Experimental design

Definition of experimental and control groups: control and Hg exposed plants

Number within each group-3

Sample

Description: leaves

Microdissection or macrodissection: not applicable

Processing procedure: samples were cut and washed in de-ionized water

If frozen, how and how quickly? Immediately after washing samples were frozen in liquid nitrogen

If fixed, with what and how quickly? Not applicable

Sample storage conditions and duration: samples were stored at -80C for 2 months and processed

Nucleic acid extraction

Procedure and/or instrumentation: Trizol reagent procedures with no modifications (Invitrogen Life Technologies, Paisley, UK)

Name of kit and details of any modifications: Trizol reagent (Invitrogen Life Technologies, Paisley, UK)

Details of DNase or RNase treatment: RNeasy Mini Kit (Quiagen GmbH, Hilden, Germany)

Contamination assessment (DNA or RNA): agarose gel

Nucleic acid quantification: spectroradiometry

Instrument and method: NanoDrop® ND-1000 UV-Vis Spectrophotometer . The absorbance was measured at 260 and 280 nm. The concentration of nucleic acid was determined using the Beer-Lambert law, which predicts a linear change in absorbance with concentration. An A260 reading of 1.0 is equivalent to about 40 µg/ml of RNA and the OD at 260 nm is used to determine the RNA concentration in a solution. RNA has its absorption maximum at 260 nm and the ratio of the absorbance at 260 nm and 280 nm was used to assess the RNA purity of an RNA preparation. Pure RNA had an A260/A280 of 2.1. NanoDrop® ND-1000 UV-Vis Spectrophotometer enables highly accurate

RNA integrity: method/instrument: microcapillary electrophoretic RNA separation

RIN/RQI or Cq of 3' and 5' transcripts: see excel files attached (GRAPHS worksheet)

Inhibition testing (Cq dilutions, spike or other): see excel files attached (GRAPHS worksheet)

Reverse transcription

Complete reaction conditions: see excel files attached (SETUP worksheet)

Amount of RNA and reaction volume: see excel files attached (SETUP worksheet)

Priming oligonucleotide (if using GSP) and concentration: Not applicable

Reverse transcriptase and concentration: Power SYBR Green PCR Master Mix (Applied Biosystems) according to manufacturer instructions

Temperature and time: see excel files attached (SETUP worksheet)

Manufacturer of reagents and catalogue numbers: Power SYBR® Green PCR Master Mix and Power SYBR® Green RT-PCR Reagents Kit Catalog Number 4368577, 4367659, 4367660, 4368706, 4368702, 4368708 (Master Mix) and 4368711 (RT-PCR Reagents Kit)

Cqs with and without reverse transcription: see excel files attached (GRAPHS worksheet)

Storage condition of cDNA: —80C

qPCR target information

Gene symbol-See word file: Genes for experiment B.doc

Sequence accession number-See word file: Genes for experiment B.doc

Amplicon length: See word file: Genes for experiment B.doc

In silico specificity screen (BLAST, and so on): See word file: Genes for experiment B.doc

Location of each primer by exon or intron (if applicable): Not applicable

What splice variants are targeted: Not applicable

qPCR oligonucleotides

Primer sequences: See word file: PRIMERS.doc

Location and identity of any modifications: not applicable

Manufacturer of oligonucleotides: Applied Biosystems

Purification method: as manufacturer indications from Applied Biosystems

qPCR protocol

Complete reaction conditions: see excel files attached (SETUP worksheet)

Reaction volume and amount of cDNA/DNA: see excel files attached (SETUP worksheet)

Primer, (probe), Mg2+, and dNTP concentrations: see excel files attached (SETUP worksheet) and Applied Biosystems indications

Polymerase identity and concentration: see excel files attached (SETUP worksheet) and Applied Biosystems indications

Buffer/kit identity and manufacturer: see excel files attached (SETUP worksheet) and Applied Biosystems indications

Additives (SYBR Green I, DMSO, and so forth): see excel files attached (SETUP worksheet) and Applied Biosystems indications

Complete thermocycling parameters: see excel files attached (SETUP worksheet) and Applied Biosystems indications

Manufacturer of qPCR instrument: 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA

qPCR validation

Specificity (gel, sequence, melt or digest): gel

For SYBR Green I, Cq of the NTC): see excel files attached (GRAPHS worksheet)

Calibration curves with slope and y intercept: see excel files attached (GRAPHS worksheet)

PCR efficiency calculated from slope: see excel files attached (GRAPHS worksheet)

R2 of calibration curve: see excel files attached (GRAPHS worksheet)

Linear dynamic range: see excel files attached (GRAPHS worksheet)

Cq variation at LOD: see excel files attached (GRAPHS worksheet)

Evidence for LOD: see excel files attached (GRAPHS worksheet)

If multiplex, efficiency and LOD of each assay: not applicable

Data analysis

qPCR analysis program (source version): Sequence Detection Systems Software version 2.3

Method of Cq determination: CT slope method (following Applied Biosystems specifications)

Outlier identification and disposition: not applicable

Results for NTCs: see excel files attached (DATA RESULTS worksheet)

Justification of number and choice of reference genes: budget

Description of normalization method: normalization to the expression of 14-3-3-LIKE PROTEIN B (14-3-3B) pir||T04406 14-3-3b protein – barley gene (endogenous control gene), and showed similar expression for all treatments in the microarray analysis

Number and stage (reverse transcription of qPCR) of technical replicates:2

Repeatability (intraassay variation): see excel files attached (DATA INPUT worksheet)

Statistical methods for results significance: analysis of variance

Software (source, version): SAS(SAS, 2004 Institute Inc., Cary, NC, USA)