

Fig. S1. Expression patterns of *CYP77A4* gene from a publicly available database.

(A and B) Expression levels of *CYP77A4* gene in various tissues (A) and embryos (B) based on microarray data taken from the eFP-browser (<http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>) (Winter *et al.*, 2007). Red indicates a relatively higher expression level.

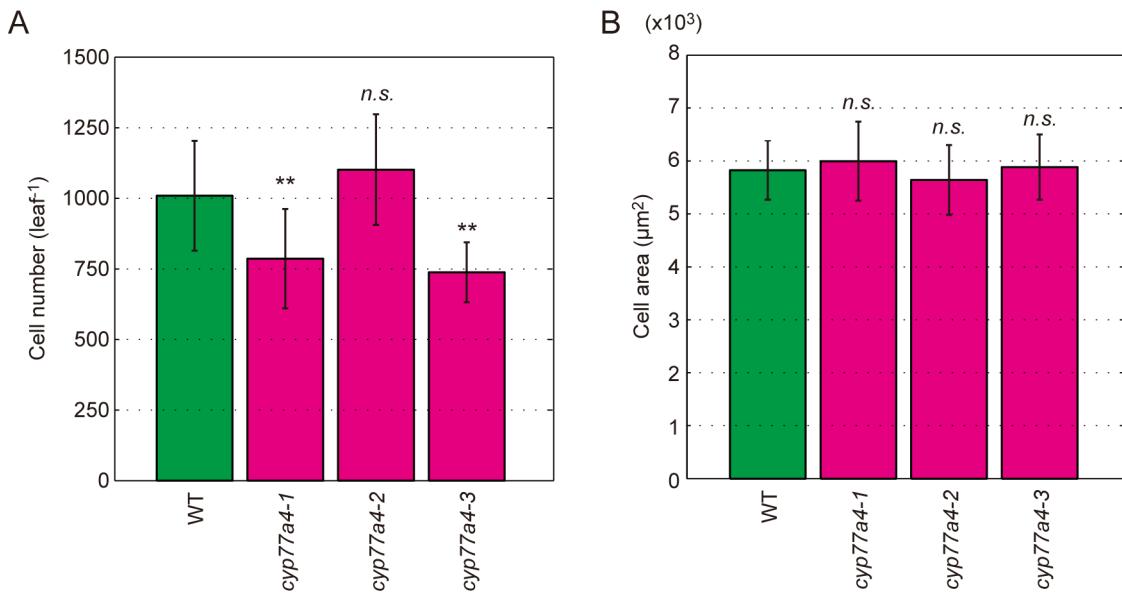


Fig. S2. Palisade cell number and size in WT and *cyp77a4* cotyledons.

(A and B) Cotyledons from 21-day-old plants were collected, fixed, and cleared for microscopic investigations. The numbers (A) and areas (B) of palisade cells in the WT and *cyp77a4* mutants. The mean \pm s.d. is shown for each line ($n = 12$ for WT, $n = 77$ for *cyp77a4-1*, $n = 69$ for *cyp77a4-2*, and $n = 68$ for *cyp77a4-3*). ** $P < 0.01$ compared with the WT (Student's *t*-test). n.s., non significant ($P > 0.05$ with Student's *t*-test).

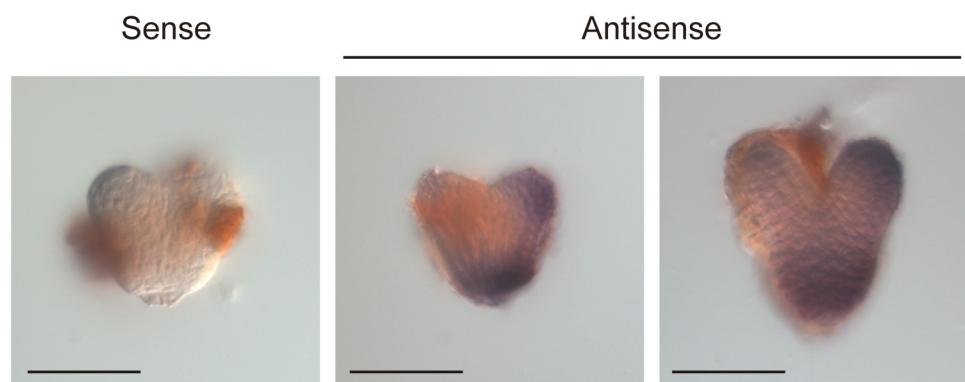


Fig. S3. Expression analysis of *CYP77A4* by whole mount *in situ* hybridization.

Accumulation of *CYP77A4* transcripts in embryos. Probe to the sense strand of the *CYP77A4* transcript was used as a negative control. Whole mount *in situ* hybridization was performed as follows: *CYP77A4* cDNA was cloned into pZErO-2 vector (Thermo Fisher Scientific) using primers listed below. DIG-labeled RNA probes of both sense and antisense strands were transcribed by DIG RNA Labeling Kit (Roche). The embryos were dissected from 5-week-old plants and fixed in a fixative containing 4% (w/v) paraformaldehyde and 1% (v/v) glutaraldehyde in PBS. The subsequent procedures were described elsewhere (Rozier *et al.*, 2014). Scale bars: 50 µm.

(Primers used)

5'-CTGCTTGAGTCAACAAGAAATAAACAG-3'

5'-AGCAAACACACCTTACAATAATCTC-3'

(Reference)

Rozier, F., Mirabet, V., Vernoux, T. and Das, P. (2014). Analysis of 3D gene expression patterns in plants using whole-mount RNA *in situ* hybridization. *Nat. Protoc.* **9(10)**, 2464-2475.

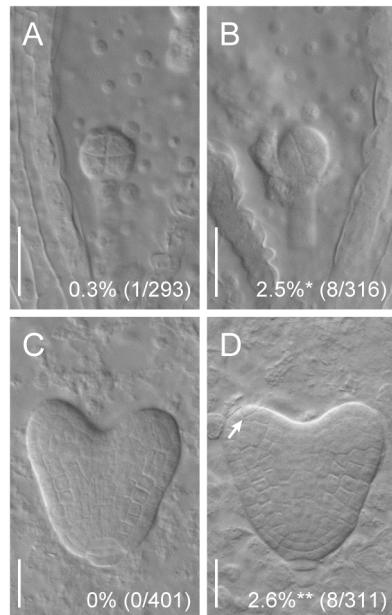


Fig. S4. Irregular cell division pattern in the *cyp77a4* mutants.

(A-D) Embryos at the earlier development stage than 32-cell and at the heart stages in the WT (A and C) and the *cyp77a4*-3 mutants (B and D). Penetrance of the irregular cell division pattern (A and B) and periclinal/oblique cell division in the protodermal tissue (C and D) were calculated as a frequency of the embryos with altered division pattern against normal embryos. An arrow indicates the irregular cell division. * $P<0.05$, ** $P<0.01$ compared with the corresponding WT (two-tailed Fisher's exact test). Differential interference contrast images of cleared immature seeds are shown. Samples were collected from 5-week-old plants. Scale bars: 25 μ m.

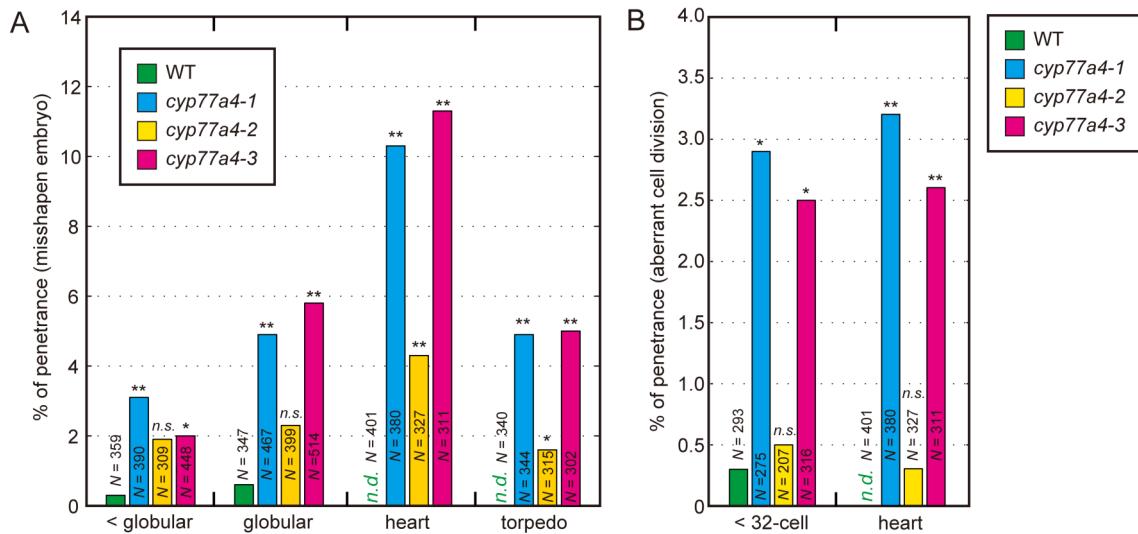


Fig. S5. Penetrance of abnormal morphology and aberrant cell division in *cyp77a4* embryos.

(A and B) Missshapen embryos at the earlier development stage than globular (< globular), globular, heart and torpedo stages (A), and irregular divisions at the earlier development stage than 32-cell (< 32-cell) and heart stages (B) were observed in *cyp77a4* mutants, particularly in the null alleles (*cyp77a4-1* and *cyp77a4-3*), when compared with the WT controls. * $P<0.05$, ** $P<0.01$ compared with the corresponding WT at each developmental stage (two-tailed Fisher's exact test). n.s., non significant ($P>0.05$). n.d., non detected.

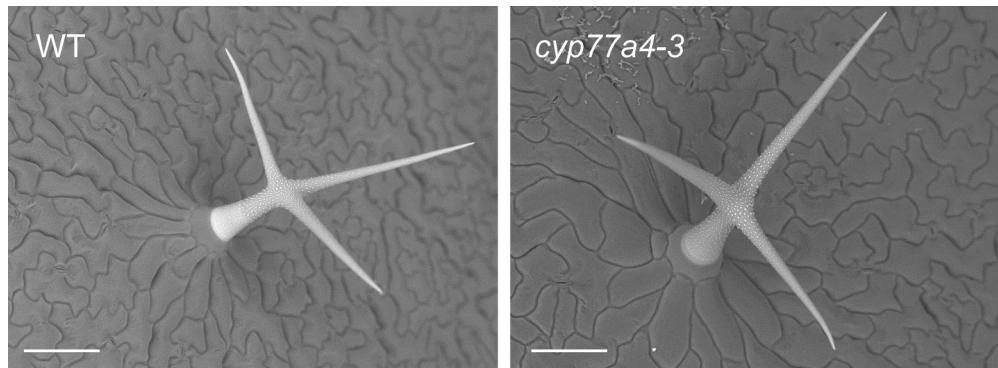


Fig. S6. Scanning electron microscopy images of trichomes.

Mature leaves of 4-week-old WT and *cyp77a4-3* plants were sampled. Scanning electron microscopy (SEM) was performed using a Hitachi TM3000 SEM microscope at 15 kV. Scale bars: 100 μ m.

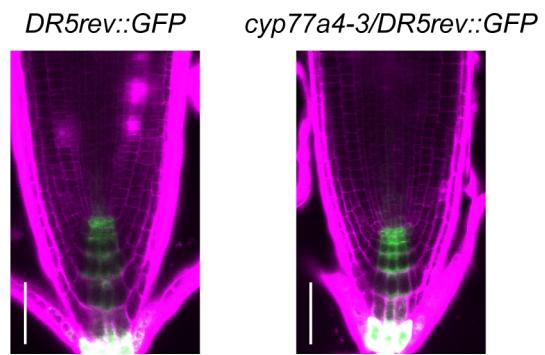


Fig. S7. Expression of *DR5_{rev}::GFP* in WT and *cyp77a4* roots.

Confocal images of the root tip in 7-day-old seedlings in *DR5_{rev}::GFP* and *cyp77a4-3/DR5_{rev}::GFP*. GFP fluorescence and propidium iodide stain are shown in green and magenta, respectively. Scale bars: 50 μ m.

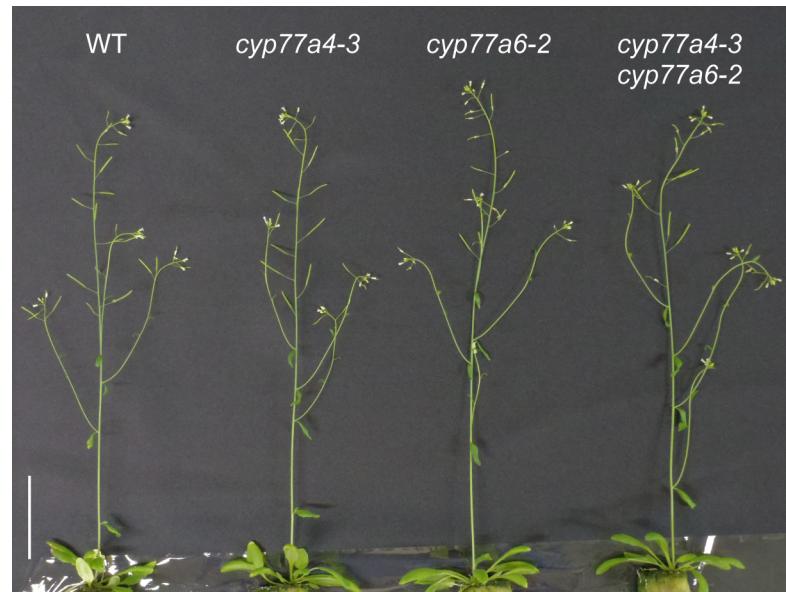


Fig. S8. Overall growth of the *cyp77a4* *cyp77a6* double mutant.

Four-week-old WT, *cyp77a4-3*, *cyp77a4-2*, and *cyp77a4-3 cyp77a6-2* plants. Scale bar: 5 cm.

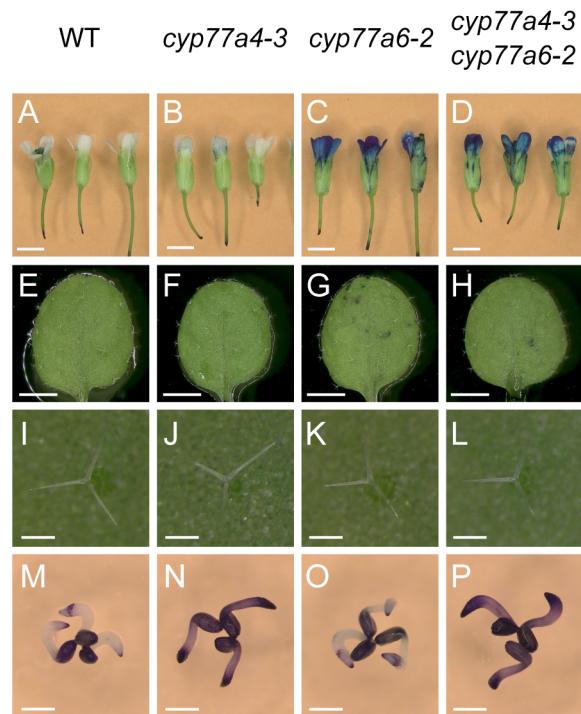


Fig. S9. Toluidine blue permeability of the *cyp77a4* mutant.

(A–P) Flowers (A–D), leaves (E–H), trichomes (I–L), and germinating embryos (M–P) of the WT (A,E,I,M), *cyp77a4-3* (B, F, J, N), *cyp77a6-2* (C, G, K, O), and *cyp77a4-3 cyp77a6-2* (D,H,L,P) stained by toluidine blue. Scale bars: 2 mm (A–H), 200 μ m (I–L) and 500 μ m (M–P).

Table S1. Arabidopsis T-DNA insertion mutants of cytochrome P450 genes

| Stock number | Locus | Gene name | Genotyping primers | |
|--------------|-----------|-----------|------------------------|------------------------|
| | | | Forward 5'-3' | Reverse 5'-3' |
| SALK_001709C | At3g48520 | CYP94B3 | TGCTCAATCCAATTGGTTTC | AAAACGAAGCGTGTGTTGAAC |
| SALK_003101C | At3g30290 | CYP702A8 | TCCCTCATATAATCATGCAACG | AACTCTCCAAACTCCTCTCCG |
| SALK_005826C | At4g00360 | CYP86A2 | ATCGAACACATGCTCAAGACC | GAATTCCAAGCAATCCTCTCC |
| SALK_006594C | At3g20110 | CYP705A20 | AGCAATGTTGACCGTTGACTC | ACGTTACGGTTGTTGATGAGC |
| SALK_008736C | At3g48290 | CYP71A24 | TCGGCATAACGTAACGTTTC | GCTCTTCTTCCCTCTCGACTG |
| SALK_009366C | At3g53300 | CYP71B31 | TCCAACGTTAGGATCACGTC | ATGCTCAAACACAAACCTTGG |
| SALK_011806C | At1g16410 | CYP79F1 | CTAGGTCCAATATTCCGCC | CACAAGCCTGTCTTCCAAC |
| SALK_012075C | At1g13110 | CYP71B7 | TCAGAGCCAACCAAACAAAG | GAGGAAGCTTCCATTTGAGG |
| SALK_019080C | At3g10570 | CYP77A6 | GCTCAATGAAAGTCCAGCATC | GCTCCGTTTATTCCAAGGAG |
| SALK_021290C | At2g46960 | CYP709B1 | GTCAGGTGCCTGAAACTTG | TGAGATGCATATCCTGGCTC |
| SALK_023343C | At3g26280 | CYP71B4 | CAGAAGGCATGGTAGTCGAAG | TCCGATGTCTTACCGTTACG |
| SALK_023674C | At3g53290 | CYP71B30P | CTCCCTCTAAACCAAACCGAC | TCCCAAGATTAACAATGTCCG |
| SALK_036476C | At3g13730 | CYP90D1 | TTTGCCTTGTGAAGGAATACG | GAAGGAATTGACAGGGAGAGCC |
| SALK_046395C | At5g04660 | CYP77A4 | TGTTTACAAAGCAATGAGGGC | TGCGAATCCTGAGATTCAATC |
| SALK_047258C | At3g14610 | CYP72A7 | CCCAACTCTGCTGAAACAAAG | TTCCCTTGCATTAGATCATC |
| SALK_048981C | At4g15110 | CYP97B3 | CCTGGAGCTCTAACATCTG | ATATTGGACAATGCGAGAAC |
| SALK_056876C | At2g45580 | CYP76C3 | CACAAACACAGTGGTTACCTG | GTACGGCACAAAGAGATTGG |

| Stock number | Locus | Gene name | Genotyping primers | |
|--------------|-----------|-----------|-------------------------|-------------------------|
| | | | Forward 5'-3' | Reverse 5'-3' |
| SALK_057638C | At3g53280 | CYP71B5 | CGATCTTGAGACTTGTAGCCG | ATGGTGTCTTGGAATGCTG |
| SALK_081643C | At3g01900 | CYP94B2 | CCACTATGCGTCTGGTCTCTC | CACCCAAACTTCATCTCATTG |
| SALK_086471C | At3g26330 | CYP71B37 | TTTGCCACTACACTCATTCTC | CCTGATGGGTTGAATCAAATG |
| SALK_087617C | At1g13090 | CYP71B28 | CTGAAGATCCACTCGATGAGC | ACACC GTGAAGATTGTTGC |
| SALK_090621C | At4g15330 | CYP705A1 | CCCATTTTATGATCAATGGG | GTGCCGA ACTGTATACGCATG |
| SALK_090743C | At3g44970 | CYP708A4 | TAGTTTAGTGGTTGCAGGG | TATTGCCGTGTTGAGGAAGTC |
| SALK_092654C | At1g79370 | CYP79C1 | CGGAGACGAAGAACATGATGTC | TAACCGGTATGATCAAGCTGG |
| SALK_094765C | At1g64950 | CYP89A5 | GAGTAGCAGAACTCACCAAAACC | AGGC GAAAGAAGAGGGAGATTG |
| SALK_096641C | At1g13080 | CYP71B2 | CACAGGAGATTGCTTCAAAGC | TAAAGGCATAATCATTGCCG |
| SALK_109844C | At1g13070 | CYP71B27 | CACAGGAGATTGCTTCAAAGC | CTCTATCGGCAATCCTCACAG |
| SALK_113348C | At4g39950 | CYP79B2 | CCCATATCGGCTAAGAAGGAC | AAGTTGTGATGACGGAACTCG |
| SALK_114536C | At2g46660 | CYP78A6 | CCGGTTAAAGAACATGGCTTAC | AACTCCAAGGATCAACCCAC |
| SALK_120416C | At1g17060 | CYP72C1 | CCGACATGTGAAGTAAGCTGG | AACAGAAAAAGCCAAAAGGC |
| SALK_129352C | At3g30180 | CYP85A2 | CCATGGGTTAAAGATCATTGG | TTGTTGTGGGAACTCTATCGG |
| SALK_130811C | At3g14630 | CYP72A9 | GCATTCTCAATTCAAAACATGG | TCCTGCATACCGTAAGAAC |
| SALK_142442C | At2g42250 | CYP712A1 | TCCATCTATTGGATTCAAGGG | CTCTGCAGAACCAAACCTCAGG |
| SALK_149325C | At4g15440 | CYP74B2 | CAACAGCTTAATTGAACCGG | ACATTTCTGGGAAACAATCG |
| SALK_150522C | At1g74550 | CYP98A9 | GTCAGAGCTCGAGACCACAAC | GTCGCACAAGTAGTAGGTGCC |

Table S2. Frequencies of cotyledon phenotypes in two *cyp77a6* alleles

| Genotype | Frequencies of cotyledon number (%) | | | | Total number |
|------------------|-------------------------------------|-----------------|--------------|-------|--------------|
| | One | Two (Irregular) | Two (Normal) | Three | |
| WT | 0 | 0 | 100.0 | 0 | 964 |
| <i>cyp77a6-1</i> | 0 | 0 | 100.0 | 0 | 949 |
| <i>cyp77a6-2</i> | 0 | 0 | 100.0 | 0 | 940 |

The unseparated cotyledon (Fig. 2D) and cup-shaped cotyledon (Fig. 2F) were counted as One in addition to the single cotyledon (Fig. 2E). Two separated cotyledons with bilateral asymmetry around the shoot apical meristem (Fig. 2C) were counted as Two (Irregular).

Table S3. Frequencies of cotyledon phenotypes in the *cyp77a4 cyp77a6* double mutant

| Genotype | Frequencies of cotyledon number (%) | | | | Total number |
|----------------------------|-------------------------------------|-----------------|--------------|-------|--------------|
| | One | Two (Irregular) | Two (Normal) | Three | |
| WT | 0 | 0 | 99.9 | 0.1 | 932 |
| <i>cyp77a4-3</i> | 1.0 | 0.6 | 98.4 | 0 | 945 |
| <i>cyp77a6-2</i> | 0 | 0 | 99.9 | 0.1 | 971 |
| <i>cyp77a4-3 cyp77a6-2</i> | 1.4 | 0.7 | 97.8 | 0 | 967 |

The unseparated cotyledon (Fig. 2D) and cup-shaped cotyledon (Fig. 2F) were counted as One in addition to the single cotyledon (Fig. 2E). Two separated cotyledons with bilateral asymmetry around the shoot apical meristem (Fig. 2C) were counted as Two (Irregular).

Table S4. Primers used for genotyping, RT-PCR, qRT-PCR and vector constructions.

| # | Primer name | Sequence 5'-3' |
|-------------------------------------|---------------------------------|------------------------------|
| T-DNA genotyping | | |
| 1 | <i>cyp77a4-1_LP_SALK_046395</i> | TGTTTACAAAGGAATGAGGGC |
| 2 | <i>cyp77a4-1_RP_SALK_046395</i> | TGCGAATCCTGAGATTCAATC |
| 3 | <i>cyp77a4-2_LP_SALK_112368</i> | GAATATCGTAACCAGCAAGCG |
| 4 | <i>cyp77a4-2_RP_SALK_112368</i> | AACGTATGGCCCGATTTTAC |
| 5 | <i>cyp77a4-3_LP_SALK_076143</i> | TGGGACACTCCTGTTAAAGC |
| 6 | <i>cyp77a4-3_RP_SALK_076143</i> | GTTTCCCGAATTCTTGAGAC |
| 7 | <i>cyp77a6-1_LP_SALK_019080</i> | GCTCAATGAAAGTCCAGCATC |
| 8 | <i>cyp77a6-1_RP_SALK_019080</i> | GCTCCGTTTATTCCAAGGAG |
| 9 | <i>cyp77a6-2_LP_SALK_023926</i> | AAATCAATTCACTTCCGGCG |
| 10 | <i>cyp77a6-2_RP_SALK_023926</i> | CTTCACCGTTAACGCTTCAG |
| Semiquantitative RT-PCR and qRT-PCR | | |
| 11 | <i>CYP77A4_RT-PCR_Fwd</i> | GAATCCGACCCGAACAATCTT |
| 12 | <i>CYP77A4_RT-PCR_Rev</i> | TCCGCGCAAGCATCAAAT |
| 13 | <i>ACTIN2_RT-PCR_Fwd</i> | TTCCTCTCCGCTTGAATTGTCTCG |
| 14 | <i>ACTIN2_RT-PCR_Rev</i> | GGATGGCATGAGGAAGAGAGAAACC |
| 15 | <i>CYP77A4_qRT-PCR_Fwd</i> | TGACGCATGCCGTTATGG |
| 16 | <i>CYP77A4_qRT-PCR_Rev</i> | TCCGCGCAAGCATCAAAT |
| Vector construction | | |
| 17 | <i>At5g04650_Intron1_Fwd</i> | caccGTGTTAAGAAATGAGTTGGTGGTT |
| 18 | <i>CYP77A4_CDS_Fwd</i> | caccATGTTCCCTAACTCCTTTCTCC |
| 19 | <i>CYP77A4_5'-UTR_Rev</i> | TTTAGCTCTGTTATTCTGTGACTC |
| 20 | <i>CYP77A4_CDS_Rev</i> | CTAAATCCTGGTTGACCATAGCT |
| 21 | <i>CYP77A4_CDS_Rev (-TAG)</i> | AATCCTGGTTGACCATAGCTCT |
| 22 | <i>CYP77A4_3'-UTR_Rev</i> | GTGTAGATTGGTACTTAATGTTATAA |