

**Plant Gene Register**

# New Cold- and Drought-Regulated Gene from *Medicago sativa*<sup>1</sup>

Serge Laberge\*, Yves Castonguay, and Louis-Philippe Vézina

Station de Recherches, Agriculture Canada, 2560 boulevard, Hochelaga, Sainte-Foy Québec, Canada G1V 2J3

Plants are known to differ in their ability to withstand freezing temperatures, but the molecular/genetic basis of this differential freezing tolerance is unclear. Exposure of plants to low, nonfreezing temperatures (cold acclimation) increases their tolerance to subsequent freezing (see recent review by Guy, 1990). Significant biochemical modifications occur during cold acclimation of plants, including changes in gene expression (Thomashow, 1990). Three cold acclimation-specific cDNAs (Mohapatra et al., 1989) and one cDNA responsive to environmental stress (cold and drought) and ABA (Mohapatra et al., 1988) have previously been isolated from alfalfa (*Medicago sativa* L. cv Apica). DNA sequence determination of four alfalfa cDNAs that were shown to be responsive to environmental stresses (low temperature and drought) and ABA indicated that they are part of a family of genes encoding Gly-rich proteins containing many repeated peptide motifs (Luo et al., 1991, 1992).

We report here the isolation of a new full-length cDNA clone (MsaciA) that is cold and drought regulated in alfalfa. Plants of the cold-tolerant alfalfa were grown at 21°C for 5 weeks and cold acclimated for 2 weeks at 2°C. A  $\lambda$ gt10 library was constructed with mRNA isolated from cold-acclimated crowns. The cDNA of a cold-inducible transcript was isolated by differential hybridization using single-strand cDNA synthesized from cold-acclimated and nonacclimated crowns. The nucleotide sequence of the full-length cDNA and the deduced amino acid sequence of MsaciA have been determined (Table I). MsaciA encodes a putative Gly-rich protein (38%), which contains many repeated motifs. This putative protein shares homology in the range of 68 to 88% (amino acid identity) with the previously isolated environmental stress- and ABA-regulated putative proteins from alfalfa (Luo et al., 1991, 1992) and, thus, represents a new member of this gene family.

## ACKNOWLEDGMENT

We thank Messrs. Lucien Pelletier, Pierre Lechasseur, and Jean Langlois for their excellent technical assistance.

Received October 28, 1992; accepted November 14, 1992.  
Copyright Clearance Center: 0032-0889/93/101/1411/02.

<sup>1</sup> This paper is contribution No. 450 of the Station de Recherches de Sainte-Foy.

\* Corresponding author; fax 1-418-648-2402.

**Table I.** Characteristics of the MsaciA cDNA from *M. sativa*

Organism:	<i>Medicago sativa</i> cv Apica.
Gene:	cDNA encoding for a Gly-rich protein.
Location on Chromosome:	Multigene family of unknown location.
Function:	Unknown.
Techniques:	cDNA library constructed in $\lambda$ gt10 with mRNA isolated from crowns of 5-week-old plants acclimated 2 weeks at 2°C, 8-h photoperiod, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Isolation by differential hybridization using single-strand cDNA probes from cold-acclimated and nonacclimated crowns. Complete sequence of forward and reverse strands.
Method of Identification:	Sequence comparison to GenBank/EMBL data base: Sequence identity to the coding region of <i>Medicago falcata</i> pSM2075 (Luo et al., 1991) and the coding region of <i>M. sativa</i> pUM90-1, pUM90-2, and pUM91-4 clones (Luo et al., 1992).
Expression Characteristics:	Abundant mRNA of approximately 0.9 kb induced by exposure of whole plants to 2°C. Induced also in plants grown at 22°C by water stress to approximately 50% of the level observed in cold-acclimated plants.
Features of cDNA Structure:	Deduced translation start site at nucleotide 33 and stop site at nucleotide 647.
G/C Content:	51.2% in coding region.
Codon Usage:	Strong bias for GGT (Gly) in the protein-coding region.
Structural Features of Protein:	Deduced amino acid sequence of 204 residues containing 38% Gly, 9% His, 9% Asn, and 7% Tyr. Absence of Pro, Trp, Phe, and Cys.
Antibodies:	Not prepared.

The GenBank/EMBL accession number for the sequence reported in this article is L03708.

## LITERATURE CITED

- Guy CL (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annu Rev Plant Physiol Plant Mol Biol* 41: 187–223
- Luo M, Lin L, Hill RD, Mohapatra SS (1991) Primary structure of

- an environmental stress and abscisic acid-inducible alfalfa protein. *Plant Mol Biol* **17**: 1267–1269
- Luo M, Liu J-H, Mohapatra S, Hill RD, Mohapatra SS (1992)** Characterization of a gene family encoding abscisic acid- and environmental stress-inducible proteins of alfalfa. *J Biol Chem* **267**: 15367–15374
- Mohapatra SS, Poole RJ, Dhindsa RS (1988)** Abscisic acid-regulated gene expression in relation to freezing tolerance in alfalfa. *Plant Physiol* **87**: 468–473
- Mohapatra SS, Wolfrain L, Poole RJ, Dhindsa RS (1989)** Molecular cloning and relationship to freezing tolerance of cold-acclimation-specific genes of alfalfa. *Plant Physiol* **89**: 375–380
- Thomashow MF (1990)** Molecular genetics of cold acclimation in higher plants. *Adv Genet* **28**: 99–131