Landes Bioscience www.landesbioscience.com

Supplemental Material to:

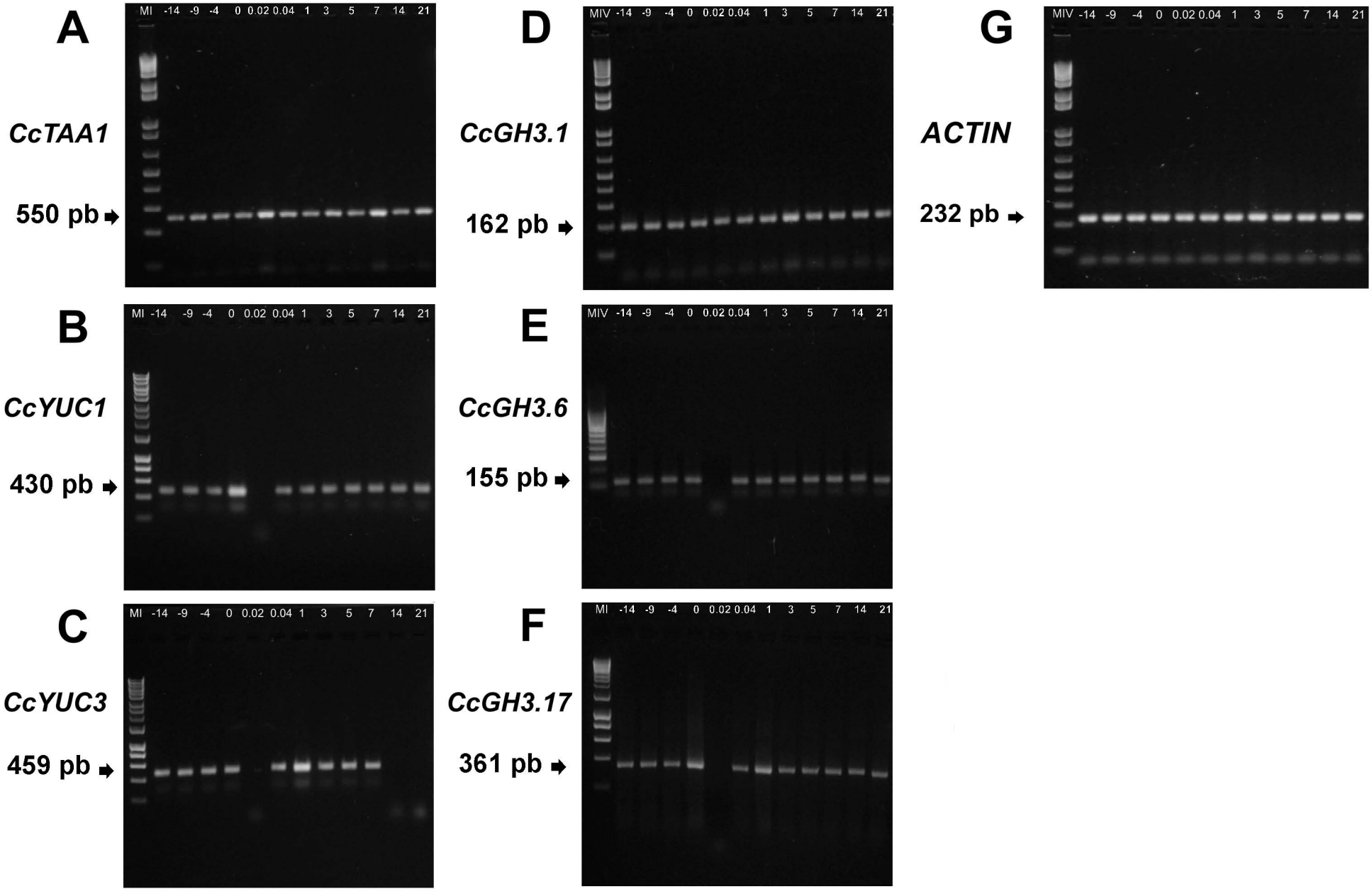
Benajmín Ayil-Gutiérrez, Rosa Galaz-Ávalos, Eduardo Peña-Cabrera, and Victor Loyola-Vargas*

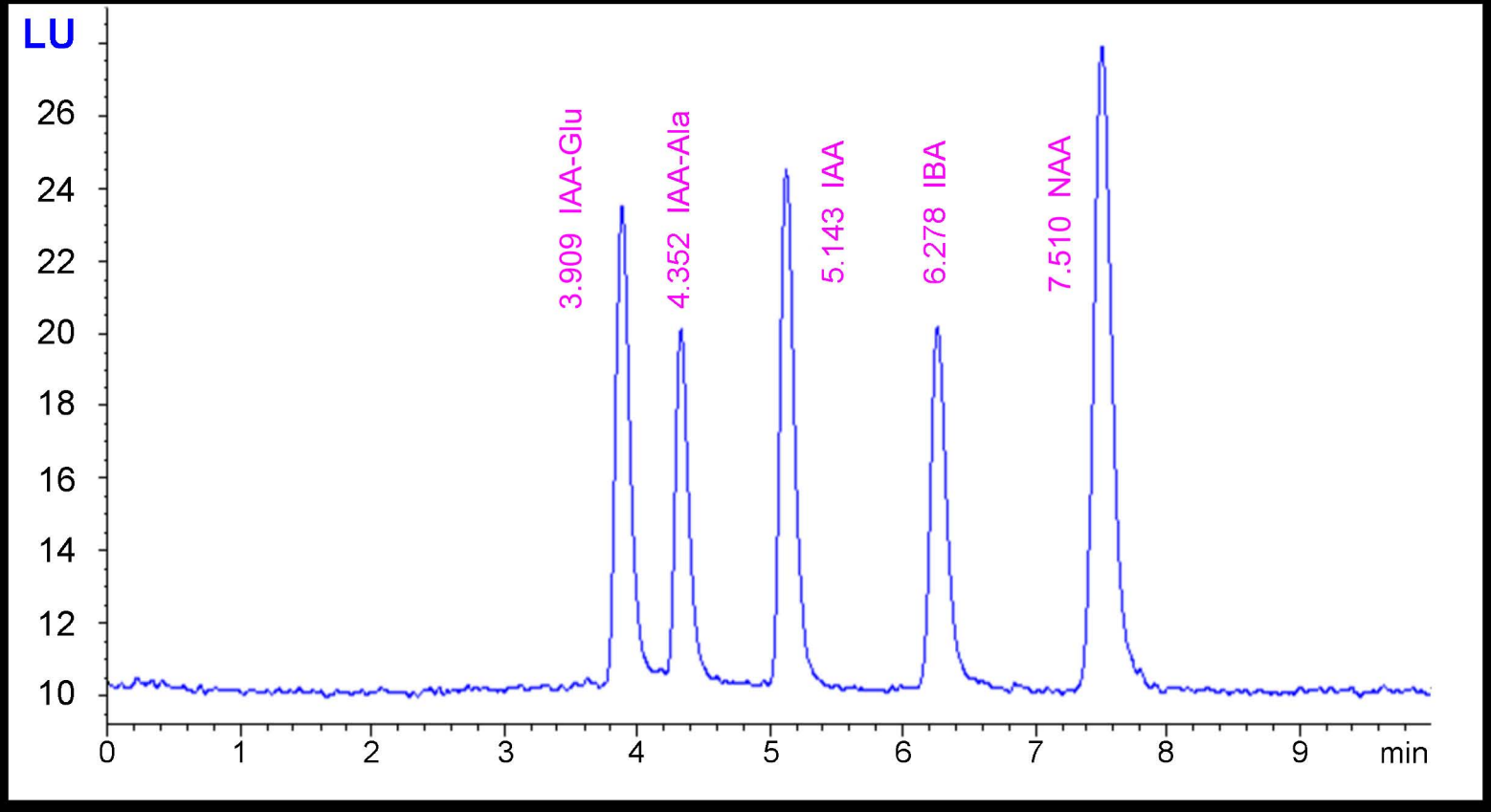
Dynamics of the concentration of IAA and some of its conjugates during the induction of somatic embryogenesis in Coffea canephora

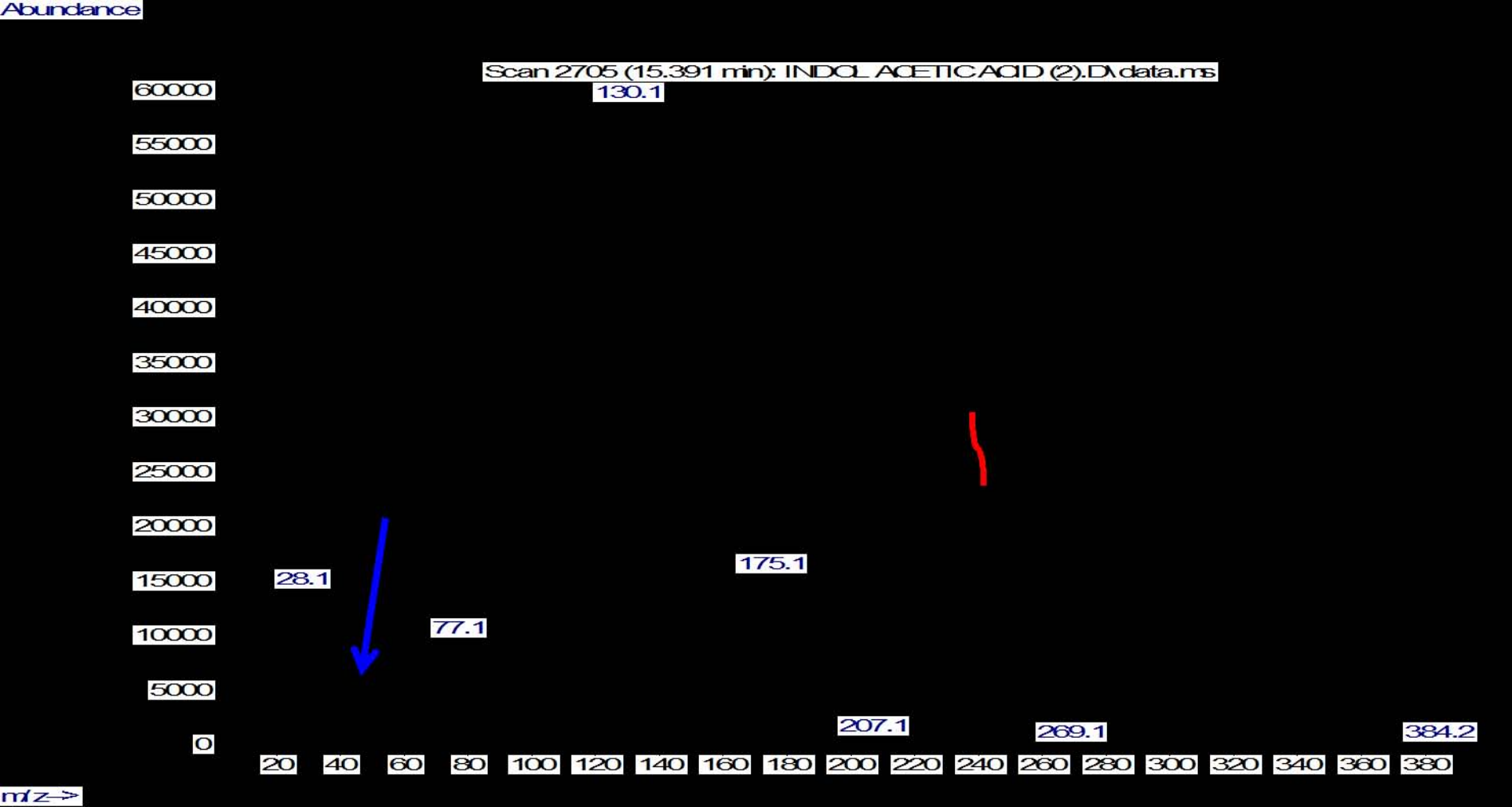
Plant Signaling & Behavior 2013; 9(1)
http://dx.doi.org/10.4161/psb.26998

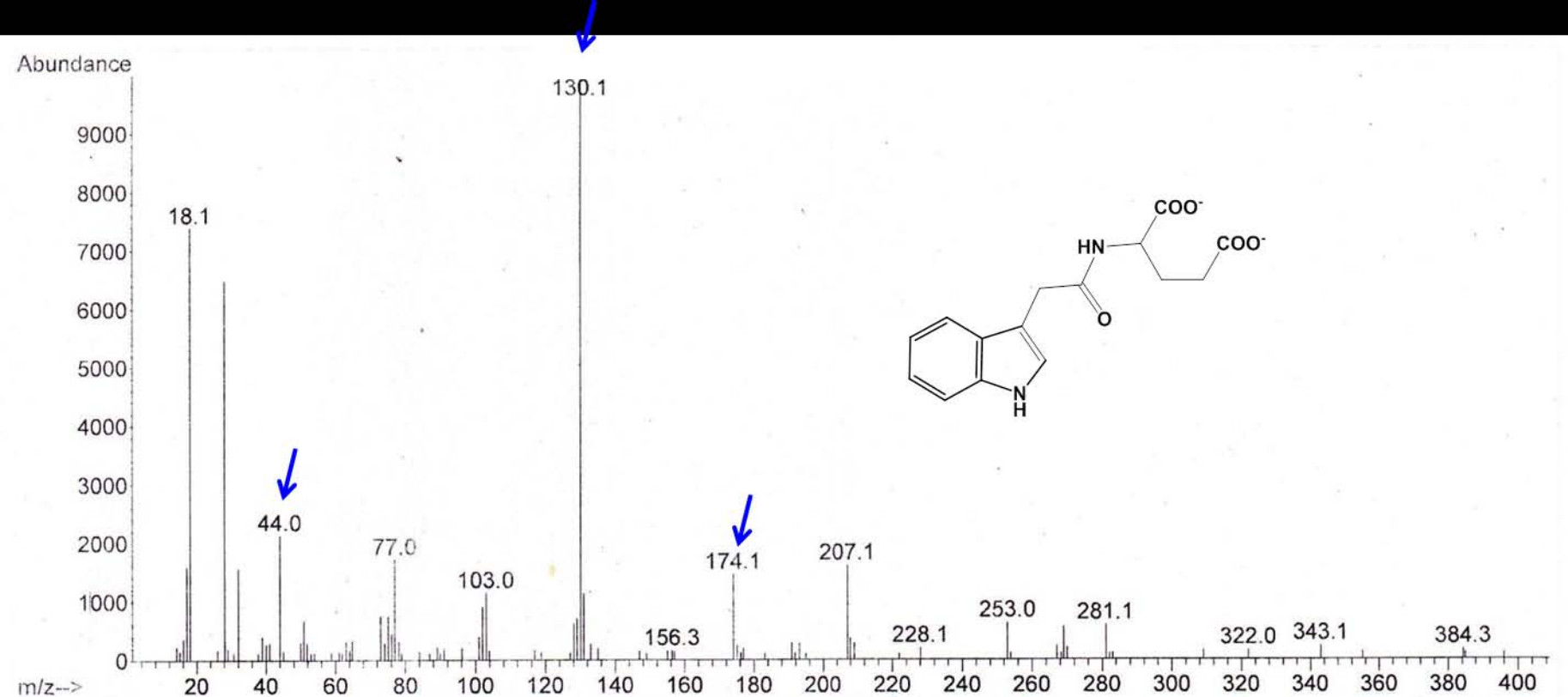
www.landesbioscience.com/journals/psb/article/26998


A
0.5 cm
B
1 cm
C
2 cm


A
CcTAA1
550 pb
MI -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
B
CcYUC1
430 pb
MI -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
C
CcYUC3
459 pb
MI -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
D
CcGH3.1
162 pb
MIV -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
E
CcGH3.6
155 pb
MIV -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
F
CcGH3.17
361 pb
MI -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21
G
ACTIN
232 pb
MIV -14 -9 -4 0 0.02 0.04 1 3 5 7 14 21


LU
26
24
22
20
18
16
14
12
10
3.909 IAA-Glu
4.352 IAA-Ala
5.143 IAA
6.278 IBA
7.510 NAA
0
1
2
3
4
5
6
7
8
9
min


Abundance
Scan 2705 (15.391 min): INDOL ACETIC ACID (2).D\data.ms
130.1
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
28.1
77.1
175.1
207.1
269.1
384.2
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
m/z-->


Abundance
130.1
18.1
44.0
77.0
103.0
174.1
207.1
156.3
228.1
253.0
281.1
322.0
343.1
384.3
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
m/z-->
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
COO-
HN
COO-
O
N
H

Supplementary figures.

Figure 1S. Germination of somatic embryos. A) Cotyledonary somatic embryos. B) Germination of the somatic embryos. From left to right, cotyledonary somatic embryo; development of the plantlets until the stage of six leaves. C) Individual *C. canephora* plantlet from a three-month-old somatic embryo.

Figure 2S. Transcript profile of auxin homeostasis-related genes during the process of somatic embryogenesis on *C. canephora*. Total RNA was extracted from foliar tissues from pre-conditioning conditions (-14,-9,-4 and 0 days) and embryogenic conditions (0.02, 0.04, 1, 3, 5, 7, 14 and 21 days), and the gene expression was determined by RT-PCR. A-C represents the transcription of genes involved in the auxin biosynthesis, D-E represents the transcription of genes involved in the auxin conjugation, and F represents the gene expression of the actin constitutive gene. The size of each fragment cloned and sequenced corresponds to the evaluated genes and was verified by electrophoresis in agarose gels at 1.5% and stained with GelRed. MI and MIV represent the molecular markers HyperLadder I (to fragments above a 200 pb) and HyperLadder IV (to fragments less than 200 pb), respectively.

Figure 3S. HPLC chromatogram profile for IAA, IBA, NAA and IAA-Glu and IAA-Asp standards. The samples were analyzed as described in Materials and Methods. The retention times are 3.86 min (IAA-Glu), 3.93 min (IAA-Asp), 4.29 min (IAA-Ala), 5.02 min (IAA), 6.12 min (IBA).

Figure 4S. HPLC chromatograms for the standardization of the IAA quantification. One hundred mg of *C. canephora* foliar tissue was extracted and analyzed as described in Materials and Methods. A) Chromatogram of the sample without any further addition. B) Chromatogram of a sample processed as in A and added with 1 mg ml^{-1} of BHT. C) Chromatogram of a sample processed as in B, but extracted in the presence of IAA.

Figure 5S. Gas chromatography–mass spectrometry identification of auxin IAA. The expected quinolinium ion with an m/z of 130, as well as the other expected fragments, were present.

Figure 6S. Gas chromatography–mass spectrometry identification of IAA-Glu conjugate. The expected quinolinium ion with an m/z of 130, as well as the others expected fragments, were present.

Supplementary tables.

Table 1S. Primers designed for qPCR and RT-PCR.

Gene	Sequences of the primers
CcTAA1	F-5'-CGGAACCATCAGAGAGACGGT-3'
	R-5'-GTCAGATCCACAACGCTCTCC-3'
CcYUC1	F-5'-AGAAGGTTTTGGTGGTGG-3'
	R-5'-CTTTTGTAACCTGTTGCTAA-3'
CcYUC3	F-5'-GGCCCWTCAGGTCTAGCC-3
	R-5'-GRGATTTRTAGTCACMAGCATG-3'
GH3.1	F-5'-TCCAGAAGATGGTCTTGGCAG-3'
	R-5'-CGATCACCGTTTTCCATACGC-3'
GH3.6	F-5'-GCTGGAGGAGATGCCTCTTT-3'
	R-5'-CATCGAGACCCCATCTCTGC-3'
GH3.17	F-5'-TTGTGCAGCGAGATGCGATA-3'
	R-5'-TAGGCTGGCGAAACTACAAGC-3'
ACTIN	F-5'-GGAGAAGAGTTATGAGCTGCCTGAC-3'
	R-5'-CATACGATCAGCAATACCAGGGAA-3'