1-10 are significantly higher than those in Nipponbare. Whereas, the grain width of Z322-1-10 is narrower than that of Nipponbare (Table 1), Therefore, Z322-1-10 has a high potential in QTL pyramiding because of these desirable traits.

Frequency distributions of traits in the F_3 population

To understand the genetic behaver of QTLs responsible for the phenotypic variation in Z322-1-10, frequency distributions of traits in the F_3 population are displayed. The values
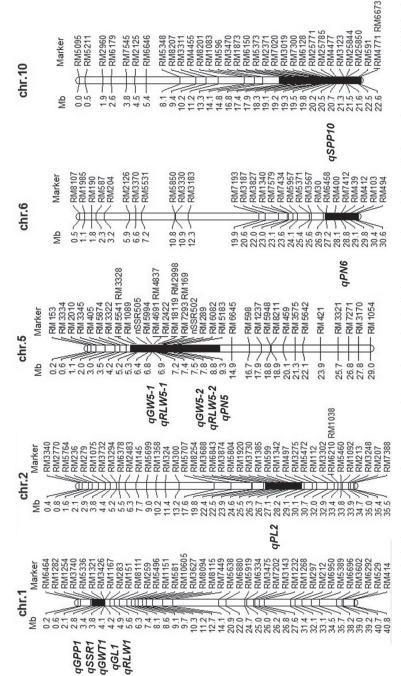
Figure 1. Chromosome substitution segments of Z322-1-10 and QTLs detected. Substitution segment intervals from Xihui18 on the genome are highlighted in dark. *qPL*: QTL for panicle length; *qSPP*: QTL for spikelets per panicle; *qGPP*: QTL for 1000-grains per panicle; *qSSR*: QTL for seed setting ratio; *qGWT*: QTL for 1000-grain weight; *qGW*: QTL for grain width; *qGL*: QTL for grain length; *qRLW*: QTL for grain length to width ratio

Table 1. Descriptive statistics of 10 important agronomic traits of Z322-1-10,
Nipponbare and their F$_3$ population

Traits	Parent		F$_3$ population			
	Nipponbare	Z322-1-10	mean±SD	Range	Skew	Kurt
PL (cm)	24.36±1.59[a]	26.32±1.78[b]	26.53±1.94	21.7–32	0.07	−0.14
PN	11.60±2.29[a]	9.70±1.79[a]	11.15±3.11	4–27	1.29	4.39
SPP	141.30±12.92[a]	184.00±24.94[b]	186.42±41.15	95–327	0.53	0.99
GPP	127.20±13.11[a]	144.40±23.48[a]	127.35±46.49	0–237	−0.53	0.42
SSR (%)	89.97±2.99[a]	78.79±10.77[b]	69.22±22.64	0–96.64	−1.18	0.85
GWT (g)	21.33±0.75[a]	23.77±1.02[b]	22.30±2.89	14.2–27	−3.29	1.37
GL (mm)	7.00±0.05[a]	8.41±0.23[b]	7.87±0.40	7.00–8.81	0.04	−0.64
GW (mm)	3.17±0.03[a]	3.06±0.02[b]	3.01±0.17	2.6–3.8	0.98	2.78
RLW	2.21±0.02[a]	2.77±0.03[b]	2.61±0.21	2.04–3.15	0.03	−0.03

Note: number followed by ([a]) and ([b]) indicate significant difference at P<0.05; PL – panicle length; PN – panicle number; SPP – number of spikelets per panicle; GPP – grains per panicle; SSR – seed setting rate; GWT – 1000-grain weight; GL – grain length; GW – grain width; RLW – ratio of length to width.

of skewness and kurtosis are important characters to evaluate for the assumed normal distributions. As shown in Table 1 and Fig. S1*, the skewness values of most traits are close to 0 and the kurtosis values are mostly positive, suggesting that the distribution of these traits except grain length and seed setting ratio are continuous and approximately normal. These results indicate that most traits in Z322-1-10 are controlled by multiple QTLs.

Among all the traits, only grain length shows a bimodal distribution (Fig. S1). The peak value of short grain length is distributed in the range of 7.00–7.53 mm with the Nipponbare value at about 7.00 mm. The peak values of long grain length ranges from 7.54 to 8.81 mm with the Xihui18 value at 8.41 mm (Fig. S1a and Table1). Chi-square test showed that the ratio of plant number with long grain length (111) to that with short grain length (38) fitted to a 3:1 segregation ratio ($\chi^2 = 0.0014 < \chi^2_{0.05,1} = 3.84$). These results suggested that grain length in Z322-1-10 is controlled by a single dominant gene.

QTL mapping for nine agronomic traits

A total of 13 QTLs were identified in the five substitution segments of Z322-1-10, including one QTL for each of the following traits: plant length, number of spikelets per panicle, grain number per panicle, seed setting ratio, 1000-grains weight and grain length; two QTLs for panicle number per plant and grain width; and three QTLs for the length to width ratio (Fig. 1 and Table 2). QTL *qPL2* is located on chromosome 2 linked with marker RM1920. This QTL has an effect of 0.79 cm and explains 17.28% of the total

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 2. QTLs identified for nine agronomic traits in rice from the F_3 population

Traits	QTL	Chr.	Linked marker	Estimated effect	Var%	P-value
Plant length	*qPL2*	2	RM1920	0.79	17.28	0.015
Panicle number	*qPN5*	5	RM289	−0.83	7.42	0.014
Panicle number	*qPN6*	6	RM7412	−0.77	6.45	0.045
Spikelets per panicle	*qSPP10*	10	RM7300	10.50	7.25	0.041
Grains per panicle	*qGPP1*	1	RM3426	8.21	3.56	0.027
Seed setting rate	*qSSR1*	1	RM3426	−3.49	2.77	0.048
Grain weight	*qGWT1*	1	RM3426	0.34	2.45	0.049
Grain length	*qGL1*	1	RM3426	0.20	34.68	<.0001
Grain width	*qGW5-1*	5	nSSR505	−0.13	44.17	<.0001
Grain width	*qGW5-2*	5	RM289	0.06	13.01	0.005
Ratio of Length-width	*qRLW1*	1	RM3426	0.05	9.27	0.002
Ratio of Length-width	*qRLW-5-1*	5	nSSR505	0.10	33.05	<.0001
Ratio of Length-width	*qRLW5-2*	5	RM289	−0.05	7.18	0.035

phenotypic variance. QTL *qPN5,* located on chromosome 5 and linked with marker RM289, controls panicle number with an effect of −0.83 that explains 7.42% of the phenotypic variance. QTL *qPN6,* located on chromosome 6 and linked with RM7412, controls panicle number with an effect of −0.77 and explaining 6.45% of the phenotype variance. QTL *qSPP10,* located on chromosome 10 and linked with RM730, is responsible for spikelets per panicle with an effect of 10.50 at the Nipponbare backgrounds and explaining 7.25% of phenotypic variance. QTL *qGPP1,* located on chromosome 1 and linked with RM3426, controls grain number per panicle, can increase grain number to 8.21 per panicle and explain 3.56% of the phenotypic variance. QTL *qSSR1* controls seed setting ratio and is located on the chromosome 1 and linked with RM3426. This QTL explains 2.77% of the phenotypic variance. Minor effect QTL *qGWT1* and major effect QTL *qGL1* are both located on the chromosome 1 and linked with RM3426. The minor QTL increases grain weight by 0.34 g and explains 2.45% of the phenotypic variance. The major QTL increase grain length by 0.20 mm and explains 34.68% of the phenotypic variance. The same substitution segment on chromosome 5 carries two QTLs with effects in opposite direction, where *qGW5-1* is linked with nSSR505 and decreases grain width by 0.13 mm per grain and it explains 44.17% of the phenotypic variance, *qGW5-2* is linked with RM289 and has an effect of 0.06 that explains13.01% of phenotypic variance. QTL *qRLW1*, located on chromosome 1 and linked with RM3426, controls the length to width ratio with an effect of 0.05 and explaining 9.27% of the phenotypic variance. QTL *qRLW-5-1*, located on the same substitution segment of chromosome 5 and linked with marker nSSR505, controls the length to width ratio with an effect of 0.10 and explaining

33.05% of the phenotypic variance. QTL *qRLW-5-2*, located on the same substitution segment on chromosome 5 and linked with marker RM289, has an effect of –0.05 on the length to width ratio and explain 7.18% of the phenotypic variance (Table 2).

Discussion

CSSLs are a set of lines developed by a series of backcrosses of a donor parent to a recipient parent until the recipient parent genome is fully recovered except for one or several chromosome segments from the donor parent (Zamir 2001; Ali et al. 2010). Thus, the use of CSSLs can simplify the study of complex genetic traits (Furuta et al. 2014). Once the CSSL libraries are developed in the genetic background of an elite cultivar, the best performing CSSLs may be released as new cultivars. Furthermore, other CSSLs can be used as parents in a breeding program to transfer the introgressed segments with superior performing genes into the elite cultivars by MAS (Ali et al. 2010). CSSLs also provide tools to broaden the genetic variation for plant breeding (Zamir 2001). They can be introgressed from wild species to cultivate relatives (Ali et al. 2010) and from one subspecies to another in rice. CSSLs developed in the background of an *indica* cultivar with donor segments from japonica varieties. These CSSLs include the one derived from '93–11' (*indica* recurrent parent) × Nipponbare (*japonica* donor) (Wang et al. 2009); Zhenshen 97B (indica recurrent parent) × Nipponbare (*japonica* donor) (Zhao et al. 2009); Examples of CSSLs containing donor segments from an *indica* cultivar in a *japonica* genetic background include: 'Asominori'(*japonica*, recurrent parent) × 'IR24'(*indica* donor) (Wan et al. 2006); 'Koshihikari' (*japonica*, recurrent parent) × 'Nona Bokra'(*indica* donor) (Takai et al. 2007). In our laboratory, we developed a series of CSSLs derived from Nipponbare (*japonica*, recurrent) × Xihui18 (*indica*, donor parent). Here, a novel elite CSSL (Z322-1-10) was identified. Z322-1-10 carries five chromosome substitution segments with a total length of 24.01 Mb and an average length of 4.80 Mb per segment. These segments are distributed among chromosomes 1, 2, 5, 6 and 10. The line carries desirable agronomic traits, such as larger panicle length, grain length, 1000-grains weight, and more spikelets per panicle than its recipient parent Nipponbare.

Understanding the genetic basis of these quantitative traits is a major challenge. Not only can CSSLs be used as basal materials to verify and characterize QTLs identified, but also can be used for additional backcross that enables us to proceed to fine mapping of QTLs (Ebitani et al. 2005). Here, Z322-1-10 was used to backcross its recurrent parent for mapping of QTLs for nine important agronomic traits. A total of thirteen QTLs were identified. Compared to previous researches, *qGW5-1* should be located in the same interval that contains the cloned *GW5* between RM3328 and RMw513 (Weng et al. 2008), QTL *qGW5-2* was mapped by Hu et al. (2012). *qLRW5-1* was previously mapped in a similar region with the *qRLW5* reported by Liu et al. (2015) and Hu et al. (2012). The *qPN6* QTL has also been reported in the same location as *ppp6.1* mapped by Cho et al. (2007). *qGPP1* might be in the same location as the cloned *Gn1* (Ashikari et al. 2005). The remaining nine QTLs were not previously reported. These QTLs will all be important for MAS breeding.

It is worth to mention that there are three major QTLs (*qGL1*, *qGW5-1* and *qRLW5-1*). Two major QTLs (*qGW5-1* and *qRLW5-1*) are closely linked with two minor QTLs (*qGW5-2* and *qRLW5-2*). This leads to a nearly normal distribution of the trait in the F_3 population. To dissect the two major QTLs, we need to further decompose the substitution segment on chromosome 5 into two substitution segments by further backcrossing. However, major QTL *qGL1* is of a single dominance inheritance, whose frequency distribution for grain length in the F_3 population is bimodal, and the ratio long grain to short grain is 3:1. To date, only two major QTLs for grain length (*GS3* and *GL3.1*) on chromosome 3 have been cloned (Fan et al. 2006; Peng et al. 2012). In addition, *qGL1* is different from any other previous mapped QTLs such as *qGL1* linked with RM1(Liu et al. 2015) and *qgl1.1-1.4*, *qgl2* (Shanmugavadivel et al. 2013), *qGL-2* (Wan et al. 2006; Liu et al. 2015), *qgl3.1-3.2* (Shanmugavadivel et al. 2013), *qGL3* (Liu et al. 2015), *qgl*5.1-5.4, *qgl6*, *qGL-9*, *qgl11.1-2*, *qgl12* (Shanmugavadivel et al. 2013; Wan et al. 2006)*, qGL7* and *qGL7-2* (Liu et al. 2015; Wan et al. 2006; Shao et al. 2010)*.* Therefore, the major *qGL1* will be important for further cloning and understanding of the molecular mechanisms in regulating yield production.

A novel CSSL that has many favorable agronomic traits for breeding was identified. It carries five chromosome substitution segments, whose average length is 4.80 Mb. Using the F_3 population derived from the cross of Nipponbare with Z322-1-10, we identified 13 QTLs whose genetic effects explain 2.45% to 44.17% of the phenotypic variance of nine agronomic traits. Three major QTLs were found in the substitution segments, of which *qGL1* as a single dominant gene and has not been cloned. The study will not only be important for fine mapping and cloning of the gene *GL1*, but also for pyramiding breeding to incorporate favorable QTL alleles.

Acknowledgements

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Figure S1*. Distribution of phenotypic traits in F_3 population. N: Nipponbare; Z: Z322-1-10