The Transcriptional Response of Barley (*Hordeum vulgare* L.) to Boron Toxicity

By

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ABSTRACT

The occurrence of Boron (B) toxicity in Australian soils is recognised as a limiting factor for cereal productivity. A number of loci conferring tolerance to B toxicity have been identified in barley and chromosomally mapped. However, a lack of knowledge relating to the physiological and molecular events that occur under B toxicity and the molecular basis for B stress tolerance has been a bottleneck in harnessing available genetic diversity in barley and wheat. The recent advances in functional genomics provided an opportunity to examine B stress in barley in more detail. The aim of this project was to analyse genes differentially expressed under B stress in tolerant and intolerant barley to identify candidate genes involved in B toxicity tolerance. Two experimental approaches, Suppression Subtractive Hybridization (SSH) and microarray were adopted.

Firstly, SSH was performed to examine gene expression in roots of selected tolerant and intolerant doubled haploid lines from a Clipper (B intolerant) X Sahara 3771 (B tolerant) mapping population, grown under moderate B stress. The SSH experiment aimed to investigate the early transcriptional response of B tolerant barley lines to B stress in order to identify the basis for B toxicity tolerance in roots.

Differential screening of the subtracted library generated from B treated plants identified a total of 111 non-redundant clones up-regulated in bulked tolerant lines. On the other hand 94 clones were differentially expressed under non-treated conditions. Among the clones identified from subtracted library generated from B treated plants, metabolism was the largest functional category, representing 21% of the clones. The largest functional category in the subtracted library generated from non treated plants was cellular transport, representing 19% of the clones. Based on sequence similarity, about 170 transcripts identified in this experiment were assigned to chromosomal segments (bins) on the three homoeologous genomes of bread wheat. In total, 36 clones from the subtracted library generated from B treated plants were analysed as candidates. Nine were genetically mapped within the region of B tolerance QTL on three chromosomes (2H, 4H and 6H). The genes mapped to 4H and 6H QTL have the highest association with these loci in the Clipper X Sahara 3771 doubled haploid mapping population. A 4H B tolerance QTL candidate gene was identified as a B transporter gene with similarity to the *Arabidopsis BOR1* gene. Genes identified to be differentially expressed in the tolerant lines from SSH suggest activation of a diverse defence response in the roots of barley plants under B stress. Data from SSH experiment indicate that cell wall-plasma membrane-

cytoskeleton continuum constitute the first action site against B toxicity and the influence of toxic B on K^+ uptake could be the key initiating factor.

In the second approach, the Affymetrix 22K Barley1 GeneChip™ was used to investigate B stress adaptation processes in barley. Gene expression was profiled in leaves of Sahara 3771 and Clipper plants grown under various B concentrations. The results show that the two genotypes respond differently to B toxicity. The B intolerance of Clipper is expressed through the induction of a high number of probe sets (2310) even at a low B concentration of 100 μM. In contrast, Sahara 3771 responded to a high B concentration (2000 μM) through the induction of only a few hundred (266) probe sets. In Sahara 3771 no change in the expression level of any probe sets was observed at 100 μM B. Altogether 286 probe sets showed differential expression in Sahara 3771 under three levels of B treatment (500, 1000 and 2000 μM). About 30% of these were down-regulated and about 70% were up-regulated in Sahara 3771 in response to B treatment. Most of the probe sets (59%) up-regulated in Sahara 3771 did not respond to B treatment in Clipper. These genes are either salt stress responsive or related to plant defense and thus could play a key role in protecting barley plants from the toxic effects of B.

Two differentially expressed probe sets annotated as B transporters were identified between Sahara 3771 and Clipper under control condition. These two B transporter probe sets did not respond to B treatment but showed opposing expression patterns in the two varieties. One of these probe sets (Contig21126_at) is similar to the B transporter gene isolated from the SSH experiment that maps to the 4H tolerance locus. The map location and expression of this B transporter gene suggest that it could be the borate anion efflux transporter predicted by the proposed efflux model of B tolerance in Sahara 3771 barley. The other B transporter gene (Contig14139_at) showed over expression in Clipper under control condition and could be contributing to high B accumulation in Clipper which needs further investigation.

Data from both experiments have indicated that B toxicity triggers oxidative stress and that jasmonate-based signaling plays a key role in B toxicity tolerance. SSH data indicate that Sahara 3771 which evolved in the harsh environment of Africa is more efficient in osmoregulation and ROS scavenging than Clipper. This trait is likely to give Sahara 3771 an edge over Clipper in tolerating toxic the effect of B. In addition to the efflux mechanism, which becomes less efficient with increasing B supply, Sahara 3771 appears to apply a number of other mechanisms for alleviating or withstanding toxic B induced stress to sustain growth. Some of these mechanisms are already known to be used by plants to cope with a number of stresses.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this copy of my thesis, when deposited in University Library, being available for loan and photocopying.

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List of Abbreviations

($B[OH]_3$) or H_3BO_3 = boric acid

(O_2^-) =superoxide

µF =microfarrad

µg = microgram

µM = micromole

12-OPDA = 12-oxo-phytodienoic acid

13S-HPOT = 13(S)-hydroperoxy-9(Z),ll(E),15(Z)- octadecatrienoic acid; a-ketol, 12-oxo-13-hydroxy-9(Z),15(Z)-octadecadienoic acid

4CL = 4-coumarate:CoA ligase

A9C = anthracene-9-carboxylic acid

ABC = ATP binding cassette

ACPFG = Australian Centre for Plant Functional Genomics

ADP = adenosine diphosphate

AE = anion exchanger

AGRF = Australian Genome Research Facility

AOC = allene oxide cyclase

AOS = allene oxide synthase

AQP9 = animal aquaporin9

ATP = adenosine 5'-triphosphate

B = boron

$B[OH]^-_4$ = borate

BAC = bacterial artificial chromosome

BADH = betaine aldehyde dehydrogenase

BLAST = Basic Local Alignment Search Tool

BOR1= boron transporter 1

Br^- = bromine ion

BSA = Bovine Serum Albumin

bZIP = Basic Leucine Zipper

C X S = Clipper X Sahara

C4H = cinnamate 4-hydroxylase

CA II = carbonic anhydrase II

Ca^{2+} = calcium ion

CaMV = Cauliflower Mosaic Virus

cDNA = complementary DNA

CDS = coding sequence

CeSA 1= cellulose synthase A catalytic subunit 1

CeSA 3= cellulose synthase A catalytic subunit 3

CEV1= constitutive expression of VSP1 protein 1

CHCA = α-cyano-4-hydroxycinnamic acid

CHS = chalcone synthase

CIMMIT = International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento del Maíz y del Trigo)

Cl^- = chlorine ion

cM = centimorgan

cm = centimeter

CPRF2 = light-inducible protein CPRF-2

cRNA = complementary RNA

CS = Chinese Spring

Cyt = cytochrome

dATP = 2-deoxyadenosine 5'-triphophate

dCTP =2-deoxycytidine 5'-triphophate

DEPC =diethylpolycarbonate

dGTP = 2-deoxyguanosine5'-triphosphate

DH = doubled haploid

DHA = dehydroascorbate

DI = deionized

DIDS = 4,4'-di-isothiocyanostilbene-2,2'-disulfonic acid

DPC = diphenylamine-2carboxilic acid

dTTP = 2-deoxythymidine 5'-triphophate

dw= dry weight

ECM = extra-cellular matrix

EDTA = ethylendiaminetetracacetic acid

EST = expressed sequence tag

FDR = false discovery rate

fw = fresh weight

GB = glycine betaine

GDH = glutamate dehydrogenase

GLP = germin-like protein

GONST = golgi nucleotide sugar transporter

GPI= glucosylphospatidyl-inositol

GPX = glutathione peroxidise

GRP94 =94-kDa glucose related protein

GSH = reduced glutathione

GST = glutathione S transferase

H^+ = hydrogen ion/ proton

H_2O_2 = hydrogen peroxide

HAC1 = histone acetyltransferase HAC1

HAK = high-affinity K^+ uptake systems

HATS = high-affinity nitrate transport system

HCO_3^- = bicarbonate ion

$HgCl_2$ = mercuric chloride

HIF = heterogeneous inbred families

HsBTR1= *Homo sapiens* bicarbonate transporter-related protein 1

HSP = heat shock protein

HVGI = TIGR Barley gene index

I^- = iodine ion

ICARDA = International Center for Agricultural Research in the Dry Areas

ICP-MS = Inductively Coupled Plasma Mass Spectrometry

ITB = intolerant boron

ITC = intolerant control

JA = jasmonic acid

JAFA = Joined Assembly of Function Annotations

kDa = kiloDalton

kg = kilogram

K_m = Michaelis-Menten kinetics coefficient

LB = Luria broth

LIMMA = Linear Models for Microarray Data Package

LOD = Log of the Odds

LOX = lipoxygenase

LRS = Likelihood Ratio Statistics

M = mole

MAPK = mitogen-activated protein kinase

MAPKK = mitogen-activated protein kinase kinase

MAPKKK = mitogen-activated protein kinase kinase kinase

MDHA = monodehydroascorbate

mg = milligram

$MgCl_2$ = magnesium chloride

MIPS = Munich Information Center for Protein Sequences

MIPs = major intrinsic proteins

mM = millimole

MOPS = 3-(N-morpholino)propanesulfonic acid

mRNA= messenger ribonucleic acid

MRP = multidrug resistance-associated protein

N = nitrogen

Na^+ = sodium ion

Na_2CO_3 = sodium carbonate

NaBC1 = sodium borate cotransporter 1

NaCl = sodium chloride

NaCl = sodium chloride

NAD = nicotinamide adenine dinucleotide

NADH = reduced form of nicotinamide adenine dinucleotide

NADP = nicotinamide adenine dinucleotide phosphate

NADPH = reduced form of nicotinamide adenine dinucleotide phosphate

NADP-ME = NADP-malic enzyme

$NaHCO_3$ = sodium bicarbonate

NCBI = National Center for Biotechnology Information

NDH = NADH-quinone oxidoreductase

NdhK = NADH-plastoquinone oxidoreductase subunit K

ng = nanogram

NHX = sodium/hydrogen exchanger

NIL = near isogenic lines

NIP = nodulin 26 like intrinsic protein

NO_3 = nitrate

nr = non-redundant

NRT = nitrate transporter

O_2 = Oxygen molecule

OH^- = hydroxyl ion

P = probability

P^{32} = phosphorus-32

PAL = phenylalanine ammonia-lyase

PCR = polymerase chain reaction

PEG = poly ethylene glycol

Pf_b = lipid permeability of boric acid

PIP = plasma membrane intrinsic protein

PIPES = piperazine-1-4-bis[2-ethanesulfonic acid]

PO_4^{3-} = phosphate ion

ppm = parts per million

PRR73 = pseudo-response regulator 73

PS = photosystem

PS1-A = photosystem I P700 apoprotein A1

qPCR = quantitative polymerase chain reaction

QTL = quantitative trait loci

RAB1C= Ras-related protein Rab-35

RAV2 = regulator of V-ATPase in vacuolar membrane protein 2

RFLP = restriction fragment length polymorphism

RG-II = rhamnogalacturonan- II

RNA = ribonucleic acid

ROS = reactive oxygen species

rpm = revolutions per minute

rRNA = ribosomal RNA

RT = room temperature

SAMDC = *S*- adenosylmethionine decarboxylase

SARDI = South Australian Research and Development Institute

SDS = sodium dodecyl sulfate

SFP = single feature polymorphism

SIP = small basic intrinsic protein

SLC4 = solute carrier family 4

SLC4A11= sodium bicarbonate transporter-like protein 11

SNP = single nucleotide polymorphism

SO_4^{2-} = sulfate ion

SOB = super-optimal broth

SOD = superoxide dismutase

SOS = salt overly sensitive

SSC = sodium chloride/ sodium citrate

SSH = Suppression Subtractive Hybridization

t = metric ton

TAE = Tris/acetate/EDTA

TB = tolerant boron

TC = tolerant control

T-DNA = transferred DNA

TIGR = The Institute for Genomic Research

TIP = tonoplast intrinsic protein

TM = trans-membrane

TPX= thiol peroxidase

Ub = ubiquitin

UDP = uridine diphosphoglucose

USPA =universal stress protein A

UTR = untranslated region

UV = ultra violet

V = voltage

V-ATPase = vacuolar type H^+-ATPase

VDAC = voltage-dependent anion-selective channel protein

VSP = vegetative storage protein

W/V = weight/volume

YNL275w = nonglycosylated anion transport protein from yeast