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Mapping QTLs for Cold Tolerance at Seedling Stage Using an *Oryza sativa* × *O. rufipogon* Backcross Inbred Line Population

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Abstract

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Tolerance to low temperature is an important factor affecting the growth and development of rice (*Oryza sativa* L.) at an early growing season in the temperate region, and at high altitudes of tropical regions. In this study, a backcross inbred line (BIL) population derived from an interspecific cross between Xieqingzao B (*O. sativa* L.) and an accession of Dongxiang wild rice (*O. rufipogon* Griff.) was used to identify quantitative trait loci (QTLs) associated with cold tolerance at the seedling stage. Seedlings were treated with a temperature of 6°C for 2 days and seedling mortality was measured for QTL mapping. QTL analysis was performed on the whole BIL population and on one subpopulation that showed Xieqingzao B homozygous at QTL detected in the whole population. One major QTL, *qSCT8*, and one QTL, *qSCT4.3*, with smaller effect was found in the whole population. The QTLs *qSCT8* and *qSCT4.3* were mapped on chromosome 8 and 4, explaining 60.96% and 8.83% of the phenotypic variance, respectively. In the subpopulation, three QTLs, *qSCT4.1*, *qSCT4.2* and *qSCT12*, accounting for 56.22%, 57.62% and 53.09% of the phenotypic variance, respectively, were detected on chromosome 4 and 12. At all five loci, the alleles introduced from the Dongxiang wild rice were effective in decreasing seedling mortality. Our results provide a basis for fine mapping and cloning of QTLs associated with cold tolerance, and the markers linked with QTLs could be used to improve the cold tolerance of rice varieties by marker-assisted selection.

Keywords: Dongxiang wild rice; QTL analysis; rice; seedling cold tolerance

Abiotic stresses such as cold, heat, drought and salinity could have a devastating impact on crop plant growth and yield. Rice (*Oryza sativa* L.), which originates from tropical or subtropical regions, is sensitive to cold stress at a temperature below 15–20°C (Yoshida *et al.* 1996; Nakagahra *et al.* 1997). At cold stress conditions, cold injuries to rice seedlings appear uneven with seedling stand establishment, seedling stunting, yellowing or withering, leaf discoloration, and increased seedling mortality (Nakagahra *et al.* 1997). The cold damage also causes poor germination, less panicles, delayed heading, spikelet sterility, and decreased yield (Kaneda & Beachell 1973; Yoshida *et al.* 1996; Andaya & Mackill 2003; Fujino *et al.* 2004; Suh *et al.* 2010). As the direct seeding cultivation has been adopted by more farmers due to labour-use efficiency and lower cost compared to transplanting, improving cold

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tolerance at the seedling stage is essential for stable yield. In particular, selection for more stress-tolerant varieties is one important strategy for increasing crop productivity through minimizing losses due to biotic and abiotic stresses (Khush 1999).

Dongxiang wild rice (*O. rufipogon* Griff., hereafter referred to as DWR), is the northernmost wild rice known in the world (116°36'E, 28°14'N). DWR is the important natural resource for genetic and breeding programs since it possesses many excellent characteristics including fertility restoration for male sterility (Chen *et al*. 2008), drought tolerance (Zhang *et al*. 2006), and cold tolerance (Chen *et al*. 1996, 2002; Liu *et al*. 2003; Li *et al*. 2010; Xia *et al*. 2010; Xiao *et al*. 2014). DWR is able to safely survive under temperature as low as –12.8°C (He *et al*. 1996), providing an invaluable genetic resource for the improvement of cold tolerance in rice. Recently, there were identified several quantitative trait loci (QTLs) conferring cold tolerance at different development stages in DWR (Chen *et al*. 2002; Liu *et al*. 2003; Xia *et al*. 2010; Zuo *et al*. 2010; Xiao *et al*. 2014, 2015).

With the development of genomics, QTLs associated with cold stress tolerance at the seedling and reproductive stages in rice have been identified, of which a few have been cloned or fine mapped (Andaya & Mackill 2003; Andaya & Tai 2006; Saito *et al*. 2010; Suh *et al*. 2010; Kim *et al*. 2014; Zhang *et al*. 2014; Xiao *et al*. 2015). In a previous research, the BC_1F_1 population derived from a cross using the *indica* cultivar Xieqingzao B (hereafter referred to as XB) as the recurrent parent and DWR as the donor parent was used to detect QTLs for cold tolerance (Chen *et al*. 2002). On the basis of single marker analysis, the SSR markers RM280 on chromosome 4 and RM337 on chromosome 8 were found and associated with cold tolerance. In the present study, a linkage map was constructed based on the BC_1F_5 population derived from the XB//XB/DWR BC_1F_1 population to identify QTLs for cold tolerance at seedling stage. QTL analysis was performed on the whole backcross inbred line (BIL) population firstly. The subpopulations that showed XB to be homozygous at QTL detected in the whole population were then employed to identify more QTLs which might be covered by the major QTLs. The present study was aimed to detect QTLs for cold tolerance in DWR for improving cold tolerance of cultivated rice by means of marker assisted selection (MAS).

MATERIAL AND METHODS

Plant material. The mapping population consisted of 202 backcross inbred lines (BILs), which were derived from the interspecific cross XB//XB/DWR, in which XB is the maintainer line of the dwarf-abortive cytoplasmic male sterile line Xieqingzao A, and DWR is an accession of *O. rufipogon* from Dongxiang, Jiangxi Province, China (Chen *et al*. 2010).

Evaluation of low-temperature tolerance. According to the methods of Wu and Lin (1990) and Chen *et al*. (2002), the BILs and the recurrent parents XB were tested in three replications. Seeds of each line were dried at 40°C for 48 h to break dormancy and then imbibed and germinated at culture dishes. When the seedlings grew to one-leaf stage, the weak seedlings were pulled out and 30 strong seedlings of each line were maintained, and treated with the low temperature at 6°C for 2 days at a growth chamber. After the cold treatment, the growth chamber was adjusted to 28°C/25°C (day/night) for recovery of normal growth for 1 day. The dead seedling percentage (number of dead seedling/total number of seedlings treated) was calculated and expressed as cold tolerance at the seedling stage.

Data analysis. A linkage map consisting of 149 DNA markers and spanning 1306.4 cM (Hu *et al.* 2016) was used for QTL mapping. QTLs were determined using Windows QTL Cartographer 2.5 (Wang *et al*. 2012). Composite interval mapping was conducted using a walking speed of 1 cM and a window size of 10 cM with backward and forward regression. A logarithm of the odds (LOD) threshold > 3.0 was used to claim a putative QTL. The QTL was named following the nomenclature recommended by McCouch and CGSNL (McCouch 2008).

The QTL analysis was performed on the whole BIL population firstly. Then the lines that showed DWR homozygous at the flanked marker for QTL detected in the whole population were eliminated and the subpopulation consisting of the remaining lines was used to further mapping.

RESULTS

Variation of cold tolerance in the BIL population. The seedling mortality of the BIL population and the recurrent parent XB are summarized in Table 1. The average seedling mortality of the BIL population was 85.97% and ranged from 0 to 100%. The seedling mortality was skewed towards the tolerant

https://doi.org/10.17221/154/2016-CJGPB

Table 1. Descriptive statistics for seedling mortality (%) in the backcross inbred line population of Xieqingzao B//Xieqingzao B/Dongxiang wild rice

Mean	SD	CV	Range (%)	Skewness	Kurtosis	Mean trait value of XB
85.97	22.31	0.26	0-100	0.17	0.34	100

SD – standard deviation; CV – coefficient of variation; XB – XieqianzaoB

type. The cold tolerance at seedling stage displayed approximately continuous variation, which indicated that major, as well as minor genes contributed to the cold tolerance traits (Figure 1).

QTL mapping in the whole BIL population. Two QTLs associated with cold tolerance at seedling stage were detected, including one major QTL on the long arm of chromosome 8, and one minor QTL on the long arm of chromosome 4 in the whole population (Table 2; Figure 2). They jointly explained 69.79% of the phenotypic variance in the BIL population. Two QTLs, including *qSCT4* and *qSCT8*, the alleles from DWR decreased seedling mortality. The major QTL *qSCT8* was mapped in the interval RM256-RM281 on the long arm of chromosome 8 with LOD score 20.40, contributing 60.96% to the phenotypic variance. The minor QTL *qSCT4* was located in the interval RM127-RG620 and explained 8.83% of the phenotypic variance (Table 2).

QTL analysis in the subpopulation. On the basis of QTL mapping in the whole population, the lines that showed DWR homozygous at any marker flanked by *qSCT4* (RM127 and RG620) and *qSCT8* (RM256 and RM281) in the whole BIL population were eliminated and the subpopulation consisting of the remaining 138 lines was used to detected more QTLs for cold tolerance at seedling stage. Three QTLs conferring cold tolerance at seedling stage were detected in the subpopulation (Table 2; Figure 3). Two of them were mapped in the interval RG449–RM142 and RM142–RM273 on the long arm of chromosome 4, explaining 56.22% and 57.62% of phenotypic variance, respectively. According to the gene nomenclature system for rice (McCouch 2008), these two QTLs were defined as *qSCT4.1* and *qSCT4.2*, respectively, and the QTL *qSCT4* detected in the whole BIL population was renamed as *qSCT4.3*. The other QTL found in the subpopulation, *qSCT12*, was located in the interval RG463–RM17 on the long arm of chromosome 12. It had a LOD score of 7.59 and contributed 53.09% to phenotypic variance. At the three loci, the DWR alleles always had the effect for decreasing seedling mortality.

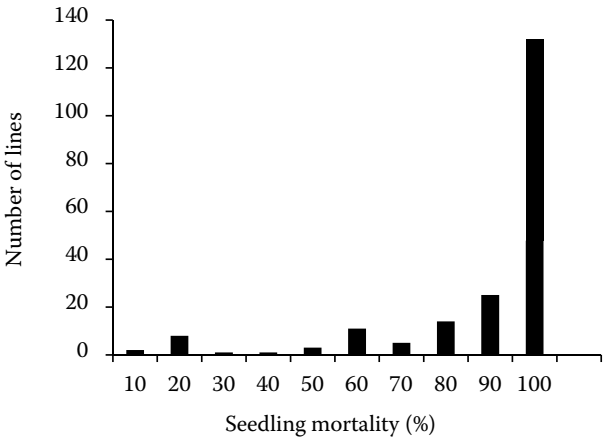

Figure 1. Frequency distribution of seedling mortality in the backcross inbred line population of Xieqingzao B//Xieqingzao B/Dongxiang wild rice

Table 2. QTLs associated with cold tolerance at seedling stage detected in the backcross inbred line population and subpopulation of rice

QTL	Interval	Whole population			Sub-population		
		LOD	R^2	A (%)	LOD	R^2	A (%)
qSCT4.1	RG449–RM142				5.13	56.22	–21.97
qSCT4.2	RM142–RM273				7.80	57.62	–21.48
qSCT4.3	RM127–RG620	3.30	8.83	–12.02			
qSCT8	RM256–RM281	20.40	60.96	–25.56			
qSCT12	RG463–RM17				7.59	53.09	–21.11

LOD – logarithm of the odds; R^2 – percentage of phenotypic variance explained by the QTL; A – additive effect of replacing an Xieqingzao B allele by a Dongxiang wild rice allele

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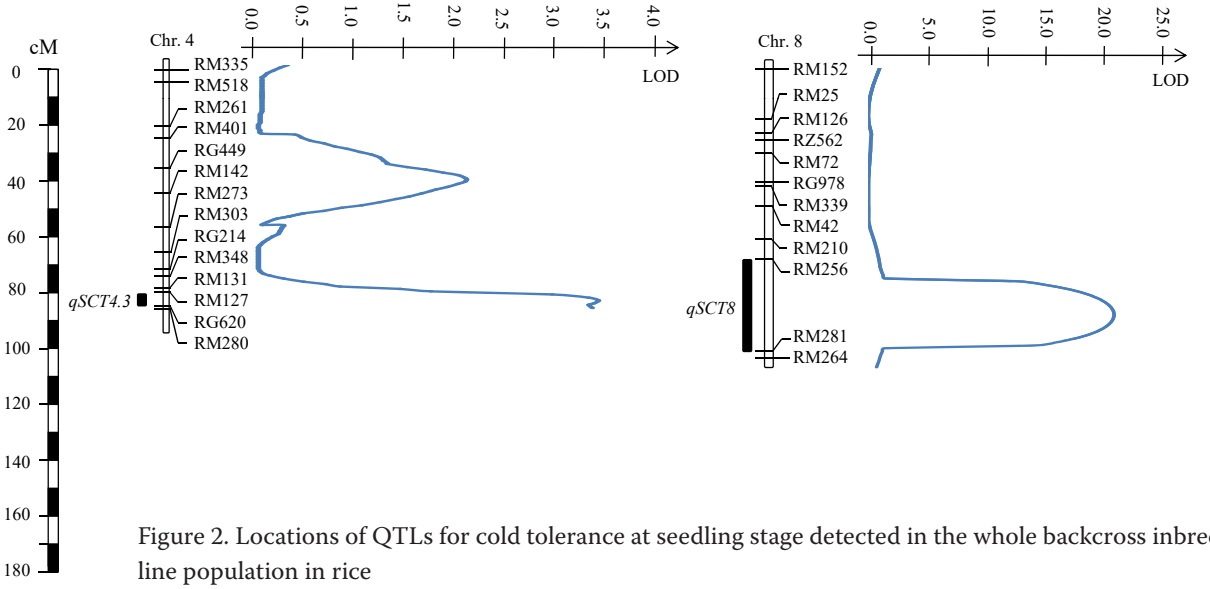

Figure 2. Locations of QTLs for cold tolerance at seedling stage detected in the whole backcross inbred line population in rice

DISCUSSION

Low-temperature tolerance is one of the major determinants for rice seedling survival which is utilized in the direct seeding method at early cropping season. In the previous study, Chen *et al.* (2002) detected QTL for cold tolerance in the BC_1F_1 population derived from the cross XB//XB/DWR using a single marker analysis. The SSR markers RM280 on chromosome 4 and RM337 on chromosome 8 were found to be associated with seedling cold tolerance. In this study, a BIL population derived from the same cross was employed. QTL analysis was performed on the whole BIL population and on one subpopulation using composite interval mapping. A total of five QTLs were detected, including *qSCT4.1*, *qSCT4.2* and *qSCT4.3* on chromosome 4, *qSCT8* on chromosome 8, and *qSCT12* on chromosome 12. Among them, *qSCT4.3* was mapped to the interval RM127–RG620 (www.gramene.org: 34529722 – 34698199 bp) on the long arm of chromosome 4, which is near the marker RM280 (www.gramene.org: 34989558 – 34989727 bp). Therefore, the *qSCT4.3* might be the same QTL detected by Chen *et al.* (2002). This suggests that *qSCT4.3* locus is a contribution to stable seedling cold tolerance. The *qSCT8* was located in interval

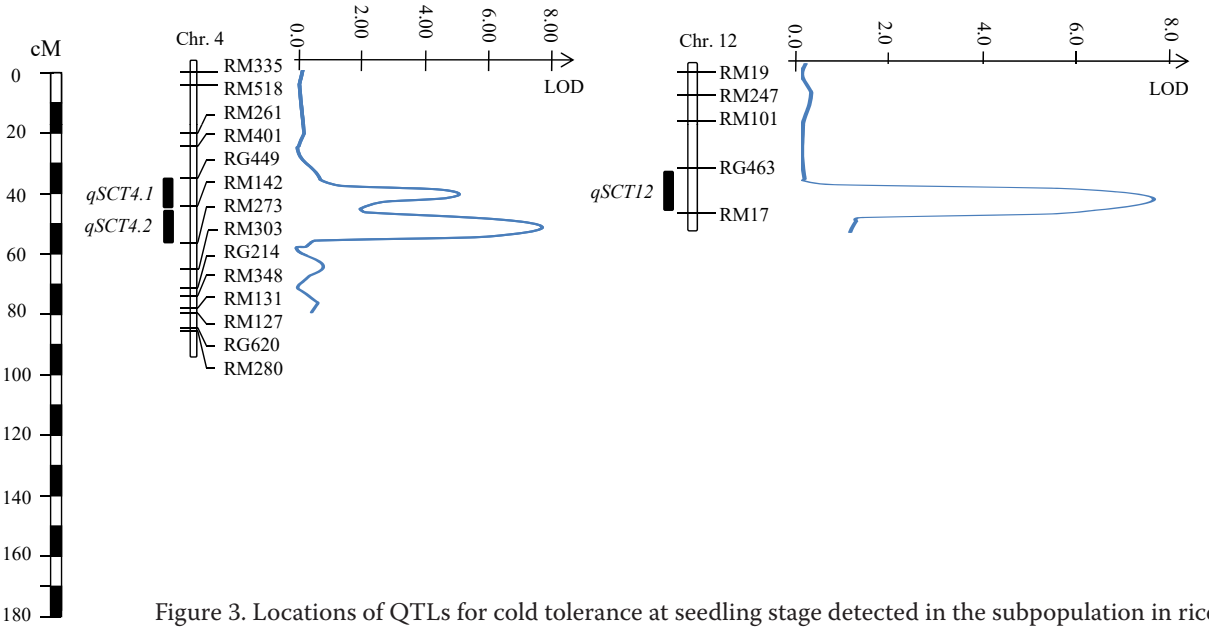

Figure 3. Locations of QTLs for cold tolerance at seedling stage detected in the subpopulation in rice

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the RM256–RM281 (www.gramene.org: 24140510-27765514 bp) on the long arm of chromosome 8, while the SSR marker RM337 (www.gramene.org: 146952-147138 bp) is on the short arm. Compared with the study of Chen *et al.* (2002), therefore, *qSCT8*, *qSCT4.1*, *qSCT4.2* and *qSCT12* loci appear to be newly identified QTLs.

Cold tolerance is a complex trait which is controlled by quantitative trait loci in rice. Many indices of cold tolerance including visual and physiological indicators are used to evaluate cold tolerance and map cold tolerance related QTLs at the seedling stage in rice. The cold tolerance score of 1 (tolerant, all leaves normal, no apparent visual injury) to 9 (susceptible, all leaves wilted, seedlings apparently dead) was commonly used to evaluate the cold stress in rice (IRRI 1988). Andaya and McKill (2003) identified a major QTL *qCTS12a* conferring cold-induced necrosis and wilting tolerance, and accounting for 41% of the phenotypic variation. *qCTS12a* has been fine mapped to a region of approximately 87kb (Andaya & Tai 2006). Qian *et al.* (2000) detected a QTL *qSCT-4* associated with seedling mortality, which was flanked by the marker interval G271-C975 on chromosome 4. The region of *qSCT-4* was partly overlapped with *qSCT4.1* and flanked by RG449-RM142 that was identified in this study. Zhang *et al.* (2005) identified a QTL *qSV-8-1* for seedling vigour trait in the region of RM223-OSR7 on chromosome 8, which is in the interval of *qSCT8* flanked by RM256-RM281. Dai *et al.* (2004) reported the existence of a QTL for cold tolerance on the long arm of chromosome 12 flanked by G370-G148, which is close to *qSCT12*. The same regions of QTLs for cold tolerance were mapped with different index and in different genetic backgrounds and environments. It indicates that the region of *qSCT4.3*, *qSCT8* and *qSCT12* which were mapped on chromosome 4, 8 and 12 could be hotspots with regard to seedling cold tolerance.

DWR, the common wild rice as the ancestor of cultivated rice, is an important natural resource of alleles for breeding program relating to cold stress tolerance. Several researches on cold tolerance related QTLs mapping, which used DWR as a cold tolerant donor parent to construct a population, have been reported. Liu *et al.* (2003) detected three QTLs for cold tolerance on chromosome 1, 6 and 11 using an advanced backcross population derived from Guichao 2 and DWR as a donor parent at booting to flowering stages. Two QTLs *qSCR3* and *qSCR11* were identified on chromosome 3 and 11, respectively, in a F_2 population from 9311/DWR (Zuo *et al.* 2010). Using root conductivity, leaf osmotic potential and plant survival rate as cold-tolerant trait indices, Xia *et al.* (2010) and Xiao *et al.* (2014, 2015) detected two QTLs *qRC10-1* and *qRC10-2* for root conductivity correlated with cold tolerance, and two QTLs *qLOP2* and *qPSR2-1* for leaf osmotic potential and plant survival related with cold-tolerance (Xia *et al.* 2010; Xiao *et al.* 2014, 2015). In the present study, a BIL population, where the donor parent is DWR, was used to identify the QTLs controlling cold tolerance at seedling stage. QTL of *qSCT4.3* for cold tolerance identified on chromosome 4 and explaining 8.83% of phenotypic variation was detected in a BC_1F_1 population by Chen *et al.* (2002) as well. Four major QTLs detected on chromosome 8 and 12 are new loci involving cold tolerance at seedling stage compared with the previous study of Chen *et al.* (2002). The QTLs or genes identified from DWR will promote improving rice cold tolerance and will contribute to the understanding of its molecular mechanisms.

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References

Andaya V.C., Mackill D.J. (2003): Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. Journal of Experimental Botany, 54: 2579–2585.

Andaya V.C., Tai T.H. (2006): Fine mapping of the *qCTS12* locus, a major QTL for seedling cold tolerance in rice. Theoretical and Applied Genetics, 113: 467–475.

Chen D.Z., Xiao Y.Q., Zhao S.X., Xiong H.J., Pi Y.H. (1996): Study on the cold tolerance of Dongxiang wildrice at stage of seedling and heading. Acta Agricualturae Jiangxi, 8: 1–6.

Chen D.Z., Zhong P.A., Xiao Y.Q., Huang Y.J., Xie J.K. (2002): Identification of QTLs for cold tolerance at seedling stage in Dongxiang wild rice (*Oryza rufiongon* Griff.) by SSR markers. Acta Agricualturae Universitatis Jiangxiensis, 24: 753–756.

Chen J., Huang D.R., Wang L., Liu G.J., Zhuang J.Y. (2010): Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* × *O. rufipogon*. Breeding Science, 60: 153–159.

Chen X.R., Yang K.S., Fu J.R., Zhu C.L., Peng X.S., He P., He H.H. (2008): Identification and genetic analysis of fertility restoration ability in Dongxiang wild rice (*Oryza rufipogon*). Rice Science, 15: 21–28.

https://doi.org/10.17221/154/2016-CJGPB

Dai L.Y., Lin X.H., Ye C.R., Ise K., Saito K., Kato A., Xu F.R., Yu T.Q., Zhang D.P. (2004): Identification of quantitative trait loci controlling cold tolerance at the reproductive sage in Yunnan landrace of rice, Kunmingxiaobaigu. Breeding Science, 54: 253–258.

Fujino K., Sekiguchi H., Sato T., Kiuchi H., Nonoue Y., Takeuchi Y., Ando T., Lin S.Y., Yano M. (2004): Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Theoretical and Applied Genetics, 108: 794–799.

He G.C., Shu L.H., Zhou Y.Q., Liao L.J. (1996). The overwintering ability of Dongxiang wild rice (*Oryza rufipogon*) at Wuhan. Journal of Wuhan University (Natural Science Edition), 42: 252–254.

Hu B.L., Huang D.R., Xiao Y.Q., Fan Y.Y., Chen D.Z., Zhuang J.Y. (2016): Mapping QTLs for mineral element contents in brown and milled rice using an *Oryza sativa* × *O. rufipogon* backcross inbred line population. Cereal Research Communications, 44: 57–68.

IRRI (1988): Standard Evaluation System for Rice. Los Baños, Internationla Rice Research Institute.

Kaneda C., Beachell H.M. (1973): Response of indica-japonica rice hybrids to low temperatures. SABRAO Journal, 6: 17–32.

Khush G.S. (1999): Green revolution: preparing for the 21st century. Genome, 42: 646–655.

Kim S.M., Suh J.P., Lee C.K., Lee J.H., Kim Y.G., Jena K.K. (2014): QTL mapping and development of candidate gene-derived DNA markers associated with seedling cold tolerance in rice (*Oryza sativa* L.). Molecular Genetics and Genomics, 289: 333–343.

Li F., Guo S.Y., Zhao Y., Chen D., Chong K., Xu Y.Y. (2010): Overexpression of a homopeptide repeat-containing bHLH protein gene (Orb-HLH001) from Dongxiang wild rice confers freezing and salt tolerance in transgenic *Arabidopsis*. Plant Cell Reports, 29: 977–986.

Liu F.X., Sun C.Q., Tan L.B., Fu Y.C., Li D.J., Wang X.K. (2003): Identification and mapping of quantitative trait loci controlling cold-tolerance of Chinese common wild rice (*O. rufipogon* Griff.) at booting to flowering stages. Chinese Science Bulletin, 48: 2068–2071.

McCouch S.R. (2008): CGSNL (Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative), gene nomenclature system for rice. Rice, 1: 72–84.

Nakagahra M., Okuno K., Vaughan D. (1997): Rice genetic resources: history, conservation, investigative characterization and use in Japan. Plant Molecular Biology, 35: 69–77.

Qian Q., Zeng D.L., He P., Zheng X.W., Chen Y., Zhu L. (2000): QTL analysis of the rice seedling cold tolerance in a double haploid population derived from anther culture of a hybrid between indica and japonica rice. Chinese Science Bulletin, 45: 448–453.

Saito K., Hayano S.Y., Kuroki M., Sato Y. (2010): Map-based cloning of the rice cold tolerance gene *Ctb1*. Plant Science, 179: 97–102.

Suh J.P., Jeung J.U., Lee J.I., Choi Y.H., Yea J.D., Virk P.S., Mackill D.J., Jena K.K. (2010): Identification and analysis of QTLs controlling cold tolerance at the reproductive stage and validation of effective QTLs in cold-tolerant genotypes of rice (*Oryza sativa* L.). Theoretical and Appllied Genetics, 120: 985–995.

Wang S.C., Basten C.J., Zeng Z.B. (2012): Windows QTL Cartographer 2.5. Department of Statistics. North CarolinaStateUniversity, Raleigh. Availble at http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Wu M.S., Lin D.H. (1990): Research on cold tolerance in wild rice. In: Collection of Research Articles on Wild Rice Germplasm. Beijing, Chinese Science Techn Press: 57–62.

Xia R.X., Xiao N., Hong Y.H., Zhang C., Su Y., Zhang X.M., Chen J.M. (2010): QTLs mapping for cold tolerance at seedling stage in Dongxiang wild rice (*Oryza rufipogon* Griff.). Scientia Agriculture Sinica, 43: 443–451.

Xiao N., Huang W.N., Zhang X.X., Gao Y., Li A.H., Dai Y., Yu L., Liu G.Q., Pan C.H., Li Y.H., Dai Z.Y., Chen J.M. (2014): Fine mapping of *qRC10-2*, a quantitative trait locus for cold tolerance of rice roots at seedling and mature stages. PLoS ONE, 9: e96046.

Xiao N., Huang W.N., Li A.H., Gao Y., Li Y.H., Pan C.H., Ji H.J., Zhang X.X. Dai Y., Dai Z.Y., Chen J.M. (2015): Fine mapping of the *qLOP2* and *qPSR2-1* loci associated with chilling stress tolerance of wild rice seedlings. Theoretical and Applied Genetics, 128: 173–185.

Yoshida R., Kanno A., Sato T., Kameya T. (1996): Cool temperature-induced chlorosis in rice plants. Plant Physiology, 110: 997–1005.

Zhang Q., Chen Q.H., Wang S.L., Hong Y.H., Wang Z.L. (2014): Rice and cold stress: methods for its evaluation and summary of cold tolerance-related quantitative trait loci. Rice (New York, N.Y.), 7: 24.

Zhang X., Zhou S., Fu Y., Su Z., Wang X.K., Sun C.Q. (2006): Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.). Plant Molecular Biology, 62: 247–259.

Zhang Z.H., Qu X.S., Wan S., Chen L.H., Zhu Y.G. (2005): Comparison of QTL controlling seedling vigour under different temperature conditions using recombinant inbred lines in rice (*Oryza sativa*). Annals of Botany, 95: 423–429.

Zuo J., Gao J., He R.H., Li D., Han X.X., Xing J., Li Q., Cao M.L. (2010): QTL mapping for cold resistance at seedling stage in Dongxiang wild rice. Hybrid Rice, 27: 56–59.

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