FUNCTIONAL GENOMIC ANALYSIS FOR SALT STRESS RELATED GENES IN BARLEY

(Hordeum spontaneum L.*)*

By

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2000

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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 Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2010.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
GA_3	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