Plant Physiology and Biochemistry 104 (2016) 174–179

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Hydrogen sulfide mediates nicotine biosynthesis in tobacco (*Nicotiana tabacum*) under high temperature conditions

Xiaodong Chen [a], Qian Chen [b], Xiaoming Zhang [b], Ruijing Li [c], Yujie Jia [c], Abd_Allah Ef [d], Aiqun Jia [a], Liwei Hu [e, **], Xiangyang Hu [b, c, *]

[a] School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing 210094, China
[b] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[c] Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, Shanghai, China
[d] Plant Production Department, College of Food and Agricultural Sciences, King Saud University, PO. Box. 2460 Riyadh 11451, Saudi Arabia
[e] Laboratory of Tobacco Agriculture, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form
14 February 2016
Accepted 23 February 2016
Available online 27 February 2016

Keywords:
H_2S
High temperature
Nicotine
Jasmonic acid

ABSTRACT

Hydrogen sulfide (H_2S) acts as a signal to induce many physiological processes in plants, but its role in controlling the biosynthesis of secondary metabolites is not well established. In this study, we found that high temperature (HT) treatment induced nicotine biosynthesis in tobacco (*Nicotiana tabacum*) and promoted the rapid accumulation of H_2S. Furthermore, HT triggered the biosynthesis of jasmonic acid (JA), a plant hormone that promotes nicotine biosynthesis. Suppression of the H_2S signal using chemical inhibitors or via RNAi suppression of L-cysteine desulphydrase (L-CD) in transgenic plants, compromised JA production and nicotine biosynthesis under HT treatments, and these inhibitory effects could be reversed by applying exogenous H_2S. Based on these data, we propose that H_2S is an important trigger of nicotine biosynthesis in tobacco under HT conditions, and that H_2S acts upstream of JA signaling by modulating the transcription of genes associated with JA biosynthesis.

1. Introduction

Hydrogen sulfide (H_2S) is a small, reactive water-soluble gas. Like other gaseous signaling molecules, such as nitric oxide (NO) and carbon monoxide (CO), H_2S functions in multiple physiological processes in animals (Boehning and Snyder, 2003), including the anti-inflammatory response, vasorelaxation, smooth muscle relaxation, neuronal excitability, and blood pressure regulation (Mok et al., 2004). In mammalian cells, H_2S is synthesized mainly via two pyridoxal-5'-phosphate-dependent enzymes; cystathionine β-synthase (CBS, EC4.2.1.22), which hydrolyses L-cysteine to L-serine, and cystathionine γ-lyase (CSE, EC 4.4.1.1), which hydrolyses L-cysteine to H_2S, pyruvate, and ammonia. Both enzymes participate in cysteine metabolism, with CSE acting as an L-Cys desulfhydrase (Yang et al., 2008). In plants, pyridoxal 5'-phosphate (PLP)-dependent D/L-cysteine desulfhydrases (D/L-CDs) are primarily responsible for H_2S generation, while another PLP-dependent enzyme, β-cyanoalanine synthase (CAS), contributes to H_2S formation by converting cysteine and cyanide to H_2S and β-cyanoalanine (Cheng et al., 2013). Recent evidence suggests that H_2S is involved in various physiological processes in plants, such as stomatal closure and drought tolerance, and is associated with miRNA biosynthesis (Wang and Song, 2008; Wang et al., 2008; Garcia-Mata and Lamattina, 2010; Shen et al., 2013). In *Arabidopsis thaliana*, the cysteine synthesis complex (CSC) is known to consume H_2S during the synthesis of L-Cys from O-acetyl serine (OAS), in a reaction that is catalyzed by the enzyme O-acetylserine(thiol)lyase (OAS-TL) (Wirtz et al., 2004; Alvarez et al., 2010). However, the mechanisms by which H_2S functions as a signaling molecule in plant responses to environmental stresses are poorly understood.

Tobacco (*Nicotiana tabacum*) synthesizes an array of alkaloids that play essential roles in defense responses against herbivore and

Abbreviations: NPA, 1-N-naphthylphthalamic acid; CO, carbon monoxide; CSC, cysteine synthesis complex; PAG, DL-propargylglycine; HT, high temperature; H_2S, Hydrogen sulfide; JA, jasmonic acid; NO, nitric oxide; L-CDs, L-cysteine desulfhydrases; PMT, putrescine N-methyltransferase; OAS, O-acetyl serine.

* Corresponding author.Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China.
** Corresponding author.
E-mail addresses: liwei.hu@gmail.com (L. Hu), huxiangyang@kib.ac.cn (X. Hu).

http://dx.doi.org/10.1016/j.plaphy.2016.02.033
0981-9428/

insect attack (Kessler and Baldwin, 2002; Steppuhn et al., 2004). Nicotine, which constitutes approximately 0.6–3% of the tobacco leaf dry weight, is the main alkaloid produced by cultivated tobacco (*N. tabacum* L.). Nicotine is synthesized in the root from ornithine and arginine via putrescine, which is in turn metabolized to higher polyamines, such as spermidine and spermine. In land plants, putrescine can conjugate with cinnamic acid derivatives or fatty acids and, in plants that produce nicotine or tropane alkaloids, it is converted into *N*-methylputrescine. Putrescine N-methyltransferase (PMT; EC 2.1.1.53) participates in the first committed step of alkaloid biosynthesis (Chattopadhyay and Ghosh, 1998; Chou and Kutchan, 1998). The product *N*-methylputrescine is then deaminated oxidatively to 4-methylaminobutanal, which spontaneously cyclizes to give the *N*-methylpyrrolinium cation. This oxidative deamination reaction is catalyzed by *N*-methylputrescine oxidase. The *N*-methyl-Δ^1-pyrrolinium cation condenses with an unidentified nicotinic acid-derived metabolite to give nicotine in tobacco (Naconsie et al., 2014). Quinolinic acid phosphoribosyltransferase (QAPRT; EC2.4.2.19) serves as the entry-point enzyme in the pyridine nucleotide cycle, which produces nicotinic acid. Following its biosynthesis in tobacco roots, nicotine is translocated to the leaves via the xylem, where it is transported into the leaf vacuoles through the action of a tonoplast-localized transporter. The accumulation of nicotine in tobacco is affected by environmental factors, cultural practices, and plant hormone levels (Shoji et al., 2008). For example, the application of nitrogen fertilizer or jasmonic acid results in a marked increase in nicotine biosynthesis (Paschold et al., 2007; Shoji et al., 2008).

In this study, we found that HT stress induced nicotine biosynthesis, JA accumulation, and the generation of H_2S. We further investigated how H_2S interacts with the JA signal to promote nicotine biosynthesis during HT stress. This work reveals a novel function for H_2S in tobacco nicotine metabolism.

2. Materials and methods

2.1. Materials and HT treatments

Sterilized tobacco (*Nicotiana. tabacum cv.* Wisconsin 38) seeds were germinated and grown to seedlings under continuous illumination on half-strength Gamborg B5 medium solidified with 2% (W/V) gellan gum and supplemented with 0.3% sucrose at 24 °C. Two-week-old plants were transferred to perlite saturated with half-strength Gamborg B5 medium, and grown for one week in the greenhouse at 24 °C before HT treatment. For the HT treatment, 3-week-old seedlings were placed in a plant growth chamber at 35 °C. After treatment for indicated time, either the roots were collected immediately for molecular analysis and alkaloid measurements, or the whole seedlings were collected for H_2S and JA content analysis or gene expression analysis. For the chemical inhibitor treatments, these chemicals at various concentrations were dissolved in 0.01% Tween-20 and sprayed on the leaves of 3-week-old tobacco plants. As a control, the leaves of plants of the same age were sprayed with 0.01% Tween-20.

2.2. H_2S content analysis

After the different treatments, seedlings were collected and immediately ground to a fine powder in liquid nitrogen, before being suspended in bi-distilled water. After vortexing the suspension for 1 min, H_2S released over a 20-min period was measured using a Micro Sulfide Ion Electrode (LIS-146AGSCM; Lazar Research Lab. Inc., Los Angeles, CA, USA) at 25 °C (Garcia-Mata and Lamattina, 2010). H_2S concentrations were determined from a calibration curve made with the H_2S donor NaHS. Each measurement was repeated at least three times.

2.3. Quantification of JA

Approximately 200 mg of seedling subjected to various treatments was ground in liquid nitrogen, and extracted with 1.5 mL of methanol containing 60 ng of 9,10-D_2-9,10-dihydrojasmonic acid as internal standards. The JA content was determined using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) as previously described (Vadassery et al., 2012).

2.4. Alkaloid analysis

After different treatment, 0.5 g of tobacco roots was collected and frozen in liquid nitrogen and lyophilized, before homogenization and resuspension in 4 ml of 0.1 M H_2SO_4. The nicotine content was measured as reported (Shoji et al., 2008).

2.5. Measurement of L-Cys desulfhydrase (L-CD) enzyme activity

Plant tissue (1 g) was ground with a mortar and pestle in liquid nitrogen. Soluble proteins were extracted with 1.5 ml of cold extraction buffer, comprised of 20 mM Tris–HCl, pH 8.0, 0.1% (w/v) dithiothreitol (DTT), and 0.2% (w/v) sodium ascorbate, as previously described (Cheng et al., 2013). The homogenate was centrifuged (13,000 *g*; 4 °C; 15 min) and the resulting supernatant was used for spectrophotometric determination of L-CD enzyme activity by measuring the H_2S released from L-cysteine, as previously described (Cheng et al., 2013).

2.6. Protein isolation and immunoblot analysis

Proteins were extracted from whole seedlings with extraction buffer containing 50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1 mM PMSF, 1× Complete Protease Inhibitor Cocktail (Roche), 5% glycerol, 1 mM EDTA, and 1 mM DTT. The protein concentration was determined using a commercial Bradford assay kit, following the manufacturer's instructions (Bio-Rad). The samples were mixed with an equal volume of 10% sodium dodecyl sulphate (SDS) sample buffer and boiled for 3 min before separation on a 10% SDS-PAGE gel. The fractionated proteins were then transferred to a PVDF membrane and immunoblot assays were performed as previously described (Hu et al., 2014), using anti-PMT1 and anti-Actin (Abmart, Shanghai, China) antibodies at dilutions of 1:3000 and 1:2000, respectively. The anti-PMT1 antibody was prepared by immunizing rabbits with peptide synthesized from the N-termination of tobacco PMT1 (MEVISTNTNGSTI).

2.7. RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

Total RNA was extracted from 3-week-old tobacco seedlings subjected to various treatments using TRIzol reagent (Invitrogen), and qRT-PCR was performed as previously described (Hu et al., 2014). The gene-specific primers used to detect the transcripts are listed in Table S1.

2.8. Cloning of the N. tabacum LCD gene and preparation of transgenic irLCD plants

Transgenic tobacco lines expressing inverted-repeats of the *LCD* gene, (*ir*)*LCD*, were generated to perform RNA interference (RNAi) suppression of the *LCD* gene. This was achieved by cloning a 200-bp fragment of the *NtLCD* gene and using this as template to amplify the sense and anti-sense fragments by RT-PCR (Fig. S1). The primer sequences are listed in Supplemental Table 1. The sense and anti-

sense fragments were then independently inserted into the *Nco*I/*Sma*I and *Bam*HI/*Xba*I sites of the pFGC5491 vector, and the resulting constructs were transformed into *Agrobacterium tumefaciens* strain LBA4404 by electroporation. *N. tabacum* plants were transformed with *A. tumefaciens* harboring the RNAi construct as described previously (Batuman et al., 2006). Homozygous transgenic lines were selected by screening the T2 generation seeds for Basta (10 mg L^{-1}) resistance. Real-time qPCR was used to select single insert transgenic lines with the highest degree of silencing, and lines *irJAZh-#3*, *irJAZh-#5*, and *irJAZh-#6* were used for subsequent experiments.

3. Results

3.1. HT treatment promotes nicotine biosynthesis

To determine whether nicotine biosynthesis in tobacco is affected by environmental factors, we previously gauged the effect of various abiotic stresses, such as salt, cold, drought, and HT, on nicotine content and found that HT markedly induced nicotine accumulation. As shown in Fig. 1A&B, we noted that various periods of HT treatment substantially increased the nicotine content in the roots of three-week-old tobacco seedlings (Fig. 1A). Immunoblot analysis revealed that the abundance of the key enzyme involved in nicotine biosynthesis in tobacco, PMT, also gradually increased after HT treatment, peaking after 2 days of HT treatment and remaining constant over the next 3 days (Fig. 1B).

3.2. HT treatment induces H_2S production and JA biosynthesis

Since JA is known to be an important signal molecule involved in nicotine biosynthesis in tobacco (Shoji et al., 2008), we then investigated the effect of HT treatment on JA levels. JA accumulation gradually increased in tobacco seedlings with increasing periods of HT treatment (Fig. 1C). We also determined that the transcript levels of *NtLOX*, *NTAOC*, *NtAOS*, and *NtOPR*, which are associated with JA biosynthesis in plant, increased in response to the HT treatment (Fig. 1D).

As H_2S was previously reported to be an essential signal involved in multiple physiological processes, we thus measured changes in H_2S content in tobacco after exposure to HT treatment. Interestingly, we found that treatment at 35 °C induced the production of H_2S in 3-week-old seedlings, and that H_2S production remained elevated after 3 days of HT exposure (Fig. 2A). The HT treatment also increased the activity of L-CD, which catalyzes the production of H_2S (Fig. 2B). These data suggest that H_2S and nicotine biosynthesis are linked in tobacco plants subjected to HT stress.

3.3. Suppressing H_2S biosynthesis compromises HT-induced JA and nicotine biosynthesis

To elucidate the potential role of H_2S in HT-induced nicotine biosynthesis, we treated tobacco seedlings with DL-propargylglycine (PAG), a specific inhibitor of L-CD. PAG treatment not only suppressed the HT-mediated induction of H_2S biosynthesis (Fig. 3A), but also suppressed JA accumulation (Fig. 3A). In accordance with this observation, expression of the JA biosynthetic genes *NtLOX*, *NtAOS*, *NtAOC*, and *NtOPR* was also suppressed by PAG treatment (Fig. S2). This inhibitory effect could be partly ameliorated by the addition of the exogenous H_2S donor, NaHS (Fig. S2). Direct treatment with NaHS also induced JA biosynthesis in the tobacco seedlings (Fig. S3) in a dose-dependent manner, and this was accompanied by an increase in the transcript levels of the four JA biosynthetic genes described above (Fig. S4). However,

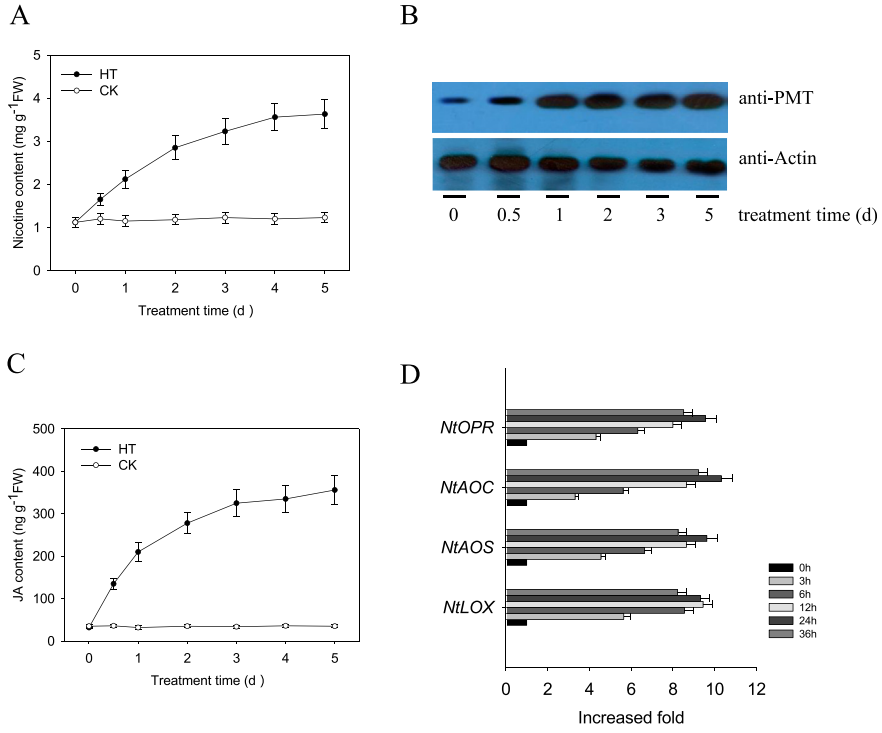

Fig. 1. High temperature (HT) treatment induces nicotine and jasmonic acid (JA) biosynthesis. Three-week-old tobacco seedlings were subjected to HT (35 °C) treatment and JA levels and the transcript levels of JA biosynthesis-related genes were measured in the seedling after the indicated periods of treatment. Nicotine content and PMT1 protein levels were measured in the root of seedlings at the indicated time points. Data represent the means of three replicate experiments (±SD). CK, control treatment (i.e., plants grown at 25 °C). (A&B) HT treatment resulted in an increase in nicotine biosynthesis (A) and PMT1 protein abundance (B). (C&D) HT treatment induced JA production (C), and increased the transcript levels of *NtLOX*, *NtAOS*, *NtAOC*, and *NtOPR* (D)

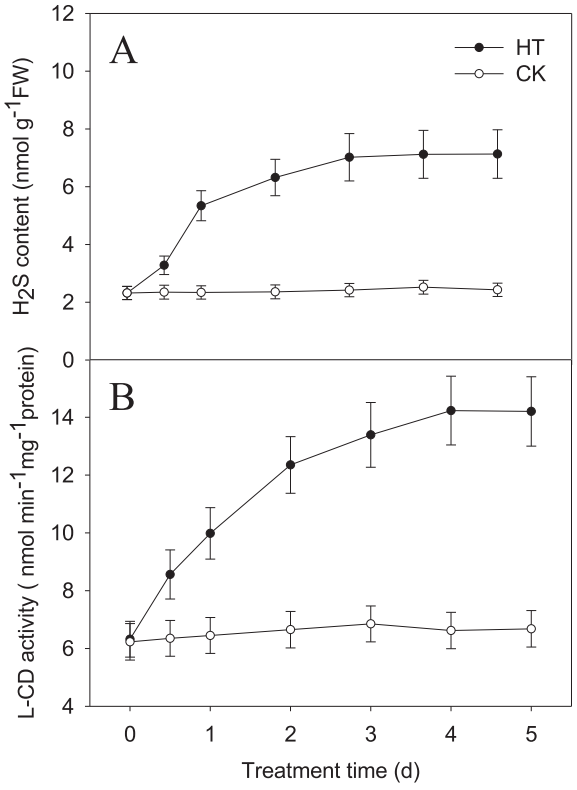

Fig. 2. High temperature (HT) treatment induces the biosynthesis of H_2S (A) and L-CD enzyme activity (B). Three-week-old tobacco seedlings were subjected to HT (35 °C) treatment, and the accumulation of H_2S (A) and L-cysteine desulphydrase (L-CD) (B) enzyme activity were measured after the indicated periods of treatment. CK: control treatment (25 °C). Data represent the means of three replicate experiments (±SD).

exogenous MeJA treatment did not have a significant effect on H_2S generation (Fig. S5). Taken together, these data suggest a role for H_2S signaling in HT-mediated JA biosynthesis, and indicate that H_2S operates upstream of JA signaling.

Treatment of tobacco seedlings with different concentrations of the H_2S donor, NaHS, also resulted in increased nicotine levels, as did treatments with MeJA or the H_2S donor GYY4137 (Fig. 3B). Consistent with the NaHS-induced nicotine accumulation, NaHS treatment also PMT1 to accumulate in a dose-dependent manner (Fig. 3C). The GYY4137 and MeJA treatments also induced PMT1 accumulation (Fig. 3C), while application of PAG suppressed both HT-induced nicotine biosynthesis (Fig. 3A) and PMT1 accumulation (Fig. 3D). These data suggest that H_2S functions as a signaling molecule that mediates HT-induced nicotine biosynthesis in tobacco.

3.4. Transgenic irLCD RNAi tobacco lines accumulate high levels of JA and nicotine under HT conditions

The above results indicate that HT treatment increased L-CD enzyme activity, which is known to be a key enzyme in the synthesis of H_2S in plants (Wirtz et al., 2004). To confirm that H_2S regulates nicotine biosynthesis under HT conditions, we used *RNAi* technology to generate transgenic tobacco plants expressing an inverted-repeat (ir) of the *L-CD* gene, named *irLCD* lines. We screened three individual lines for low levels of *L-CD* transcript (Fig. S6) and L-CD enzyme activity (Fig. S7) after HT treatment. We found that the increase in H_2S production in response to HT treatment was far lower in the *irLCD* lines than in the non-transgenic control plants (Fig. 4A), as was the increase in L-CD activity (Fig. S7), indicating that suppression of *L-CD* expression impaired H_2S production following HT treatment.

We similarly evaluated JA and nicotine levels in the transgenic lines following HT treatment and found that HT treatment resulted in less of an increase in the content of either compound in the *irLCD* lines than in the wild-type plants (Fig. 4B&C). However, this effect could be partially rescued by application of the H_2S donor, NaHS. These data further support a role for H_2S in HT-induced nicotine biosynthesis.

4. Discussion

In this study, we observed that HT treatment induced H_2S generation, L-CD activity, and nicotine biosynthesis (Figs. 1 and 2). This effect could be suppressed by treatment with the L-CD enzyme inhibitor, PAG (Fig. 3A), while addition of the exogenous H_2S donor, NaHS, increased nicotine biosynthesis and PMT1 accumulation (Fig. 3). These observations support the notion that H_2S acts as a signaling molecule that mediates nicotine biosynthesis under HT conditions. Indeed, H_2S was previously reported to enhance abiotic and biotic stress tolerance, including the tolerance of maize seedlings to heat stress (Li et al., 2013). Other small messengers, including reactive oxygen species (ROS) and nitric oxide (NO), are also associated with the plant's response to heat stress (Li et al., 2015). H_2S was shown to trigger stomatal closure in Arabidopsis, and NO was found to be necessary for this process (Scuffi et al., 2014). Furthermore, H_2S regulates the plant's response to abiotic or biotic stress by activating the ascorbate–glutathione cycle and reestablishing ROS homeostasis (Shi et al., 2015; Singh et al., 2015). Here we found that HT induced H_2S generation, which indicates possible crosstalk among H_2S, ROS, and NO during the plant's response to HT stress.

It has been reported that heat stress can induce JA biosynthesis in *A. thaliana*, and the *cpr5-1* mutant, which exhibits constitutive activation of the JA signaling pathway, displays increased heat stress tolerance (Clarke et al., 2009). Considerable evidence also shows that JA acts as a signal that induces nicotine biosynthesis in tobacco (Shoji et al., 2008). We found that HT treatment induced JA accumulation and increased the transcript levels of genes involved in the JA biosynthetic pathway, specifically *NtLOX*, *NtAOC*, *NtAOS*, and *NtOPR* (Fig. 1C&D). Additionally, suppressing H_2S biosynthesis using specific inhibitors (Fig. 3A) or RNAi in transgenic plants (Fig. 4) caused a reduction in the expression of these genes and reduced JA biosynthesis following HT treatment. We also determined that suppression of H_2S biosynthesis reduced JA production (Fig. 3A), while, conversely, MeJA treatment did not affect H_2S production after HT treatment (Fig. S5). Taken together, these data suggest that H_2S plays a role in HT-induced JA biosynthesis in tobacco, and is congruent with previous results suggesting that JA promotes nicotine biosynthesis (Shoji et al., 2008). In a previous study, H_2S treatment alleviated gibberellin acid-triggered programmed cell death in *Triticum aestivum* (wheat) aleurone cells (Xie et al., 2014). High levels of H_2S inhibit auxin transport and alter root system development in Arabidopsis and *Solanum lycopersicum* (tomato) (Fang et al., 2014; Jia et al., 2015). Similarly, our data indicate potential crosstalk between the JA and H_2S signaling pathways in HT-induced nicotine biosynthesis, where H_2S may act upstream of the JA signal.

In conclusion, we found that HT treatment induced nicotine biosynthesis, and that this was accompanied by the production of H_2S, which acts as a signal that triggers JA biosynthesis and nicotine accumulation. Suppressing L-CD enzyme activity by using a specific inhibitor or by RNAi in transgenic plants resulted in reduced levels of H_2S and JA production and nicotine biosynthesis following HT treatment. Based on these data, we propose a model in which H_2S

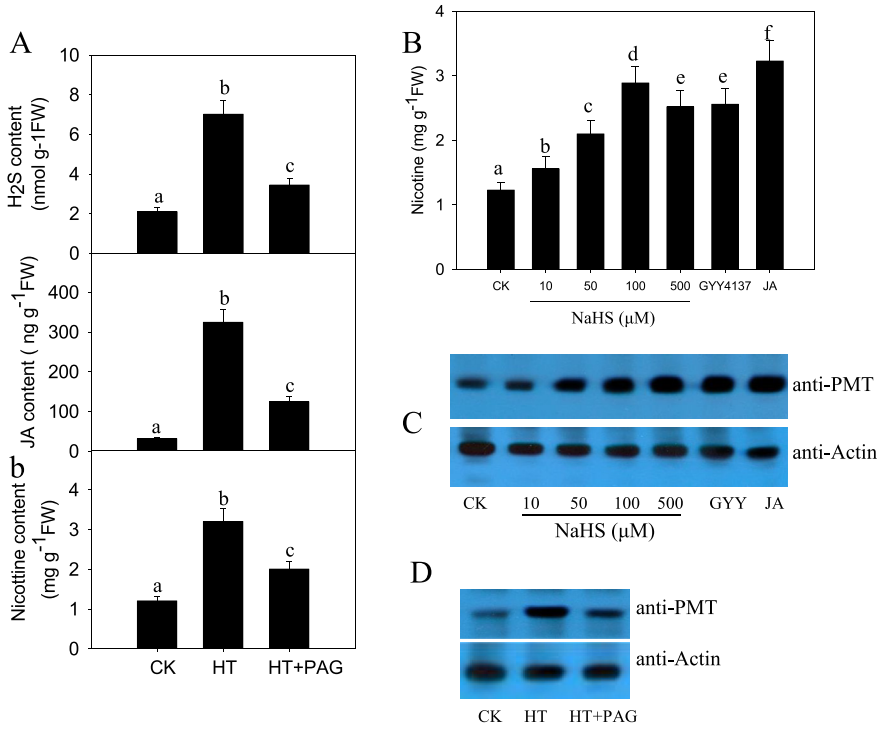

Fig. 3. The effect of an H_2S donor (NaHS) and L-CD inhibitor (PAG) on H_2S, jasmonic acid (JA), and nicotine biosynthesis in tobacco following high temperature (HT) treatment. Data represent the means of three replicate experiments (±SD). The means denoted by different letters indicate significant differences according to Tukey's test ($p < 0.05$). (A) The effect of HT, DL-propargylglycine (PAG), and 1-N-naphthylphthalamic acid (NPA) on the production of H_2S and JA, and on nicotine biosynthesis in tobacco plants subjected to HT treatment. Three-week-old seedlings underwent HT treatment (35 °C) for 3 days, and the H_2S, JA, and nicotine contents were measured. For the inhibitor treatments, the seedlings were treated with 1 mM PAG or 10 μM NPA prior to the HT treatment. (B) The effects of the H_2S donors NaHS, GYY4137, and methyl jasmonic acid (MeJA) on nicotine biosynthesis. Three-week-old seedlings were treated with the indicated dose of NaHS, 100 μM GYY4237, or 50 μM MeJA for 3 days, and the nicotine content was measured. (C&D) The effect of the H_2S donors NaHS, GYY4137, and MeJA on PMT1 abundance. Three-week-old seedlings were treated with NaHS at the indicated dose, 100 μM GYY4237, or 50 μM MeJA for 1 day, and PMT1 abundance was assessed by immunoblot analysis using an anti-PMT1 antibody. The experiments were repeated three times with similar results.

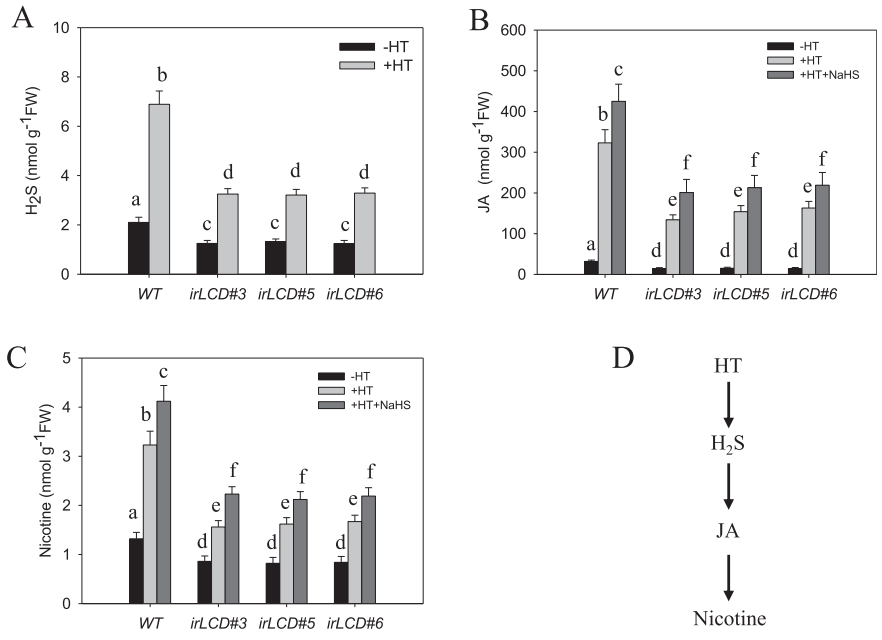

Fig. 4. High temperature (HT) treatment does not substantially induce the production of H_2S, jasmonic acid (JA), or nicotine in transgenic irLCD RNAi lines. Three three-week-old irLCD lines were exposed to HT (35 °C), or HT plus 100 μM NaHS (HT + NaHS), or no treatment (control, -HT) for 3 days; The production of H_2S (A) and JA (B) in the seedlings, and nicotine (C) in the roots of seedlings, was measured. A possible model explaining the role of H_2S in HT-induced JA and nicotine biosynthesis (D). Data represent the means of three replicate experiments (±SD). The means denoted by different letters indicate significant differences according to Tukey's test ($p < 0.05$).

functions in HT-mediated nicotine biosynthesis (Fig. 4D). In this model, HT-induced H_2S production occurs upstream of JA to trigger

nicotine biosynthesis. Our findings provide insight into the function of H_2S signaling in plant metabolism, and raise the possibility that H_2S signaling controls nicotine biosynthesis in tobacco.

Author contribution

LH and XH conceived and designed the experiments. XC, QC, RL, YJ and XZ conducted the experiments, AJ, LH, AE and XH analyzed the data and wrote the manuscript. AJ revised the manuscript and advised on the preparation of nicotine analysis.

Acknowledgments

This research was supported by the Major Science and Technology Program NOs.110201101003-TS-03, 2011YN02 and 2011YN03. The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at king Saud University for its funding this Research Group NO (RG-1435-014).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2016.02.033.

References

Alvarez, C., Calo, L., Romero, L.C., Garcia, I., Gotor, C., 2010. An O-acetylserine(thiol) lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in arabidopsis. Plant Physiol. 152, 656–669.
Batuman, O., Mawassi, M., Bar-Joseph, M., 2006. Transgenes consisting of a dsRNA of an RNAi suppressor plus the 3' UTR provide resistance to citrus tristeza virus sequences in *Nicotiana benthamiana* but not in citrus. Virus Genes 33, 319–327.
Boehning, D., Snyder, S.H., 2003. Novel neural modulators. Annu. Rev. Neurosci. 26, 105–131.
Chattopadhyay, M.K., Ghosh, B., 1998. Molecular analysis of polyamine biosynthesis in higher plants. Curr. Sci. India 74, 517–522.
Cheng, W., Zhang, L., Jiao, C., Su, M., Yang, T., Zhou, L., Peng, R., Wang, R., Wang, C., 2013. Hydrogen sulfide alleviates hypoxia-induced root tip death in *Pisum sativum*. Plant Physiol. Biochem. PPB Societe francaise de physiologie Veg. 70, 278–286.
Chou, W.M., Kutchan, T.M., 1998. Enzymatic oxidations in the biosynthesis of complex alkaloids. Plant J. 15, 289–300.
Clarke, S.M., Cristescu, S.M., Miersch, O., Harren, F.J., Wasternack, C., Mur, L.A., 2009. Jasmonates act with salicylic acid to confer basal thermotolerance in *Arabidopsis thaliana*. New Phytol. 182, 175–187.
Fang, T., Cao, Z., Li, J., Shen, W., Huang, L., 2014. Auxin-induced hydrogen sulfide generation is involved in lateral root formation in tomato. Plant Physiol. Biochem. 76, 44–51.
Garcia-Mata, C., Lamattina, L., 2010. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytol. 188, 977–984.
Hu, X., Kong, X., Wang, C., Ma, L., Zhao, J., Wei, J., Zhang, X., Loake, G.J., Zhang, T., Huang, J., Yang, Y., 2014. Proteasome-mediated degradation of FRIGIDA modulates flowering time in Arabidopsis during vernalization. Plant cell 26, 4763–4781.
Jia, H., Hu, Y., Fan, T., Li, J., 2015. Hydrogen sulfide modulates actin-dependent auxin transport via regulating ABPs results in changing of root development in Arabidopsis. Sci. Rep. 5, 8251.
Kessler, A., Baldwin, I.T., 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annu. Rev. Plant Biol. 53, 299–328.
Li, Z.G., Yang, S.Z., Long, W.B., Yang, G.X., Shen, Z.Z., 2013. Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (Zea mays L.) seedlings. Plant Cell Environ. 36, 1564–1572.
Li, Z.G., Xie, L.R., Li, X.J., 2015. Hydrogen sulfide acts as a downstream signal molecule in salicylic acid-induced heat tolerance in maize (Zea mays L.) seedlings. J. Plant Physiol. 177, 121–127.
Mok, Y.Y., Atan, M.S., Yoke Ping, C., Zhong Jing, W., Bhatia, M., Moochhala, S., Moore, P.K., 2004. Role of hydrogen sulphide in haemorrhagic shock in the rat: protective effect of inhibitors of hydrogen sulphide biosynthesis. Br. J. Pharmacol. 143, 881–889.
Naconsie, M., Kato, K., Shoji, T., Hashimoto, T., 2014. Molecular evolution of N-methylputrescine oxidase in tobacco. Plant Cell Physiol. 55, 436–444.
Paschold, A., Halitschke, R., Baldwin, I.T., 2007. Co(i)-ordinating defenses: NaCOI1 mediates herbivore- induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. Plant J. 51, 79–91.
Scuffi, D., Alvarez, C., Laspina, N., Gotor, C., Lamattina, L., Garcia-Mata, C., 2014. Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. Plant Physiol. 166, 2065–2076.
Shen, J., Xing, T., Yuan, H., Liu, Z., Jin, Z., Zhang, L., Pei, Y., 2013. Hydrogen sulfide improves drought tolerance in *Arabidopsis thaliana* by microRNA expressions. PloS one 8, e77047.
Shi, H., Ye, T., Han, N., Bian, H., Liu, X., Chan, Z., 2015. Hydrogen sulfide regulates abiotic stress tolerance and biotic stress resistance in Arabidopsis. J. Plant Physiol. 57, 628–640.
Shoji, T., Ogawa, T., Hashimoto, T., 2008. Jasmonate-induced nicotine formation in tobacco is mediated by tobacco COI1 and JAZ genes. Plant Cell Physiol. 49, 1003–1012.
Singh, V.P., Singh, S., Kumar, J., Prasad, S.M., 2015. Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate-glutathione cycle: possible involvement of nitric oxide. J. Plant Physiol. 181, 20–29.
Steppuhn, A., Gase, K., Krock, B., Halitschke, R., Baldwin, I.T., 2004. Nicotine's defensive function in nature. PLoS Biol. 2, E217.
Vadassery, J., Reichelt, M., Hause, B., Gershenzon, J., Boland, W., Mithofer, A., 2012. CML42-mediated calcium signaling coordinates responses to Spodoptera herbivory and abiotic stresses in Arabidopsis. Plant Physiol. 159, 1159–1175.
Wang, P., Song, C.P., 2008. Guard-cell signalling for hydrogen peroxide and abscisic acid. New Phytol. 178, 703–718.
Wang, S.S., Shi, Q.M., Li, W.Q., Niu, J.F., Li, C.J., Zhang, F.S., 2008. Nicotine concentration in leaves of flue-cured tobacco plants as affected by removal of the shoot apex and lateral buds. J. Integra. Plant Biol. 50, 958–964.
Wirtz, M., Droux, M., Hell, R., 2004. O-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. J. Exp. Bot. 55, 1785–1798.
Xie, Y., Zhang, C., Lai, D., Sun, Y., Samma, M.K., Zhang, J., Shen, W., 2014. Hydrogen sulfide delays GA-triggered programmed cell death in wheat aleurone layers by the modulation of glutathione homeostasis and heme oxygenase-1 expression. J. Plant Physiol. 171, 53–62.
Yang, G., Wu, L., Jiang, B., Yang, W., Qi, J., Cao, K., Meng, Q., Mustafa, A.K., Mu, W., Zhang, S., Snyder, S.H., Wang, R., 2008. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science 322, 587–590.

Further readings

Sun, J.Q., Xu, Y.X., Ye, S.Q., Jiang, H.L., Chen, Q., Liu, F., Zhou, W.K., Chen, R., Li, X.G., Tietz, O., Wu, X.Y., Cohen, J.D., Palme, K., Li, C.Y., 2009. Arabidopsis ASA1 Is Important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. Plant cell 21, 1495–1511.