Genetic analysis and mapping of genes controlling freezing tolerance in oilseed *Brassica*

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Abstract

Freezing tolerance is the ability of plants to survive subfreezing temperatures and is a major component of winter survival. In order to study the genetic regulation of freezing tolerance, an F2 population of *Brassica rapa* and a doubled haploid population of *Brassica napus* were assayed in vitro for relative freezing tolerance of acclimated and nonacclimated plants. Linkage maps developed previously were used to identify putative quantitative trait loci (QTL). Genomic regions with significant effects on freezing tolerance were not found for the *B. napus* population, but for *B. rapa* four regions were associated with acclimated freezing tolerance (FTA) and acclimation ability (FTB), and two unlinked regions were associated with nonacclimated freezing tolerance (FTN). Acclimation ability was regulated by genes with very small additive effects and both positive and negative dominance effects. The allele from the winter parent at the FTN QTL had positive additive effects, but negative dominance effects. RFLP loci detected by a cold-induced and a stress-related cDNA from *Arabidopsis thaliana* mapped near two QTL for FTA/FTB. Further tests are needed to determine if alleles at these loci are responsible for the QTL effects we detected.

Introduction

The ability of plants to tolerate frost is a major component of winter survival in herbaceous perennial or biennial crop plants, such as oilseed *Brassica*. In many crop species, freezing tolerance increases dramatically when plants are acclimated to low temperatures before freezing. The freezing tolerance of cold-acclimated plants, measured by an in vitro assay, correlated with the winter survival of *Brassica* cultivars, but there was no correlation between nonacclimated and acclimated freezing tolerances [42]. This suggested that the two tolerances were controlled by separate ge-
nthetic mechanisms, but very little is known about the genetic control of freezing tolerance in *Brassica* species.

The genetic regulation of freezing tolerance and winter hardiness is probably complex in most or all crop species. Studies with winter wheat have suggested polygenic inheritance of winter hardiness \[11, 13\] and several chromosomes containing genes for frost resistance have been identified using monosomic \[42\] or chromosome substitution \[20, 41\] lines. Segregation in a cross of diploid *Solanum* species suggested that nonacclimated freezing tolerance and capacity to acclimate were controlled by only a few genes \[40\]. In barley, only one genomic region was associated with both winter hardness and cold tolerance \[12\], however, the presence of transgressive segregants suggested that other regions were involved but were not mapped in this study. The reported gene action for frost tolerance has varied from recessive \[31\] to partially dominant \[9\] in winter wheat, largely additive \[4\] to partially dominant \[30\] in alfalfa, and partially recessive in potato \[40\]. These differences could be due to variability in genotypes, or to frost test conditions since the severity of the winter determined whether there was dominant or recessive control of hardiness in oats \[24\].

Cold acclimation induces many changes in plants, including altered gene expression, and cold-induced cDNA clones have been isolated from several species, including *Arabidopsis thaliana* \[10, 18, 22, 25, 26\] and *B. napus* \[27, 28, 32, 49\]. The functions of these protein products in improving cold tolerance are not known, however, the cold-regulated (COR) genes code for boiling-stable, hydrophilic proteins which may function to keep critical proteins hydrated during freezing \[see 46 for review\]. The possible function of some other cold-induced genes, such as NADPH-aldose reductase, phosphoglucomutase \[21\], alcohol dehydrogenase \[15\], and lipid transfer proteins \[14\] are not readily evident. Other stress treatments, such as desiccation \[37\], salinity \[35\], and ABA treatment \[2, 16\], also can induce freezing tolerance, suggesting that some common mechanism may be induced by these different stresses. ABA-responsive genes have been isolated from cold-acclimated tissues and some cold-induced genes have sequence homology to the dehydrins and late embryogenesis-abundant proteins \[3, 5, 8, 19, 48, 50\], but it is not clear whether their expression is required for cold tolerance or a response to ABA accumulation caused by freezing-induced dehydration.

In this study, the genetic control of freezing tolerance was investigated in oilseed *Brassica rapa* and *B. napus* using molecular marker and quantitative trait locus (QTL) mapping. In addition, RFLPs detected by cold-induced genes or genes involved in plant stress responses in *B. napus* or *A. thaliana* were mapped in *B. rapa* and QTL analysis was used to determine whether segregation at the candidate loci was associated with variation for freezing tolerance.

**Materials and methods**

**Plant population and RFLP linkage maps**

A *B. napus* doubled haploid (DH) population was generated by microspore culture of a single F1 hybrid plant of cv. Major (biennial rapeseed) crossed with a DH line of cv. Stellar (annual canola) \[7\]. These parents differed in growth habit, freezing tolerance, and winter survival \[43\]. S2 plants from 105 lines were used to extract DNA for RFLP analysis and for trait measurements. A linkage map of 138 RFLP loci was constructed as reported previously \[7\].

A *B. rapa* F2 population was generated by self-pollination of a single F1 plant of cv. Per (biennial) crossed with cv. R500 (annual). These parents differed for growth habit, freezing tolerance, and winter survival \[43\]. F3 families from self-pollination of 85 F2 plants were used for RFLP analysis and for trait measurements. A linkage map of 143 loci was constructed using the 85 F2 genotypes which were a subset of 91 F2 genotypes used previously for map construction \[44\]. The maps had similar distances and identical locus orders, except for linkage group 2 (LG 2) which contained additional loci and was reordered.