

Pyramiding QTL for yield-related traits and grain shape in rice using single-segment substitution lines

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Abstract

In this study, six Secondary Single-segment substitution lines (SSSLs) and five double-segment substitution lines (DSSLs) that had higher yield were produced by selective crossing of seven primary SSSLs. The DSSL-D3 had significantly higher (by 9.44%) yield than Huajingxian74, the recurrent parent. With pyramided QTL *qGL3*, *qGWT3*, *qPN* and the *Waxy* locus, the DSSL D2 showed a higher yield (by 8.70%) combined with long and high-quality grain. These results indicated that pyramiding of favourable QTL using SSSLs is feasible to improve rice yield and quality.

Key words: Rice, single-segment substitution lines, yield, grain shape, pyramiding

Gene pyramiding is an important strategy for germplasm improvement. However, it is difficult for quantitative traits. Most of the QTL that have been identified so far are QTL that have a relatively large effect [1]. Individuals of a mapping population are difficult to use directly as parents in a pyramiding program [2]. To address these problems, novel methods have been applied [3, 4]. To date, a large single segment substitution lines (SSSLs) library in rice was constructed [5]. We obtained six SSSLs and five DSSLs whose yields were higher than HJX74 in 2008. The DSSLs were derived from crosses among seven primary SSSLs that carry QTL for yield-related traits and grain shape [6, 7]. In the study, we analyzed the influence on yield of QTL additivity and epistatic interactions between QTL after QTL pyramiding. Six SSSLs and five DSSLs and the recipient rice cultivar

HJX74 were planted in the experimental field of Southwest University, Chongqing, China, on 8 March, 2009, in a randomized complete block design. Each plot was 1.65 m × 1.06 m in dimension. Seedlings were transplanted between plants of 26.4 cm × 16.5 cm. Ten hills in the middle of each plot were sampled for the measurements of panicle number per plant, thousand-grain weight, number of grains per panicle, number of spikelets per panicle, seed set, grain length, grain width after harvest. Finally, all plants in plot were harvested and, together with the sampled 10 plants to measure yield.

Statistical analyses were performed with the software SPSS 12.0 (SPSS Inc., Chicago, IL) and MS Excel. QTL detection referred to the methods of Eshed and Zamir [4]. The additive effect values were calculated as half of the difference for a given parameter between each SSSL and HJX74 [4]. Interaction analysis between QTLs in the DSSLs was performed in accordance with the method of Eshed and Zamir [8]. The interaction effect was estimated as $(HJX74 + DSSL_{ab}) - (SSSL_a + SSSL_b)$ and its significance was determined with Student's *t*-test ($P < 0.01$).

Two QTL for thousand-grain weight, *qTGW3* and *qTGW4*, were located single segment substitution lines S2 and S3, respectively. Two QTL for panicle number per plant, *qPN2* and *qPN6*, were detected on chromosomes 2 and 6, respectively. Two QTL for grain length, *qGL3* and *qGL4*, were detected on

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chromosomes 3 and 4, respectively. Their additive effects are all positive, increasing corresponding traits relative to that of HJX74 (Table 1). In the D3, in which a QTL for thousand-grain weight (*qTGW3*) with an additive effect of 1.15 g on chromosome 3 was pyramided with QTL (*qTGW4*) with an additive effect of 0.87 g, the interaction effect between the two QTLs was -0.85. This indicated that the genetic effect of the double segmental substitution in D3 increased the thousand-grain weight by (1.15 + 0.87 - 0.85) g, namely 1.17 g, relative to HJX74, which was somewhat larger than the increases of 1.15 g from *qTGW3* and 0.87 g from *qTGW4*. In D5, in which a QTL for panicle number per plant (*qPN6*) with an additive effect of 1.61 on chromosome 6 was pyramided with another substitution segment without a significant additive effect on chromosome 4, the interaction effect between the two QTL was -1.35. Therefore, the increase in panicle number per plant was 1.61-1.35, i.e., 0.26. Similarly, after interaction of *qGL3* with *qGL4*, the genetic effects in D3 increased grain length by (0.50 + 0.27 - 0.44) mm, namely 0.33 mm (Table 2).

Only one DSSL (D3) showed a significantly increased yield in 2009. In D3, genetic effects of increasing 1.17g for *qTGW3* and *qTGW4*, together with their interaction effects, were responsible for the increased yield (Table 3). The yield of all the DSSLs was higher than that of the corresponding SSSLs used in the pyramiding (Table 3). For example, the yield of D2 (8.70% increase yield) was higher than that of S2 (4.05% increase yield) or S5 (1.17% increase yield). These results showed that pyramiding favourable genes in a common genetic background is effective, especially for QTL with main effects. In the current study, we identified nine QTL for yield-related traits and grain shape. Owing to the fact that different QTL for yield-related traits with positive additive effects existed in the genetic background of HJX74 in different SSSLs (S1-S6), the increase in yields ranged from 0.19% to 4.05%. Consequently, additive effects of QTL for yield-related traits have an important influence on rice yield.

Interaction between QTL is also important [8].

Table 1. QTL detected for yield traits and grain shape using single-segment substitution lines

Code	Chr.	Substitution interval	Trait	QTL	Mean±SD	A	Probability value
HJX74			Thousand-grain weight (g)		20.87±0.42		
S2	3	RM6146-PSM127		<i>qTGW3</i>	23.17±0.29	1.15	1.28×10 ⁻³
S3	4	RM3217-RM3276		<i>qTGW4</i>	22.60±0.36	0.87	4.45×10 ⁻³
HJX74			Panicle number/plant		5.33±0.08		
S1	2	RM138-PSM125		<i>qPN2</i>	7.33±0.27	1	2.42×10 ⁻⁴
S6	6	RM587-RM510		<i>qPN6</i>	8.67±1.25	1.67	9.79×10 ⁻³
S5	6	RM170-RM510		<i>qPN6</i>	8.56±1.13	1.61	7.99×10 ⁻³
HJX74			Grain length		8.37±0.09		
S2	3	RM6146- PSM127		<i>qGL3</i>	9.36±0.12	0.50	1.38×10 ⁻⁴
S3	4	RM3217-RM3276		<i>qGL4</i>	8.90±0.07	0.27	1.56×10 ⁻³
S4	4	RM451- RM3276		<i>qGL4</i>	8.69±0.05	0.16	5.65×10 ⁻³
HJX74			Grain length-to-width ratio		3.19±0.05		
S2	3	RM6146- PSM127		<i>qRLW3</i>	3.49±0.03	0.15	2.55×10 ⁻⁴
S3	4	RM3217-RM3276		<i>qRLW4</i>	3.47±0.04	0.14	3.26×10 ⁻³
S4	4	RM451-RM3276		<i>qRLW4</i>	3.40±0.01	0.11	3.21×10 ⁻³

A indicates the additive effect

Table 2. Epistatic effects between QTL detected in the double-segment substitution lines

Trait	Code	Chr.	Substitution interval i	Chr.	Substitution interval j	A _i ¹⁾	A _j ²⁾	A _{ij} ³⁾	Probability value
Thousand-grain weight (g)	D3	3	RM6146-PSM127	4	RM3217-RM3276	1.15	0.87	-0.85	2.30×10 ⁻³
Panicle number per plant	D5	6	RM170- RM510	4	RM451-RM3276	1.61	ii	-1.35	8.79×10 ⁻³
Grain length	D2	3	RM6146-PSM127	6	RM170- RM510	0.50	ii	-0.24	7.08×10 ⁻³
Grain length	D3	3	RM6146-PSM127	4	RM3217-RM3276	0.50	0.27	-0.44	4.27×10 ⁻³
Grain length-to-width ratio	D4	3	RM6146-PSM127	4	RM451-RM3276	0.15	0.11	-0.12	3.78×10 ⁻³

Additive effect of substitution segment "i"; 2) additive effect of substitution segment "j"; 3) the epistatic effect of additive-by-additive interaction between substitution segments i and j

Table 3. Multiple comparisons of yield and analysis of QTL pyramiding

Material	Substitution interval		Yield±SD (kg/ha)	Increase (%)	Genetic effect of detected QTL in substitution segment ¹		
					TGW	PN	GL
D3	RM6146-PSM127	RM3217-RM3276	8832.2±92.6a	9.44	1.17		0.33
D2	RM6146-PSM127	RM170- RM510	8772.0±148.9ab	8.70	0.51	1.00	0.26
D1	RM6146-PSM127	RM587-RM510	8563.5±182.9ab	6.12	0.18	0.44	0.41
D4	RM6146-PSM127	RM451- RM3276	8506.0±98.6ab	5.40	0.34		0.16
S2	RM6146-PSM127		8397.0±140.9ab	4.05	1.15		0.50
S3	RM3217-RM3276		8333.0±96.0ab	3.27	0.87		0.27
D5	RM170-RM510	RM451-RM3276	8327.0±124.8ab	3.18		0.26	0.07
S6	RM587-RM510		8271.0±98.6ab	2.49		1.67	
S5	RM170-RM510		8164.5±128.5ab	1.17		1.61	
S1	RM138-Psm125		8111.5±160.3ab	0.51		1.00	
S4	RM451- RM3276		8085.5±177.38ab	0.19			0.16
HJ74			8070.0±174.24b	0.00			

1) PN, panicle number per plant; TGW, thousand-grain weight; GL, grain length;

At least one additive effect existed in all five pairs of digenic interactions detected among the DSSLs (Table 2). Owing to epistatic interactions between the two loci, the additivity of a single QTL effect had a diminishing impact [8]. The present results indicated that the pyramiding of favourable main-effect QTL in a common genetic background is effective if significant negative epistatic interactions are absent. In our study, a significantly increased yield was only displayed in D3 (S2 and S3 pyramided), for which the yield increased by 9.44% relative to that of HJX74. Furthermore, although the yield of the other DSSLs was not increased significantly, some DSSLs showed combined benefits of pyramiding two substitution segments. For example, by pyramiding S2, which carried QTL for grain length (Table 1), and S5, which carried the *Waxy* gene, a regulator of amylose content [9], D2 displayed long grain length and high grain quality simultaneously. These results indicated that pyramiding favourable QTLs using SSSLs is feasible to improve rice yield and quality.

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