Plants with genetically encoded autoluminescence

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Autoluminescent plants engineered to express a bacterial bioluminescence gene cluster in plastids have not been widely adopted because of low light output. We engineered tobacco plants with a fungal bioluminescence system that converts caffeic acid (present in all plants) into luciferin and report self-sustained luminescence that is visible to the naked eye. Our findings could underpin development of a suite of imaging tools for plants.

Bioluminescent reporters have not been broadly applied in plants because exogenous addition of luciferin is expensive and can be toxic. Although bacterial bioluminescence genes can be targeted to plastids to engineer autoluminescence, it is technically cumbersome and fails to produce sufficient light. The caffeic acid cycle, which is a metabolic pathway responsible for luminescence in fungi, was recently characterized. We report light emission in Nicotiana tabacum and Nicotiana benthamiana plants without the addition of any exogenous substrate by engineering fungal bioluminescence genes into the plant nuclear genome.

Caffeic acid is an intermediate in the phenylpropanoid pathway, which produces lignin and other metabolites in vascular plants. We reasoned that it might be feasible to integrate the fungal caffeic acid cycle into plant metabolism. Moreover, the green luminescence produced by the caffeic acid cycle fits well with the optical transparency window of pigmented plant tissues (Fig. 1a). Although caffeic acid is not native to animals, autonomous luminescence could also be enabled in animals by including two additional enzymes needed for its biosynthesis from tyrosine—tyrosine ammonia lyase and coumarate 3-hydroxylase—or their functional equivalents (Fig. 1b and Supplementary Fig. 1).

We engineered autonomously glowing N. tabacum plants by random-site genome integration using Agrobacterium-mediated transformation of DNA cassettes comprising codon-optimized versions of four Neonothopus nambi bioluminescence genes: nlduz (luciferase), nhsigs (hispidin synthase), nh3h (hispidin-3-hydroxylase) and ncpf (caffeoyl pyruvate hydrodrolase) (Fig. 1, Methods, Supplementary Fig. 2 and Supplementary Note 1).

Fifteen independently obtained plant lines had confirmed genome integration events. The overall phenotype, chlorophyll and carotenoid content, flowering time and seed germination did not differ from wild-type tobacco in the greenhouse, with the exception of a 12% increase in median height of transgenic plants (Supplementary Fig. 3 and Supplementary Note 2). This suggests that, unlike expression of bacterial bioluminescence system, expression of caffeic acid cycle is not toxic in plants and does not impose an obvious burden on plant growth, at least in the greenhouse. Light emission at all developmental stages was visible to the naked eye, with intensity from the flowers reaching 10^10 photons per minute (Supplementary Table 1). This level of brightness allowed us to capture detailed images on consumer-grade cameras with exposure times of 0.5–30 s, providing similar quality to that of more expensive luminescence imaging equipment (Fig. 2 and Supplementary Figs. 3–8).

To identify metabolites that might limit light emission, we infused leaves of glowing plants with luciferin or its precursors. We found that bright luminescence developed instantly after injection of luciferin or hispidin, whereas low intensity was produced more slowly if leaves were supplemented with caffeic acid (Supplementary Video 1). Because engineered N. tabacum lines did not retain infused exogenous precursors at the injection site, we created a glowing line of N. benthamiana. In evaluation of all-but-one mixtures of hispidin precursors, caffeic acid produced increased luminescence, whereas malonyl-CoA, CoA or ATP, added individually or as a mixture, did not (Supplementary Note 3 and Supplementary Fig. 9). Taken together, these experiments suggest that caffeic acid limits hispidin biosynthesis (Supplementary Note 4 and Supplementary Video 1).

Consistent with a link between caffeic acid availability and luminescence intensity, the distribution of luminescence resembled reported expression patterns of enzymes involved in the phenylpropanoid pathway. During seed germination, there was increased luminescence at the tips of cotyledons and roots (Fig. 2a and Supplementary Video 2). Roots also glowed brightly at branching points (Fig. 2d), often hours before visible evidence of lateral root
initiation (Supplementary Videos 3 and 4). As plants developed, luminescence increased at the transition zone between the root and the stem. Young shoots were brightest at the terminal and axillary buds and at the upper part of the stem; older parts of the shoot dimmed as plants matured (Supplementary Video 5). Flowers produced the most luminescence (Fig. 2c,e, Supplementary Fig. 10 and Supplementary Video 6).

Increased light emission under conditions known to activate production of phenylpropanoids was observed using time-lapse luminescent imaging. Moreover, the spatial and temporal patterns of luminescence of tobacco plants were characterized (Supplementary Notes 5–7). In injured leaves, we observed a sustained increase in light emission at the injury site. We also discerned luminescence spreading from an injury site via small veins at approximately 2 μm s⁻¹ (Supplementary Fig. 11 and Supplementary Video 7). Apical shoot removal resulted in sustained bright luminescence in lateral shoots proximal to the cut site (Supplementary Fig. 12 and Supplementary Video 8). Aging leaves, reported to have gradually reducing caffeic acid content until late senescence, generally exhibited decreased light emission. Nevertheless, some leaves displayed waves of intense light emission during the final stages of senescence (Supplementary Video 5), possibly reflecting age-related nutrient remobilization. Finally, plants treated with methyl jasmonate or ripe banana skin (which emits ethylene, among other compounds) responded with massively increased luminescence throughout the plant (Supplementary Fig. 13a,b).

We have established the feasibility of using fungal bioluminescence genes to produce glowing plants that are at least an order of magnitude brighter than was previously achieved using a bacterial bioluminescence system (Supplementary Table 1 and Supplementary Figs. 6 and 7). By enabling autonomous light emission, dynamic processes in plants can be monitored, including development and pathogenesis, responses to environmental conditions and effects of chemical treatment. Screening methods should also be enabled by the simplicity and efficiency of acquiring luminescent data. By removing the need for exogenous addition of luciferin or other substrates, these luminescent capabilities should be particularly useful for experiments with plants grown in the soil.

Online content
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Fig. 2 | Bioluminescent plants during development. Light emission from N. tabacum plants at germination (a), vegetative (b) and flowering (c) stages; light emission from roots (d) and cross section of flowers (e). Photos were captured on a Sony Alpha ILCE-7M3 camera (Methods). The 110 seedlings depicted in a are representative of three independent experiments. Images of plants in vegetative (b, 3 weeks) and flowering (c, 8 weeks) stages, as well as individual flowers (e) are representative of 100 plants followed from in vitro to flowering in four separate experiments. The age of plants is stated relative to transfer from in vitro to the greenhouse. The image of roots of an individual plant depicted in d is representative of three independent imaging experiments on six plants.

References
1. Krichevsky, A., Meyers, B., Vainstein, A., Maliga, P. & Citovsky, V. PLoS ONE 5, e15461 (2010).
2. Kotlobay, A. A. et al. Proc. Natl Acad. Sci. USA 115, 12728–12732 (2018).
3. Yan, Y. & Lin, Y. Biosynthesis of caffeic acid and caffeic acid derivatives by recombinant microorganisms. US patent 8809028B2 (2012).
4. Kawamata, S. et al. Plant Cell Physiol. 38, 792–803 (1997).
5. Gaquerel, E., Gulati, J. & Baldwin, I. T. Plant J. 79, 679–692 (2014).
6. Toyota, M. et al. Science 361, 1112–1115 (2018).
7. Singh, S. K. et al. Sci. Rep. 5, 18148 (2015).
8. Li, L. et al. Sci. Rep. 6, 37976 (2016).
9. Li, W. et al. Sci. Rep. 7, 12126 (2017).
10. Woo, H. R., Kim, H. J., Nam, H. G. & Lim, P. O. J. Cell Sci. 126, 4823–4833 (2013).
11. Pauwels, L. et al. Proc. Natl Acad. Sci. USA 105, 1380–1385 (2008).
12. Bernard, M. A. & Bästrup-Spohr, L. Induced Plant Resistance to Herbivory (Springer, 2008).
13. Singh, R., Rastogi, S. & Dwivedi, U. N. Compr. Rev. Food Sci. Food Saf. 9, 398–416 (2010).

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Methods

Assembly of plasmids for plant transformation. Coding sequences of the ntluz, nlnhisp, nln3h and nnlcph genes from N. namibi were codon optimized for expression in N. tabacum and ordered synthetically from Evrogen. Synthetic genes were flanked by Bsall restriction sites designed to leave AATT-GCTT overhangs, compatible with the existing modular cloning standard described in ref. 13. Each gene was then cloned into Level 1-like vector, under the control of the constitutive 35S promoter from cauliflower mosaic virus and ocs terminator from Agrobacterium tumefaciens. These Level 1 plasmids were then digested by Bpfl and assembled together into a Level 2-like backbone in the following order: nlnhisps-nln3h-ntluz-nnlcph or, in the case of cph-less version, nlnhisps-nln3h-ntluz. This gene cluster was preceded by a kanamycin resistance cassette for selection in plants. The entire construct, consisting of the kanamycin cassette plus lucines gene, was flanked by A. tumefaciens terminators from the construct into plant genomes (Supplementary Fig. 2).

All clonings described above were performed according to established Golden Gate cloning methods, wherein digestion and ligation are performed together in a single step. All reactions were performed in 1x T4 ligase buffer.

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Agrobacterium-mediated transformation of plants. Assembled plasmids were transferred into A. tumefaciens strain AGL10 (ref. 14). Bacteria were grown in flasks on a shaker overnight at 28 °C in L medium supplemented with 25 mg l−1 rifampicin and 50 mg l−1 kanamycin. Bacterial cultures were diluted in liquid Murashige and Skoog (MS) medium to an optical density of 0.6 at 600 nm.

Leaf explants used for transformation experiments were cut from 2-week-old tobacco plants (N. tabacum cv. Petit Havana SR1, N. benthamiana) and incubated with bacterial culture for 20 min. Leaf explants were then placed onto filter paper overlaid on MS medium (MS salts, MS vitamin, 30 g l−1 sucrose, 8 g l−1 agar). PH 5.8 supplemented with 1 mg l−1 6-benzylaminopurine and 0.1 mg l−1 indole acetic acid. Twenty-four hours after transfection, the medium was changed to MEM supplemented with 20 mM HEPES, and luminescence was analyzed by IVIS Spectrum CT (PerkinElmer). For the analysis, the background luminescence signal from the empty wells was subtracted from the luminescence signal of wells with control and autocluminescent cells.

Chlorophyll content in leaves. Next, 0.5 g of fresh plant leaf sample was homogenized in tissue homogenizer with 10 ml of 95% ethanol. Homogenized sample mixture was centrifuged at 10,000 r.p.m. for 15 min. An aliquot of the supernatant (0.5 ml) was mixed with 95% ethanol (4.5 ml). The solution mixture in a glass cuvette was analyzed for chlorophyll-a, chlorophyll-b and carotenoids content at 649, 664 and 470 nm.

Imaging of leaf injuries. Plants were cultivated in a greenhouse for 6 weeks. Leaves of N. tabacum were wounded with a blade, causing a cut across the vein.

Absorption spectra of tobacco leaves. The leaves from adult wild-type N. tabacum plants were collected and measured directly by spectrophotometer (Cary 100 Bio, Varian).

Imaging of leaf injuries. Plants were cultivated in a greenhouse for 6 weeks. Leaves of N. tabacum were wounded with a blade, causing a cut across the vein.

Treatement with methyl jasmonate. Three-week-old transgenic bioluminescent N. tabacum plants were treated with methyl jasmonate (5 mM in 10 mM MES buffer, pH 7.0) by spraying. Control plants were treated with buffer (10 mM MES buffer, pH 7.0). Plants were then imaged in closed glass jars for 3 d in the dark.

Incubation with banana skin. Three-week-old transgenic bioluminescent N. tabacum plants were imaged with ripe banana skin in closed glass jars for 24 h.
Quantitative PCR. In experiments aimed to determine whether expression of ntluz gene oscillates during the day, we collected the third leaf counting from the apical bud from 27 2-day-old transgenic glowing plants. The leaves were collected with 3-h intervals during 24 h, and leaf samples from three plants were collected at each time point. From each plant, we collected leaves only once. All leaves were flash frozen in liquid nitrogen and homogenized for RNA extraction with TRIzol kit (Thermo Fisher Scientific). Synthesis of the first cDNA strand was carried out with an MMLV kit (Evrigen). Quantitative PCR was performed with qPCRmix-HS SYBR+ LowROX kit (Evrigen) on a 7500 Real-Time PCR machine (Applied Biosystems) with primers annealing at the ntluz transcript: GACACGAGGATCCCGG and CTGGGCAATTTCGACAAATCTTA with the following program: 95 °C for 1 min and then 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 15 s.

Infiltration of tobacco leaves with hispidin precursors. For experiments with infiltration of transgenic N. benthamiana leaves, we prepared 100 μM solutions of caffeic acid, malonyl-CoA, ATP and coenzyme A in 10 mM MES buffer (pH 7.0). We also prepared 100 μM mixtures of these compounds in the same buffer: Mix 1, (full caffeic acid, malonyl-CoA, CoA, ATP); Mix 2 without caffeic acid (malonyl-CoA, CoA, ATP); Mix 3 without malonyl-CoA (caffeic acid, CoA, ATP); Mix 4 without CoA (caffeic acid, malonyl-CoA, ATP); and Mix 5 without ATP (caffeic acid, malonyl-CoA, CoA). The solutions were injected into the blades of N. benthamiana leaves, and leaves were imaged for 15 min after injections. The analysis of the frame at 1 min after injection is presented in Supplementary Fig. 9. Similar experiment design was followed for the injection of luciferin precursors into N. tabacum leaves, followed by 16 h of imaging (Supplementary Video 1).

LC-MS/MS analysis. Analytical standard (≥ 98.0%) caffeic acid and acetic acid were purchased from Sigma-Aldrich. Hispidin was synthesized by Planta (≥ 95.0%). HPLC-grade acetonitrile was purchased from J.T. Baker. Deionized water was obtained from a Mill-Q System.

We analyzed several groups of samples: leaves and flowers of the wild-type N. tabacum (NT900) and two transgenic lines of plants (NT001 and NT078). Immediately after collection, the samples were frozen in liquid nitrogen and manually ground in a mortar. To reduce biological variability, we mixed plant material from three different organs of the same group. For each sample, about 1 g of the frozen tissue was lyophilized in 50-ml Falcon tubes, and freeze-dried material was stored at −20 °C. Each sample was prepared and analyzed in three replicates.

For the analysis, about 50 mg of lyophilized powder was weighed and treated with 7 ml of 70% methanol for 30 min in an ultrasonic bath and then centrifuged for 10 min at 4,000 rpm. The supernatant was collected, filtered with Phenum GF/PVDF syringe filter (diameter 30 mm, pore size 0.45 μm) and analyzed on an LC/MS instrument. Analyses were performed by a Shimadzu 8030 system consisting of HPLC coupled to PDA and triple quadrupole mass spectrometer (HPLC–DAD–ESI–TQ MS). The chromatographic separation was performed on Discovery C18 column 4.6 × 150 mm, 5 μm in a gradient mode with mobile phase components A (0.3% acetic acid in water) and B (acetonitrile). The gradient run was performed in the following way: 0–4 min 10–40% B, 4–5 min 40–80%, 5–10.5 min, isocratic elution with 100% B and then returned to the initial condition. The column temperature was 40 °C, the flow rate was 1 ml/min and the sample injection volume was 20 μl.

The electrospray ionization (ESI) source was set in negative ionization mode. Multiple reaction monitoring was used to perform mass spectrometric quantification. MS conditions: interface voltage 3,500 V (ESI mode. Multiple reaction monitoring was used to perform mass spectrometric analysis. The precursor and product ions (m/z) of target analytes were 178.95 and 134.95 for caffeic acid and 245.05 and 159.00 for hispidin; S41587-020-0500-9.

References
14. Weber, E., Engler, C., Gruetzmacher, R., Werner, S. & Marillenon, S. PLoS ONE 6, e16765 (2011).
15. Lamson, S. V., Haddock, T. L., Beal, J. & Densmore, D. M. ACS Synth. Biol. 5, 99–103 (2016).
16. Lazo, G. R., Stein, P. A. & Ludwig, R. A. Biotechnology 9, 963–967 (1991).
17. Rogers, S. O. & Bendich, A. J. Plant Molecular Biology Manual (Springer, 1994).

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Author contributions
T.M., A.S.M., L.G.S., T.V.C., E.B.G., T.A.K., N.M.M., S.V.C., A.S.T., L.I.F., K.A.P., E.S.S., Y.V.Y., V.V.N., S.A.D., P.V.S., O.A.M., V.O.S., S.M.D., A.I.B., A.S.P. and K.S.S. performed experiments. T.M., A.S.M., L.G.S., T.V.C., E.B.G., T.A.K., N.M.M., S.V.C., A.S.T., L.I.F., K.A.P., E.S.S., Y.V.Y., V.V.N., S.A.D., P.V.S., O.A.M., V.O.S., S.M.D., A.I.B., A.S.P., V.V.C., S.V.D., F.A.K., I.Y.V. and K.S.S. performed data analysis. A.S.M. designed imaging setup, planned and performed experiments, analyzed data and wrote the paper. I.Y.V. and K.S.S. proposed and directed the study, planned experimentation and wrote the paper. All authors reviewed and commented on the paper draft.

Competing interests
This work was supported by Planta LLC. I.Y.V. and K.S.S. are shareholders and employees of Planta. Planta has filed patent applications related to use of components of the fungal bioluminescent system and development of glowing transgenic organisms.

Additional information
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