Plant Science 269 (2018) 75–84

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Genetic mapping of a lobed-leaf gene associated with salt tolerance in *Brassica napus* L.

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ARTICLE INFO

Keywords:
Lobed leaf
QTL
Candidate gene
Cloning
Expression
Salt tolerance

ABSTRACT

Lobed leaf is a common trait, which is related with photosynthesis and plant stress resistance in crops. In order to fine map and isolate the lobed-leaf gene in *Brassica napus*, an $F_{2:3}$ population derived from 2205 (salt tolerance) and 1423 (salt sensitive) was constructed, and the quantitative trait locus (QTL) technology was adopted to identify the QTLs related to lobed leaf formation. As a result, one major QTL was identified on LG10, and two intron polymorphic (IP) markers and one sequence characterized amplified region (SCAR) marker were successfully developed in QTL region. The lobed-leaf gene was mapped to a region from 15.701 to 15.817 M on A10. In light of annotations of the genes in candidate region, a leaf morphological development related gene, *Bra009510*, was primary identified as the candidate gene. The full length of the candidate gene was 1390 bp containing three exons and two introns in the two parents. The open reading frame (ORF) was 693 bp and encoded a protein of 229 amino acids. Eight amino acid differences between the two parents in CDS (coding sequences) region were identified. qRT-PCR analysis showed that the expression of the candidate gene was significantly different between the two parents under salt stress. These results showed that the candidate gene might be related to leaf morphological development and abiotic stresses. Our study will lay a solid foundation for studying lobed leaf mechanism in *B. napus* L.

1. Introduction

Leaf shape in *Brassica napus* is classified into round leaf, semi-lobed leaf and lobed leaf [1], which is easily identified by visual inspection. Thus, it can be used as an ideal morphological marker in rapeseed breeding, especially in the identification of hybrid authenticity, purity identification, as ornamental plants and other aspects [2,3]. Moreover, rapeseed leaves are the vegetative organs for photosynthesis, which provide energy for the activities of plants [4]. Studies have shown that appropriate dense planting is an important measure to increase crop yield, and reasonable plant types can effectively increase plant photosynthesis [5–7]. In general, for the plants with larger leaves, when they are densely planted, only the upper leaves can effectively carry out photosynthesis, and the photosynthetic efficiency of the lower leaves is poor, resulting in low yield of the plants [6–8]. On the contrary, because of the small leaf area and no accumulation of leaves for lobed leaves, they can make full use of light, and have higher photosynthetic efficiency and higher plant yield [6,8]. Specific leaf area (SLA) reflects the ability of plant leaves to obtain light resources, and thus, it is positively related to net photosynthetic rate (NPR) and dark respiration [9]. The leaf index influences the exchange of material and energy on the leaf surface by influencing the size of the leaf area [10,11]. In addition, some studies also showed that leaf shape is related to plant stress resistance, such as drought resistance and cold resistance [12–14]. Similar studies have been reported in maize, leaf shape and leaf direction were positively correlated with drought resistance [15]; Leaf area is relatively easy to be measured, which has a significant correlation with drought resistance [16].

Lobed leaf is related to the heterosis utilization, stress resistance and photosynthetic utilization. Studies on lobed leaves have been carried out. In *Brassica*, the studies are mainly focused on the inheritance and gene mapping of lobed leaf. Several studies showed that lobed leaf formation was controlled by one pair of incompletely dominant genes in *B. rapa* and *B. napus*, and the lobed leaf is dominant over the round

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Abbreviations: QTL, quantitative trait locus; IP, intron polymorphic; SCAR, sequence characterized amplified region; CDS, coding sequences; ORF, open reading frame; SLA, specific leaf area; NPR, net photosynthetic rate; LS, leaf shape; LA, leaf area; RL, root length; LFW, leaf fresh weight; LDW, leaf dry weight; RDW, root dry weight; EC, electrical conductivity; SOD, superoxide dismutase; SP, soluble protein; SPAD, chlorophyll content; STR, salt tolerance rating; CIM, composite interval mapping; LOD, log likelihood of the odds

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https://doi.org/10.1016/j.plantsci.2018.01.005
Received 28 October 2017; Received in revised form 11 January 2018; Accepted 14 January 2018

leaf [1,17,18]. Some molecular markers linked to lobed-leaf genes were identified. For example, a SCAR marker linked to the lobed-leaf gene was developed in Chinese cabbage [19]; two QTLs related to the lobed leaf were identified in *B. rapa* [20]; and a lobed-leaf gene was mapped to A10 in *B. napus* [18]. However, a few studies on the molecular mechanism of lobed leaf formation in *B. napus*, especially on the relationship between the lobed-leaf and photosynthesis and stress resistance were reported [18,21]. With the publication of the genome sequence of *B. napus* and *B. rapa*, it is possible to finely map and clone the candidate genes, which brought great convenience for studying the mechanism of lobed leaves.

In a previous study, a lobed-leaf *B. napus* inbred line 1423 was identified, which is a restorer line (530C) of two *B. napus* varieties 'Shanyou 16′ and 'Shanyou 107′. These two varieties have some advantages, such as lobed leaf, high yield, stress resistance, and short stem. A genetic map derived from lines 2205 and 1423 has been constructed, and some QTLs related to salt tolerance have been identified [22]. These results provided a good basis for studying the correlation between lobed leaves and salt tolerance. However, the studies on lobed-leaf gene mapping and cloning, and relationship of lobed leaf and salt tolerance have not been reported. Thus, the purpose of our study is that: 1) analyzing the relationship between lobed leaf characteristics and plant photosynthetic efficiency; 2) studying the correlation between lobed leaf and salt tolerance; 3) fine mapping the lobed-leaf gene; 4) cloning the candidate gene. This research will lay a foundation for studying the mechanism of lobed leaf formation and the utilization of lobed leaf in *B. napus.*

2. Materials and methods

2.1. Materials

An F_2 population including 196 individuals derived from two *B. napus* lines, 2205 (round leaf, salt-tolerant) and 1423 (lobed leaf, salt-sensitive) was constructed for lobed-leaf gene mapping (Fig. 1). Each individual of F_2 was selfed to generate 196 $F_{2:3}$ lines, which were used to determine the leaf shape (LS), net photosynthetic rate (NPR) and leaf area (LA). Ten plants of each $F_{2:3}$ line were measured per replicate. In order to study inheritance of lobed leaves and finely map the lobed-leaf gene, line 2205 crossed with line 1423 to produce F_1, and then, F_1 was selfed to produce an F_2. F_1 backcrossed to lines 2205 and 1423, respectively, to produce two BC_1 populations. These four populations were used to study the inheritance of lobed leaves. And the BC_1 population derived from F_1 and line 2205 was developed to finely map the lobed-leaf gene.

2.2. Correlation between lobed leaf and photosynthesis

The LS of $F_{2:3}$ constructed by Lang et al. (2017) was determined by a visual method. The plants of $F_{2:3}$ were assessed individually for leaf shape using a 1–3 scale, where 1 = round leaf; 2 = semi-lobed leaf; 3 = lobed leaf. The third leaf of seedlings in the 6–7 leaf stage was selected, the NPR of leaves was measured by the LI-6400XT portable photosynthesis system, and the LA of leaves was determined by the Yaxin-1242 portable leaf area meter. Each leaf was measured for three times. And the r-test [23] was conducted to analyze the correlation among LS, NPR and LA by the SPSS 20.0 software.

2.3. Correlation between lobed leaf and salt tolerance

Since the morphological and physiological indexes have been measured in the previous study using the $F_{2:3}$ population [22], including root length (RL), leaf fresh weight (LFW), leaf dry weight (LDW), root dry weight (RDW), electrical conductivity (EC), superoxide dismutase(SOD), soluble protein (SP), chlorophyll content (SPAD), and salt tolerance rating (STR). Therefore, in this study, this $F_{2:3}$ population was used to study the relationship between the leaf shape and these salt tolerance related indexes by the SPSS 20.0 software.

2.4. QTL mapping of lobed leaf

The genetic linkage map of *B. napus* was constructed by Lang et al. (2017). The QTLs related to lobed leaves were mapped using the composite interval mapping (CIM) function of the Win QTL Cartographer v.2.5 [24]. The LOD thresholds of QTL were determined by a 1000 permutation test at a 95% confidence level. The QTL mapping was performed followed the method of Fan et al. [25].

Fig. 1. Performance of the two parents 2205 and 1423 at seedling stage.