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Research Paper

Genetic analysis for rice seedling vigor and fine mapping of a major QTL *qSSL1b* for seedling shoot length

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Seedling vigor is an important agricultural trait as direct-seeded rice technology becomes widely applied. In order to investigate the genetic mechanisms underlying seedling vigor in rice, seeds of 132 recombinant inbred lines (RILs) derived from 93-11 and PA64s, harvested from Lingshui and Hangzhou were cultivated in the nutrient solution, and four indices for seedling vigor were measured including seedling shoot length (SSL), seedling root length (SRL), seedling wet weight (SWW) and seedling dry weight (SDW). Significant correlations were observed among the indices, and also between 1000-seed weight (TSW) and SWW or SDW. Combined with a high-resolution genetic map generated from sequencing of the RILs, 65 quantitative trait loci (QTLs) were detected on all chromosomes with interval of 1.93 Mb on average. Among 57 QTLs for seedling vigor, 28 were detected from seeds harvested in both sites and 33 were first identified. With BC_3F_2 derived from 93-11 and a CSSL harboring segments from PA64s in 93-11 background, a major QTL for SSL, *qSSL1b* was fine mapped within 80.5 kb between two InDel markers. Our study provides a platform for further cloning of the QTL and dissecting the molecular basis for seedling vigor at early seedling stage in rice.

Key Words: rice, QTL, seedling vigor, *qSSL1b*, early seedling stage.

Introduction

Rice direct seeding technology is widely applied in Southeast Asia these years as it requires lower labor costs compared to the conventional transplantation (Abe *et al*. 2012). However, there are many factors limiting the growth and development of direct-seeding rice, such as low emergence rate, difficult weeding and lodging in later growth period (Yang *et al*. 2015). Seedling vigor is the ability of a seed to emerge rapidly from soil or water, mainly reference to seed germination rate and early seedling growth (Huang *et al*. 2004). Seeds with high vigor is important for rice production because it can not only significantly enhance seedling establishment (Luo *et al*. 2007), but also improve the capability to compete against weeds at seedling stage (Rao *et al*. 2007). Therefore, seedling vigor has been paid more attention respect to cultivation techniques and genetic analysis in recent years.

Seedling vigor is a complex agronomic trait with several indicators, such as germination rate, final germination percentage and germination index during seed germination stage (Wang *et al*. 2010), and root length, shoot length, wet weight and dry weight in the early seedling growth process (Redoña and Mackill 1996, Regan *et al*. 1992). Quantitative trait loci (QTL) analysis has been demonstrated a effective way to study complex traits, for example, yield associated traits (Huang *et al*. 2009, Song *et al*. 2007, Wang *et al*. 2015). Recently, the method has also been employed to study seedling vigor in rice. Han *et al*. (2006) identified several QTLs for low-temperature vigor of germination at 14°C using the $F_{2:3}$ populations and deduced that the gene action of low-temperature vigor was most likely to be partially dominant. Germination rate, final germination percentage and germination index were evaluated by Wang *et al*. (2010) with recombinant inbred line (RIL) population, and most of the detected QTLs for rice seedling vigor were found coincide with QTLs for seed weight, seed size or seed dormancy. In Dang's study, 27 QTLs were identified for seedling vigor, and 15 elite parental combinations were designed to improve seedling vigor in rice (Dang *et al*. 2014). Xie *et al*. (2014) mapped eight quantitative trait loci (QTLs) for seedling vigor by using a RIL population, and narrowed two major QTLs, *qSV-1* and *qSV-5c* to 1.13-Mbp and 400-kbp genomic regions, respectively. A major QTL for seedling height on the long arm of chromosome 3 was also fine mapped, including the candidate gene *OsGA20ox1* (Abe

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et al. 2012). In all, these studies have provided useful information for seedling vigor, but there still more researches to be performed in this area.

In this study, we grown 132 RILs derived from 93-11 (*Oryza sativa ssp. indica*) × PA64s (*Oryza sativa ssp. indica*) in a nutrient solution for two weeks, and conducted seedling vigor evaluations by measurement of seedlings' shoot length, root length, wet weight and dry weight. With the help of a high quality genetic map based on the SNPs generated from deep sequencing of the RIL genomes (Gao *et al*. 2013), a total of 57 QTLs were found. Several co-located QTLs share the same location with previous studies. The major QTL, *qSSL1b* was fine mapped within 80.5 kb between two insertion-deletion (InDel) markers. These results give more information of the genetic and molecular basis underlie seedling vigor in rice.

Materials and Methods

Plant materials

The recombinant inbred lines (RILs) presented in this study were developed by a cross between an elite paternal inbred *Oryza sativa ssp. indica* cv. 93-11 and the maternal inbred *Oryza sativa ssp. indica* cv. PA64s (a photo-thermo-sensitive male sterile line). The provided population was developed in the experimental fields at China National Rice Research Institute in Lingshui, Hainan Province, and in Hangzhou, Zhejiang Province, China. To develop a Chromosome segment substitution line (CSSL) containing the QTL for SSL, *qSSL1b* detected both in Lingshui and Hangzhou on chromosome 1, a line of RILs with PA64s genotype in the *qSSL1b* region was selected to backcross with recurrent parent 93-11. Two markers SNP1-202 and SNP1-257 (**Table 4**) were used for marker assisted selection (MAS) of each generation. As a result, a BC_3F_1 line, with 93-11 genetic background exhibiting heterozygous across the entire *qSSL1b* region, was constructed. After self-crossing, a BC_3F_2 population was obtained for fine mapping of *qSSL1b*.

Phenotypic evaluation of rice seedling vigor

Mature seeds of parents and 132 core RILs were harvested in Lingshui (2012) and Hangzhou (2013) and soaked in deionized water overnight at 30°C in the dark. After germination, seeds were put into the holes of 96-well plates and transferred to 35 L plastic pots containing an nutrient solution (pH 5.6) containing 1.5 mM NH_4NO_3, 1.0 mM $CaCl_2$, 1.6 mM $MgSO_4$, 0.5 mM K_2SO_4, 0.3 mM NaH_2PO_4, 0.1 mM Fe-EDTA, 9.5 uM $MnCl_2$, 20 uM H_3BO_3, 0.2 uM $ZnSO_4$, 0.2 uM $CuSO_4$, 0.05 uM Na_2MoO_4 and 0.01 uM Na_2SiO_3. The plants were grown in a greenhouse and the solution was changed once per 3 days. After 15 days, plants were collected and four indices were employed to evaluate seedling vigor: seedling shoot length (SSL), seedling root length (SRL), seedling wet weight (SWW) and seedling dry weight (SDW). For SSL and SRL, three plants of each line were measured. Six plants were gathered for measurement of SWW and SDW. Before measuring SDW, the samples were packed in individual paper bags and kept in an oven at 75°C for two days.

Data analyses and QTL detection

Statistical analysis of the parents and RILs was conducted for each index by SAS (version 8.01). Pearson correlation coefficients were calculated between each pair of all four indices and 1000-seed weight. A recombinant bin map with 2,262 high-quality polymorphic SNP markers was constructed by the re-sequencing parents and 132 core RIL lines (Gao *et al*. 2013). QTL analysis was performed with the R/qtl_1.26-14 (http://www.rqtl.org/) using Composite Interval Mapping (CIM). LOD threshold for each dataset was set based on a permutation test (1,000 permutation, P = 0.05). It was considered as a major effect QTL when its LOD score was larger than 2.5. PEV was estimated by ANOVA, and the QTL nomenclature followed the suggestion of McCouch *et al.* (2008).

Results

Phenotypic variations for seedling vigor and 1000-seed weight of the parents and RILs

For SSL and SRL of seedlings, no significant difference existed between two parents from the seeds harvested in Lingshui while statistically significant difference was found in Hangzhou (**Table 1**, **Fig. 1A–1C**). The SWW and SDW

Table 1. Means (standard deviation), min, max, skewness and kurtosis for SSL, SRL, SWW and SDW during seedling stage in parents and RIL lines

Harvested location	Trait	Parents		RIL population				
		93-11	PA64s	Mean ± SD	Min	Max	Skewness	Kurtosis
Lingshui	SSL	22.74 ± 0.38	23.41 ± 0.39	25.83 ± 4.14	17.05	38.52	0.65	0.54
	SRL	5.13 ± 0.03	5.26 ± 0.08	5.85 ± 1.20	3.82	9.56	0.67	0.56
	SWW	2.02 ± 0.09	1.75 ± 0.07	2.06 ± 0.58	0.95	4.43	1.12	2.22
	SDW	0.24 ± 0.02	0.19 ± 0.01	0.25 ± 0.08	0.11	0.61	1.33	3.80
Hangzhou	SSL	20.25 ± 0.37	25.10 ± 1.84	23.24 ± 3.56	15.80	33.04	0.69	0.43
	SRL	5.51 ± 1.08	7.21 ± 1.08	6.55 ± 1.53	3.34	12.10	0.89	1.04
	SWW	1.84 ± 0.08	1.49 ± 0.06	1.60 ± 0.43	0.53	2.95	0.67	0.97
	SDW	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.05	0.08	0.36	0.71	0.72

SSL: seedling shoot length, SRL: seedling root length, SWW: seedling wet weight, SDW: seedling dry weight. The results of SSL and SRL in parents are presented as the means ± SD of triplicate samples. Results of SDW and SWW are shown as sum of 6 plants.

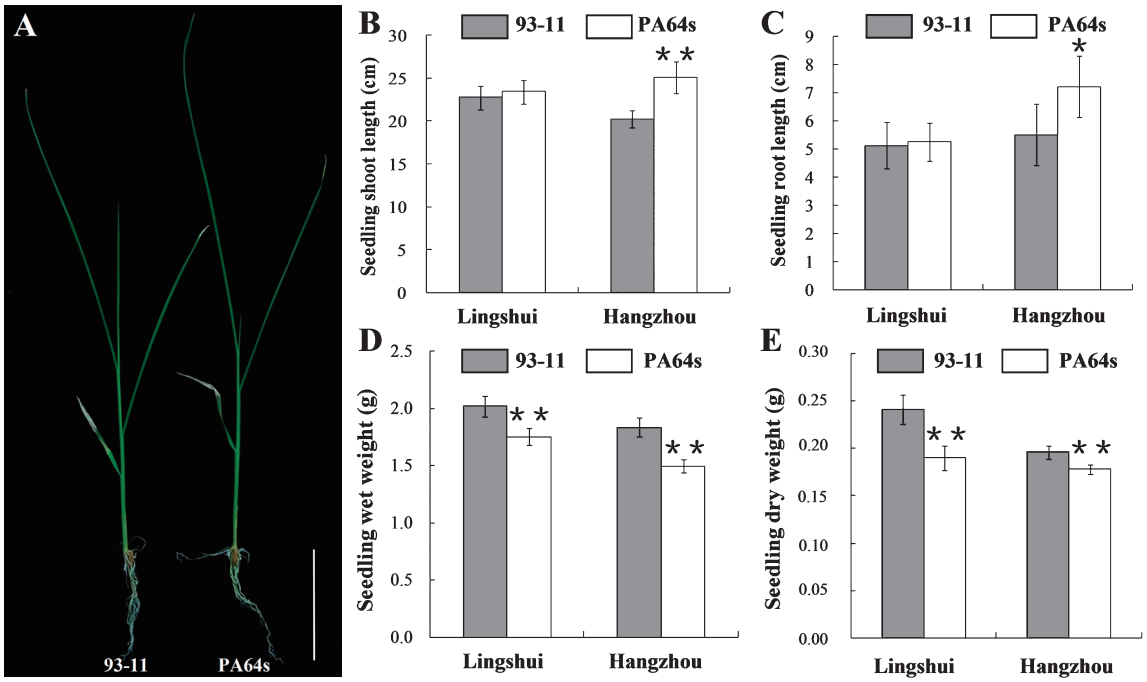

Fig. 1. A. Seedling phenotype of 93-11 and PA64s at early seedling stage from Hangzhou. Bar = 5 cm. B–E. Four seedling vigor indices of 93-11 and PA64s from Lingshui and Hangzhou. Results of SDW and SWW are shown as a total of 6 plants. ** and * on the bars indicate significant difference at the 1% and 5% level, respectively according to *t* test.

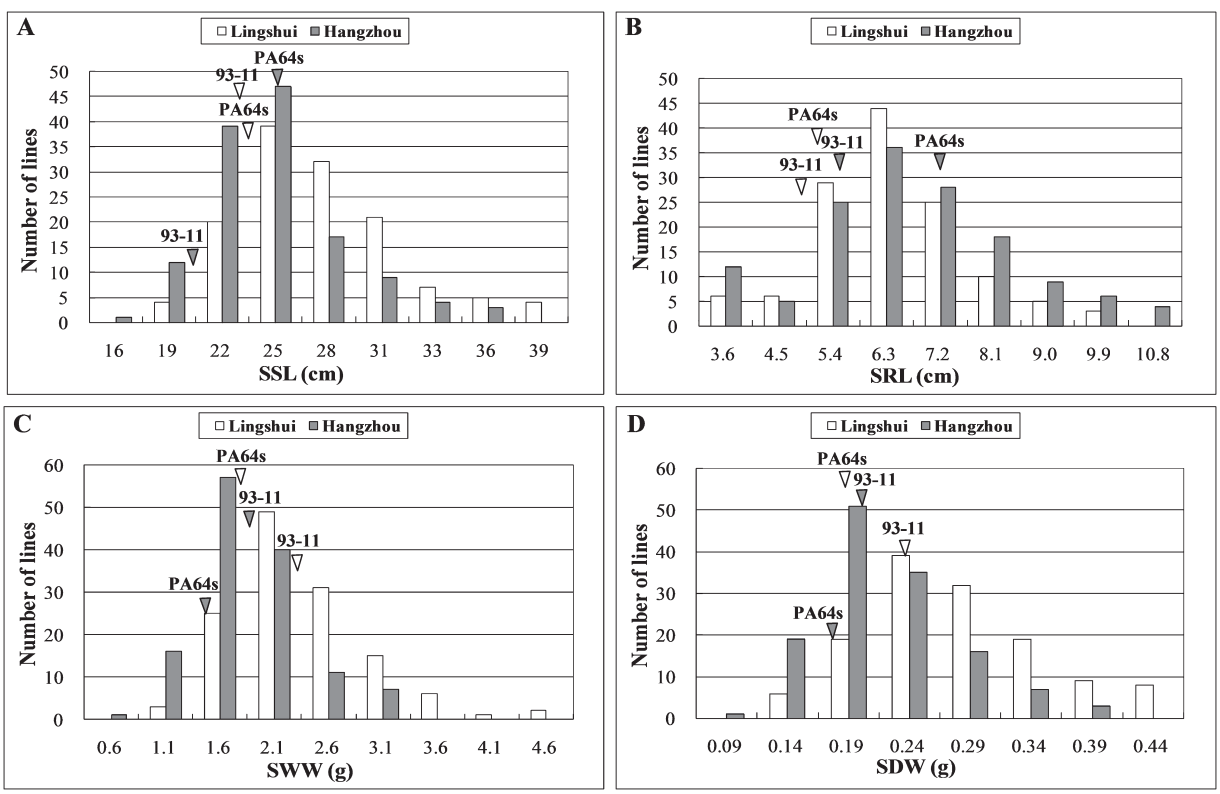

Fig. 2. Frequency distributions of seedling shoot length (SSL) (A), seedling root length (SRL) (B), seedling wet weight (SWW) (C) and seedling dry weight (SDW) (D) among RILs with seeds from Lingshui and Hangzhou in early seedling growth stage. Results of SDW and SWW are shown as a total of 6 plants. The white patterns represent phenotype collected in Lingshui and the grey ones represent those in Hangzhou. White triangles and grey triangles indicate SSL, SRL, SWW and SDW of 93-11 and PA64s from Lingshui and Hangzhou, respectively.

of PA64s seedlings from seeds in both sites reached 80%–90% of 93-11, illustrating seedlings of 93-11 were stronger than PA64s (**Table 1**, **Fig. 1D**, **1E**). The RIL population also showed significant divergence for all the evaluated traits. The mean value of SSL was about 24.50 cm, with the maximum value 38.52 cm and the minimum value 15.80 cm. The minimum value of SRL, SWW and SDW were around 20% of their mean value and the maximum value were about 170% of the mean value in the RIL population (**Table 1**). And the continuous distributions of four seedling vigor traits among the RILs showed significantly transgressive segregation with values either larger or smaller than those of the parents, which revealed that seedling vigor at early seedling stage was controlled by polygene (**Table 1**, **Fig. 2**).

The differences in 1000-seed weight between 9311 and PA64s are displayed in **Fig. 3A** and **3B**. Significant differences of seeds from Lingshui and Hangzhou were found between 9311 and PA64s with respect to 1000-seed weight. Nearly normal distributions were observed in the RIL population for 1000-seed weight from Lingshui and Hangzhou, respectively, indicating the trait was controlled by multigenes (**Fig. 3C**).

Correlation among the four indices and 1000-seed weight

Pair-wise correlation coefficients among the four indices and those indices with 1000-seed weight were presented in **Table 2**. Significant correlations were observed among the four parameters. Considering previous study suggested that seed weight might be correlated with seedling vigor (Cui *et al*. 2002), we also calculated correlation coefficients between 1000-seed weight with the four indices in the RILs and found significant correlations between 1000-seed weight and SSL, SWW, SDW from Hangzhou, and also between 1000-seed weight and SWW, SDW from Lingshui, indicating seed weight contribute to seedling vigor at early seedling stage in this population (**Table 2**).

QTL identification for seedling vigor

As shown in **Table 3** and **Fig. 4**, combined with a high-resolution genetic map based on the SNPs generated from sequencing of the RILs (Gao *et al*. 2013), 57 QTLs well-distributed on all 12 chromosomes were identified for the four indices, including SSL, SRL, SWW and SDW. Among them, 28 QTLs were detected from seeds in both Lingshui and Hangzhou, and 33 QTLs were unreported so far.

Eighteen QTLs were responsible for SSL, and nine of them were detected from two sites. The phenotypic variance explained by a single QTL ranged from 1.1% to 13.6%. One major QTL *qSSL1b* had highest LOD (5.48) from Lingshui and relative higher LOD (3.77) from Hangzhou, with R^2 of 10.5% and 11.2%, respectively. The additive effect of *qSSL1b* showed negative, indicating that the positive allele from PA64s contributed to the length of seedling shoot. Genetic distance of *qSSL1b* was mapped between 117.12 and 133.43 cM, the same locus with *qCSH1*, a previously reported major QTL for seedling height (Han *et al*. 2007).

Seven QTLs associated with SRL were detected on chromosomes 2, 3, 5, 7, 8 and 10, respectively. Most of the positive alleles of QTLs were from 93-11. As seedling root length of seeds from Hangzhou was much longer than that from Lingshui (**Table 1**), only two QTLs were identified from two sites, indicating seedling vigor affected by seeds

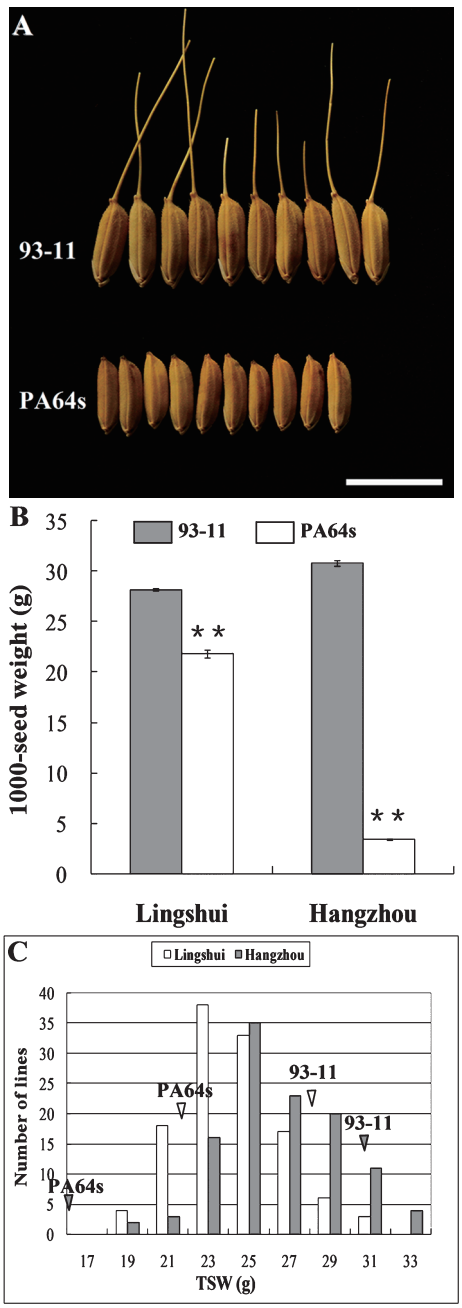

Fig. 3. A. Seeds of 93-11 and PA64s harvested from Lingshui. Bar = 10 mm. B. Comparison of 1000-seed weight of 93-11 and PA64s from Lingshui and Hangzhou. C. Frequency distributions of 1000-seed weight among RILs with seeds harvested from Lingshui and Hangzhou. White triangles and grey triangles indicate 1000-seed weight of 93-11 and PA64s in Lingshui and Hangzhou, respectively.

Table 2. Correlation coefficients among indices of seed vigor measured during seedling stage and 1000-seed weight in 132 RIL lines derived from 93-11 × PA64s

Trait	SSL	SRL	SWW	SDW	TSW
SSL		0.46**	0.87**	0.87**	0.19
SRL	0.60**		0.51**	0.50**	0.07
SWW	0.83**	0.58**		0.95**	0.24*
SDW	0.85**	0.57**	0.94**		0.25*
TSW	0.33*	0.21	0.38**	0.43**	

SSL: seedling shoot length, SRL: seedling root length, SDW: seedling dry weight, SWW: seedling wet weight. TSW: 1000-seed weight. * and ** indicate the 5% and 1% significant level, respectively. Seeds from Lingshui are shown in above diagonal and Hangzhou in below diagonal.

harvested in different environments.

SWW and SDW are closely related traits, and correlation coefficient parameter of these two traits reached more than 90% (**Table 2**). As a result, though 14 QTLs for SWW and 18 QTLs for SDW were identified, most of them shared the same locus. Cluster of *qSWW1c* and *qSDW1c* detected from two sites also contained the major QTL *qSSL1b*.

Detection of QTLs for 1000-seed weight

A total of 8 QTLs were detected for 1000-seed weight in Lingshui and/or Hangzhou, distributing on chromosomes 1, 2, 3, 4, 8, 9 and 11 (**Table 3**, **Fig. 4**). Only one QTL *qTSW2* was identified in both sites, which revealed the trait was environment dependent. The QTL *qTSW1* was mapped to the locus overlapped with *qSWW1a*, consistent with significant correlation between 1000-seed weight and SWW.

Fine mapping of qSSL1b

We produced a CSSL carrying the major QTL *qSSL1b* from PA64s in 93-11 background by a repeated backcrossing to 93-11. Then phenotypic character was measured in F_2 population including 1,233 individuals derived from a CSSL-*qSSL1b* BC_3F_1 line exhibiting heterozygous across the entire *qSSL1b* region screened with markers SNP1-202 and SNP1-257. By comparing the sequences of the parents, four InDel markers were developed (**Table 4**). Combining the genotype and phenotype of homozygous individuals, the QTL was fine mapped between two InDel markers IND1-3 and IND1-4 within around 80.5-kb region of the long arm of chromosome 1 (**Fig. 5**), where 16 annotated genes were identified by Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/), including one encoding retrotransposon and four coding for expressed protein (**Table 5**).

Discussion

Seedling vigor is mainly determined by genetic inheritance, conditions of seed storage and environments of germination and early seedlings growth stage (Sun *et al*. 2007, Yang *et al*. 2015). In this study, seeds of the 132 RILs from Lingshui and Hangzhou were stored at 4°C before germination, and seedlings were grown in a nutrient solution in greenhouse. By these measurements, the influence of non-genetic factors on QTL detection for seedling vigor can be greatly reduced. McKenzie *et al*. (1980) reported that seedling traits measured under controlled laboratory conditions were correlated with those under field conditions, demonstrating that QTLs identified for seedling vigor in laboratory could be

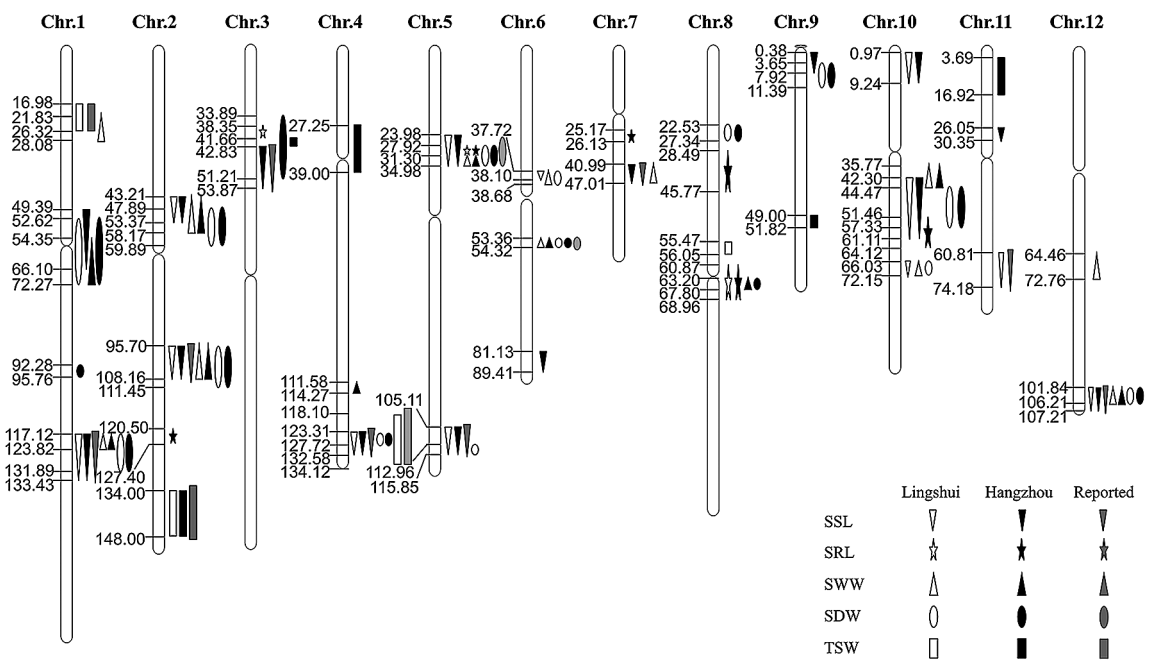

Fig. 4. Location of QTLs for SSL, SRL, SWW, SDW and TSW from Lingshui and Hangzhou on the genetic map. SSL: seedling shoot length, SRL: seedling root length, SDW: seedling dry weight, SWW: seedling wet weight, TSW: 1000-seed weight.

Table 3. QTLs for SSL, SRL, SDW and SWW during seedling stage and QTLs for TSW of seeds from Lingshui and Hangzhou

Trait	QTL	Chr.	Genetic distance (cM)	Seeds from 2012 Lingshui			Seeds from 2013 Hangzhou			Reference
				LOD	%Var	Add.	LOD	%Var	Add.	
SSL	*qSSL1a*	1	49.39–66.10				4.42	13.6	1.233	
	unnamed	1								http://archive.gramene.org/db/qtl/
	qSSL1b	1	117.12–133.43	5.48	10.5	–1.039	3.77	11.2	–1.193	
	qCSH1	1								Han *et al*. 2007
	qSSL2a	2	43.21–53.37	2.64	1.8	0.542	2.77	1.1	0.324	
	qSSL2b	2	95.70–108.16	3.00	3.6	–0.789	3.23	2.1	–0.374	
	qCSH2	2								Han *et al*. 2007
	qSSL3	3	42.83–53.87				2.70	4.9	0.787	
	qSL3-2	3								Cao *et al*. 2002
	qSSL4	4	123.31–132.58	2.55	3.1	0.228	4.36	3.5	0.606	
	qPHS4	4								Abe *et al*. 2012
	qSSL5a	5	23.98–34.98	4.20	4.3	–0.677	3.10	3.3	–0.614	
	qSV-5	5								http://archive.gramene.org/db/qtl/
	qSSL5b	5	105.11–115.85	2.71	6.2	–1.047	2.73	2.9	–0.632	
	unnamed	5								Dang *et al*. 2014
	qSSL6a	6	37.72–38.10	2.72	2.9	–0.747				
	qSSL6b	6	81.13–89.41				2.87	2.6	–0.574	
	qSSL7	7	40.99–47.01				2.55	4.3	0.732	
	unnamed	7								Anandan *et al*. 2016
	qSSL9	9	0.377–7.92				2.75	3.1	0.605	
	qSSL10a	10	0.97–9.24	3.47	7.7	1.221	2.96	7.8	1.038	
	qSSL10b	10	42.30–61.11	2.82	2.5	0.637	3.05	5.2	0.790	
	qSSL10c	10	66.41–72.15	3.43	9.7	1.298				
	qSSL11a	11	26.05–30.35				2.90	5.7	0.848	
	qSSL11b	11	60.81–74.18	2.70	9.2	–1.272				
	qSL11									Cao *et al*. 2002
	qSSL12	12	101.84–107.21	3.70	3.7	–0.804	2.94	7.4	–0.844	
	qCSH12	12								Han *et al*. 2007
SRL	*qSRL2*	2	120.50–127.40				3.18	6.4	–0.377	
	unnamed	2								http://archive.gramene.org/db/qtl/
	qSRL3	3	38.35–41.66	2.91	4.4	0.025				
	qSRL5	5	27.92–31.30	3.72	6.1	0.376	3.54	5.4	0.356	
	unnamed	5								http://archive.gramene.org/db/qtl/
	qSRL7	7	25.17–26.13				3.02	3.9	–0.252	
	unnamed	7								http://archive.gramene.org/db/qtl/
	qSRL8a	8	28.49–45.77				2.50	6.6	0.411	
	qSRL8b	8	56.05–68.96	3.36	4.6	0.255	2.53	3.8	0.303	
	qSRL10	10	51.46–64.12				3.59	11.7	0.569	
SWW	*qSWW1a*	1	21.83–28.08	3.20	7.2	0.154				
	unnamed	1								http://archive.gramene.org/db/qtl/
	qSWW1b	1	54.35–72.27				2.87	6.6	0.112	
	unnamed	1								http://archive.gramene.org/db/qtl/
	qSWW1c	1	117.12–123.82	4.72	5.5	–0.138	3.77	3.7	–0.079	
	unnamed	1								http://archive.gramene.org/db/qtl/
	qSWW2a	2	43.21–58.17	4.42	1.8	0.075	2.59	1.2	0.046	
	qSWW2b	2	95.70–108.16	4.46	2.6	–0.093	2.82	1.9	–0.058	
	unnamed	2								http://archive.gramene.org/db/qtl/
	qSWW4	4	111.58–114.27				3.08	4.8	0.091	
	unnamed	4								http://archive.gramene.org/db/qtl/
	qSWW5	5	31.49–34.98	5.73	1.5	–0.020	2.89	3.1	–0.033	
	qSWW6a	6	37.72–38.68	4.48	3.2	–0.109				
	qSWW6b	6	53.36–54.32	2.95	4.1	–0.127	3.14	3.5	–0.034	
	unnamed	6								http://archive.gramene.org/db/qtl/
	qSWW7	7	40.68–47.11				2.74	3.8	0.082	
	unnamed	7								http://archive.gramene.org/db/qtl/
	qSWW8	8	63.20–67.80				2.55	3.6	0.086	
	qSWW10a	10	35.77–44.47	4.41	4.3	0.076	2.91	4.9	0.030	
	qSWW10b	10	66.41–72.15	4.33	13.8	0.218				
	qSWW12	12	101.84–106.21	3.23	6.9	–0.061	3.12	5.3	–0.101	

Table 3. (continued)

Trait	QTL	Chr.	Genetic distance (cM)	Seeds from 2012 Lingshui			Seeds from 2013 Hangzhou			Reference
				LOD	%Var	Add.	LOD	%Var	Add.	
SDW	*qSDW1a*	1	52.62–72.27				3.12	7.7	0.015	
	unnamed	1								http://archive.gramene.org/db/qtl/
	qSDW1b	1	92.28–95.76				2.66	6.2	–0.013	
	qSDW1c	1	117.12–131.89	5.27	7.5	–0.018	4.13	8.1	–0.011	
	qSDW2a	2	47.89–59.89	5.56	1.2	0.008	3.04	1.3	0.006	
	qSDW2b	2	95.70–111.45	5.81	2.7	–0.013	5,22	2.4	–0.011	
	unnamed	2								http://archive.gramene.org/db/qtl/
	qSDW3	3	33.89–51.21				4.49	2.3	0.012	
	qSDW4	4	123.31–127.72	5.35	3.5	0.005	4.15	3.4	0.009	
	qSDW5a	5	27.92–34.00	6.08	5.1	–0.001	3.85	10.2	–0.004	
	unnamed	5								Huang *et al*. 2004
	qSDW5b	5	112.96–115.27	2.98	5.9	–0.019				
	qSDW6a	6	37.91–38.68	5.81	3.3	–0.015				
	qSDW6b	6	53.36–54.32	3.70	3.1	–0.015	3.31	2.7	–0.004	
	qTDW6-2	6								Cui *et al*. 2002
	qSDW8a	8	22.53–27.34	6.33	1.7	0.001	2.50	2.2	0.001	
	qSDW8b	8	63.96–64.93				3.30	4.1	0.011	
	qSDW9	9	3.65–11.39	5.30	2.8	0.002	2.59	3.2	0.009	
	qSDW10a	10	44.47–57.33	5.79	1.9	0.010	2.83	1.9	0.007	
	qSDW10b	10	66.03–72.15	3.75	11.9	0.027				
	qSDW12a	12	64.46–72.76	3.99	10.2	–0.017				
	qSDW12b	12	101.84–106.21	4.20	2.0	–0.011	3.38	6.4	–0.013	
TSW	*qTSW1*	1	16.98–26.32	3.11	6.9	0.633				
	gw1a	1								Hua *et al*. 2002
	qTSW2	2	134.00–148.00	3.61	12.9	0.896	3.52	12.9	0.972	
	qTGWT-2-2	2								Zhuang *et al*. 2002
	qTSW3	3	41.85–42.63				3.41	4.8	0.597	
	qTSW4a	4	27.25–39.00				2.80	5.3	0.611	
	qTSW4b	4	118.10–134.12	2.84	13.1	0.884				
	tgwt4	4								Lin *et al*. 1996
	qTSW8	8	55.47–60.87	2.62	3.1	0.435				
	qTSW9	9	49.00–51.82				2.97	2.2	–0.405	
	qTSW11	11	3.69–16.92				3.23	8.8	0.788	

SSL: seedling shoot length, SRL: seedling root length, SDW: seedling dry weight, SWW: seedling wet weight, TSW: 1000-seed weight.

Table 4. Primers for InDel and SNP markers developed

Primer	Forward (5′-3′)	Reverse (5′-3′)	Type
SNP1-202	TGGATGGGAACTGTCAATTTG	TGTTCAAGGCAGCAAAGAGG	SNP
IND1-1	ACATGGGCTAGCTGACAGGCGATC	TGGTGAGAACCCGGCACAACG	InDel
IND1-2	GTGGGACAGACAGCCTCAGC	GCAATAATTCAGGAAATAATTGGG	InDel
IND1-3	TTCCAGCTGACTGGTGACTGCTC	AGCTTGAGCTGCAATCCCACAG	InDel
IND1-4	TGGTATCAATCGTTGATGGATGC	CAGATCCATGGATGTCTCCAAAC	InDel
SNP1-257	GTTTGGACCAGGAGTACGAGG	TCAAGACCAGCATGAGCATATAGAG	SNP

applied in the field. In addition, experiments conducted in laboratory have its advantages: environment factors can be easily controlled and experiments can be conducted at any time. However, owing to different environmental conditions in Lingshui and Hangzhou during filling stage, only 28 of 57 QTLs for seedling vigor were shared by the seeds harvested from two locations.

It was proposed that seedling vigor is greatly influenced by seed weight or seed size (Milosevic *et al*. 2010). Therefore, correlation coefficients between 1000-seed weight and the four indices were analyzed and significant correlations were found between 1000-seed weight and SWW or SDW, suggesting seed weight may contribute to seedling vigor at seedling stage (**Table 2**). And we found 15 QTL clusters for seedling vigor and TSW, revealing they may be controlled by the same locus.

Seedling vigor is defined as seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings (McDonald 1993). Therefore, the most crucial step in QTL mapping for seedling vigor is the evaluation and screening of the quantitative traits (Wang *et al*. 2010). We only measured SSL, SRL, SWW and SDW during early seedling growth stage because they were identified as representative indicators for seedling vigor (Redoña

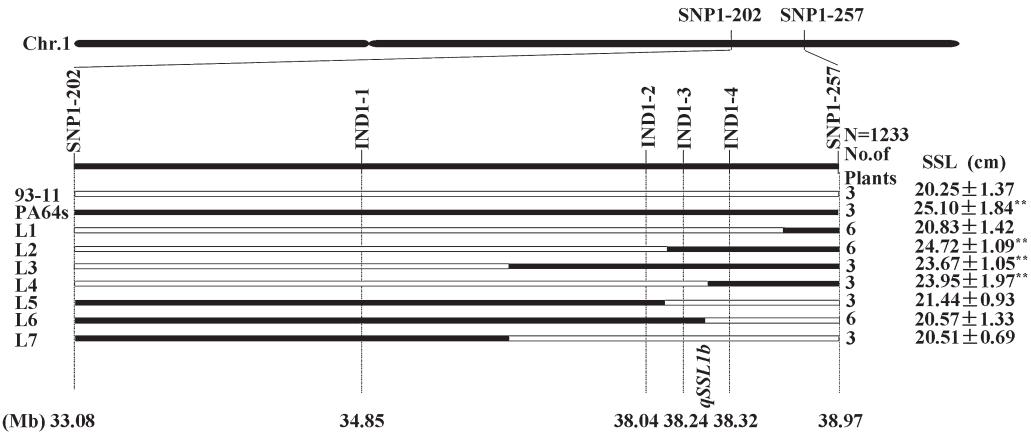

Fig. 5. Graphical genotypes of BC_3F_2 generation and location of *qSSL1b*. White and black boxes indicate homozygous regions for 93-11 and PA64s, respectively. Mean seedling shoot length (SSL) and standard deviation values were included. ** indicate significant difference ($P < 0.01$) between 93-11 and PA64s or BC_3F_2 individual by *t* test.

Table 5. Annotated genes included in the 80.5 kb region for *qSSL1b*

Gene ID	Annotation
LOC_Os01g65870	expressed protein
LOC_Os01g65880	nodulin MtN3 family protein, putative, expressed
LOC_Os01g65890	DNA repair metallo-beta-lactamase, putative, expressed
LOC_Os01g65900	chitin-inducible gibberellin-responsive protein, putative, expressed
LOC_Os01g65902	apocytochrome f precursor, putative, expressed
LOC_Os01g65904	expressed protein
LOC_Os01g65920	F-box/LRR-repeat protein 2, putative, expressed
LOC_Os01g65940	expressed protein
LOC_Os01g65950	thioesterase family protein, putative, expressed
LOC_Os01g65970	transcription factor HBP-1b, putative, expressed
LOC_Os01g65980	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
LOC_Os01g65986	DUF803 domain containing, putative, expressed
LOC_Os01g65992	expressed protein
LOC_Os01g66000	NADH dehydrogenase I subunit N, putative, expressed
LOC_Os01g66010	amino acid transporter, putative, expressed
LOC_Os01g66020	protein kinase family protein, putative, expressed

and Mackill 1996, Regan *et al*. 1992). Previous study also found that germination rate (speed) and early seedling growth were correlated in rice (Cui *et al*. 2002).

Based on a high quality genetic map from sequencing of the RIL genomes (Gao *et al*. 2013), a total of 57 QTLs were identified for seedling vigor during early seedling growth stage. So far, other researchers have identified several major QTLs for seedling vigor authentically (Dang *et al*. 2014, Huang *et al*. 2004, Liu *et al*. 2014, Wang *et al*. 2010) and some QTLs were fine mapped to a narrow region and even cloned (Abe *et al*. 2012, Fujino *et al*. 2008, 2011, Xie *et al*. 2014, Yano *et al*. 2012). Actually, 24 QTLs detected in our study shared the same loci with previously identified QTLs. For example, *qSSL4*, a QTL detected for seedling shoot length was consistent with *qPHS4*, a QTL for height of seedlings mapped between markers RM3534 and RM349 in Abe's study (Abe *et al*. 2012). The QTL for seedling dry weight on chromosome 6, named as *qSDW6b*, shared the same locus with *qTDW6-2* reported by Cui *et al.* (2002). In addition, 33 QTLs were first detected here for seedling vigor, such as loci for SWW and SDW on chromosome 12. Owing to high density of SNP markers in our genetic map, most QTLs were detected in a relatively narrower region compared with previous studies (1.93 Mb vs 8.80 Mb on average). However, we did not detect a QTL covering *OsGA20ox1*, a gene reported to increase plant height and leaf sheath length at the initial growth stage (Yano *et al*. 2012).

In the study, we identified and delimited a major QTL for seedling shoot length, *qSSL1b* within 80.5 kb region on chromosome 1, where 16 genes were annotated (**Table 5**), including the candidate gene for *ph1*, a major locus affecting plant height in rice encoding a chitin-inducible gibberellin-responsive protein (Kovi *et al*. 2011). Certainly, a larger genetic population is required for further fine mapping and cloning of the QTL. Meanwhile, series of InDel markers developed here will help improve rice seedling vigor by marker assisted selection (MAS) in future.

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