FUNCTIONAL GENOMIC ANALYSIS FOR SALT STRESS RELATED GENES IN BARLEY

(Hordeum spontaneum L.)

Ву

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ABSTRACT

Lamyaa Mostafa Kamal: Functional Genomic Analysis for Salt Stress Related Genes in Barley (*Hordeum spontaneum* L.). Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2010.

Barley (*Hordeum vulgare* L.) is a salt-tolerant crop species with considerable economic importance in salinity-affected arid and semiarid regions of the world. In this work, barley cultivar (*Hordeum spontaneum* L.) was used to isolate gene (*HVP1*) analogue which is responsible for the expression of H⁺-inorganic pyrophosphatase (V-PPase) found in plant vacuolar membranes translocates H⁺ into the vacuoles in conjunction with the vacuolar H⁺-ATPase (V-ATPase). Furthermore, the isolated gene was cloned, sequenced and compared with the H⁺-inorganic pyrophosphatase genes isolated and identified from other organisms.

Key Words:

Barley, Abiotic stresses, Salinity, vacuolar H⁺- inorganic pyrophosphatase (*HVP1*).

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LIST OF ABBREVIATIONS

2, 4-D Auxin (2,4-dichlorophenoxyacetic acid)

ABA Abscisic acid hormone

AGERI Agriculture genetic engineering research institute

AVP Vacuolar H +-inorganic pyrophosphatase isolated

from Arabidopsis thaliana

BVP Vacuolar H +-inorganic pyrophosphatase isolated

from Beta vulgaris

DEAD box Genes encoding eukaryotic initiation factor 4A

ESTs Expressed sequence tags

GA₃ Gibberellic acid

HVP Vacuolar H⁺-inorganic pyrophosphatase isolated

from *Hordeum vulgare*

IF Internal forward primer

IR Internal reverse primer

MS Molecular size
MW Molecular weight

NCBI National center for biotechnology information

OVP Vacuolar H +-inorganic pyrophosphatase isolated

from Oryza sativa

PCR Polymerase chain reaction

PPi Inorganic pyrophosphate

ROS Reactive oxygen species

RT-PCR Reverse transcription (cDNA-PCR)

SOS Salt overly sensitive

SP Spacific primer

STS Sequence tagged sites

TAE Tris-Acetic acid glacial-EDTA

TE buffer Tris-EDTA buffer
TF Total forward primer
TR Total reverse primer

TVP Vacuolar H⁺-inorganic pyrophosphatase isolated

from Nicotiana tabacum

V-PPase Vacuolar H⁺-inorganic pyrophosphatase enzyme

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I. INTRODUCTION

Abiotic stresses can severely impair plant growth and development. Environmental factors, such as drought, extreme temperatures, low or high, and salinity are also responsible for significant yield reductions in cultivated areas world-wide. Thus, the responses of plants to various stresses for decades have been the focus of physiological studies and, more recently, of molecular and reverse genetics studies and transgenic experimentation (Oztruk et al., 2002).

Salinity is one of the major environmental factors that limit the worldwide crop productivity. Salt tolerance of barley has been of interest for a long time and has resulted in a considerable body of data from studies using physiological, genetic, cytogenetic approaches and more recently, of molecular and reverse genetics studies. Recent molecular characterization of salinity stress in plants has indicated the involvement of multiple genes responsive to salinity. Large-scale approaches including microarrays and differential display have been employed to identify genes, transcripts and proteins responding to salinity stress in plants (Walia et al., 2006).

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world after wheat, maize and rice and is widely grown in the arid and semiarid regions of the Mediterranean for forage purposes and as a grain crop. Due to its low water demand and its tolerance to drought and salinity, it was considered as a successful candidate for cultivation in arid and newly reclaimed lands in Egypt. This crop has been growing in Egypt since the ninth millennium B.C. and Egypt is considered as one of the centers of origins of cultivated barley, so attention has been given to the Egyptian barley germplasm (**Wendrof** *et al.*, 1979 and **Zohary and Hope**, 1988).

Wild barley (Hordeum spontaneum L.) shows a large morphological and phenotypic variation, which is associated with