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Supplementary Information for

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

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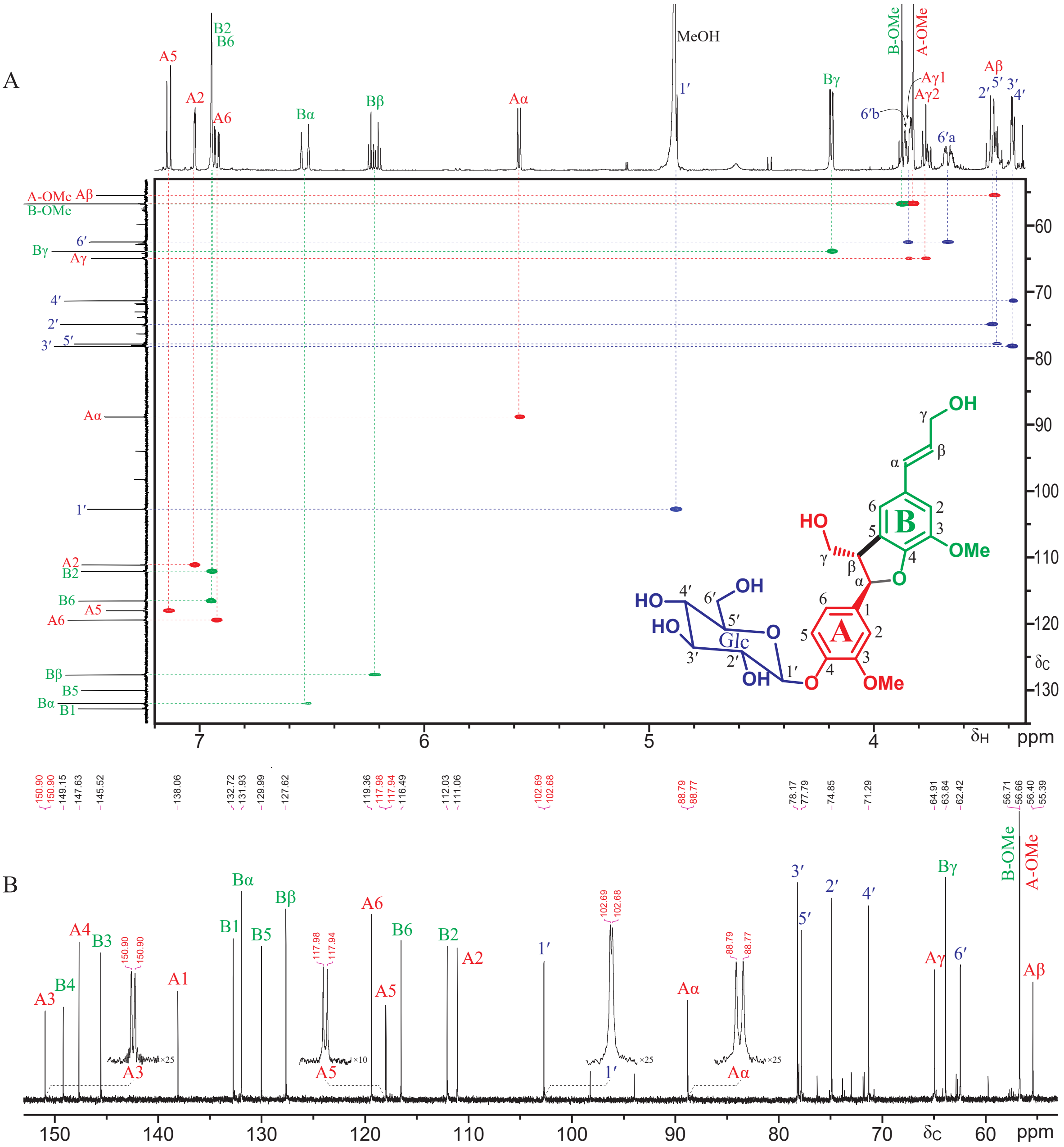

Figure S1: DCG NMR spectra at 500 MHz, sample in MeOH-d$_4$

(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 diastereomers of *trans*-DCG.

The NMR data is also shared on figshare (https://figshare.com/) with DOI "10.6084/m9.figshare.21971948". The NMR file is named "hki75T2 -2020" and contains the raw Bruker Topspin dataset for DCG, including the 1D ^{1}H and ^{13}C NMR spectra, as well as the 2D HSQC, HMBC, and COSY spectra; the structure is included with the datasets as well as 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 in both dimensions using the stored parameters, and magnitude calculation).

2D COSY Data

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

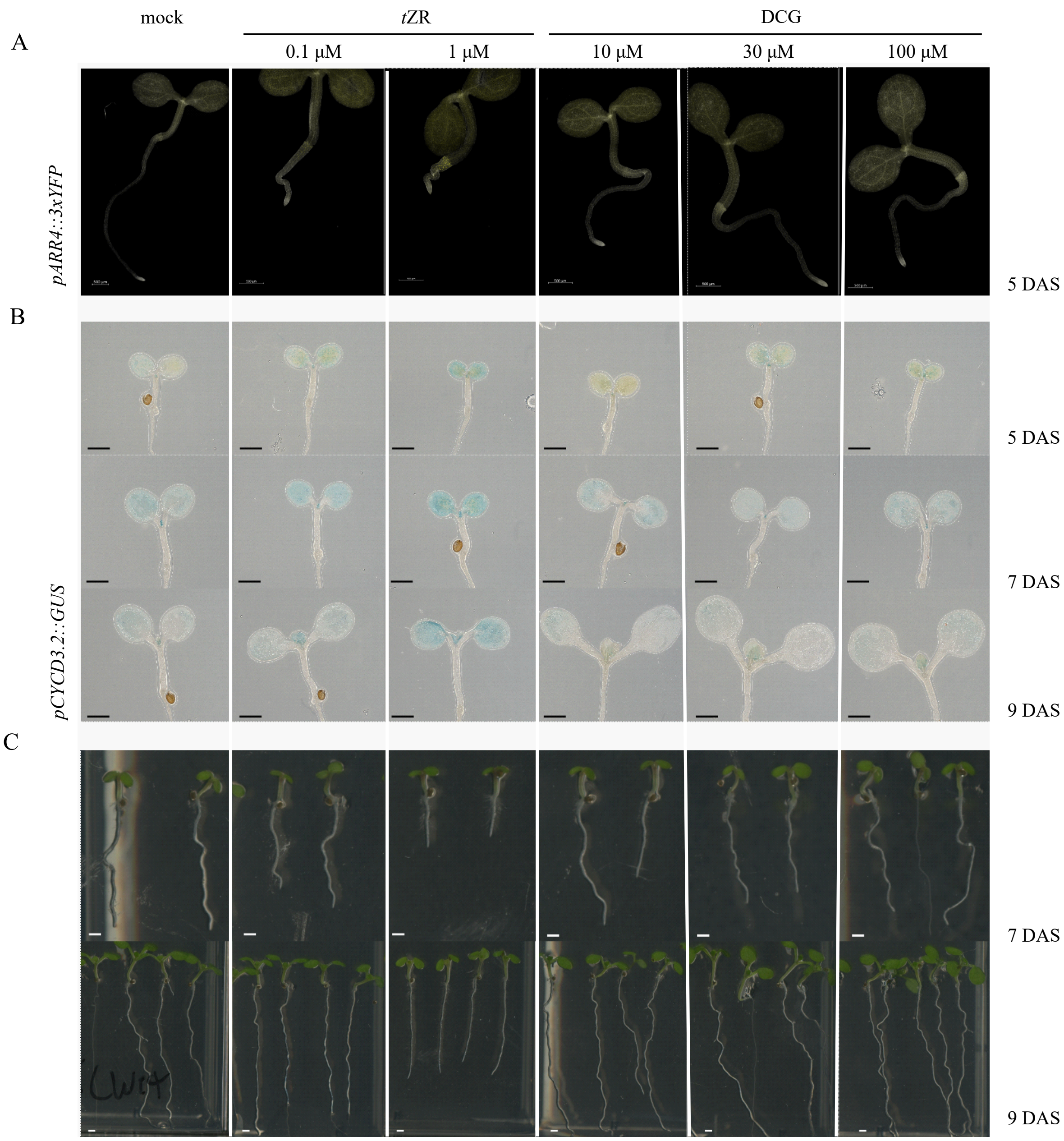

Figure S2: Effect of DCG on seedling phenotype and gene expression.

(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 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.

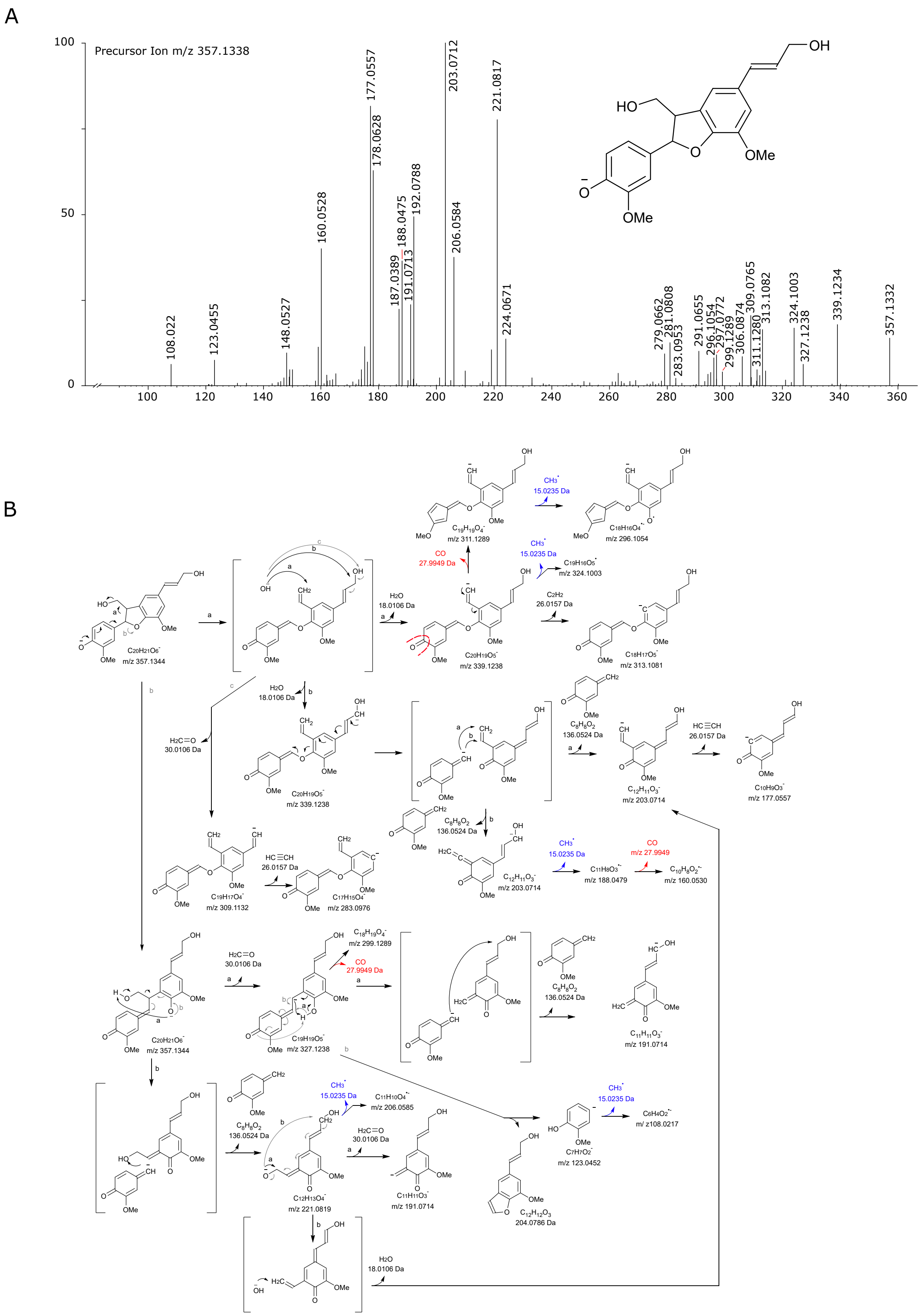

Figure S3: MS/MS fragmentation spectrum of DDC.

(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. Fresh weight of callus isolated from tobacco leaf explants, 21 days after explanting on LS medium supplemented with either *t*ZR (1 µm) or DCG (10 or 30 µm) in combination with NAA (1 µm).

1 µm NAA (mg)	1 µm NAA + 1 µm *t* ZA (mg)	1 µm NAA + 10 µm DCG (mg)	1 µm NAA + 30 µm DCG (mg)
0.0	178.3	0.0	18.0
0.0	0.0	0.0	0.0
9.1	307.9	0.0	0.0
0.0	18.3	0.0	25.2
10.0	476.4	0.0	0.0
0.0	49.9	0.0	0.0
0.0	0.0	0.0	8.0
12.6	47.2	0.0	0.0
0.0	402.9	0.0	0.0
0.0	47.1	0.0	17.6
0.0			0.0
0.0			27.3
			0.0
			0.0

Table S2. Fresh weight of tobacco pith explants, 21 days after explanting on LS medium supplemented with either 1 µm *t*ZR or 30 µm DCG in combination with 1 µm NAA.

1 µm NAA (mg)	1 µm NAA + 1 µm *t* ZA (mg)	1 µm NAA + 10 µm DCG (mg)	1 µm NAA + 30 µm DCG (mg)
499.3	352.0	266.5	369.8
488.4	711.8	125.7	577.5
242.8	928.5	274.2	475.0
221.5	200.6	588.9	546.4
224.0	301.5	417.6	282.9
224.4	362.2	284.8	493.4
320.1	768.4	346.8	423.8
477.4	677.0	549.0	605.0
301.3	642.0	410.6	377.4
	364.3	207.0	113.1
	907.8		103.2
	540.2		124.4
	364.9		700.7
	735.4		
	688.2		

Table S3. Fresh weight of callus isolated from tobacco leaf explants, 21 days after explanting on LS medium supplemented with either 1 µm *t*ZR or 30 µm DCG in combination with 1 µm NAA.

1 µm NAA (mg)	1 µm NAA + 1 µm *t*ZA (mg)	1 µm NAA + 30 µm DCG (mg)
8.6	136.2	29.1
0.0	116.9	0.3
0.0	75.4	4.9
7.1	119.3	18.9
21.8	133.9	4.7
1.0	52.2	36.1
9.8	181.8	26.9
14.4	144.4	13.4
22.2	168.1	20.9
6.8	112.5	8.8
4.3	107.4	10.1
8.4	239.5	0.5
7.4	228.1	6.5
20.7	152.8	45.1
21.4	217.2	0.0
2.4	216.6	21.3
4.6	166.1	0.0
0.6	151.0	14.3
1.9	293.0	19.4
8.4	178.4	10.5
12.3	110.2	28.0
21.5	226.5	11.6
28.1	313.6	0.0
6.1	155.8	26.4
0.0	99.6	0.0
2.9	190.0	17.1
7.0	198.2	0.0
5.1	105.8	0.0
21.6	179.2	0.0
2.7	191.1	26.1
6.6	202.5	14.5
10.7	28.6	6.0
0.4		20.5
0.0		25.6
9.6		11.8
18.8		20.5
0.5		0.5
23.1		19.2
27.6		
5.4		

Table S4. Fresh weight of tobacco pith explants, 21 days after explanting on LS medium supplemented with either 1 µm *t*ZR or 30 µm DCG in combination with 1 µm NAA.

1 µm NAA (mg)	1 µm NAA + 1 µm *t* ZA (mg)	1 µm NAA + 30 µm DCG (mg)
192.6	576.6	227.6
282.6	1257.6	315.6
333.6	300.6	247.6
27.6	228.6	83.6
218.6	195.6	14.6
275.6	532.6	206.6
494.6	634.6	286.6
437.6	574.6	114.6
	640.6	57.6
	853.6	335.6
	294.6	303.6
	338.6	741.6
		551.6
		430.6
		476.6
		305.6
		180.6
		115.6
		425.6

Table S5. Primer list

Number	Name	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