**Expanded screening of abiotic stress responses in *Nicotiana benthamiana*: evidence for NbPhyt1-mediated osmotic signaling**

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Abiotic stress driven by global climate change is a major threat to agricultural productivity [1]. Plants adapt to these stresses through complex molecular mechanisms, in which proteases are key regulators. Phytaspases, aspartate-specific serine subtilases, are well characterized for their roles in drought stress responses in *Solanum lycopersicum* and *Arabidopsis thaliana* [2,3]. However, the functional involvement of *Nicotiana benthamiana* phytaspase 1 (NbPhyt1) in abiotic stress signaling remains unelucidated. This study aimed to perform an expanded screening of NbPhyt1 responses to drought, temperature fluctuations, light deprivation, and D-mannitol-induced osmotic stress to define its precise regulatory niche in *N. benthamiana*.

*N. benthamiana* soil-grown plants and sterile seedlings grown on MS/2 medium were subjected to selected abiotic stress treatments: drought (34 days of water withholding, 10 mL/3 days maintenance), heat (37°C overnight or 30°C for 5 days), cool (18°C for 15 days), darkness (2 days of continuous dark), and osmotic stress (150 mM D-mannitol for 11 days). Following these treatments, NbPhyt1 enzymatic activity was quantified in leaf extracts fluorometrically, and *NbPhyt1* gene expression level was analyzed by quantitative real-time PCR (qPCR).

Our results demonstrate that in adult plants, NbPhyt1 activity and expression remain unaffected by drought and temperature fluctuations; In seedlings, NbPhyt1 is unaffected by light deprivation. However, a specific response to osmotic stress was identified at the seedling stage: seedlings maintained on D-mannitol-supplemented media exhibited reproducibly higher NbPhyt1 activity compared to the control group, a difference further supported by distinct gene expression levels. These findings indicate a selective requirement for this phytaspase during osmotic shock in early development, distinguishing it from other abiotic stress signaling pathways. This study defines the functional boundaries of NbPhyt1, showing it is specifically involved in osmotic stress adaptation during early seedling development, but not in drought, temperature, or dark stress responses. These findings provide new insights into phytaspase regulation and highlight its potential role in enhancing plant resilience to osmotic-related challenges, such as soil salinity.

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**References**

1. Iqbal H, et al. From lab to field: harnessing $H_2O_2$-mediated upregulation of plant capacities under abiotic stresses. *Physiol Plant*, 2025, 177(5):e70488.
2. Wang S, et al. A regulatory network involving calmodulin controls phytosulfokine peptide processing during drought-induced flower abscission. *Plant Cell*, 2025, 37(1):koaf013.
3. Stührwohldt N, et al. Phytosulfokine (PSK) precursor processing by subtilase SBT3.8 and PSK signaling improve drought stress tolerance in Arabidopsis. *J Exp Bot*, 2021, 72(9):3427-3440.