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Role of nitric oxide in thermotolerance

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AtCaM3 is a key factor in heat shock (HS) signal transduction. Nitric oxide (NO) is believed to mediate a variety of resistant reactions against environmental factors. Our experiments indicate that under heat stress NO induces thermotolerance. In order to do so, NO is signal molecule acting upstream of AtCaM3, stimulating the DNA-binding activity of HS transcription factors as well as the accumulation of heat shock proteins. As a novel HS signaling molecule, NO signal pathway is little known and several unexpected results are emerging. Herein we are discussing them and conclude that in order to obtain a more profound understanding of this new role of NO, detailed research will be needed in the future.

Key words: *Arabidopsis thaliana*, nitric oxide, thermotolerance, heat shock protein

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Nitric oxide is a signaling molecule extensively involving in plant normal growth and development in a concentration-dependent manner. The involvement of NO in thermotolerance was reported by Lee et al.[1] In this paper, they found that *hot5* (encodes S-nitrosoglutathione reductase)-null mutants showed increased nitrate and nitroso species levels and the heat sensitivity of both missense and null alleles was associated with increased NO species levels. Heat sensitivity was enhanced in wild-type and mutant plants by NO donors and the heat sensitivity of the *hot5* mutants was successfully rescued by an NO scavenger. Thus, they proposed that enhancement of the NO level induced heat sensitivity in Arabidopsis.[1] In previous works, NO was always found to enhance plant resistance against environmental factors, including drought, salt stress, disease, etc.[2] Low and high NO concentration might induce the ambivalent reaction in plant tissue. So we have tried a series of low concentrations of sodium nitroprusside (SNP) as NO donor to treat Arabidopsis seedlings. The result is surprising and exciting that application of SNP greatly increased survival rates of the seedlings, even 100 μM SNP for nearly 100% survival rate (**Fig. 1**), indicating the possibility of NO stimulating thermotolerance.

To verify this suggestion, *noa1(rif1)* (for nitric oxide associated1/resistant to inhibition by fosmidomycin1), which exhibits reduced endogenous NO levels, was used to examine the relationship between NO and heat stress. Our data indicate that NO functions as a second messenger in the induction of thermotolerance through AtCaM3, which is dependent on the enhancement of HSF DNA binding activity and HSP accumulation.[3] In combination with the results of high NO level reducing thermotolerance from Lee et al.,[1] we suggest that NO homeostasis is a key factor affecting the initiation of resistant reactions to heat stress.

Also, we found a strange phenomenon that different sensitivity to NO existed between wild-type and *noa1(rif1)* mutant seedlings under HS. Under 20 μM SNP treatment, survival rate of *noa1(rif1)* seedlings reached the maximum value (as shown in **Fig. 2** of the original paper), whereas it of wild-type seedlings was the highest in the present of 100 μM SNP (**Fig. 1**). This may be due to the nature of NOA1(RIF1), which is only associated with NO biosynthesis,[4,5] such that supplementation with NO alone could not fully rescue the thermotolerance of the *noa1(rif1)* mutant equal to that of wild type.

We used NO inhibitors, N^{ω}-nitro-l-arginine and 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxyde, to treat the

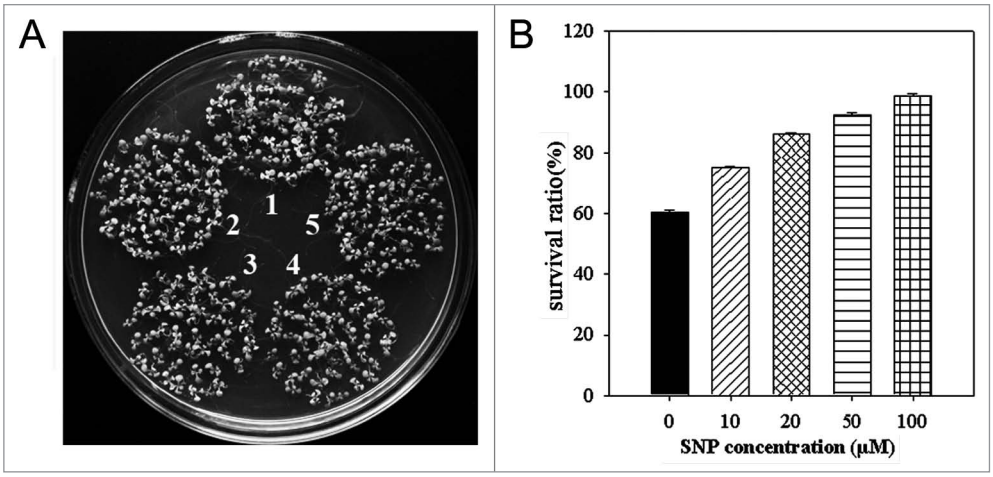

Figure 1. Effect of SNP on survival rates of Arabidopsis seedlings. (A) Six-day-old wild-type seedlings grown at 22°C were pretreated with 1 mL of a different concentrations (1, 0 mM; 2, 10 mM; 3, 20 mM; 4, 50 mM; or 5, 100 mM) of SNP as an NO donor. After 24 h, they were exposed to 45°C for 60 min, then returned to 22°C and photographed 6 d later. (B) Survival ratios of the seedlings. The data are the mean ± SE of at least four independent experiments, with 50 seedlings per experiment.

seedlings and expected to find their inhibition to thermotolerance as the reverse proof of the supplement of NO. However, the result showed that they could not decrease survival rates of the seedlings even the rather high concentrations (data not shown). Later experiments indicated that it might be due to increased NOA1(RIF1) expression to keep a fixed NO concentration in plant (data not shown), indicating that a stable internal NO level is crucial at this special developmental stage.

Though we have documented the involvement of NO in HS, there are several important issues awaiting further studies such as its signaling pathways, source(s) of NO, interactions of NO with cytosolic Ca^{2+} and effects of NO on adult plants. These need our further and detailed research.

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