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Supplementary Information for

Reassessing the claimed cytokinin-substituting activity of dehydrodiconiferyl alcohol glucoside (DCG)

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Supplementary text
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Material and methods

Chemicals

All compounds were purchased from Sigma-Aldrich unless mentioned otherwise. All concentrated stock solutions were made in dimethylsulfoxide (DMSO; commercial grade).

Chemical synthesis

Dehydrodiconiferyl alcohol glucoside (DCG)

DCG was prepared by coupling of diacetoxy-dehydrodiconiferyl alcohol and acetobromo-α-D-glucose (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide) followed by deacetylation.

Dehydrodiconiferyl alcohol (DDC)

To synthesize DDC, diethyl (8-5)-dehydrodiferulate was first prepared by radical coupling of ethyl ferulate using horseradish peroxidase and hydrogen peroxide in pH 4.0 acetate buffer (1). The dehydrodiferulate ester (1 g, 2.26 mmol) was completely dissolved in anhydrous toluene (200 mL), under nitrogen, and cooled in an ice bath for 30 min. Diisobutylaluminum hydride (DIBAL-H, 1 M in toluene, 13.56 mmol) was slowly added *via* syringe and stirred for 1 h. The reaction was carefully quenched with ethanol. The reaction mixture was poured into EtOAc and washed with RO water (× 1) and satd. NH_4Cl (× 3). The collected EtOAc layer was dried over anhydrous magnesium sulfate ($MgSO_4$), the inorganics were filtered off, and the solution evaporated. The crude product was purified by flash column chromatography using hexane and EtOAc as eluant to obtain 414 mg (1.15 mmol, 51%) of the pure product as a colorless oil.

Diacetoxy-dehydrodiconiferyl alcohol

DDC (850 mg, 2.37 mmol) was acetylated in acetic anhydride (15 mL) and pyridine (15 mL) overnight at room temperature. The reaction solution was directly dried on an evaporator to obtain a yellow oil in a quantitative yield of the peracetylated DDC. The triacetoxy-dehydrodiconiferyl alcohol (1.1 g, 2.27 mmol) was dissolved in pyrrolidine (20 mL) with shaking and poured into 250 mL of EtOAc. The organic layer was washed with 1 M H_2SO_4 (× 3), dried over anhydrous $MgSO_4$, and the solvent removed under vacuum. Purification by flash column chromatography provided 810 mg (1.82 mmol, 80%) of the final product as a colorless oil. ^{1}H NMR (360 MHz, acetone-d_6): δ 7.63 (br s, 1H, A4-OH), 7.05 (d, J = 1.9 Hz, 1H, A2), 7.033 (br s, 1H, B2), 7.017 (br s, 1H, B6), 6.89 (dd, J = 8.1, 1.9 Hz, 1H, A6), 6.83 (d, J = 8.1 Hz, 1H, A5), 6.63 (dt, J = 15.8, 1.1 Hz, 1H, Bα), 6.23 (dt, J = 15.8, 6.5 Hz, 1H, Bβ), 5.50 (d, J = 7.3 Hz, 1H, Aα), 4.66 (dd, J = 6.5, 1.1 Hz, 2H, Bγ), 4.42 (dd, J = 11.1, 5.5 Hz, 1H, Aγ1), 4.32 (dd, J = 11.1, 7.4 Hz, 1H, Aγ2), 3.86 (s, 3H, B-OMe), 3.82 (s, 3H, A-OMe), 3.77 (m, 1H, Aβ), 2.02 (s, 3H, Bγ-OAc), 1.99 (s, 3H, Aγ-OAc); ^{13}C NMR: δ 170.9 (Aγ-OAc), 170.8 (Bγ-OAc), 149.3 (B4), 148.4 (A3), 147.5 (A4), 145.3 (B3), 134.8 (Bα), 133.3 (A1), 131.3 (B1), 129.2 (B5), 122.1 (Bβ), 119.9 (A6), 116.3 (B6), 115.7 (A5), 112.1 (B2), 110.5 (A2), 89.0 (Aα), 65.9 (Aγ), 65.5 (Bγ), 56.4 (B-OMe), 56.3 (A-OMe), 51.1 (Aβ), 20.8 (Bγ-OAc), 20.7 (Aγ-OAc).

Diacetoxy-dehydrodiconiferyl alcohol tetra-O-acetyl-β-D-glucopyranoside

The coupling approach between diacetoxy-dehydrodiconiferyl alcohol and tetra-*O*-acetyl-β-D-glucopyranosyl bromide was based on a previous study (2). Diacetoxy-dehydrodiconiferyl alcohol (920 mg, 2.08 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide (2.56 g, 6.23 mmol) were dissolved in quinoline (10 mL), and silver oxide (Ag_2O, 482 mg, 2.08 mmol) was added. The reaction mixture was stirred for 4 h at room temperature. The reaction mixture was then poured into EtOAc (200 mL) in a separatory funnel and washed with 1 M H_2SO_4

(× 3) and sat. NH_4Cl (× 3) before being dried over anhydrous $MgSO_4$. The inorganics were filtered off and the solvent evaporated under vacuum resulting in the crude product as an oil. Purification by flash-chromatography produced 1.37 g (1.77 mmol, 85%) of the final product. ^{1}H NMR (360 MHz, acetone-d_6): δ 7.19 (d, *J* = 8.3 Hz, 1H, A5), 7.11 (d, *J* = 2.0 Hz, 1H, A2), 7.05 (br s, 1H, B2), 7.03 (br s, 1H, B6), 6.96 (dd, *J* = 8.3, 2.0 Hz, 1H, A6), 6.64 (dt, *J* = 15.9 Hz, 1.2, 1H, Bα), 6.23 (dt, *J* = 15.9 Hz, 6.5, 1H, Bβ), 5.57 (d, *J* = 6.7 Hz, 1H, Aα), 5.35 (dd, *J* = 9.4, 9.3 Hz, 1H, 3′), 5.24 (dd, *J* = 7.9, 1.6 Hz, 1H, 1′), 5.18 (dd, *J* = 9.3, 7.9 Hz, 1H, 2′), 5.10 (dd, *J* = 9.7, 9.6 Hz, 1H, 4′), 4.66 (dt, *J* = 6.5, 1.2 Hz, 2H, Bγ), 4.44 (dd, *J* = 11.1, 5.5 Hz, 1H, Aγ1), 4.33 (dd, *J* = 11.1, 7.5 Hz, 1H, Aγ2), 4.28 (m, 1H, 6′b), 4.14 (dd, *J* = 12.3, 2.4 Hz, 1H, 6′a), 4.09 (m, 1H, 5′), 3.88 (s, 3H, B-OMe), 3.81 (s, 3H, A-OMe), 3.76 (m, 1H, Aβ), 2.03 to 1.95 (6s, 18H); ^{13}C NMR: δ 170.9−169.6 (six acetate carbonyl peaks), 151.6 (A3), 149.3 (B4), 147.2 (A4), 145.4 (B3), 138.3 (A1), 134.7 (Bα), 131.6 (B1), 129.0 (B5), 122.3 (Bβ), 120.0 (A5), 119.1 (A6), 116.3 (B6), 112.2 (B2), 111.7 (A2), 100.9 (1′), 88.4 (Aα), 73.3 (3′), 72.6 (5′), 72.0 (2′), 69.4 (4′), 65.9 (Aγ), 65.5 (Bγ), 62.7 (6′), 56.6 (A-OMe), 56.4 (B-OMe), 51.3 (Aβ), 20.79−20.51 (six acetate methyl peaks).

Dehydrodiconiferyl alcohol 4-O-glucopyranoside [Glc-G(8–5)G, (*E*)-2-(hydroxymethyl)-6-(4-(3-(hydroxymethyl)-5-(3-hydroxyprop-1-en-1-yl)-7-methoxy-2,3-dihydrobenzofuran-2-yl)-2-methoxy-phenoxy)tetrahydro-2*H*-pyran-3,4,5-triol]

Diacetoxy-dehydrodiconiferyl alcohol tetra-*O*-acetyl-β-D-glucopyranoside (333 mg, 0.43 mmol) was dissolved in 100 mL of THF, and the solution was kept in an ice bath for 30 min. The reaction flask was maintained under nitrogen before 1 M DIBAL-H (4.3 mmol) in toluene was slowly added, and stirred for 1 h. The reaction solution was quenched with ethanol, and the solvent directly removed on a rotary evaporator. The obtained yellow solid was treated with hot water (100 mL × 3) and the product was extracted from the solid by vacuum filtration. Following water removal by evaporation, a colorless oil was obtained. Purification by TLC (EtOAc: MeOH, 10:1, v/v) afforded the required dehydrodiconiferyl alcohol 4-*O*-glucopyranoside (139 mg, 0.26 mmol, 62%). ^{1}H NMR (500 MHz, MeOH-d_4): δ 7.14 (d, *J* = 8.4 Hz, 1H, A5), 7.02 (d, *J* = 1.9 Hz, 1H, A2), 6.95 (br s, 2H, B2 & 6), 6.92 (dd, *J* = 8.4, 1.9 Hz, 1H, A6), 6.53 (dt, *J* = 15.8, 1.1 Hz, 1H, Bα), 6.22 (dt, *J* = 15.8, 6.0 Hz, 1H, Bβ), 5.58 (d, *J* = 6.0 Hz, 1H, Aα), 4.89 (1H, 1′), 4.19 (dd, *J* = 5.8, 1.1 Hz, 2H, Bγ), 3.88 (s, 3H, B-OMe), 3.87 (m, 1H, 6′b), 3.83 (m, 1H, Aγ1), 3.82 (s, 3H, A-OMe), 3.76 (m, 1H, Aγ2), 3.67 (m, 1H, 6′a), 3.46 (m, 3H, Aβ & 2′ & 5′), 3.38 (m, 2H, 3′ & 4′); ^{13}C NMR: δ 150.8 (A3), 149.1 (B4), 147.5 (A4), 145.4 (B3), 138.0 (A1), 132.7 (B5), 131.9 (Bα), 130.0 (B1), 127.7 (Bβ), 119.4 (A6), 118.0 (A5), 116.5 (B6), 112.0 (B2), 111.2 (A2), 102.6 (1′), 88.7 (Aα), 78.0 (3′), 77.7 (5′), 74.8 (2′), 71.2 (4′), 64.9 (Aγ), 63.8 (Bγ), 62.4 (6′), 56.8 (B-OMe), 56.7 (A-OMe), 55.2 (Aβ).

Solution-state nuclear magnetic resonance (NMR) experiments

NMR experiments for the compounds were performed in acetone-d_6 or MeOH-d_4. NMR spectra were acquired on a Bruker Biospin (Billerica, MA) Avance 360 MHz with a room temperature probe or an Avance 500 MHz spectrometer equipped with a 5mm TCI ^{1}H/^{13}C/^{15}N cryoprobe with inverse geometry (proton coils closest to the sample). The central acetone or MeOH solvent peaks were used as the internal reference (δ_C 29.8 and δ_H 2.04 ppm for acetone; δ_C 49.0 and δ_H 3.30 ppm for MeOH). Typical standard Bruker implementations of the traditional suite of 1D (^{1}H and ^{13}C) and 2D NMR experiments (DEPT-135, COSY, HSQC, and HMBC) were used to elucidate/validate the structures. A small line broadening (LB) value (0.1 Hz) in the Window Function and zero-filling (SI = 256K) were applied to see the clear splitting of A3, A5, and Aα peaks from the DDC moiety, as well as the glucoside 1′ peak.

Plant growth conditions

Arabidopsis thaliana Col-0 seeds were surface sterilized overnight with chlorine gas in a closed container. The chlorine gas was generated by mixing 150 mL NaOCl (12-14%) with 8 mL 37% HCl. Sterile seeds were sown on plates containing 0.5x Murashige and Skoog (MS) agar medium (1.5 $g.L^{-1}$ MS basal mixture powder (Duchefa), 10 $g.L^{-1}$ sucrose, 0.5 $g.L^{-1}$ MES monohydrate, 8 $g.L^{-1}$ Plant Tissue Culture Agar (Negogen); pH 5.7 with KOH). After sowing, seeds were stratified for 48 h at 4 °C, whereupon plates were transferred to a growth chamber (21 °C; 16 h light/8 h dark photoperiod). *Nicotiana tabacum* cv. Havana 425 seeds were sown in 13-cm pots containing universal potting soil (Saniflor). Plants were grown under long day greenhouse conditions (21 °C; 16 h light/8 h dark photoperiod).

Projected rosette area measurement

A segregating *c3'h* population was grown on horizontal plates (12 x 12 cm) containing 0.5x MS medium as described above. 10 days after stratification (DAS), pictures were taken using a Nikon D3200 camera mounted in a vertical setup and the projected rosette area of individual plants was measured using ImageJ software (3). The latter was done by converting the picture to binary values, after which the 'magic stick' tool was used to select the pixels that represent the seedling's rosette. Subsequently, one cotyledon of every plant was harvested for genotyping by PCR. DNA was extracted using the Edwards DNA extraction protocol (4). The primers used for genotyping are listen in table S1. Every PCR mixture had a total volume of 25 µL, consisting of 5 µL DNA template, 2.5 µL 5X Green GoTaq® Flexi Reaction Buffer (Promega), 1.25 µL $MgCl_2$, 0.5 µL dNTP Mix (Promega), 1 µL of each primer (10 µM), 0.25 µL GoTaq® G2 Flexi DNA Polymerase (Promega), and 13.5 µL miliQ water. Following PCR program was used; 5 min of initial denaturation (95 °C), followed by 37 consecutive cycles of 30 s denaturation (95 °C), 30 s annealing (55 °C), 1 min extending (72 °C). PCR products were visualized by agarose gel electrophoresis.

Quantifying DCG content

After confirmation of the genotype as described above, plants homozygous for the presence and for the absence of the T-DNA insert were harvested for metabolic analysis. For wild-type and mutant samples, respectively 3 and 5 seedlings were pooled together in 2-mL Eppendorf tubes. In case leaf explants were used, one leaf disc was transferred to a 2-mL Eppendorf tube. The tubes with plant biomass were flash-frozen in liquid nitrogen and the plant material was subsequently ground with two 3-mm iron beads using a Retsch MM200 mixer mill (1 min, 30 Hz). For metabolite extraction 1 mL 100% methanol was added to the ground and frozen tissue, whereupon the samples were incubated for 20 min at room temperature in an Eppendorf Thermomixer (470 rpm). Next, samples were centrifuged for 3 min in a table top centrifuge (Eppendorf) at 14 000 rpm, whereupon 800 µL of the supernatants was transferred to a fresh 2-mL Eppendorf tube. Samples were subsequently dried in a SpeedVac 7810010 (Labconco), and the dried pellet was resuspended in 100 µL cyclohexane. MiliQ water (100 µL) was added and thoroughly mixed. Samples were centrifugated (15 min, 14 000 rpm), whereupon the liquid (lower) phase was used for metabolite analysis. Samples were subjected to Ultra Performance Liquid Chromatography High Resolution Mass Spectrometry (UPLC-HRMS) at the VIB Metabolomics Core Ghent (VIB-MCG). 10 ul was injected on a Waters Acquity UHPLC device connected to a Vion HDMS Q-TOF mass spectrometer (Waters,

Manchester, UK). Chromatographic separation was carried out on an ACQUITY UPLC BEH C18 (150 × 2.1 mm, 1.7 µm) column (Waters, USA), column temperature was maintained at 40 °C. A gradient of two buffers was used for separation: buffer A (100:0.1 water:formic acid, pH 3) and buffer B (100:0.1 acetonitrile:formic acid, pH 3), as follows: 99% A for 0 min decreased to 50% A in 30 min, decreased to 30% from 30 to 35 minutes, and decreased to 0% from 35 to 37 minutes. The flow rate was set to 0.35 $mL.min^{-1}$. Electrospray Ionization (ESI) was applied, LockSpray ion source was operated in negative ionization mode under the following specific conditions: capillary voltage, 2.5 kV; reference capillary voltage, 3 kV; source temperature, 120 °C; desolvation gas temperature, 550 °C; desolvation gas flow, 800 $L.h^{-1}$; and cone gas flow, 50 $L.h^{-1}$. The collision energy for full MS scan was set at 6 eV for low energy settings, for high energy settings (MSe) it was ramped from 20 to 70 eV. For DDA-MS/MS energy settings were ramped from 10-35eV for low mass, and from 35-70eV for high mass. Mass range was set from 50 to 1500 Da, scan time was set at 0.1s. Nitrogen (greater than 99.5%) was employed as desolvation and cone gas. Leucine-enkephalin (100 $pg.\mu L^{-1}$ solubilized in water:acetonitrile 1:1 [v/v], with 0.1% formic acid) was used for the lock mass calibration, with scanning every 2 min at a scan time of 0.1 s. Profile data was recorded through Unifi Workstation v2.0 (Waters). Data processing was done with Progenesis QI v2.4 (Waters).

Identifying and quantifying DDC

The method for DDC detection was as described for DCG detection.

RT-qPCR

A. thaliana Col-0 plants were grown on 0.5x MS medium as described above. 7 DAS, seedlings were transferred to 12-well plates (VWR) containing liquid 0.5x MS medium supplemented with a compound of interest. DMSO was used for mock treated controls. The liquid 0.5x MS medium was identical to the solid medium from which agar was omitted. Four hours after transfer, four plants of a particular treatment were pooled in a 2-mL Eppendorf tube flash-frozen in liquid nitrogen and subsequently ground with two 3 mm iron beads using a Retsch MM200 mixer mill (1 min, 30 Hz). For each treatment, eight biological replicates were used. RNA was extracted using Reliaprep™ RNA tissue miniprep kit (Promega) according to the manufacturer's instructions, which included a DNase treatment. Extracted RNA was eluted from the spin column using 30 µL of RNAse free water whereupon the eluted total RNA was quantified using a ND-1000 Nanodrop spectrophotometer (Thermo Fisher Scientific). One µg total RNA was converted to cDNA using the qScript® cDNA SuperMix (Quantbio) according to the manufacturer's instructions; 4 µL qScript cDNA SuperMix (5x) was combined with a volume that contains 1 µg RNA, topped off with water so the solution's total volume was 20 µL. Relative expression of the selected genes was determined with the Roche LightCycler 480 combined with the SYBR Green I master Kit (Roche Diagnostics) using following PCR protocol: one activation cycle of 10 min (95 °C); 45 amplification cycles of 10 s (95 °C), 10 s (60 °C) and 10 s (72 °C). Each biological repeat sample was run in triplicate to identify potential technical variation that could have occurred during pipetting. Every sample had a total volume of 20 µL, consisting of 10 µL 2x SYBR Green mix, 8 µL primer mix (1 µM), and 2 µL cDNA. Fluorescence values were exported from the Lightcycler 480 program, after which *C*t values, normalization factors and primer efficiencies were calculated according to (5) using two reference genes: *POLYUBIQUITIN10* (*UBQ10*; AT4G05320) and

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE C-2 (*GAPC2*; AT1G13440). Primers used for RT-qPCR can be found in table S1.

Reporter line assays

All reporter lines were grown on solid 0.5x MS medium containing the compound of interest as described above. After respectively 5, 7 and 9 DAS *pARR4::3xYFP* seedlings were fixated and cleared using a ClearSee solution as described in (6). Cell walls of these samples were subsequently counterstained with 0.1% Calcofluor white M2R (Fluorescent brightener 28) (Polysciences, CAT#4359) solution for 1 h. After washing twice with ClearSee solution, Calcofluor White and YFP fluorescence signal was analyzed using 405 nm and 509 nm excitation and detection at 425-475 nm and 527 nm, respectively. *pCYCD3.2::GUS* seedlings were sampled at similar timepoints and cleared overnight in 80% ice-cold acetone. After washing the samples twice, seedlings were incubated for 20 minutes at 37 °C in X-gluc buffer (98% P-buffer, 1% 0.12 M X-glux, 2 mM $K_3[Fe(CN)_6]$, 2 mM $K_4[Fe(CN)_6]\cdot 3H_2O$). After overnight de-staining in 70% ethanol, GUS staining was inspected using the Keyence VHX-7000 digital microscope.

Explant assays

The callus assays were performed according to (7) following the technical details as described in the original paper as close as possible. Briefly, 12 to 15 cm long leaves were cut from 35 to 45 cm tall soil-grown *N. tabacum* cv. Havana 425 plants. Youngest- and oldest leaves were not sampled to ensure uniform sample collection. After washing the leaves with soap-containing milliQ (MQ) water, they were surface sterilized by three cycles of consecutive 7% bleach (NaOCl), water, and 70% ethanol (1 min each). The sterilization was followed by three consecutive rinses with sterile MQ water. To create leaf explants, 1 cm diameter circular explants were taken from the sterilized leaves, avoiding major veins. Fresh explants were immediately transferred to round petri dishes (diameter 3 cm) containing solid LS medium (4.4 $g.L^{-1}$ LS basal mixture powder (Duchefa), 30 $g.L^{-1}$ sucrose, 10 $g.L^{-1}$ Plant Tissue Culture Agar (Neogen); pH 5.7 with KOH). The plates were sealed with two layers of parafilm (Bemis) and subsequently transferred to a tissue culture room where they were incubated under a 16h light/8h dark regime at 24 °C. After a three weeks incubation period explants were photographed using a Nikon D3200 camera whereupon the callus tissue was removed from the leaf discs and the callus biomass was quantified using a micro balance (Mettler Toledo). For the pith assays, pith tissue was obtained from mature internodes of 35 and 45 cm tall soil-grown *N. tabacum* cv. Havana 425 plants. Internode tissue was sterilized as described above for leaves. Pith tissue was dissected from sterilized internodes, and subsequently cut into sections of approximately 10 mg. After measuring the weight of the explants using a micro balance (Mettler Toledo), they were transferred to solid LS medium, on which they were incubated as described earlier for the leaf explants. Three weeks after explanting, explants were imaged using a Nikon D3200 camera whereupon their biomass was qunatified using a micro balance (Mettler Toledo). The start and end values allowed to calculate the net increases in biomass during the three week incubation period.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.2 software. In case of 2 groups of independent data, we first checked the equality of variance using an F-test, and subsequently performed a two-sided Student t-test.

When given more than 2 groups of independent data, determined by one factor, we checked the normality and equality of variance. If the data fulfilled both these criteria, an ordinary one-way ANOVA test, followed by a POST-HOC test (Tukey or Dunnett multiple comparison) was performed. If not, non-parametric tests were used to test the null-hypothesis whereupon multiple comparison tests were performed. For normal data determined by more than one factor, a two-way ANOVA test combined with a Dunnett's multiple comparisons test was performed.

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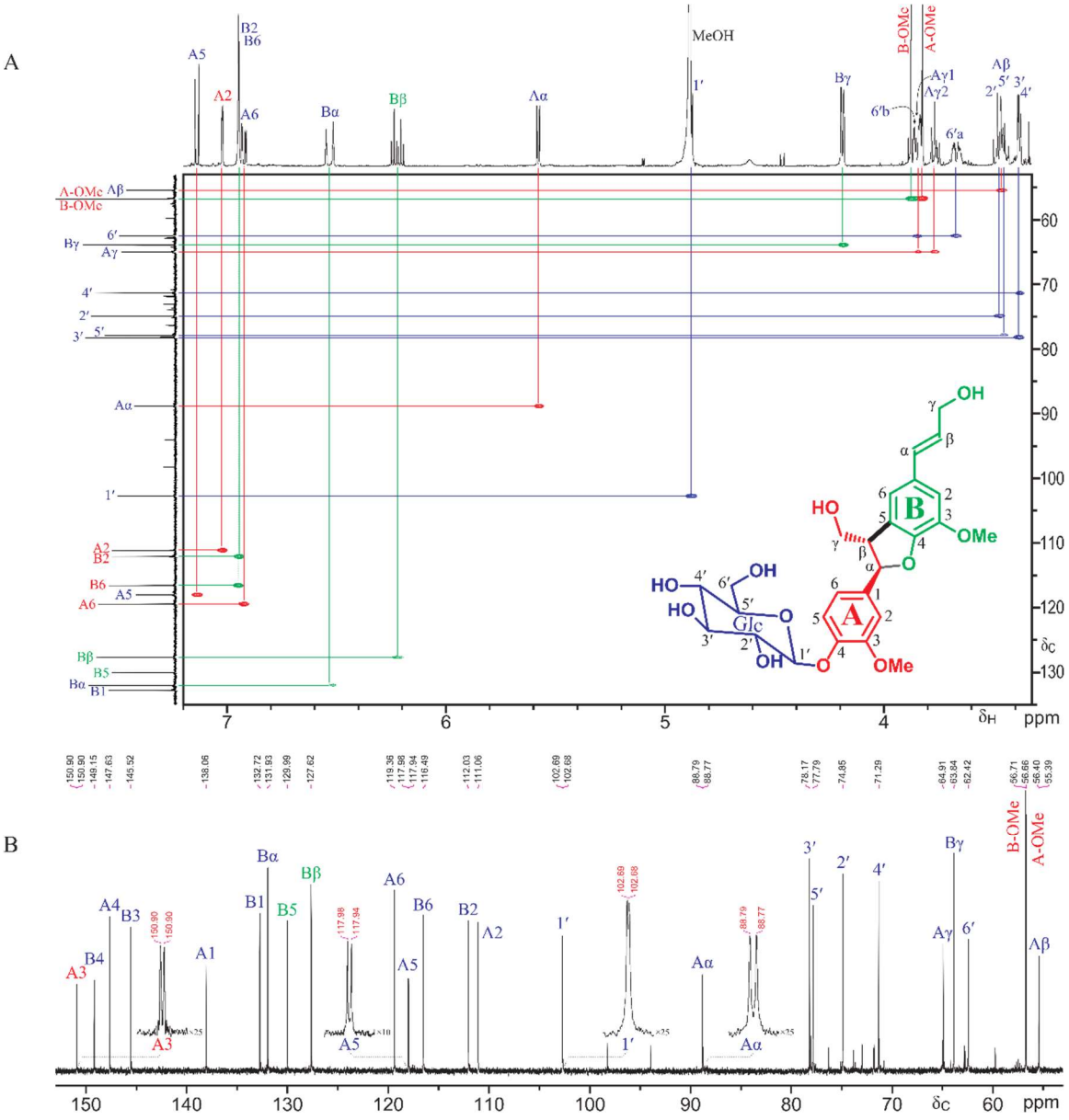

Figures S1: DCG NMR spectra at 500 MHz, sample in MeOH-d_4

(A) 2D HSQC spectrum with 1D proton and carbon projections and assignments. (B) Carbon NMR with expansions of 4 carbons showing their split nature due to the equal presence of the two stereoisomers of *trans*-DCG.

Also deposited as Supplementary Data ["DCG NMR data (PNAS).zip"; the NMR file is named "hki75T2-2020"] are the raw Bruker the raw Bruker Topspin dataset for DCG, including the 1D 1H and 13C NMR spectra, as well as the 2D HSQC, HMBC, and COSY spectra; the structure is included with the datasets as well as, as is the Bruker standard, all the parameters for processing. Processed data is not included with the file (to save space) but the full

detail saved with the processed spectra can be used to quickly regain the processed data used here in the following way.

1D ^{1}H NMR data

1. gfp (Resolution-enhancement via Gaussian apodization using the parameters stored followed by Fourier transformation and phase correction using the stored parameters and phases)
2. abs (automated baseline correction)

If the non-resolution-enhanced spectrum is required…

1. Set lb to 0.1 (or as desired)
2. efp (exponential multiplication followed by Fourier transform and phase correction)
3. abs (automated baseline correction)

1D ^{13}C NMR data

1. efp (exponential multiplication followed by Fourier transform and phase correction using the stored parameters). [LB is set to 0.1 Hz for resolution of stereoisomer peaks; LB can be set to 1 Hz if such resolution is not required]
2. abs (automated baseline correction)

2D HSQC Data

1. xfb (Fourier transformation and phase correction using the stored parameters in both dimensions).
2. abs2 (automated baseline correction in f2, the proton dimension)
3. abs1 (automated baseline correction in f1, the carbon dimension)

2D HMBC Data

1. xfb (Fourier transformation and in both dimensions using the stored parameters, and magnitude calculation).

2D COSY Data

1. xfb (Fourier transformation and in both dimensions using the stored parameters, and magnitude calculation)

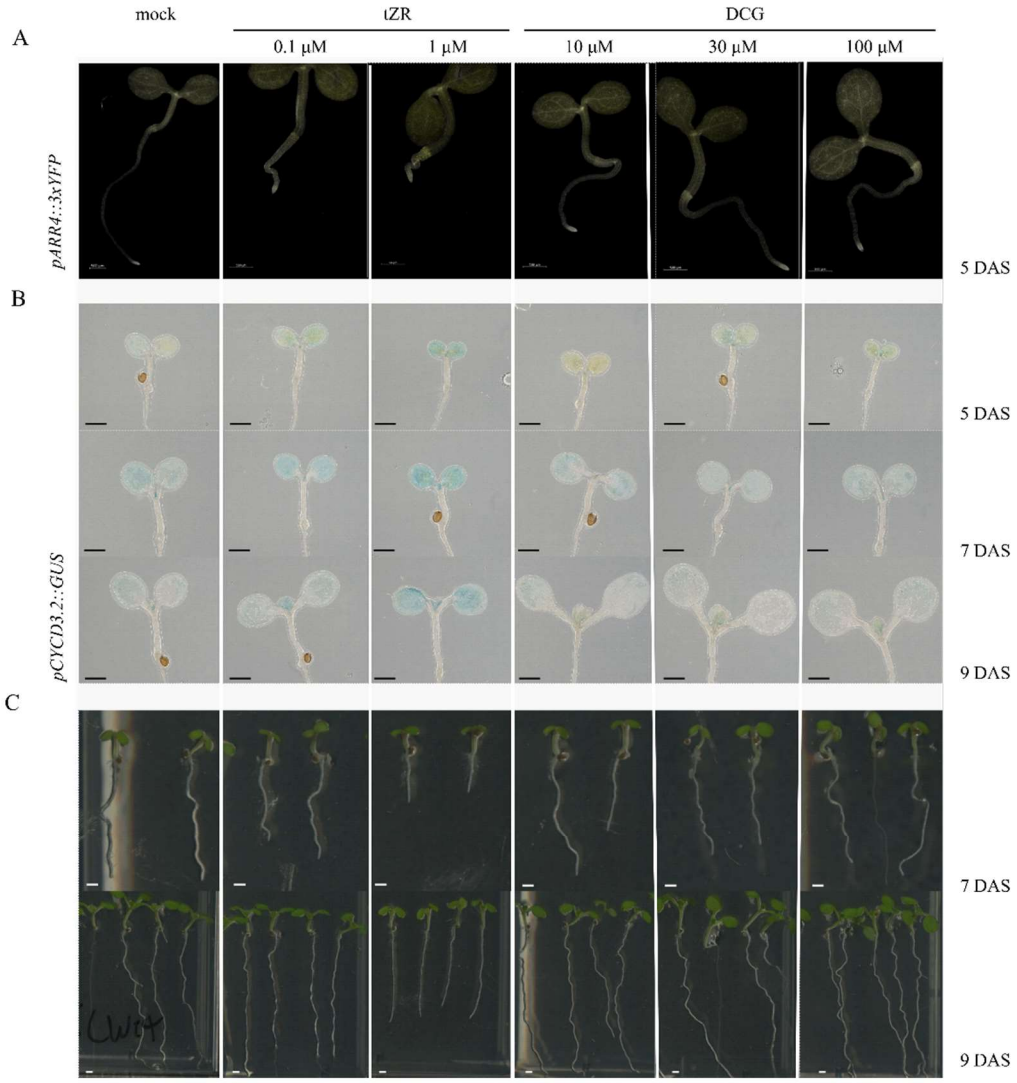

Figure S2: Effect of DCG on seedling phenotype and gene expression(A) Arabidopsis *pARR4::3xYFP* cytokinin reporter seedlings visualized using confocal microscopy 5 days after stratification (DAS). (B) Arabidopsis *pCYCD3.2::GUS* cell division reporter seedlings visualized using GUS-staining respectively 5, 7, and 9 DAS. (C) Arabidopsis wild-type seedlings 7 and 9 DAS. scale bars in (A), (B) and (C), represent 0.5, 1 and 1 mm respectively.

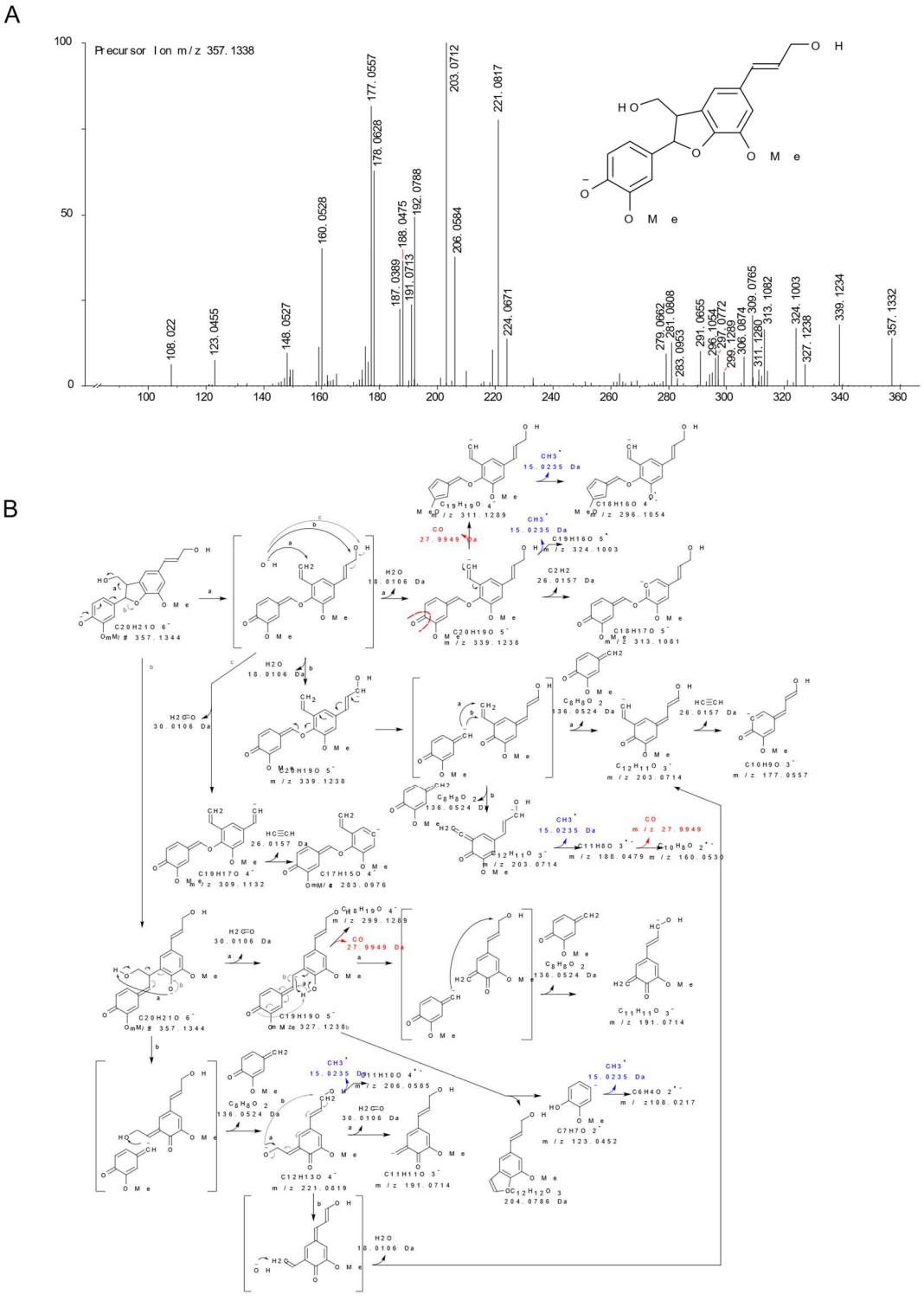

Figure S3: MS/MS fragmentation spectrum of DDC.

(A) Negative electrospray ionization (ESI) fragmentation pattern of DDC, precursor ion m/z 357.1337. (B) Proposed charge-driven collision-induced dissociation pathways of DDC.

Table S1. Primer list

Primer number	Primer name	Primer sequence
1	C3'H_fw	TCGGTCTGGATCGGTTCAATTCTAAACG
2	C3'H_rev	GTTGGATACTATGGCCTTGAACTCAAGC
3	*c3'h*_fw	GGAACAGATCGACGGAAGCATTTAGC
4	*c3'h*_rev	TTGCTTTCGCCTATAAATACGACGGATC
5	ARR4_fw	AGCCGTTGATGACAGTCTCGTTG
6	ARR4_rev	CCACTATCTACCGCCGTAACTTTG
7	ARR5_fw	AGTTCGGTTGGATTTGAGGATCTG
8	ARR5_rev	TCCAGTCATCCCAGGCATAGAG
9	CYCD3.2_fw	TCTCAGCTTGTTGCTGTGGCTTC
10	CYCD3.2_rev	TCTTGCTTCTTCCACTTGGAGGTC
11	UBQ10_fw	GAAGTTCAATGTTTCGTTTCATGT
12	UBQ10_rev	GGATTATACAAGGCCCCAAAA
13	ACT8_fw	GGATCTCTAAGGCAGAGTATGA
14	ACT8_rev	TCTCCAAACGCTGTAACCGGA
15	GPC2_fw	ACCACTGTCCACTCTATCACTGC
16	GPC2_rev	TGAGGGATGGCAACACTTTCCC