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Hydrogen Sulfide Inhibits Flowering but Hastens New Leaf Growth in Bok Choy (Chinese Cabbage)

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Summary

Hydrogen sulfide (H_2S) is a colorless, toxic, and flammable gas with the smell of rotten eggs. High concentrations of H_2S are toxic, but a very small amount of H_2S can help plants and our bodies in many ways. The unexpected flowering (bolting) of leafy greens inhibits leaf growth and reduces the leaf quality, and the effect of H_2S on plant bolting is still unknown. Here we investigated the effects of H_2S on flowering and leaf growth by using the hearts of bok choy (*Brassica rapa* subsp. *Chinensis*), a type of Chinese cabbage. Incubation of bok choy hearts for 16 days in a solution of sodium hydrosulfide (NaHS), a H_2S donor, significantly repressed bok choy flowering and shortened flower stems. In contrast, the flowering of bok choy in the tap water and sodium chloride (NaCl) groups was normal, with longer flower stems and many flowers. NaHS had no effect on the growth of leaf stems or on leaf chlorophyll contents. In addition, NaHS significantly reduced pollen viability but induced the growth of new leaves from the bottom part of bok choy hearts, in comparison with the two control groups, tap water and NaCl. These observations indicate that H_2S inhibits flowering but hastens new leaf growth in bok choy. Not only can this discovery help grow green vegetables, but it can also potentially help control weeds.

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Introduction

Hydrogen sulfide (H_2S) is a gas with a distinctive rotten egg smell that is widely found in the environment from volcanoes, sulfur springs, undersea vents, swamps, municipal sewers, and in crude petroleum and natural gas (1). H_2S is toxic at high concentrations, but at very small amounts, it can help plants and our bodies in many ways, including the improvement of plant germination and drought resistance, protection against cardiovascular diseases, and stimulation of memory (2-9). Plants can assimilate sulfate from the soil and then reduce sulfate to H_2S (2,3). Plants also can enzymatically generate H_2S using the organic compound cysteine as a substrate (3). The ability to reduce sulfate to H_2S and express H_2S-generating enzymes is plant-type specific, which reflects the different response of plants to H_2S (7,8). Garlic is reported to be extremely healthy for the body, because H_2S is released when people eat it (9). H_2S has been proposed to be an important gaseous signal molecule in our bodies (2,4,6).

Bok choy (*Brassica rapa* subsp.*Chinensis*), one kind of Chinese cabbage, is one of the most popular leafy green vegetables around the world (10,11). Premature flowering or development of a flowering stem, termed bolting, can prevent normal leaf growth and reduce the leaf quality (12). Many factors affect vegetable bolting, including temperature, water, hormones, and transplanting (12-14). Nitric oxide, another important gaseous signal molecule, was reported to repress plant floral transition (2,15). However, the effect of H_2S on plant flowering is still unknown. We hypothesize that H_2S at a physiological concentration can inhibit plant flowering but hasten new leaf growth in bok choy (4,7). Sodium hydrosulfide (NaHS) is a commonly used H_2S donor in biological systems. When NaHS is added into tap water, NaHS dissociates into a sodium cation (Na^+) and bisulfide anion (HS^-), thus freeing HS^- to interact with free protons (H^+) to produce H_2S (4,7,10). In this study, we used NaHS as a H_2S donor.

Our experiments demonstrate that incubation of bok choy heart with NaHS in tap water, but not sodium chloride (NaCl) in tap water or tap water alone, inhibits bok choy flowering and reduces pollen viability. We also observed that NaHS induces the growth of new leaves.

Results

To observe the effects of H_2S on bok choy heart flowering, the bok choy hearts were partially immersed in solutions containing NaHS at different concentrations (0.5 mg/L, 5.0 mg/L, and 50 mg/L). The solutions of NaCl (5.0 mg/L) and tap water acted as controls. In each group, three bok choy hearts were tested. The physiological concentration of H_2S inside the plant or mammalian blood has been reported to be in the range of 1–100 μM, which is equal to 0.056–5.6 mg/L (4,10). When NaHS is dissolved in water, only 1/3 of H_2S is released (4), so the concentrations of NaHS used in this study are

physiologically relevant, and H_2S at these concentrations has no toxic effect on plants. After 16 days, flowering of bok choy in the tap water and NaCl groups exhibited similar stem length and flower number, while NaHS at all three concentrations significantly repressed bok choy flowering and led to shorter flower stems (**Figure 1**) The height of flower stems in the 5 mg/L NaHS group was only 24% of that in the tap water group ($p < 0.05$). The growth of leaf stems showed no difference between all five groups.

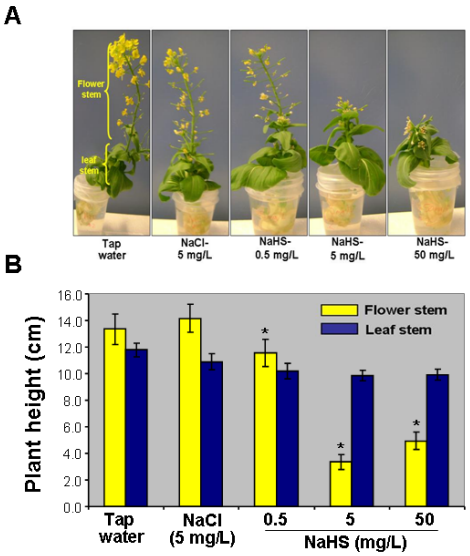

Figure 1: Average height of plants from each group at day 16. A) Representative image from each of the 5 groups, and **B)** the statistical analysis of the height of flower stem (yellow) and leaf stem (blue) among different groups. *, $p<0.05$ versus either the tap water or NaCl groups.

Pollen viability is usually positively correlated with flowering, while leaf growth is often negatively correlated. We found that NaHS significantly inhibited pollen viability, as measured using standard methods (16). NaHS at 0.5, 5.0, and 50 mg/L reduced pollen viability by 8%, 20%, and 39%, respectively, when compared with the tap water group ($p < 0.05$). NaCl did not affect pollen viability (**Figure 2**). In contrast, H_2S induced the growth of new leaves from the bottom part of bok choy hearts. The average number of new leaves from each bok choy heart in the tap water and NaCl groups was only 1.0, but it was 4.5, 5.5, and 5.0 in the NaHS 0.5, 5.0 and 50 mg/L groups, respectively ($p < 0.05$, **Figure 3**). These results suggest that H_2S redirects the flowering energy to growing new leaves.

Chlorophyll, a green pigment found in the chloroplasts of plants, is critical in photosynthesis and allows plants to absorb energy from light. H_2S has been shown to improve chlorophyll contents in the leaves of oilseed rape under lead stress (10). We found that the chlorophyll contents increased with time; however, there was no difference between the chlorophyll contents of all five groups (**Figure 4**).

Discussion

Many leafy green vegetables, including bok choy, tend to bolt in response to environmental changes (13). Bok choy, also called pak choi, bok choi, or pak choy, is a type of Chinese cabbage and now is harvested in America and other countries as well (11). Bok choy bolting involves growing a long stalk bearing flowers, which turns the leafy green vegetables from their vegetative stage to their reproductive stage and severely reduces their value as agricultural products (13,14). Here we observed a beneficial effect of NaHS: they had more new leaves but shorter flower stems and fewer flowers than the tap water and NaCl groups. A high concentration of NaHS is not needed to inhibit flowering, because the NaHS 5 mg/L group showed a more significant effect on inhibiting flower stem height than the NaHS 50 mg/L group. The anti-bolting effect of NaHS should not be attributed to Na^+, because NaCl incubation of the bok choy has no effect on flowering and new leaf growth. Similarly, NaCl dissociates to Na^+ and Cl^- when dissolved in solution.

In addition, lower pollen viability in bok choy hearts from the NaHS group was noted. This means that H_2S can be used to inhibit seed formation. This discovery might not only help grow green vegetables, but it can also potentially be used to control weeds. If dandelions cannot flower, they will not be able to produce any pollen to germinate and produce new seeds.

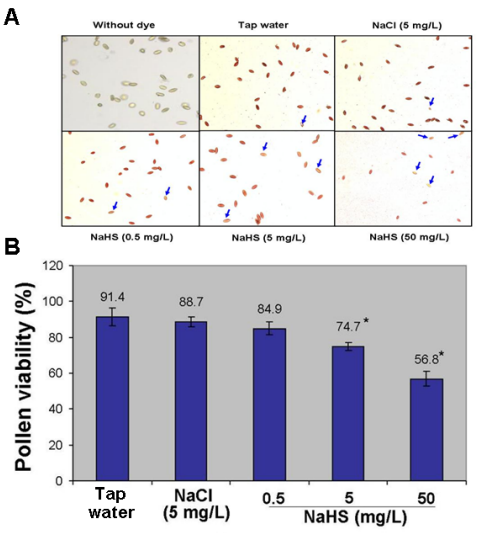

Figure 2: Pollen viability of plants at day 16. A) Pollen grains from each of the 5 groups, after being dyed. Arrows point to unviable pollens, or pollen that does not take in the dye due to lack of proteins and lipids. **B)** Statistical analysis of pollen viability among different groups. *, $p<0.05$ versus tap water or NaCl groups.

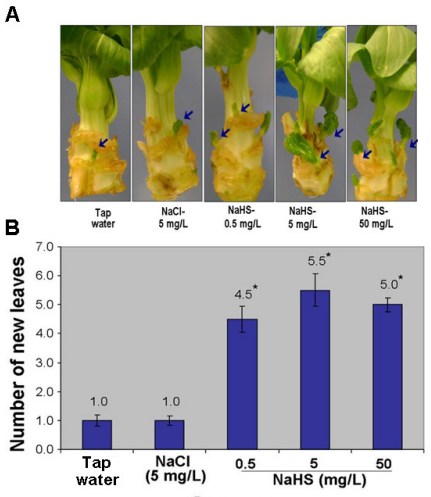

Figure 3: Number of new leaves of each bok choy heart at day 16. A) Representative image from each of the 5 groups, with the arrows pointing to some, or all of the plant's new leaves. **B)** Statistical analysis of new leaf numbers among different groups. * $p<0.05$ versus tap water or NaCl groups.

The chlorophyll contents remained fairly constant between all five groups; this suggests that H_2S does not damage the health of the leaves. We did observe that the chlorophyll content increased with time, suggesting higher chlorophyll content is required for photosynthesis to support leaf growth.

Although we included three bok choy hearts of equal size in each group, the data collected from the experiments may still have some minor errors. H_2S, as a gas, will evaporate when formed in solution. We used parafilm to cover the cups, but this approach may not completely prevent the evaporation of H_2S from the cup, so the exact concentration of H_2S in the solutions was not known. In addition, the lighting quality may have had some limitations. Although we tried to place the tray with cups and bok choy hearts at the same place after changing the solution every two days, the cups located in the center of the tray may have received more light.

Further research should be conducted to determine the underlying mechanism by which H_2S inhibits flowering and stimulates the growth of new leaves. It is well known that various environmental factors, including day length, cold temperature, and other stress factors, affect flowering through different genetic pathways (13,14). It is worthwhile to explore whether H_2S regulates the signal pathways involved in the process of bolting and flowering. H_2S has been shown to directly modify proteins and alter protein function and activity, so it is highly possible that H_2S may inhibit the activity of flowering-related proteins (2). The bolting is believed to be induced by an increase in the levels of gibberellin, which is a plant hormone that regulates flowering. The effect of H_2S on the synthesis and transformation of gibberellin and/or other hormones may therefore be worth studying in relation to premature blossoming and bolting in bok choy (13). More research needs to be done to test the effect of NaHS on other plant types, such as dandelions. Inhibition of dandelion flowering may prevent seed formation enough to reduce herbicide application. Finally, field research should be conducted to determine the anti-bolting activity of H_2S.

In conclusion, the present study demonstrated that that H_2S inhibits flowering but hastens new leaf growth in bok choy.

Methods

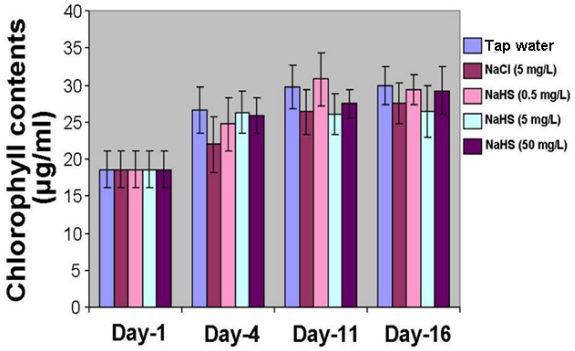

Figure 4: Concentration of chlorophyll in each plant group. Measured every 4-7 days, chlorophyll content remained relatively the same between groups every day.

Bok choy was purchased from the Real Canadian Superstore (Thunder Bay, ON, Canada). All the chemicals used in this experiment, including NaCl and NaHS, were purchased from Sigma (St. Louis, MO, USA). Tap water was used to dilute other solutions. NaHS, which can release H_2S in minutes, was used as a H_2S donor (4,10). Planting cups (40 mL for early stage and 100 mL for late stage) were used to hold the bok choy hearts.

On the first day, a tray (35 cm × 60 cm) was prepared with five columns, one for each group, and three cups were labeled for each group. These five groups were: Tap water, NaCl (5 mg/L), NaHS (0.5 mg/L), NaHS (5 mg/L), and NaHS (50 mg/L). Leaves from the bok choy were peeled off until only 3-4 leaves and the heart containing the flower stalk remained. A total of 15 bok choy samples of equal size were chosen for the experiments and placed in each cup. The tray was placed at room temperature under a photoperiod of 14 hours of light and 10 hours of darkness provided by fluorescent and incandescent lamps.

Every two days, the solution was replaced with a fresh solution, and the cups were washed before the plants were placed back into them. Parafilm would then be wrapped around the opening of the cup to prevent

evaporation. The height of leaf stem and flower stem were measured using a ruler. General observations of each sample, including health, leaf color, flowering status, seed formation, were also recorded. Every three to seven days, the chlorophyll contents from the leaves were measured. The pollen viability of each group of plants was also measured on day 16.

To measure chlorophyll contents, a small leaf clipping (approximately 20 mg) was collected and put into a test tube. 1 mL of 80% acetone was then added for every 20 mg of leaf clipping (17). Using a small plastic q-tip-like rod, the mixture was mashed up to extract chloroplast (17). After 5 minutes, 1 mL of this mixture was added into a spectrophotometer cuvette. The tubes were labeled and placed inside the spectrophotometer (GENESYS™ 10S UV-Vis Spectrophotometers, Thermo Scientific, ON) that was first set up with a light wavelength of 665 nm. After these numbers were recorded, the machine was then set up with a wavelength of 645 nm, and again the numbers were recorded. An equation was then used to determine the chlorophyll concentration of each plant: chlorophyll = 12.25 × A665 - 2.79 × A645 = µg/mL (17).

The pollen viability of each group of plants was measured by gently removing pollen from a fresh flower using tweezers, and putting the pollen onto a microscope slide (16). 20 µl of 0.3% Oil red O dye and 15 µl of glycerol (to thicken the dye) was added and left for 5 minutes to dye the pollen. A cover slip was then put on the slide and the slide was placed under a light microscope (Olympus Microscopes IX70, Japan) at 100X magnification. Pollen grains that take in the dye are healthy and viable, because they have many dye absorbing proteins and lipid. Pollen viability was expressed as the percentage pollen grains that took in the dye.

Microsoft Excel was used to calculate the results and run the statistical analysis via student t-test (18). The data were expressed as the standard error from three repeats per group, and the significance level was set at $p < 0.05$.

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