بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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MONA MAGHRABY

IDENTIFICATION OF SOME SALINITY RELATED GENES IN WILD BARLEY

By

SHAIMAA RADWAN MAHMOUD MOHAMED KHALIL

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2007
M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2015

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Identification of Some Salinity Related Genes in Wild Barley

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ABSTRACT

Wild barley (*Hordeum spontaneum*) is the progenitor of the cultivated barley (*Hordeum vulgare L.*). In an attempt to evaluate the potential use of the Egyptian wild barley as a source of salt tolerance related genes, its response to salt stress was assessed at the physiological and molecular levels. The physiological evaluation was conducted at 10 levels of salinity along with the control by measuring some photosynthesis parameters (OJIP, *Fv/Fm* and PI). Photosynthesis efficiency began to be affected by salt stress at 125 mM and continued at 150, 175, 200, 225 and 250 mM NaCl. The DDRT- PCR of wild barley from plants treated with 0, 125 and 200 mM NaCl using 14 arbitrary primers revealed the overexpression of seven genes (*thi4, B2, vps29, lhc3, oee1, shmt* and *sbt*). Validation of the identified up-regulated genes was performed using the Real-time PCR analysis. Out of the seven genes, *B2* revealed the highest up-regulated expression followed by *shmt*. This study was extended to evaluate the response of ten Egyptian barley cultivars (Giza 129, 132, 123, 127, 2000, 130, 126, 135, 128 and 133) to salinity stress. The ten cultivars were assessed under open field conditions by measuring the OJIP, *Fv/Fm* and PI. In addition, the growth parameters (No. of leaves, plant height and total leaves area) and other photosynthesis parameters (RC/CS, TFm, RC/ABS and ABS/RC) at 0,100 and 200 mM NaCl were assessed. Giza 129 was identified as the most salt tolerant and Giza 123 as the most salt sensitive. Both cultivars along with Giza 132 (moderately salt tolerant) were used to evaluate the relative expression of the *B2* and *shmt* genes at 0,100 and 200 mM NaCl. The expression of both genes increased in the tested cultivars upon salinity treatment, while at 200 mM NaCl the fold increase in gene expression in Giza 129 was significantly higher followed by Giza 123 and 132. Repeating the same experiment for Giza 129, 132 and 123 along with wild barley under growth chamber revealed that the effect of salinity was more vigorous at the physiological and morphological levels. In addition, the gene expression was in general higher than that of the open field conditions although exhibiting similar trend of expression pattern. Based on the results, the *B2* of the Egyptian wild barley was chosen as a promising candidate gene for salt stress tolerance. The partial sequence (823 bp) identified by the DDRT- PCR at the 3' end of the *B2* gene of the Egyptian wild barely was employed to design gene- specific primers to obtain the *B2* 5' end (831bp) *via* RLM- RACE. The full length sequence of the *B2* gene (1449 bp) was successfully assembled and deposited in the GenBank under the accession number MT249004. Compared to its published homologue in the cultivated barley, the nucleotide sequence of the isolated *B2* gene was 23 bp longer and its deduced amino acids sequence 5 a.a. residues shorter (354 a.a. *vs*. 359 a.a.) with 96.1 % identity.

Keywords: *Hordeum spontaneum*, DDRT- PCR, *Hordeum vulgare*, salinity, chlorophyll fluorescence, Real-time PCR and RLM- RACE.

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my mother, to my father, to my sister (Walaa), to my nephews (Mydi, Zain, Ali, Sharaf and Lila) and to my brother (Ataa), for their endless support along the period of my post-graduation.

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