SUPPORTING INFORMATION

Spermine deficiency shifts the balance between Jasmonic Acid and Salicylic Acid-mediated defense responses in Arabidopsis

Chi Zhang¹, Kostadin E. Atanasov¹, Ester Murillo¹, Vicente Vives-Peris², Jiaqi Zhao¹, Cuiyun Deng³, Aurelio Gómez-Cadenas² and Rubén Alcázar^{1,*}

SUPPLEMENTARY FIGURES

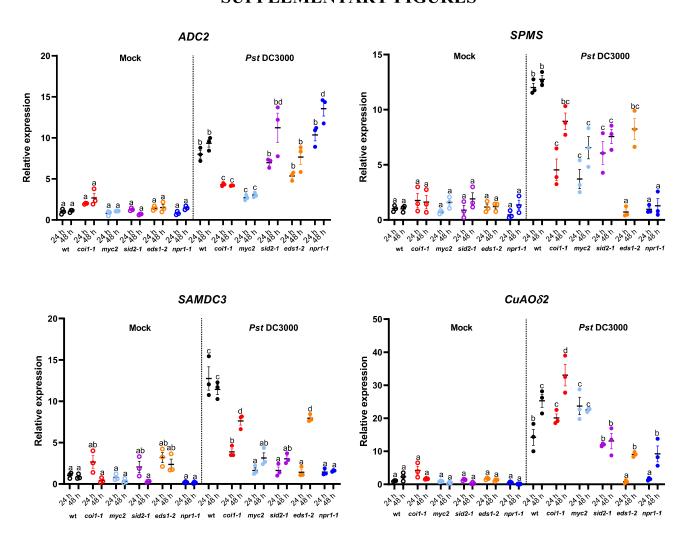


Figure S1. Expression analyses of *ADC2*, *SPMS*, *SAMDC3* and *CuAO* δ 2 in wild-type, *coi1-1*, *myc2*, *sid2-1*, *eds1-2* and *npr1-1* mutants in response to *Pst* DC3000 (OD₆₀₀=0.001) and mock (10 mM MgCl₂) infiltration at 24 h and 48 h of treatment. Expression values are relative to wild-type (mock) treatment and represent the mean \pm standard deviation from three biological replicates per genotype and treatment. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

¹ Department of Biology, Healthcare and Environment. Section of Plant Physiology, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Av. Joan XXIII 27-31, 08028 Barcelona, Spain.

² Departamento de Biología, Bioquímica y Ciencias Naturales, Universitat Jaume I, Av. Sos Baynat s/n, 12071 Castelló de la Plana, Spain.

³ Plant Synthetic Biology and Metabolic Engineering Program, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola, 08193 Barcelona, Spain.

^{*} For correspondence (email: <u>ralcazar@ub.edu</u>)

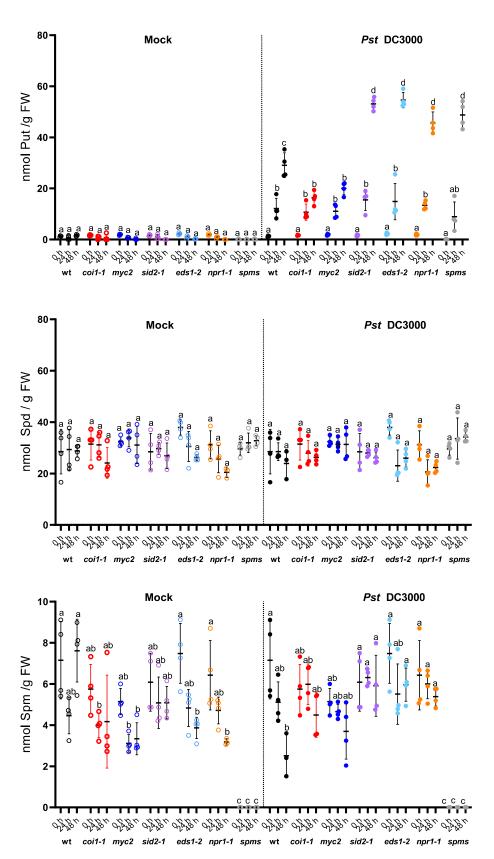


Figure S2. Determination of polyamine contents in wild-type (wt), coi1-1, myc2, sid2-1, eds1-2 and npr1-1 mutants in response to Pst DC3000 (OD₆₀₀=0.001) and mock (10 mM MgCl₂) infiltration at 0 h, 24 h and 48 h of treatment. Values represent the mean \pm standard deviation from four biological replicates per genotype. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

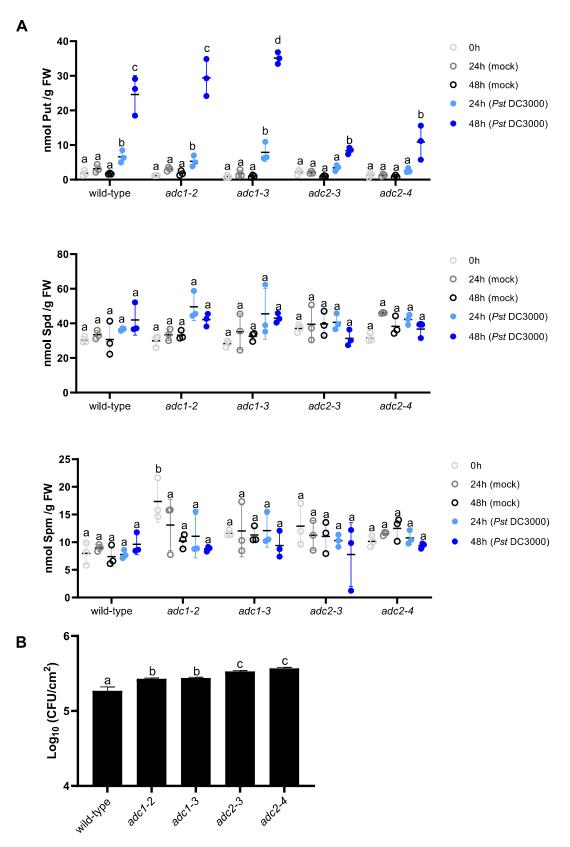


Figure S3. (A) Determination of polyamine contents in wild-type, adc1-2, adc1-3, adc2-3 and adc2-4 mutants in response to Pst DC3000 (OD₆₀₀=0.001) and mock (10 mM MgCl₂) infiltration at 0 h, 24 h and 48 h of treatment. Values represent the mean \pm standard deviation from three biological replicates per genotype. **(B)** Determination of Pst DC3000 growth in wild-type, adc1 and adc2 mutants at 48 h of infiltration. Bacterial numbers are expressed as colony forming units (CFU) per cm² of leaf area. Values are the mean from six biological replicates \pm SD. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

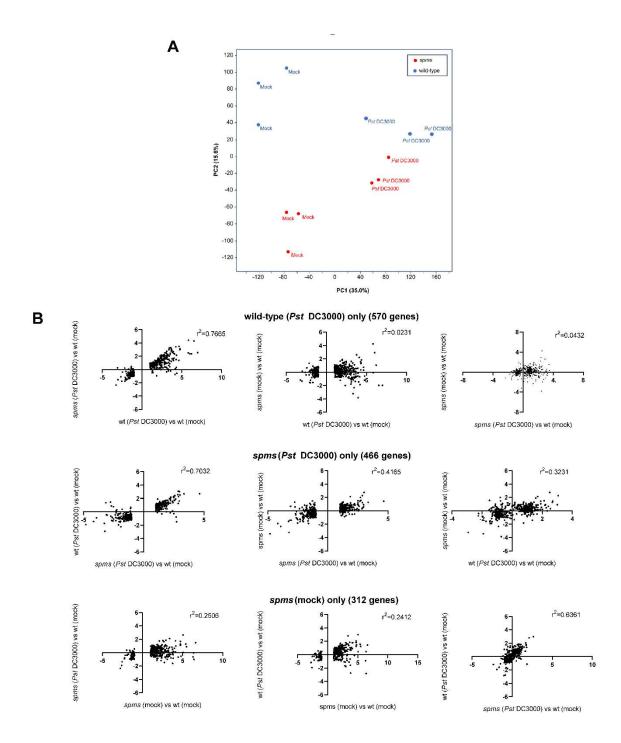


Figure S4. (A) Principal component analysis (PCA) of RNA-seq gene expression data at 24 h of *Pst* DC3000 (OD₆₀₀=0.001) and mock (10 mM MgCl₂) inoculation in *spms* and wild-type plants. (B) Expression correlation analyses between wild-type (wt) and *spms* in the 'wild-type (*Pst* DC3000) only' sector (genes deregulated only in the comparison between wt *Pst* DC3000 vs wt mock), 'spms (*Pst* DC3000) only' sector (genes deregulated only in the comparison between *spms Pst* DC3000 vs wt mock) and 'spms (mock) only' sector (genes deregulated only in the comparison between *spms* mock vs wt mock).

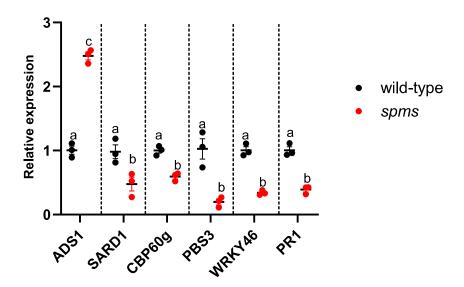


Figure S5. Basal expression of *ADS1*, *SARD1*, *CBP60g*, *PBS3*, *WRKY46* and *PR1* in wild-type and *spms* mutant plants determined by qRT-PCR. Expression values are relative to the wild-type and represent the mean \pm standard deviation from three biological replicates per genotype. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

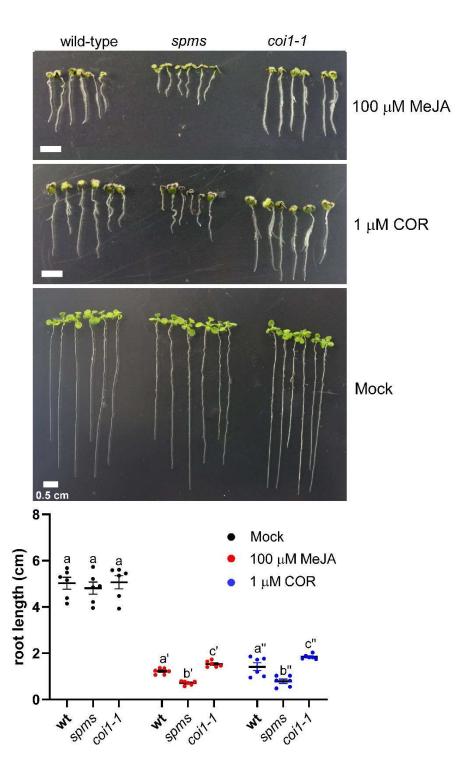


Figure S6. Root growth inhibition assays in response to MeJA and COR. Wild-type (wt), *spms* and *coi1-1* seeds were germinated and grown on vertical plates containing half-strength MS supplemented with 1% sucrose and 100 μ M methyl jasmonate (MeJA), 1 μ M coronatine (COR) or mock (0.1% DMSO in water) at 16 h light/8 h dark cycles, 20 – 22 °C and 100–125 μ mol photons m⁻² s⁻¹ of light intensity. Pictures were taken 12 days after germination for the measurement of the primary root length. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

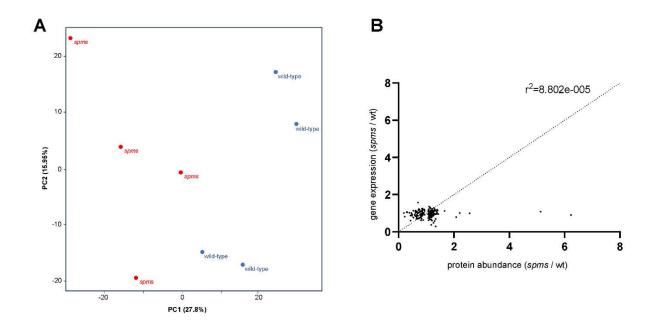


Figure S7. (A) Principal component analysis (PCA) of the proteomics data in *spms* and wild-type under basal conditions. **(B)** Correlation analysis between protein abundance and gene expression in *spms* under basal conditions. Values of proteins exhibiting >1.3-fold difference between *spms* and wild-type are shown.

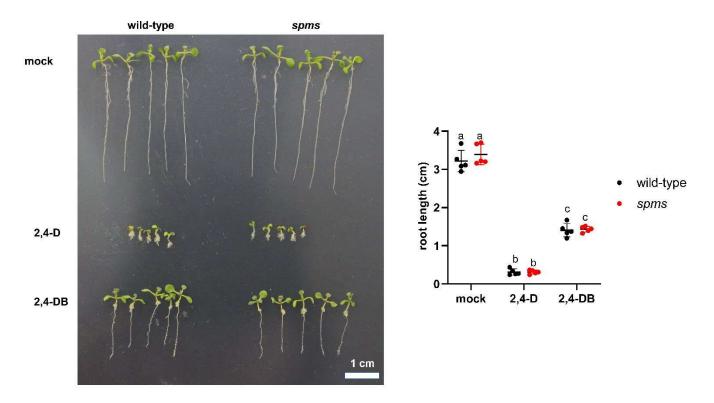


Figure S8. Root growth phenotypes of 12-day-old wild-type and *spms* seedlings germinated and grown on half-strength MS supplemented with 1 % sucrose and 0.05 μ g/ml 2,4-dichlorophenoxyacetic acid (2,4-D), 0.2 μ g/ml 2,4-dichlorophenoxybutyric acid (2,4-DB) or mock (0.1% DMSO in water). Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

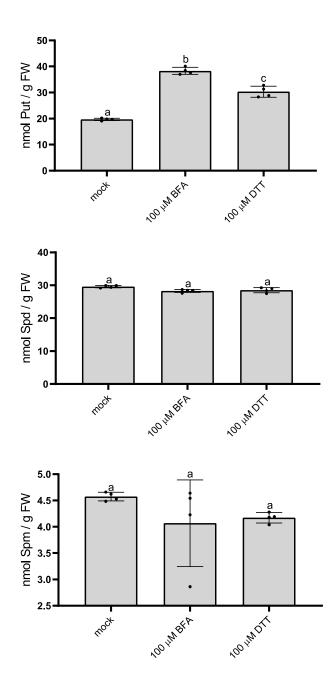


Figure S9. Polyamine levels (Put, putrescine; Spd, spermidine; Spm, spermine) in 10-day-old wild-type seedlings at 6 h of treatment with 100 μ M Brefeldin A (BFA), 100 μ M DTT (dithiothreitol) or mock (0.1% DMSO in water). Values represent the mean \pm standard deviation from four biological replicates per treatment. Different letters indicate significant differences (p<0.05) according to two-way ANOVA followed by Tukey's post-hoc test.

SUPPLEMENTARY TABLES

- **Table S1.** List of oligonucleotides used in this work.
- **Table S2.1.** List of 1867 differentially expressed genes (fold-change>2; Bonferroni corrected p-value <0.05) in the comparison between wild-type inoculated with *Pst* DC3000 and wild-type inoculated with mock (10 mM MgCl₂) at 24 h of treatment.
- **Table S2.2.** List of 1793 differentially expressed in the comparison between *spms* inoculated with *Pst* DC3000 and wild-type inoculated with mock (10 mM MgCl₂) at 24 h of treatment.
- **Table S2.3.** List of 1078 common differentially expressed genes between [*spms* (*Pst* DC3000) vs wild-type (mock)] and [wild-type (*Pst* DC3000) vs wild-type (mock)] at 24 h of treatment.
- **Table S2.4.** Gene ontology (GO) enrichment analysis of genes in Table S1.3
- **Table S2.5.** List of 570 genes differentially expressed only in the wild-type inoculated with *Pst* DC3000 at 24 h of treatment ('wild-type *Pst* DC3000 only')
- Table S2.6. Gene ontology (GO) enrichment analysis of genes in Table S1.5
- **Table S2.7.** List of 466 genes differentially expressed only in the spms mutant inoculated with *Pst* DC3000 at 24 h of treatment ('spms Pst DC3000 only')
- **Table S2.8.** Gene ontology (GO) enrichment analysis of genes in Table S1.7
- **Table S2.9.** List of 608 differentially expressed in the comparison between *spms* inoculated with mock (10 mM MgCl₂) vs wild-type inoculated with mock (10 mM MgCl₂) at 24 h of treatment.
- **Table S2.10**. Gene ontology (GO) enrichment analysis of genes in Table S1.9
- **Table S2.11**. List of 312 genes differentially expressed only in the *spms* mutant inoculated with mock (10 mM MgCl₂) at 24 h of treatment ('*spms* mock only').
- **Table S2.12**. Gene ontology (GO) enrichment analysis of genes in Table S1.11
- **Table S2.13.** List of 172 common differentially expressed genes in the comparisons between [spms (Pst DC3000) vs wild-type (mock)], [wild-type (Pst DC3000) vs wild-type (mock)] and [spms (mock) vs wild-type (mock)] at 24 h of treatment.
- **Table S2.14.** List of 77 common differentially expressed genes in the comparisons between [spms (Pst DC3000) vs wild-type (mock)] and [spms (mock) vs wild-type (mock)] at 24 h of treatment.
- **Table S2.15.** List of 47 common differentially expressed genes in the comparisons between [wild-type (*Pst* DC3000) vs wild-type (mock)] and [*spms* (mock) vs wild-type (mock)] at 24 h of treatment.
- **Table S2.16.** Complete list of genes and their expression in the comparison between [*spms* (*Pst* DC3000) vs wild-type (mock)] at 24 h of treatment.
- **Table S2.17.** Complete list of genes and their expression in the comparison between [*spms* (mock) vs wild-type (mock)] at 24 h of treatment.

- **Table S2.18.** Complete list of genes and their expression in the comparison between [wild-type (*Pst* DC3000) vs wild-type (mock)] at 24 h of treatment.
- **Table S3.1.** List of differentially expressed genes (fold-change>2; Bonferroni corrected p-value <0.05) in the comparison between spms and wild-type under basal conditions.
- Table S3.2. Gene ontology (GO) enrichment analysis of genes in Table S2.1
- **Table S4.1.** List of differentially accumulating proteins between *spms* and the wild-type.
- **Table S4.2.** GO terms of differentially accumulating proteins in Table S3.1.
- **Table S5.** Untargeted lipidomics analysis of *spms* and wild-type plants under basal conditions.