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RESEARCH ARTICLE

Linkage map construction and QTL mapping for cold tolerance in *Oryza rufipogon* Griff. at early seedling stage

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Abstract

Cold stress is one of the major restraints for rice production. Cold tolerance is controlled by complex genetic factor. In this study, a backcross inbred lines (BILs) population derived from an inter-specific cross (*Oryza sativa* L.×*O. rufipogon* Griff.) was used for genetic linkage map construction and quantitative trait locus (QTL) mapping. A linkage map consisting of 153 markers was constructed, spanning 1 596.8 cM with an average distance of 11.32 cM between the adjacent markers. Phenotypic evaluation of the parents and BILs under (6±1)°C cold stress revealed that the ability of cold tolerance in BILs at early seedling obeyed a skewed normal and continuous distribution. Fifteen QTLs on chromosomes 6, 7, 8, 11, and 12 were identified using survival percent (SP) and non death percent (NDP) as indicators of cold tolerance, which could explain 5.99 to 40.07% of the phenotypic variance, of which the LOD values ranged from 3.04 to 11.32. Four QTLs on chromosomes 3, 5 and 7 were detected using leaf conductivity (LC) and root conductivity (RC) as indicators of cold tolerance, ranging from 19.54 to 33.53% for the phenotypic variance explained and 2.54 to 6.12 for the LOD values. These results suggested that there might be multi major QTLs in *O. rufipogon* and some useful genes for cold tolerance have been transferred into cultivated rice, which would be helpful for cloning and utilizing the cold tolerance-responsive genes from wild rice.

Keywords: common wild rice, cold tolerance, quantitative trait loci (QTL), introgression

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crop plants and provides the staple food for more than 50% of the world's population (Tan *et al*. 2007; Zhang *et al*. 2009).

Cold stress is one of the major limiting factors affecting global rice production since cold stress can cause poor germination, slow growth, discoloration, withering, and anthers injury of rice plants (Andaya and Tai 2007). There are about 15 million ha of rice fields in 24 different countries suffered from cold damage, especially in Japan, Korea and China (Lou *et al*. 2007). In Australia, that the yield lost 1–2 t ha^{-1} due to low temperature during the reproductive stage was also reported in 1995–1996; in China, it was recorded that the annual yield lost 3–5 million t (Farrell *et al*. 2001; Xu *et al*. 2008). In temperate area, rice cultivation usually involves transplanting seedling from nursery to paddy field because of low temperature. And such cultivation approach is expensive, time consuming and labor intensive. Consequently,

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it is an important objective to broaden the genetic variation related to cold tolerance and understand its genetic basis and molecular mechanisms, which would facilitate to develop unique rice varieties with strong ability of cold tolerance.

Cold tolerance in rice is a quantitative trait controlled by complex genetic factor (Andaya and Mackill 2003). And the final cold damage was influenced by too many processions and factors (Lou *et al*. 2007). So, development of the rice cultivar with strong cold tolerance is very difficult through traditional breeding technique or program. With the rapid development of molecular biology, QTL analysis is a key tool for illuminating the genetic basis of complex traits associated with cold tolerance, since it can explain the multiple QTLs into single exercisable Mendel factor. Using a set of recombinant inbred lines (RILs) that were derived from a cross between M202 and IR50 (*indica*, highly sensitive to cold stress), Andaya and Mackill (2003) mapped a major QTL (*qCTS12a*) on chromosome 12 which accounted for 41% of the phenotypic variation in seedling growth after cold stress. Zhang *et al*. (2005) detected three QTLs on chromosomes 3, 7 and 11 for cold tolerance at the early seedling stage with RILs derived from a cross between a tropical *japonica* and an *indica* cultivar. Lou *et al*. (2007) identified a major QTL, *qCTS-2*, on chromosome 2 in doubled haploid (DH) lines that were derived from a cross between a cold-tolerant *japonica* variety and a cold sensitive *indica* cultivar. Recently, a number of QTLs involved in different responses to cold stress have been identified continuously, including at seedling and booting stages (Zeng *et al*. 2009; Suh *et al*. 2012; Kim *et al*. 2014; Xiao *et al*. 2014; Zhang *et al*. 2014). Using genome-wide association studies, a total of 132 loci were identified at the seedling stage (Lü *et al*. 2015). These studies provide helpful information for molecular breeding in cold tolerant enhancement. To our best knowledge, however, previous QTL analyses on cold tolerance are mainly limited to cultivated rice, and the major QTL of cold tolerance in cultivated rice is insufficiency (Huang *et al*. 2008; Zhang *et al*. 2014). Therefore, understanding the genetic control of cold tolerance in wild rice is very important for utilizing the unique genes of cold tolerance from related wild species.

Dongxiang common wild rice (*Oryza rufipogon* Griff., DWR) is recognized to be the northernmost habitats for common wild rice (*O. rufipogon*) populations in the world, which belongs to one of the second class national protected plants (Chen *et al*. 2008; Xie *et al*. 2010a). DWR possesses many excellent characteristics, such as strong cold tolerance (Liu *et al*. 2003; Luo *et al*. 2012), drought tolerance (Zhang *et al*. 2006; Xie *et al*. 2010a), fertility restoring gene for male sterility (Chen *et al*. 2008), and the information of origin and evolution for cultivated rice (Xie *et al*. 2010). Among these useful agronomic characteristics, the cold tolerance is the most significant feature (Luo *et al*. 2012). It can overwintering successful in Wuhan City, Hubei Province, China, where the minimum temperature reaches to –12°C in winter (Liu *et al*. 2003). So, the characteristic of strong cold tolerance in DWR has been attracted much attention. Koseki *et al*. (2010) mapped a major QTL, *qCtss11*, to chromosome 12 for cold tolerance at seedling stage with a genetic mapping population derived from a cross between a cold tolerant wild rice and a sensitive *indica* cultivar. Mao *et al*. (2015) identified at least 13 QTLs on chromosomes 2, 3, 7, 8, 9, 11, and 12 for seedling cold tolerance in Dongxiang common wild rice through a combination of interval mapping and single locus analysis in two genetic populations. So far, about 25 cold-tolerance QTLs were identified in DWR (Chen *et al*. 2002; Liu *et al*. 2003; Xia *et al*. 2010; Mao *et al*. 2015). However, to date, the genetic and molecular mechanism for cold tolerance genes in DWR is still unclear, resulting in limited application of DWR in rice breeding programs.

In order to identify novel alleles conferring cold tolerance from wild rice, and ultimately to transfer the genes into cultivated rice, we developed a set of BC_1F_{10} backcross inbred lines (BILs) of 229 lines using the cultivated rice as recipient and the common wild rice as donor. In this study, the previously constructed 229 BILs are used to evaluate and detect cold tolerance QTL in DWR at early seedling to unlock the hidden information, which is the premise and base of exploiting and utilizing of the useful alien genes from DWR. These results would be helpful for cloning and utilizing the cold tolerance-responsive genes from DWR.

2. Materials and methods

2.1. Plant materials

The mapping population consists of 229 BILs derived from the inter-specific cross Xieqingzao B//Xieqingzao B/DWR (Chen *et al*. 2006), in which *O. sativa* ssp. *indica* var. Xieqingzao B (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 County, Jiangxi Province, China. Cultivated rice XB (recurrent parent) and common wild rice DWR (donor parent) were used to produce F_1 hybrid. Afterward, the hybrid F_1 was backcrossed to XB to create BC_1F_1 population. Finally, we used BC_1F_1 population selfed consecutively to obtain BC_1F_{10} inbred lines (ILs) using single-seed descent method. The BC_1F_{10} population are used to cold tolerance assessment and QTL analysis.

2.2. Phenotypic variation evaluation for cold tolerance

About 50 seeds were selected from each BC_1F_{10} BILs, they were stored at 50°C for 3 d to break dormancy, using

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