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BRIEF COMMUNICATION

Freezing sensitivity in the *gigantea* mutant of *Arabidopsis* is associated with sugar deficiency

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Abstract

The freezing sensitivity in the *gi-3* mutant (an allele of the *gigantea* mutant) was associated with a constitutive reduction in soluble sugar content. Although sugar accumulation was evident in wild-type plants in response to cold treatment, the *gi-3* mutant showed a constitutive reduction in soluble sugar content. There were no significant differences in the proline content and the transcript levels of cold-responsive gene *RD29A* and abscisic acid-responsive gene *RAB18* between the wild type and the *gi-3* mutant in response to cold treatment. These results suggest that freezing sensitivity in the *gi-3* mutant is associated with sugar deficiency.

Additional key words: cold acclimation, gene expression, *gi-3* mutant, proline content.

Plants from temperate regions can increase their freezing tolerance by cold acclimation (Guy 1990). Cold acclimation is associated with complex biochemical and physiological changes in plants, including changes in gene expression (Thomashow 1999, Fowler and Thomashow 2002), leaf ultrastructure (Ristic and Ashworth 1993), membrane lipid composition (Miquel *et al.* 1993), enzyme activities, contents of sugars and polyamines and abscisic acid (ABA) (Strand *et al.* 1997, Shen *et al.* 2000, Uemura *et al.* 2003, Li *et al.* 2005), and ion channel activities (Knight *et al.* 1996).

Several lines of evidence suggest that cold-induced sugar accumulation enhances the degree of plant freezing tolerance. In *Arabidopsis* rosettes, a large increase in the degree of freezing tolerance that occurs within 1 d at 2 °C is positively correlated with soluble sugar content (Wanner and Junttila 1999), and the heterosis of leaf freezing tolerance generated by crossing between different ecotypes is positively correlated with leaf sugar content (Rohde *et al.* 2004). Increased sucrose content in transgenic *Arabidopsis* plants overexpressing a gene for sucrose phosphate synthase paralleled the freezing tolerance (Strand *et al.* 2003). Conversely, *sensitive to freezing 4* (*sfr4*) mutants exhibited an impaired cold acclimation capacity due to a reduced accumulation of glucose and sucrose at low temperature relative to the wild type (McKown *et al.* 1996). The impaired cold acclimation capacity of *sfr4* protoplasts was restored by providing sugars before protoplast isolation, and this decreased the incidence of freeze-induced protoplast membrane lesions (Uemura *et al.* 2003). Also, increased sugar level in transgenic *Arabidopsis* plants overexpressing *CBF3/DREB1A* suggested a possible link between the CBF/DREB1 pathway and cold-induced sugar accumulation (Gilmour *et al.* 2000). Recently, starch-related α-glucan/water dikinase, encoded by *Arabidopsis STARCH EXCESS 1* (*SEX1*), has been shown to be involved in the cold-induced development of freezing tolerance, indicating that starch degradation is important for enhanced freezing tolerance during an early phase of cold acclimation (Yano *et al.* 2005).

GIGANTEA (*GI*) is a gene encoding a novel nuclear-

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Abbreviations: ABA - abscisic acid; *ACTIN* - *Actin11*; f.m. - fresh mass; *GI* - *GIGANTEA*; RT-PCR - reverse transcriptase-polymerase chain reaction; *SEX1- STARCH EXCESS 1; sfr4 - sensitive to freezing 4.*

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localized protein (Huq *et al.* 2000), which has no homology with any proteins of known function in the databases (Fowler *et al.* 1999). Although the function of the GI protein is unknown, mutations in the *GI* gene cause a pleiotropic phenotype with effects on flowering in response to photoperiod, phytochrome B signaling, the circadian clock, and sugar metabolism (Eimert *et al.* 1995, Fowler *et al.* 1999, Huq *et al.* 2000). Recently, we found that *GI* gene is involved in the regulation of the cold stress response in *Arabidopsis* (Cao *et al.* 2005), however, the underlying mechanism is unclear. In this study, we provide evidence that freezing sensitivity in the *gi-3* mutant (an allele of the *gi* mutant) of *Arabidopsis* is associated with a constitutive reduction in soluble sugar content.

Wild-type *Arabidopsis* ecotype Landsberg *erecta* (Ler, obtained from *Lehle Seeds*, Round Rock, TX, USA) and *gi-3* (Ler ecotype, obtained from *Maarten Koornneef*, Wageningen, The Netherlands) were used in this study. Seeds were sowed in square pots with soil presoaked with PNS medium (Estelle and Somerville 1987). The plants were then raised for 18 d at 22 °C under continuous irradiance at a photon flux density of ~100 μmol m^{-2} s^{-1}. For the cold treatment, 18-d-old plants were incubated at 4 °C under continuous irradiance of 35 μmol m^{-2} s^{-1} for 2 d.

The third to fifth leaves from 18-d-old plants treated with cold for indicated time were sampled for analyses of soluble sugar and proline contents as well as semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Soluble sugar content was determined according to Zhang (1985). Briefly, total soluble sugars were extracted from lyophilized leaf material (20 mg) in 80 % (v/v) ethanol (2 cm^3) at 80 °C for 15 min. The samples were shaken for approximately 1 h at room temperature and allowed to stand overnight at 4 °C. Extracts were filtered through glass wool and chlorophyll removed by shaking samples (0.4 cm^3) with water (0.4 cm^3) and chloroform (0.4 cm^3). The aqueous extract was assayed for sugar content using the phenol-sulfuric acid assay. Proline content was measured according to Zhang *et al.* (1990). Briefly, lyophilized leaf material (30 mg) was extracted with 3 cm^3 of deionized water at 80 °C for 15 min. The samples were shaken for approximately 1 h at room temperature and then allowed to stand overnight at 4 °C. The extracts were filtered through glass wool and analyzed for proline content using the acid ninhydrin method. Total RNA extraction and RT-PCR were performed according to the method described by Cao *et al.* (2005). The following primers were designed for gene-specific transcript amplifications:

Actin11 (*ACTIN*, At3g12110):
fw-5'-GATTTGGCATCACACTTTCTACAATG-3' and
rv-5'-GTTCCACCACTGAGCACAATG-3';
RD29A (At5g52310):
fw-5'-ATCACTTGGCTCCACTGTTGTTC-3' and
rv-5'-ACAAAACACACATAAACATCCAAAGT-3';
RAB18 (At5g66400):
5'-GTGGTGGCTTGGGAGGAATGCTTCA-3' and
5'-ATGCGACTGCGTTACAAACCCTCA-3'.

Data are the means ± SE of three independent replicates. The analyses of variance were computed on statistically significant differences ($P < 0.05$) determined based on the appropriate *F*-tests. The mean differences were compared using Duncan's multiple range test.

Previous studies showed that the *gi* mutant manifests an elevated starch content in leaves (Eimert *et al.* 1995), and we found that the *gi* mutant displays an increased sensitivity to freezing stress (Cao *et al.* 2005). Therefore, it is interesting to investigate whether freezing sensitivity in the *gi* mutant is associated with sugar deficiency. We determined the content of total soluble sugar in the third to fifth leaves of wild-type and *gi-3* plants during incubation at 4 °C for 0, 3, 6, 12, 24, and 48 h. Sugar accumulation was evident within a few hours after the transfer of wild-type plants to 4 °C, and soluble sugar content increased steadily during 48 h. However, the *gi-3* mutant showed a constitutive reduction in soluble sugar content, although sugar accumulation was also detected in *gi-3* plants in response to cold treatment (Fig. 1). In addition, sucrose-pretreated leaves of *gi-3* plants also showed a significant increase in freezing tolerance (data not shown). These results suggest that sugar deficiency is

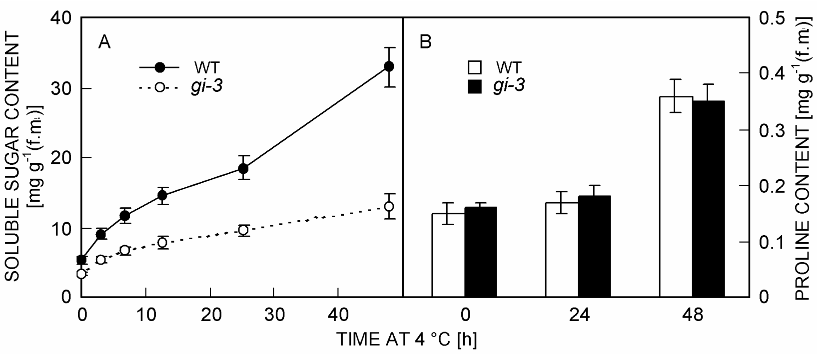

Fig. 1. Accumulation of soluble sugar (*A*) and proline (*B*) in leaves of wild-type and *gi-3* plants in response to cold treatment. The third to fifth leaves from 18-d-old wild-type and *gi-3* plants treated at 4 °C for indicated time were sampled for measurements of soluble sugar and proline contents. Data are the means ± SE of three independent replicates.

the primary cause of freezing sensitivity in the *gi-3* mutant. Our findings are consistent with the observations that the *sex1* mutant exhibits both an elevated starch content and an impaired freezing tolerance (Eimert *et al.* 1995, Yano *et al.* 2005), and that freezing sensitivity in the *sfr4* mutant of *Arabidopsis* is due to low sugar content (Uemura *et al.* 2003).

Proline is one of several compounds that act as compatible osmolytes to ameliorate the effect of dehydration that occurs during freezing and drought stress, and increases in the proline content occur in many plant species during cold acclimation (Xin and Browse 1998). To test whether the *gi-3* mutation also affects the proline content, we determined proline content in the third to fifth leaves of wild-type and *gi-3* plants during incubation at 4 °C for 0, 24, and 48 h (Fig. 1*B*). There was no significant difference in the proline content between wild-type and *gi-3* leaves during incubation at 4 °C for 0, 24, and 48 h. Such data are comparable with those obtained in the other study of cold acclimation in *Arabidopsis* (Wanner and Junttila 1999). This suggests that the *gi-3* mutation does not affect the proline content in leaves of *Arabidopsis* at low temperature.

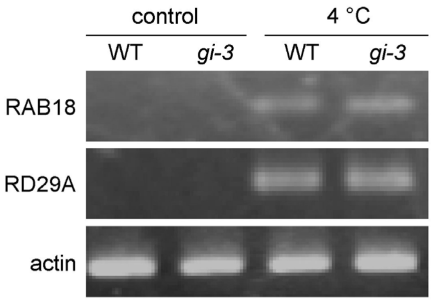

Fig. 2. Transcript levels of stress-induced genes in wild-type and *gi-3* plants before and after cold treatment. The third to fifth leaves from 18-d-old wild-type and *gi-3* plants treated at 4 °C for 0 (control) and 48 h were sampled for RT-PCR

Although it seems very unlikely, the above results did not exclude the possibility that freezing sensitivity in the *gi-3* mutant was caused by an inhibition of stress-inducible signalling pathways such as CBF/DREB1- or ABA-dependent pathways. To eliminate this possibility, we performed RT-PCR analyses for two representative stress-inducible genes (Fig. 2). There was no significant difference in the transcript level of the cold-responsive gene *RD29A* between wild-type and *gi-3* plants, which is consistent with our pervious results (Cao *et al.* 2005). In addition, the transcript level of the ABA-responsive gene *RAB18* was not significantly different between wild-type and *gi-3* plants (Fig. 2). These results suggest that the *gi-3* mutation does not affect the transcript levels of stress-responsive genes, and that the impaired freezing tolerance in the *gi-3* mutant is not related to any defect in the CBF/DREB1- or ABA-signalling pathways.

In conclusion, the results presented here suggest that freezing sensitivity in the *gi-3* mutant of *Arabidopsis* is associated with a constitutive reduction in soluble sugar content. Our results provide further evidence that sugar plays an important role in the regulation of the cold stress response in *Arabidopsis*. However, the precise mechanisms underlying the regulation of *GI* gene in the cold stress response remain to further be determined.

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Rieger, M.: **Introduction to Fruit Crops.** - Food Products Press, an Imprint of the Haworth Press, Inc., New York - London - Oxford 2006. 462 pp. Softbound USD 69.95. ISBN 1-56022-259-0.

The term fruit crop is defined by the author as ”a perennial, edible crop where the economic product is the true botanical fruit or is derived therefrom”. This eliminates annuals, such as tomato and melons and includes atypical fruit crops, such as coffee, cocoa, oil palm and coconut, because of edible parts of true botanical fruit. Fruit crops under this definition cover worldwide over 40 million hectares and add tens of billions of USD per year to the global economy.

The present volume provides in encyclopedic form extensive review of tropical, subtropical, as well as temperate fruits. Descriptions of the crops follow an uniform outline covering taxonomy, origin and history of cultivation, folklore, medical properties and nonfood usage, world production, botanical description, aspects of general culture, harvest and postharvest handling, contribution to diet and bibliography. The introductory chapter deals with the general concepts and terminology related to each section of the outline. Fruit species are reviewed in alphabetical order in 29 chapters including almond, apple, apricot, banana and plantain, blackberry and raspberry, blueberry, cacao, cashew, cherry, citrus fruits, coconut, coffee, cranberry, date, grape, haselnut or filbert, macadamia, mango, oil palm, olive, papaya, peach, pecan, pineapple, pistachio, plum, strawberry, walnut. The text is accompanied with a number of useful tables and figures and includes 30 first-rate color plates.

The book is written clearly and concisely, covers an enormous amount of material and requires only basic knowledge of plant biology and horticulture. Special technical terms are explained in the glossary at the end of the book. This together with a list of common and scientific names of fruit crops, conversion factors of units and a subject index enhance the value of the book. This unique text and reference source will certainly be of great interest to a wide horticultural audience, especially to students, teachers and commercial fruit growers.

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