THIOREDOXIN-REGULATED β-AMYLASE (BAM1) TRIGGERS DIURNAL STARCH DEGRADATION IN GUARD CELLS, AND IN MESOPHYLL CELLS UNDER OSMOTIC STRESS

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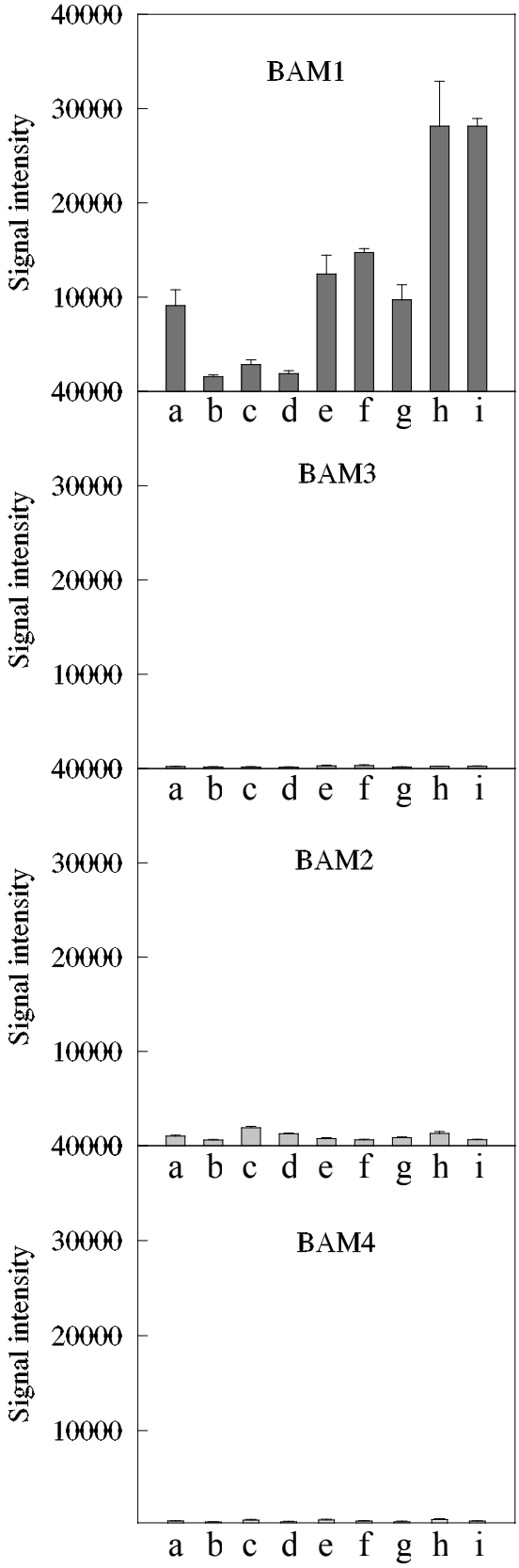
Figure S1



a: total root

b: root hair zone

c: root tip

d: elongation zone

e: endodermis

f: endodermis+cortex

g: epid. atrichoblasts

h: lateral root cap

i: stele

Figure S2

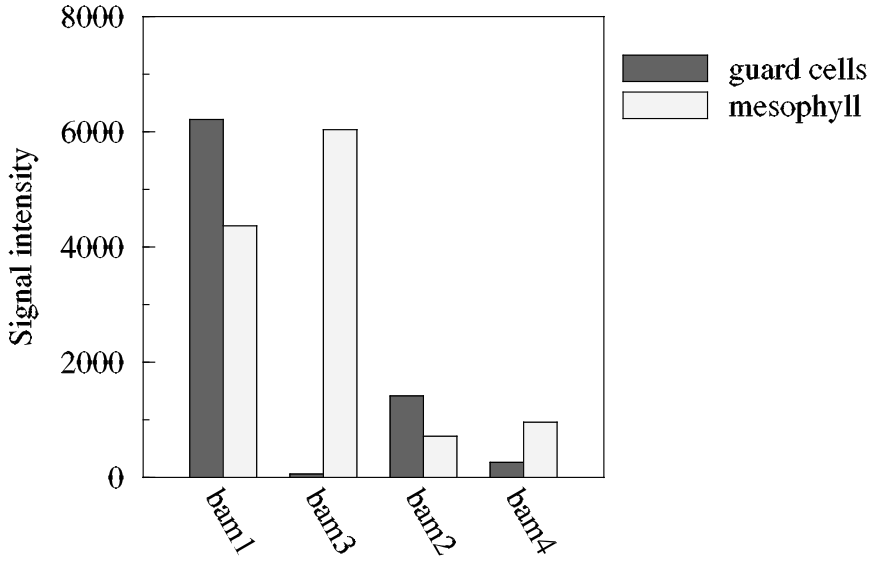

8000
6000
4000
2000
0
Signal intensity
guard cells
mesophyll
bam1
bam3
bam2
bam4

Figure S3

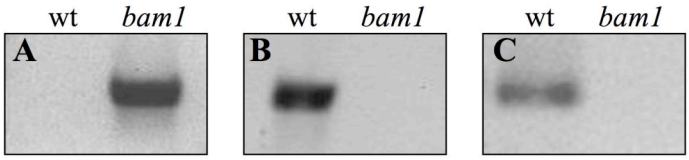


Figure S4

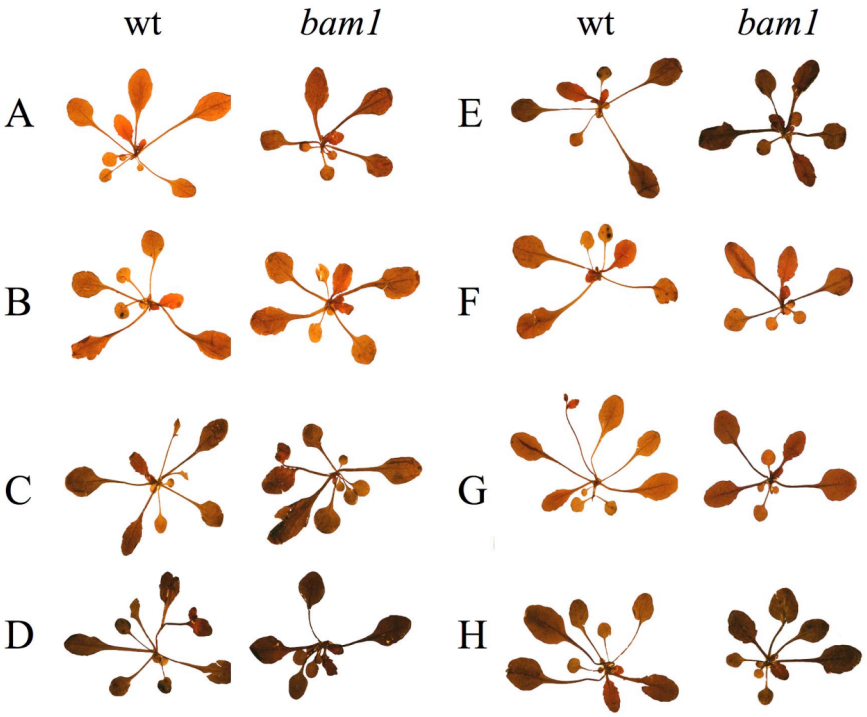


Figure S5

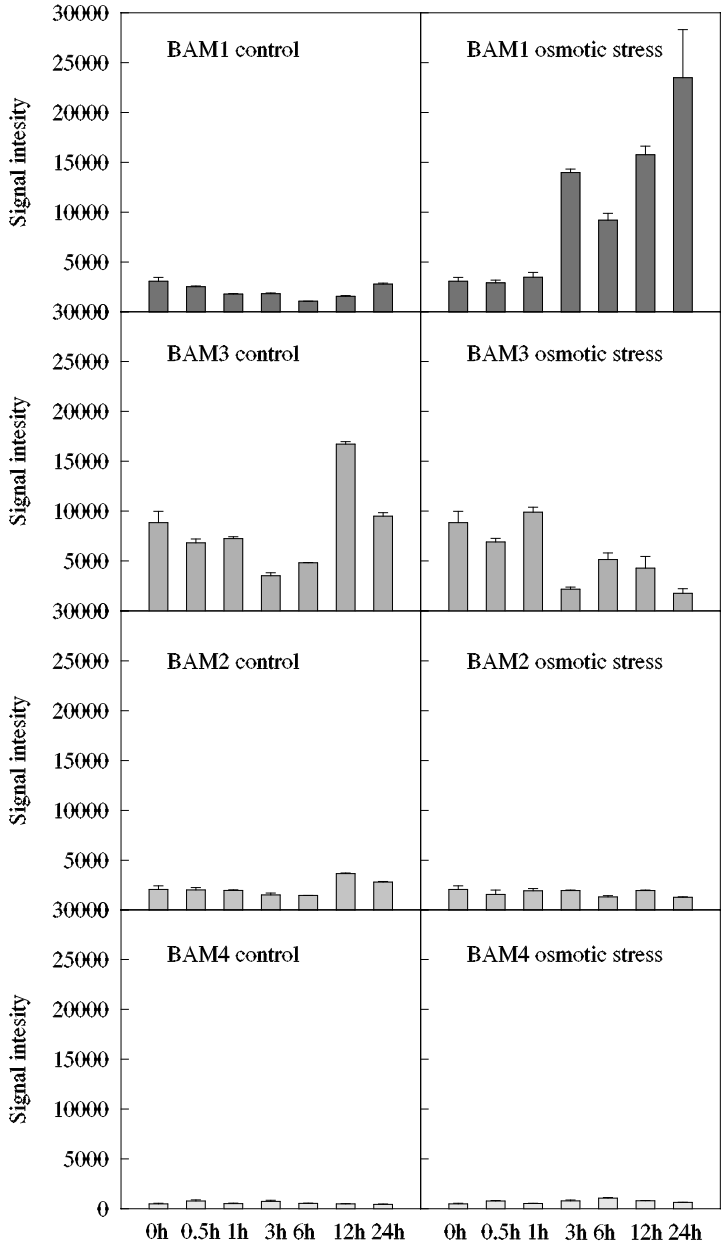
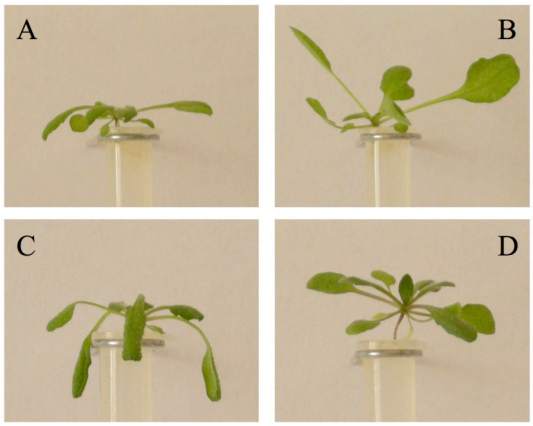

BAM1 control
BAM1 osmotic stress
BAM3 control
BAM3 osmotic stress
BAM2 control
BAM2 osmotic stress
BAM4 control
BAM4 osmotic stress
Signal intesity
30000
25000
20000
15000
10000
5000
0
0h 0.5h 1h 3h 6h 12h 24h

Figure S6



SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. NASCarray data from root expression profile. Original data are available at www.genevestigator.ethz.ch, experiments no. 191 (**Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN.** 2003. A gene expression map of the Arabidopsis root. *Science* **302,** 1956-1960). Five separate GFP transgenic lines of Arabidopsis were produced expressing in stele, endodermis, endodermis + cortex, epidermal atrichoblast cells and lateral root cap. Roots from 6-day-old plants were cut off about 1 cm from their tip and protoplasted. GFP expressing cells were isolated on a fluorescence activated cell sorter and then used for gene expression profiling. Root radial zone expression profiles (from inner stele to outer lateral root cap) and root developmental stage profiles (from the initial radial expansion of the root tip to the full elongation of root hairs) were performed. All profiles were in triplicate. Results of both radial zone and developmental stage profiling were combined in the graph. Values are means ± SD.

Fig. S2. Selected data from Affymetrix expression arrays obtained from guard and mesophyll cells (**Yang Y, Costa A, Leonhardt N, Siegel RS, Schroeder JI.** 2008. Isolation of a strong Arabidopsis guard cell promoter and its potential as a research tool.
Plant Methods **4**, 1-15). Mesophyll and guard cell protoplasts were isolated from 5 weeks-old Arabidopsis plants. Analyses were repeated in duplicate, one with and one without the addition of transcriptional inhibitors during protoplasting. Here only data with inhibitors are shown.

Fig. S3. Analysis of Arabidopsis line (SALK_039895) carrying T-DNA insertion in *BAM1* gene sequence. Analysis was performed by PCR on genomic DNA, and by RT-PCR on cDNA obtained by total RNA retrotranscription. A: PCR on genomic DNA with combination of T-DNA and *BAM1* specific primers; B: PCR on genomic DNA with *BAM1* specific primers annealing on the opposite sides of the T-DNA insertion site; C: RT-PCR on total mRNA with *BAM1* specific primers.

Fig. S4. Starch content in wild type and *bam1* plants. Two independent experiments were performed on 26-day-old plants. Figure represents the typically observed pattern of starch accumulation and breakdown. In the light, starting from the third hour of illumination, plants were collected 30 minutes after (A), 2 hours after (B), 6 hours after (C). After light was switched off, plants were collected after 3 hours (D), after 7 hours (E), 11 hours (F) of darkness. In panel G and H plants were illuminated again for 3 and 6 hours respectively

Fig. S5. Selected microarray data from the AtGenExpress abiotic stress series (osmotic stress and salt stress time course, Kudla Lab). Original data are available at www.genevestigator.ethz.ch (**Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W.** 2004. GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. *Plant Physiology* **136,** 2621–2632); experiments no. 120. The stress treatements with 300 mM mannitol or 150 mM NaCl were performed in parallel on 18-day-old Arabidopsis plants. For each time point two samples serving as replicates were collected. Only data from shoots are shown. Values are means ± SD.

Fig. S6. Mannitol effect on wild type and *bam1* plants. Representative pictures from two independent experiments with 10 plants for each condition. A and B, *bam1* and wild type plants, respectively, in absence of mannitol; C and D, *bam1* and wild type plants, respectively, after 6 hours of 450 mM mannitol treatment. Wild type plants can better stand the stress than *bam1* plants.