

**TRANSCRIPTION FACTORS INVOLVED IN
COLD RESPONSE IN PLANTS**

by

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Declaration

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Abstract

Studies in *Arabidopsis* have shown cold stress tolerance can be enhanced by manipulation of the *CBF/DREB* and *ICE* transcription factor genes. To date, few studies have investigated *CBF* and *ICE* genes in crop species such as barley. Using a C-repeat element as bait, two *CBF* genes were isolated from a cold-stressed barley cDNA library, *HvCBF16* and *HvCBF23*. *HvCBF16* was induced by cold treatment but not by other abiotic stresses. *HvCBF23* was constitutively expressed and was not induced by cold treatment. The analysis of transgenic plants expressing these genes will determine their importance in cold tolerance.

Transgenic barley plants expressing the barley gene *HvCBF2A* were found to be more cold tolerant in controlled temperature trials, and hence were assayed to determine the basis of their acquired phenotype. Northern and qRT-PCR analysis showed that four genes known to be involved in cold tolerance were significantly upregulated. Importantly the increased expression was proportional to the level of transgene expression and levels were higher following cold treatment.

A homolog of the *Arabidopsis ICE* transcription factor was isolated from a freezing-tolerant barley variety (*Hordeum vulgare* L. cv. Haruna Nijo) and transcript analysis of *HvICE2* under various abiotic stresses showed that expression of *HvICE2* was induced at low temperatures, particularly in floral tissues. *HvICE2* was over-expressed using the maize *ubiquitin* constitutive promoter in transgenic barley. Expression analysis of putative downstream genes, including various *COR* genes, in the transgenic plants before and during cold treatment did not reveal any alteration in expression. This suggests *HvICE2* that the *COR* genes studied are not targets of *HvICE2* or that additional factors or conditions are required for effective function of *HvICE2*. Transgenic *Arabidopsis* plants were produced with over- or reduced-expression of the uncharacterised *ICE* gene, *AtICE2*. The cold tolerance of the *AtICE2* transgenic lines was not significantly different from wild type plants.

Abbreviations

AP2	Apetala 2	LB	Luria-Bertani
<i>At</i>	<i>Arabidopsis thaliana</i>	M	Molar
bp	Base pair	min	Minutes
BSA	Bovine serum albumin	ml	Millilitre
CBF	C-repeat binding factor	mM	Millimolar
cDNA	Complementary DNA	mRNA	Messenger RNA
COR	Cold-responsive	n	Nano
CRT	C-repeat	PCR	Polymerase Chain Reaction
cv	Cultivar	QTL	Quantitative trait locus
DAP	Days after pollination	RACE	Randomly amplified cDNA ends
dCTP	Deoxycytidine triphosphate	RNA	Ribose Nucleic Acid
DNA	Deoxyribonucleic acid	RNase	Ribonuclease
dNTP	Deoxyribonucleotide triphosphate	rpm	Revolutions per minute
DRE	Dehydration response element	sec	Seconds
DREB	Dehydration response element binding (protein)	SD	Synthetic dropout
ERF	Ethylene responsive factor	<i>Ta</i>	<i>Triticum aestivum</i>
EST	Expressed sequence tag	Taq	<i>Thermus aquaticus</i>
HPLC	High performance liquid chromatography	<i>Tm</i>	<i>Triticum monococcum</i>
hrs	Hours	U	Enzymatic units
<i>Hv</i>	<i>Hordeum vulgare</i>	UV	Ultra violet
ICE	Inducer of CBF expression	v/v	Volume per volume
Kb	Kilo base	V	Volts
L	Litre	w/v	Weight per volume
		Y1H	Yeast 1-hybrid
		YPD	Yeast potato dextrose