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News & Views

# Diversity of hydrogen sulfide concentration in plant: a little spark to start a prairie fire

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The ubiquitous and versatile signal-transduction role of hydrogen sulfide ($H_2S$) has been revealed in mammals and plants in recent years [1–5]. It is very important to grasp the physiological concentrations of $H_2S$ *in vivo* because of its toxic characteristics at high concentrations. However, the reported data fail to reach an agreement owing to the use of different methods and technique limitations. In fact, this is a common problem for gasotransmitters. What is the exact boundary between the physiological and toxicological concentration? What is the accurate endogenous content? These problems have been plaguing us even though there are a great number of research papers that explore $H_2S$ signals. Different $H_2S$ concentrations were reported due to different methods and materials used by different research groups, as follows: ~8 nmol $min^{-1}$ g fresh weight $(FW)^{-1}$ that was light dependent [6] and 0.02 to 0.2 ng $g^{-1}$ dry weight $s^{-1}$ after fumigation with $SO_2$ in cucumber (*Cucumis sativus* L.) [7]; ~80 nmol/g FW [8] and 1–5 μmol/L [9] in *Vicia faba* L.; and 1–5 μmol/L in *Arabidopsis thaliana* [10]. Additionally, 100 μmol/L NaHS was applied in the range of the physiological concentration and became toxic at concentrations ≥500 μmol/L [9,11].

To systematically determine the physiological concentration of $H_2S$ in plants, we selected 17 typical species as experimental materials in this study (Fig. 1a and b). Simultaneously, two widely adopted methods, methylene blue and electrode, were compared. The former is the most classic and commonly used [11–13], and the latter (TBR4100) is real-time and more sensitive [14,15].

Determining the $H_2S$ contents of different species is important. It is expected that $H_2S$ exists in all plant materials, but there is no significant difference among the species as assessed by the methylene blue method. The whole $H_2S$ content ranges from 0.010 to 0.199 μmol $g^{-1}$ F W (Fig. 1a). Using the electrode method, the $H_2S$ content showed a wider range, from 0.177 to 0.708 μmol $g^{-1}$ FW, in which *Platycladus sp*. had the highest level and tobacco had the lowest level (Fig. 1b). However, there was still no obvious pattern change between lower plants and higher plants, among materials from different growth environments, species or developmental stages. This suggested that the $H_2S$ content in plants *in vivo* was maintained at a stable level, probably in the range from 0.010 to 0.708 μmol $g^{-1}$ FW, which corroborates the results of previous reports [6–11].

Different stages from 2-week (w) to 10-w rosette leaves of *Arabidopsis* were used to determine the development-specific $H_2S$ content. Using the methylene blue method, the $H_2S$ content in rosette leaves was concentrated at 0.045–0.117 μmol $g^{-1}$ FW. It was the highest in 2-w-old seedlings, decreased gradually and then remained stable in 4–10-w-old plants. There was a significant difference in that the $H_2S$ content of the 2-w-old plants was 2.6 times that of the 6-w-old plants (Fig. 1c). The electrode method's data showed the same trends, with the $H_2S$ content decreasing gradually with development, and the content was concentrated in the range of 0.67–1.978 μmol $g^{-1}$ FW. The $H_2S$ content of 2-w-old plants was 3.3 times that of 6-w-old plants (Fig. 1d). In our previous study, the expression levels of $H_2S$-producing genes increased during plant development [11,16]. This indicated that the transcription of $H_2S$-producing genes and the $H_2S$ emissions in vivo are not synchronized.

Different organs of 8-w-old *Arabidopsis* were used to determine tissue-specific $H_2S$ contents. With a range of 0.0089–0.035 μmol $g^{-1}$ FW, the highest content in flowers was 3.94 times the lowest in rosette leaves as assessed by the methylene blue method (Fig. 1e). A similar trend was found in the results of the electrode method (Fig. 1f). The highest content of $H_2S$ in flower, 0.515 μmol $g^{-1}$ FW, was 11 times that of the lowest in cauline leaves, 0.046 μmol $g^{-1}$ FW. Thus, the $H_2S$ content showed tissue-specificity, being higher in the flowers, seeds and roots than in other parts. This trend did not match the expression pattern of $H_2S$-producing genes [11]. The $H_2S$ content in reproductive organs was higher than in vegetative organs, which implied that $H_2S$ might play an important role during development. This also explained why the $H_2S$ content in rosette leaves gradually decreased with development (Fig. 1c and d).

The data of chemical method are indirect results obtained through a series of step pattern "reaction–absorption–reaction–color–detection" according to its measurement principle. Meanwhile, the electrode method requires only one electron exchange step which generates a current showing the real-time change of $H_2S$ concentration. It seems that electrode method is more sensitive and the data is more accurate. Anyway, the emission pattern of $H_2S$ was similar whether using the chemical or electrode

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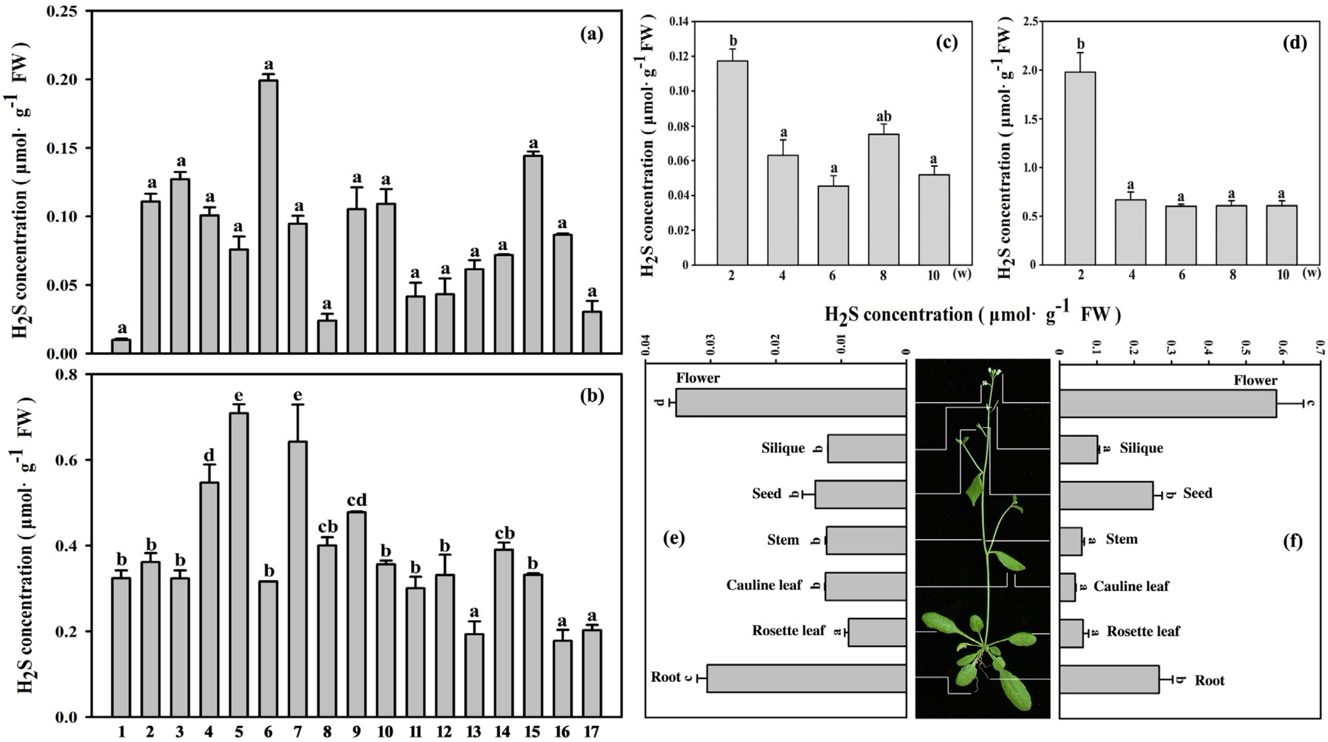


**Fig. 1.** (Color online) $H_2S$ concentrations of 17 species as assessed by the methylene blue (a) and electrode (b) methods; $H_2S$ concentrations in different developmental stages (c and d) and different organs (e and f) of *Arabidopsis* as assessed by the methylene blue (c and e) and electrode (d and f) methods. 1: chlorella (*Cladophora aegagrophila*); 2: funaria (*Funaria hygrometrica*); 3: moss (*Fantinalis antipyretica*); 4: juniper (*Juniperus formosana*); 5: platycladus (*platycladus orientalis*); 6: scutellaria (*Scutellaria baicalensis*); 7: tomato (*Lycopersicon esculentum*); 8: green bean (*Phaseolus*); 9: wheat (*Triticum aestivum*); 10: cabbage (*Brassica campestris*); 11: pumpkin (*Cucurbita moschata*); 12: cucumber (*Cucumis sativus*); 13: clove (*Syringa oblata* ); 14: willow (*Salix babylonica*); 15: millet (*Setaria italica*); 16: tobacco (*Nicotiana tabacum*); 17: Arabidopsis (*Arabidopsis thaliana*). Leaves (4, 5, 13, 14) or the whole seedlings (1–3; 6–12; 15–17) were collected to detect. Rosette leaves were collected from the plant of 2-w-, 4-w-, 6-w-, 8-w- and 10-w-old *Arabidopsis* (c and d); The root, stem, rosette leaf, cauline leaf, flower, seed, silique samples were isolated from 8-w-old *Arabidopsis* (e and f). Each experiment was performed with three independent repetitions. The results were expressed as the mean ± SE. Data was analyzed using SPSS (version 19, IBM SPSS, Chicago, IL, USA), and Lowercase letters indicate statistical differences as determined by an ANOVA ($P < 0.05$) and Tukey's multiple range *test*.

method. $H_2S$ was widely presented in all plants and showed a spatial–temporal pattern. The physiological concentration range of $H_2S$ remained 0.0089–1.978 μmol $g^{-1}$ FW independent of species, organs and developmental stages. As a result, the $H_2S$ content *in vivo* is on a microscale, which explains the difficulty in developing fluorescent probes. Here, a sharp contrast was found between trace $H_2S$ concentrations and its versatile physiological functions.

## Conflict of interest

The authors declare that they have no conflict of interest.

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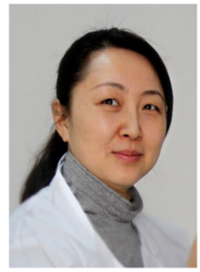

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