Characterization of biosynthetic pathways for the production of the volatile DMNT and TMTT in *Zea mays*

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Supplemental Figure 1: Analysis of purified maize TPS2 and TPS3 by SDS-PAGE.

(A) empty vector pASK-IBA37plus.

(B) TPS2 allele of inbred line Tzi8 with predicted weight of 65.24 kDa.

(C) TPS2 allele of inbred line B73 with predicted weight 65.44 kDa.

(D) TPS3 allele of inbred line B73 with predicted weight 64.79 kDa. Lane 1, purified Eluat-I; lane 2, purified Eluat-II; lane 3, purified Eluat-III; lane 4, purified Eluat-IV; M, pre-stained protein ladder, 10 to 250 kDa (Thermo Scientific 26619); lane 3, protein extract of FPPS3 in pASK-IBA37plus; lane 4, protein extract of FPPS2 in pASK-IBA37plus; lane 5, empty vector pASK-IBA37plus; lane S2, unstained protein marker (Thermo Scientific 26614).



Supplemental Figure 2: Chiral GC-MS analysis of the sesquiterpene and diterpene products from recombinant expressed TPS2.

(A) The products formed after incubation with FDP substrate were subjected to a chiral GC-MS analysis which allows the separation and identification of the (S) and (R) enantiomers nerolidol in comparison to an authentic nerolidol standard.

(B) The products formed after incubation with GGDP were subjected to a chiral GC-MS analysis which allows the separation of the enantiomers of geranyllinalool in comparison to an authentic geranyllinalool standard. For the analysis of chiral compounds, a Rt-bDex sm column (Restek, Bad Homburg, Germany) was used with the following conditions: GC-program: 50 °C for 1 min, first ramp 2 °C/min to 170°C, second ramp 100° C/min to 220 °C, final 2 min hold. GC–MS carrier gas: hydrogen (1 mL/min).



Supplemental Figure 3: Expression of *tps2* is induced by *S. littoralis* regurgitate. Leaves of 14 d-old plants of the inbred line B73 were wounded and treated with caterpillar regurgitate for 24 h or left undamaged (control). Transcript abundance of *tps2* was determined relative to the *APT1* reference gene. One-way-ANOVA was used to calculate the significance based on three independently treated plants. Error bars represent standard error. Triple asterisks represent significant difference from the control at P < 0.01.



Supplemental Figure 4: Quantification of the major terpene compounds in the maize inbred lines B73 and Tzi8 after wounding and treatment with indanone-derivative (elicitor). Relative peak areas of volatiles were determined by gas chromatography coupled to an FID detector. Averages and standard errors of data from six plants are shown. Triple asterisks represent significant difference from the control at P < 0.01.



Supplemental Figure 5: Quantification of the major terpene compounds in the maize inbred lines B73 and the transposon insertion mutant MuIII after wounding and treatment with indanone-derivative (elicitor). Relative peak areas of volatiles were determined by gas chromatography coupled to an FID detector. Averages and standard errors of data from three plants are shown. Triple asterisks represent significant difference from the control at P < 0.01.

Supplemental Table 1. Sequences of Nucleotide Primers.

Gene	Primer sequence 5´-3´	function	direction
tps3	CAAATCATCAACCTAATCACTAC	Cloning in pCR4-TOPO	Forward
tps3	CAAATCATCAACCTAATCACTAC	Cloning in pCR4-TOPO	reverse
tps2	GATGCATATCACTCACTC	Cloning in pCR4-TOPO	forward
tps2	CTTTCATATCTGCGGATCGGAG	Cloning in pCR4-TOPO	reverse
tps2	AAGCAGACCTGCACGAGGTT	qRT-PCR	reverse
tps2	GAAGGAGCATGGATCTAACCATG	qRT-PCR	forward
tps3	AAGGAGACCTGCGCGATGTC	qRT-PCR	reverse
tps3	CAAGGAGCATGGATCTAACCCTA	qRT-PCR	forward
tps2/3	ATGGTAGGTCTCAGCGCATGTACTCTCTACCAGGAGC	Cloning in pASK37/33plus	forward
tps2/3	ATGGTAGGTCTCATATCAAAGCACCAACATCATCGCC GCG	Cloning in pASK37/33plus	reverse
tps2 (Tzi8)	ATGGTAGGTCTCAGCGCATGTACTCTCTACCAGGAGC	Cloning in pASK37/33plus	forward
tps2 (Tzi8)	ATGGTAGGTCTCATATCAAAGCACCAACATCATCGCC GCG	Cloning in pASK37/33plus	reverse
cyp92C5	ACGACCTTCACGACCATTTC	qRT-PCR	forward
cyp92C5	CCTCATCCAGGACATCATCG	qRT-PCR	reverse
cyp92C6	AGAAGTGGTCGGGCTGCTAC	qRT-PCR	forward
cyp92C6	CTCGAAGAAGCGGTCGTACT	qRT-PCR	reverse
cyp92C5	tccggatccgagcatggagctggcatcaa	Cloning in pESC-Leu2d	forward
cyp92C5	CTACTCGAGTCATTCGGTCGCGCTGTAG	Cloning in pESC-Leu2d	reverse
cyp92C6	tccggatccgagcATGCACCACGAAACAC	Cloning in pESC-Leu2d	forward
cyp92C6	gccaagcttTCAGAACACGAGCTTGTAC	Cloning in pESC-Leu2d	reverse
APT1_ (GRMZM2 G131907)	AGGCGTTCCGTGACACCATC	qRT-PCR	forward
APT1 (GRMZM2 G131907)	CTGGCAACTTCTTCGGCTTCC	qRT-PCR	reverse