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Gene Expression during Cold Acclimation in Strawberry

Christian NDong, François Ouellet, Mario Houde and Fathey Sarhan

Département des Sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale "Centre-Ville", Montréal, Québec, Canada. H3C 3P8

To elucidate the molecular basis of cold acclimation in strawberry (*Fragaria × anannassa*), we have begun studies to identify genes associated with low temperature (LT) acclimation. Differential screening of a cDNA library prepared from cold-acclimated strawberry plants allowed us to isolate several cDNAs showing differential expression at LT. Northern analysis showed that the transcript level of *Fcor1* (*Fragaria* cold-regulated) peaked after 2 days of LT exposure while that of *Fcor2* peaked after 2 weeks. On the other hand, the level of *Fcor3* transcript decreased within 24 hours of LT exposure and remained low during the 8 weeks acclimation period. *Fcor1* and *Fcor2* are expressed in all tissues while *Fcor3* is specific to leaves. The *Fcor1*-encoded protein has a compositional bias for leucine, isoleucine, glycine, proline and serine. This protein shares homology with the proteins encoded by *blt101*, a LT-responsive gene from barley, and *ESI3*, a gene induced by salt stress in *Lophopyrum*. The FCOR2 protein is rich in lysine, leucine, valine, alanine and arginine, and shows no homology with any known gene products. The partial *Fcor3* cDNA clone encodes a polypeptide that shows a very high identity with the spinach PSI subunit V and with the PSI PsaG polypeptide from barley. The level of *Fcor1* transcript accumulation is correlated with the freezing tolerance of the strawberry cultivars used in our study. This suggests that *Fcor1* may be useful as a molecular marker to select for this trait in related species of the *Rosaceae* family.

Key words: Cold acclimation — Freezing tolerance — Gene expression — RNA extraction — *Rosaceae* — Strawberry.

Freezing tolerance (FT) is induced in perennial and winter annual plants by low temperature (LT) and/or a short photoperiod, which are characteristics of autumn (McKersie and Ya'acov 1994). Freezing temperature is one of the major causes of crop damage, and the plant's ability to acclimate and develop FT is a determining factor in its survival. Understanding the mechanisms involved in FT is still a great challenge. During the exposure to low, non-freezing temperatures, many biochemical and physiological modifications are triggered. In hardy plants, these modifications lead to an increased capacity to withstand freezing temperatures. This metabolically active process is regulated at the gene expression level and is associated with the induction of specific proteins (Danyluk 1996, Guy 1990, Hughes and Dunn 1996, Thomashow 1990). However, the mechanism by which this altered gene expression allows plants to increase their FT is still unclear. To elucidate this mechanism, it is necessary to identify genes that are regulated by LT and positively correlated to the degree of FT. Characterization of these genes and their products will allow us to determine their implication in the overall acclimation process.

Cultivated strawberry (*Fragaria × anannassa*) is a moderately hardy species. It is one of the most important small fruit crop production in several northern parts of the world such as Canada. This crop is often damaged when snow coverage is low and thus, selection for elite hardy varieties of strawberry plants is needed. To achieve this, a better understanding of the genetics of this important trait is required. Towards this goal, we initiated studies to isolate and characterize genes associated with FT in this important fruit crop. Strawberry also represents a good model for the economically important *Rosaceae* family which includes apple, peach, pear and roses. Information gained from this study will be useful for the development of a strategy to improve and select for FT in these species. Using differential screening of a cDNA library prepared from a cold-acclimated tolerant cultivar of strawberry, we have isolated and characterized 3 cDNA clones that are regulated by LT exposure. The potential implication of these genes in the acquisition of FT is discussed.

Materials and Methods

Plant material and growth conditions—In this study, we used the hardy strawberry cultivars Chambly, Red Coat and Oka (*Fragaria × anannassa*, LT_{50} −12°C), a hardy wild strawberry (*Fragaria virginiania*, LT_{50} −16°C), and the freezing-sensitive Chandler cultivar (LT_{50} −2°C). FT is expressed as the temperature killing 50% of the plants (LT_{50}) as determined by the regrowth test (Perras and Sarhan 1989). Strawberry plants were grown for three months at 25°C/20°C (day/night) with a 14-h light period (250 μmol m^{-2} s^{-1}) in a mixture of soil : peat : vermiculite (1 : 1 : 1). At this age, the plants are at a developmental stage allowing them to acclimate and develop freezing tolerance. Control (non-acclimated) plants were maintained under the same conditions while cold acclimation was performed by subjecting the plants to 4°C/2°C with a 10-h light period. Short daylight conditions are important for successful cold acclimation and induction of freezing tolerance. Field-acclimated plants were removed

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Abbreviations: FT, freezing tolerance; LT, low temperature.

from the soil, for analysis and freezing tolerance tests, at mid november when the plants were fully acclimated. For ABA treatment, plants were watered daily for 4 d with a nutrient solution containing 0.1 mM ABA. Water stress was induced by withholding water until plants became visibly wilted. Salt-stressed plants were grown for 20 h in a solution containing 250 mM NaCl. ABA, water-stressed and salt-treated plants showed a typical physiological response for each stress and treatment. For deacclimation, cold-acclimated plants were returned to normal growth conditions for 1 week.

Extraction of total and poly(A)$^+$ RNA—Isolation of good quality RNA from strawberry plants is problematic due to the presence of high amounts of polysaccharides and phenolic compounds. The procedure described by Pawlowski et al. (1994) has been adapted to extract good quality total RNA from strawberry plants. Plant material (4 g) was ground to a fine powder in dry ice using a mortar and pestle. The powder was mixed with 19 volumes of a buffer containing 200 mM Tris-HCl, pH 8.5, 300 mM LiCl, 10 mM EDTA, 4% sodium deoxycholate, 2% NP-40 and insoluble PVP (1 g per g of fresh tissue). The homogenate obtained was stirred on ice for 10 min and mixed with a blender. The suspension was adjusted to 1.36 M ammonium acetate and mixed again. Cell debris and PVP were removed by centrifugation at 5,000 × *g* for 25 min at 4°C and the supernatant was filtered through Miracloth (Calbiochem). Sodium acetate 3 M (0.1 vol., pH not adjusted) and isopropanol (one vol.) were then added. Total nucleic acids were pelleted by centrifugation at 5,000 × *g* for 25 min, resuspended in 20 ml DEPC-treated H_2O and extracted with phenol-chloroform (1 : 1) until no interface was left and then extracted once with chloroform. Total RNA was precipitated with 0.25 vol. of 10 M LiCl overnight on ice and collected by centrifugation at 10,000 × *g* for 20 min. The pellet was resuspended in 1 ml DEPC-treated H_2O and extracted again with phenol-chloroform and chloroform. Finally, the RNA was precipitated with 0.1 vol. of 3 M sodium acetate pH 5.2 and 2.5 vol. of ethanol at −80°C for 15 min, centrifuged, and resuspended in 0.2 ml of H_2O.

The poly(A)$^+$ RNA was prepared using the dynabeads mRNA DIRECT kit as described by the manufacturer (Dynal). In brief, the total RNA was mixed with 0.2 ml of dynabeads (Oligo dT) and annealed for 5 min at room temperature. The tube was placed in the Magnetic Particle Concentrator (MPC) for 2 min and the supernatant was removed. The beads were washed 3 times with 1 ml of the washing buffer containing LiDS at room temperature, making sure to remove the supernatant completely between steps. Elution was performed by adding 20 μl of elution solution and heating at 65°C for 2 min. The tube was placed in the MPC and the supernatant containing the mRNA was transferred to a new tube and stored at −80°C.

Construction and screening of the cDNA library—Poly(A)$^+$ RNA was isolated from two-week cold-acclimated strawberry plants cv. Chambly and used to construct a cDNA library in lambda ZAPII (Stratagene). The cDNA was synthesized using the Amersham kit and ligated to *Eco*RI-*Not*I adaptors. The cDNA was purified using a sephacryl S-300 column according to the instructions from Pharmacia and ligated to λZAPII *Eco*RI/CIAP. The library was screened with ^{32}P-labeled cDNA probes prepared from poly(A)$^+$ RNA isolated from cold-acclimated and non-acclimated strawberry plants. The plaques showing a differential hybridization were selected and purified.

Northern blotting—Total RNA (10 μg) samples were mixed with ethidium bromide and separated on formaldehyde agarose gels as described (Rosen and Villa-Komaroff 1990). After electrophoresis, RNA was transferred to nitrocellulose membranes in 20 × SSC. The filters were air-dried and baked for 2 h at 80°C prior to hybridization with the ^{32}P-labeled cDNA inserts. Filters were washed at 65°C with several buffer changes of decreasing SSC concentrations (5 to 0.5 ×) and autoradiographed on Kodak XRP films with intensifying screens at −80°C.

DNA sequence analysis—Deletion subclones were generated for both strands using exonuclease III and VII as described (Yanish-Perron et al. 1985). Sequencing was performed by the dideoxy chain termination method (Sanger et al. 1977) using the T7 sequencing kit (Pharmacia). A computer-aided search of nucleotide and protein sequence databases was carried out with the Genetic Computer Group's Sequence Analysis Software Package, version 8.0 (Michigan State University).

Results

RNA extraction and purification—The adaptation of the procedure described by Pawlowski et al. (1994) for RNA extraction was found to be very efficient in eliminating the large amounts of polysaccharides and phenolic compounds present in strawberry tissues. The RNA obtained was of a high quality, as judged by its integrity on gel, and the yield was significantly higher (125 μg per g of fresh tissue) than that obtained with the other methods tested. The poly(A)$^+$ obtained was thus of the quality required to construct the cDNA library. This method could be suitable for RNA isolation from most plant tissues rich in polysaccharides and phenolic compounds, which are known to inhibit cDNA synthesis. The use of 2-butoxyethanol to remove these substances, as suggested by Manning (1991), did not result in their complete elimination and made it very difficult to quantitate the RNA due to other UV absorbing material still present in the preparation. Furthermore, the presence of phenolic compounds did not allow us to obtain good poly(A)$^+$ RNA to produce a high quality cDNA library, as judged by the low phage titer obtained.

Isolation of cold-regulated cDNAs—Three cold-regulated cDNA clones were isolated and designated *Fcor1, 2* and *3* for Fragaria cold-regulated genes. Northern blot analysis (Fig. 1A) shows that the 600 b transcript corresponding to *Fcor1* accumulated rapidly (within 24 h) and reached a high level after 2 d of LT exposure. The expression of this gene was low after 2 weeks of LT exposure and remained stable thereafter. This gene was expressed preferentially in the roots and to a lower extent in the leaves, while only a faint signal was detected in the crown (Fig. 1B). The transcript accumulated to similar levels in the hardy cultivars Chambly, Oka and Red Coat which possess a similar LT_{50} (results not shown). The abundance of the *Fcor1* mRNA after 2 d of cold acclimation was genotype-dependent since it accumulated to the highest level in the very hardy wild strawberry (*F. virginiania*) compared to the hardy cultivar Chambly and to the less freezing-tolerant cultivar Chandler (Fig. 1C). The *Fcor1* transcript level was higher in strawberry plants acclimated under field conditions, compared to plants acclimated under controll-

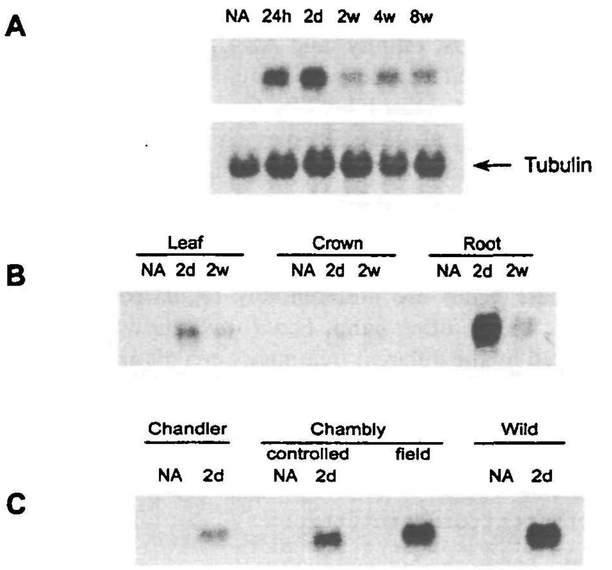

Fig. 1 *Fcor1* expression during cold acclimation of strawberry. Total RNA (10 μg per lane) was separated by agarose gel electrophoresis in the presence of formaldehyde and transferred to nitrocellulose membranes. The blots were probed with the ^{32}P-labeled cDNA insert from plasmid p*Fcor1*. The final wash was at 65°C in 0.5 × SSC containing 0.1% SDS. The bands were visualized by autoradiography. Tubulin was used to control the equal loading and quality of RNA. A: Kinetic analysis of mRNA accumulation during LT exposure in cv. Chambly. B: Tissue specificity of expression in leaf, crown and root tissues in cv. Chambly. C: Analysis of mRNA accumulation in cultivars having different FT. NA, non-acclimated plants; 24 h, 2 d, 2 w, 4 w, 8 w, cold-acclimated for 24 h, 2 d, 2, 4, and 8 weeks; controlled, cv. Chambly cold-acclimated in controlled conditions; field, cv. Chambly cold-acclimated under natural conditions in the field; Chandler, cv. Chandler grown under controlled conditions; Wild, *Fragaria virginiania* grown under controlled conditions.

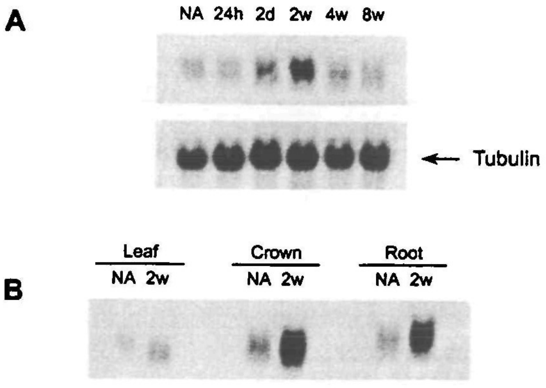

Fig. 2 *Fcor2* expression during cold acclimation of strawberry. Total RNA was separated and probed with the insert from p*Fcor2* as described in Figure 1. Tubulin was used to verify the equal loading and quality of RNA. A: Kinetic analysis of mRNA accumulation during LT exposure in cv. Chambly. B: Tissue specificity of expression in leaf, crown, and root tissues in cv. Chambly. Symbols as in Fig. 1.

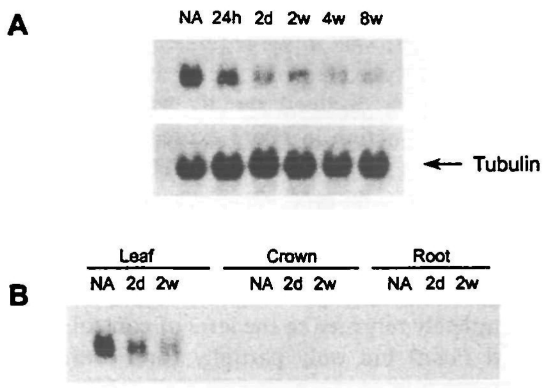

Fig. 3 *Fcor3* expression during cold acclimation of strawberry. Total RNA was separated and probed with the insert from p*Fcor3* as described in Fig. 1. Tubulin was used to verify the equal loading and quality of RNA. A: Kinetic analysis of mRNA accumulation during LT exposure in cv. Chambly. B: Tissue specificity of expression in leaf, crown, and root tissues in cv. Chambly. Symbols as in Fig. 1.

ed environment (Fig. 1C). These results indicate that the controlled conditions cannot completely substitute for the acclimation under field conditions. The *Fcor2* transcript of 550 b accumulated to its maximum level after 2 weeks of LT exposure and then declined to the control level (Fig. 2A). The transcript was found mainly in the crown and roots (Fig. 2B). The expression was not correlated with FT since the transcript accumulated to similar levels in the

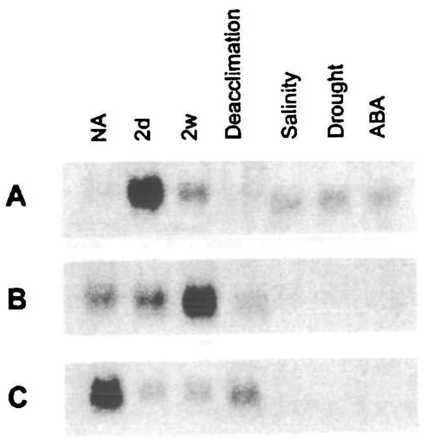

Fig. 4 Northern analysis of mRNA hybridized with *Fcor1* (A), *Fcor2* (B) and *Fcor3* (C) during cold acclimation, deacclimation and after different treatments. Total RNA was separated and probed as described in Fig. 1. NA, non-acclimated control plants grown at 24°C; 2 d and 2 w, plants acclimated at 4°C for 2 d and 2 weeks; Deacclimation, plants acclimated 2 d at 4°C and returned to normal growth conditions for 1 week; Salinity, plants treated with a solution containing 250 mM NaCl for 20 h; Drought, water-stressed plants visibly wilted; ABA, plants treated with a solution containing 0.1 mM ABA.

hardy and sensitive genotypes (results not shown). The third clone, *Fcor3*, was down-regulated by LT (Fig. 3A) and was specifically expressed in the leaves (Fig. 3B). The transcript of 600 b declined rapidly within 24 h of LT exposure. The reduction in *Fcor3* expression in the different genotypes was not correlated with FT.

Specificity of induction—To determine whether gene expression is regulated by temperature shifts, LT-treated plants were returned to the normal growth temperature for 7 days. The results in Fig. 4 show that the message abundance completely returned to the level of control plants for *Fcor1* and *Fcor2* but only partially for *Fcor3*. This indicates that the expression of *Fcor1* and *Fcor2* is more tightly regulated by temperature shifts than that of *Fcor3*.

Water stress, salinity, and ABA are known to induce some LT-regulated genes in different plant systems (Dallaire et al. 1994, Danyluk 1996, Guy 1990, Mohapatra et al. 1988, Orr et al. 1986, Reaney and Gusta 1987, Welin et al. 1994). Results in Figures 4A and B show that the expression of *Fcor1* and *Fcor2* are weakly affected by other stresses and exogenous application of ABA (slight accumulation for *Fcor1* and slight decrease for *Fcor2*), suggesting that these genes are preferentially regulated by LT exposure. On the other hand, *Fcor3* was significantly down-regulated by the different treatments in a manner similar to that of LT (Fig. 4C), indicating that expression of *Fcor3*

A

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  1      cacaaatccacacacagcagttaccaacttcttcttcttcttctttacta
 52      tctgtttctaagtatgggtgcagcaacctgcgtcgacatcatcatcgccat
 (1)                  M  G  A  A  T  C  V  D  I  I  I  A  I
                      α  α  α  α  α  α  α  β  β  β  β  β  β
103      cctcttgcctcctcttggggtcttcctcaggtttggctgcggggtggagtt
(14)     L  L  P  P  L  G  V  F  L  R  F  G  C  G  V  E  F
         β  .  .  ↲  ↲  β  β  β  β  β  ↲  ↲  ↲  ↲  ↲  ↲  ↲
154      ctggatctgtttgattttgactatttgtggtaccttcctgggatcatctat
(31)     W  I  C  L  I  L  T  I  C  G  T  F  L  G  S  S  M
         ↲  ↲  ↲  ↲  ↲  ↲  ↲  ↲  ↲  ↲  .  .  ↲  ↲  .  .  .
205      gctatctgggccatcaccaagtgatgatcagccactcgtacttcattttct
(48)     L  S  G  P  S  P  S  D  D  Q  P  L  V  L  H  F  L
         .  .  .  .  ↲  ↲  ↲  β  β  β  β  β  β  β  ↲  ↲  .
256      gtggtcgtacagatgtgaagggcacataaggcctatgtagttttcaccgtt
(65)     W  S  Y  R  C  E  G  H  I  R  P  M  *
         ↲  ↲  ↲  ↲  ↲  ↲  ↲  ↲  .  .  ↲  ↲
307      ggattgcatagtactttgtttggtgttagccaataatgtagtgcgtcgttt
358      ctcctttgtatttagttcattgtgtttccctatcttgtctatctcttgtga
409      agggtgggtggggttttggttggtattatggattaatgtctctttaaacat
460      tgataatttatttaattattctcaatgtggatttgatttaattttcaatgt
511      gattctcatccacccgacccagtagggtttggagggtatcactagctttat
580      gaataatattgagtgctcc
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B

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                  10        20        30        40
  FCOR1   MGAATCVDIIIAILLPPLGVFLRFGCGVEFWICLILTICGTFLGSSMLS
          ||:||  :::|:||:|||:|||||:   ||||||||:||| |   |
 BLT101   MGSATVLEVILAIILPPVGVFLRYKLGVEFWICLLLTILGYIPGIIYAV
                  10        20        30        40
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C

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                  10        20        30        40
  FCOR1   MGAATCVDIIIAILLPPLGVFLRFGCGVEFWICLILTICGTFLGSSMLS
          ||:||  :::|:||:|||:|||||:   ||||||||:||| |   |
   ESI3   MGSATVLEVILAIILPPVGVFLRYKLGVEFWICLLLTILGYIPGIIYAV
                  10        20        30        40
```

Fig. 5 Sequence analysis of *Fcor1*. A: Nucleotide and deduced amino acid sequence. The predicted polypeptide is 76 amino acids in length, with a calculated molecular mass of 8 kDa and a pI of 6.05. The underlined sequences show the hydrophobic regions. On the third line, secondary structure predictions were made by the method of Garnier et al. (1978). Symbols are *β*, *β* sheet; *α*, *α*-helix; ↲ , turns. B: Amino acid alignment of FCOR1 with BLT101 from barley. C: Amino acid alignment of FCOR1 with ESI3 from *L. elongatum*. Alignment was obtained using the Gap and pretty programs of the Genetics Computer Group software package.

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  1     ctctcacacacttcaccgcaaccaaccatgcctttcaagaggtacgtcgag
 (1)                               M  P  F  K  R  Y  V  E
                                   .  .  .  .  β  β  β  β
 52     atcggaagggtggctctcgtcaactacggcgaggactacggcaagctcgtc
 (9)    I  G  R  V  A  L  V  N  Y  G  E  D  Y  G  K  L  V
        β  β  β  β  β  β  β  β  ┘  ┘  ┘  ┘  ┘  ┘  β  β  β
103     gtcatcgtcgacgtcatcgaccagaacagagctcttgtcgatgcccctgat
(26)    V  I  V  D  V  I  D  Q  N  R  A  L  V  D  A  P  D
        β  β  β  β  β  β  β  .  .  β  β  β  β  .  .  α  α
154     atggtgagatctcagatgaatttcaagaggctttctctcactgacatcaaa
(43)    M  V  R  S  Q  M  N  F  K  R  L  S  L  T  D  I  K
        α  α  α  α  α  α  α  .  α  α  α  α  α  α  α  α  α
205     attgacatcaagagggtccccaagaagaaggaattgctggctgcaatggag
(60)    I  D  I  K  R  V  P  K  K  K  E  L  L  A  A  M  E
        α  α  α  α  α  α  α  α  α  α  α  α  α  α  α  α  α
256     gctgctgatgtgaagaagaagtgggaaagtagctcttggggtaggaagctt
(77)    A  A  D  V  K  K  K  W  E  S  S  S  W  G  R  K  L
        α  α  α  α  α  α  α  α  α  .  .  ┘  ┘  .  .  .  β
307     attgttcagaagcgaagggctaatcttaatgactttgatcgcttcaagctt
(94)    I  V  Q  K  R  R  A  N  L  N  D  F  D  R  F  K  L
        β  β  β  β  β  ┘  ┘  .  .  .  .  α  α  α  α  α  α
358     atgttggctaagatcaagagggctggcttggttaagagccagcttgcaaaa
(111)   M  L  A  K  I  K  R  A  G  L  V  K  S  Q  L  A  K
        α  α  α  α  α  α  α  α  α  α  α  α  α  α  α  α  α
409     ctgaagaagcagagcgcttaggcaatatttgatgttaggtttacaattttg
(128)   L  K  K  Q  S  A  *
        α  α  α  α  α  α
460     taatggattttctttagaactgatttcggattgtaatcccttgttccttct
535     atcaatttctagtctttttcagatt
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Fig. 6 Nucleotide sequence and deduced amino acid sequence of *Fcor2*. The predicted polypeptide is 133 amino acids in length, with a calculated molecular mass of 15 kDa and a pI of +11. On the third line, secondary structure predictions were made by the method of Garnier et al. (1978). Symbols as in Fig. 5.

may be sensitive to various stress conditions.

Nucleotide sequences of Fcor1, Fcor2 and Fcor3 and predicted amino acid sequences—The *Fcor1* cDNA insert is 580 bp long while the mRNA detected on northern blots is ca. 600 b, indicating that *Fcor1* is a near full length clone. The nucleotide sequence revealed a short ORF of 76 amino acids with an ATG codon at nucleotide 65 (Fig. 5A). The calculated molecular mass of the deduced polypeptide is 8,000 Da with an isoelectric point of 5.5. The predicted protein has a compositional bias for Leu (14.5%), Ile and Gly (10.5% each), Ser and Pro (7.9% each), whereas Asn and Lys residues are absent. The hydropathy profile, calculated according to Kyte and Doolittle (1982), suggests that the predicted 76 amino acid sequence encodes a hydrophobic protein (Leu and Ile constitute 25% of the protein) with two hydrophobic domains from amino acids 1 to 41 and from 60 to 66 (Fig. 5A, underlined sequences). A search of the Genbank database revealed that *Fcor1* shares 57.8% homology with *ESI3*, a gene induced by salt stress in *Lophopyrum elongatum* (Gulick et al. 1994) and 67.8% with *blt101*, a low temperature specific gene of barley (Goddar et al. 1993). At the amino acid level, there is 68.4% identity between FCOR1, BLT101 and ESI3 (Fig. 5B, C). These three proteins share their homology in the first N-terminal half, which may represent a targeting signal. Starting from amino acid residue 41, there was no homology between FCOR1 and BLT101 or ESI3 suggesting that the C-terminal half could encode distinct proteins.

The nucleotide sequence of the *Fcor2* cDNA insert (535 bp) contains an ORF of 133 amino acids with an ATG codon at nucleotide 28 (Fig. 6). The clone detects a 550 b transcript on northern blots, indicating that it is probably full length. This gene encodes a protein with a molecular mass of 15,300 Da with a very high isoelectric point of 11.0 and a net positive charge of +16. The predicted protein is lysine-rich (15.0%) and has a high content in Leu (10.5%), Ala (9.0%) and Arg (8.3%), with no Cys or His residues. The regions from amino acids 41 to 85 and from 105 to 133 have a high propensity to form an α-helix. The hydropathy profile indicates that most of the protein is hydrophilic. The database search revealed no homology with any known genes.

The *Fcor3* cDNA insert (196 bp) detects a transcript of 600 b on northern blots and is thus incomplete. The longest ORF encodes for 34 amino acids with a compositional bias for Ile (11.8%) Ala, Asp, Gly, Phe, Ser, Val and Tyr (8.9% each) (Fig. 7A). A database search based on the predicted amino acid sequence of the partial gene product showed a very high sequence identity (91.4%) with the photosystem I (PSI) subunit V from spinach (Steppuhn et al.

A

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  1   gttcaacattgtggatgtttggcatggggctcaattggtcacatagtggc
        F  N  I  V  D  V  L  A  W  G  S  I  G  H  I  V  A
 52   ttactacattttggcaacctccagcaacggctatgatcccaagttctttga
        Y  Y  I  L  A  T  S  S  N  G  Y  D  P  K  F  F  D
103   ctgatgtgatttcattttgatccaccctgttgtgttctttctcttgcttgt
        *
154   aatttgtatagcatatcaatgaatcattagttgaagagcttc
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B

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                 1                               34
FCOR3   .........FNIVDVLAWGSIGHIVAYYILATSSNGYDPKFFD*
                 |||||||||||||||||||||||:||||||:|||
SUB V   LLKSNDPVGFNIVDVLAWGSIGHIVAYYILATASNGYDPSFF*
                     150
```

C

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                 1                               34
FCOR3   .........FNIVDVLAWGSIGHIVAYYILATSSNGYDPKFFD*
                 ||:|||||||||||||||||||||:||||||:||
PsaG    ILKSNDPVGFNLVDVLAWGSIGHIVAYYILATTSNGYDPPFFG*
      101                                       143
```

Fig. 7 Sequence analysis of *Fcor3*. A: Nucleotide sequence and deduced amino acid sequence. B: Amino acid alignment of FCOR3 with subunit V from spinach photosystem I mRNA. C: Amino acid alignment of FCOR3 with PsaG from barley. Alignment was obtained using the Gap and pretty programs of the Genetics Computer Group software package.

1988) and with the PSI PsaG polypeptide from barley (Okkels et al. 1992) (Fig. 7B, C).

Discussion

A cDNA library was prepared from cold-acclimated strawberry plants to identify genes regulated by LT exposure. Three cDNA clones were characterized and northern analyses indicated that expression of two of these cDNAs (*Fcor1* and *Fcor2*) is up-regulated by LT while the other (*Fcor3*) is down-regulated.

The accumulation of the *Fcor1* transcript was found to be higher in the hardy cultivars compared to the less tolerant ones. This correlation between the RNA level and FT suggests that *Fcor1* may be involved in the acclimation process and that it could be useful as a molecular marker to select for the FT trait in new strawberry cultivars and other related species of the *Rosaceae* family. The lower level of expression of *Fcor1* in the sensitive cultivar could be explained by a different genomic organization, gene copy number, mRNA stability, or differential gene regulation. In cereals, it has been suggested that FT is determined by the degree and duration of LT-induced gene expression. The response pattern of the LT-induced *Wcs120* gene family in wheat indicates that the inability to maintain LT-induced gene expression in an up-regulated state is the main reason why spring habit cultivars do not achieve a level of FT similar to those of winter habit cultivars (Fowler et al. 1996). The regulatory mechanism responsible for this differential response is still not understood.

A computer search of databanks revealed that *Fcor1* shares homology with the barley LT-specific gene *blt101*. The *blt101* transcript accumulates in shoot meristems upon transfer of plants from control to LT and other stress conditions. *Fcor1*, unlike *blt101*, is not significantly induced by drought, salinity, or ABA treatment. However, this does not rule out that both *Fcor1* and *blt101* may be regulated in the same manner by LT. The *blt101* gene encodes a 5.9 kDa polypeptide of 54 amino acids which is predominantly hydrophobic. The homology between BLT101 and FCOR1 is found over the first N-terminal 40 amino acids. Among this sequence, the first 20 amino acids are homologous to a consensus signal present in several extracellular proteins (Goddar et al. 1993). The database search revealed that *Fcor1* also has significant homology with *ESI3*, a gene induced early and to a high level during salt stress in the roots of *Lophopyrum elongatum*. Since BLT101 and ESI3 are identical proteins, they may play a similar role in barley and *Lophopyrum*. Gulick et al. (1994) have proposed that ESI3 may be an integral membrane protein that could play a role in membrane function or in the maintenance of membrane integrity in water-stressed tissues. The homology with BLT101 and ESI3 suggests that FCOR1 is either a membrane protein or an extracellular protein. Subcellular localization of this protein is required to determine its effective location.

Fcor2 has no similarity with any genes in the databanks. The basic nature of the encoded polypeptide suggests that FCOR2 may play a role similar to the highly basic histones. It could interact with DNA or with other negatively charged molecules such as RNA and phospholipids. The characterization of FCOR1 and FCOR2 is in progress. The expression of the cDNAs in *E. coli* and the production of the proteins for antibody preparation will allow us to determine the cellular location of these proteins and to better understand their possible function during LT exposure.

The *Fcor3*-encoded polypeptide shares a very high similarity with the nuclear-encoded subunit V of photosystem I (PSI) from spinach, and with the precursor of the PsaG polypeptide of PSI from barley. Steppuhn et al. (1988) have suggested that this protein would primarily fulfill a structural function and would not be directly involved in the binding of Fe^{2+} or in electron transport. PsaG (also known as PSI-G) and PSI-K are two similar polypeptides. PSI-K has been identified in a wide range of organisms from cyanobacteria to higher plants (Chitnis et al. 1995). It is more closely associated with the PSI-A/PSI-B heterodimer than any other small PSI subunits. In barley, the PSI-G polypeptide has been shown to be tightly bound to this dimer (Okkels et al. 1992). This may suggest a possible role of PSI-G and PSI-K in the organization of the PSI core. It was recently suggested that PSI-G has a function in binding light harvesting complex (LHCI) to the PSI (Nielsen et al. 1996, Chitnis et al. 1995). The similarity of FCOR3 with the PSI subunit PSIG and the tissue specificity of expression suggest that this gene may represent a candidate to study the role of PSI in modulating stress conditions, a role that is poorly understood. It is now well established that growth at LT not only induces FT of winter cereals, but also induces an increased resistance to LT-induced photoinhibition of photosynthesis (Öquist et al. 1993). LT modulation of the photosynthetic apparatus may thus be an important factor during the induction of FT in cereals. The decrease of *Fcor3* mRNA level during LT exposure could reflect a metabolic adjustment needed to protect some components of the PSI machinery. For example, the reduced level of FCOR3 protein could lower the amount of electrons transferred by the antennae to this photosystem. Such a reduction in electron flow may prevent the destruction of the LHCI which could be saturated due to the lower rate of thermochemical reactions during photosynthesis at LT. On the other hand, it is possible that FCOR3 may be related to a stress response since the transcript level is affected by stresses other than LT exposure and that it does not completely return to the control level after 1 week of deacclimation. Isolation of the complete cDNA and characterization of the purified protein are needed to understand the possible function of this protein in PSI.

Based on the available evidence, we cannot yet assign a definite role to the gene products corresponding to the cDNAs isolated in this study. Taken together, our results suggest that the *Fcor1* gene or its product may be used as potential molecular markers to select for the FT trait in the *Rosaceae* family. We plan a more detailed physicochemical characterization of these proteins to determine their possible function. It would also be of great importance to understand, at the molecular level, how the differential gene expression is regulated by low temperature and how this affects freezing tolerance.

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