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Synergistic effect of antioxidant system and osmolyte in hydrogen sulfide and salicylic acid crosstalk-induced heat tolerance in maize (*Zea* mays L.) seedlings

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Salicylic acid (SA), is a plant hormone with multifunction that is involved in plant growth, development and the acquisition of stress tolerance. Hydrogen sulfide (H_2S) is emerging similar functions, but crosstalk between SA and H_2S in the acquisition of heat tolerance is not clear. Our recent study firstly reported that SA treatment enhanced the activity of L-cysteine desulfhydrase (L-DES), a key enzyme in H_2S biosynthesis, followed by induced endogenous H_2S accumulation, which in turn improved the heat tolerance of maize seedlings.[1] In addition, NaHS, a H_2S donor, enhanced SA-induced heat tolerance, while its biosynthesis inhibitor DL-propargylglycine (PAG) and scavenger hydroxylamine (HT) weakened SA-induced heat tolerance. Also, NaHS had no significant effect on SA accumulation and its biosynthesis enzymes phenylalanine ammonia lyase (PAL) and benzoic-acid-2-hydroxylase (BA2H) activities, as well as significant difference was not observed in NaHS-induced heat tolerance of maize seedlings by SA biosynthesis inhibitors paclobutrazol (PAC) and 2-aminoindan-2-phosph- onic acid (AIP) treatment.[1] Further study displayed that SA induced osmolytes (proline, betaine and trehalose) accumulation and enhancement in activity of antioxidant system in maize seedlings. These results showed that antioxidant system and osmolyte play a synergistic role in SA and H_2S crosstalk-induced heat tolerance of maize seedlings.

Salicylic acid (SA), a plant hormone with multifunction that is involved in plant growth, development and the acquisition of stress tolerance, is mainly synthesized through the phenylalanine (Phe) route localized in the cytoplasm, phenylalanine ammonia lyase (PAL) and benzoic-acid-2-hydroxylase (BA2H) are key enzymes in SA biosynthesis in plants.[2-5] SA has long been considered as an important endogenous immune signal in the induction of disease resistance response in plants.[3,6]

In addition, SA has also functions in responses to abiotic stress factors such as heavy metal toxicity, salt, drought, chilling and heat.[3-4] Spraying potato plants with an acetyl SA can improve the resistance of plants to heat stress in a concentration manner.[2] In addition, treatment with SA led to an increase in the level of endogenous H_2O_2 by reduction in catalase activity, similar to heat hardening at 45°C for 1 h.[7] In maize, treatment of seeds with SA not only could markedly enhanced chilling tolerance, but also heat tolerance, and the acquisition of this tolerance is closely relative to enhancement in the activity of antioxidant system.[8] Our previous work firstly found that exogenous SA enhanced the activity of L-cysteine desulfhydrase (L-DES), a key enzyme in H_2S biosynthesis, followed by induced endogenous H_2S accumulation, which in turn improved the heat tolerance of maize seedlings, NaHS (H_2S donor) enhanced SA-induced heat tolerance, indicated that H_2S might be a downstream signal molecule in SA-induced heat tolerance of maize seedlings,[1] but the mechanism of SA and H_2S interaction-induced heat tolerance is not completely clear.

Hydrogen sulfide (H_2S) has recently been identified as a third endogenous gaseous transmitter after nitric oxide (NO) and reactive oxygen species (ROS).[9] In plants, L-cysteine desulfhydrase (L-DES, E.C. 4.4.1.1.) is considered to be a key enzyme in H_2S biosynthesis.[9,10] Recently, many positive effects of H_2S is being emerged in multiple physiological processes, including seed germination, organogenesis, stomata movement, osmotic stress, salt stress, chilling stress, oxidative stress and heavy metal stress.[11-17] Our previous research results also showed that NaHS treatment can improve heat tolerance of tobacco cells, maize and wheat seedlings.[1,18-24]

Enhancement in antioxidant defense system and osmotic adjustment are common mechanisms of plants adapt to adverse environments including heat stress by scavenging reactive oxygen species (ROS); buffering redox; stabilizing protein, nucleic acid and biomembrane; regulating acidity in cytoplasm.[25,26] Further

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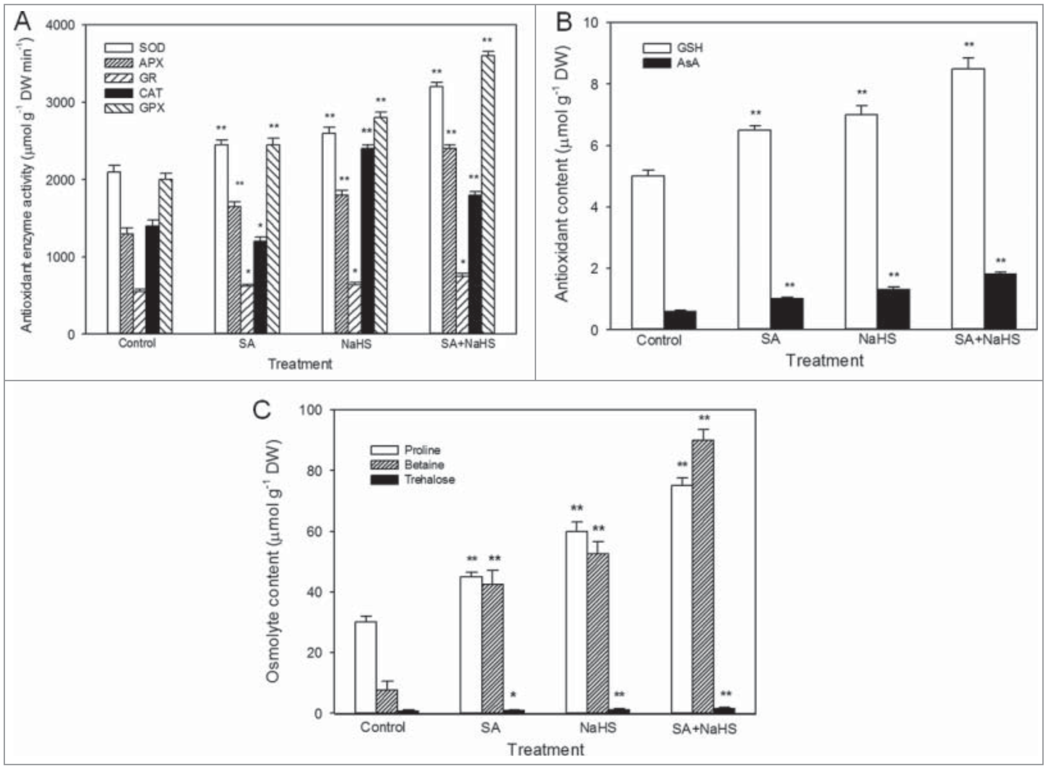

Figure 1. Effects of SA pretreatment on the accumulation of osmolyte (**A**), antioxidant enzyme activity (**B**) and antioxidant content (**C**) in maize seedlings under normal culture conditions. Error bars represent standard error and each data in figure represents the mean ± SE of 3 experiments, and asterisk and double asterisks indicate significant difference ($P < 0.05$) and very significant difference ($P < 0.01$) from the control without SA or NaHS treatment, respectively.

study showed that SA and NaHS treatment alone or in combination significantly increased the activity of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT, SA treatment alone inhibited CT activity) and guaiacol peroxidase (GPX), and the content of antioxidants ascorbic acid (AsA) and glutathione (GSH), as well as the accumulation of endogenous osmolytes such as proline, betaine and trehalose in maize seedlings (**Fig. 1**). In addition, these effects induced by SA were enhanced by NaHS treatment (**Fig. 1**). These data indicated that antioxidant system and osmolyte exert a very important role in SA and H_2S interaction-induced heat tolerance of maize seedlings.

In conclusion, pretreatment of maize seedlings with SA significantly stimulated an increase in L-DES activity in maize seedlings, followed by induced endogenous H_2S accumulation, which in turn increased the heat tolerance of maize seedlings (**Fig. 2**).[1] In addition, SA-induced heat tolerance was enhanced by NaHS, weakened by PAG and HT (**Fig. 2**),[1] suggesting that crosstalk between SA and H_2S exist in the heat tolerance of maize seedlings. Further research showed that SA and H_2S interaction induced an increase in the activity of antioxidant system and the accumulation of osmolyte in maize seedlings, implied that antioxidant system and osmolyte play a synergistic role in SA and H_2S crosstalk-induced heat tolerance of maize seedlings (**Fig. 2**). However, plant heat tolerance is involved in crosstalk among Ca^{2+}, H_2O_2, NO, H_2S and plant hormones, which needs to be further investigated in the future.

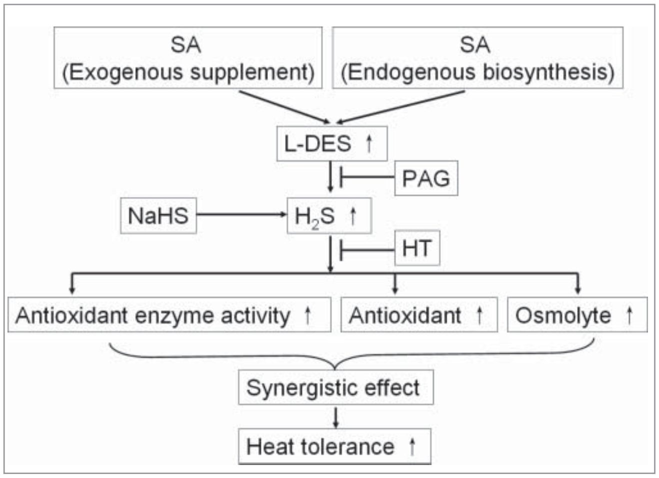

Figure 2. A possible model of crosstalk between SA and H_2S in the acquisition of heat tolerance in plants. Arrows (→) indicate positive effects, arrows (↑) represent increase in index, blunt line (├) indicates negative effect. H_2S, hydrogen sulfide; HT, hydroxylamine; L-DES, L-cysteine desulfhydrase; PAG, DL-propargylglycine; SA, salicylic acid (adapted from Li et al.[1]).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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