CLONING SOME SALINITY STRESS RELATED GENE(S) IN BARLEY

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ABSTRACT

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The main task of the present work was to isolate and characterize abiotic stress-related gene(s) from barley (*Hordeum spontaneum* L.) through polymerase chain reaction (PCR). Two salt-responsive cDNA fragments were characterized *via* differential display-polymerase chain reaction (DDR-PCR) from wild barley (*Hordeum spontaneum* L.) under salt stress (250mM NaCl) were pre selected as the most tolerant against salt stress (**Eissa *et al.*, (2007**). Polymerase chain reaction (PCR) was adopted to amplify *RAP1/4* and *SCP11/1* related sequences using specific primers and genomic DNA isolated from wild barley samples. PCR amplified products were purified, cloned, transformed into *E. coli* and sequenced using ABI PRISM 310 Gene Analyzer. DNA sequencing were detected and then subjected to homology searching *via* computer software. The results indicated that the fragment of RAP ¼ B contained many conserved domains and its nucleic acid sequence was similar to the *DREB1/CBF3* from barley (*Hordeum vulgare* L.) with significant homology of 84%. Semi-quantitative RT-PCR showed that *HsCBF3* was induced in the Barley by NaCl (250 mM) treatments. These results suggested that the novel *CBF3* gene might play an important role in response to high salinity through binding to the DRE *cis*-element.

Key words: Barley, *Hordeum spontaneum* L., salt stress, cloning, function analysis, Semi quantitative-PCR, transcription factor, CBF3 gene.

LIST OF ABBREVIATION

ABA	Abscisic acid
ACP	Annealing-control-primer
Blast	Basic local alignment search tool
CBF	C-repeat binding factors
Cd	Conserved domain
DREB	Dehydration response element binding protein
FAO	Food and Agriculture Organization
HS	*Hordeum spontaneum*
HV	*Hordeum vulgare*
LEA	Late embryogenesis abundant
MCS	Multiple cloning site
RT-PCR	Reverse transcriptase-polymerase chain reaction
ROS	Reactive Oxygen Specie

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I. INTRODUCTION

Barley (*Hordeum vulgar* L.) is one of the main cereals of the Mediterranean agriculture belt and is a founder crop of old world Neolithic food production and one of the earliest crops domesticated **(Harlan and Zohary, 1966; Mohammed and Takao 2007)**.

Barley (*Hordeum vulgare* L.) is the fourth important cereal crop in Egypt after wheat, maize and rice **(Salem *et al.,* 2009)**. Barley is the main crop grown on a large scale in the low rainfed northern coastal region (100-200 mm annual rainfall), in newly reclaimed land and in regions affected by salinity or where irrigation water is limited. It is grown in both rainfed and irrigated conditions, though in the more favourable irrigated soils of the Nile Valley, barley gives way to more valuable crops. Barley production is severely affected by rainfall, as a consequence, the total barley area fluctuates dramatically in the world, *e.g*. 34,000 hectares (ha) in 1970-1974 to 188,000 ha in 1994/1995 **(Forster *et al.,* 2004).**

Drought, high-salt and low-temperature stresses are important factors affecting the development of plants and the yields of crops. When exposed to these abiotic stresses, plants will undergo a series of physiological and biochemical changes, which lead to the activation or inactivation of some specific endocellular signaling pathways in response to stimuli (**Shinozaki and Yamaguchi-Shinozaki, 1996, Liu *et al.,* 1998**). There are mainly two sorts of genes involved in these processes. Some encode proteins that can either participate in protecting plant cells from the stresses or repair injuries caused by the stresses, and the others are the protein kinases that respond to and transduct the stress induced signals or transcription activators (**Shinozaki and Yamaguchi-Shinozaki, 1996**). Salinity affects approximately 20% of the world's arable land and approximately 40% of irrigated land to various

degrees (**Sahi *et al*., 2006**). High salinity is one of the most important environmental stresses that can lead to changes in plant growth and development. Severe stress may threaten survival and finally undermine crop productivity. Improving the response of crop plants requires a thorough understanding of the cellular mechanisms that allow certain species to grow at high salinity. Plants respond to abiotic stress by altering the expression of many genes, which in turn affect major mechanism leading to adaptation and survival during periods of stress (**Hasegawa *et al*., 2000**). Salt-stress response is shown to encompass large number of genes including those linked to different pathways, which leads finally to a response on the whole plant level.

CBF/DREB (C-repeat binding factor/dehydration responsive element binding factor) family of transcription factors in plants is reported to be associated with regulation of gene expression under stress conditions.

Many genes that respond to drought, high-salt and low-temperature conditions were discovered to have DRE or CRT (C-repeat element) in their promoter regions (**Yamaguchi-Shinozaki and Shinozaki, 1994; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000**). Five transcription activators, *i.e.* DREB1 A-C and DREB2 A-B, were obtained by screening the Arabidopsis cDNA library and by using the yeast one-hybrid method, which specifically bind to DRE and regulate the expression of stress-resistant genes. DREB1s are induced by low-temperature, whereas DREB2 is induced by drought and high-salt conditions. DREB1 and DREB2 independently function as transcription activators in low-temperature and drought-induced signaling pathways, respectively **(Liu *et al*., 1998**)

Furthermore the dehydration-responsive transcription factors (DREB) and C-repeat binding factors (CBF) bind to DRE and CRT

cis-acting elements that contain the same motif (CCGAC). Members of the CBF/DREB1 family, such as CBF1, CBF2, and CBF3 (or DREB1B, DREB1C, and DREB1A, respectively) are themselves stress-inducible. DREB/CBF proteins are encoded by AP2/EREBP multigene families and mediate the transcription of several genes such as rd29A, rd17, cor6.6, cor15a, erd10, kin1, kin2 and others in response to cold and water stress **(Ingram and Bartels 1996; Stockinger *et al.,* 1997; Gilmour *et al.,* 1998; Liu *et al.,* 1998; Seki *et al.,* 2001; Thomashow *et al.,* 2001)**.

Barley (*Hordeum vulgare*) Cbf3 is located on barley chromosome 5H between markers WG364b and saflp58 on the barley cv Dicktoo _ barley cv Morex genetic linkage map. This position is some 40 to 50 cM proximal to the winter hardiness quantitative trait locus that includes the Vrn-1H gene, but may coincide with the wheat 5A Rcg1 locus, which governs the threshold temperature at which cor genes are induced. From this, it remains possible that HvCbf3 is the basis of a minor quantitative trait locus in some genetic backgrounds, though that possibility remains to be thoroughly explored **(Dong *et al*., 2002)**

The objectives of the present work is the following:

1. Isolation and cloning the stress tolerance gene(s) with emphases to salinity.
2. Sequencing and characterization of the isolated gene(s) *via* published genetic data base.
2. Study the effects of salt stress in barley leaves on gene expression profiling related to salt stress; DREB-CBF3-like gene using semi-quantitative RT-PCR.