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Mapping of quantitative trait loci related to cold resistance in *Brassica napus* L.

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ABSTRACT

Cold stress is one of the major abiotic stresses that seriously limit rapeseed production worldwide. However, few studies on the mechanism of cold resistance in *Brassica napus* have been reported. In this study, an $F_{2:3}$ population including 147 lines was developed to identify the quantitative trait loci (QTLs) related to cold resistance in *B. napus*. As a result, a genetic linkage map based on 333 simple sequence repeat (SSR) markers covering 1317.70 cM was constructed. Up to 11 QTLs for four indicators were identified in the two locations. These QTLs accounted for 1.09% to 42.50% of the phenotypic variations, and six major QTLs accounted for more than 10% of the phenotypic variations. Three QTLs, qSPADYL-6, qSPADYS-6, and qMDAYS-6, were mapped to the same region of linkage group 6 (LG6). Blast analysis indicated that the sequences of the markers related to these three QTLs showed great collinearity with those on the A08 chromosome of *Brassica rapa*, and that the target genes might exist in the region from 1.069 to 15.652 M on A08. Two genes, *BnaA08g05330D* and *BnaA08g15470D*, encoding the respective cold-regulated proteins in *B. napus*, were identified. They exhibited high similarity with *Bra039858* and *Bra010579* (stress-responsive proteins) in the candidate region. RT-qPCR analysis showed a significant difference in gene expression between the two parents. These two genes were hence identified as the genes responsible for cold resistance.

1. Introduction

Cold stress is one of the typical abiotic stresses that seriously affect crop growth in the world. Recently, abnormal or extreme weather such as cold spells in late spring have been occurring more often than usual due to climate change. Almost every year, the rapeseed acreage has been reduced because of frost damage, which has negatively impacted the production of the edible oil in China (Zhang et al., 2008). In recent years, the Chinese government has been investing significant financial resources towards increasing the cultivation of rapeseed in northern China. However, northern China's low temperature in the winter makes it is difficult for *B. napus* varieties to survive. Some studies have shown that *B. rapa* has better cold resistance than *B. napus* (Sun et al., 2007, 2013), and several *B. rapa* varieties have been successfully cultivated in northern China. However, these varieties are rarely 'double low' (low erucic acid and low glucosinolate) and therefore, its expansion has been limited. *B. napus* is the most important type of rapeseed, with the largest sowing area in China. So far, very few cold resistance varieties of *B. napus* have been authorized by the Chinese government; thus, it is urgent to cultivate more varieties of *B. napus* that are cold resistant.

In order to cultivate cold-resistant varieties, it is necessary to study the cold resistance of plants. The cold resistance of plants is determined by many complex, interacting factors (Ma et al., 2016). Numerous studies have shown that cold resistance is closely related to plant cell membranes, enzymes, and a physiologically active defense system (Deng and Chen, 2001; Jia and Guan, 2012; Pu and Sun, 2010). In an environment where the temperature remains below zero for long periods of time, cold stress is induced in the plants, and the growth and development of these plants are seriously affected (Chen and Xu, 1998). When plants suffer frost damage, the stability of cell membranes is maintained to resist low-temperature damage. Studies have shown that the stability of cell membranes is determined by the fatty acid composition of the membrane lipids (Ilker et al., 1979). When the ratio of unsaturated fatty acid to saturated fatty acid is high, or the phase transition temperature of the plant cell membrane is low, the plant's ability to resist chilling injury is strong (Roughan, 1985). A positive correlation between the stability of cell membranes and cold resistance has been observed (Jian et al., 1994; Wang et al., 2006). Studies have

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also shown that, under low temperature, the permeability of cell membranes and electrolyte leakage increase, resulting in an increase in the relative conductivity of tissues (Yang et al., 2004). Therefore, electrical conductivity can be used as an index to determine the degree of frost damage. Boyer and Westgate (2004) also indicated that the plant cell enzyme system plays an important role in cold damage: the continuous decrease of temperature leads to structural changes in the enzymes in the cell, leading to a decrease in enzyme activity.

Under low temperature, green plants often use more energy to resist cold damage. Most of this energy comes from respiration, resulting in increased respiration and increased dry matter loss. When the outside temperature continues to decrease, the structure of the chloroplasts changes and the dorsal membrane of the chloroplasts is broken down, causing a dysfunction in the chloroplasts and hindering the normal growth of plants (Lee et al., 2002). The disappearance of starch grains in chloroplasts affects the chloroplasts' normal synthesis and the utilization of light energy, resulting in reduction of photosynthetic efficiency of plants (Kratsch and Wise, 2000; Fracheboud et al., 2004). Under low-temperature stress, excessive accumulation of reactive oxygen species in plants may also cause plant damage. Sometimes the reactive oxygen content in plants is at a low level. However, when subjected to low temperature, the plants produce an increased amount of reactive oxygen contents, resulting in the peroxidation of membrane phospholipids, structural rearrangements of plasma membrane enzymes, and subsequent changes in the catalytic function of the membrane proteins (Boyer and Westgate, 2004). Therefore, in order to enhance the cold resistance of plants, an important step is to reduce the accumulation of reactive oxygen species. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are important protective enzymes in plants, which can remove excess reactive oxygen species and free radicals produced during chilling damage and maintain the normal growth of plants (Kuk et al., 2003; Demiral and Türkan, 2004; Türkan et al., 2005). Plants also rely on a variety of substances related to osmotic adjustment to resist adverse changes. Soluble sugar, soluble protein (SP), malondialdehyde (MDA), and proline (Pro) are the most common substances related to osmotic adjustments in plants. Although the content of soluble sugar in most plants is positively correlated with cold resistance, in some plants there is no correlation between the soluble sugar content and cold resistance (Lindow and Arny, 1978; Wang et al., 1998). Several studies have shown that MDA and electric conductivity (EC) have a significant negative correlation with cold resistance in *B. napus* (Fechner et al., 1986; Huang et al., 2014). The varieties with a high MDA content have higher levels of membrane lipid peroxidation, resulting in a lower cold resistance (Fechner et al., 1986; Liu et al., 2012; Lin et al., 2013). By contrast, the content of Pro is positively related to the cold resistance of plants, increasing significantly under low temperature (Zhao et al., 2010). Therefore, soluble sugar, SP, MDA, and Pro can be used as important indicators of the cold resistance of plants.

To study the mechanism of cold resistance, many studies have been constructed and a number of cold-resistance genes have been cloned and studied in *Arabidopsis* and *Brassica*. *Cor15a*, a gene encoding a chloroplast-targeted polypeptide in *Arabidopsis thaliana*, has been identified (Lin et al., 1992). It mainly functions in stabilizing thylakoid membranes against freezing-induced damage, and increasing cold tolerance in *Arabidopsis* (Artus et al., 1996). *Cor15a* has been cloned in canola, wheat, and other plants (Cui et al., 2003; Zhong et al., 2006; Zhang et al., 2016). In addition, two genes, *BnCBF5* and *BnCBF17*, which increase the constitutive freezing tolerance, and regulate the chloroplast development to improve photochemical efficiency and photosynthetic capacity, have been identified in *B. napus* (Savitch et al., 2005). Some other cold-resistance genes, such as *RCI3,* and transcription factors, such as *AP2* and *NAC,* were also cloned (Ishiguro and Nakamura, 1994; Llorente et al., 2002; Zhang et al., 2008). By contrast, only a few studies have been published on the genetics of cold resistance in *Brassica*. Wu et al. (2017) identified 24 QTLs related to cold resistance in *B. rapa*, which account for 11.18%–81.18% of the phenotypic variations. However, studies on gene map-based cloning of cold tolerance in *B. napus* have yet to be constructed.

Several cold-resistant breeding lines were identified in our laboratory (not published). For example, a *B. napus* line that shows great cold resistance, GZ hui, derived from the maintainer line of a *B. napus* cultivar 'Shanyou 0913', was discovered through successive years of investigation. Therefore, this line has been used as the source of cold resistance in *B. napus*. The objective of this study was to construct a genetic map of *B. napus*, identify the QTLs related to cold resistance, and primarily analyze the candidate genes in the QTL mapping region. In addition, new cold-resistance-related markers were developed for marker-assisted selection (MAS) of cold resistance.

2. Materials and methods

2.1. Plant materials and stress treatments

An F_2 population derived from GZ hui (*B. napus*, cold-resistant) and 10B (*B. napus*, cold-sensitive) was constructed for QTL mapping. Each individual of F_2 was selfed to generate 147 $F_{2:3}$ lines, which were used to measure morphological and physiological indexes. The F_2 and parental plants were grown on an experimental farm of Northwest Agriculture and Forestry University (Yangling, Shaanxi) in 2014. The $F_{2:3}$ lines were grown in two locations, Yangling and Yongshou, in 2015. Yangling is located in the central Shaanxi in China, which belongs to the hinterland of Guanzhong plain. The minimum temperature in Yangling in 2016 was −8 °C, and the number of days with temperatures below 0 °C was more than 30 days, mainly in January and February. The samples were taken for morphological and physiological index measurement on January 20, 2016. The minimum temperature was about −5 °C and the maximum temperature was 8 °C at that time. Yongshou is located north of Yangling and belongs to a part of the loess plateau of China. The temperature there is colder than in Yangling. In 2016, the lowest temperature was −13 °C, and the days below 0 °C reached 40 in January and February. The samples were taken for morphological and physiological index measurement on January 18, 2016. The minimum temperature was about −9 °C, and the maximum temperature was 4 °C A total of 15–20 plants of each $F_{2:3}$ line was measured per replicate. Three replicates were assessed per location. Additionally, the two parents (GZ hui and 10B) were treated with 25 °C, 0 °C, −5 °C, and −10 °C at three-leaf stage, respectively. The roots and leaves of the two parents were sampled after exposure to these respective temperatures for three days. Ten individuals for each parent were mixed for one treatment, and three replicates were assessed. The samples were stored at −80℃ for RNA extraction.

2.2. Evaluation of morphological and physiological indexes

To assess the frost damage, the frost damage index (FDI) of $F_{2:3}$ families was determined. A total of 15–20 plants in each $F_{2:3}$ family were investigated for frost damage on March 1, 2016. The plants of $F_{2:3}$ were assessed individually for frost damage using a 0–5 scale, where 0 = no frost damage; 1 = frost damage on several large leaves; 2 = frost damage on half of the leaves, with central leaves undamaged; 3 = frost damage on most of the leaves, with central leaves undamaged; 4 = frost damage on all leaves including central leaves; 5 = dead. For statistical analysis, a frost damage index was calculated using the following formula: FDI = $(1 \times S1 + 2 \times S2 + 3 \times S3 + 4 \times S4 + 5 \times S5)/(\text{total number of plants} \times 5)$, where S1–S5 represent the number of plants classified into grades 1–5, respectively. The specific method is described by Liu (1985).

Cold resistance of the $F_{2:3}$ families was evaluated by five indexes: leaf electric conductivity (EC), leaf relative water content (RWC), chlorophyll content (SPAD), soluble protein (SP), and malonaldehyde (MDA). EC was measured by the method of Li (Li, 2000); SOD was