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Plants with genetically encoded autoluminescence

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Abstract

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TM, ASM, LGS, TVC, EBG, TAK, NMM, SVC, AST, LIF, KAP, ESS, YVG, DS, VVN, SAD, PVS, OAM, VOS, SMD, AIB, ASP, and KSS performed experiments. TM, ASM, LGS, TVC, EBG, TAK, NMM, SVC, AST, LIF, KAP, ESS, YVG, VVN, SAD, PVS, OAM, VOS, SMD, AIB, ASP, VVC, SVD, FAK, IVY and KSS performed data analysis. ASM designed imaging setup, planned and performed experiments, analysed data and wrote the paper. IVY and KSS proposed and directed the study, planned experimentation and wrote the paper. All authors reviewed and commented on the paper draft.

Conflict of interest statement

This work was supported by Planta LLC. IVY and KSS are shareholders and employees of Planta. Planta filed patent applications related to use of components of fungal bioluminescent system and development of glowing transgenic organisms.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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Autoluminescent plants that express a bacterial bioluminescence gene cluster[1] have not been widely adopted due to requisite expression in plastids and low light output. Alternatively, we have engineered tobacco lines expressing a fungal bioluminescent system[2], which converts caffeic acid present in all plants into luciferin, and report self-sustained luminescence easily visible to the naked eye. Our findings might underpin development of a suite of imaging tools for plants.

Bioluminescent reporters have not been broadly applied in plants because exogenous addition of luciferin can be toxic, technically cumbersome and expensive. Although the metabolic pathway underlying fungal bioluminescence (the caffeic acid cycle) was recently reported[2], applying this to luminescent imaging in multicellular organisms had only been demonstrated with externally-supplied substrate. Here, we report light emission in *Nicotiana tabacum* and *benthamiana* plants without the need for exogenous substrate, by integrating fungal bioluminescence genes into the nuclear genome.

Caffeic acid is a key component of the phenylpropanoid pathway in vascular plants, leading to lignin and a host of other secondary metabolites. Thus, the caffeic acid cycle in luminous fungi is well suited for integration into plant metabolism. Moreover, the green luminescence fits within an optical transparency window of pigmented plant tissues (Figure 1a). Although caffeic acid is not native to animals, autonomous luminescence can be enabled by including two additional enzymes needed for its biosynthesis from tyrosine — tyrosine ammonia lyase and coumarate 3-hydroxylase, or their functional equivalents (Figure 1b, Supplementary Figure 1)[3].

We engineered the autonomously glowing tobacco plants via random-site genome integration using Agrobacterium-mediated transformation. DNA cassettes were used containing codon-optimised versions of the four previously reported *Neonothopanus nambi* bioluminescence genes: *nnluz* (luciferase), *nnhisps* (hispidin synthase), *nnh3h* (hispidin-3-hydroxylase), and *nncph* (caffeoyl pyruvate hydrolase) (Figure 1, Online Methods, Supplementary Figure 2, Supplementary Note 1).

Fifteen plant lines were independently obtained with confirmed genome integrations. The overall phenotype, chlorophyll and carotenoid content, flowering time, and seed germination did not differ from the wild-type tobacco, with the exception of a 12% increase in median height of transgenic plants (Supplementary Figure 3; Supplementary Note 2). This suggests that unlike bacterial bioluminescence, expression of the caffeic acid cycle is not toxic and does not impose an obvious burden on plant growth. Light emission at all developmental stages was visible to the naked eye, with intensity from the flowers reaching 10^{10} photons/min (Supplementary Table 1). The brightness allowed us to capture detailed images on consumer-grade cameras with exposure times of 0.5–30 seconds, providing comparable quality to that of more expensive luminescence imaging equipment (Figure 2, Supplementary Figures 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 following injections of luciferin or hispidin, while lower intensity was produced more slowly with caffeic acid (Supplementary Video 1). In further experimentation, because *N. tabacum*

did not retain the precursors at the place of injection, we created a similar glowing strain of *N. benthamiana*. In evaluation of all-but-one mixtures of hispidin precursors, caffeic acid produced increased luminescence, whereas malonyl-CoA, CoA, or ATP (individually or as a mixture) did not (Supplementary Note 3, Supplementary Figure 9). This suggests that caffeic acid limits hispidin biosynthesis (Supplementary Note 4).

Consistent with this linkage to caffeic acid availability, the distribution of luminescence resembled reported expression patterns of enzymes involved in the phenylpropanoid pathway[4]. Luminescence evident upon seed germination had greater intensity at the tips of cotyledons and roots (Figure 2a, Supplementary Video 2). Roots also glowed brightly at branching points (Figure 2d), often hours before visible evidence of lateral root initiation (Supplementary Videos 3–4). As plants developed, luminescence increased at the transition zone between the root and stem. Young shoots were brightest at the terminal and axillary buds, and upper part of the stem; older parts of the shoot dimmed as plants matured (Supplementary video 5). Flowers produced the greatest luminescence (Figure 2e, 2c, Supplementary figure 10, Supplementary video 6).

Increased light emission under conditions known to activate production of phenylpropanoids was revealed using time-lapse luminescent imaging. Moreover, the spatial and temporal patterns of luminescence of tobacco plants were revealed (Supplementary Notes 5, 6, 7). In injured leaves[5,6], we observed a sustained increase in light emission at the injury site. We have also discerned luminescence spreading from the injury site by small veins at approximately 2 μm/sec (Supplementary Figure 11, Supplementary Video 7). Apical shoot removal[7] resulted in sustained bright luminescence in lateral shoots proximal to the cut site (Supplementary Figure 12, Supplementary Video 8). Aging leaves, reported to have gradually reducing caffeic acid content until late senescence[8], 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[9,10]. Finally, plants treated with methyl jasmonate[11,12] or ripe banana skin (emits ethylene among other compounds)[13] responded with dramatically increased luminescence throughout the plant (Supplementary Figure 13ab).

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 possible using a bacterial bioluminescence system (Supplementary Table 1, Supplementary Figures 6, 7)[1]. 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 supported due to the efficiency of acquiring luminescent data. By not requiring exogenous addition of luciferin or other substrates, these luminescent capabilities should be particularly useful for plants grown in soil.

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Online Methods

Assembly of plasmids for plant transformation

Coding sequences of the *nnluz*, *nnhisps*, *nnh3h* and *nncph* genes from *Neonothopanus nambi* were codon-optimized for expression in *Nicotiana tabacum* and ordered synthetically from Evrogen (Moscow, Russia). Synthetic genes were flanked by BsaI restriction sites designed to leave AATG-GCTT overhangs, compatible with the existing modular cloning standard described in ref.[14]. Each gene was then cloned into Level1-like vector, under the control of the constitutive 35s promoter from cauliflower mosaic virus, and ocs terminator from *Agrobacterium tumefaciens*. These Level1 plasmids were then digested by BpiI and assembled together into a Level2-like backbone in the following order: *nnhisps-nnh3h-nnluz-nncph*, or, in the case of *cph*-less version: *nnhisps-nnh3h-nnluz*. This gene cluster was preceded by a kanamycin resistance cassette for selection in plants. The entire construct, consisting of the kanamycin cassette plus luminescence genes, was flanked by *A. tumefaciens* insertion sequences to facilitate *Agrobacterium*-mediated random integration of the construct into plant genomes (Supplementary Figure 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 (ThermoFisher) containing 10U of T4 ligase, 20U of either BsaI or BpiI (ThermoFisher), and 100ng of DNA of each DNA part. Golden Gate reactions were performed according to “troubleshooting” cycling conditions described in ref.[15]: 25 cycles between 37°C and 16°C (90 sec at 37°C, 180 sec at 16°C), then 5 min at 50°C and 10 min at 80°C.

Correct sequences of all plasmids were confirmed with Sanger and Illumina sequencing prior to use. Plasmid maps are available from Figshare: http://doi.org/10.6084/m9.figshare.11871888.

Assembly of plasmid for mammalian cells

DNA coding for RcTAL, HpaB, HpaC, nnHispS, NpgA, nnH3H, nnCPH, nnLuz was ordered synthetically (Evrogen, Russia) and cloned into the pKatushka2S-C1 vector (Evrogen) instead of Katushka2S coding sequence under the control of CMV promoter.

Expression in cultured mammalian cells and luminescence imaging

HEK293T cell line was transfected with a mixture of all eight plasmids by FuGENE HD transfection reagent (Promega). Transfected cells were grown in DMEM (Paneco) supplemented with 10% fetal bovine serum (HyClone), 4 mM L-glutamine, 10 u ml-1 penicillin, 10 μg ml-1 streptomycin, at 37°C, 5% CO2. 24 hours after transfection, the medium was changed to MEM supplemented with 20 mM HEPES, and luminescence was analysed 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 autoluminescent cells.

Agrobacterium-mediated transformation of plants

Assembled plasmids were transferred into *Agrobacterium tumefaciens* strain AGL0[16]. Bacteria were grown on a shaker overnight at 28°C in LB medium supplemented with 25 mg/l rifampicin and 50 mg/l kanamycin. Bacterial cultures were diluted in liquid Murashige and Skoog (MS) medium to the optical density of 0.6 at 600 nm.

Leaf explants used for transformation experiments were cut from two-week-old tobacco plants (*Nicotiana tabacum* cv. Petit Havana SR1, *Nicotiana benthamiana*) and incubated with bacterial culture for 20 minutes. Leaf explants were then placed onto filter paper overlaid on MS medium (MS salts, MS vitamin, 30 g/L sucrose, 8 g/L agar, pH 5.8) supplemented with 1 mg/L 6-benzylaminopurine and 0.1 mg/L indolyl acetic acid. Two days after inoculation, explants were transferred to the same medium supplemented with 500 mg/L cefotaxime and 75 mg/L kanamycin. Regeneration shoots were cut and grown on MS medium with antibiotics.

Molecular analysis of transgenic plants

Genomic DNA was extracted from young leaves of greenhouse-grown plantlets using cetyltrimethylammonium bromide method[17]. The presence of each of the transferred genes was confirmed by PCR with gene-specific primers (Supplementary Table 2).

For Southern blot, 30 μg of plant genomic DNA was digested overnight at 37°C by 100U of EcoRV, a restriction enzyme that cuts T-DNA constructs used in this study at a single position inside nnHispS coding region. After gel electrophoresis, digestion products were transferred onto Amersham Hybond-N+ membrane (GE Healthcare, UK) and immobilized. The DNA probe was constructed by PCR using cloned synthetic *nnluz* gene as the template and *nnluz*-specific primers listed in Supplementary Table 2. Probe DNA was labeled with alkaline phosphatase using the AlkPhos Direct Labeling Kit (GE Healthcare, UK). Prehybridization, hybridization (overnight at 60°C) with alkaline phosphatase-labeled probe, and subsequent washings of the membrane were carried out according to the AlkPhos Direct Labeling Kit protocol. Detection was performed using Amersham CDP-Star detection reagent following the manufacturer's protocol (GE Healthcare, UK). The signal from the membrane was accumulated on X-ray film (XBE blue sensitive, Retina, USA) in film cassette at room temperature for 24 hours. X-ray films were scanned on Amersham imager 600 (GE Healthcare Life Sciences, Japan).

Plant growth conditions

The plant transgenesis and cultivation was carried out at the artificial climate station Biotron N2-2.9 (branch of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Pushchino, Russia). Tobacco plants were propagated on Murashige and Skoog (MS) medium supplemented with 30 g/l sucrose and 0.8 w/v agar (Panreac, Spain). *In vitro* cultures were incubated at 24±1°C with 12-16-day photoperiod, with mixed cool white and red light (Cool White and Grolux fluorescent lamps) at light intensity 40 μmol / sec*m^2. After root development, plantlets were transferred to 9 cm pots with sterilized soil (1:3 w/w mixture of sand and peat). Potted plants were placed in the greenhouse at 22±2°C under neutral day conditions (12h light / 12h dark; 150 μmol m-2s-1)

and 75% relative humidity. For time-lapse imaging of germinating seeds (Supplementary Video 2), seeds were sterilized in sodium hypochlorite (25%/15 min) and then propagated on Murashige and Skoog (MS) medium supplemented with 30 g/l sucrose and 0.3 w/v Gellan Gum Powder (MP Biomedicals,LLC). The same medium was used for luminescence imaging of roots (panel iv in Figure 2, Supplementary Videos 3 and 4).

Plant imaging setup with photo cameras

We used Sony Alpha ILCE-7M3 camera to capture all photos and videos presented in this paper, except those taken on a smartphone (Supplementary Fig. 8) and a long-term time-lapse filmed on Nikon D800 (Supplementary video 5). Depending on the experimental setup, lens aperture and other considerations, a range of ISO values from 3200 to 40000 was used, with exposure times from 5 seconds (leaf injury) to 20 minutes (root microscopy). Most of the photos were captured with 30-second exposure time.

We used SEL50M28 lens (Sony, f/2.8), or 35mm T1.5 ED AS UMC VDSLR lens (Samyang, ~f/1.4). Long-term timelapse of growing tobacco plants (Supplementary Figure 13c, Supplementary video 5) was captured with Nikon D800 camera and Sigma AF 35mm f/1.4 DG HSM Art at ISO 8063 and 30-second shutter speed. Root microscopy was performed with Sony Alpha ILCE-7M3 camera with Meiji MA833 U. Plan 20X Objective lens was mounted on the camera via a custom-made adaptor. For quantitative comparison, XLS-4 (PerkinElmer) calibrated light source was used as a reference (emits $1.6x10^9$ photons/sec at 525 nm).

The photos were then processed in the following way. First, a raw photo obtained in the dark with the same settings was per-channel subtracted from a raw photo of plants (LibRaw version 0.19.2, 4channels tool) to remove hot pixels and reduce noise. Optionally, an ImageJ plugin was applied (https://imagej.nih.gov/ij/source/ij/plugin/filter/RankFilters.java) to remove outliers (hot pixels). For most photos, only the green channels (G and G2) were kept in the final image. Final images were rendered in pseudocolor either with “Green” or “Fire” linear lookup tables from ImageJ.

Plant imaging on IVIS Spectrum CT

Plant imaging on IVIS Spectrum CT was performed without filters in front of the camera, with 1 min exposure and without binning. The samples were acquired with “C” settings of field of view. Ambient light image was taken after the luminescence measurements. Other settings were left at defaults.

Chlorophyll content in leaves

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 rpm 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 664, 649 and 470 nm.

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Plant imaging on a smartphone

We used smartphone Huawei P30 Pro for photography. To capture the photo displayed on Supplementary Figure 8b, we used the following settings: 30 sec exposure time, ISO 6400, aperture 1.6.

Absorption spectra of tobacco leaves

The leaves from adult wild type *Nicotiana tabacum* plants were collected and measured directly by spectrophotometer Cary 100 Bio (Varian).

Imaging of leaf injuries

Plants were cultivated in greenhouse for six weeks. Leaves of *Nicotiana tabacum* were wounded with a blade causing cut across the midvein.

Treatment with methyl jasmonate

3 week old transgenic bioluminescent *Nicotiana 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 days in the dark.

Incubation with banana skin

3 week old transgenic bioluminescent *Nicotiana tabacum* plants were imaged with ripe banana skin in closed glass jars for 24 hours.

Quantitative PCR

In experiments aimed to determine whether expression of *nnluz* gene oscillates during the day, we collected the third leave counting from apical bud from twenty seven 25-day-old transgenic glowing plants. The leaves were collected with three-hour intervals during 24 hours, leaves from three plants were collected at each time point. From each plant we collected leaves only once. All leaves were flash-freezed in liquid nitrogen and homogenized for RNA extraction with TRIzol kit (Thermo Fisher Scientific, USA). Synthesis of the first cDNA strand was carried out with MMLV kit (Evrogen, Russia). Quantitative PCR was performed with qPCRmix-HS SYBR+LowROX kit (Evrogen, Russia) on 7500 Real-Time PCR machine (Applied Biosystems, USA) with primers annealing at *nnluz* transcript: GGACCAGGAGTCCCAGGC and CTTGGCATTTTCGACAATCTTA with following program: 95°C - 1 min, then 40 cycles of (95°C - 15 sec, 60°C - 15 sec, 72°C - 15 sec).

Infiltration of tobacco leaves with hispidin precursors

For experiments with infiltration of transgenic *Nicotiana benthamiana* leaves, we prepared 100 uM solutions of caffeic acid, malonyl-CoA, ATP and coenzyme A in 10 mM MES buffer (pH 7.0). We also prepared 100 uM 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), and *Mix 4 without CoA* (caffeic acid, malonyl-CoA, ATP), *Mix 5 without ATP* (caffeic acid, malonyl-CoA, CoA). The solutions were injected into the blades of cut *Nicotiana benthamiana* leaves, and

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leaves were imaged for 15 min following injections. The analysis of the frame at one minute after the injection is presented on Supplementary Figure 9. Similar experiment design was followed for the injection of luciferin precursors into *Nicotiana tabacum* leaves, followed by 16 hours 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 synthesised by Planta (≥95.0 %). HPLC-grade-acetonitrile was purchased from J.T. Baker. Deionized water was obtained from a Milli-Q System (USA).

We analysed several groups of samples: leaves and flowers of the wild type *Nicotiana tabacum* (NT000) and two transgenic lines of plants (NT001, 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 organisms of the same group. For each sample, about 1 g of the frozen tissue was lyophilized in 50 ml falcon, 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 70% methanol for 30 min in an ultrasonic bath, then centrifuged for 10 min at 4,000 rpm. The supernatant was collected, filtered with Phenex GF/PVDF syringe filter (Ø 30 mm, 0.45 μm) and analysed on LCMS instrument. Analyses were performed by 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, the sample injection volume was 20 μL.

The ESI source was set in negative ionization mode. Multiple reaction monitoring was used to perform mass spectrometric quantification. MS conditions: interface voltage 3500V (ESI −), nebulizer gas (nitrogen) flow 2.5 l/min, drying gas (nitrogen) flow 15 l/min, CID gas pressure 60 kPa, DL temperature 250°C, heat block temperature 400°C. High purity argon was used as collision gas. The precursor and product ions (m/z) of target analytes were 178.95 and 134.95 for caffeic acid, 245.05 and 159.00 for hispidin; collision energy was 35V for both compounds.

Due to the lack of isotope-labeled standards, we added standards to samples to account for substantial matrix effect. Each sample was analysed twice, with and without the addition of standards. After the first analysis, a solution with known amount of caffeic acid and hispidin was added. Assuming a linear relation between the observed signal and concentration of compounds, concentration of the extract was calculated as $C_{extr} = C_{ad} * S_{extr} / (S_{tot} – S_{extr})$, where C_{ad} – concentration of the added compound in the extract, S_{extr} and S_{tot} – analyte peak area in the first and second analyses.

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Statistics

Data are plotted as box plots implemented in Seaborn (https://seaborn.pydata.org/) package (version 0.10, Python version 3.6). The boxes extend from the lower to upper quartile values of the data, horizontal line represents the median. Whiskers represent a full data range. Two-tailored Mann-Whitney U tests (Supplementary figure 3) were computed with scipy.stats package (https://www.scipy.org/, SciPy version 1.3.1). Scikit-posthocs Python package (https://pypi.org/project/scikit-posthocs/, version 0.6.2) was used for multiple pairwise post-hoc Mann-Whitney U-test with p-values corrected by step-down method using Sidak adjustments (Supplementary figure 9). Sample numbers (n), statistical tests used and exact P values can be found in figure legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data and material availability

Plasmids, plant lines and datasets generated in the current study are available from the corresponding authors upon reasonable request. Unprocessed images of luminescent flowers captured on Sony Alpha ILCE-7M3 camera and IVIS Spectrum CT are available from Figshare.

References

1. Krichevsky A, Meyers B, Vainstein A, Maliga P, Citovsky V. PLoS One. 2010; 5:e15461. [PubMed: 21103397]
2. Kotlobay AA, et al. Proc Natl Acad Sci U S A. 2018; 115:12728–12732. [PubMed: 30478037]
3. Yan Y, Yuheng LIN. US Patent. 2014
4. Kawamata S, et al. Plant Cell Physiol. 1997; 38:792–803. [PubMed: 9297845]
5. Gaquerel E, Gulati J, Baldwin IT. Plant J. 2014; 79:679–692. [PubMed: 24617849]
6. Toyota M, et al. Science. 2018; 361:1112–1115. [PubMed: 30213912]
7. Singh SK, et al. Sci Rep. 2015; 5:18148. [PubMed: 26670135]
8. Li L, et al. Sci Rep. 2016; 6:37976. [PubMed: 27897248]

9. Li W, et al. Sci Rep. 2017; 7:12126. [PubMed: 28935979]
10. Woo HR, Kim HJ, Nam HG, Lim PO. Journal of Cell Science. 2013; 126:4823–4833. [PubMed: 24144694]
11. Pauwels L, et al. Proc Natl Acad Sci U S A. 2008; 105:1380–1385. [PubMed: 18216250]
12. Bernards MA, Båstrup-Spohr L. Induced Plant Resistance to Herbivory. 2008:189–211.
13. Singh R, Rastogi S, Dwivedi UN. Compr Rev Food Sci Food Saf. 2010
14. Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S. PLoS One. 2011; 6:e16765. [PubMed: 21364738]
15. Iverson SV, Haddock TL, Beal J, Densmore DM. ACS Synth Biol. 2016; 5:99–103. [PubMed: 26479688]
16. Lazo GR, Stein PA, Ludwig RA. Biotechnology. 1991; 9:963–967. [PubMed: 1368724]
17. Rogers SO, Bendich AJ. Plant Molecular Biology Manual. 1994:183–190.

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Editors summary

Luminescence is engineered in whole plants, without an exogenous substrate, using a fungal gene cluster.

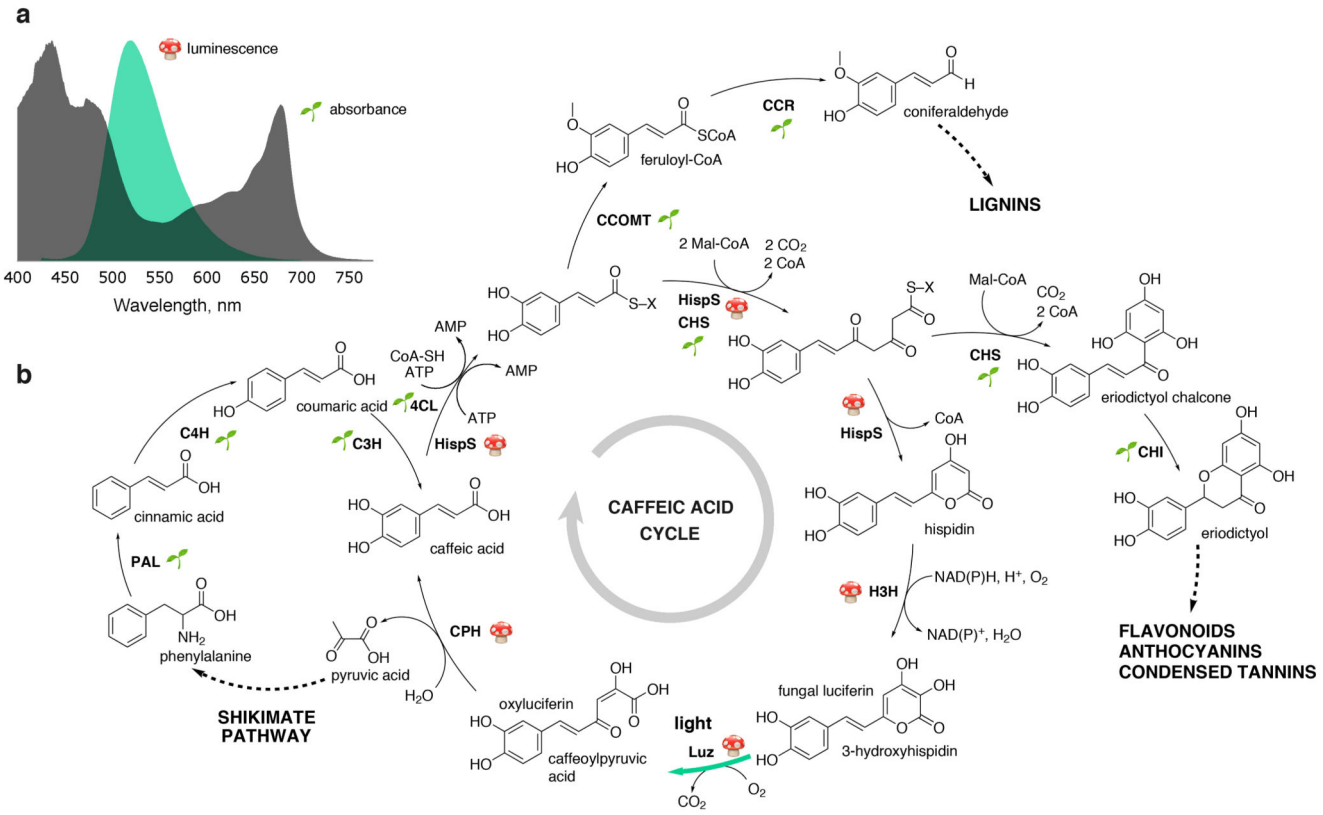

Figure 1. Features of the fungal bioluminescence system.

a. Spectrum of fungal bioluminescence (*Neonothopanus nambi*, in green) overlaid onto the absorbance spectrum of plant leaves (*Nicotiana tabacum*, in dark gray). **b.** The caffeic acid cycle shares metabolites with some of the major plant biosynthetic pathways. The fungal or plant origin of enzymes is indicated with mushroom and plantlet symbols, respectively. Abbreviations: 4CL — 4-coumarate:CoA ligase; C3H — p-coumaric acid 3-hydroxylase; C4H — cinnamic acid 4-hydroxylase; CCOMT — caffeoyl-CoA 3-O-methyltransferase; CCR — cinnamoyl-CoA reductase; CHI — chalcone isomerase; CHS — chalcone synthase; CPH — putative caffeoyl pyruvate hydrolase; H3H — hispidin-3-hydroxylase; HispS — hispidin synthase; Luz — luciferase; PAL — phenylalanine ammonia-lyase. Absorbance spectrum of leave is representative of experiment performed on three leaves. Luminescence spectrum is rendered from dataset published in Ref. 3

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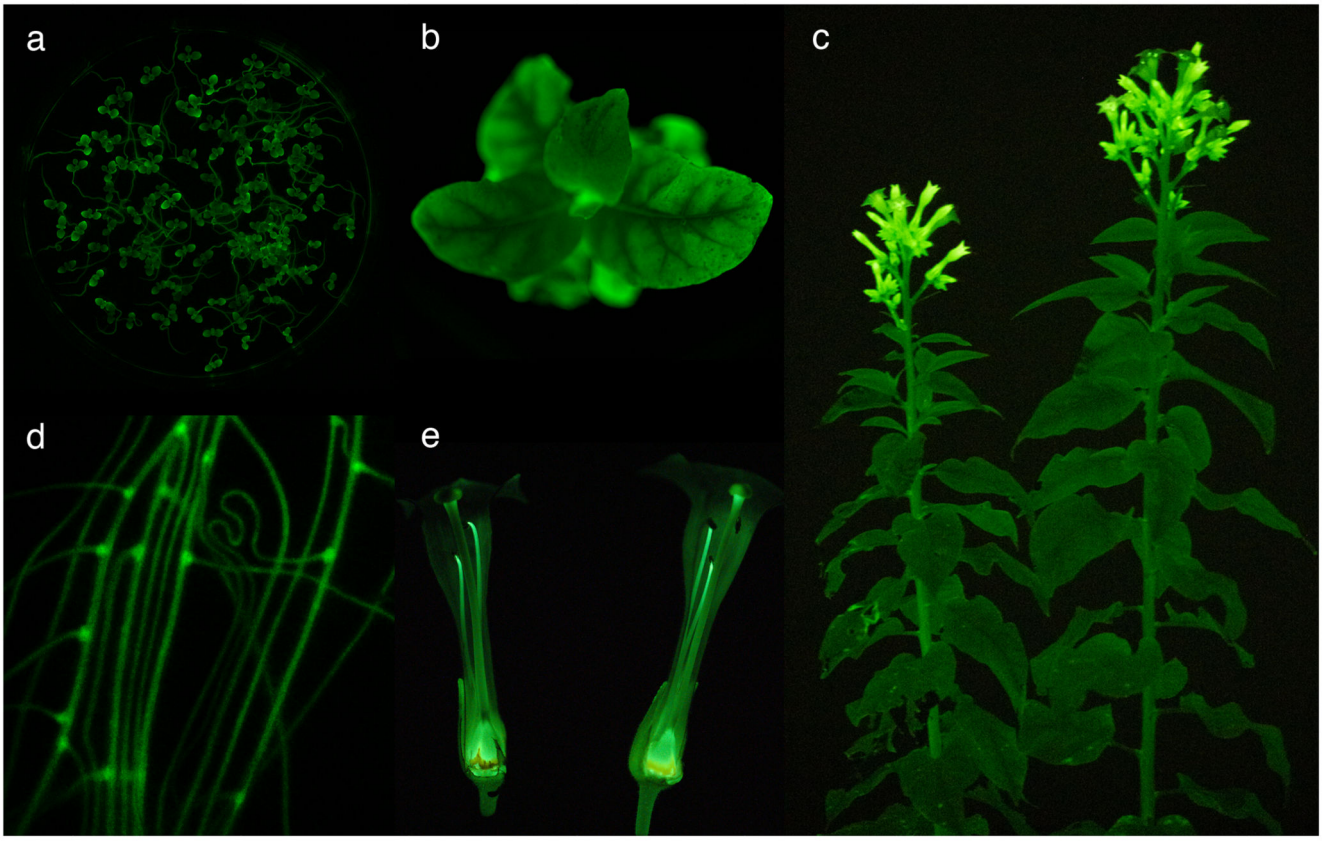

Figure 2. Bioluminescent plants at various stages of development.

a. Light emission from *N. tabacum* plants at germination (**i**), vegetative (**ii**) and flowering (**iii**) stages; light emission from roots (**iv**) and cross section of flowers (v). Photos were captured on Sony Alpha ILCE-7M3 (Online Methods). The 110 seedlings depicted on panel (i) are representative of three independent experiments. Images of plants in vegetative (ii, 3 weeks) and flowering (iii, 8 weeks) stages, as well as individual flowers (v) 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 on panel (iv) is representative of three independent imaging experiments on six plants.