**Supplementary Material**

**Jedelská et al. – S-nitrosation in tomato root under abiotic stress**

**Supplementary Table S1. Primers used for gene expression analysis by qPCR.**

| Genes | GenBank | Primers | Product length (bp) |
|---|---|---|---|
| *EF1α* | NM_001247106 | fwd: 5′-GGTCATCATCATGAACCATCC-3′<br>rev: 5′-CATACCAGCATCACCGTTCTT-3′ | 175 |
| *GAPDH* | U93208 | fwd: 5′-AACCGGTGTCTTCACTGACAAGGA-3’<br>rev: 5’-CACCCACAACAAACATGGGAGCAT-3’ | 110 |
| *APX* | AY974805 | fwd: 5′-GAGGACCTGATGTTCCCTTTC-3′<br>rev: 5′-AAGGTATGGGCACCAGAGAGT-3′ | 169 |
| *NADPH oxidase* | AF088276 | fwd: 5′-CGGATGGAATGAAGTTGAAAA-3′<br>rev: 5′-AAGCATCAAACAATTCCAACG-3′ | 206 |

**Supplementary Table S2. Comparison of physiological and biochemical parameters determined in 9-day seedlings of *Solanum* spp. genotypes.** Data represent means ± SD ($n \geq 3$).

| **Parameter** | ***S. lycopersicum* cv. Amateur** | ***S. habrochaites*** |
|---|---|---|
| Epicotyl length (cm) | 5.05 ± 0.71 | 2.41 ± 0.27 |
| Root length (cm) | 9.65 ± 0.56 | 7.67 ± 0.43 |
| Root weight (g) | 0.18 ± 0.01 | 0.06 ± 0.003 |
| NO level (rel. u.) | 32.24 ± 2.76 | 19.64 ± 1.28 |
| $ONOO^-$ level (rel. u.) | 23.17 ± 3.08 | 21.01 ± 0.73 |
| ROS level (rel. u.) | 6.93 ± 0.13 | 8.63 ± 0.46 |
| GSNOR activity ($nmol \cdot min^{-1} \cdot g^{-1}$ FW) | 11.02 ± 1.28 | 5.89 ± 0.72 |
| Nitrosothiols level ($nmol \cdot mg^{-1}$ protein) | 20.00 ± 1.89 | 8.09 ± 2.18 |
| APX activity ($\mu mol \cdot min^{-1} \cdot g^{-1}$ FW) | 242.29 ± 21.92 | 224.43 ± 16.12 |
| NADPH oxidase activity ($\mu mol \cdot min^{-1} \cdot g^{-1}$ FW) | 87.48 ± 0.12 | 69.99 ± 0.29 |
| *APX* expression | 1.28 ± 0.04 | 1.06 ± 0.01 |
| *NADPH oxidase* expression | 0.78 ± 0.03* | 1.48 ± 0.02* |

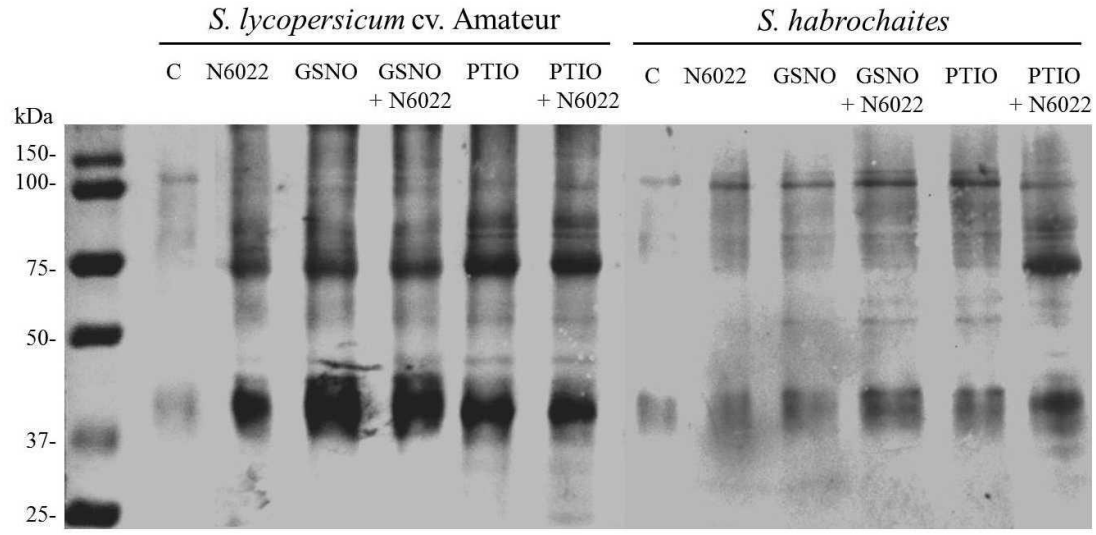
* gene expression was normalized to the expression levels of *GAPDH* and *EF1-α*

**Supplementary Figure S1**. **Representative images of 9-day seedlings of *Solanum* spp. genotypes.** (A) *Solanum lycopersicum* cv. Amateur; (B) *Solanum habrochaites*.

A B

**Supplementary Figure S2. Effect of RNS modulators supplementation to the growth media on protein nitration in roots of *Solanum* spp..** Plants of *Solanum* spp. were grown on media supplemented with 1 μM N6022; 100 μM GSNO; 100 μM GSNO + 1 μM N6022; 100 μM PTIO; or 100 μM PTIO + 1 μM N6022. (A) Levels of nitrated proteins were analysed in root extracts (100 μg of protein per lane) by SDS-PAGE and Western blot analysis using a mouse polyclonal antibody against 3-nitrotyrosine. Commercial nitrated BSA ($NO_2$-BSA, 10 μg), served as the positive control (not shown). (B) Band intensities on Western blot images were analysed using ImageJ 1.33 software. Values indicate a relative increase of each immunoreactive band compared to the control, to which the value of 1 was assigned. N.d., not detected. Data represent means ± SD (n ≥ 3).

A




B

***S. lycopersicum* cv. Amateur**

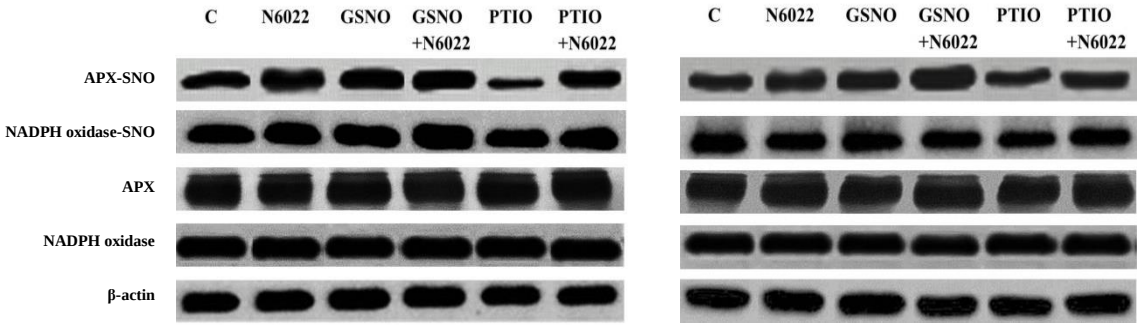
| Band (kDa) | N6022 | GSNO | GSNO +N6022 | PTIO | PTIO +N6022 |
|---|---|---|---|---|---|
| **100** | n.d. | n.d. | n.d. | n.d. | n.d. |
| **75** | 2.8 ± 0.06 | 2.5 ± 0.17 | 3.5 ± 0.01 | 2.7 ± 0.01 | 3 ± 0.30 |
| **40** | 3.6 ± 0.05 | 2.2 ± 0.2 | 2.8 ± 0.01 | 4.2 ± 0.30 | 2.5 ± 0.08 |

***S. habrochaites***

| Band (kDa) | N6022 | GSNO | GSNO +N6022 | PTIO | PTIO +N6022 |
|---|---|---|---|---|---|
| **100** | 1.6 ± 0.05 | 2.3 ± 0.05 | 4 ± 0.06 | 1.9 ± 0.02 | 5.2 ± 0.40 |
| **75** | 1.11 ± 0.02 | 1.3 ± 0.02 | 1.2 ± 0.03 | 3.8 ± 0.20 | 1.2 ± 0.04 |
| **40** | 1.2 ± 0.01 | 1.1 ± 0.09 | 1.7 ± 0.03 | 2.1 ± 0.01 | 1.4 ± 0.02 |

**Supplementary Figure S3. Detection of S-nitrosated and total APX and NADPH oxidase protein levels in tomato roots exposed to RNS modulators**. For the purification of the S-nitrosated proteins from tomato roots, proteins extracted from tomato roots (5 mg) were subjected to the biotin switch method, followed by purification using neutravidin-affinity chromatography. Purified fractions were then analysed by SDS-PAGE (100 μg of protein per lane), followed by Western blotting and immunodetection with anti-APX (1:1000) or anti-NADPH-oxidase (1:1000) antibodies. Detection of total APX and NADPH oxidase protein level was performed by immunoblot analysis using the corresponding antibody with the same dilution as for APX-SNO or NADPH-oxidase-SNO. Roots of tomato genotypes (A) *S. lycopersicum* cv. Amateur, (B) *S. habrochaites* were grown on medium supplemented with 1 μM N6022, 100 μM GSNO, 100 μM PTIO or 1 μM N6022+100 μM GSNO, 1 μM N6022+100 μM PTIO for 9 days. Actin is included as a protein loading control. (C, D) Band intensities were analysed using ImageJ 1.33 software. Values indicate a relative increase of each immunoreactive band compared to the control, to which the value of 1 was assigned. Significantly different means from the control are denoted by asterisks (ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

A B

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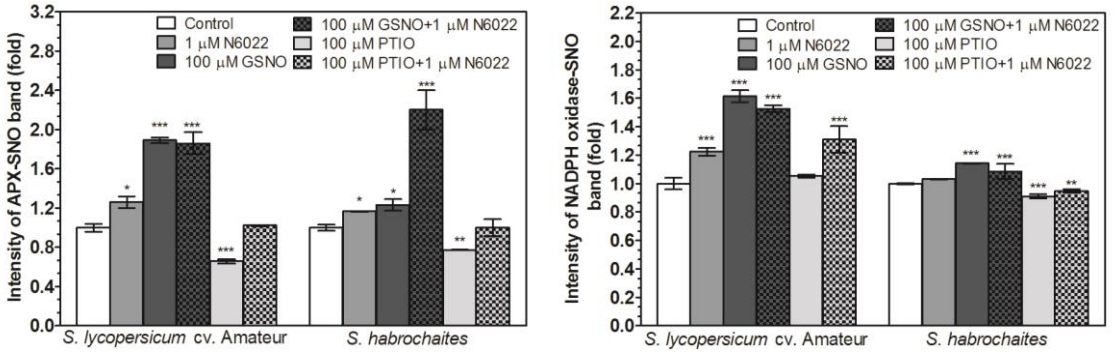


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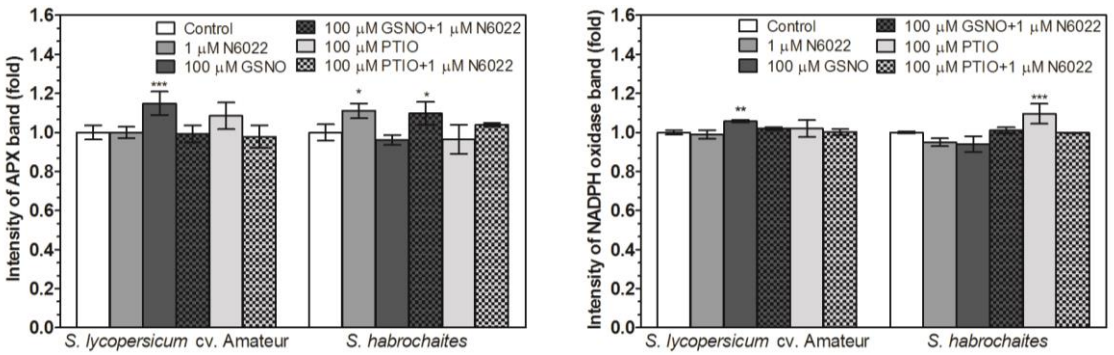
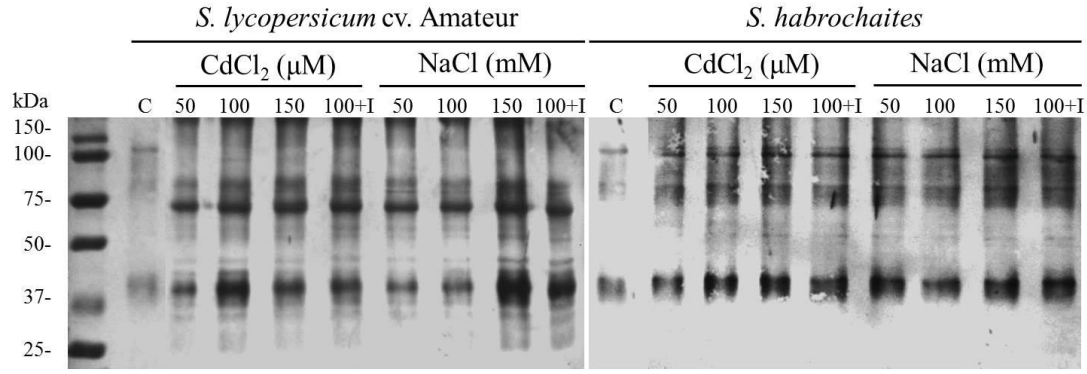


**Supplementary Figure S4. Protein nitration in roots of *Solanum* spp. genotypes exposed to cadmium or salinity stress**. (A) Levels of nitrated proteins were analysed in root extracts (100 µg of protein per lane) from plants of *Solanum* spp. exposed to 50-100-150 µM $CdCl_2$ and 100 µM $CdCl_2$+1 µM N6022, or 50-100-150 mM NaCl and 100 mM NaCl+1 µM N6022, by SDS-PAGE and Western blot analysis using a mouse polyclonal antibody against 3-nitrotyrosine. Commercial nitrated BSA ($NO_2$-BSA, 10 µg), served as the positive control (not shown). (B) Band intensities on Western blots were analysed using ImageJ 1.33 software. Values indicate a relative increase of each immunoreactive band compared to the control, to which the value of 1 was assigned. N.d., not detected. Data represent means ± SD (n ≥ 3).

A




B

| | ***S. lycopersicum* cv. Amateur** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **$CdCl_2$** | | | | **NaCl** | | | |
| **Band (kDa)** | **50 µM** | **100 µM** | **150 µM** | **100 µM + 1 µM N6022** | **50 mM** | **100 mM** | **150 mM** | **100 mM + 1 µM N6022** |
| 100 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 75 | 1.9 ± 0.01 | 2.8 ± 0.3 | 2.3 ± 0.1 | 2.8 ± 0.2 | 1.9 ± 0.05 | 2.2 ± 0.02 | 3.2 ± 0.5 | 2.3 ± 0.05 |
| 40 | 1.3 ± 0.01 | 2.1 ± 0.08 | 1.6 ± 0.01 | 1.6 ± 0.2 | 1.1 ± 0.07 | 1.2 ± 0.01 | 2.6 ± 0.09 | 2.8 ± 0.2 |

| | ***S. habrochaites*** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **$CdCl_2$** | | | | **NaCl** | | | |
| **Band (kDa)** | **50 µM** | **100 µM** | **150 µM** | **100 µM + 1 µM N6022** | **50 mM** | **100 mM** | **150 mM** | **100 mM + 1 µM N6022** |
| 100 | 1.6 ± 0.08 | 2 ± 0.03 | 2.7 ± 0.12 | 2.2 ± 0.01 | 2.7 ± 0.1 | 2.9 ± 0.01 | 2.8 ± 0.2 | 3.1 ± 0.2 |
| 75 | 1.7 ± 0.03 | 2.1 ± 0.07 | 2.2 ± 0.06 | 1.9 ± 0.01 | 2.3 ± 0.02 | 1.9 ± 0.04 | 2.9 ± 0.04 | 2.7 ± 0.02 |
| 40 | 1.2 ± 0.03 | 1.6 ± 0.05 | 1.8 ± 0.1 | 1.7 ± 0.01 | 2.1 ± 0.2 | 1.5 ±0.1 | 2 ± 0.1 | 2.2 ± 0.06 |

**Supplementary Figure S5. Detection of S-nitrosated and total APX and NADPH oxidase protein levels in tomato roots exposed to cadmium stress**. For the purification of S-nitrosated proteins from tomato roots, extracted proteins (5 mg) were subjected to the biotin switch method, followed by purification using neutravidin-affinity chromatography. Purified fractions were then analysed by SDS-PAGE (100 µg of protein per lane), followed by immunoblotting with anti-APX (1:1000) or anti-NADPH-oxidase (1:1000) antibodies. Detection of total APX and NADPH oxidase protein expression level was performed by immunoblot analysis using corresponding antibodies with the same dilution as for APX-SNO or NADPH-oxidase-SNO. Roots of tomato genotypes (A) *S. lycopersicum* cv. Amateur, (B) *S. habrochaites* were grown on medium supplemented with 50, 100, 150 µM $CdCl_2$ or 100 µM $CdCl_2$+1 µM N6022 for 9 days. Actin was included as a protein loading control. (C, D) Band intensities on Western blots were analysed using ImageJ 1.33 software. Values indicate a relative increase of each immunoreactive band compared to unstressed control, to which the value of 1 was assigned. Significantly different means from the control are denoted by asterisks (ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

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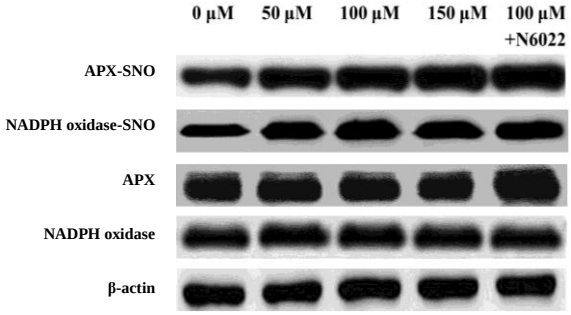


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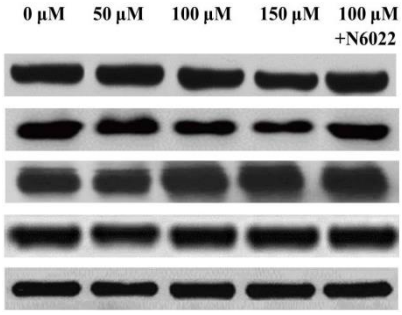


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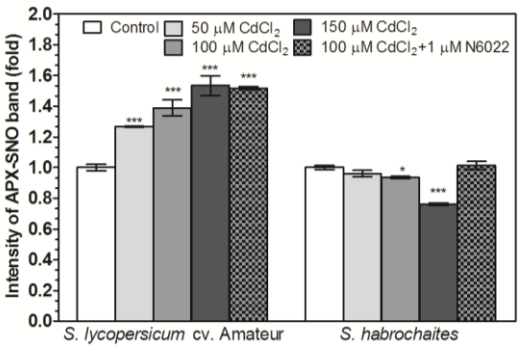


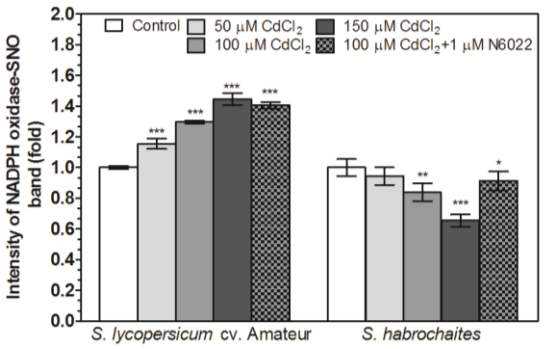


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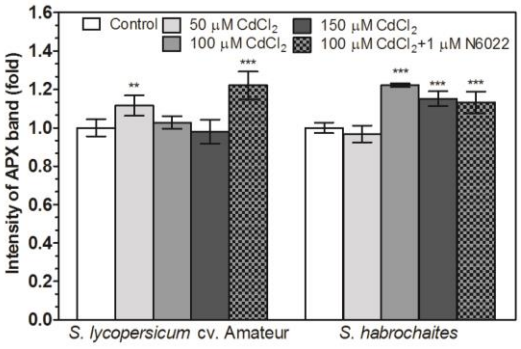


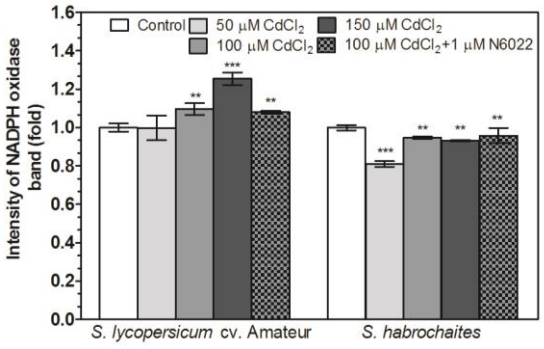

**Supplementary Figure S6. Detection of S-nitrosated and total APX and NADPH oxidase protein levels in tomato roots exposed to salinity stress**. For the purification of S-nitrosated proteins from tomato roots, extracted proteins (5 mg) were subjected to the biotin switch method, followed by purification using neutravidin affinity chromatography. Purified fractions were then analysed by SDS-PAGE (100 µg of protein per lane), followed by immunoblotting with anti-APX (1:1000) or anti-NADPH-oxidase (1:1000) antibodies. Detection of total APX and NADPH oxidase protein expression level was performed by immunoblot analysis using corresponding antibodies with the same dilution as for APX-SNO or NADPH-oxidase-SNO. Roots of tomato genotypes (A) *S. lycopersicum* cv. Amateur, (B) *S. habrochaites* were grown on medium supplemented with 50, 100, 150 mM NaCl or 100 mM NaCl+1 µM N6022 for 9 days. Actin was included as a protein loading control. (C, D) Band intensities on Western blots were analysed using ImageJ 1.33 software. Values indicate a relative increase of each immunoreactive band compared to the unstressed control, to which the value of 1 was assigned. Significantly different means from the control are denoted by asterisks (ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

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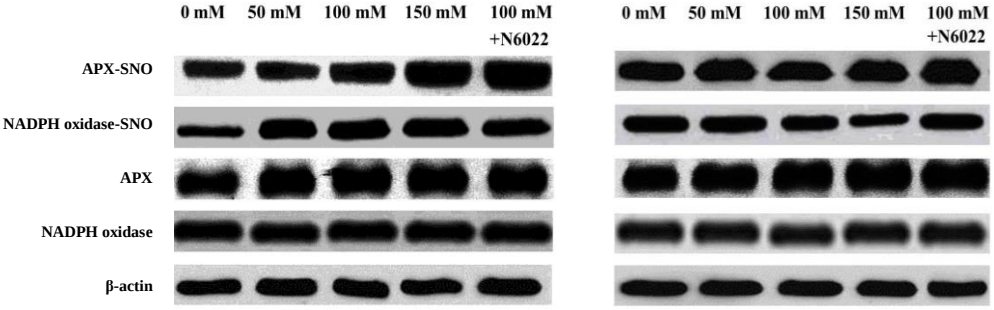


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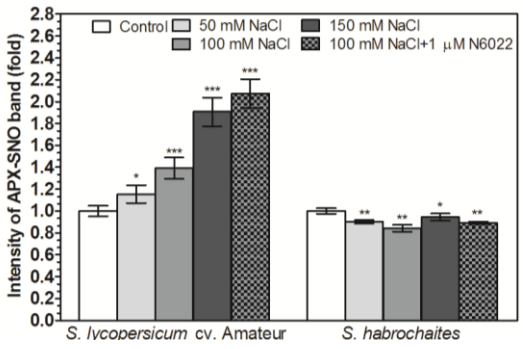


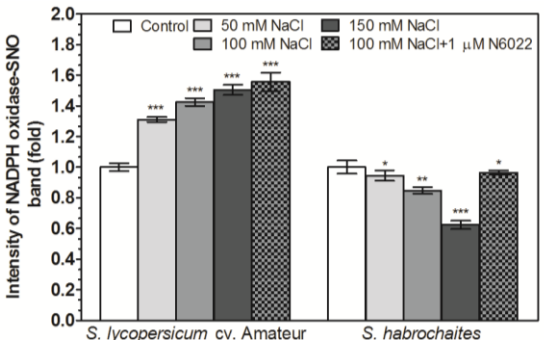


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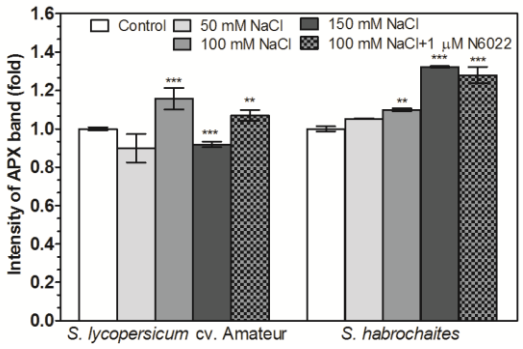


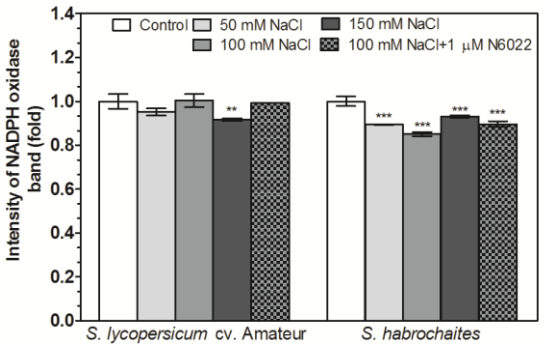

**Supplementary Figure S7. ROS levels in roots of *Solanum* spp. genotypes exposed to cadmium or salinity stress.** Fluorescent probe $H_2$DCF DA in 20 µM concentration was used for the detection of ROS in apical parts of roots. Green fluorescence signal detected by fluorescence microscopy corresponds to intracellular ROS levels. As a negative control, roots were incubated with 20 mM ascorbate (images not shown). Scale bar = 200 µm.