Supplemental Data. Moschou et al. (2008). Spermidine Exodus and Oxidation in the Apoplast Induced by Abiotic Stress is Responsible for H_2O_2 Signatures that Direct Tolerance Responses in Tobacco.



Supplemental Figure 1. Molecular and biochemical analysis of the A-*pao* plants. (A) Southern analysis for the transgene incorporation into the nuclear DNA (A2, A3 and A4 lines); (B) PAO protein levels; (C) PAO activity levels, and (D) S-, SH- and PH-PA titers (A2, A3, A4, A4.2, A6, A8 and A9 lines). PAs are expressed in nmol gFW⁻¹. Putrescine (Put), closed bars; Spermidine (Spd), open bars; Spermine (Spm), striped bars. Data are the means (\pm SE) of three independent experiments, and asterisks indicate statistical significance at *P*<0.05.

Α 3.0 * * * I 2.5 Ι I 2.0 gFW 1.5 1.0 0.5 0 100 0

В



WΤ

A2

AG

A8

300

100

200

NaCl (mM)

Supplemental Figure 2. Biomass of WT, A2, A6 and A8 transgenic lines exposed to salt stress and PA titers. (A) Biomass of WT, A2, A6 and A8 transgenic lines treated with 0, 100, 200 and 300 mM NaCl; (B) S-PA titers 48 h (% Difference compared to untreated plants) after the onset of 100 mM NaCl stress in WT, A2, A6 and A8 transgenic lines. Data are the means of three independent experiments, vertical bars represent \pm SE and asterisks indicate statistical significance at *P*<0.05.



Supplemental Figure 3. Difference in the activity levels of the PA biosynthetic enzymes and PA accumulation in WT, S16-4, PS-124 and PS-144 transgenic lines. (A) ADC, ODC, SAMDC, SPDS and SPMS activities (% Difference compared to untreated plants) 72 h after the onset of 200 mM salt stress, in WT, S16-4, PS-124 and PS-144 transgenic lines; (B) PA titers (Put, Spd, Spm and Total) (% Difference compared to untreated plants) 72 h after the on set of 200 mM salt stress, in WT, S16-4, PS-124 and PS-144 transgenic lines; (B) PA titers (Put, Spd, Spm and Total) (% Difference compared to untreated plants) 72 h after the on set of 200 mM salt stress, in WT, S16-4, PS-124 and PS-144 transgenic lines. Data are the means (\pm SE) of three independent experiments, and asterisks indicate statistical significance at *P*<0.05.



Supplemental Figure 4. Quantitative RT-PCR analysis of the *actin* and the Nt *pao* genes within the exponential phase. (A) Relative density of the *actin* transcript following RT-PCR; (B) Relative density of the Nt *pao* transcript following RT-PCR. Products in both cases were gel analyzed, Southern blotted and hybridized against the corresponding ³²P-probes. In both cases products analyzed from 30-35 cycles were within the exponential phase. Data are the means (\pm SE) of three independent experiments, and asterisks indicate statistical significance at *P*<0.05.