

Supplementary Materials for

Convergent evolution of a metabolic switch between aphid and caterpillar resistance in cereals

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This PDF file includes:

- Fig. S1. Phenotyping of *ZmBx12*-overexpressing plants I.
- Fig. S2. Phenotyping of *ZmBx12*-overexpressing plants II.
- Fig. S3. HDMBOA-Glc and MBOA levels upon DIMBOA and DIMBOA-Glc infiltration.
- Fig. S4. Specificity of benzoxazinoid- and glucosinolate-induced callose deposition.
- Fig. S5. Impact of DIMBOA-Glc O-methylation on wheat pathogen resistance.
- Fig. S6. Aphids do not induce benzoxazinoids in wheat leaves.
- Fig. S7. Identification of DIMBOA-Glc OMT candidate genes.
- Fig. S8. Phylogenetic tree of maize *OMT* genes similar to *Bx7* and wheat *OMT* genes that were found to be up-regulated after herbivory in wheat seedlings (RNA sequencing).
- Fig. S9. Sequence comparison of maize BX7 and BX10 with herbivore-induced OMT proteins from wheat.
- Fig. S10. Phylogenetic tree of maize and wheat *OMT* genes similar to *Bx7*.
- Fig. S11. Identification of *TaBx10* as a functional DIMBOA-Glc OMT.
- Fig. S12. No influence of *ZmBx12* overexpression on *TaBx10* expression.
- Fig. S13. Phylogenetic tree of Poaceae *OMT* genes similar to *Bx7*.
- Fig. S14. Phylogenetic tree of maize, wheat, and *Arabidopsis* *OMT* genes similar to *Bx7*.
- Table S1. Wheat *OMT* genes up-regulated after herbivory (RNA sequencing).

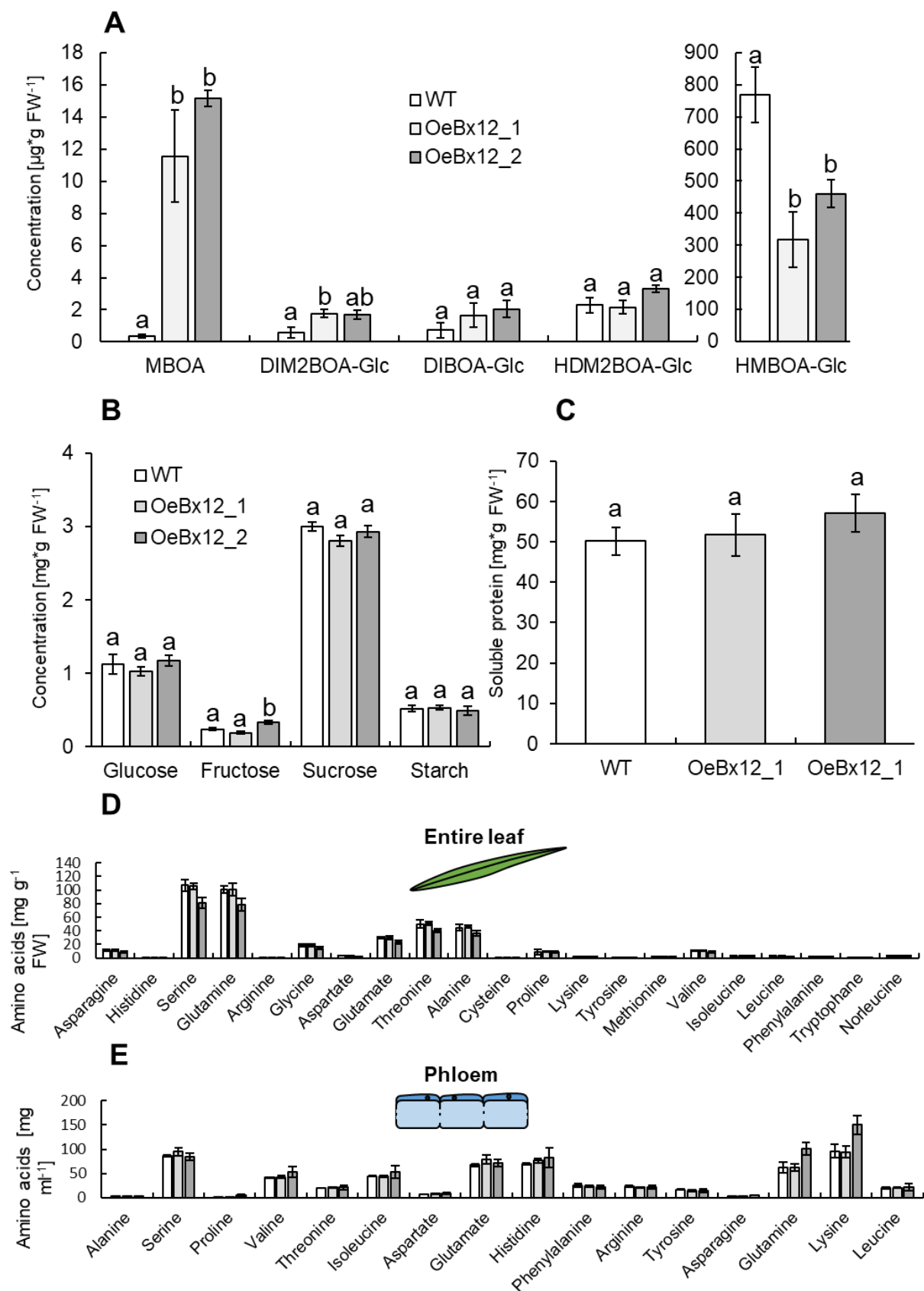


Fig. S1. Phenotyping of *ZmBx12*-overexpressing plants I. Concentrations of benzoxazinoids (A), sugars and starch (B), soluble protein (C), and free amino acids (D,E) in wild type (WT) and transgenic lines are shown. Different letters indicate significant differences between wheat lines (ANOVA followed by Holm-Sidak Post-Hoc tests, $p < 0.05$).

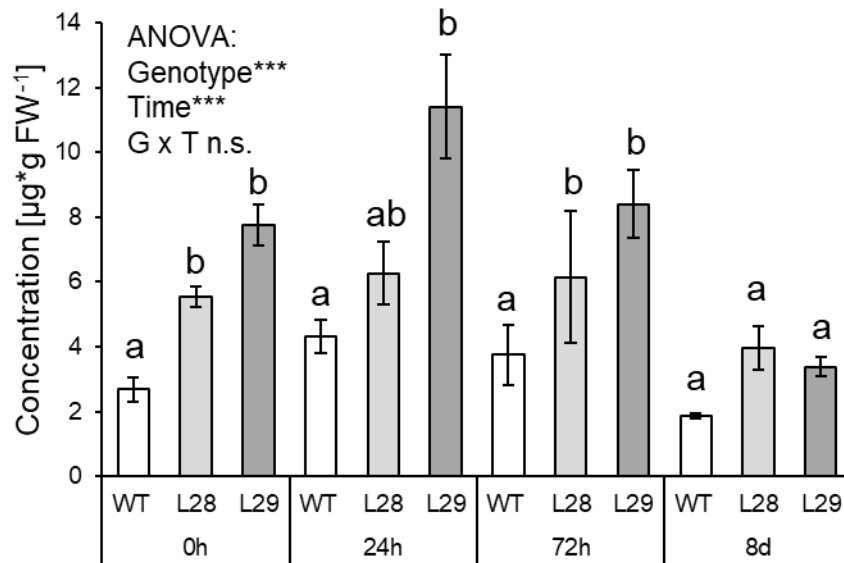


Fig. S2. Phenotyping of *ZmBx12*-overexpressing plants II. Concentrations of ferulic acid in wild type (WT) and transgenic lines in non-induced plants (0h) and *S. littoralis*-induced plants at different time points are shown. Chlorogenic acid, caffeic acid, coumaric acid and sinapic acid were below the limit of detection. Significance levels for Two-Way ANOVA factors are shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Letters indicate significant differences between lines within time points (ANOVA followed by Holm-Sidak Post-Hoc tests, $p < 0.05$). N.s. not significant.

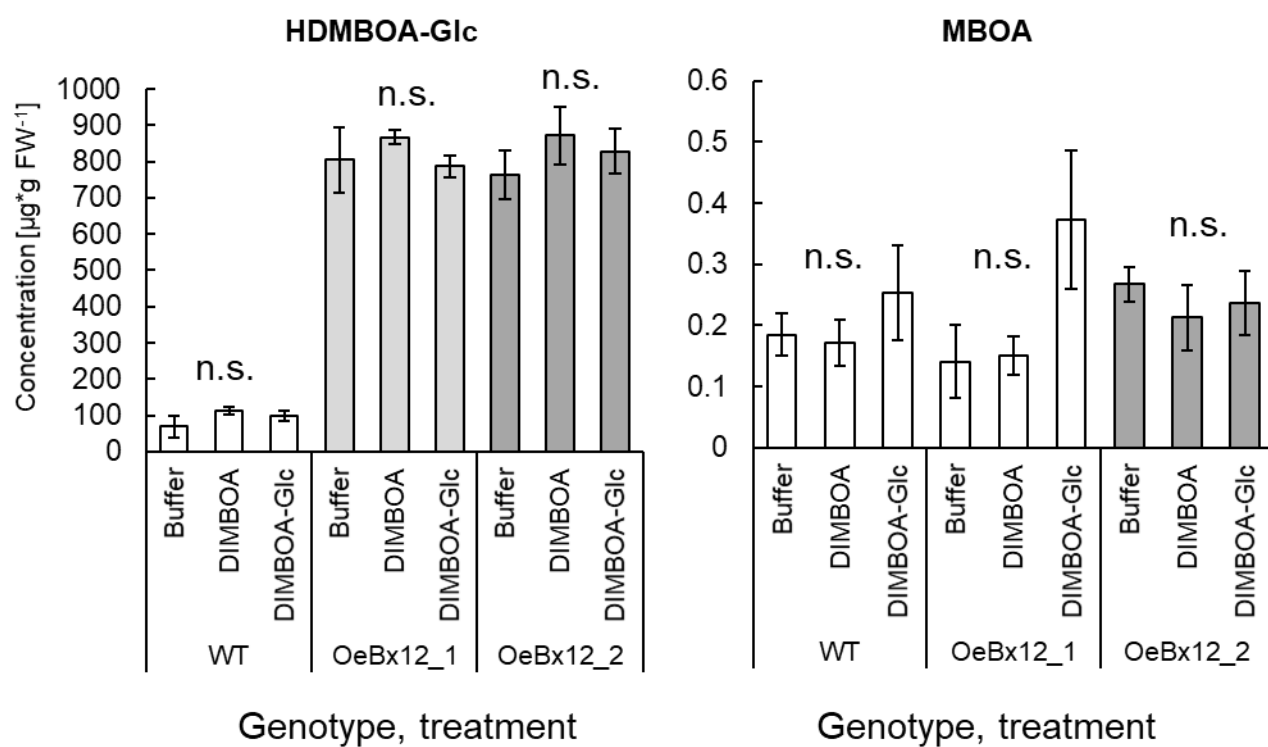


Fig. S3. HDMBOA-Glc and MBOA levels upon DIMBOA and DIMBOA-Glc infiltration. No significant differences between treatments within genotypes were detected.

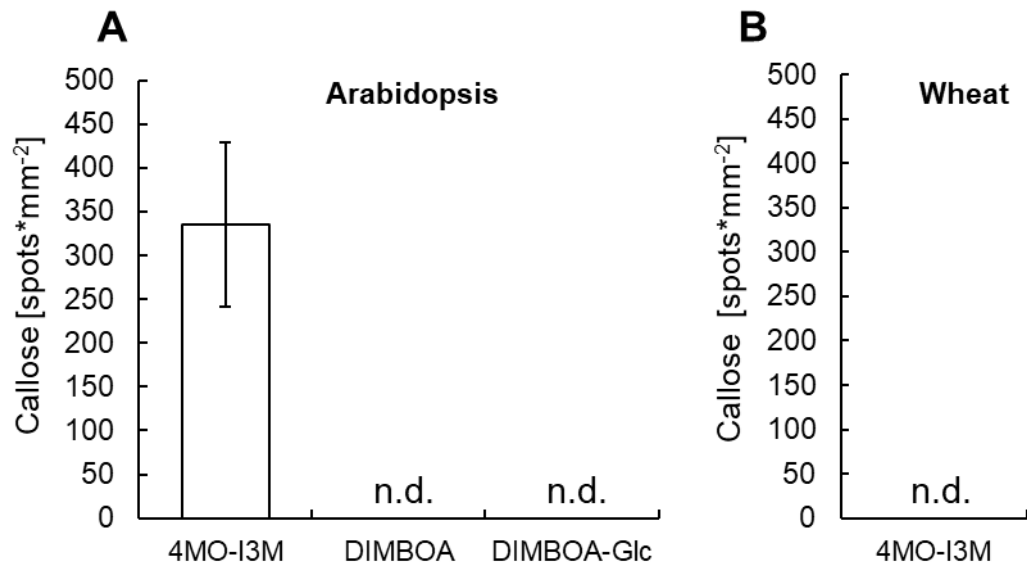


Fig. S4. Specificity of benzoxazinoid- and glucosinolate-induced callose deposition. Callose deposition in wheat (**A**) and *Arabidopsis thaliana* leaves (**B**) infused with the glucosinolate 4MO-I3M or the benzoxazinoids DIMBOA and DIMBOA-Glc (n=6). N.d. not detected.

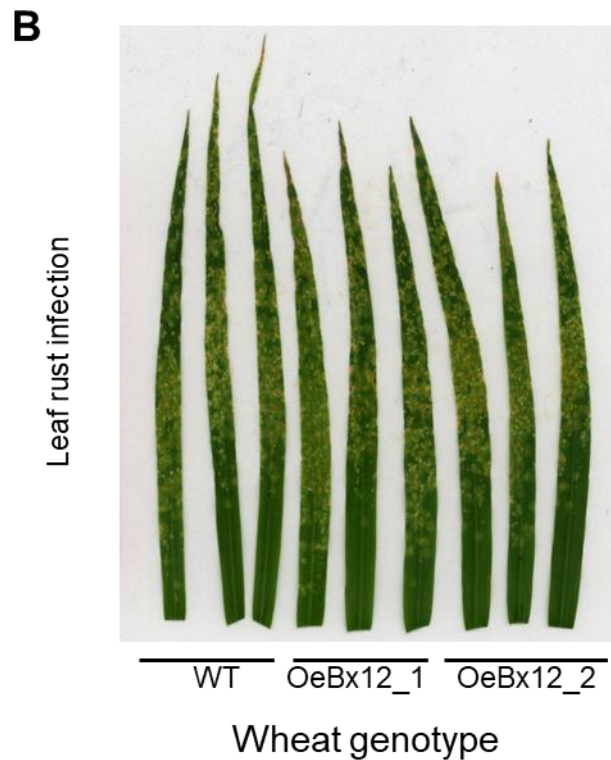
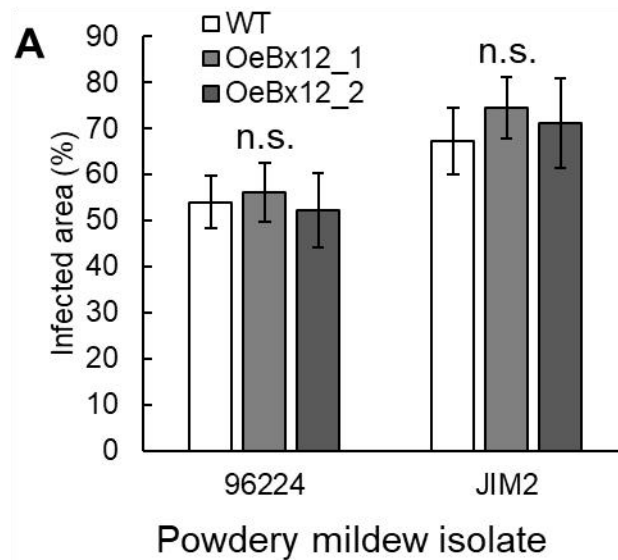


Fig. S5. Impact of DIMBOA-Glc O-methylation on wheat pathogen resistance. (A) infection severity caused by two powdery mildew (*Blumeria graminis* f. sp. tritici) isolates. No differences between wild type (WT) and transgenic lines were found (ANOVA followed by Holm-Sidak Post-Hoc tests, $p > 0.05$). (B) representative photographs of leaf rust (*Puccinia recondita* f.sp.tritici) infested wheat leaves. Visual inspection revealed no differences in infection severity between the different lines. Photo credit for (B): Ping Yang, University of Zürich.

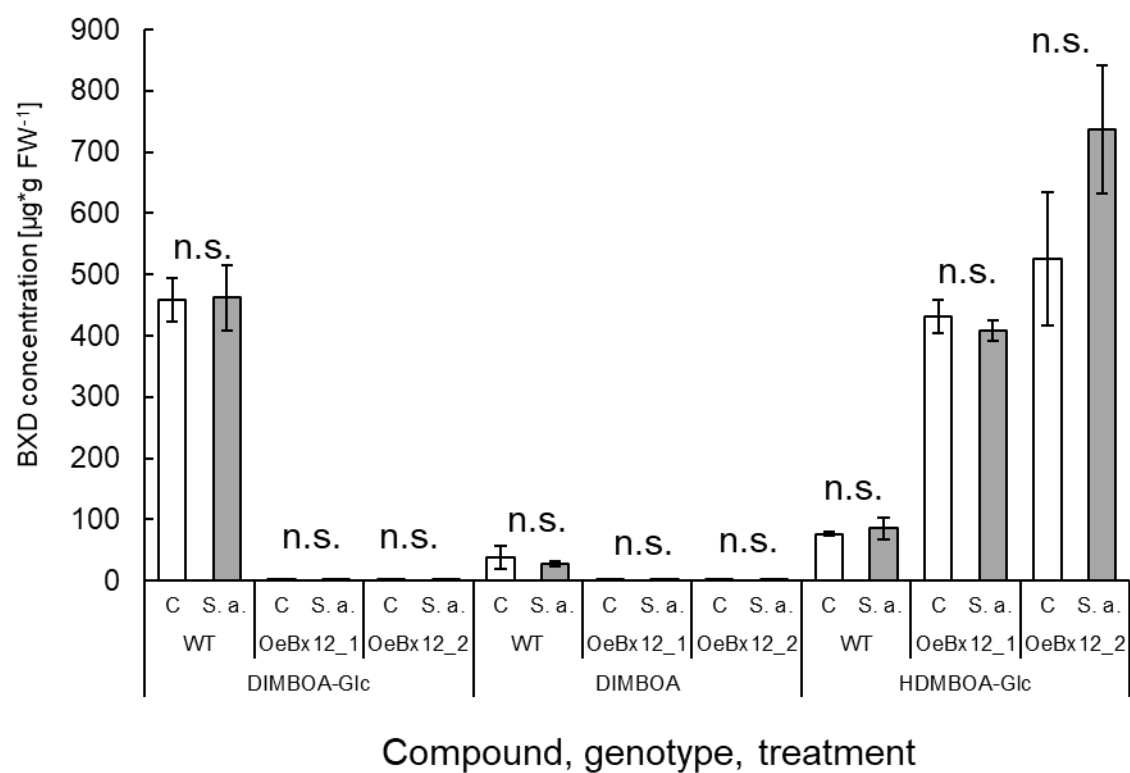


Fig. S6. Aphids do not induce benzoxazinoids in wheat leaves. Major benzoxazinoids in wild type (WT) and *ZmBx12* overexpressing leaves of control plants (C) and plants infested with *Sitobion avenae* aphids (S.a.). No significant differences between treatments within genotypes were detected.

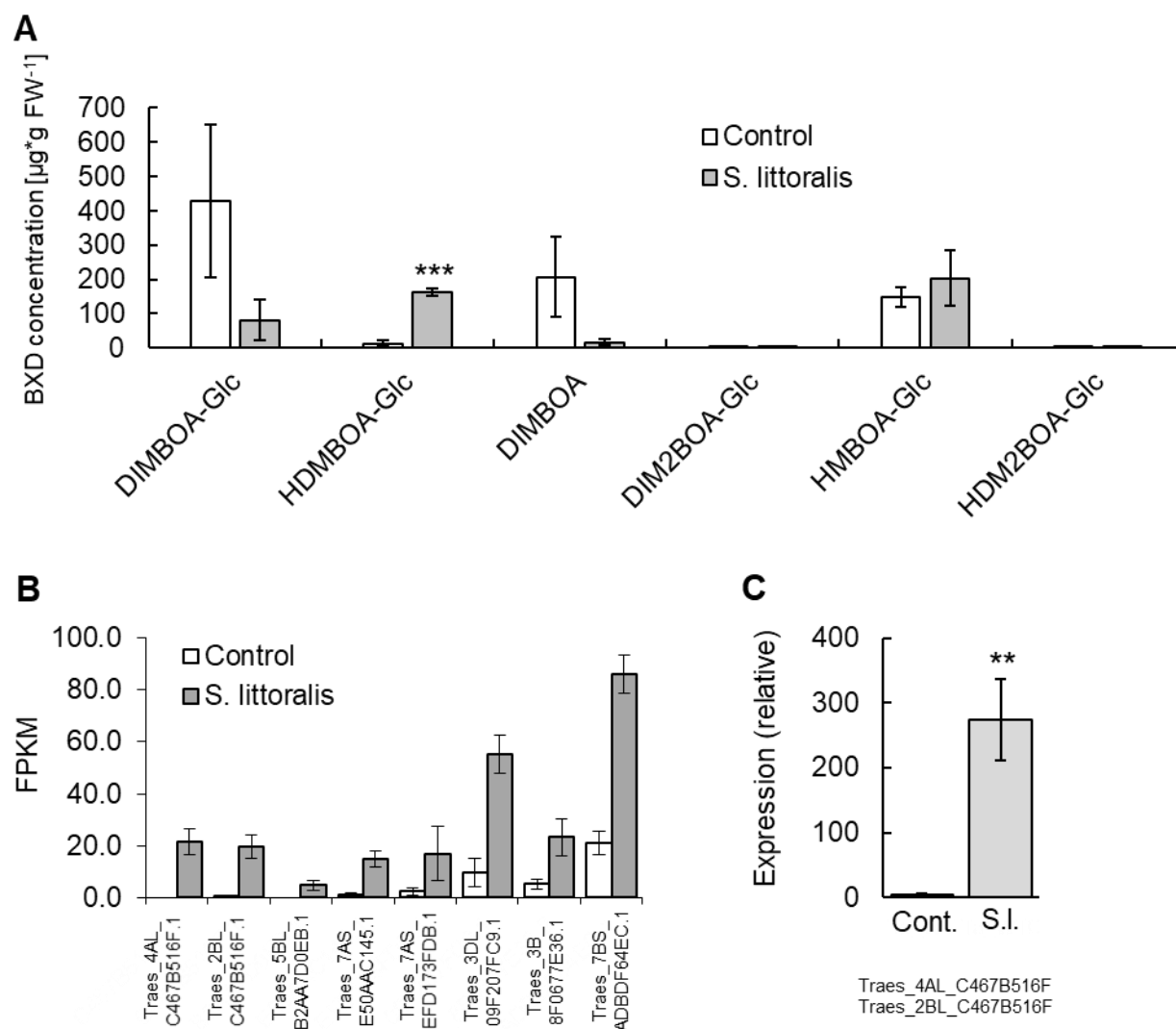


Fig. S7. Identification of DIMBOA-Glc OMT candidate genes. (A) Benzoxazinoid levels in control and *S. littoralis* induced wheat leaves (n=3). (B) The transcriptomes of the same samples were sequenced, and the obtained sequences were mapped to the primary gene models of the *T. aestivum* v2.2 genome. Differential gene expression was analyzed using the EDGE test implemented in the CLC genomics workbench package. Genes with a fold change > 3.5 and $P < 0.001$ were considered as upregulated. Means and SE are shown (n=3). (C) qRT-PCR analysis of Traes 4AL C467B516F/Traes 2BL C467B516F gene expression in herbivore-damaged wheat leaves. Means and SE are shown (n=6-7). Because 4AL C467B516F and Traes 2BL C467B516F share 100 % nucleotide identity, they could not be distinguished from each other in the qRT-PCR. Cont.: Control. S.I.: *Spodoptera littoralis*.

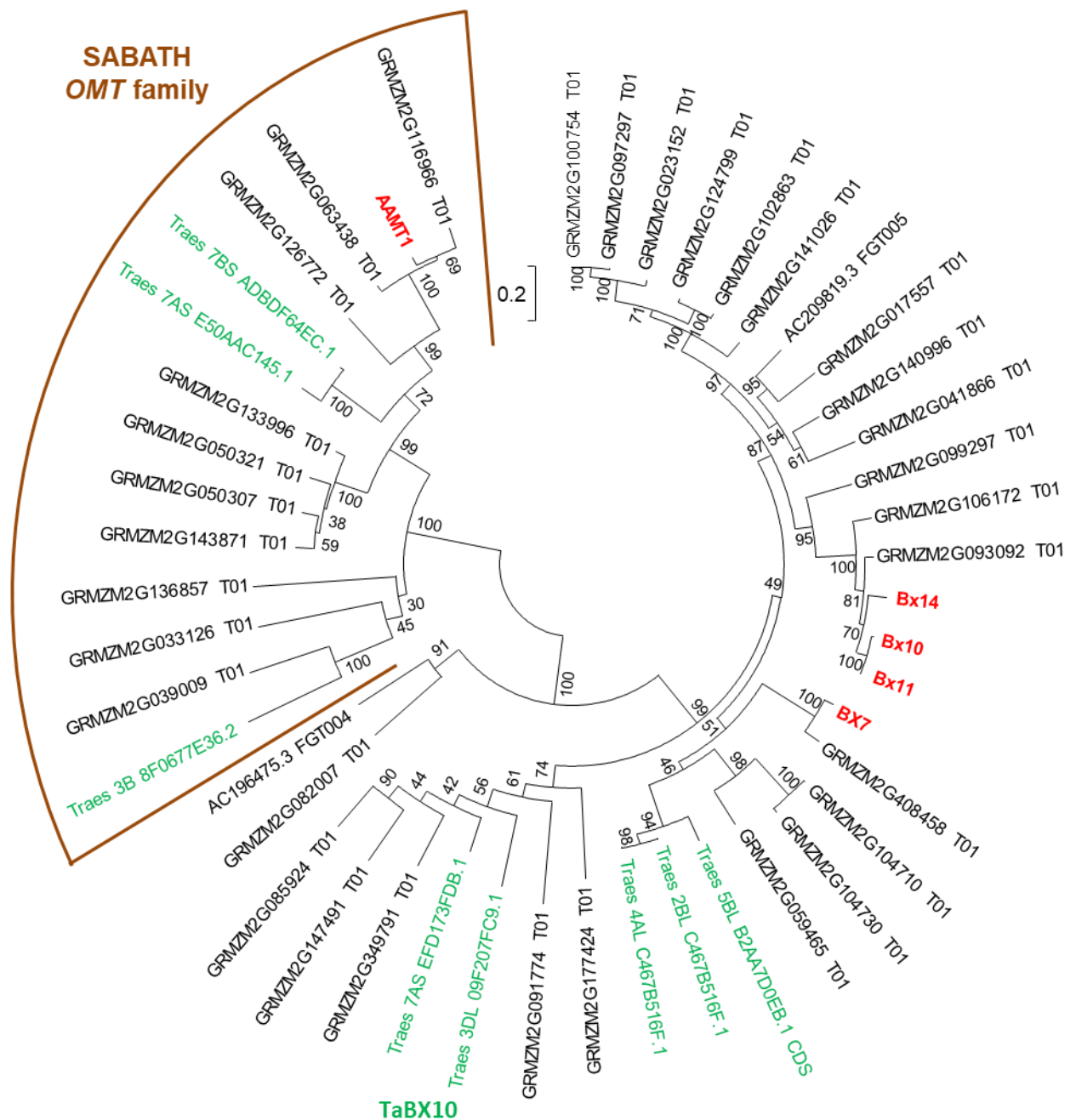


Fig. S8. Phylogenetic tree of maize OMT genes similar to Bx7 and wheat OMT genes that were found to be up-regulated after herbivory in wheat seedlings (RNA sequencing). The tree was inferred by using the Maximum Likelihood method based on the General Time Reversible model. Bootstrap values (n = 1000 replicates) are shown next to each node. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. GRMZM, *Zea mays* genes (black); Traes, *Triticum aestivum* genes (green). Maize AAMT1 (anthranilic acid carboxyl methyltransferase 1) has been described as a member of the SABATH family (Köllner et al., 2010, Herbivore-Induced SABATH Methyltransferases of Maize That Methylate Anthranilic Acid Using S-Adenosyl-L-Methionine. *Plant Physiology* 153, 1795-1807).

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BX7      MGHQAQHGTD-----DTEELTAHRCWCHALGYVKSMAKCAADLRIPDTHRC--GSATIGELLAAEISASN
BX10     MALMQES-----SSQDLLOAHDELHHSSTCFKSLALAVADLRIPDAIHHCAGCATILOILAEALHESK
Traes_2BL_C467B516F.1 MPAAQHIERDQDLAM-----SSDELLCAOLELYHHCIAFVKSALKAADLRIPDAIHRC--CAATISELASDTCIHETK
Traes_4AL_C467B516F.1 MPAAQHIERDQDLAM-----SSDELLCAOLELYHHCIAFVKSALKAADLRIPDAIHRC--CAATISELASDTCIHETK
Traes_5BL_B2AA7D0EB.1 GRSKKQVAASIPHSIDHTHTPCRPRSTSNETKTELLCAOLELYHHCIAFVKSALKAADLRIPDAIHRC--CAATISELASDTCIHETK
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      HDVLRVRVRLTAMRIFAASH-----DPAKADAAAISSYQTPASRLVSSSSSVDDAAGASKENTTTPSILPNIAHLVRPNTIS
BX10     LRALRRIMRVLTAVTGIESVVEQPPAGGGDSTVHTSDAEVVVYRITAAARFLVSD-----DVSTATLAPFVSLALQPIAAC
Traes_2BL_C467B516F.1 RSNLRRVVRVLTAVTGIESVVEQPPAGGGDSTVHTSDAEVVVYRITAAARFLVSD-----ERSPHNLSPMVGTIVNTLCWT
Traes_4AL_C467B516F.1 RSNLRRVVRVLTAVTGIESVVEQPPAGGGDSTVHTSDAEVVVYRITAAARFLVSD-----ERSPHNLSPMVGTIVNTLCWT
Traes_5BL_B2AA7D0EB.1 RSNLRRVVRVLTAVTGIESVVEQPPAGGGDSTVHTSDAEVVVYRITAAARFLVSD-----ERSPHNLSPMVGTIVNTLCWT
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      L-LFSMGEMWK----DESAASVSLYETVHRQGMWACVEDDAANRASFYESMDADTLVLMQAVVRCRPHVFDGK-SVVDVGCGRGTAAAA
BX10     PHALGISAWFRQEQHEPSYGLAFRQTP---TIMEHADDDVN---ALLNKGMAADSRFLMPVIRECGETFRGID-SVVDVGCGRGGGAAAT
Traes_2BL_C467B516F.1 S-LLKMPFWFTQGQEGESAQSHSLVQLANGCTFWDTTKVDG---GLFNDGMAADSRITAMKVLKEHGGAFAEVKSSIVDIGCNHGATASA
Traes_4AL_C467B516F.1 S-LLKMPFWFTQGQEGESAQSHSLVQLANGCTFWDTTKVDG---GLFNDGMAADSRITAMKVLKEHGGAFAEVKSSIVDIGCNHGATASA
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      NSLHVVCASNLSQWLKAPEDLTRNRIP---AFNIDEHARREMLPMVREAYEQQFRRKDFKLFTELRAKELVSGGRMVISLVCTRSDVIAS
BX10     RSVHFFHSSYSILMWRKVPPEELSSCTHLNEGNIYIGKTTTP---PMVIKLFQEQFQKDFELFETLRFKELVSGGRMLTFLCRKNEEMMT
Traes_2BL_C467B516F.1 -----
Traes_4AL_C467B516F.1 -----
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      VVAFFPHIQRCTVMDLPHVVAEAPAGTAGLSHHGGDMFEHIPISADALMLKWLHLDWDEDCKIKIMRCKEFAIGGKAGGKVIHIDTVLGS
BX10     IAAAFPHL-KCSVLDLPHVVAGAPSDGNV-QSVAGNMFESIPPATAVFLKTLHLDWGDDECVKILKNCKOAI SPRDAGGKVIHIDTVVVG
Traes_2BL_C467B516F.1 VARAFPHL-KCSVLDLPHVVAAPASDIL-TEVAGNMFYVPPADAVLLKWLHLDWKHEDCVKILRCKEALPAKAGGKVIHIDTVVVG
Traes_4AL_C467B516F.1 VARAFPHL-KCSVLDLPHVVAAPASDIL-TEVAGNMFYVPPADAVLLKWLHLDWKHEDCVKILRCKEALPAKAGGKVIHIDTVVVG
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      EFSIFPGI--LAQILSVMAEGVIDKAKFDSYVPLHGPSIEEVREIIEEGSES-----HKEAFVHDPATAE-----MNIALSS
BX10     HGEVGTLYELVAESLSIVLKGRVEKEKLDSENVPIYTPSVKEVRELINRSRLFD-----EHARLFESNWDPODDSDGDVVLDYA-GS
Traes_2BL_C467B516F.1 -----
Traes_4AL_C467B516F.1 -----
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      RADDHDDDKTCRETYVLDLHILSFVNCABRDEHEWRRIFLAGFRDYKITHTRC--IPSTIEVVP*
BX10     RQ---SNIKHQTQVMPDLYMMA-VNGVERDEQEKKIFTEAGFKDYKILPVICDVSVIEVVP*
Traes_2BL_C467B516F.1 PVTQPNHKK--EAQVLDDIYMMG-SDGMRERENWSLIFSEAGFSYDYKITPTNG--IRSLIEVVP*
Traes_4AL_C467B516F.1 PVTQPNHKK--EAQVLDDIYMMG-SDGMRERENWSLIFSEAGFSYDYKITPTNG--IRSLIEVVP*
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      GANVANCIKRAVMEPLIVHFGEDIID-----DLFVVVFASIVAKHLEKAKAKYPIVLSLKKAT
BX10     -----ASGLMLETHHLVMDLKLVMTRCRORDEKVDSDIFMKAGESEYKIFKKVC-ARAVIEVVP*
Traes_2BL_C467B516F.1 -----
Traes_4AL_C467B516F.1 -----
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

BX7      PSKFVNLIALFEPIIVQHFGEVMEDEFVRAALHWSL--DVDCLSRQERARTSRAMLVVSLLAKA*
BX10     -----
Traes_2BL_C467B516F.1 -----
Traes_4AL_C467B516F.1 -----
Traes_5BL_B2AA7D0EB.1 -----
Traes_7AS_E50AAC145.1 -----
Traes_7AS_EFD173FDB.1 -----
Traes_3DL_09F207FC9.1 -----
Traes_3B_8F0677E36.2 -----
Traes_7BS_ADBDF64EC.1 -----

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Fig. S9. Sequence comparison of maize BX7 and BX10 with herbivore-induced OMT proteins from wheat. Amino acids identical in at least 5 out of 10 sequences are marked by black boxes and amino acids with similar side chains are marked by gray boxes. The genes encoding Traes_2BL_C467B516F.1 and Traes_4AL_C467B516F.1 have identical nucleotide sequences and were both designated as *TaBX10* (shown in red).

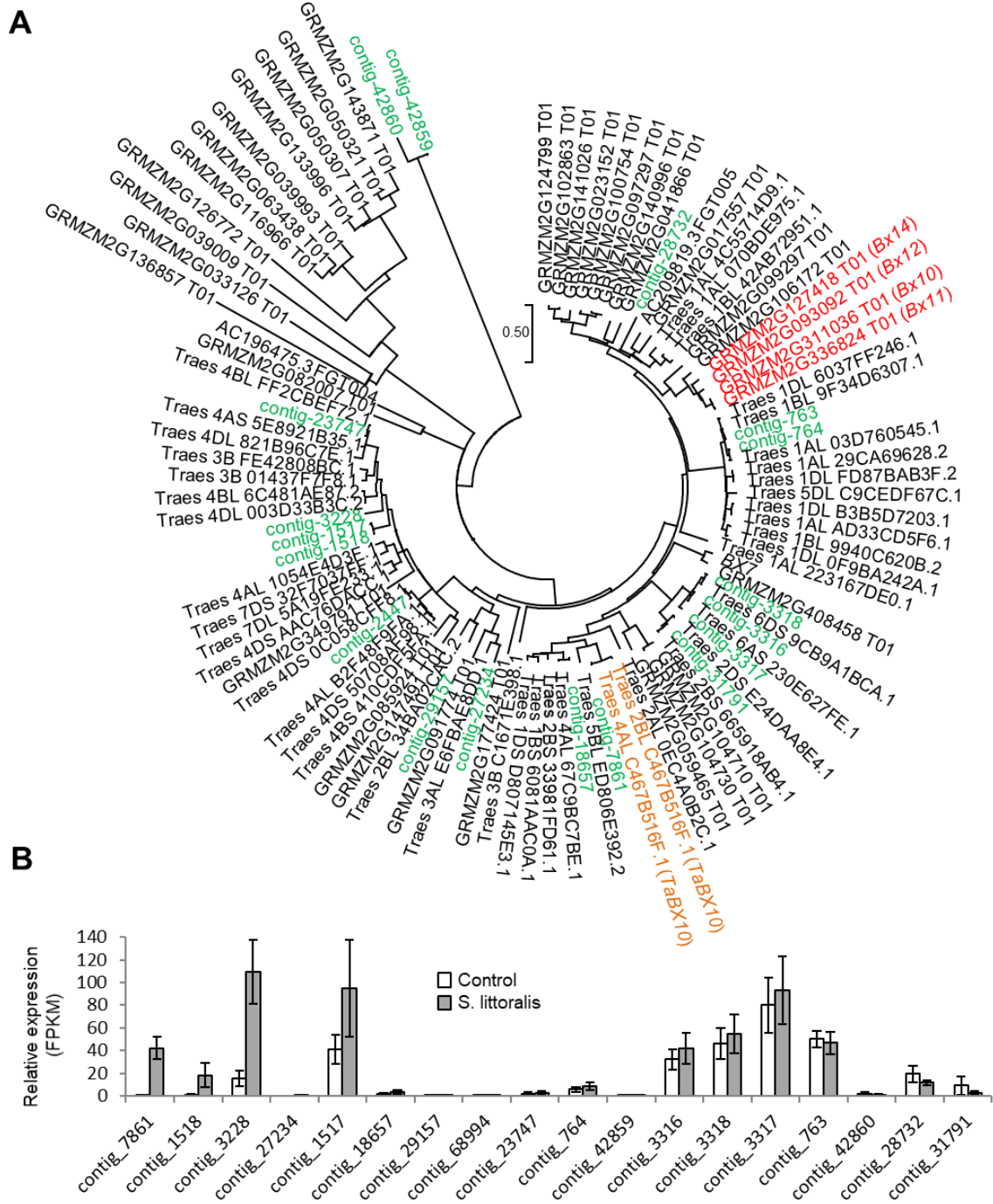


Fig. S10. Phylogenetic tree of maize and wheat OMT genes similar to Bx7. (A) and expression values of OMT contigs extracted from a *de novo* transcriptome of herbivore-damaged wheat leaves **(B)**. OMTs were identified using a BLASTP analysis with maize BX7 as query and the maize and wheat protein datasets in Phytozome 12.1 (<https://phytozome.jgi.doe.gov>) as template. In addition, a *de novo* transcriptome of *Spodoptera littoralis* damaged wheat leaves was used as template for a TBLASTN analysis with Bx10 as query. Genes with ORFs > 1000 nucleotides were considered as ‘full-length’ and used for phylogenetic analysis. Maize Bx10,11,12,14, wheat TaBX10, and wheat OMT contigs found in the *de novo* transcriptome are shown in red, brown, and green, respectively. The tree was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. GRMZM, *Zea mays*; Traes, *Triticum aestivum*; contig, contig number in the *de novo* transcriptome.

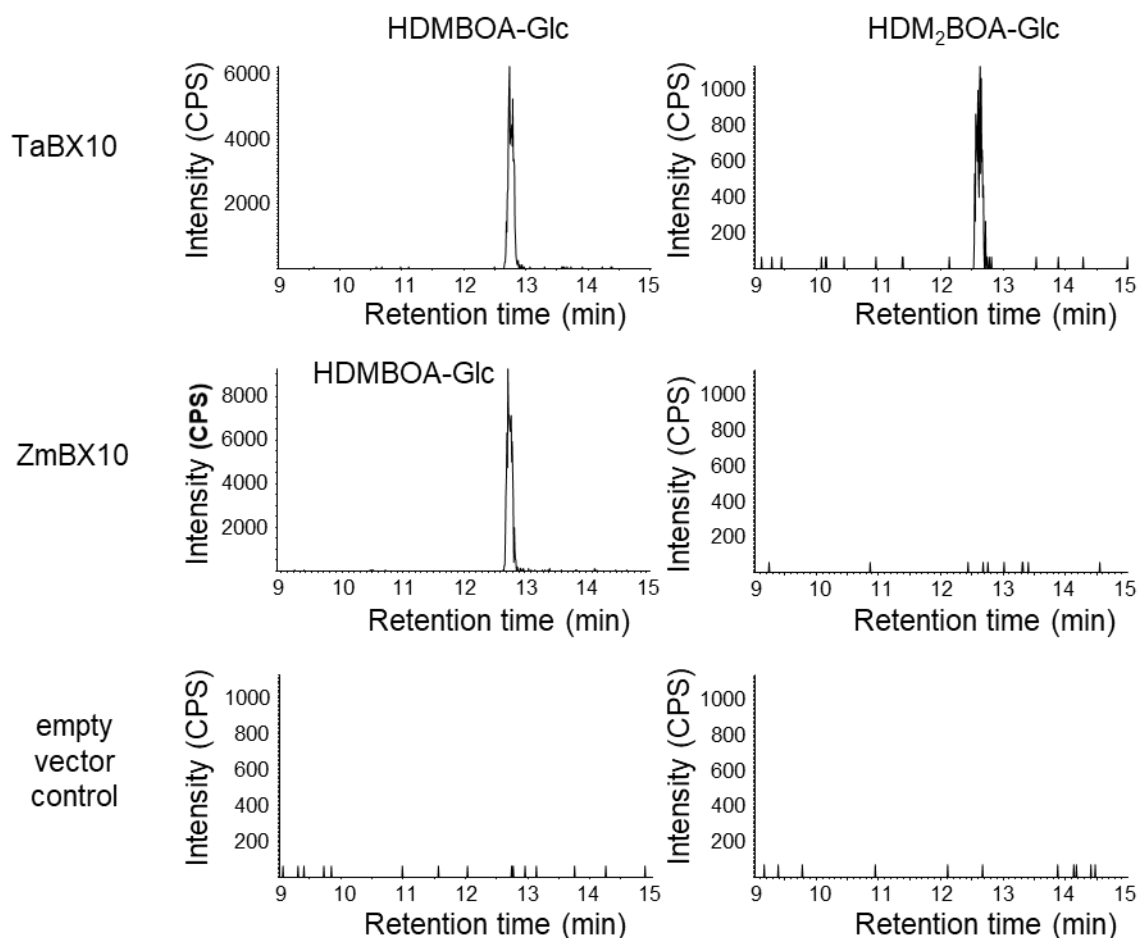


Fig. S11. Identification of *TaBx10* as a functional DIMBOA-Glc OMT. Recombinant Traes 4AL C467B516F (*TaBx10*) accepts DIMBOA-Glc and DIM₂BOA-Glc as substrate and produces HDMBOA-Glc and HDM₂BOA-Glc, respectively. The enzymes were heterologously expressed in *E. coli*. Purified recombinant proteins were incubated with a mixture of DIMBOA-Glc and DIM₂BOA-Glc as substrate and SAM as co-substrate. Enzyme products were analyzed using LC-MS/MS. Maize *ZmBx10* was included as positive control and an *E. coli* strain expressing an empty vector was used as negative control.

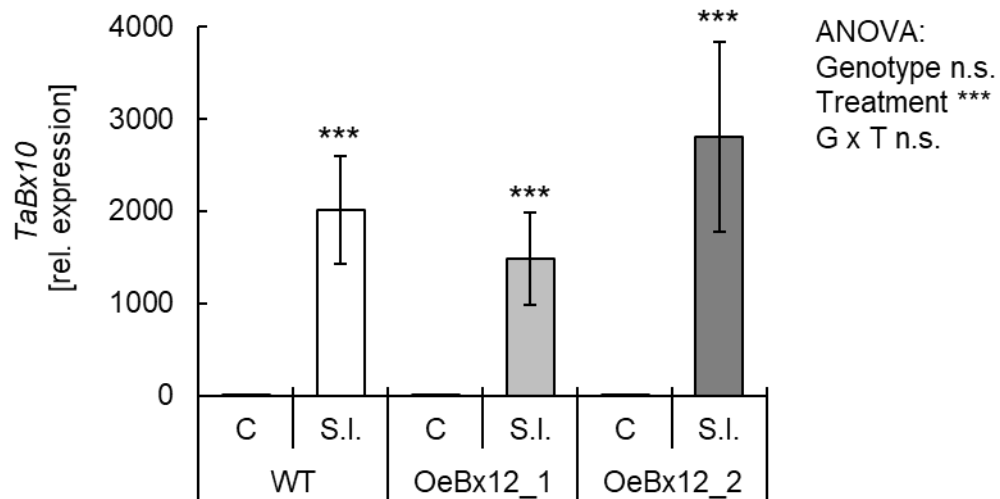


Fig. S12. No influence of *ZmBx12* overexpression on *TaBx10* expression. Relative expression of *TaBx10* in the leaves of WT and OeBx12 plants that were uninfested or infested with *S. littoralis* larvae for 24 h ($n=5-6$). Stars above bars indicate a significant difference between treatments within genotypes (Holm-Sidak Post-Hoc tests, $*p<0.05$; $**p<0.01$; $***p<0.001$). Significance levels for ANOVA factors are shown on the right ($*p<0.05$; $**p<0.01$; $***p<0.001$). C: Control. S.I.: *Spodoptera littoralis*.

The detailed tree of this
clade is shown in figure 3

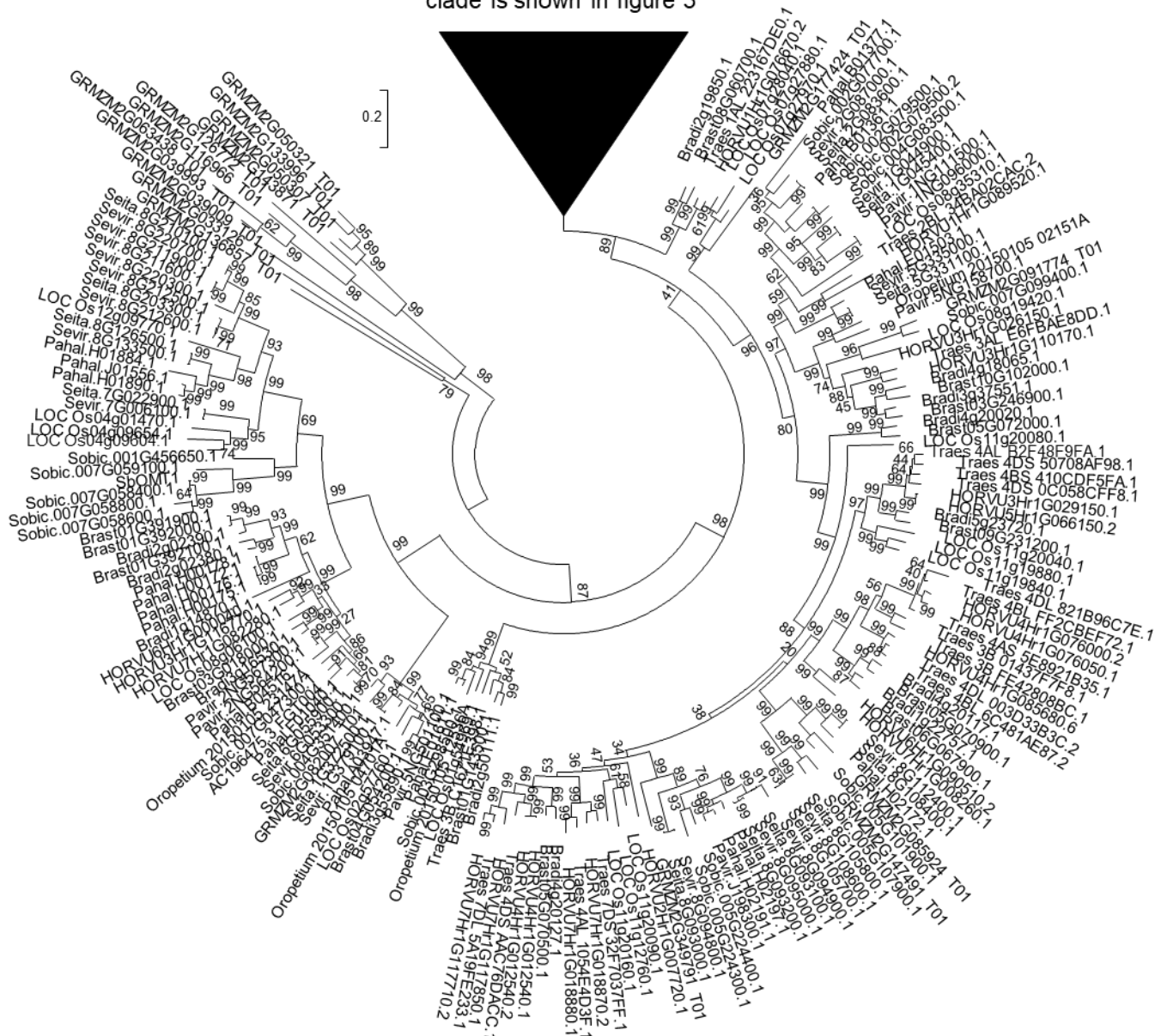


Fig. S13. Phylogenetic tree of Poaceae OMT genes similar to Bx7. OMTs were identified using a BLASTP analysis with maize BX7 as query and all available Poaceae protein datasets in Phytozome 12.1 (<https://phytozome.jgi.doe.gov>) as template. Genes with ORFs > 1000 nucleotides were considered as ‘full-length’ and used for dendrogram analysis. The tree was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. Bootstrap values (n = 1000 replicates) are shown next to each node. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. GRMZM, *Zea mays*; Sobic, *Sorghum bicolor*; Sevir, *Setaria viridis*; Seita, *S. italica*; Pavir, *Panicum virgatum*; Pahal, *P. hallii*; Oropetium, *Oropetium thomaeum*; Traes, *Triticum aestivum*; HORVU, *Hordeum vulgare*; Bradi, *Brachypodium distachyon*; Brast, *B. stacei*; LOC Os, *Oryza sativa*.

Table S1. Wheat *OMT* genes up-regulated after herbivory (RNA sequencing). Wheat seedlings were subjected to herbivory by *Spodoptera littoralis*. The transcriptomes of three damaged and three undamaged plants were sequenced and compared to identify herbivory-induced *O*-methyltransferase genes. All identified *OMT* genes with a fold change > 3.0 and a *P*-value < 0.001 are shown.

Feature ID	EDGE test Fold change	EDGE test P-value	EDGE test Weighted difference	leaf ctr - 2644_A FPKM	leaf ctr - 2644_C FPKM	leaf ctr - 2644_B FPKM	leaf - 2644_E FPKM	leaf - 2644_D FPKM	leaf - 2644_F FPKM	leaf ctr - Means FPKM	leaf herb Means FPKM	annotation
Traes_4AL_C467B516F.1	161.6	1E-13	1.58E-05	0.0	0.0	0.0	16.5	18.9	29.4	0.0	21.6	PTHR11746//PTHR11746:SF108 - O-METHYLTRANSFERASE
Traes_2BL_C467B516F.1	96.9	1E-11	1.44E-05	0.0	0.0	0.2	15.7	16.6	27.1	0.1	19.8	PTHR11746//PTHR11746:SF108 - O-METHYLTRANSFERASE
Traes_5BL_B2AA7D0EB.1	35.7	0.0001	3.42E-06	0.0	0.0	0.0	1.9	5.0	7.3	0.0	4.7	PTHR11746//PTHR11746:SF108 - O-METHYLTRANSFERASE
Traes_7AS_E50AAC145.1	11.5	5E-07	9.89E-06	2.1	0.6	0.3	14.1	10.9	19.4	1.0	14.8	PTHR31009//PTHR31009:SF16 - S-ADENOSYL-L-METHIONINE:CARBOXYL METHYLTRANSFERASE
Traes_7AS_EFD173FDB.1	6.3	0.0029	1.1E-05	2.5	0.0	4.4	0.8	19.8	30.4	2.3	17.0	2.1.1.240 - Trans-resveratrol di-O-methyltransferase / Resveratrol O-methyltransferase
Traes_3DL_09F207FC9.1	4.7	2E-07	3.19E-05	7.1	3.7	18.3	53.3	46.2	66.4	9.7	55.3	PTHR11746//PTHR11746:SF91 - O-METHYLTRANSFERASE
Traes_3B_8F0677E36.2	3.8	0.0002	1.28E-05	5.7	2.0	7.8	12.1	25.9	31.7	5.2	23.2	PTHR31009//PTHR31009:SF12 - S-ADENOSYL-L-METHIONINE:CARBOXYL METHYLTRANSFERASE