

NPR1-dependent salicylic acid signaling is not involved in elevated CO₂-induced heat stress tolerance in *Arabidopsis thaliana*

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Abbreviations: ABA, abscisic acid; Fv/Fm, maximum photochemical efficiency of PSII; HS, heat stress; HSPs, heat-shock proteins; MAPK, mitogen activated protein kinase; *npr1*, *nonexpressor of pathogenesis-related gene 1*, PSII; photosystems II, SA, salicylic acid.

Elevated CO₂ can protect plants from heat stress (HS); however, the underlying mechanisms are largely unknown. Here, we used a set of *Arabidopsis* mutants such as salicylic acid (SA) signaling mutants *nonexpressor of pathogenesis-related gene 1* (*npr1-1* and *npr1-5*) and heat-shock proteins (HSPs) mutants (*hsp21* and *hsp70-1*) to understand the requirement of SA signaling and HSPs in elevated CO₂-induced HS tolerance. Under ambient CO₂ (380 μmol mol⁻¹) conditions, HS (42°C, 24 h) drastically decreased maximum photochemical efficiency of PSII (Fv/Fm) in all studied plant groups. Enrichment of CO₂ (800 μmol mol⁻¹) with HS remarkably increased the Fv/Fm value in all plant groups except *hsp70-1*, indicating that NPR1-dependent SA signaling is not involved in the elevated CO₂-induced HS tolerance. These results also suggest an essentiality of HSP70-1, but not HSP21 in elevated CO₂-induced HS mitigation.

High temperature, one of the major abiotic factors affecting plant growth is consistently threatening global food security due to ongoing climate change.¹ Heat stress (HS) primarily targets photosynthesis for its high sensitivity and thus minimizing plant productivity. It has been reported that HS could inhibit or inactivate photosystem II (PSII), by degrading the reaction center-binding protein D1 of PSII.² Plant responses to HS often involve production and accumulation of heat-shock proteins (HSPs) which are required for heat acclimation and subsequent tolerance.^{1,3} Small HSPs could combine with thylakoid and protect oxygen evolving complex proteins of PSII against HS.¹ It has also been suggested that HSPs mainly function to prevent the damage by serving as molecular chaperons, but do not participate to reverse protein denaturation and aggregation.^{1,4} In addition, as a phytohormone, salicylic acid (SA) has been implicated in basal thermotolerance, exogenous application of SA in *Arabidopsis* could induce HSPs gene expression *in vivo*.^{3,5} In wheat, SA pretreatment increases the protein kinase activity and retards the degradation of D1 protein under heat and high light stress. SA accelerates the recovery of D1 protein after termination of stress.⁶

Besides a threatening rise in global temperature, climate change is also attributed to increases in atmospheric CO₂ concentration, which may offer interactive effects of HS and elevated CO₂ concentration on plant growth and productivity.⁷ Previous studies indicate that elevated CO₂ could minimize HS-induced deleterious effects on plants.^{7,8} Nonetheless, the mechanisms of

elevated CO₂-induced HS mitigation are unknown. Previously, we have demonstrated that elevated CO₂-induced HS mitigation does not involve abscisic acid-dependent process in tomato.⁷ In the present study, we used a set of *Arabidopsis* mutants such as *nonexpressor of pathogenesis-related gene 1* (*npr1-1*, *npr1-5*; SA signaling blockage mutant) and HSPs gene mutants (*hsp21* and *hsp70-1*) to understand the requirement of SA signaling and HSPs in elevated CO₂-induced HS tolerance. The performance of the photosynthetic machinery was monitored by chlorophyll *a* fluorescence through determining the photochemical efficiency of PSII (Fv/Fm) which is well recognized as a sensitive HS indicator.⁷

Under normal temperature conditions, no obvious differences were noticed among the mutants and wild-type (Col-0) *Arabidopsis* plants in terms of Fv/Fm value (Fig. 1). After the heat shock (42°C, 24 h) in ambient CO₂ (380 μmol mol⁻¹) conditions, all groups of seedlings displayed a significant decline in Fv/Fm. HS decreased Fv/Fm by 29.11, 38.12, 37.80, 39.74 and 42.35% in Col-0, *npr1-1*, *npr1-5*, *hsp21* and *hsp70-1*, respectively compared to their respective non-stress controls. Among all studied plant groups, *hsp21* showed characteristically dwarf phenotype; however, it demonstrated similar heat sensitivity like other mutants. Administration of CO₂ enrichment along with HS remarkably increased the Fv/Fm value in all plant groups except *hsp70-1*. CO₂ enrichment increased Fv/Fm value by 33.61, 35.20, 34.16 and 38.87 % in

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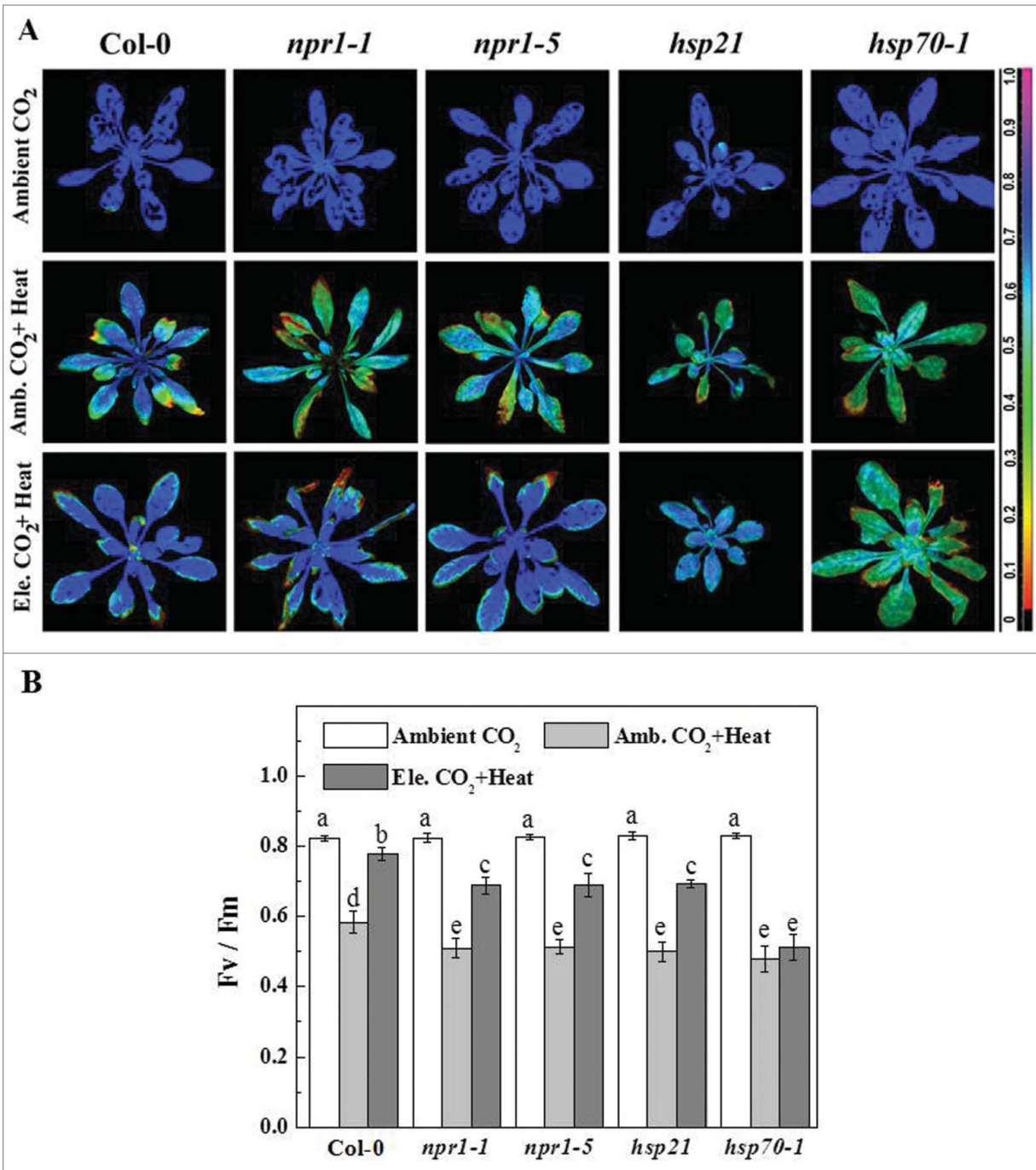


Figure 1. Effect of heat stress and elevated CO₂ on maximum photochemical efficiency of PSII (Fv/Fm) in Arabidopsis. Chlorophyll *a* fluorescence imaging was used to determine photosynthetic performance of wild-type Col-0, SA signaling blockage mutants and *hsp* mutant lines. Four weeks old Arabidopsis plants (wild-type and mutants) were exposed to either ambient CO₂ (Amb., 380 μmol mol⁻¹) or elevated CO₂ (Ele., 800 μmol mol⁻¹) for 5 d and then challenged with high temperature (42°C) for 24 h under ambient or elevated CO₂. Fv/Fm was measured after heat stress as previously described elsewhere.⁷ **(A)** Pseudo-color images of Fv/Fm where the false color code depicts ranges vertically from 0.0 (black) to 1.0 (purple). **(B)** Fv/Fm values averaged over the whole rosette area; values presented are mean ± SD from 4 rosettes. The means denoted by the same letters did not significantly differ at *P* < 0.05 according to Tukey's test.

Col-0, *npr1-1*, *npr1-5* and *hsp21*, respectively compared with their respective only heat treatment counterparts. It can be explained that *NPRI-1*, *NPRI-5* and *HSP21* genes might not be involved in the elevated CO₂-induced improvement in

photosynthetic efficiency. By contrast, the data indicate that *HSP70-1* is the most important component required for HS tolerance under both ambient and elevated CO₂ concentration in Arabidopsis.

NPR1 is the key transducer of the SA signal. Previous studies showed that NPR1 is required for the basal thermotolerance which support our current observation on *npr1-1* and *npr1-5* under ambient CO₂.⁹ Interestingly, we found that elevated CO₂ can still increase the thermotolerance of *npr1* mutants, which indicates NPR1-independent process for HS mitigation in response to elevated CO₂ (Fig. 1). Elevated CO₂ might stimulate cellular redox homeostasis and MAPK activity and thus ameliorate HS through NPR1-independent process in *npr1-1* and *npr1-5* mutants.^{7,8,10,11} HSPs protect various important proteins from irreversible heat-induced damage through preventing denaturation as well as assisting the refolding of damaged protein.^{1,4,9} The inability of *hsp21* and *hsp70-1* to produce respective proteins aggravated HS-induced damage to photosynthetic apparatus (Fig. 1). Administration of elevated CO₂ could protect photosynthetic apparatus of *hsp21* mutants, but not of *hsp70-1* mutants. Information on effects of elevated CO₂ on HSPs production and accumulation is scanty. A recent study shows that elevated CO₂-induced HSPs accumulation varies with the plant species as well as nutrient status (e.g. nitrogen) under HS.¹² In corn (*Zea mays* L.) with high nitrogen supply, elevated CO₂-induced photosynthetic thermotolerance might be partly mediated by HSPs (such as HSP60 and HSP70).¹² Extreme thermosensitivity of *hsp70-1* mutants and its inability to mitigate HS following CO₂ enrichment suggest that elevated CO₂-induced HS tolerance largely depends on HSP70-1 in Arabidopsis. However, for *hsp21* mutants, other protective processes such as efficient reactive oxygen species (ROS) detoxification, improved redox homeostasis and decreased photorespiration as

consequences of CO₂ enrichment might occur.⁸ Together, this work indicates that NPR1-mediated SA signaling is important for basal thermotolerance; however, NPR1 is not required for elevated CO₂-induced HS mitigation in Arabidopsis. Elevated CO₂ could ameliorate HS-induced deleterious effect on photosynthetic apparatus without involvement of HSP21, but basal thermotolerance as well as elevated CO₂-induced HS tolerance largely depends on HSP70-1 in Arabidopsis. Further study using advanced physiological, biochemical and molecular-genetic approaches may unveil in-depth mechanisms of elevated CO₂-induced HS mitigation and involvement of SA in this process.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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