**Supplemental data. Köllner, T.G. et al. (2008).**
**A Maize (*E*)-β-Caryophyllene Synthase Implicated in Indirect Defense Responses Against Herbivores is Not Expressed in Most American Maize Varieties.**

## Supplemental Figure 1.

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                          20                  40                  60
TPS23-Del  MAADEARSVSRLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSH
TPS23-Mex  MAADEARSVSRLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSH
TPS23-Hue  MAADEARSVSRLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYSIMKDNVKIMKEEVKKMLLDVGSSDLSH
TPS23-Par  MAADEARSVSRLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSH
TPS23-Per  MAADEARSVSKLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSH
TPS23-Dip  MAADEARSVSRLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDKVKIMKEEVKKMLLDVGSSDLSH
TPS23-Lux  MAADEARSVSRLHGEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSH

                80                  100                 120                 140
TPS23-Del  KLDCIDTLERLGLDYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDI
TPS23-Mex  KLDCIDTLERLGLDYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDI
TPS23-Hue  KLECIDTLERLGLDYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHVSSDVFLKFGDDKGDI
TPS23-Par  KLDCIDTLERLGLDYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDI
TPS23-Per  KLDCIDTLERLGLGYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDI
TPS23-Dip  KLDCINTLERLGLDYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHVSSDVFLKFRDDKGDI
TPS23-Lux  KLDCIDTLGRLGLDYHYTKEIDELMCNVFEAMDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDI

                            160                 180                 200
TPS23-Del  VTNDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Mex  VTNDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Hue  VTDDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Par  VTNDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Per  VTDDARCLLRMYEAAHVRVNGEEILDNILSHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Dip  VTDDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ
TPS23-Lux  VTNDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQ

                220                 240                 260                 280
TPS23-Del  FISTYEKSTTRINMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANTTIYARDRMVEMHFWMMGVFF
TPS23-Mex  FISTYEKSTTRNNMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANTTIYARDRMVEMHFWMMGVFF
TPS23-Hue  FISTYEKSTMRNNMLLEFSKLDFNILLTLYCEELKDLTMWWKEFQAQANTTIYARDRMVEMHFWMMGVFF
TPS23-Par  FISTYEKSTTRNNMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANTTIYARDRMVEMHFWMMGVFF
TPS23-Per  FISTYEKSTTRNNMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANTAIYARDRMVEMHFWMMGVFF
TPS23-Dip  FISTYEKSTTRNNMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANTAIYARDRMVEMHFWMMGVFF
TPS23-Lux  FISTYEKSTTRNNMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANMTIYARDRMVEMHFWMMGVFF

                            300                 320                 340
TPS23-Del  EPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNAFTAALQRWDEEGVEQCPTYLRTLYTNIRATIKAI
TPS23-Mex  EPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNAFTAALQRWDEEGVEQCPTYLRTLYTNIRATVKAI
TPS23-Hue  EPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNAFTAALQRWDEEGVEQCPTYLRTLYTNIRATVKAI
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TPS23-Dip  EPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNTFTAALQRWDEEGVEQCPTYLRTLYTNIRATVKAI
TPS23-Lux  EPQYSYSRKMLTRLFMIVSVLDDLYDSHCTTEEGNAFTAALQRWDEEGVEQCPTYLRTLYTNIRATVKAI

                360                 380                 400                 420
TPS23-Del  EEDLNFQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Mex  EEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Hue  EEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Par  EEDLNFQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Per  EEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Dip  EEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA
TPS23-Lux  EEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMHLVSQGFISMGDVA

                            440                 460                 480
TPS23-Del  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Mex  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Hue  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Par  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Per  TSETLEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Dip  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA
TPS23-Lux  TSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTTTVEQAIEKIRELIEEA

                500                 520                 540
TPS23-Del  WMDITHECLRQPQPKALLERAVNLARTMDFLYKDADGYTDSRSIKGILDSLYVHLID
TPS23-Mex  WMDITHECLRQPQPMALLERAVNLARTMDFLYKDVDGYTDSRSIKGILDSLYVHLID
TPS23-Hue  WMDITHECLRQPQPMALLERAVNLARTMDFLYKDVDGYTDSWSIKGILDSLYVHLID
TPS23-Par  WMDITHECLRQPQPKALLERAVNLARTMDFLYKDADGYTDSRSIKGILDSLYVHLID
TPS23-Per  WMDVTHECLRQPQPKALLERAVNLARTMDFLYKDVDGYTDSRSIKGILDSLYVHLID
TPS23-Dip  WMDITHECLRQPQPKALLERAVNLARTMDFLYKDVDGYTDSRSIKGILDSLYVHLID
TPS23-Lux  WMDITHECLRQPQPKALLERAVNLARTMDFLYKDVDGYTDSRSIKGILDSLYVHLID
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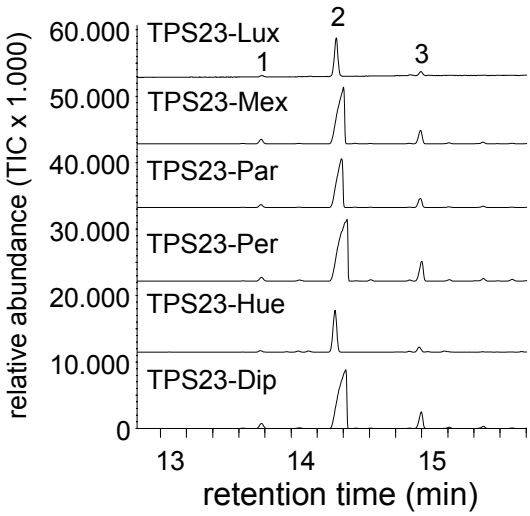
**Supplemental Figure 1. Amino acid alignment of the putative TPS23 orthologs from maize and the teosinte species *Z. m. parviglumis, Z. luxurians*, *Z. m. mexicana*, *Z. diploperennis*, *Z. perennis*, *Z. m. huehuetenangensis*.** The complete ORFs were aligned using CLUSTAL W (see methods section). Amino acids identical in all sequences are marked by black boxes and amino acids identical in at least four sequences are marked by grey boxes.




**Supplemental Figure 2**. **Sesquiterpene products of the putative TPS23 orthologs.** Putative orthologs of TPS23 were cloned from the teosinte species *Z. m. parviglumis* (Par), *Z. luxurians* (Lux), *Z. m. mexicana* (Mex), *Z. diploperennis* (Dip), *Z. perennis* (Per), *Z. m. huehuetenangensis* (Hue). The genes were expressed in *E. coli*, extracted, partially purified and incubated with the substrate (*E,E*)-FPP. The resulting terpene products were collected with a SPME fiber and analyzed by GC-MS. The products were identified as δ-elemene (1), (*E*)-β-caryophyllene (2) and α-humulene (3) by comparison of their retention times and mass spectra to those of authentic standards.

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LRFVYSSSDYDDK---------DLYVVSLRFYLLRKHGHCVSSDVFTSFK
DEEG--NFV---VDDTKCLLSLYNAAYVRTHGEKVLDEAITFTRRQLEAS
LLD-PL----EPALADEVHLTLQTPLFRRLRILEAINYI-----------
--PIYGKEAGRNEAILELAKLNFNLAQLIYCEELKEVTLWWKQLNVETNL
S-FIRDRIVECHFWMTGACCEPQYSLSRVIATKMTALITVLDDMMDTYST
TEEAMLLAEAIYRWEENAAELLP--RYMKDFYLYLLKTIDSCGDELGPN-
RSFRTFYLKEMLKVLVRGSSQEIKWRNENYVPKTISEHLEHSGPTVGAFQ
VACSSFVGMGD-SITKESFEWLLTYPELAKSLMNISRLLNDTASTKREQN
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LGR-LLP---ESPFAKEVSSSLRTPLFRRVGILEARNYI-----------
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MCNVFEARDQDL----------DLTTTSQLFYLLRKHGYHISSDVFLKFR
DDKG--DIV---TNDARCLLRMYEAAHVRVNGEEILDNILIHTKRQLQCI
VDD-L-----EPTLQEEVRYALETPLFRRLNRVQARQFI-----------
--STYEKSTTRINMLLEFSKLDFNILLTLYCEELKDLTLWWKEFQAQANT
TIYARDRMVEMHFWMMGVFFEPQYSYSRKMLTQLFMIVSVLDDLYDSHCT
TEEGNAFTAALQRWDEEGVEQCP--TYLRTLYTNIRATIKAIEEDLNFQ-
NNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEHLKISARSSGCMH
LVSQGFISMGDVA-TSEALEWASTYPKIVRAVCIIARLANDIMSYKREAS
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DENG--KFKGHLVTDAYGMLSLYEAAQWGTHGEDIIDEALAFSRSHLEEI
S---SRS---SPHLAIRIKNALKHPYHKGISRIETRQYI-----------
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--SYYEEEESCDPTLLEFAKIDFNLLQILHREELACVTRWHHEMEFKSKV
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MEELELFTDAMDEWLPVVPDEIPIPDSMKFIYNVTVEFYDKLDEELEKEG
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LIAMTFVRMTDVA-KLDAFEWLNSHPKIRVASEIISRFTDDISSYEFEHK
REHVATGIDCYMQQFGVS-KERAVEVMGNIVSDAWKDLNQELMRPHV--F
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LEHMYVSYNNSLLFSSNDSQDDDLHISALLFRLLRQHGYRISCDIFLKFM
DNNG--KFKESLVEDERGILSLYEASHMRGHGEALLEEALEFTTTHLKAY
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--EIYQQQPSHNETLLTFSKLDFNILQKLHQKEIAEICRWWKDLNVSTNF
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REHIVSAVECYMSNHGRS-EDETCAELLKQVEDAWKTINECCLHPMN--V
PMPFLICLLNLTRVMALLYSHEDGYTNSKGRTKLLIQSLLIDPLHL----
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KEDG--SFKESLTNDVEGLLELYEATYLRVQGEGVLDDALVFTRTCLEKI
AKDLVHT---NPTLSTYIQEALKQPLHKRLTRLEALRYI----------
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AYLAYVLEGTRNLKDWDLIVKYQRKNGSLFDSPATTAAAFTQFGNDGCLR
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LDETYRYWLRGDEEICL-----DLATCALAFRLLLAHGYDVSYDPLKPFA
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TLYCEELKDLTLWWKEFQAQANTAIYARDRMVEMHFWMMGVFFEPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNT
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LKISARSSGCMHLVSQGFISMGDVATSEALEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTT
TVEQAIEKIRELIEEAWMDITHECLRQPQPKALLERAVNLARTMDFLYKDADGYTDSRSIKGILDSLYVHLID.
>"TPS23 Zea mays perennis.pro" Translate DNA Sequence TPS23 Zea mays perennis.seq(1,1644)
MAADEARSVSKLHSEEDMHGKHHSTLWGDFFLHHVPCRPGQYLIMKDNVEIMKEEVKKMLLDVGSSDLSHKLDCIDTLE
RLGLGYHYTKEIDELMCNVFEARDQDLDLTTTSQLFYLLRKHGYHISSDVFLKFRDDKGDIVTDDARCLLRMYEAAHVR
VNGEEILDNILSHTKRQLQCIVDDLEPTLQEEVRYALETPLFRRLNRVQARQFISTYEKSTTRNNMLLEFSKLDFNILL
TLYCEELKDLTLWWKEFQAQANTAIYARDRMVEMHFWMMGVFFEPQYSYSRKMLTQLFMIVSVLDDLYDSHCTTEEGNT
FTAALQRWDEEGVEQCPTYLRTLYTNIRATVKAIEEDLNLQNNKHAKLVKGLIIDMAMCYNAETEWRDKKYVPATVDEH
LKISARSSGCMHLVSQGFISMGDVATSETLEWASTYPKIVRAVCIIARLANDIMSYKREASNNTMVSTVQTCAKEYGTT
TVEQAIEKIRELIEEAWMDVTHECLRQPQPKALLERAVNLARTMDFLYKDVDGYTDSRSIKGILDSLYVHLID.