

# An atypical dual-specificity protein tyrosine phosphatase PFA-DSP3 is involved in plant salt response through modulating MPK3 and MPK6

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## Abstract

Protein phosphorylation, especially serine/threonine and tyrosine phosphorylation, plays significant roles in signaling processes during plant growth and development as well as their responses to biotic or abiotic stresses. The dual-specificity protein tyrosine phosphatases are important to de-phosphorylate and inactivate the signaling components. In this study, we reported an atypical dual specificity protein tyrosine phosphatase ATPFA-DSP3 (DSP3), which loss-of-function mutant was insensitive to salt treatment, played a negative role in plant's response to salinity in Arabidopsis. DSP3 protein was primarily localized in nuclei and degraded after salt treatment. The level of ROS accumulation was lower in dsp3 mutant and higher in DSP3 over-expresser than wild type control, indicating DSP3 positively affect ROS production. DSP3 can directly interact with MPK3 and MPK6, and the phosphorylated MPK3 and MPK6 over accumulate in dsp3 mutant. Moreover, the phosphatase activity of DSP3 was required for its salt response. These results provide evidences showing that DSP3 negatively mediates plant salt response by directly modulating the accumulation of phosphorylated MPK3 and MPK6.

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**FIGURE 1.** *DSP3* mutation confers salt tolerance in *Arabidopsis*. (a) Upper: Schematic diagram of *dsp3* T-DNA insertion site in the mutant. Exons and introns are depicted to scale by boxes and lines, respectively. T-DNA insertion site is marked by inverted triangle, with arrow indicating the left-border. Bottom: Level of *DSP3* transcript in 7-day-old wild type and *dsp3* mutant seedlings analyzed by semi-quantitative RT-PCR. *UBQ5* was used as internal control. (b) Transcript level of *DSP3* in *dsp3* mutant analyzed by qRT-PCR. *PP2A* was used as internal control. (c) Germination analysis of wild type and *dsp3* mutant after 3 days of growth on ½ MS medium with or without 175 mM NaCl. (d-f) Salt sensitivity analysis of 3-week-old soil-grown Col and *dsp3* seedlings in terms of survival rate (e) and chlorophyll content (d, f). Seedlings were treated with 200 mM NaCl for 7 days for chlorophyll content analysis and 10 days for survival rate analysis. Error bars in (b), (c), (e) and (f) indicate standard deviation (SD) from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ( $P < 0.05$ ) from one-way (b, e) or two-way (c, f) ANOVA.

**FIGURE 2.** The salt-insensitive phenotype of *dsp3* can be complemented by *pDSP3::DSP3-GUS* transgene. (a, b) Seed germination phenotype (a) and quantification (b) of *dsp3* mutant and complementation lines (*Com8-2* and *Com 4-15*) after 4 days of growth on ½ MS medium with or without 175 mM NaCl. (c-f) Salt sensitivity analysis of *dsp3* and complementation lines in 3-week-old soil-grown seedlings after 200 mM NaCl treatment. (c, d) Chlorophyll content after salt treatment for 8 days. (e) Survival rate after salt treatment for 12 days. (f) Ion leakage after salt treatment for 12 days. Error bars in (b), (d), (e) and (f) indicate SD from three biological repeats. The different lowercase letters over each bar represent statistically significant difference ( $P < 0.05$ ) from two-way ANOVA.

**FIGURE 3.** Altering the expression of *DSP3* will result in the expression change of stress responsive genes under salt stress. The expression level of *RAB18*, *RD29B*, *KIN1*, *P5CS1*,

**Figure 1**

