

Figure 1. Identification of UGT74E2 as an IBA glycosyltransferase.

(A) Relative conversion rates of different plant hormones to their glucosylated form by recombinant UGT74E2. The naturally occurring auxin IBA is the preferred substrate. Error bars are SE (n=2).

(B) Separation by liquid chromatography of buffered samples containing UDP-Glc and IBA without (left) and with (right) addition of recombinant UGT74E2. Peak identity was determined by mass spectrometry. A significant proportion of IBA was glucosylated only in the presence of UGT74E2.

(C) Separation by liquid chromatography of buffered samples containing UDP-Glc and IAA (top) or IBA (bottom) in short-term incubation assays (10 min). No presence of IAA-Glc could be detected, whereas a significant proportion of IBA was glucosylated.

ABA, abscisic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; IBA-glc, IBA-glucose; JA, jasmonic acid; NAA, naphthaleneacetic acid; 2,4-D, dichlorophenoxyacetic acid; SA, salicylic acid.

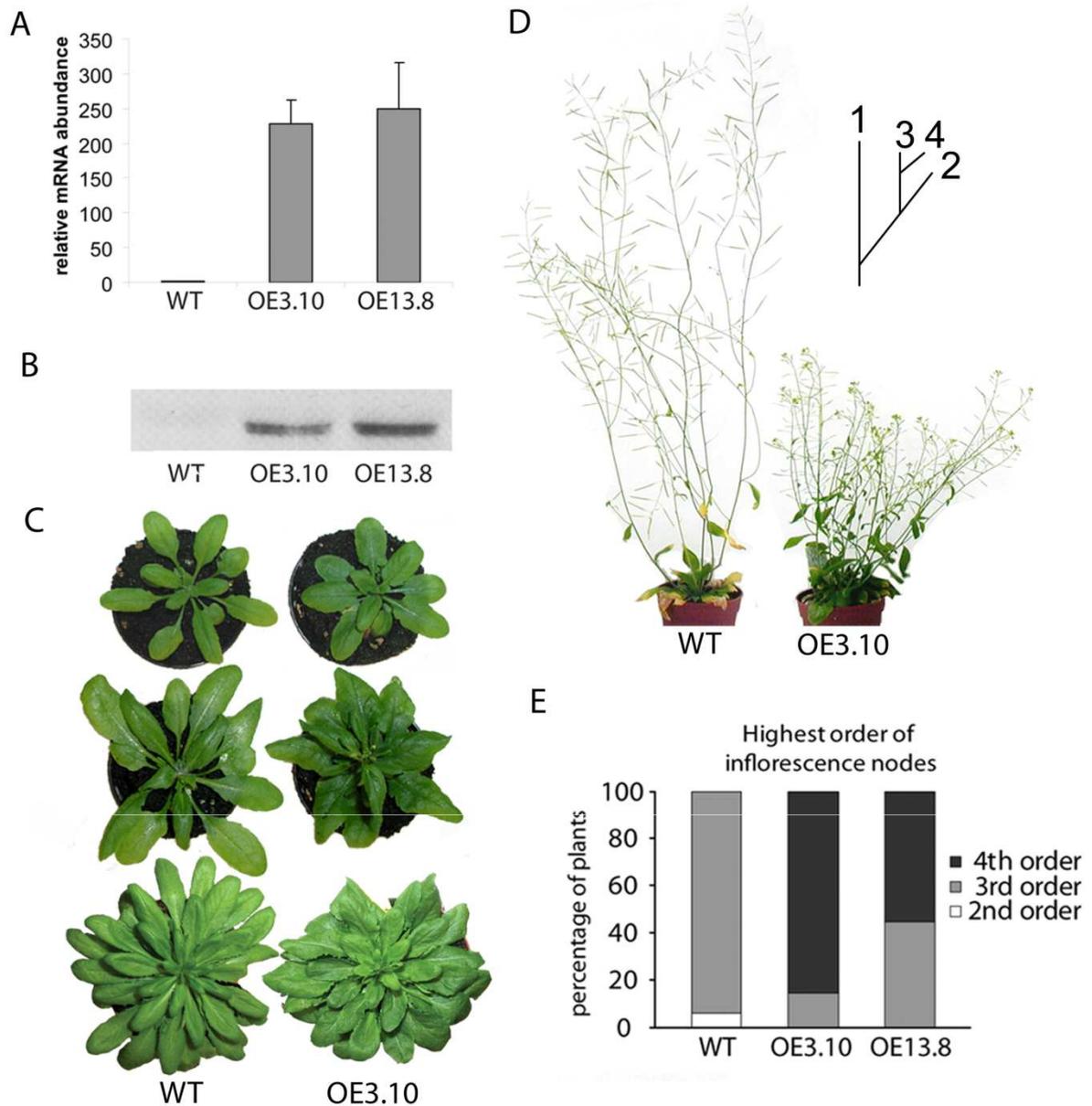


Figure 2. Modulation of *Arabidopsis* architecture by overexpression of *UGT74E2*.

(A) *UGT74E2* mRNA levels in wild-type and two lines of overexpressing *UGT74E2*OE plants assessed by real-time RT-PCR. Error bars are SE (n=3).

(B) *UGT74E2* protein levels in wild-type and *UGT74E2*OE plants assessed by protein gel blot with leaves from 1-month-old plants. For each lane, 200 μ g of soluble protein was loaded.

(C) Rosette shapes of 3-, 4-, and 5-week-old wild-type and *UGT74E2*OE plants growing under long-day (top, middle) or short-day (bottom) regimes, respectively. Note the intense green color of *UGT74E2*OE plants and the compressed rosette structure.

(D) Mature *UGT74E2*OE plants that were shorter and displayed a higher level of shoot branching than the wild type. Diagram illustrates the concept of inflorescence node order, in which 1 is the primary inflorescence and 2-4 are the subsequent orders of axillary nodes.

(E) Higher order of inflorescence branching of *UGT74E2*OE plants compared to wild-type plants ($P < 0.001$, n=9-16).

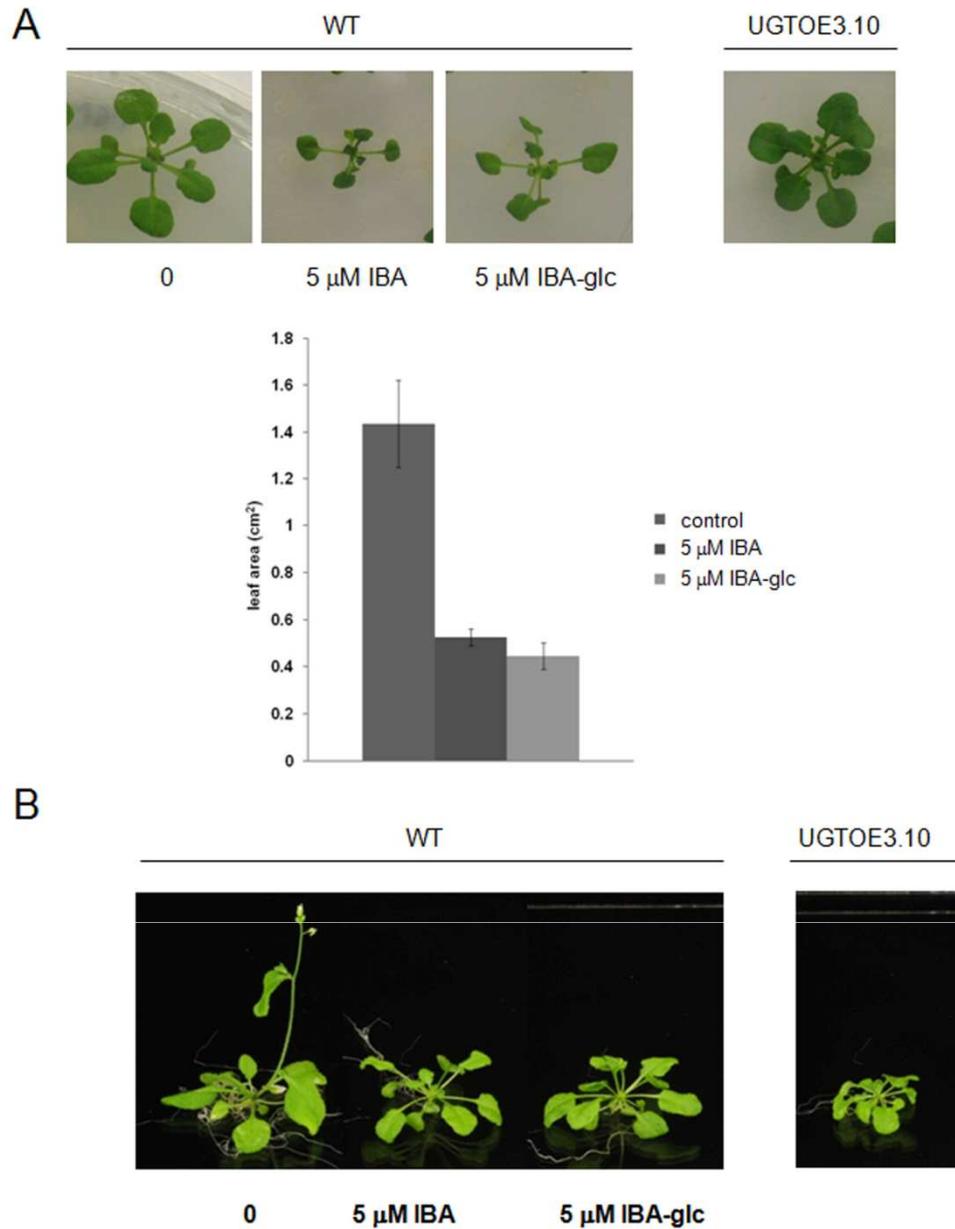


Figure3. Auxin effect on plant morphology. (A) 1-month-old (B) wild type seedlings grown on MS 0.5% sucrose plates supplemented with the indicated concentration of IBA or IBA-glc. Leaf area were calculated with the ImageJ program from NIH and expressed as cm². Error bars represent +/- SE of 3 independent experiments, n=10. (B) Effect of auxin on flowering time in 48-day-old plants grown on MS 0.5% sucrose magenta boxes supplemented with auxin. Late-flowering transition phenotype of the UGT74E2OE plants compared to wild-type plants.

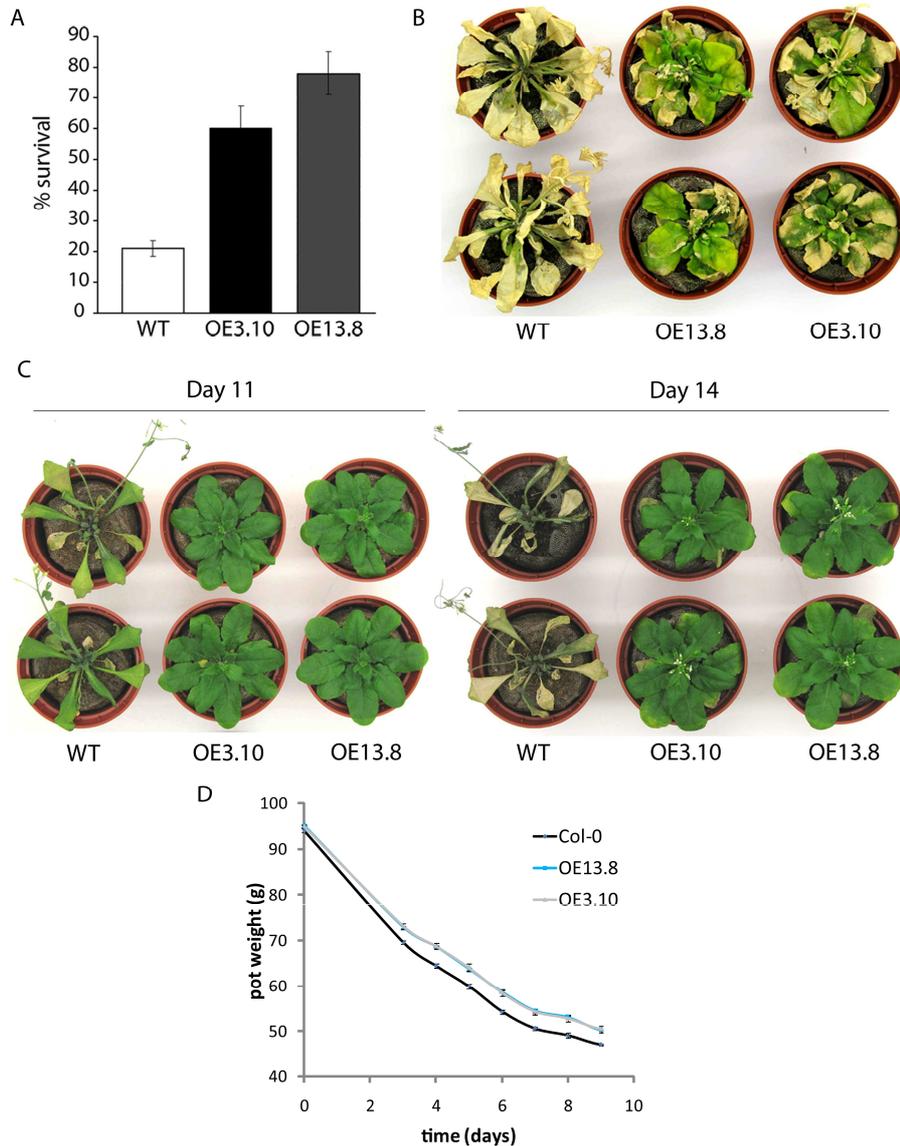


Figure 4. Increased tolerance of UGT74E2OE plants against osmotic stress.

(A) One-month-old wild-type and overexpressing transgenic seedlings grown on MS agar plates and transferred to plates supplemented with 150 mM NaCl. Survival was assessed 8 days after transfer. Overexpressing lines showed significantly increased survival compared to wild-type plants ($P < 0.05$, Student *t*-test). Measurements were made on two independent replicates of 20 seedlings each. Error bars indicate SE.

(B) Plants of wild-type Col-0 and two independent UGT74E2OE lines grown under a controlled watering regime for 3.5 weeks followed by watering for 3 weeks with 500 mM NaCl.

(C) Plants of wild-type Col-0 and two independent UGT74E2OE lines grown under a controlled watering regime for 3.5 weeks and deprived from further watering for 12 days. On day 13, plants were rewatered and observed for recovery on day 14.

(D) Plants were grown with 1.5 g H₂O/g dry soil for 3.5 weeks, after which both groups were not watered for 9 days. The total weight (pot + soil + plant) was recorded at different times. During the dehydration period, the water economy of the UGT74E2OE plants was significantly better ($P < 0.05$, Student *t*-test) than that of the wild-type plants already after 3 days. Error bars indicate SE ($n = 6$).

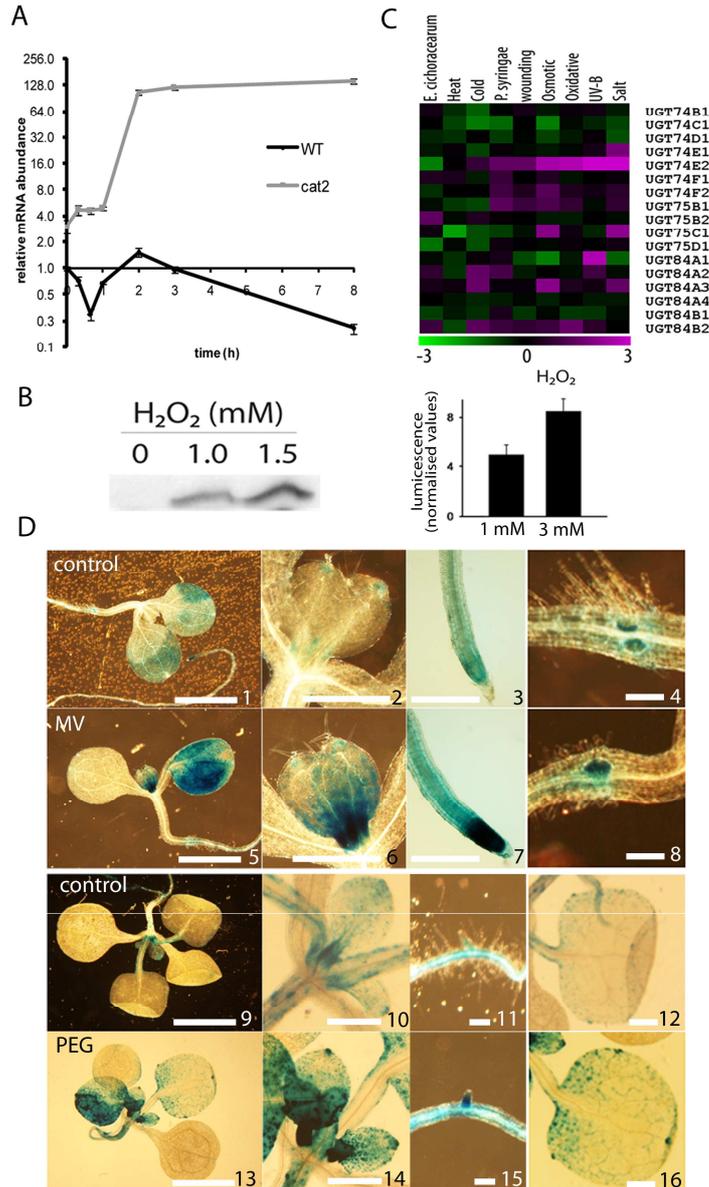


Figure 5. Induction of UGT74E2 by H₂O₂ and environmental stresses.

(A) Rapid induction of the *UGT74E2* transcript by high-light treatment in catalase-deficient (*cat2*) and wild type (WT) background as determined by qPCR. Error bars are SE (n=3).

(B) Left side : Induction of *UGT74E2* protein levels in 14-day-old wild-type seedlings exposed for 5 days to increased concentrations of exogenous H₂O₂ as determined by protein immunoblot. Right side: *UGT74E2* transcript induction was quantified in *pUGT74E2:LUC* seedlings treated with 1 mM and 3 mM H₂O₂ for 5 days. Error bars are SE (n=3).

(C) Stress-related expression patterns of the *Arabidopsis* group L glycosyltransferases. Log₂-transformed relative expression values of the group L glycosyltransferases in publicly available microarray data on biotic and abiotic stress treatments (Genevestigator). Magenta, green, and black indicate up-regulation, down-regulation, and no change vs. control experiments, respectively.

(D) Histochemical GUS staining of *pUGT74E2:GUS* seedlings under control conditions (1-4, 9-12) or exposed for 5 h to 50 mM methyl viologen (MV; 5-8) or 500 mM polyethylene glycol (PEG; 13-16). On 12-day-old seedlings (top), *pUGT74E2:GUS* expression is visible in the distal tip and base of leaf primordia (1,2), mesophyll of cotyledons (1), first formed lateral root primordia in the hypocotyl--root junction (4) and root tip (1, 3). On 16-day-old seedlings (bottom), GUS staining is prominent in young leaf primordia and developing leaf tips and stipules (9, 10), in hydathodes of developed leaves and on margins of petioles and bases (12), and in roots tips and lateral root primordia (11). MV or PEG stress increased *pUGT74E2:GUS* expression in these tissues (5-8 and 13-16). Scale bars= 2 mm (1, 2, 5, 6, 9, and 13), 0.5 mm (3, 7, 10, 12, 14, and 16), and 0.1 mm (4, 8, 11, and 15).

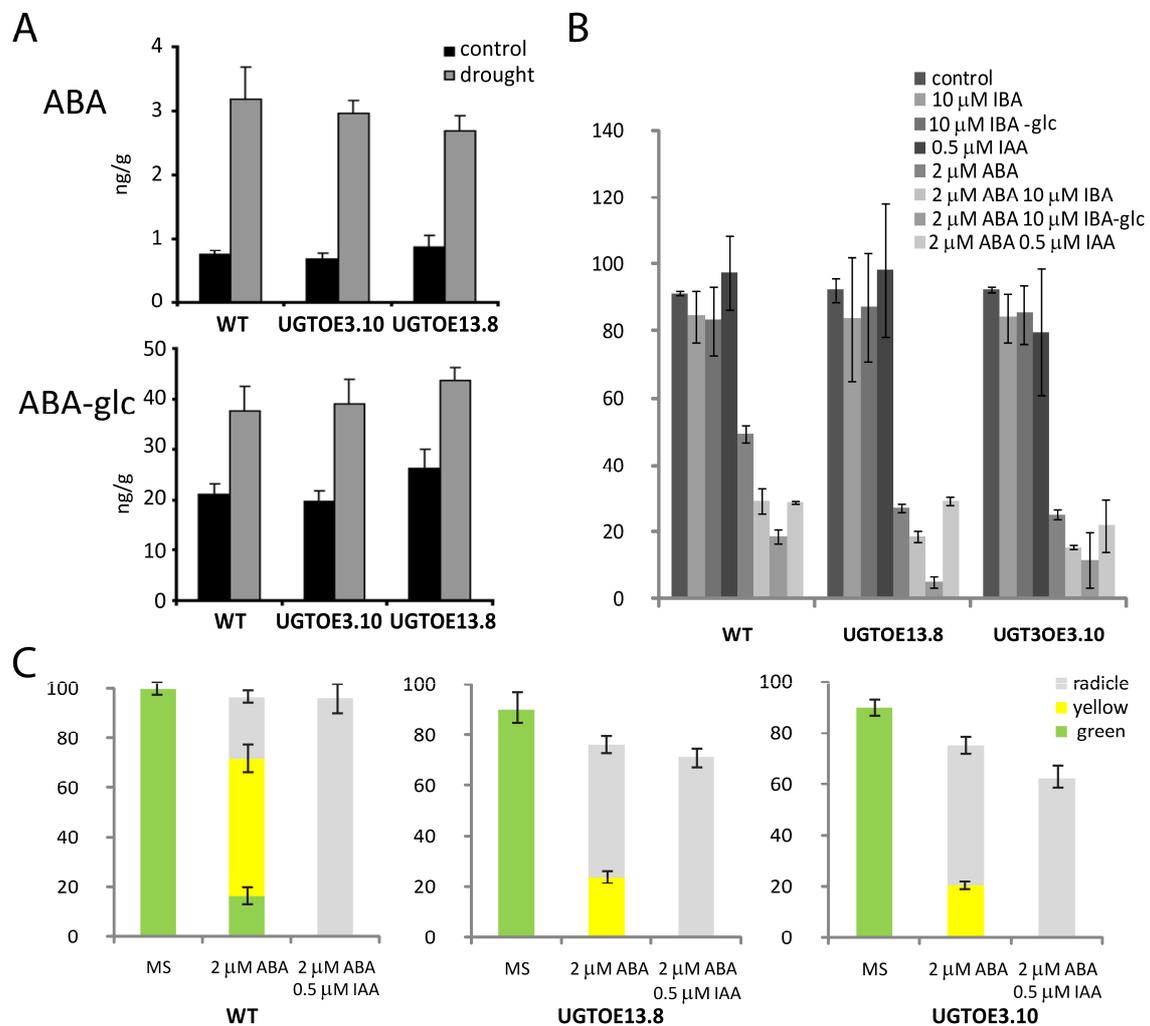


Figure 6. ABA homeostasis and response in transgenic lines.

(A) Relative abundance of ABA and ABA-Glc per unit fresh weight of wild-type and UGT74E2OE plants quantified by HPLC-MS under control and dehydrated conditions. Data are based on three biological independent replicates (means \pm SE).

(B) Percentage of emerged radicles for wild-type and UGT74E2OE lines scored 3 days after seed imbibition in the absence (control) or presence of 2 μ M ABA, 10 μ M IBA, 10 μ M IBA-Glc, 0.5 μ M IAA, or a combination of each auxin with ABA at the same concentration as mentioned before. Data are means \pm S.E and based on three biological replicates of 50 seeds each and $P < 0.05$.

(C) Percentage of emerged green cotyledons 8 days after imbibition in wild-type and transgenic lines grown as described in (B). Data are means \pm SE and based on three replicates of 50100 seeds each and $P < 0.05$.

Table 1. Disturbed auxin levels in *UGT74E2*-overexpressing plants

Auxins	Col 0	UGTOE3.10	UGTOE13.8
Experiment 1: Control			
IBA*	0.48 ± 0.14	1.03 ± 0.27	1.37 ± 0.33
IBA-glc*	1980 ± 330	3287 ± 462	5196 ± 768
IAA	88.98 ± 18.07	82.75 ± 8.76	88.60 ± 5.73
IAA-glc	304.59 ± 29.45	193.51 ± 30.04	224.24 ± 6.27
oxIAA	305.29 ± 27.91	370.39 ± 52.50	407.48 ± 18.79
IAA-Glu	13.94 ± 3.00	19.65 ± 4.51	21.68 ± 4.48
IAA-Asp	6.22 ± 1.32	6.83 ± 1.40	7.89 ± 1.24
IAA-Ala	5.01 ± 1.61	4.06 ± 0.74	5.21 ± 0.57
metIAA	85.79 ± 12.48	82.97 ± 8.65	99.52 ± 4.99
Experiment 2: PEG			
IBA	5.67 ± 0.24	5.95 ± 2.05	7.33 ± 1.89
IBA-glc*	21002.09 ± 4319.61	29964.16 ± 15223.65	31770.65 ± 4721.77
IAA	58.21 ± 4.03	97.68 ± 2.69	111.47 ± 7.97
IAA-glc*	2081.62 ± 358.24	1233.26 ± 114.94	1003.56 ± 71.46

Measurements of endogenous auxin content (pmol g⁻¹ tissue fresh weight) under control or PEG-induced stress conditions. Five replicates were tested statistically by analysis of variance. Data are means ± SE. Differences that were statistically significant are indicated in bold (n=5, p<0.05). *For experiment 1, IBA and IBA-Glc measurements (n=9-10) were p=0.06 for IBA, p=0.004 for IBA-Glc. Hormone levels for wild-type and transgenic lines grown under control conditions for experiment 2 were of similar magnitude as observed in experiment 1; p=5x10⁻⁴ for IBA-glc, p=9x10⁻⁶ for IAA-glc.

Table 2. Phenotypic comparison of wild type and UGT74E2OE plants. Wild-type plants and transformants grown in soil for 51 days in the growth chamber under short-day conditions. Gas exchange in 1-month-old leaves at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and chlorophyll *a* fluorescence were measured as described (Methods). Data are means \pm SE (n =3). Differences that were statistically significant are indicated in bold ($P < 0.005$).

	Wild type	UGTOE3.10	UGTOE13.8
Chlorophyll <i>a+b</i> ($\mu\text{g cm}^{-1}$)	12.5 \pm 0.2	17.6 \pm 0.3	15 \pm 0.2
Height (cm)	18.1 \pm 0.6	9.2 \pm 0.05	9.0 \pm 0.6
Net CO ₂ assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	12.8 \pm 0.6	12.3 \pm 0.8	13.5 \pm 0.4
Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.23 \pm 0.01	0.22 \pm 0.02	0.26 \pm 0.02
Rosette fresh weight (g)	14.9 \pm 0.8	13.8 \pm 1.5	13.4 \pm 0.7
Root length on MS without sucrose after 10 days (n=5)	3.45 \pm 0.1	2.2 \pm 0.2	1.6 \pm 0.1

Table 3. Auxin effect on photosynthesis. Wild-type and transgenic plants grown on MS 0.5% agar plates were transferred to new plates supplemented with polyethylene glycol (PEG), mannitol (M), or both PEG and auxin: M+IBA, M+IBA-glc, and PEG+IAA. Seedlings were also germinated on plates supplemented with IBA, IBA-glc, or IAA and, after 2 weeks, transferred to new PEG or M plates supplemented with IBA (M+IBA-IBA treated), IBA-glc (M+IBA-glc-IBA-glc treated) or IAA (PEG+IAA-IAA treated). Chlorophyll *a* fluorescence was measured after 8 days of stress as described (Methods). Data are means \pm SE of two independent experiments (n=25). Statistically significant differences ($\alpha=0.05$) were obtained by weighted ANOVA analyses (see Methods). ^{*1}P <0.01 between UGTOE and wild type; ^{*2}P <0.01 between PEG or M and control growth conditions; ^{*3}P <0.01 between auxin treatment and UGTOE lines under PEG or M treatment; ^{*4}P <0.01 between auxin treatment and wild-type plants under PEG or M treatments. ETRmax: maximal electron transfer rate.

Lines and treatments	ETRmax
Wild type control	35.0 \pm 1.1
UGTOE13.8 control	33.2 \pm 1.2
UGTOE3.10 control	36.1 \pm 0.5
Wild-type M	24.4 \pm 0.7 ^{*1*2}
UGTOE13.8 M	30.0 \pm 1.0 ^{*1}
UGTOE3.10 M	32.6 \pm 1.0 ^{*1}
Wild type M+IBA	12.2 \pm 0.5 ^{*3*4}
Wild tpe M+IBA-IBA treated	15.9 \pm 0.2 ^{*3*4}
Wild type M+IBA-glc	20.8 \pm 1.1 ^{*3}
Wild type M+IBA-glc-IBA-glc treated	18.5 \pm 0.7 ^{*3*4}
Wild type PEG	26.3 \pm 1.3 ^{*1*2}
UGTOE13.8 PEG	34.5 \pm 0.8 ^{*1}
UGTOE3.10 PEG	32.7 \pm 0.4 ^{*1}
Wild type PEG+IAA	22.1 \pm 1.0 ^{*3}
Wild type PEG+IAA-IAA treated	32.7 \pm 1.1