GENETIC ENGINEERING APPROACHES TO IMPROVE ABIOTIC STRESS RESISTANCE IN EGYPTIAN WHEAT

BY

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 1999

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ABSTRACT

Ahmed Mohammed Mohammed Ramadan, Genetic engineering approaches to improve abiotic stress resistance in Egyptian wheat Unpublished master of Science Thesis, Genetics Dept., Fac. Agric., Ain Shams Univ., 2005.

The bacterial gene, *mtlD*, that encodes mannitol-1-phosphate dehydrogenase,for mannitol accumulation was introduced into Egyptian bread wheat cv. Giza 163. The transformation was carried out by particle bombardment of immature embryos using the biolistic device. A plant expression vector was constructed in which *mtlD* gene was driven by the *ubi* promoter, and the selectable marker *bar* encoding the phosphinothricin acetyl transferase gene for herbicide resistance was driven by the *CaMV 35S* promoter. Two T_0 plants were regenerated and submitted to preliminary evaluation by leaf painting with the herbicide basta (1 g/L) and showed no symptoms of necrosis. The two putatively transgenic plants were submitted to PCR and Southern analysis, in which the latter showed that one copy of the *mtlD* gene has been incorporated. Subsequent molecular testing was carried out to determine gene expression in transformed T_1 wheat plants using northern analysis. Salt stress experiment in sand culture indicated that one of the two transgenic events could tolerate intermediate concentration of salt (8000 ppm 3 NaCl/1 CaCl2). Under salt stress, flag leaf area in transgenic plants was reduced by 60%, plant height by 35%, peduncle length by 24%, 1000 grain weight by 26%, biological yield by 74%, and grain yield by 40%. In wild type, flag leaf area was reduced by 53%, plant height by 40%, peduncle length by 46%, 1000-grain weight by 58%, biological yield by 71%, and grain yield by 66%. Finally, we concluded that this approach can be used in improving abiotic stress tolerance in wheat

Key words: Wheat, immature embryos, transformation, biolistic bombardment, drought tolerance, Mannitol, leaf-painting, bialaphos, PCR, genomic Southern, Northern.

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List of abbreviations

AP1	Proteinase inhibitor
Bip	Endoplasmic reticulum response binding protein
Camv	Cauliflower mosaic virus
CMO	Choline mono oxygenase
DEX	Dexmethasone
DON	Deoxynivalenol
DRE	Dehydration response element
EPSPs	5-enolpyruvylshikimate-3-phosphate synthase
Fad7	Omega-3-fatty acid desaturase
FHB	Fusarium head blight
G163	Giza163
GPD	Glycerol-3-phosphate dehydrogenase
GUS gene	β- glucourindase
HMW	High molecular weight glutenin
hpt	Hygromycin phosphor transferase
HVA1	One of a late embryogenesis abundant (LEA) protein genes
KP4	Antifungal protein from *Ustilago maydis* infection virus
LMW	Low molecular weight glutenin
MAPKKK	Mitogene activated protein kinase kinase kinase
MS media	Murashige and skoog media
mtlD	Mannitol-1-phosphate dehydrogenase
NMR	Nuclear magnetic resonance
nptII	Kanamycin resistance gene
osCDPK	Calcium-dependent protein kinase
OSML	Osmotin-like protein genes
otsA and otsB	Trehalose biosynthetic genes
PPT	Phosphinothircin
PR	Pathogenesis related protein
P-ubi	Ubiquitin promoter
RM1	Cytokinin containing media
SA	Salicylic acid
SacB	Fructan accumulation gene
SOD	Super oxide dismutase
WSMV	Wheat streak mosaic virus
WUE	Water use efficiency

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