New Phytologist

***New Phytologist* Supporting Information**

Article title: **Occurrence and conversion of progestogens and androgens are conserved in land plants.**

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The following Supporting Information is available for this article:

Figure S1 Standard curves of steroid ions used as quantifiers. The graphs show the peak area of the specific ions used as quantifier blotted against the amount of steroid standard in ng mL^{-1}.

Figure S2 Standard curves of steroid ions used as qualifiers. The graphs show the peak area of the specific ions used as quantifier blotted against the amount of steroid standard in ng mL^{-1}.

Figure S3 Constructs for plant transformation. The figure shows constructs used for expression of human CYP11A1 in *A. thaliana* and *P. patens*.

Figure S4 qPCR analysis of 35S:poCYP11A1 expressing *A. thaliana* plants. The figure shows the results of qPCR experiments with poCYP11A1 expressing plants.

Figure S5 qPCR analysis of Ubi:poCYP11A1 expressing protonemata of *P. patens*. The figure shows qPCR experiments and steroid profile of *P. patens* expressing poCYP11A1.

Table S1 Cultivation media used for plant tissue culture and steroid biotransformation.

Table S2 Nucleic acid and amino acid sequence of the plant-optimized, human Cyp11A1 (poCyp11A1). The table shows the nucleic acid and amino sequence of poCyp11A1 used for plant transformation.

Table S3 Primer list. The primers used in this study can be found here in 5´-3´orientation.

Table S4 Steroid solutions used for the validation of the UHPLC-MS-based quantification of progestogens and androgens in plants. Each solution was measured pure and used for the extraction of 3 technical replicates of plant material.

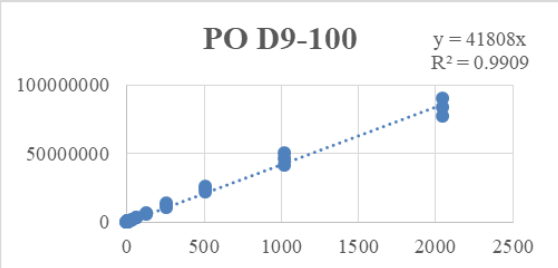
Table S5 Influence of different plant matrices on signal intensity (as peak area in counts) of nonadeuterated progesterone-D9 (PO-D9) quantifier ion. We here depict the signal intensities

of PO-D9 in form of peak area as counts (n = 3) to ensure that matrix effects do not influence measurements between the used organisms.

Table S6 Statistical analysis of steroid conversion. Plant species were treated with progestogens or androgens. The resulting steroids were analyzed, quantified and compared to the mock treated control (con). We here show the results of a statistical analysis using Bernoulli generalized linear models, factor level reduction and the Welch two sample t-test.

Table S7 Steroid and cholesterol contents in plants. The table shows the contents of pregnenolone (PR), progesterone (PO), 17α-hydroxypregnenolone (17α-OHPR), 17α-hydroxyprogesterone (17α-OHPO), androstenedione (AD), testosterone (TO) and 5α-dihydrotestosterone (DHT) and cholesterol in the analyzed plant species.

Fig. S1 Standard curves of steroid ions used as quantifiers. A dilution series (Table S2) was prepared for the different steroids used in this study. The graphs show the peak area of the specific ions used as quantifier blotted against the amount of steroid standard in ng mL^{-1}. PO-D9 = Deuterated Progesterone; PR = Pregnenolone; PO = progesterone; 17α-OHPR = 17α-Hydroxypregnenolone; 17α-OHPO = 17α-Hydroxyprogesterone; DHEA = Dehydroepiandrosterone; AD = Androstenedione; TO = Testosterone; DHP = 5α-Dihydroprogesterone; DHT = 5α-Dihydrotestosterone. Measurements of three technical replicates are shown as individual spots. Response factors of steroids and internal standards were calculated with a previous data set.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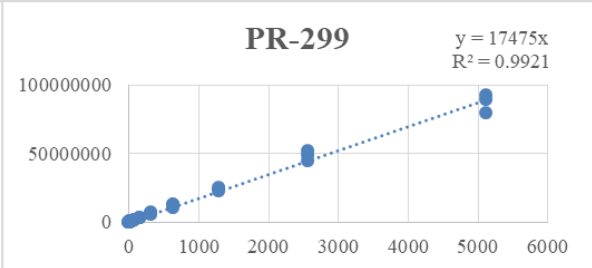
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0
0
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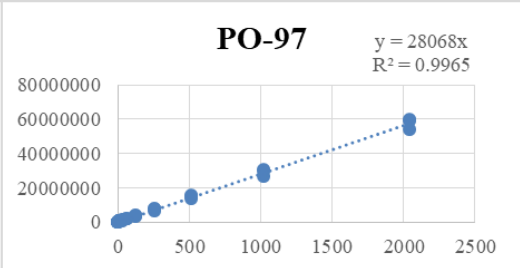
2000
3000
4000
5000
6000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20000000
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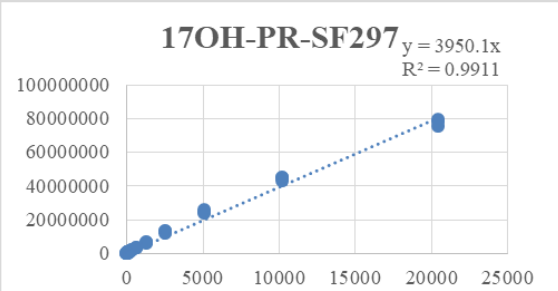
0
500
1000
1500
2000
2500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60000000
40000000
20000000
0
0
5000
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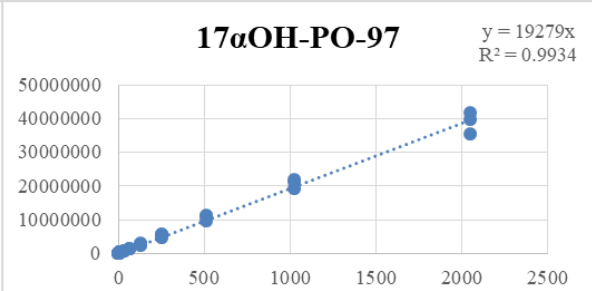
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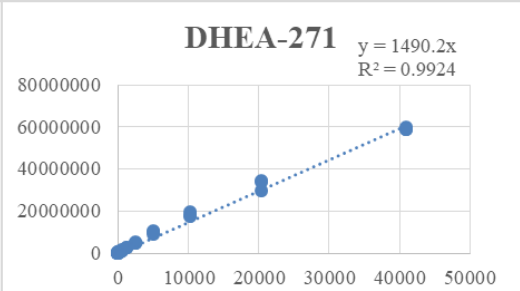
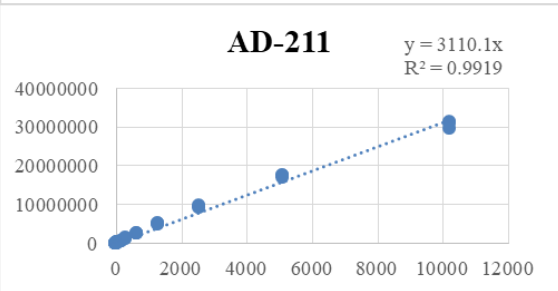
0
500
1000
1500
2000
2500


DHEA-271
y = 1490.2x
R² = 0.9924
80000000
60000000
40000000
20000000
0
0
10000
20000
30000
40000
50000


AD-211
y = 3110.1x
R² = 0.9919
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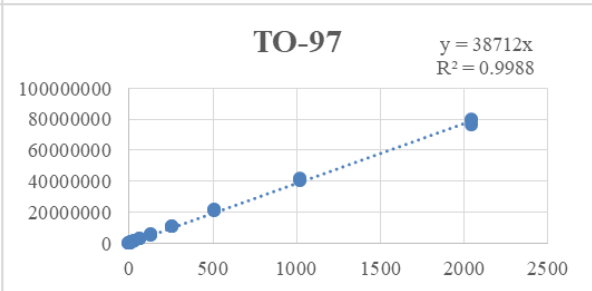
30000000
20000000
10000000
0
0
2000
4000
6000
8000
10000
12000


TO-97
y = 38712x
R² = 0.9988
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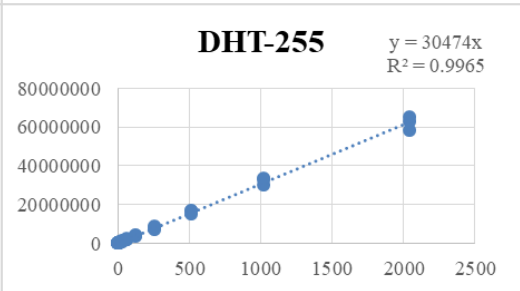
80000000
60000000
40000000
20000000
0
0
500
1000
1500
2000
2500


DHT-255
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R² = 0.9965
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60000000
40000000
20000000
0
0
500
1000
1500
2000
2500

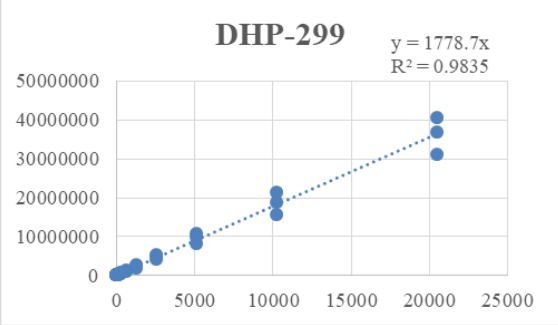
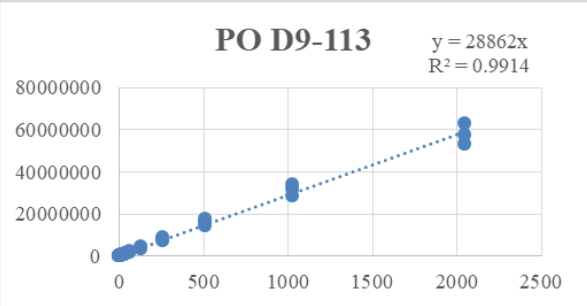

DHP-299
y = 1778.7x
R² = 0.9835
50000000
40000000
30000000
20000000
10000000
0
0
5000
10000
15000
20000
25000

Fig. S2 Standard curves of steroid ions used as qualifiers. A dilution series (Tab.S2) was prepared for the different steroids used in this study. The graphs show the peak area of the specific ions used as qualifier blotted against the amount of steroid standard in ng mL^{-1}. PO-D9 = Deuterated Progesterone; PR = Pregnenolone; PO = progesterone; 17α-OHPR = 17α-Hydroxypregnenolone; 17α-OHPO = 17α-Hydroxyprogesterone; DHEA = Dehydroepiandrosterone; AD = Androstenedione; TO = Testosterone; DHP = 5α-Dihydroprogesterone; DHT = 5α-Dihydrotestosterone. Measurements of three technical replicates are shown as individual spots. Response factors of steroids and internal standards were calculated with a previous data set.


PO D9-113
y = 28862x
R² = 0.9914
80000000
60000000
40000000
20000000
0
0
500
1000
1500
2000
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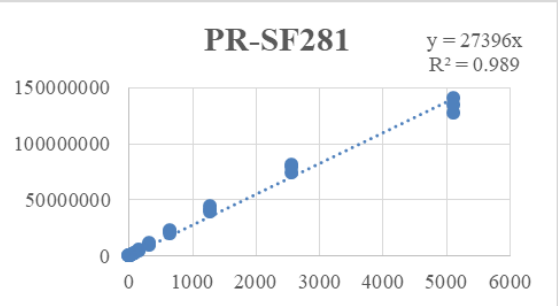
PR-SF281
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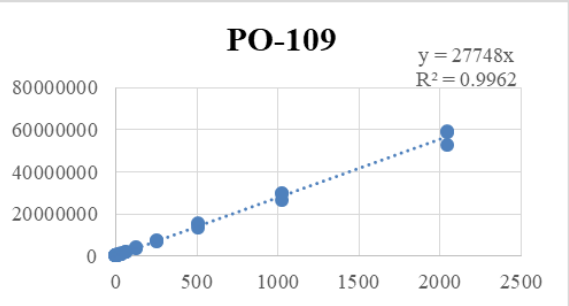
0
1000
2000
3000
4000
5000
6000


PO-109
y = 27748x
R² = 0.9962
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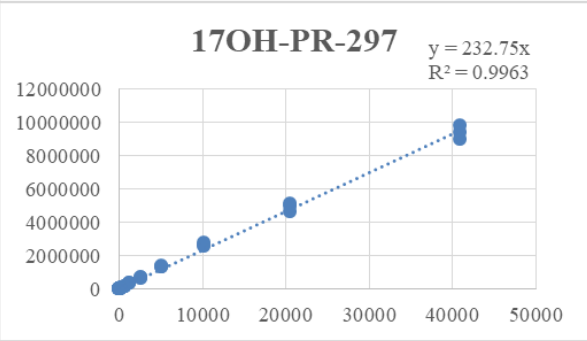
60000000
40000000
20000000
0
0
500
1000
1500
2000
2500


17OH-PR-297
y = 232.75x
R² = 0.9963
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2000000
0
0
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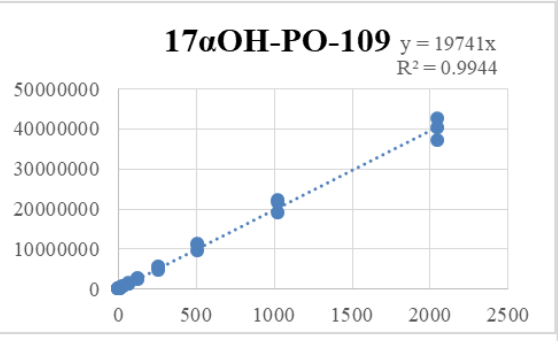
20000
30000
40000
50000


17αOH-PO-109
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500
1000
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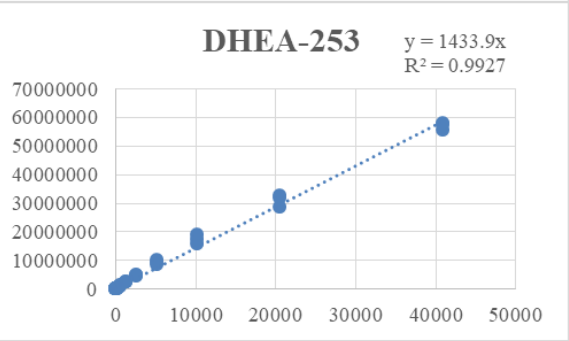
2000
2500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R² = 0.9927
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40000000
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20000000
10000000
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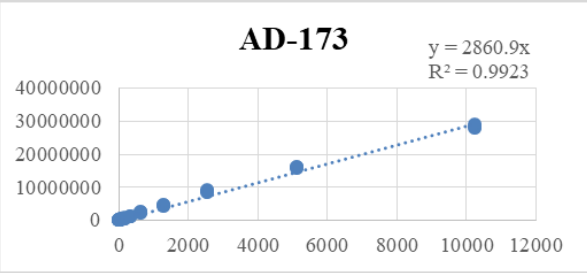
10000
20000
30000
40000
50000


AD-173
y = 2860.9x
R² = 0.9923
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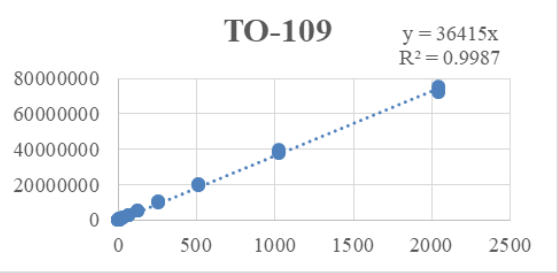
30000000
20000000
10000000
0
0
2000
4000
6000
8000
10000
12000


TO-109
y = 36415x
R² = 0.9987
80000000
60000000
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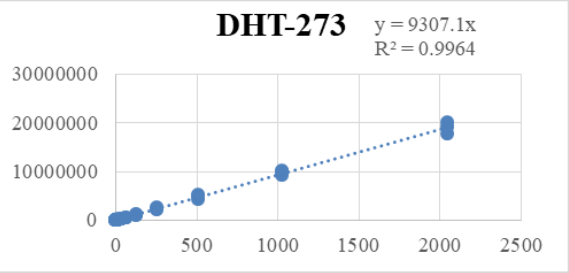
20000000
0
0
500
1000
1500
2000
2500


DHT-273
y = 9307.1x
R² = 0.9964
30000000
20000000
10000000
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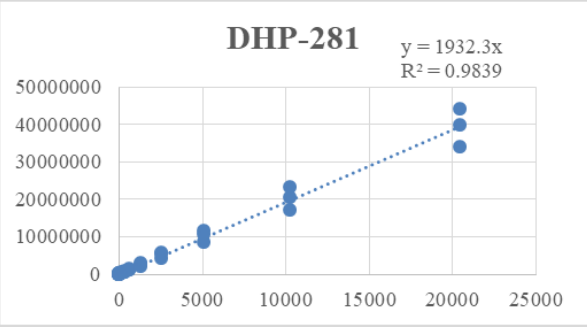
500
1000
1500
2000
2500


DHP-281
y = 1932.3x
R² = 0.9839
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40000000
30000000
20000000
10000000
0
0
5000
10000
15000
20000
25000

Fig. S3 Constructs for plant transformation. The figure shows constructs used for expression of an plant-optimized version of human CYP11A1 gene (poCYP11A1). pCAMBIA1301 (a) was used for the transformation of *A. thaliana*, while pFAU580 (b) was used to transform *P. patens*.

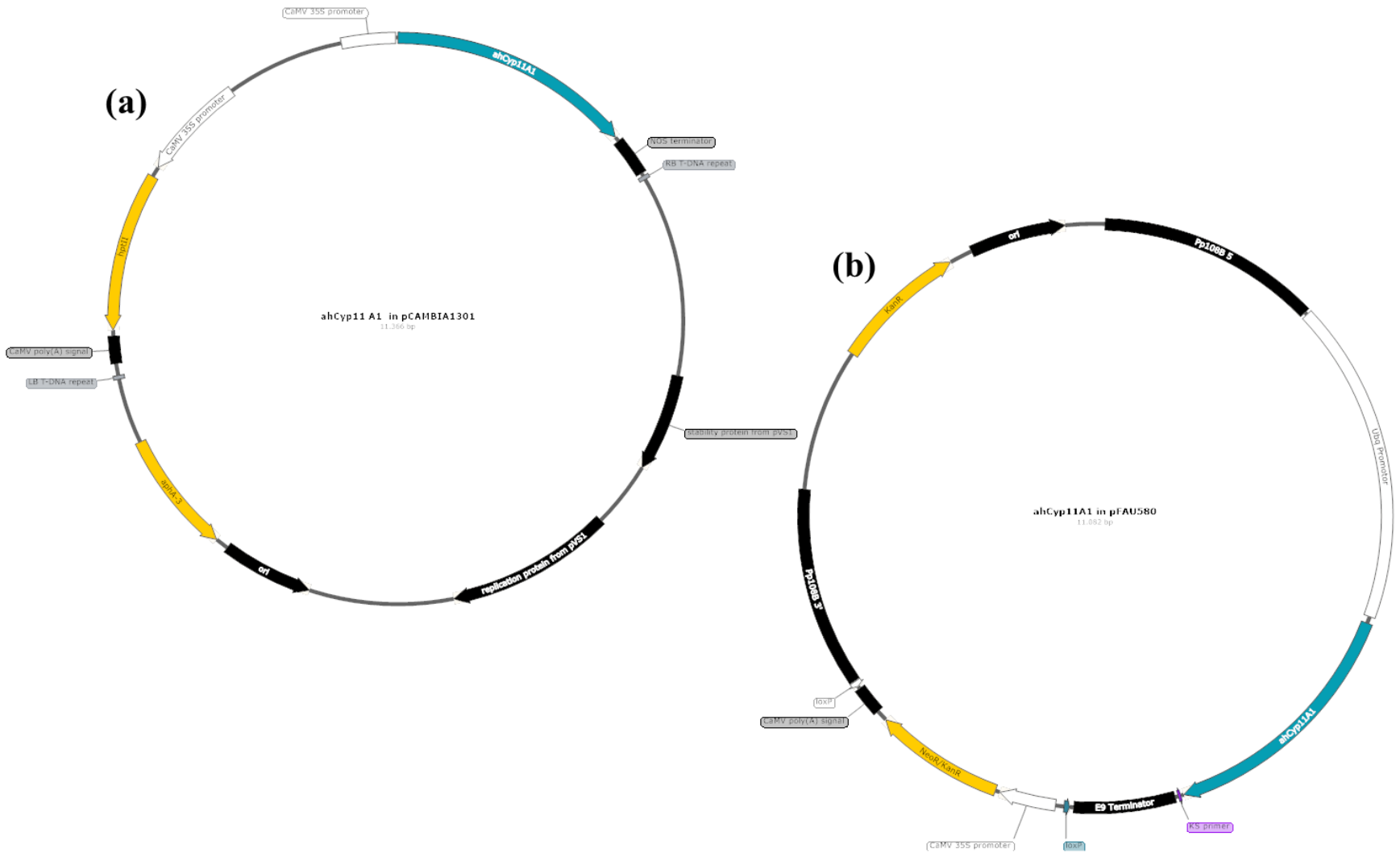
(a)
CaMV 35S promoter
ahCyp11A1
NOS terminator
RB T-DNA repeat
hptII
CaMV 35S promoter
ahCyp11 A1 in pCAMBIA1301
11,366 bp
CaMV poly(A) signal
LB T-DNA repeat
stability protein from pVS1
aadA3
ori
replication protein from pVS1
(b)
ori
Pp1088 S
KanR
Ubq Promoter
ahCyp11A1 in pFAU580
11,662 bp
Pp1088 SF
ahCyp11A1
loxP
CaMV poly(A) signal
NeoR/KanR
E9 Terminator
M5 primer
CaMV 35S promoter
loxP

Fig. S4 qPCR analysis of 35S:poCYP11A1 expressing *A. thaliana* plants. The figure shows melting curves of qPCR experiments (a – c) and poCYP11A1 expression levels of transgenic A. *thaliana* leaves (d). We could show that only one fragment was produced by qPCR using primers against reference gene RPS18 (a) and against poCYP11A1 (b). poCYP11A1 fragments could not be detected in cDNA of wildtype plants and non-template controls (c). All transgenic plants showed an expression of poCYP11A1 under control of CaMV 35S promoter. In contrast to poCYP11A1 expressing protonema of *P. patens*, transgenic *A. thaliana* plants did not show enhanced values of pregnenolone, progesterone or other steroids.

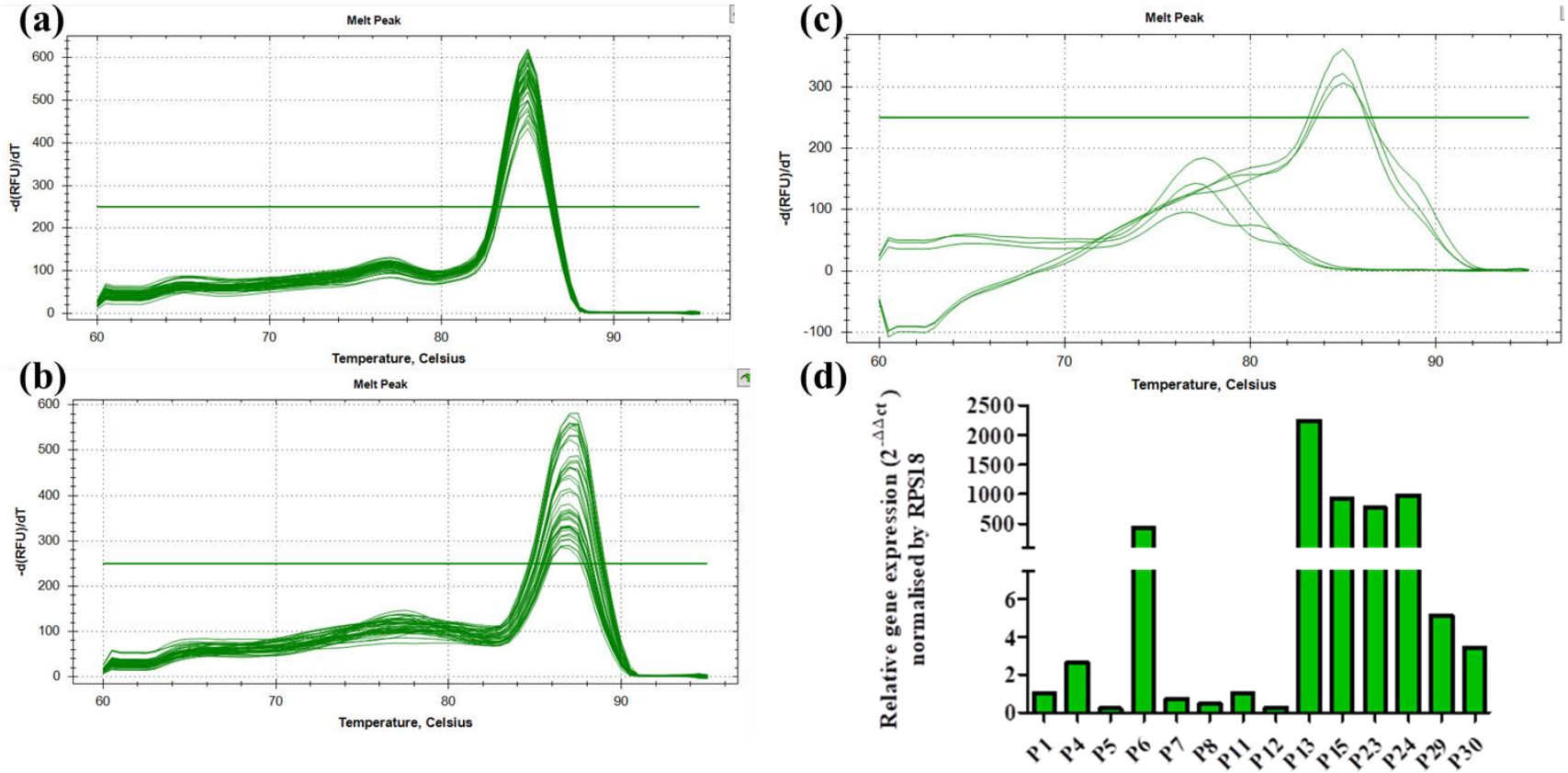
(a)
Melt Peak
-d(RFU)/dT
Temperature, Celsius
(b)
Melt Peak
-d(RFU)/dT
Temperature, Celsius
(c)
Melt Peak
-d(RFU)/dT
Temperature, Celsius
(d)
Relative gene expression ($2^{-\Delta\Delta ct}$) normalised by RPS18
P1
P4
P5
P6
P7
P8
P11
P12
P13
P15
P23
P24
P29
P30

Fig. S5 qPCR analysis of Ubi:poCYP11A1 expressing protonemata of *P. patens*. The figure shows melting curves of qPCR experiments (a – b) and poCYP11A1 expression levels of transgenic protonema of *P. patens* (c). We could show that only one fragment was produced by qPCR using primers against reference gene ubiquitin (a) and against poCYP11A1 (b). All transgenic lines showed a expression of poCyp11A1 under control of ubiquitin promoter. These lines were used for further analyses of steroids. While neither pregnenolone (d), progesterone (e), nor 5α-pregnane-3,20-dione (f) was found in wild-type protonema, all these steroids accumulated in detectable values in Ubi:poCYP11A1 lines L1, and L4. Ubi:poCYP11A1. L3 shows also high values of PR and DHP, but the PO level was not stimulated. The analyzed transgenic *A. thaliana* lines did not contain elevated PR, PO, or DHP levels. Graphs show means and SEM (n = 3).

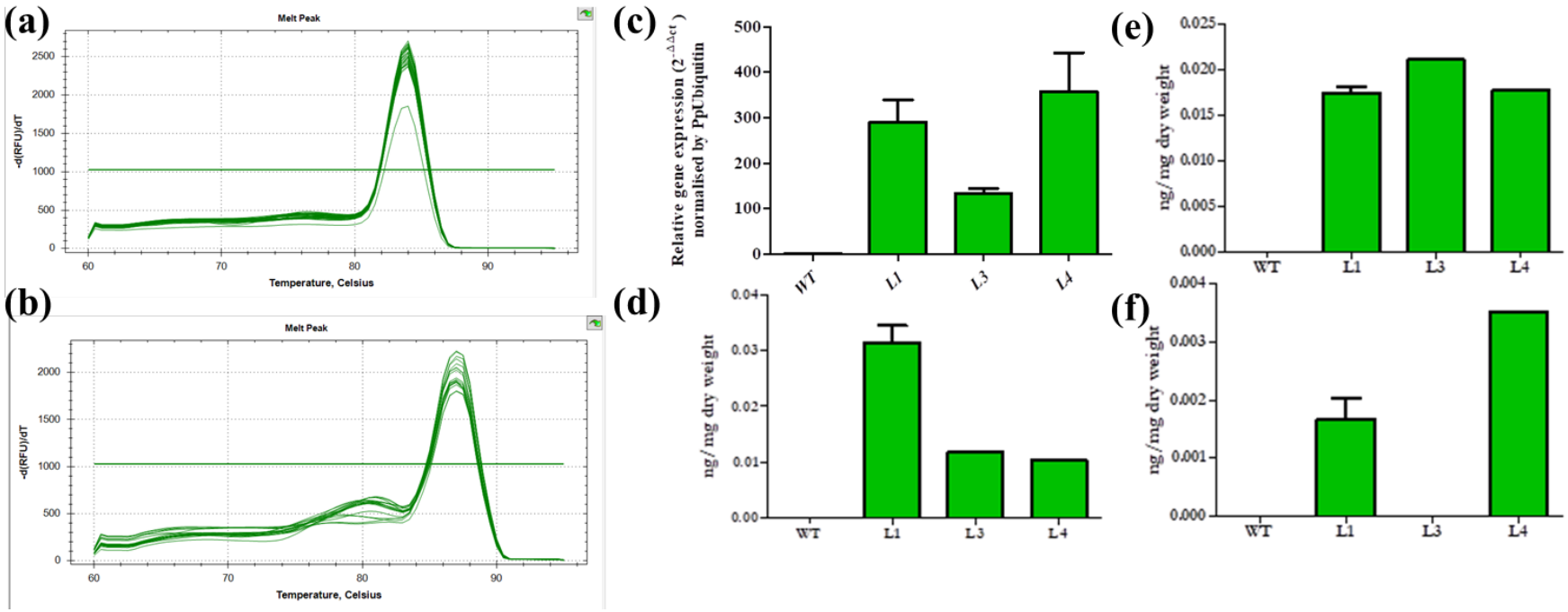
(a)
Melt Peak
-d(RFU)/dT
Temperature, Celsius
(b)
Melt Peak
-d(RFU)/dT
Temperature, Celsius
(c)
Relative gene expression ($2^{-\Delta\Delta ct}$) normalised by PpUbiquitin
WT
L1
L3
L4
(d)
ng/mg dry weight
WT
L1
L3
L4
(e)
ng/mg dry weight
WT
L1
L3
L4
(f)
ng/mg dry weight
WT
L1
L3
L4

Table S1 Cultivation media used for plant tissue culture and steroid biotransformation.

Composition of MSG1 medium for *L. decidua*, modified according to Kraft and Kadolsky (Kraft and Kadolsky, 2018).

Chemicals	Final concentration [mg l^{-1}]
$MgSO_4$ x 7 H_2O	320
KH_2PO_4	170
$CaCl_2$ x H_2O	440
KCl	745
KNO_3	100
$MnSO_4$ x H_20	16.9
H_3BO_3	6.2
$ZnSO_4$ x 7 H_2O	8.6
KI	0.83
Na_2MoO_4 x 2 H_2O	0.25
$CuSO_4$ x 5 H_2O	0.025
$CoCl_2$ x 6 H_2O	0.025
Myo-Inositol	100
$FeSO_4$ x 7 H_2O	27.8
Na_2-EDTA	37.3
Sucrose	20,000
Thiamine-HCl	1
Nicotinic acid	0.5
Pyridoxin-HCl	0.5
2,4-Dichlorophenoxyacetic acid	2
Benzo(a)pyrene	0.5
Gelrite	3,300
Adjust pH to 5.7 and autoclave for 20 min at 121 °C.	
L-Glutamine (filter-sterilized from stock)	1,460

Composition of Sugar-free nutrient medium for *Lemnaceae* according to Appenroth (Appenroth et al., 1996).

Chemicals	Final concentration [mg l^{-1}]
KH_2PO_4	4,083
$Ca(NO_3)2$ x 4 H_2O	47,230
KNO_3	161,800
H_3BO_3	61.8
$MnCl_2$ x 4 H_2O	514.5
Na_2MoO_4 x 2 H_2O	19.4
$MgSO_4$ x 7 H_2O	49,300
Fe(III)-EDTA	1,725
Autoclave for 20 min at 121 °C.	

Composition of BCDA medium for *P. patens* according to Bail (Le Bail et al., 2013).

Chemicals	Final concentration [mg l^{-1}]
Diamonium tartrate	920
$MgSO_4$ x 7 H_2O	250
KH_2PO_4	250
KNO_3	1,010
$Al_2(SO_4)_3$ K_2SO_4 x 24 H_2O	0.055
$CoCl_2$ x 6 H_2O	0.055
$CuSO_4$ x 5 H_2O	0.055
H_3BO_3	0.614
KBr	0.028
KI	0.028
LiCl	0.028
$MnCl_2$ x 4 H_2O	0.389
$SnCl_2$ x 2 H_2O	0.028
$ZnSO_4$ x 7 H_2O	0.055
$FeSO_4$ x 7 H_2O	12.5
Autoclave solution for 20 min at 121 °C.	
Add 1 ml of 1 M $CaCl_2$ from filter-sterilized stock before pouring the plates.	

Composition of MS-medium for *A. thaliana* according to Murashige & Skoog (Murashige & Skoog, 1962).

Chemicals	Final concentration [mg l^{-1}]
MS-salt	4,400
MES	500
Sucrose	13,700
Adjust pH to 5.7 with KOH.	
Gel-Rite or Agar	3,000 7,000
Autoclave for 20 min at 121 °C.	

Composition of medium stock solutions for *C. reinhardtii* according to Kropat (Kropat et al., 2011)

Preparation of concentrated stock solutions		
Stock name	**Chemicals**	**V_{final} [ml]**
EDTA (Na_2 salt)	13,959 mg Na_2-EDTA (Na_2-EDTA is first dissolved in 250 ml dH_20. Afterwards, pH is adjusted to 8.0 with KOH. Volume is then filled up to 300 ml)	300
$(NH_4)_6Mo_7O_{24}$	88 mg $(NH_4)_6Mo_7O_{24}$	250
Na_2SeO_3	43 mg Na_2SeO_3	250
Preparation of individual stock solutions		
Stock name	Chemicals	V_{final} [ml]
TAP (Beijernick) salts	16,000 mg NH_4Cl 4,000 mg $MgSO_4$ x 7 H_2O 2,000 mg $CaCl_2$ x H_20	1000
TAP phosphates	3,774 mg KH_2PO_4 x 3 H_2O 144 mg KH_2PO_4	100
TAP Trace 1	50 ml of concentrated stock EDTA (Na_2 salt)	250
TAP Trace 2	25 ml of concentrated stock $(NH_4)_6Mo_7O_{24}$	250
TAP Trace 3	25 ml of concentrated stock Na_2SeO_3	250
TAP Trace 4	180 mg $ZnSO_4$ x 7 H_2O 5.5 ml of concentrated stock EDTA (Na_2 salt)	250
TAP Trace 5	297 mg $MnCl_2$ x 4 H_2O 12 ml of concentrated stock EDTA (Na_2 salt) (pH < 6.8 to prevent precipitation)	250
TAP Trace 6	1,350 mg $FeCl_3$ x 6 H_2O 2,050 mg Na_2-EDTA 580 mg Na_2CO_3 x 2 H_2O (First dissolve and mix Na_2-EDTA and Na_2CO_3 x 2 H_2O. Add $FeCl_3$ x 6 H_2O last.)	250
TAP Trace 7	85 mg $CuCl_2$ 4 ml of concentrated stock EDTA (Na_2 salt)	250
Filter-sterilize all stock solutions and store at 4 °C.		

Composition of medium for *C. reinhardtii* according to Kropat (Kropat et al., 2011) from stock solutions of the table above.

Stock name	Amount of stock solution [µl l^{-1}]
TAP (Beijernick) salts	25,000
TAP phosphates	250
TAP Trace 1	1000
TAP Trace 2	1000
TAP Trace 3	1000
TAP Trace 4	1000
TAP Trace 5	1000
TAP Trace 6	1000
TAP Trace 7	1000
TRIS 0.2 M	2.42 g
For solid medium: Agar	7,000 mg
Adjust pH to 7.0 with glacial acid and autoclave for 20 min at 121 °C.	

Table S2 Nucleic acid and amino acid sequence of the plant-optimized, human Cyp11A1 (poCyp11A1). We designed a plant-optimized version of human Cyp11A1 (poCyp11A1). This sequence was cloned into pCAMBIA1301 and pFAU580 and used for the transformation of *A. thaliana* and *P. patens*. We show here the nucleic acid and amino sequence of poCyp11A1 in Fasta-Format.

>Nucleic acid sequence of poCyp11A1

atgctagcgaaaggtctccccccgcgctccgttctcgtgaaaggatgtcaaaccttcctatccgcaccccgggagggtttggggcgtctgc
gcgtgcctaccggggagggcgccggaatctccacgagatctccacggcccttcaacgagataccttctccaggagacaatggctggctca
atttgtatcacttctggagggagaccggcacacacaaggttcacctccaccatgtacaaaatttccagaagtatggacctatttatcgcgaaaa
gcttggaaatgttgaatctgtatacgtgatagatcctgaagatgttgcgcttctcttcaaatctgaaggtccaaatcctgagcgcttcctgattcc
gccgtgggttgcataccatcagtactatcagcgaccaatcggcgtattactcaagaagtccgccgcgtggaagaaggatagggtggcattg
aaccaggaggtgatggctccggaggcgacgaagaactttcttccgcttctggacgcggtgtcgagggactttgtgtcagtgctgcatcgcc
gtatcaagaaagctggttcaggaaactatagcggcgatatttctgacgacctgtttagatttgctttcgaaagcatcaccaatgttattttcggcg
agcgccaagggatgttggaggaggtcgttaacccagaagcgcaaaggtttatcgacgccatctaccaaatgttccacacatcagttccaatg
ctgaaccttcccccctgaccttttcagacttttccgtactaaaacatggaaagaccatgtggcggcatgggacgtcatttttttcaaaggctgatat
atacactcaaaacttctactgggagctccgacagaaggggagtgtccaccatgattatagggggatcctgtaccggctgctcggcgacagc
aagatgagctttgaggatatcaaagcaaatgtgacggagatgttggccggcggggtcgacacgacgtccatgactttgcagtggcaccttta
cgagatggcgcggaatctcaaggttcaagatatgcttagggccgaggtcttagccgccagacaccaggctcagggcgatatggctactat
gcttcagctggtccctctcctcaaggcttcgataaaggaaacactgcggctgcatccgataagcgtgacgctccagcggtacctcgtcaatg
acctggtcctccgtgactacatgattccggcgaagactttggtccaagtagccatttatgccttgggccgcgagccgacatttttctttgacccc
gaaaacttcgatcccactaggtggctaagtaaagataagaacattacctacttcaggaatttgggctttggttgggggtccgccagtgcctg
ggtagaagaatagccgaactggagatgacaatcttcctcatcaacatgcttgaaaattttagagtggaaatccagcatttgtcggatgtcgga
accacattcaacctgatcctcatgcccgagaaaccaattagctttaccttctggccattcaaccaagaggcaacccagcagtga

>Amino acid sequence of poCyp11A1

MLAKGLPPRSVLVKGCQTFLSAPREGLGRLRVPTGEGAGISTRSPRPFNEIPSPGDNGWL
NLYHFWRETGTHKVHLHHVQNFQKYGPIYREKLGNVESVYVIDPEDVALLFKSEGPNPE
RFLIPPWVAYHQYYQRPIGVLLKKSAAWKKDRVALNQEVMAPEATKNFLPLLDAVSRD
FVSVLHRRIKKAGSGNYSGDISDDLFRFAFESITNVIFGERQGMLEEVVNPEAQRFIDAIY
QMFHTSVPMLNLPPDLFRLFRTKTWKDHVAAWDVIFSKADIYTQNFYWELRQKGSVH
HDYRGILYRLLGDSKMSFEDIKANVTEMLAGGVDTTSMTLQWHLYEMARNLKVQDML
RAEVLAARHQAQGDMATMLQLVPLLKASIKETLRLHPISVTLQRYLVNDLVLRDYMIP
AKTLVQVAIYALGREPTFFFDPENFDPTRWLSKDKNITYFRNLGFGWGVRQCLGRRIAE
LEMTIFLINMLENFRVEIQHLSDVGTTFNLILMPEKPISFTFWPFNQEATQQ*

Table S3 Primer list. The primers used in this study can be found here in 5´-3´orientation.

Name of the primer	Primer sequence	Usage
JK_hptII_for:	5´GATGTTGGCGACCTCGTATT´3	Identification of transformants of *A. thaliana*
JK_hptII_rev:	5´GATGTAGGAGGGCGTGGATAT´3	
JK_pFAU580_CYP11A1_for:	5´TATAGCGGCCGCATGCTAGCGAAAGGTCTCCCC´3	Cloning of *ahCYP11A1* in pFAU580 for expression in *P. patens*.
JK_pFAU580_CYP11A1_rev:	5´GAGGAGGAATTCTCACTGCTGGGTTGCCTCT´3	
FAU-C123:	5´GCAAGATGTGCCGTCTCAGT´3	Amplification of RIC in gDNA of *P. patens*.
FAU-B757:	5´AGCTTTCCCCACTTTCCAGG´3	
JK_qahCyp11A1_for:	5´CGCAAAGGTTTATCGACGCC´3	Quantification of *ahCYP11A1* mRNAs in *A.thaliana*/ *P.patens*
JK_qahCyp11A1_rev:	5´TGCCACTGCAAAGTCATGGA´3	
RPS18_for:	5´GTCTCCAATGCCCTTGACAT´3	Reference gene for qPCR experiments with *A.thaliana*.
RPS18_rev:	5´ TCTTTCCTCTGCGACCAGTT´3	
FAU_88:	5´TACGGACCCTAATCCAGATGAC´3	Reference gene for qPCR experiments with *P.patens.*
FAU_28:	5´CAACCCATTGCATACTTCTGAG´3	

Table S4 Steroid solutions used for the validation of the UHPLC-MS-based quantification of progestogens and androgens in plants. Each solution was measured pure and used for the extraction of 3 technical replicates of plant material.

	Steroids in ng/ mL									
Solution	**PR**	**PO**	**17α-OHPR**	**17α-OHPO**	**DHEA**	**AD**	**TO**	**DHT**	**DHP**	**D9-PO**
MeOH	0	0	0	0	0	0	0	0	0	0
A	5	1	10	1	10	10	1	1	5	1
B	10	2	20	2	20	20	2	2	10	2
C	20	4	40	4	40	40	4	4	20	4
D	40	8	80	8	80	80	8	8	40	8
E	80	16	160	16	160	160	16	16	80	16
F	160	32	320	32	320	320	32	32	160	32
G	320	64	640	64	640	640	64	64	320	64
H	640	128	1280	128	1280	1280	128	128	640	128
I	1280	256	2560	256	2560	2560	256	256	1280	256
J	2560	512	5120	512	5120	5120	512	512	2560	512
K	5120	1024	10240	1024	10240	10240	1024	1024	5120	1024
L	10240	2048	20480	2048	20480	20480	2048	2048	10240	2048
M	20480	4096	40960	4096	40960	40960	4096	4096	20480	4096

Table S5 Influence of different plant matrices on signal intensity (as peak area in counts) of nonadeuterated progesterone-D9 (PO-D9) quantifier ion. 1 mL MeOH (80%) with PO-D9 (100 ng mL-1) was used for steroid extraction of 20 mg dry weight of the following plants: A. thaliana leaves, A. thaliana roots, H. vulgare leaves, H. vulgare roots, S. polyrhiza shoots, somatic embryos of L. decidua, P. patens protonema and cells of C. reinhardtii. We here depict the signal intensities of PO-D9 in form of peak area as counts (n = 3).

Plant matrix	**PO-D_9 signal intensity ± standard deviation**
MeOH + 100 ng PO-D_9	7811667 ± 685461
C. reinhardtii cells	5756667 ± 119304
P. patens protonema	7480000 ± 65574
L. decidua somatic embryos	7763333 ± 32146
S. polyrhiza plants	7130000 ± 122882
H. vulgare shoots	7523333 ± 87369
H. vulgare roots	7810000 ± 65574
A. thaliana shoots	7323333 ± 141892
A. thaliana roots	7716667 ± 15275

Table S6 Statistical analysis of steroid conversion. *Chlamydomonas reinhardtii* (*C.r*), *Physcomitrella patens* (*P.p*), *Larix decidua* (*L.d*), *A. thaliana* (*A.t*), *Hordeum vulgare* (*H.v*) and *Spirodela polyrhiza* (*S.p*) were treated with progestogens (pregnenolone = PR, progesterone = PO, 17α-hydroxypregnenolone = 17α-OHPR, 17α-hydroxyprogesterone = 17α-OHPO) or androgens (dehydroepiandrosterone = DHEA, androstenedione = AD, testosterone = TO). The resulting steroids were analyzed ,quantified and compared to the mock treated control (con). In cases where the analyte of interest could not be detected in plants of a certain treatment present / absent data were used and Bernoulli generalized linear models were applied. Significance values were obtained by comparing the model with the explanatory variable with the model without the variable with a likelihood ratio test (Zuur et al. 2009). In cases of more than two factor levels per treatment factor level reduction was applied to test which treatments differ to the control plants (Crawley, 2013). In case plants of all treatments contained a certain analyte, actual concentrations were used, and the Welch two sample t-test (for comparison of one treatment against a control), an anova (for comparisons of more than two treatments), or the Kruskal-Wallis rank sum test (for comparisons of more than two treatments, if normality of the residuals or variance homogeneity was not fulfilled) were applied. Here we show the statistical analysis of our results.

enzyme / analyte	plant	data	method	deviance	t-value	χ^2-value	F-value	p-value	
17α-OHPO accu-mulation	*C.r*	presence / absence	Bernoulli glm	11.457				**0.003**	con < PR = PO
	P.p	presence / absence	Bernoulli glm	11.457				**0.003**	con < PR = PO
	L.d	presence / absence	Bernoulli glm	2.46				0.292	
	A.t	presence / absence	Bernoulli glm	11.457				**0.003**	con < PR = PO
	H.v	presence / absence	Bernoulli glm	8.546				**0.014**	con (a), PR (ab), PO (b)
	S.p	presence / absence	Bernoulli glm	11.457				**0.003**	con < PR = PO
17,20-Lyase of 17α-OHPR	*C.r*	presence / absence	Welch Two Sample t-test		-1.215			0.335	
	P.p		not analyzed						
	L.d		not analyzed						
	A.t	concentrations	Welch Two Sample t-test		2.412			0.059	
	H.v	concentrations	Welch Two Sample t-test		-1.294			0.268	
	S.p	presence / absence	Bernoulli glm	8.318				**0.004**	con < 17α-OHPR

17,20-Lyase of 17α-OHPO	*C.r*		not analyzed			
	P.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPO
	L.d		not analyzed			
	A.t	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPO
	H.v	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPO
	S.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPO
3β-HSD Progesterone pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318	**0.004**	con < PR
	P.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < PR
	L.d	presence / absence	Bernoulli glm	8.318	**0.004**	con < PR
	A.t	presence / absence	Bernoulli glm	8.318	**0.004**	con < PR
	H.v	log(concentrations)	Welch Two Sample t-test	-12.72	**< 0.001**	con < PR
	S.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < PR
3β-HSD 17-OHPO pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
	P.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
	L.d	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
	A.t	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
	H.v	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
	S.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < 17α-OHPR
3β-HSD AD pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA
	P.p	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA
	L.d	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA
	A.t	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA
	H.v	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA
	S. p	presence / absence	Bernoulli glm	8.318	**0.004**	con < DHEA

3β-HSD PR pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318		**0.004**	con < PO
	P.p		not analyzed				
	L.d		not analyzed				
	A.t	presence / absence	Bernoulli glm	8.318		**0.004**	con < PO
	H.v		not analyzed				
	S.p		not analyzed				
3β-HSD DHEA pro-duction	*C.r*	concentrations	Welch Two Sample t-test		1.177	0.355	
	P.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
	L.d		not analyzed				
	A.t	concentrations	Welch Two Sample t-test		13.602	**0.001**	con < AD
	H.v	log(concentrations)	Welch Two Sample t-test		6.451	**0.006**	con < AD
	S.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
17β-HSD TO pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
	P.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
	L.d	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
	A.t	concentrations	Welch Two Sample t-test		26.537	**0.001**	con < AD
	H.v	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
	S.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < AD
17β-HSD AD pro-duction	*C.r*	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO
	P.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO
	L.d	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO
	A.t	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO
	H.v	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO
	S.p	presence / absence	Bernoulli glm	8.318		**0.004**	con < TO

TO production after treatment with diverse steroids	*C.r*	presence / absence	Bernoulli glm	16.22		**0.006**	con = PR = PO = 17α-OHPO = 17α-OHPR < DHEA
	P.p	presence / absence	Bernoulli glm	16.22		**0.006**	con = PR = PO = 17α-OHPO = 17α-OHPR < DHEA
	L.d	presence / absence	Bernoulli glm	16.22		**0.006**	con = PR = PO = 17α-OHPO = 17α-OHPR < DHEA
	A.t	presence / absence	Bernoulli glm	22.915		**< 0.001**	17α-OHPO = 17α-OHPR < con = PR = PO = DHEA
	A.t	concentrations	Kruskal-Wallis rank sum test		10.385	**0.016**	
	H.v	presence / absence	Bernoulli glm	15.250		**0.009**	con (a), DHEA (ab), preg = prog = 17α-OHPO = 17α-OHPR (b)
	H.v	concentrations	Kruskal-Wallis rank sum test		7.933	0.094	
	S.p	presence / absence	Bernoulli glm	24.953		**< 0.001**	con = PR = PO < 17α-OHPO = 17α-OHPR = DHEA
	S.p	concentrations	anova		74.230	**< 0.001**	17α-OHPO = 17α-OHPR < DHEA
5α-reduction DHP production	*C.r*	presence / absence	Bernoulli glm	26.992		**< 0.001**	con = 17α-OHPO = 17α-OHPR = AD = DHEA = TO < PR = PO
	P.p	presence / absence	Bernoulli glm	26.992		**< 0.001**	con = 17α-OHPO = 17α-OHPR = AD = DHEA = TO < PR = PO
	L.d	presence / absence	Bernoulli glm	26.992		**< 0.001**	con = 17α-OHPO = 17α-OHPR = AD = DHEA = TO < PR = PO
	A.t	concentrations	Kruskal-Wallis rank sum test		22.343	**0.002**	
	H.v	presence / absence	Bernoulli glm	26.992		**< 0.001**	con = 17α-OHPO = 17α-OHPR = AD = DHEA = TO < PR = PO
	S.p	presence / absence	Bernoulli glm	26.992		**< 0.001**	con = 17α-OHPO = 17α-OHPR = AD = DHEA = TO < PR = PO

5α-reduction DHT production	*C.r*	presence / absence	Bernoulli glm	20.744		**0.004**	PO (a), PR (ab), 17α-OHPO = 17α-OHPR = AD = DHEA = con = TO (b)
	C.r	concentrations	Kruskal-Wallis rank sum test		13.844	**0.031**	
	P.p	presence / absence	Bernoulli glm	18.085		**0.012**	17α-OHPO = 17α-OHPR = AD = DHEA = con = PO = PR < TO
	L.d	presence / absence	Bernoulli glm	31.755		**< 0.001**	17α-OHPO = 17α-OHPR = con = PO = PR < AD = DHEA = TO
	A.t	presence / absence	Bernoulli glm	31.755		**< 0.001**	17α-OHPO = 17α-OHPR = con < PO = PR = AD = DHEA = TO
	H.v	presence / absence	Bernoulli glm	21.336		**0.003**	17α-OHPO = 17α-OHPR = con = PO = PR (a), AD = DHEA (ab), TO (b)
	S.p	presence / absence	Bernoulli glm	31.755		**< 0.001**	17α-OHPO = 17α-OHPR = con = PO = PR < AD = DHEA = TO

Table S7 Steroid and cholesterol contents in plants. The table shows the contents of steroids: pregnenolone (PR), progesterone (PO), 17α-hydroxypregnenolone (17α-OHPR), 17α-hydroxyprogesterone (17α-OHPO), androstenedione (AD), testosterone (TO) and 5α-dihydrotestosterone (DHT). Values of 3 independent measurements are given in ng/g dry weight. Additionally, the contents of cholesterol (CO) are depicted. Cholesterol values are given as mean± Std. [%]. "0" indicates that the analyte was not detectable, i.e. no or too low signal (S/N < 3) or absence of the respective qualifier signal. Cholesterol data are calculated in mg/ 100 g dry weight.

Species	PR [ng g^{-1}]	PO [ng g^{-1}]	17α-OHPR [ng g^{-1}]	DHEA [ng g^{-1}]	17α-OHPO [ng g^{-1}]	AD [ng g^{-1}]	TO [ng g^{-1}]	DHT [ng g^{-1}]	CO [mg $100g^{-1}$]
Chlorella vulgaris	0	0	0	2130	0	0	0	0	3.2±2
Chlorella vulgaris	0	0	0	2406	0	0	0	0	
Chlorella vulgaris	0	0	0	3140	0	0	0	0	
Physcomitrella patens:	0	5	0	5894	0	0	14	0	20.5±5
Physcomitrella patens:	0	6	0	5464	0	0	13	0	
Physcomitrella patens:	0	6	0	5845	0	0	14	0	
Azolla filiculoides	119	28	0	416	0	0	1	0	10.6±5
Azolla filiculoides	64	12	0	472	0	0	1	0	
Azolla filiculoides	402	7	0	627	0	0	2	0	
Lycopodium clavatum	0	34	0	27712	0	0	2	9	4.7±0.6
Lycopodium clavatum	0	52	0	69045	0	0	4	26	
Lycopodium clavatum	0	80	0	66835	0	0	9	25	
Equisetum aarvense	0	0	0	8159	0	0	0	30	7.4±13
Equisetum aarvense	0	0	0	9644	0	0	0	29	
Equisetum aarvense	0	0	0	8446	0	0	0	30	
Pinus pinea	7365	6	0	0	0	0	0	0	7.3±3
Pinus pinea	13601	9	0	0	0	0	0	0	
Pinus pinea	11351	15	0	0	0	0	0	0	

Ginkgo biloba	0	0	0	80415	19	0	0	91	7.4±1
Ginkgo biloba	0	0	0	77964	20	0	0	66	
Ginkgo biloba	0	0	0	81253	19	0	0	70	
Cycas revoluta	98	12	0	0	0	0	0	0	4.8±5
Cycas revoluta	101	12	0	0	0	0	0	0	
Cycas revoluta	115	12	0	0	0	0	0	0	
Gnemon gnetum	0	0	0	3272	0	0	0	80	12±0.2
Gnemon gnetum	0	0	0	3335	0	0	0	84	
Gnemon gnetum	0	0	0	3115	0	0	0	89	
Hordeum vulgare	0	32	0	6050	0	0	0	0	7.4±3
Hordeum vulgare	0	32	0	7122	0	0	0	0	
Hordeum vulgare	0	29	0	5645	0	0	0	0	
Allium schoenoprasum	0	3	0	985	0	0	0	0	13±4
Allium schoenoprasum	0	1	0	1021	0	0	0	0	
Allium schoenoprasum	0	1	0	1163	0	0	0	0	
Spirodela polyrhiza	0	0	0	0	0	0	0	0	8.5±1
Spirodela polyrhiza	0	0	0	0	0	0	0	0	
Spirodela polyrhiza	0	0	0	0	0	0	0	0	
Tulpia gesneriana	0	0	0	0	0	0	1	0	10±14
Tulpia gesneriana	0	0	0	0	0	0	1	0	
Tulpia gesneriana	0	0	0	0	0	0	1	0	
Dioscorea bulbifera	0	0	0	19587	0	0	0	120	46.9±12
Dioscorea bulbifera	0	0	0	19995	0	0	0	121	
Dioscorea bulbifera	0	0	0	21942	0	0	0	128	
Tacca chantrieri	0	0	0	0	0	0	0	0	9.7±1
Tacca chantrieri	0	0	0	0	0	0	0	0	
Tacca chantrieri	0	0	0	0	0	0	0	0	
Freycinetia cumingiana	0	3	0	3695	0	0	6	115	8.8±1
Freycinetia cumingiana	0	1	0	2961	0	0	6	104	
Freycinetia cumingiana	0	2	0	3616	0	0	5	116	
Plantago lanceolata	1191	77	0	473	0	0	0	1	11±2
Plantago lanceolata	1197	82	0	628	0	0	0	2	
Plantago lanceolata	1039	73	0	728	0	0	0	1	
Digitalis grandiflora	186	68	3539	398	0	0	82	0	15.5±8
Digitalis grandiflora	225	69	3637	422	0	0	90	0	
Digitalis grandiflora	214	71	3693	485	0	0	81	0	
Olea europaea	0	0	0	2460	0	0	0	19	3.9±4
Olea europaea	0	0	0	2652	0	0	0	15	
Olea europaea	0	0	0	3182	0	0	0	21	

Erysimum crepidifolium	0	4	0	0	0	0	2	0	11±0.3
Erysimum crepidifolium	0	3	0	0	0	0	3	0	
Erysimum crepidifolium	0	4	0	0	0	0	3	0	
A. thaliana	0	100	0	450	0	0	1990	500	5.9±1
A. thaliana	0	70	0	520	0	0	2460	520	
A. thaliana	0	30	0	529	0	0	2620	530	
Melilotus officinalis	0	48	0	187076	0	0	0	56	5.2±33
Melilotus officinalis	0	38	0	165435	0	0	0	49	
Melilotus officinalis	0	59	0	167540	0	0	0	55	
Vicia faba	0	0	0	597	0	0	0	0	7.3±3
Vicia faba	0	0	0	574	0	0	0	0	
Vicia faba	0	0	0	447	0	0	0	0	
Galium odoratum	271	68	0	1379027	0	0	0	42	3±8
Galium odoratum	82	47	0	145631	0	0	0	33	
Galium odoratum	1443	44	0	146803	0	0	0	32	
Salix alba	993	56	0	163481	0	0	18	733	8.4±37
Salix alba	895	53	0	161306	0	0	15	748	
Salix alba	855	59	0	163262	0	0	10	771	
Betula pendula	60	35	0	30847	0	0	0	240	4.6±13
Betula pendula	130	33	0	29910	0	0	0	235	
Betula pendula	103	32	0	26651	0	0	0	211	
Fagopyrum esculentum	547	38	0	239234	0	0	0	40	9.8±6
Fagopyrum esculentum	514	50	0	258597	0	0	0	64	
Fagopyrum esculentum	636	53	0	282632	0	0	0	78	
Eucalyptus globus	0	0	0	34615	0	0	0	26	6.7±6
Eucalyptus globus	0	0	0	38290	0	0	0	23	
Eucalyptus globus	0	0	0	36191	0	0	0	22	
Vaccinium myrtillus	727	34	0	64236	0	0	0	20	0.6±2
Vaccinium myrtillus	552	35	0	59135	0	0	0	22	
Vaccinium myrtillus	570	41	0	65408	0	0	0	22	
Althaea officinalis	0	7	0	204737	0	0	0	69	8.9±0.4
Althaea officinalis	0	10	0	198856	0	0	0	82	
Althaea officinalis	0	9	0	195008	0	0	0	63	
Rubus fruticosus agg.	197	8	0	29588	0	0	0	37	7.1±5
Rubus fruticosus agg.	76	18	0	26205	0	0	0	34	
Rubus fruticosus agg.	121	10	0	26898	0	0	0	28	
Petroselinum crispum	0	0	0	1718	0	0	0	0	2.8±8
Petroselinum crispum	0	0	0	2024	0	0	0	0	
Petroselinum crispum	0	0	0	1738	0	0	0	0	
Taraxacum *sect.* ***Ruderalia***	0	4	0	460	0	0	0	0	6.4±6
Taraxacum *sect.* ***Ruderalia***	0	5	0	320	0	0	0	0	
Taraxacum *sect.* ***Ruderalia***	0	3	0	192	0	0	0	0	

Laurus nobilis	0	3373	0	17711	0	0	0	21	9.5±3
Laurus nobilis	0	227	0	16591	0	0	0	14	
Laurus nobilis	0	207	0	20276	0	0	0	23	
Nymphaea hybrida	0	2	0	0	0	0	4	0	6.2±8
Nymphaea hybrida	0	1	0	0	0	0	6	0	
Nymphaea hybrida	0	1	0	0	0	0	7	0	

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