

## Supplementary Material

### **Enhancing antioxidant systems by exogenous spermine and spermidine in wheat (*Triticum aestivum*) seedlings exposed to salt stress**

*Abdelaleim I. ElSayed*<sup>A,F</sup>, *Mohammed S. Rafudeen*<sup>B</sup>, *Mohamed A. M. El-hamahmy*<sup>C</sup>, *Dennis C. Odero*<sup>D</sup>  
and *M. Sazzad Hossain*<sup>E</sup>

<sup>A</sup>Biochemistry Department, Faculty of Agriculture, Zagazig University, 44519 Zagazig, Egypt.

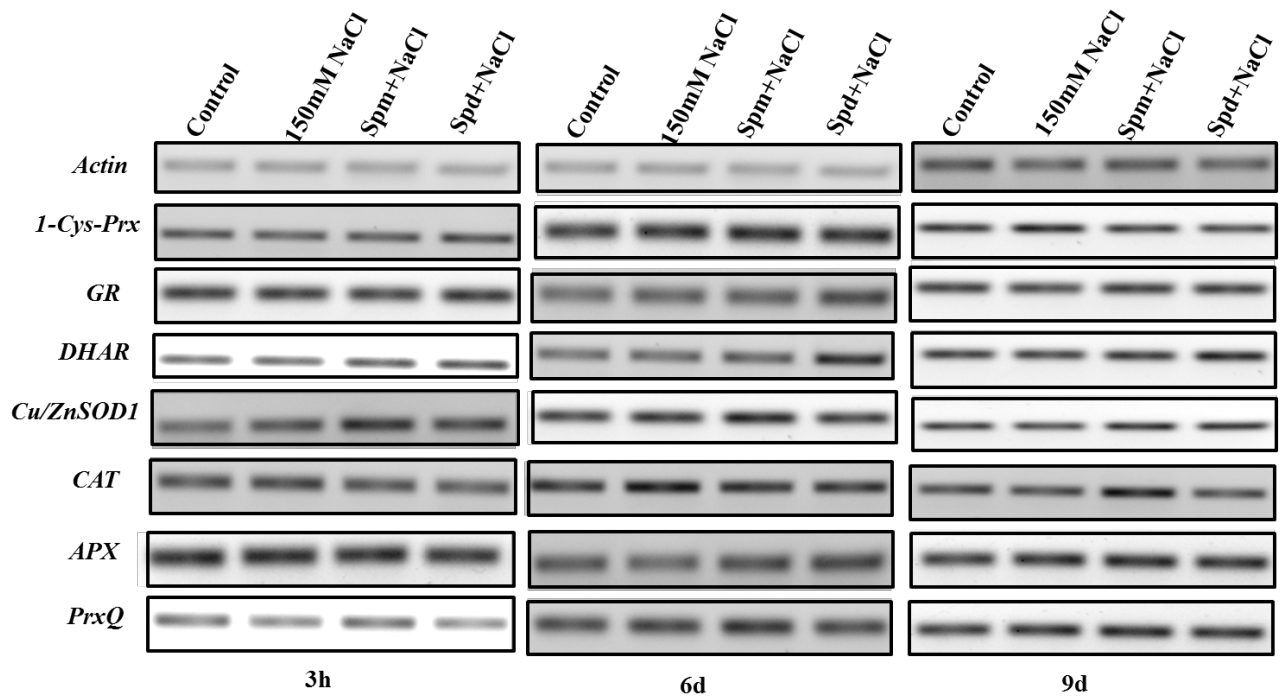
<sup>B</sup>Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa.

<sup>C</sup>Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt.

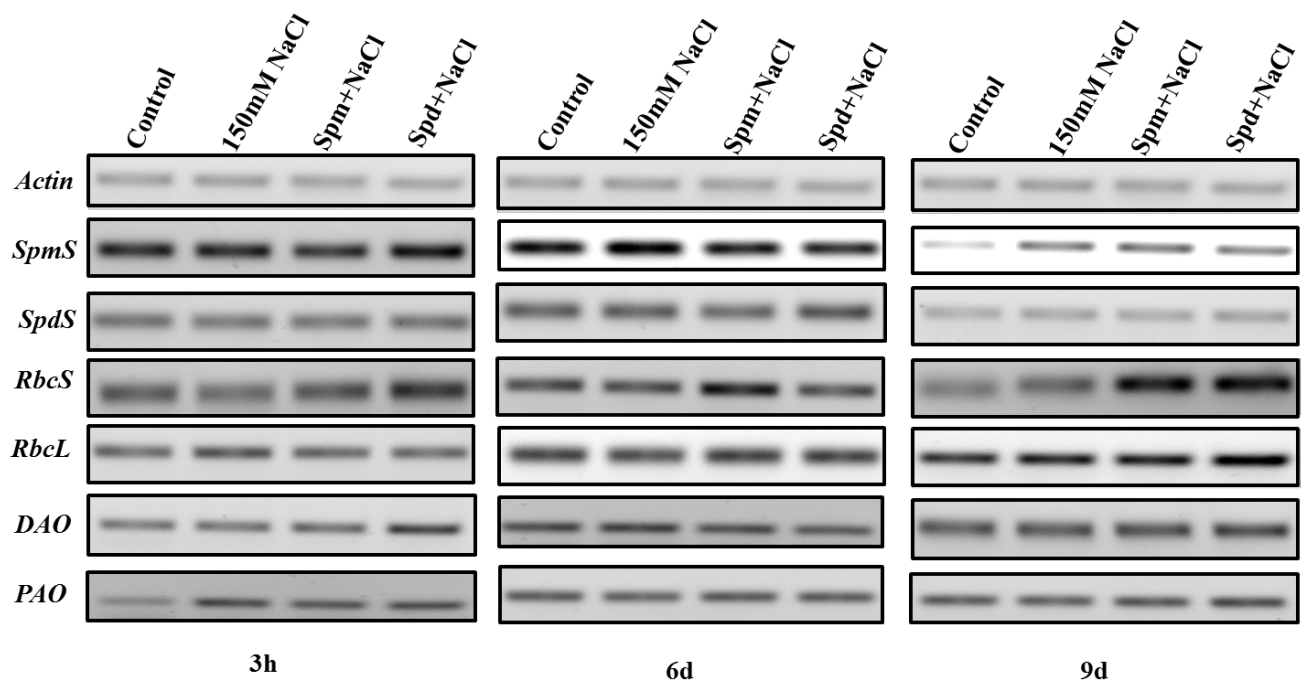
<sup>D</sup>Everglades Research and Education Centre, University of Florida-IFAS, 3200 East Palm Beach Road, Belle Glade, FL 33430, USA.

<sup>E</sup>Department of Biochemistry and Physiology of Plants, Faculty of Biology, Bielefeld University, Universitätsstr.25, D-33615, Bielefeld, Germany.

<sup>F</sup>Corresponding author. Email: aelsayed@zu.edu.eg



**Fig. S1.** Semi-quantitative RT-PCR of antioxidant enzymes genes *CAT*, *GR*, *DAHR*, *Cu/ZnSOD1* and *APX*, additionally, expression of *I-Cys-Prx* and *PrxQ* from cDNA of wheat seedlings treatments with exogenous Spm and Spd under salt stress compared with control plants at different time points. Samples standardized to Actin. RT-PCR product were separated on agarose gels (1.5%) and visualized by ethidium bromide stain. *DHAR* dehydroascorbate reductase; *Cu-ZnSOD1* Copper-Zinc superoxide dismutase; *CAT* catalase; *APX* ascorbate peroxidase; *GR* glutathione reductase; *POD* peroxidase; *I-Cys-Prx* 1-cysteine peroxiredoxin; *PrxQ* peroxiredoxin Q.



**Fig. S2.** Semi-quantitative RT-PCR of *RbcL*, *RbcS*, *SpmS* and *SpdS* from cDNA of wheat seedlings treatments with exogenous Spm and Spd under salt stress compared with control plants at different time points. Samples made relative to *Actin* levels. RT-PCR product were separated on agarose gels (1.5%) and visualized by ethidium bromide stain. *RbcL* Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit; *RbcS* ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit; *SpdS* spermidine synthase; *SpmS* spermine synthase; *DAO* diamine oxidases; *PAO* polyamine oxidases.

**Table S1. Primers sequences for semi quantitative and quantitative RT-PCR of the salt-related genes in wheat (*Triticum aestivum* L.) seedlings**

Gene name	Reference sequence	5' - 3' primer sequence	T <sub>A</sub>
<i>Actin</i>	AB181991	CTCTGACAATTTCCCGCTCA ACACGCTTCCTCATGCTATCC	58 °C
<i>DHAR</i>	AY074784	TCAAGAACGTGACAAAGGTGG TAACGGTGGTGATGGCAAAT	58 °C
<i>Cu-Zn SOD1</i>	U69536	ACCTCCATGAGTTCGGTGAC CATTAGGGCCAGTCAAAGGA	58 °C
<i>CAT</i>	GU984379	GGCTGCTTGAAGTTGTTCTCCT CTGCTAGTACCTCCTGATCCGTT	58 °C
<i>APX</i>	EF555121	CATCCCTGGAAGACGTGATT CAAACCCAGACCTTTCAGGA	58 °C
<i>GR</i>	AY364467	CCAATAGGCTGAACCTGGAA CGAAGCAAGTAGCCTCCATC	58 °C
<i>l-Cys-Prx</i>	AY304482	AATGGACCAAGGACATCGAG GACGGGTACAGGAAGCTCAG	58 °C
<i>PrxQ</i>	AY789643	ACTTCACGCTCAAGGACCAG CCGCCTTCTTGTACTTCTCG	58 °C
<i>RbcL</i>	AY328025	GCTGCCGAATCTTCTACTGG AGAGCACGTAGGGCTTTGAA	58 °C
<i>RbcS</i>	AB020957	TGCAGAACATCCCCAATGTA TAGAATGCGCACAGATGCTC	58 °C
<i>SPDS</i>	KF900082.1	CCGACTTCATGTTGGTGATG TGCAGCCACATACTTTCAGC	58 °C
<i>SPMS</i>	XM_003572046.3	CATCCTGTTTCATCGTCATGC TAACCAATACGGTTCGCAACA	58 °C
<i>DAO</i>	AJ583529.1	TCCACGTGCCGGAATTGTAA ATGAGACCGTGGAGAGACCA	58 °C
<i>PAO</i>	NM_105256.4	CTAGTGACACGGTGGGGAAC TAAGAAAGCTCCGTGAGCGG	58 °C

**Table S2. Spermidine synthase and spermine synthase sequences used in the phylogenetic analysis.**

<b>Gene name</b>	<b>Plant species</b>	<b>GenBank accession number</b>
Spermidine synthase	<i>Triticum aestivum</i>	AHJ14574.1
Spermidine synthase	<i>Triticum aestivum</i>	AHJ14572.1
Spermidine synthase	<i>Triticum aestivum</i>	AHJ14573.1
Spermidine synthase	<i>Triticum aestivum</i>	AEL33692.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_010227639.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_014752641.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_010227638.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_010227637.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_003563796.1
Spermine synthase	<i>Oryza brachyantha</i>	XP_006656137.1
Spermine synthase	<i>Oryza brachyantha</i>	XP_015693724.1
Spermine synthase	<i>Oryza brachyantha</i>	XP_015644436.1
Spermine synthase	<i>Setaria italica</i>	XP_004965517.1
Spermine synthase	<i>Setaria italica</i>	XP_004965516.1
Spermidine synthase	<i>Zea mays</i>	ACG48418.1
Spermidine synthase	<i>Zea mays</i>	XP_008659039.1
Spermidine synthase	<i>Zea mays</i>	XP_008659038.1
Spermidine synthase	<i>Zea mays</i>	XP_008659037.1
Spermine synthase	<i>Brachypodium distachyon</i>	XP_003572094.1