

SHORT COMMUNICATION



## Patellin1 negatively regulates plant salt tolerance by attenuating nitric oxide accumulation in Arabidopsis

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### ABSTRACT

Salt stress adversely affects plant growth and development. Multiple adaptive mechanisms have been used for plant salt tolerance. We previously reported that membrane trafficking-related protein patellin1 (PATL1) negatively regulates plant salt tolerance. Here, we characterized that Arabidopsis PATL1 negatively modulates nitric oxide (NO) accumulation upon salt exposure. Our work revealed a functional link between salt response and NO signaling.

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As sessile organisms, plants encounter various environmental stresses during their life cycle. Soil salinization is one of the major adverse factor that limits crop growth and production.<sup>1</sup> Plants have evolved multiple signaling networks for effective salt tolerance regulation, including ionic homeostasis maintenance, osmotic adjustment as well as other mechanisms.<sup>1</sup> It is believed that elucidation of the mechanisms by which plants cope with salt stress will definitely promote the generation of salt-tolerant crops, thus providing a possibility to make full use of saline soil widespread globally.

Nitric oxide (NO) can be induced by numerous environmental stimuli in plants and functions as a versatile messenger in multiple signaling networks.<sup>2,3</sup> In plants, the production of NO occurs mainly through two different enzymatic pathway.<sup>4</sup> Subsequently, NO reacts with GSH to form GSNO, a major bioactive NO species, which is then reversibly metabolized by a highly conserved GSNOR (*S*-nitrosoglutathione reductase), making the NO level being dynamically modulated.<sup>5,6</sup> The principal bioactivity of NO is executed through *S*-nitrosylation by covalently adding a NO group on the reactive cysteine (Cys) thiol of a substrate protein, resulting in *S*-nitrosothiols which might alter the functions of target proteins by diverse mechanisms.<sup>7,8</sup> Interestingly, Arabidopsis GSNOR1 itself also undergoes NO-mediated *S*-nitrosylation at Cys10, causing local conformational changes and inducing selective autophagy of GSNOR1 through directly interacting with ATG8.<sup>9</sup> *S*-nitrosylation is a redox-based post-translational modification (PTM) on proteins, and it also plays a role in reactive oxygen species (ROS) production in plants. This reciprocal regulation of ROS and NO are critical for early signaling in both abiotic and biotic stress responses.<sup>7,8,10,11</sup> As aforementioned, GSNOR is the master regulator of NO germination in plants, and mutations in the single copied *GSNOR1/HOT5/PAR2* gene in Arabidopsis genome cause elevated NO concentration and enhanced *S*-nitrosylation level, leading to severe growth defects and

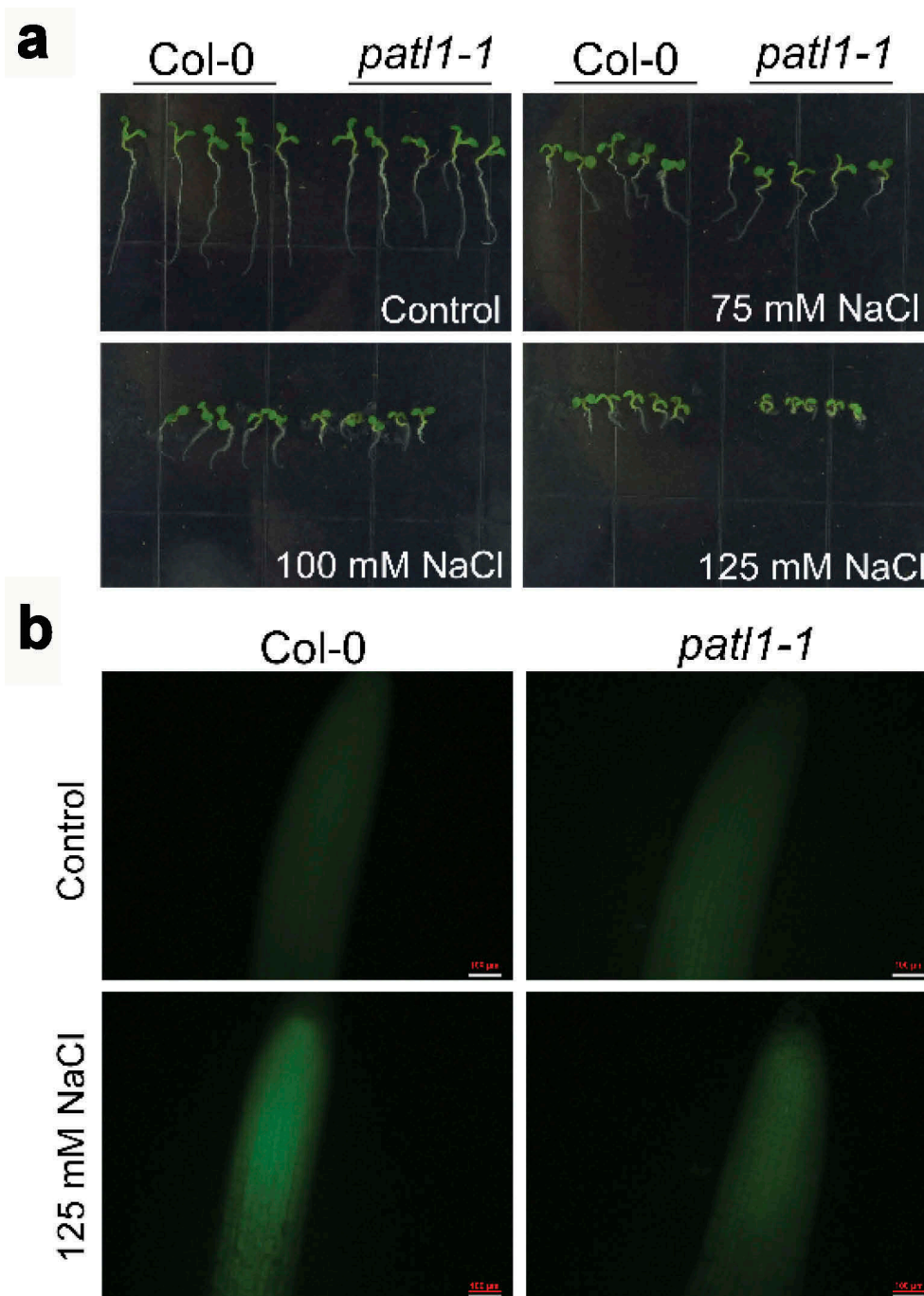
altered responses environmental stresses.<sup>12–14</sup> Specifically, GSNOR1 loss-of-function mutant *gsnor1-3* exhibits a salt-tolerant phenotype with low Na<sup>+</sup>/K<sup>+</sup> ratio under salt stress condition, and salt-induced Ca<sup>2+</sup> and calcium-binding protein CaM4 have been reported to function in GSNOR-mediated salt tolerance regulation.<sup>15</sup> In fact, salt stress can effectively induce endogenous NO germination, which then results in salt resistance by increasing K<sup>+</sup>/Na<sup>+</sup> ratio in two ecotypes of reed.<sup>16</sup> Exogenous application of GSNO has been found to effectively enhance tonoplast H<sup>+</sup>-ATPase and H<sup>+</sup>-PPase activity, resulting in elevated Na<sup>+</sup>/H<sup>+</sup> exchange activity across vacuole membrane and lower the Na<sup>+</sup> toxicity in cytoplasm.<sup>17</sup> In Arabidopsis, NOA1-dependent NO production and heme oxygenase1 expression are also associated with plant salt response.<sup>18,19</sup> These studies together support a conclusion that NO and its dynamic regulation play a crucial role in plant salt stress signaling networks.

Arabidopsis patellin (PATL) protein family members are putative membrane trafficking-related proteins.<sup>20,21</sup> These proteins are characterized by two conserved domains universally observed in membrane trafficking-related proteins: a Sec14 lipid-binding domain and a Golgi dynamics domain (GOLD) followed the N-terminal variable domain.<sup>20,21</sup> Arabidopsis PATLs are expressed in distinct but partly overlapping patterns, which might represent the redundant and diversified functions of PATLs in Arabidopsis.<sup>21</sup> Recently, we have characterized PATL1 as a novel negative regulator of plant salt tolerance.<sup>22</sup> There exists a direct interaction between PATL1 and SOS1, a major component of Salt Overly Sensitive (SOS) pathway. PATL1 is implicated in ion homeostasis regulation in plants possibly through SOS1, and it also functions in cellular redox homeostasis during salt stress.<sup>22</sup> PATL1 also forms a complex with calmodulin4 (CaM4) and they two together play a regulatory role in plant freezing response.<sup>23</sup> Interestingly, CaM4 is reported to participate in plant salt tolerance regulation through activating GSNOR and

promoting NO accumulation upon salt exposure.<sup>15</sup> Thus there might exist a possible unidentified mechanism that PATL1 regulates plant salt tolerance through the action of GSNOR and NO.

In the present study, we demonstrate that Arabidopsis PATL1 negatively modulates plant salt tolerance through attenuating NO accumulation. Consistent to our previous study,<sup>22</sup> data showed that the *patl1-1* mutant (SALK\_103668) with elevated PATL1

transcription exhibits salt-sensitive phenotype (Figure 1(a)). To further determine the underlying mechanisms for the negative effect of PATL1 on plant salt tolerance, we thus examined intracellular NO accumulation in Col-0 and *patl1* mutants at the seedling stage using the method of 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) staining. Data revealed that the NO levels were remarkably increased in both tested plants in the presence of 125 mM NaCl (Figure 1(b)).



**Figure 1.** Patellin1 negatively regulates plant salt tolerance by attenuating NO accumulation in Arabidopsis.

(a) Salt sensitivity analysis of 7-d-old seedlings of Col-0 and *patl1-1* grown on MS medium under control condition or supplemented with different concentrations of NaCl (75, 100 and 125 mM). Photographs were taken when phenotypic differences were observed. (b) NO accumulation in the roots of 7-d-old seedlings. Col-0 and *patl1-1* plants were incubated in  $\frac{1}{2}$  liquid MS medium (pH = 5.8) with 10  $\mu$ M DAF-FM DA for 20 min. After incubation, the roots were washed three times for 15 min each in  $\frac{1}{2}$  liquid MS medium prior to observation using a fluorescence microscope. Bar = 100  $\mu$ m.

Interestingly, NO accumulated to a significantly lower level in *pat11-1* seedlings than that in Col-0 (Figure 1(b)), which is consistent to the salt-sensitive phenotype of *pat11* plants.

Considering that GSNOR1 is implicated in NO homeostasis and there exists a possible link between PATL1 and GSNOR1, we propose that PATL1 might interact GSNOR directly and significantly enhances its activity, leading to decreased accumulation of NO under salt stress condition. Future assays to determine whether PATL1 and GSNOR1 together participate in plant salt tolerance are required. Since our data have shown that PATL1 plays a negative role in NO production upon salt exposure (Figure 1) and GSNOR1 serves as a negative regulator of plant salt tolerance,<sup>15</sup> we therefore hypothesized that PATL1 might play a positive regulatory role on GSNOR activity, and that PATL1 attenuates NO accumulation through the action of GSNOR activity upon salt exposure. NO is significantly induced upon salt exposure, indicating a regulatory role of NO in plant salt tolerance. Previous studies have reported that NO alleviates salt toxicity in reeds and maize through the activation of H<sup>+</sup>-ATPase localized in the PM and vacuole membrane, promoting Na<sup>+</sup> efflux to the apoplast and/or vacuolar Na<sup>+</sup> compartmentation.<sup>17,24</sup> In Arabidopsis, NO is associated with salt resistance via attenuation of the salt-induced Na<sup>+</sup>/K<sup>+</sup> ratio increase.<sup>15</sup> However, there exists no direct evidence concerning whether specific Na<sup>+</sup> transporters are involved in NO-mediated salt tolerance network. Considering that PATL1 is linked to PM Na<sup>+</sup>/H<sup>+</sup> antiporter SOS1 activity regulation as well as NO signaling, it is possible that NO might play a regulatory role in SOS1-mediated Na<sup>+</sup> efflux.

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