Mapping of QTLs controlling seedling establishment using a direct seeding method in rice

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Low-temperature is one of the most common environmental stresses that affect plant growth and development and places a major limit on plant productivity. Tolerance to low-temperature is an important objective of rice breeding programs in temperate areas and at high altitudes in tropical and sub-tropical areas. Vigorous rice growth during the seedling stage at low-temperature is an important characteristic for stable seedling establishment (SES) in direct seeding methods, in which rice is sown directly into a flooded field. The aim of this study was to identify QTLs controlling SES using 3 mapping populations of backcrossed inbred lines (BILs) with Arroz Da Terra and Italica Livorno as the donor parents. SES was evaluated by a field evaluation system in this study, which is used in current rice breeding programs because of the complexity of environmental conditions in SES in rice cultivation in a paddy field. A total of 9 chromosomal regions for SES were identified, which explained 10.9% to 25.6% of total phenotypic variation. For all of QTLs except for *qSES5-2*, alleles from the donor parents increased phenotypic values. These QTLs should be useful for the improvement of SES in rice breeding programs in low-temperature regions.

Key Words: direct seeding method, *Oryza sativa* L., QTL, seedling establishment.

Introduction

Undesirable environmental conditions cause critical damage to plant growth and yield. These conditions could induce a wide range of plant responses, including altered gene expression and cellular metabolism, retarded growth, and reduced yield. Low-temperature is one of the most common environmental stresses that affect plant growth and development and places a major limit on plant productivity. The improvement of tolerance to low-temperature is an important objective in plant breeding programs.

Rice, *Oryza sativa* L., originated in a tropical region. Tolerance to low-temperature is an important objective of rice breeding programs in temperate areas, at high altitudes in tropical and sub-tropical areas, and in areas with a cold irrigation water supply (Nakagahra *et al.* 1997). Low-temperature causes the retardation of rice growth, from seed germination

to grain-filling stages. In the seedling (Peterson *et al.* 1978) and booting stages (Dai *et al.* 2004, Kuroki *et al.* 2007, Saito *et al.* 1995) in particular, rice plants are sensitive to low-temperature, leading to the decreased rice yields.

Tolerance to low-temperature is a quantitative trait, which is controlled by many genes and affected by natural environmental conditions. Many QTLs have been identified as being involved in tolerance and response to low-temperature in rice, including seed germination stage (Fujino *et al.* 2004, Ji *et al.* 2009, Jiang *et al.* 2006, Miura *et al.* 2001, Zhang *et al.* 2005a), seedling stage (Andaya and Mackill 2003a, Baruah *et al.* 2009, Jiang *et al.* 2008, Lou *et al.* 2007, Zhang *et al.* 2005b), and booting stage (Andaya and Mackill 2003b, Dai *et al.* 2004, Kuroki *et al.* 2007, Saito *et al.* 1995, Suh *et al.* 2010, Takeuchi *et al.* 2001, Zhou *et al.* 2010). These QTLs could enhance tolerance to low-temperature in rice. However, it is not clear which is the most effective QTL for the improvement of tolerance to low-temperature for rice varieties in local regions, and which of them contribute to the variation of this trait in particular regions under local environmental conditions. For the application of QTLs identified using different genetic backgrounds under different natural

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environmental conditions in rice breeding programs in particular regions, it is necessary to validate them under local environmental conditions. Consequently, an important aim of rice breeding programs in local regions is to evaluate the response of a genotype under local environmental conditions in which the cultivars will be grown.

Hokkaido (42–45°N latitude) is the northernmost region of Japan and one of the northern-limits of rice cultivation in the world. Cool temperature during rice growth is a typical climatic condition in Hokkaido. In rice breeding programs in this region, tolerance to low-temperature during rice growth is the most important objective. Vigorous rice growth during the seedling stage under low-temperature is an important character for stable seedling establishment (SES) in direct seeding methods, in which rice is sown directly into flooded fields in Hokkaido. Seeds are sown in early May, when the temperature is still low for rice growth. Delayed and decreased emergence of rice seedlings from water because of low-temperature greatly increases seedling mortality (Peterson *et al.* 1978). Low SES leads to decreases in the number of plants grown in a paddy field, resulting in serious decreases in yield and grain quality.

For the development of new cultivars for direct seeding methods in local regions with low-temperature, introduction of stable SES is a priority in rice breeding programs. The phenotype of SES is involved in continuous growth from seed germination to early seedling growth. Furthermore, not

only low-temperature but also other environmental factors, including soil conditions and anaerobic conditions due to flooding, cause a decrease in SES in rice production. However, it is unclear which environmental factors play major roles in mortality during SES.

The identification of the number and magnitude of gene effects could contribute to a better understanding of the genetic control of SES, facilitating the development of new cultivars with vigorous SES. The objective of this study was to identify QTLs controlling SES using 3 mapping populations of backcrossed inbred lines (BILs) with Arroz Da Terra and Italica Livorno as the donor parents. In this study, SES was evaluated by the field evaluation system used in the current rice breeding programs in Hokkaido. A total of 9 chromosomal regions for SES were identified. The relationships between the 9 QTLs identified in this study and known QTLs are discussed.

Materials and Methods

Plant materials

To evaluate varietal differences in SES, 24 rice varieties, including landrace and breeding types from Hokkaido, were used (Table 1). In addition, 3 varieties from Europe were used. These varieties were cultivated in a paddy field at Kamikawa Agricultural Experimental Station in 2009 (Pippu, Hokkaido, Japan).

Table 1. Variations of emergence of seedlings in soil (ESS) and emergence of seedlings from water (ESW) among rice varieties from Hokkaido

Variety	Origin	Breeding	ESS (%)		ESW (%)		Group
			NG	G	NG	G	
Arroz Da Terra	Portugal	—	84.7 ± 3.1	89.3 ± 4.6	72.0 ± 4.0	83.3 ± 4.2	I
Dunguang Shali	Hungary	—	77.3 ± 4.2	87.3 ± 1.2	70.0 ± 9.2	82.7 ± 4.2	I
Italica Livorno	Italy	—	66.0 ± 2.0	64.0 ± 5.3	60.7 ± 3.1	60.0 ± 7.2	I
Akage	Hokkaido	Landrace	60.7 ± 8.1	61.3 ± 5.0	54.0 ± 8.0	52.7 ± 8.1	II
Fukoku	Hokkaido	1935	56.7 ± 1.2	76.0 ± 6.9	50.0 ± 5.3	63.3 ± 12.2	II
Iburiwase	Hokkaido	Landrace	52.0 ± 2.0	55.3 ± 5.0	46.0 ± 2.0	55.3 ± 6.1	II
Kitaibuki	Hokkaido	1993	66.7 ± 7.0	74.7 ± 7.6	46.0 ± 2.0	66.0 ± 4.0	II
Tannemochi	Hokkaido	1983	63.3 ± 9.0	70.0 ± 7.2	42.7 ± 12.1	48.0 ± 5.3	III
Chinkobouzu	Hokkaido	1924	62.7 ± 3.1	66.7 ± 4.2	42.0 ± 5.3	58.7 ± 6.1	III
Daichinohoshi	Hokkaido	2003	48.0 ± 7.2	56.0 ± 14.0	40.0 ± 7.2	54.0 ± 8.7	III
Yukimaru	Hokkaido	1993	69.3 ± 15.0	83.3 ± 7.6	39.3 ± 13.6	66.0 ± 10.6	III
Kirara397	Hokkaido	1988	55.3 ± 5.0	58.7 ± 4.2	39.3 ± 4.2	47.3 ± 4.6	III
Hoshinoyume	Hokkaido	1996	44.0 ± 5.3	70.0 ± 6.9	35.3 ± 2.3	61.3 ± 5.0	III
Hayamasari	Hokkaido	1988	60.0 ± 15.1	78.7 ± 8.1	34.7 ± 19.2	69.3 ± 8.1	III
Himehonami	Hokkaido	1966	47.3 ± 11.7	68.7 ± 7.0	34.7 ± 12.2	60.0 ± 3.5	III
Kitaake	Hokkaido	1983	53.3 ± 2.3	77.3 ± 7.0	34.7 ± 4.6	72.7 ± 8.1	III
Nanatsuboshi	Hokkaido	2001	50.7 ± 11.0	77.3 ± 11.5	34.0 ± 9.2	64.7 ± 4.2	III
Nourin No.20	Hokkaido	1941	66.0 ± 10.0	70.7 ± 1.2	33.3 ± 8.1	58.7 ± 4.2	III
Eiko	Hokkaido	1942	44.7 ± 4.2	69.3 ± 11.7	30.0 ± 5.3	56.7 ± 15.3	III
Shiokari	Hokkaido	1963	44.0 ± 3.5	74.7 ± 8.1	29.3 ± 7.6	63.3 ± 9.0	III
Hoshimaru	Hokkaido	2006	42.7 ± 3.1	63.0 ± 7.1	29.3 ± 4.2	55.3 ± 7.6	III
Shinsetsu	Hokkaido	1954	50.7 ± 5.0	73.3 ± 3.1	29.3 ± 8.1	62.0 ± 3.5	III
Ishikari	Hokkaido	1971	52.0 ± 18.3	60.0 ± 6.0	26.0 ± 6.9	41.7 ± 13.1	III

Data represent mean ± SD of triplicates. The evaluation was performed in 2010.

Breeding varieties are shown with their year of registration.

To identify QTLs controlling SES, we used 3 mapping populations of BILs using *Italica Livorno* and *Arroz Da Terra* as the donor parents and *Hayamasari* and *Hoshinoyume* as the recipient parents. Three populations of BILs were developed by the single-seed descent (SSD) method using the procedure described by Fujino *et al.* (2004). In brief, the F_1 plants between the recipient and donor parents were backcrossed with the recipient to produce BC_1F_1 seeds. BILs developed from the resultant BC_1F_1 plants by the SSD method. The HyI population, BC_1F_6 , was derived from the cross between *Hayamasari* showing weak SES and *Italica Livorno* that exhibits vigorous SES, *Hayamasari/Italica Livorno//Hayamasari*, and consisted of 122 lines (Fujino *et al.* 2004). The HsA population, BC_1F_6 , was derived from the cross between *Hoshinoyume* showing weak SES and *Arroz Da Terra* with vigorous SES, *Hoshinoyume/Arroz Da Terra//Hoshinoyume*, and consisted of 89 lines (Fujino and Sekiguchi 2005). The HsI population, BC_1F_6 , was derived from the cross between *Hoshinoyume* and *Italica Livorno*, *Hoshinoyume/Italica Livorno//Hoshinoyume*, and consisted of 96 lines, which was developed in this study. In 2007 and 2008, these populations and their parents were cultivated in a paddy field at HOKUREN Agricultural Research Institute (Naganuma, Hokkaido, Japan). Because each BIL showed large variation in heading date (Fujino and Sekiguchi 2005), only lines that reached seed maturity were used for SES

evaluation, with 82, 71, and 83 lines in the HyI, HsA, and HsI populations, respectively. All seeds were stored at room temperature until use.

Evaluation of SES

Evaluation of SES was performed in 2008, 2009, and 2010 in a field at Kamikawa Agricultural Experimental Station (Pippu, Hokkaido, Japan). This field evaluation system is used in the current breeding programs at Kamikawa Agricultural Experimental Station (Fig. 1). Fifty seeds of each line were subjected to the experiment. Seeds were wrapped by seedertape at 10 seeds per 10 cm (Fig. 1A). The seeds were sown in experimental trays (30 cm $\times$ 60 cm) filled with paddy-field soil to a depth of 1 cm in early May (Fig. 1B). Then, the tray was placed in a paddy field (Fig. 1C). After setting the tray, irrigation water was allowed to flood the field for the period of the experiment. For the evaluation of SES, seeds with or without pre-germination treatment were used to elucidate the effect of pre-germination treatment on SES, denoted hereafter as G- and NG-, respectively. Firstly, seeds were soaked in water at 11°C for 6 days for all experiments. Then, for pre-germination treatment, seeds were placed in an incubator at 28°C for 14 hours before sowing. To evaluate SES, we measured 2 traits, emergence of seedlings in soil (ESS) and emergence of seedlings from water (ESW), because growth stages of individuals varied from

Fig. 1. Photograph of seedling establishment in the field evaluation system. (A) Seeds were wrapped by seeder tape at 10 seeds per 10 cm. (B) The seeds were sown in experimental trays. (C) The tray was placed in a paddy field. (D) Ten and (E) 30 days after sowing. (F) Phenotype of seedling in each SES group. Left to right: *Arroz Da Terra*, *Italica Livorno*, *Akage*, *Iburiwase*, *Hoshinoyume*, and *Hayamasari*.

2-leaf to 3-leaf seedlings in the evaluation. The degree of tolerance for ESS was measured on the basis of the number of plants for which second leaves emerged from the soil surface. The degree of tolerance for ESW was measured on the basis of the number of plants where third leaves emerged above the flood water surface. Thirty days after sowing, a total of 4 traits were measured for SES: G-ESS, G-ESW, NG-ESS, and NG-ESW (Fig. 1E). The evaluation of varietal difference among rice varieties from Hokkaido was performed in 2010. The evaluations of SES in the HyI, HsA, and HsI populations were performed in 2008/2009, 2008/2009, and 2009, respectively.

DNA analysis

Total DNA was extracted from the leaves of the BILs and parental lines using the CTAB method with modifications (Murry and Thompson 1980). PCR amplification, gel electrophoresis, and detection were performed in accordance with methods described by Fujino *et al.* (2004). A linkage map of the HyI population was constructed previously using 139 RFLP and 47 SSR markers (Fujino *et al.* 2004). Those of the HsA and HsI populations were constructed during the present study using 131 and 129 SSR markers, respectively, distributed over the whole rice genome (Supplemental Fig. 1). Linkage analyses were performed with MAPMAKER/EXP using the Kosambi function (Kosambi 1944, Lander *et al.* 1987).

QTL analysis

Detection of QTLs was conducted by composite interval mapping with QTL Cartographer 2.5 (Wang *et al.* 2010). The threshold to detect QTLs was determined by 1000 permutation tests at a probability level of 0.05. To normalize the variance, the percentage rate of ESS and ESW was arc-sine-transformed and used for QTL analysis. In this study, QTLs for SES located within the 10 Mb chromosomal region were treated for the same QTL because of the resolution potential by QTL analysis using the primary mapping population.

Results

Varietal differences in SES among varieties from Hokkaido

Large variations in SES were detected among the varieties tested (Fig. 1F and Table 1). The mean value of NG-ESS was 57.5%, ranging from 44.0% to 84.7%, while the mean value of NG-ESW was 41.3%, ranging from 26.0% to 72.0%. The values of G-traits were higher than those of NG-traits, suggesting that pre-germination treatment would enhance SES. On the basis of the phenotypic values of each trait, correlations among traits, and the pedigree of each variety, the degree of SES in the varieties was divided into 3 groups, I, II and III (Table 1). Group I showed vigorous SES and included 3 varieties from Europe. Group II showed intermediate SES and included 4 varieties. Group III showed weak SES and included 17 varieties from the current breeding programs. On the basis of these results, we developed 3 mapping populations of BILs using Arroz Da Terra and

Italica Livorno in group I as donor parents.

Phenotypic variations of ESS and ESW in the 3 mapping populations

Continuous variations were observed in 4 traits in each mapping population (Table 2, Fig. 2 and Supplemental Fig. 2). The phenotypic variations in 4 traits in the HsA population in 2008 are shown in Fig. 2A. G-ESS and G-ESW of Arroz Da Terra were 77% and 73%, respectively, which were higher than those of Hoshinoyume at 27% and 12%, respectively. G-ESS and G-ESW of the BILs varied continuously from 6% to 66% and from 2% to 54%, respectively. NG-ESS and NG-ESW of the BILs showed similar variation patterns to G-ESS and G-ESW. Different variation patterns were detected in the HsI population in 2009 (Fig. 2B). G-ESS and G-ESW of Italica Livorno were 67% and 61%, respectively, which were higher than those of Hoshinoyume at 42% and 32%, respectively. G-ESS and G-ESW of the BILs varied continuously from 8% to 64% and from 6% to 56%, respectively. There were transgressive segregants showing weaker values than Hoshinoyume in all traits. In NG-traits, similar variations were observed with weaker transgressive segregants. Similar variation patterns were also detected in another experiment (Supplemental Fig. 2).

QTLs for SES

A total of 9 chromosomal regions controlling SES were detected, which explained 10.9% to 25.6% of total phenotypic variation (Fig. 3 and Table 3). For all of the QTLs except for *qSES5-2*, alleles from the donor parents increased phenotypic values. Among the 9 chromosomal regions, *qSES11* on chromosome 11 was the most effective, which explained 25.6% of total phenotypic variation for NG-SEW in the HsA population. *qSES11* also showed higher effects on G-ESS and NG-ESS in the HsA population and on NG-SES in the HyI population.

qSES1 on chromosome 1 explained 23.0% of the total phenotypic variation for G-SES in the HsA population. *qSES2* on chromosome 2 was identified for NG-SEW in the HsA and HyI populations, and explained 19.3% and 18.1% of the total phenotypic variation, respectively. Two QTLs, *qSES5-1* and *qSES5-2*, were detected on chromosome 5 because the peaks of the LOD curves were more than 13 Mb apart and the LOD curves were independent. *qSES5-1* explained 13.0% to 16.6% of the total phenotypic variation. *qSES5-2* explained 13.8% of the total phenotypic variation for NG-ESS in the HsA population. *qSES6* on chromosome 6 explained 19.3% of the total phenotypic variation for G-SEW in the HsA population. Two QTLs, *qSES7-1* and *qSES7-2*, were detected on chromosome 7 on the basis of LOD curves. *qSES7-1* explained 15.3% of the total phenotypic variance for G-ESW, and *qSES7-2* explained 10.9% for G-SEW and 17.5% for G-ESS of the total phenotypic variation in the HsA population. *qSES10* on chromosome 10 explained 20.6% of the total phenotypic variance for G-SEW in the HsI population.

Table 2. Phenotypic value of 3 mapping populations and their parents in the evaluations in 2008 and 2009

Year	Population	Trait	Parent (%)		Population (%)		
			Donor	Recipient	Maximum	Minimum	Mean
2008	HsA	G-ESS	79.5	24.5	66.0	6.0	36.6
		NG-ESS	71.0	7.5	54.0	2.0	26.3
		G-ESW	71.5	15.0	54.0	2.0	21.3
		NG-ESW	58.5	6.0	44.0	0.0	14.9
	Hyl	G-ESS	51.0	31.0	60.0	12.0	30.8
		NG-ESS	48.0	29.5	66.0	0.0	30.8
		G-ESW	45.0	17.0	44.0	6.0	21.9
		NG-ESW	43.0	13.0	50.0	0.0	21.2
2009	HsA	G-ESS	86.0	42.0	86.0	16.0	46.1
		NG-ESS	79.3	32.0	66.0	8.0	34.9
		G-ESW	82.0	32.0	76.0	12.0	37.8
		NG-ESW	77.3	28.7	56.0	6.0	27.4
	Hyl	G-ESS	67.3	60.0	76.0	14.0	44.2
		NG-ESS	72.0	48.7	82.0	16.0	44.6
		G-ESW	60.7	40.7	70.0	12.0	37.2
		NG-ESW	69.0	36.0	82.0	12.0	39.5
	HsI	G-ESS	67.3	42.0	64.0	8.0	40.6
		NG-ESS	72.0	32.0	72.0	4.0	37.4
		G-ESW	60.7	32.0	56.0	6.0	29.9
		NG-ESW	69.0	28.7	58.0	0.0	31.6

Populations of HsA, Hyl, and HsI indicate the crosses between Hoshinoyume and Arroz Da Terra, Hayamasari and Italica Livorno, and Hoshinoyume and Italica Livorno, respectively.

Fig. 2. Frequency distributions of emergence of seedlings in soil (ESS) and emergence of seedlings from water (ESW). (A) HsA population ($n = 71$) in the evaluation in 2008; (B) HsI population ($n = 83$) in the evaluation in 2009. Closed and open arrowheads indicate the mean values for the donor parents and Hoshinoyume, respectively, with ranges (bar).

Discussion

During the seedling stage of rice, low-temperature causes poor and slow germination, resulting in reduced seedling establishment, non-uniform growth, and reduced yield. Stable SES under natural environmental conditions is an essential trait for rice varieties used for direct seedling methods, especially in local regions with low-temperature. In spite of the significance of tolerance to low-temperature during the seedling stage in direct seeding methods, understanding of the genetic basis of these traits is limited. In Hokkaido, low-

temperature in spring causes seedling mortality in direct seeding methods. Not only low-temperature but also other environmental factors cause decreases in SES. In this study, SES was examined by the field evaluation system used in rice breeding programs for continuous growth from seed germination to seedling development. Using Arroz Da Terra and Italica Livorno as the donor parents, a total of 9 chromosomal regions controlling SES have been identified. These QTLs are useful for the improvement of SES in rice breeding programs in local regions with low-temperature, such as Hokkaido.

Fig. 3. Chromosomal locations of QTLs for seedling establishment (SES). Closed, open, and hatched triangles indicate the positions of the LOD peaks detected in the HsA, HsI, and HyI populations, respectively. Bars indicate the interval with a decrease of 0.5 from the peak LOD value. Black boxes on the chromosome indicate the chromosomal regions of QTLs for SES. The mapped markers were positioned using pseudo-molecule 3.0 in RAP-DB.

SES was evaluated by the field system used in the current rice breeding programs in this study. QTLs for SES identified in this study will be useful for the introduction of high-level SES QTLs by marker-assisted selection (MAS) in rice breeding programs. However, most of these QTLs were detected only in a single year and a single population. The results strongly suggested that the expression of these QTLs was sensitive to environmental conditions during the evaluation of SES and the seed maturing stage. It was also unclear which trait is the most important for SES. In this study, we evaluated the 4 traits as indicators of SES. Because SES is a highly complex trait, it is quite difficult to elucidate the relationships between gene (QTL) effects and the evaluation conditions. More comprehensive characterizations of these QTLs identified in this study will be necessary to determine the genetic basis of each QTL, the interactions between these QTLs, and the response to environmental conditions. Previously, we identified QTLs controlling low-temperature germinability (Fujino *et al.* 2004). A near isogenic line for a major QTL, *qLTG3-1*, clearly showed more vigorous low-temperature germinability than the recurrent parent (Fujino *et al.* 2008, Iwata and Fujino 2010). SES is involved in the continuous growth from seed germination to seedling development. Combining these QTLs may enable establishment of stable SES.

Many QTLs for tolerance to low-temperature in seed germination and seedling stages have been identified using various kinds of mapping populations and various evaluation methods (Andaya and Mackill 2003a, Baruah *et al.* 2009, Fujino *et al.* 2004, Ji *et al.* 2009, Jiang *et al.* 2006, 2008, Lou *et al.* 2007, Miura *et al.* 2001, Zhang *et al.* 2005a, 2005b). To identify QTLs for tolerance to low-temperature

Table 3. QTLs for seedling establishment (SES) in the 3 mapping populations using Italcia Livorno and Arroz Da Terra as the donor parents

Population	Traits	QTL	Marker interval	Chromosome	2008			2009		
					LOD	AE ^a	PVE ^b	LOD	AE ^a	PVE ^b
HsA	G-ESS	<i>qSES6</i>	RM20368-RM5314*	6	4.9	4.2	19.3			
		<i>qSES7-2</i>	RM3670-RM5543*	7	3.1	3.3	10.9			
		<i>qSES11</i>	RM26981-RM5997*	11	5.1	4.8	17.2			
	NG-ESS	<i>qSES5-2</i>	RM4691-RM6229*	5	3.6	-3.7	13.8			
		<i>qSES11</i>	RM26981-RM5997*	11	5.6	4.8	20.5			
		<i>qSES11</i>	RM1355*-RM26871	11				3.4	4.2	19.3
	G-ESW	<i>qSES1</i>	RM6451*-RM1032	1	3.9	5.5	23.0			
		<i>qSES7-2</i>	RM5543*-RM6394	7	3.6	4.5	17.5			
		<i>qSES7-1</i>	RM7161*-RM3635	7				3.5	4.1	15.3
	NG-ESW	<i>qSES2</i>	RM3515*-RM5706	2				2.9	3.2	12.4
		<i>qSES5-1</i>	RM4777*-RM1024	5				2.9	2.8	13.0
		<i>qSES11</i>	RM26981-RM5997*	11	6.2	5.2	25.6			
HyI	NG-ESS	<i>qSES5-1</i>	RM4777*-RM3906	5				4.4	7.1	16.6
		<i>qSES2</i>	C601*-C370	2	4.5	4.0	18.1			
	NG-ESW	<i>qSES5-1</i>	RM4777*-RM3906	5				4.0	6.3	14.1
		<i>qSES11</i>	G202-C6*	11				3.6	5.1	13.9
HsI	G-ESW	<i>qSES10</i>	RM5147-RM1873*	10				4.7	5.0	20.5

^a Additive effect of alleles from donor parents based on arc-sine-transformation of phenotypic rate.

^b Percentage of total phenotypic variance explained by the QTL.

*Nearest marker to the QTL.

at the seedling stage, seedlings in various developmental stages grown under optimum conditions were subjected to low-temperature (Andaya and Mackill 2003a, Baruah *et al.* 2009, Jiang *et al.* 2008, Lou *et al.* 2007, Zhang *et al.* 2005b). In contrast, SES in this study was evaluated as the growth from seed germination to seedling development at continuous low-temperature. Comparison of the chromosomal locations of these QTLs with 9 QTLs for SES identified in this study could help to elucidate the relationships between them as the genetic basis of tolerance to low-temperature in rice. Several QTLs were co-located on a chromosomal region of the most effective locus *qSES11*. *qLTG-11* (Miura *et al.* 2001), *qLTG-11-1*, and *qLTG-11-2* (Jiang *et al.* 2006) were identified in the chromosomal region of *qSES11*, which controlled seed germination at a temperature of 15°C. *qSCT-11* (Zhang *et al.* 2005b), *qCTS11* and *qCTP11* (Baruah *et al.* 2009), and *qCTS11-2* (Andaya and Mackill 2003a) were identified, which controlled response to low-temperature in different developmental stages.

As with the chromosomal region of *qSES11*, QTLs for tolerance to low-temperature have been identified on the same chromosomal regions as other QTLs for SES identified in this study. *qGR-2* on chromosome 2 (Ji *et al.* 2009) for *qSES2*, *qLTG-5* (Miura *et al.* 2001) and *qLTG-5-1* (Jiang *et al.* 2006) on chromosome 5 for *qSES5-1*, *qLTG-5-2* and *qLTG-5-3* on chromosome 5 (Jiang *et al.* 2006) for *qSES5-2*, *qLTG-7* (Jiang *et al.* 2006) on chromosome 7 for *qSES7-1*, and *qLTG-10* on chromosome 10 (Jiang *et al.* 2006) for *qSES10* were identified previously as having roles in seed germination at low-temperature. In addition, *qCTS-1-a* on chromosome 1 (Lou *et al.* 2007) for *qSES1*, *qCTS5* on chromosome 5 (Baruah *et al.* 2009) for *qSES5-1*, *qSCT-5* on chromosome 5 (Jiang *et al.* 2008) for *qSES5-2*, *qSCT-6* (Jiang *et al.* 2008) and *qCTS6-2* (Andaya and Mackill 2003a) on chromosome 6 for *qSES6*, and *qCTS10* (Andaya and Mackill 2003a) on chromosome 10 for *qSES10* were identified previously as controlling responses to low-temperature in different developmental stages. Only *qSES7-2* from Arroz Da Terra identified in this study appeared to be a novel QTL.

Due to the lack of precise chromosomal locations for these QTLs, the genetic relationships between these QTLs are still unclear as to whether they represent the pleiotropic effect of a single QTL or whether different QTLs are tightly linked to each other. SES in field conditions of Hokkaido involves various kinds of environmental conditions, such as the temperature of soil and flood water, soil quality, and anaerobic conditions caused by flood. The relationships between these QTLs suggested that tolerance to low-temperature is highly important for stable SES in Hokkaido.

Substantial efforts in rice breeding programs for the improvement of tolerance to low-temperature have made rice production stable under natural environmental conditions in Hokkaido. Because of intensive breeding efforts, the genetic diversity of populations in local regions has been reduced (Cuevas-Perez *et al.* 1992, Dilday 1990), especially in vari-

eties from Japan (Yamamoto *et al.* 2010). The small variation of SES among varieties from Hokkaido identified in this study might be maximized to their genetic potential. QTLs for SES from European varieties identified in this study could be useful in rice breeding programs in Hokkaido. However, it is difficult to introduce vigorous SES from exotic germplasm into commercial rice cultivars due to the large amount of effort required for the elimination of undesirable traits or genes from the germplasm. Both Italica Livorno and Arroz Da Terra have many undesirable traits for rice cultivars in Hokkaido, such as poor straw strength, maturity, shattering, and poor grain quality and shape. The genetic relationships between QTLs for undesirable traits and SES identified in this study could facilitate the utilization of QTLs for SES from this exotic germplasm.

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