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Supplemental information

**Horizontal transfer and evolution of the biosynthetic gene cluster for
benzoxazinoids in plants**

Dongya Wu, Bowen Jiang, Chu-Yu Ye, Michael P. Timko, and Longjiang Fan

Horizontal Transfer and Evolution of the Biosynthetic Gene Cluster for Benzoxazinoid in Plants

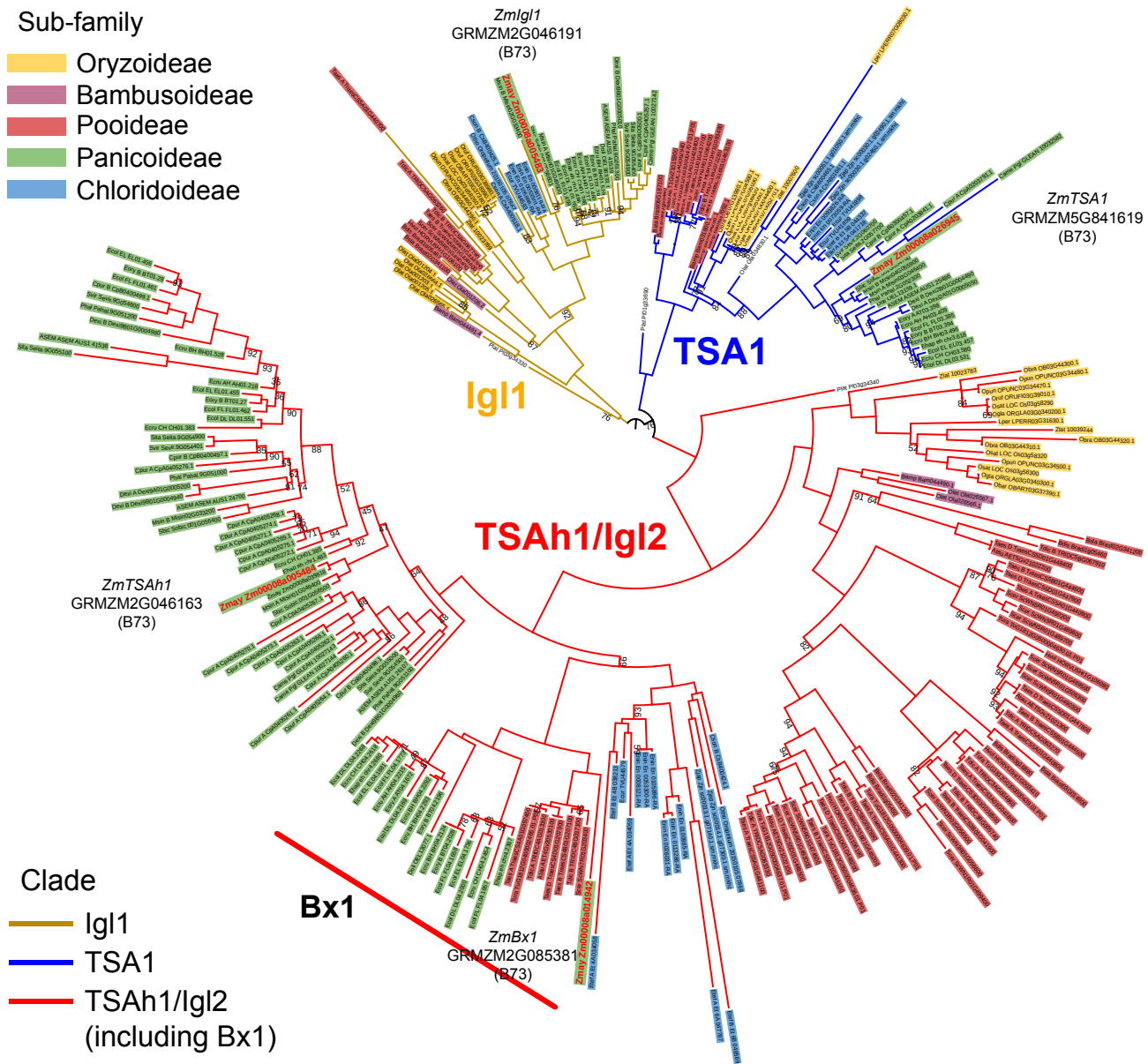
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Supplemental Figures and Tables

Sub-family

- Oryzoideae
- Bambusoideae
- Pooideae
- Panicoideae
- Chloridoideae



Clade

- Igl1
- TSA1
- TSAh1/Igl2 (including Bx1)

Supplemental Figure 1 Maximum-likelihood phylogenetic tree of *Bx1* homologs. Three clades (Igl1, TSA1 and TSAh1) are indicated by branch colors. The sub-family information is shown by the background colors of labels. The Bx1 clade is highlighted by a red bold line. Bootstrap values less than 95 are shown on branches.

```

*           20           40           60           *
N1 : MAFALKASPYLSSSSAAASS-----SSSPALLPLPGQHAS-----ARVSRPQAAITAPLAMGQA : 55
N2 : MATTTMKASP---MSASSSS-----APVLRRCVAVQPAR---VAAARRLAAAAASVALEASVPVA : 53
N3 : MAYA-VKASP---SSSAPS-----LPFRRRAAGAVV---TAGRRVKVRAALAAAALDPAAPA : 51
N4 : MAFALKASPSSTSSSSAPSHPRLRSAAAMAMPFGPAPASRAVA---AASAAASLEPAVVVPSPDSYWR : 68
N5 : MAF-ALKAS--PSTSSSSLAVQ-----SOLERRAAAVATM---PARRRAAAVRVMAITAAAPFA : 53
N6 : MAF-ALKAASYS--FSPADQ-----PSLSLAATAAVKMPAGRGKAAAAVVIRAVAAAAPLS : 58
N7 : MALFAVQAASSTSSSSPAVLQ-----QSPLPSRAAAAAVKMPQR--KKAADVRAVAAPPAPV : 62
N8 : MAS-ALKAASSTSSSSPAAVH-----SSPLSKLPAAVA---MPGR-RRSVATVRAVAAPPAPFA : 58
B1 : -----
B2 : MAF-ALNLS--CYPSFS-----SLLPWMAAAVMI---PRRRNVLPV--TKAVAVAPPA : 47
B3 : MAF-ALKTS--FSASFSQAGPSS-----SLLPRR-----RNGVSV--IRAVATVSTS : 43
B4 : MAF-SLNLN--SSPSFQAQSS-----SLLPRRLSAAVVTK---PRPRNVFV--IRAVATVVS-- : 49
      ma          a                      r

*           80           100          120          140
N1 : ---AAAEKCN-VSQTFSRLRKCKGTAFIPYITAGDPDIAATTAALKLLDYCGADVIELGIPYSDLELDG : 123
N2 : A---AAAVERRMSVSOTMSKLKEKGKTAFIPIYITAGDPDMGTTAEALRLLDAGCGADVIELGVPSDFYADG : 122
N3 : P---AAAGKRGRLSVSOTLSSHREKGTAFIPYITAGDPDLETTAEALRLLDAGCGADVIELGVPSDFYADG : 120
N4 : PLPLTPASERIPVAQAMSRARAHGKTAFIPIYITAGDPDLETTAEALRLLDAGCGADVIELGVPSDFYADG : 140
N5 : BAPARPAAGRC-LPVSOTFAKLKAKGKTAFIPIYITAGDPDIAATTAELRVLDAAGCGADVIELGVPSDFYADG : 124
N6 : BAPARSAAGRC-LPVSOTMARLKAQKGTAFIPYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 129
N7 : BGPFPKNGERCRLPVSOTMSRLKAQKGTAFIPYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 134
N8 : KLTAG-AGGRC-LPVSOTMSRLRAQKGTAFIPYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 128
B1 : -----MAAMAKGKTAFIPIYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 54
B2 : BAPAKPFAVRSR-PVSVTMAKIMAKGKTAFIPIYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 118
B3 : BAPAKPFAVTTLPVSEETIAKIMAKGKTAFIPIYITAGDPDIAATTAELRLLDAGCGADVIELGVPSDFYADG : 115
B4 : -BAPAKPFAVRCR--PVSDTIAKIMAKGKVVVCTLYVA-----HVVGS-TLELCIT- : 94
      a          vs t l Gkta ipy tagdp ttaeal ld cGadv6ELG6p sdp dg

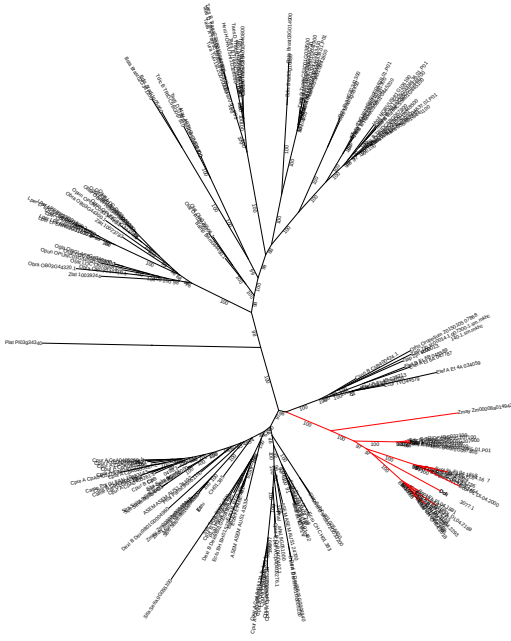
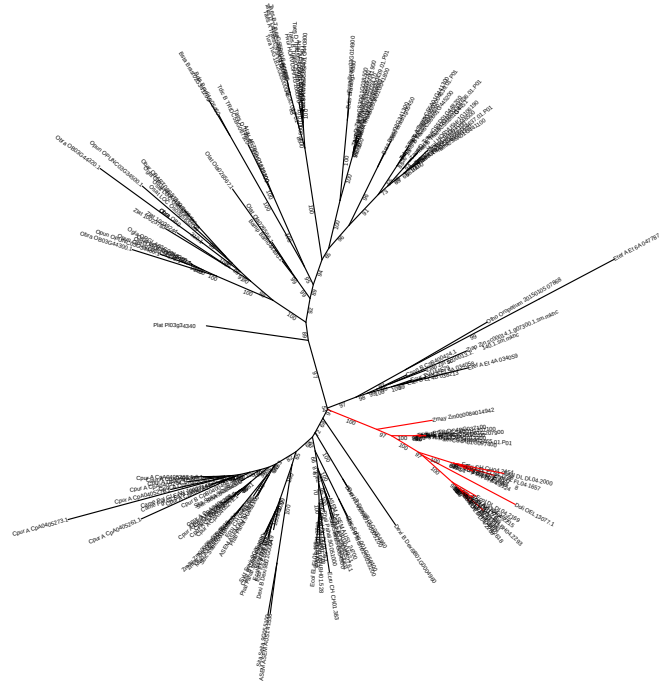
*           160          180          200          *
N1 : PVIQASASRALAKC--ATDITMAMLKEVVEHLSCPVVLTYYNPIVRRGVAANTAAAKBAGAHGLIVPDLF : 193
N2 : PVIQASASRALAAG--ATPEAVLSMLKEVTEHLSCPVVLSYLGPIVRRGVAANTAAAKBAGVQGLIVPDLF : 192
N3 : PVIQASASRALAGG--ATADGVMSMLKEVTEHLSCPVVLSYFGPIVRRGVAASVTAAVKEAGVQGLIVPDLF : 190
N4 : AVIQASAKRALAAG--ATDAMVMSMLKEVTEHLSCPVVLSYFNPIVRRGTRSFAAAARBAGVKGGLIIPDLF : 210
N5 : PVIQASASRALASG--TTFDGVLMMLKEVTEHLSCPVVLSYFKPILCRGTSDFATAAKBAGHGLIVPDLF : 194
N6 : PVIQASARALASG--TTFDGVLMMLKEVTEHLSCPVVLSYFNPIVRRGADFAAAAKBAGAHGLIVPDLF : 199
N7 : PVIQASMARARTTCGGATPDGVLMMLKEVTEHLSCPVVLSYFNPIVRRGTPGFAAAAVKBAGAHGLIVPDLF : 206
N8 : PVIQASARALASG--TTFDGVLMMLKEVTEHLSCPVVLSYFNPIVRRGADFAAAAVKBAGVHGLIVPDLF : 198
B1 : PVIQASARALASG--TMDAVLMMLKEVTEHLSCPVVLSYMKPIVRRG-----AEMKBAGVHGLIVPDLF : 120
B2 : PVIQASARALAGG--ATMDGVLMMLKEVTEHLSCPVVLSYMRPILCRG-----AETKBAGVHGLIVPDLF : 184
B3 : PVIQASARALAGG--TMDGVLMMLKEVTEHLSCPVVLSYMKPIFCQGI-----AETKBAGVHGLIVPDLF : 181
B4 : PVIQASARALAGG--TMDGVLMMLKEVTEHLSCPVVLSYMKPIIFKGI-----AETKBAGVHGLIVPDLF : 160
      p 6QAS RAla G T d v6 ML4EvtFeLSCPv66f3Y PI g A 4eAG GL66PDLF

*           220          240          260          *
N1 : LENAGIL--RNSIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVQVHLLQIHK : 263
N2 : YVDTCTF--RSEAIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERPKVNRVHLLQIHL : 262
N3 : YVETCEF--RNSIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVQVHLLQIHR : 260
N4 : HDEIRAF--RKEAIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVQVHLLQIHR : 280
N5 : CVAECTL--KSEAMKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANLNSRVOSLQIHKV : 264
N6 : YGATCAL--RSEAIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVOSLQIHKV : 269
N7 : YADTCAL--RSEAIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGSRADVNRVOSLQIHKV : 276
N8 : YGNSCALTLRTEAIKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVOSLQIHKV : 270
B1 : YVAASHL--WSEAKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVOSLQIHKV : 190
B2 : YVAAHAL--WSEAKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVOSLQIHKV : 254
B3 : YVAAHAM--WSEAKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNRVOSLQIHKV : 251
B4 : YVAAHAL--WSDAKNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVNGVTGERANVNSHVSLLQIHKV : 230
      y          ea n lELVLLTTP tP RM It As GF6Ylv36 GVTG R 6N rv l6qe64

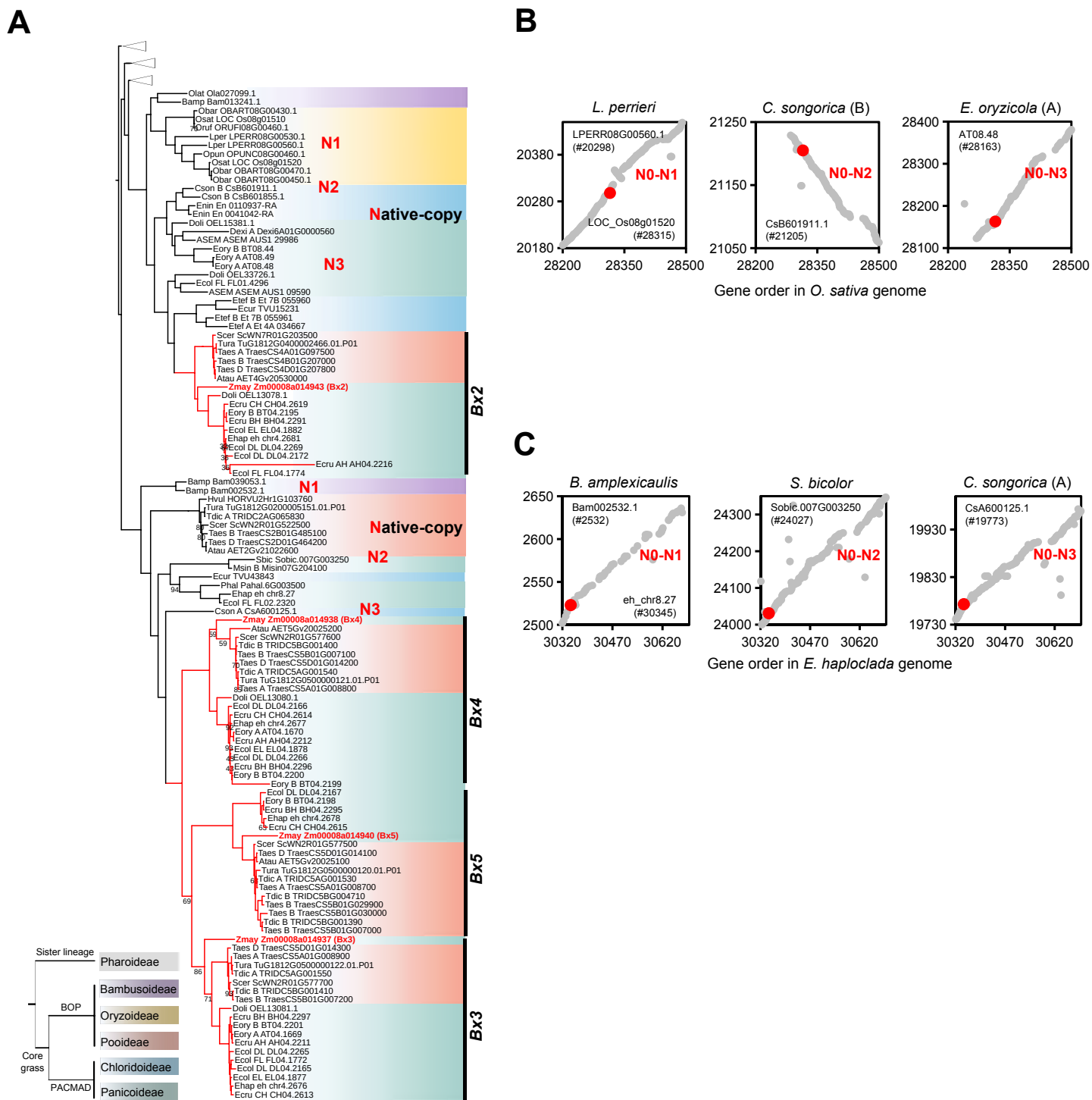
*           300          320          340          *
N1 : VTDKAVAVGFGISTPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLRR----- : 314
N2 : VTDKAVAVGFGISTPEHVRQIAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 313
N3 : VTDKAVAVGFGISTPEHVRQIAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLRR----- : 311
N4 : VTDKELAVGFGISTPEHVSQISEWAGADGVIIIGSAMVROLGEAASPRE-----GLKR----- : 331
N5 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLERLALS--HRGPTSRHARTRMLYSKFRVRRNLFI : 336
N6 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 320
N7 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 327
N8 : VTDIPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 321
B1 : VTNKPVAVGFGISKPEHVQ-----OLGEAASPKQ-----GLRR----- : 223
B2 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPRE-----GLKR----- : 305
B3 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 302
B4 : VTDKPVAVGFGISKPEHVQQAQWAGADGVIIIGSAMVROLGEAASPKQ-----GLKR----- : 281
      VT k 6avGFGIS P HV q a wgaDgviigsamv Qlgea sp G64R

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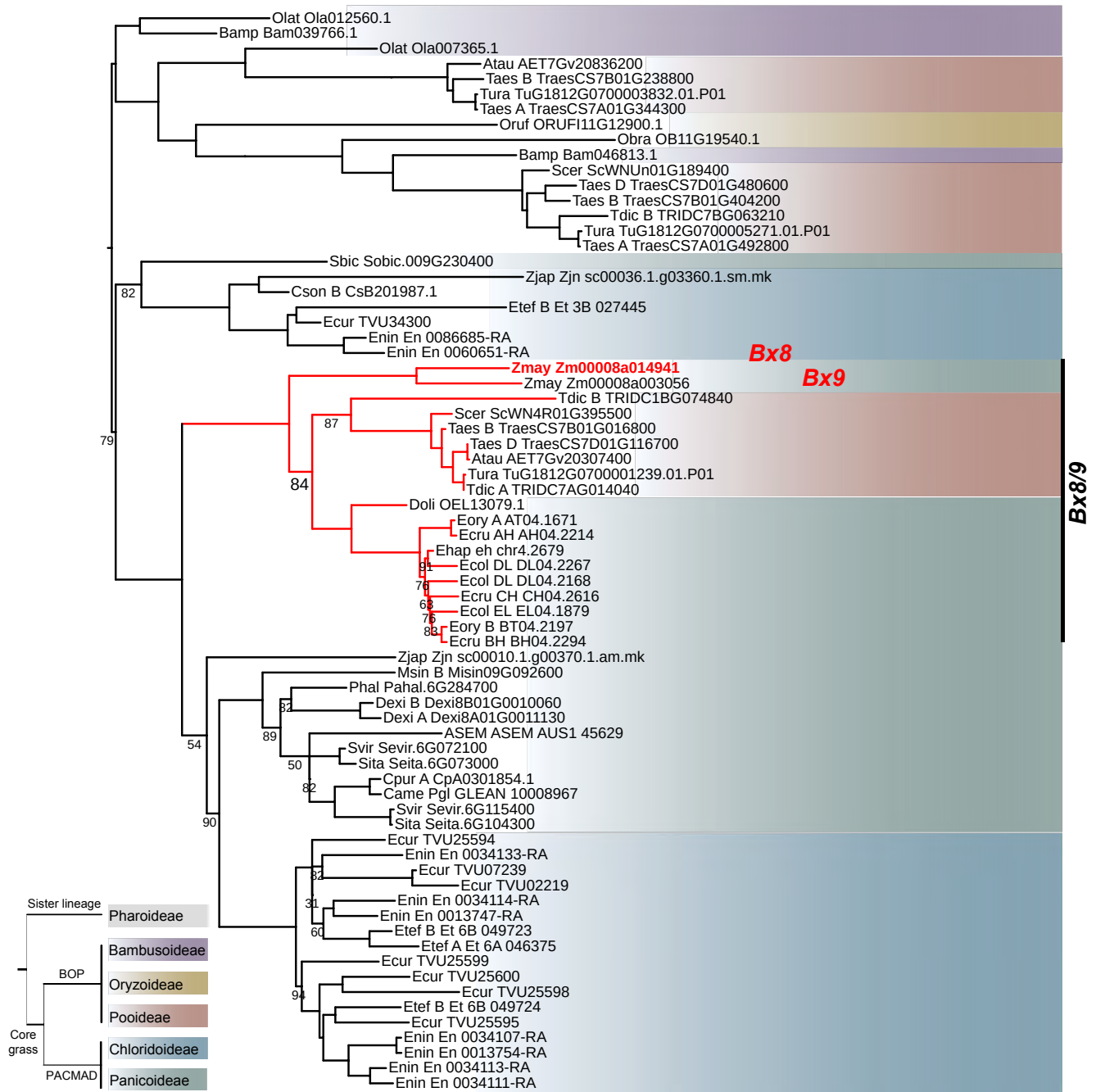
Supplemental Figure 2 Alignment of amino acid sequences of *Bx1* genes (B) and native homologs (N). See detailed sequences ID in Figure 2A.

A**B**

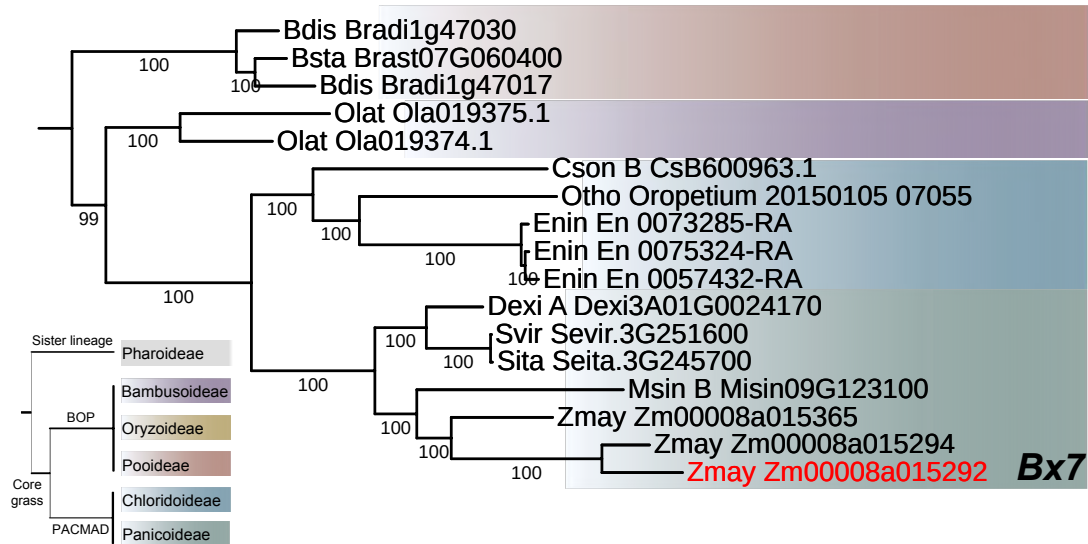
Supplemental Figure 3 Phylogenies of *Bx1* based on CDS (A) and codon12 (B) datasets. Red branches indicate the *Bx1* clade. Bootstrap values are shown on branches.



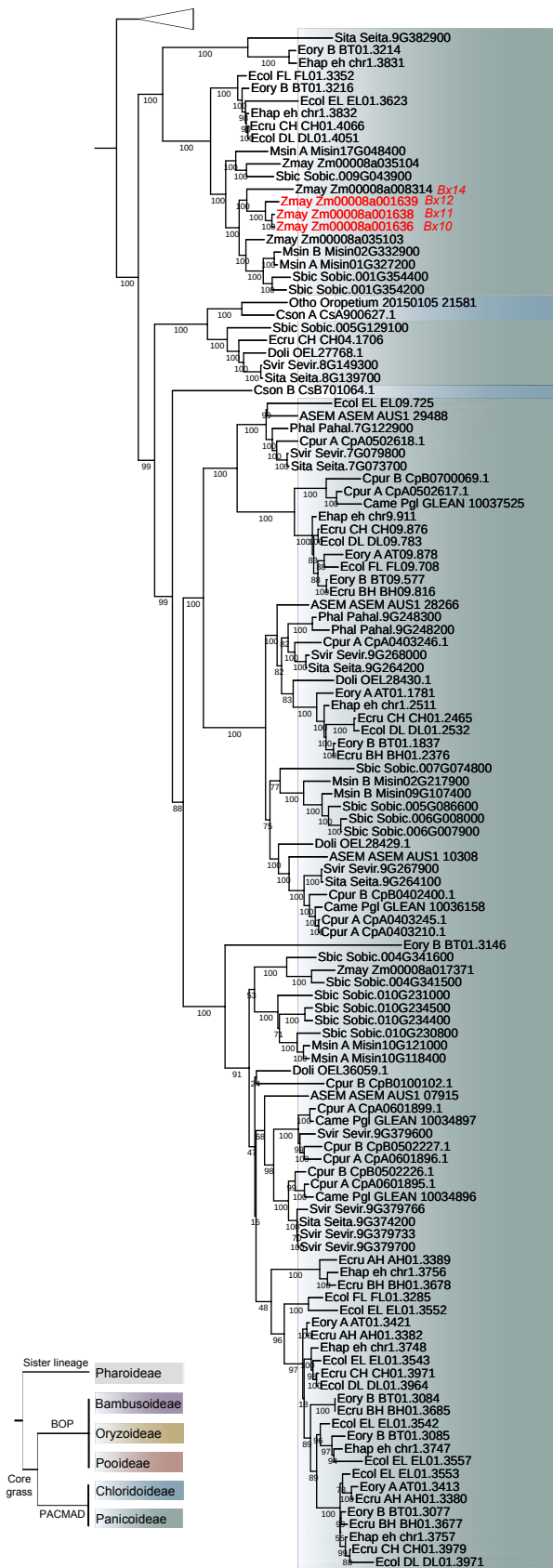
Supplemental Figure 4 Phylogeny of *Bx2* to *Bx5* homologs (A) and genomic synteny of *Bx2* (B) and *Bx5* (C) regions across the grass family. The branches of *Bx2* and *Bx3*-*Bx5* clades are highlighted by red. Bootstrap values less than 95 are shown on branches.



Supplemental Figure 5 Phylogeny of *Bx8* and *Bx9* homologs across the whole grass family. The branches of *Bx8/Bx9* clade are highlighted by red. Bootstrap values less than 95 are shown on branches.

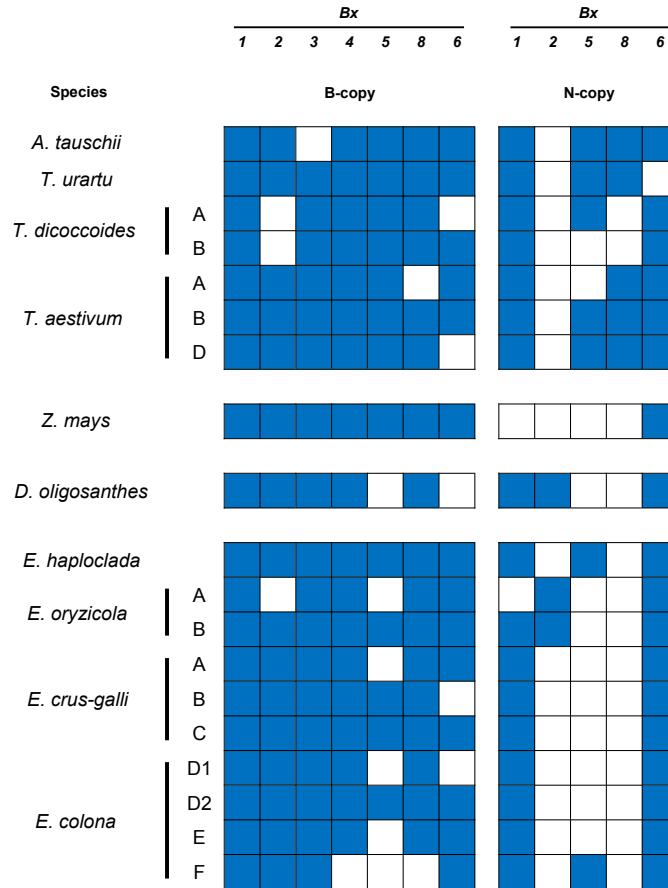


Supplemental Figure 6 Phylogeny of *Bx7* homologs across the grass family.

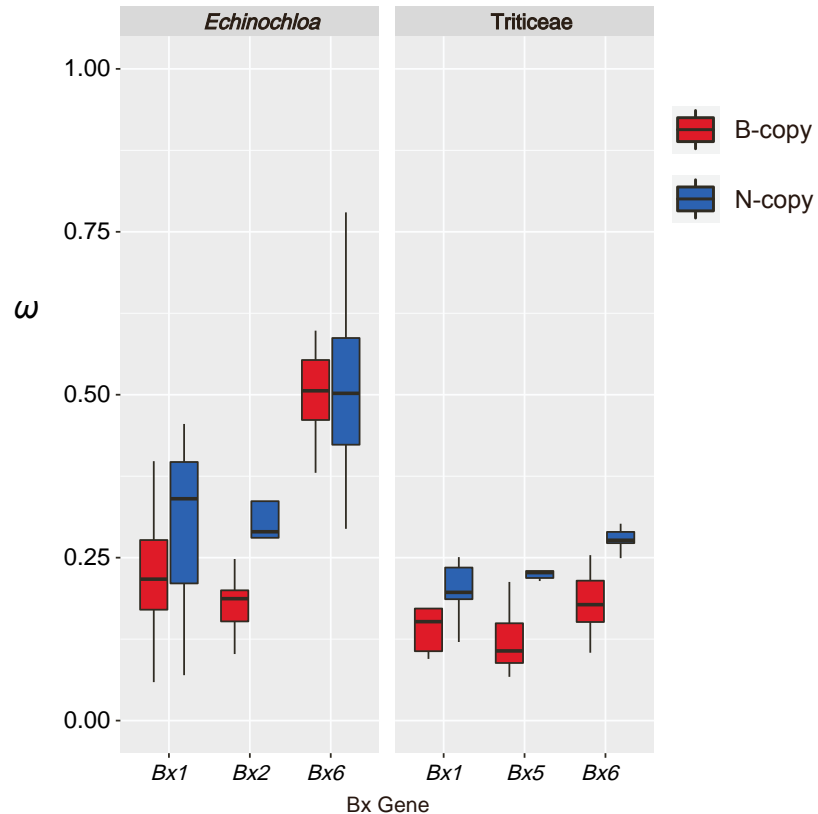


Bx10/11/12/14

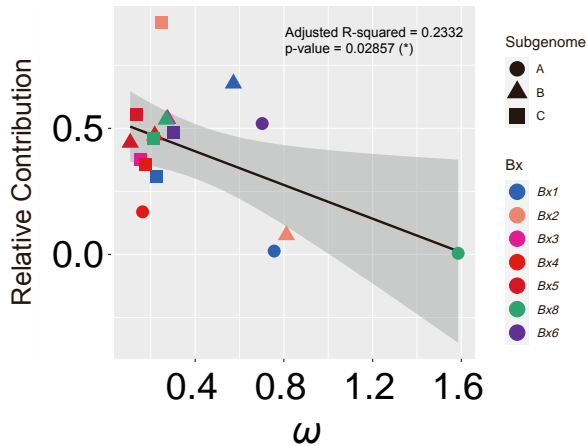
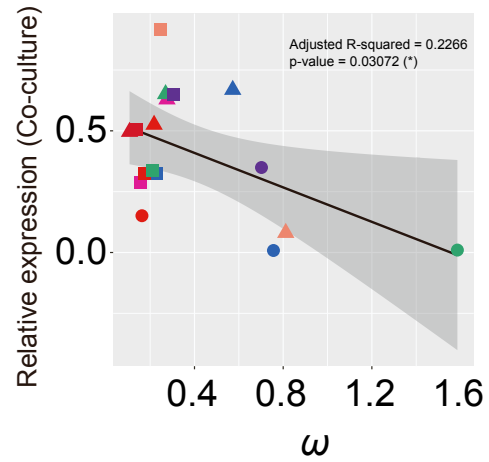
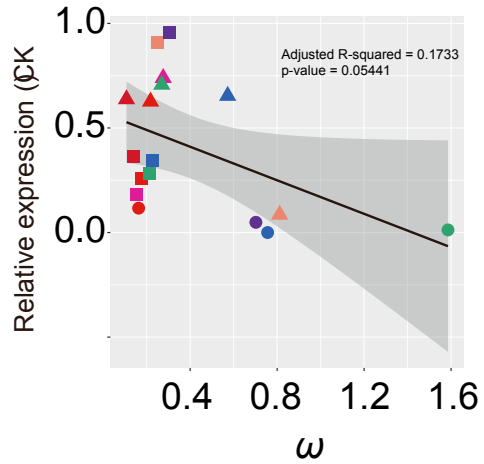
Supplemental Figure 7 Phylogeny of Bx10 to Bx12 and Bx14 homologs across the whole grass family.



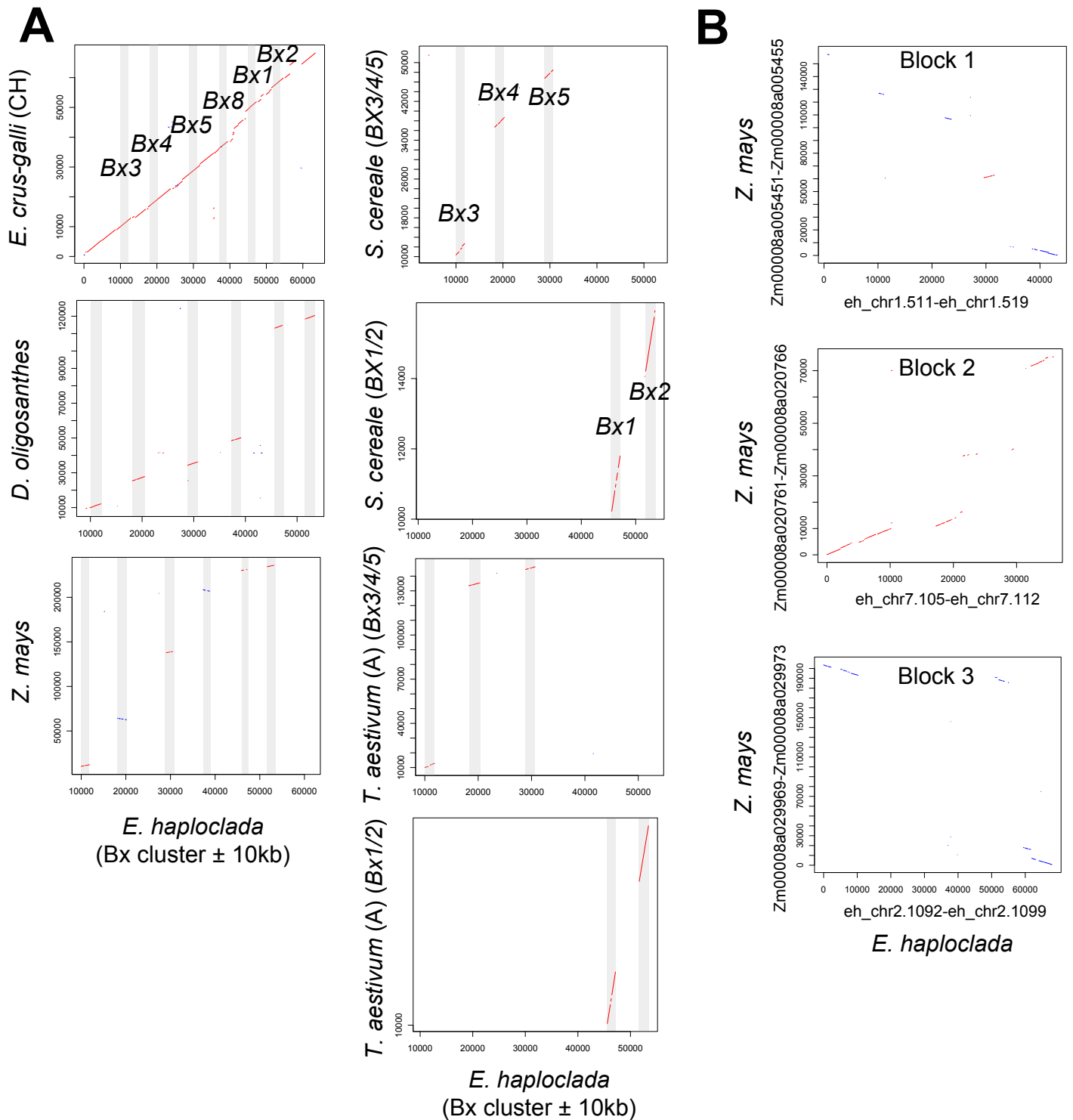
Supplemental Figure 8 Presence and absence of Bx genes (B-copy) and native homologs (N-copy) in Triticeae and Panicoideae. Blue grids represent presence and white represent absence.



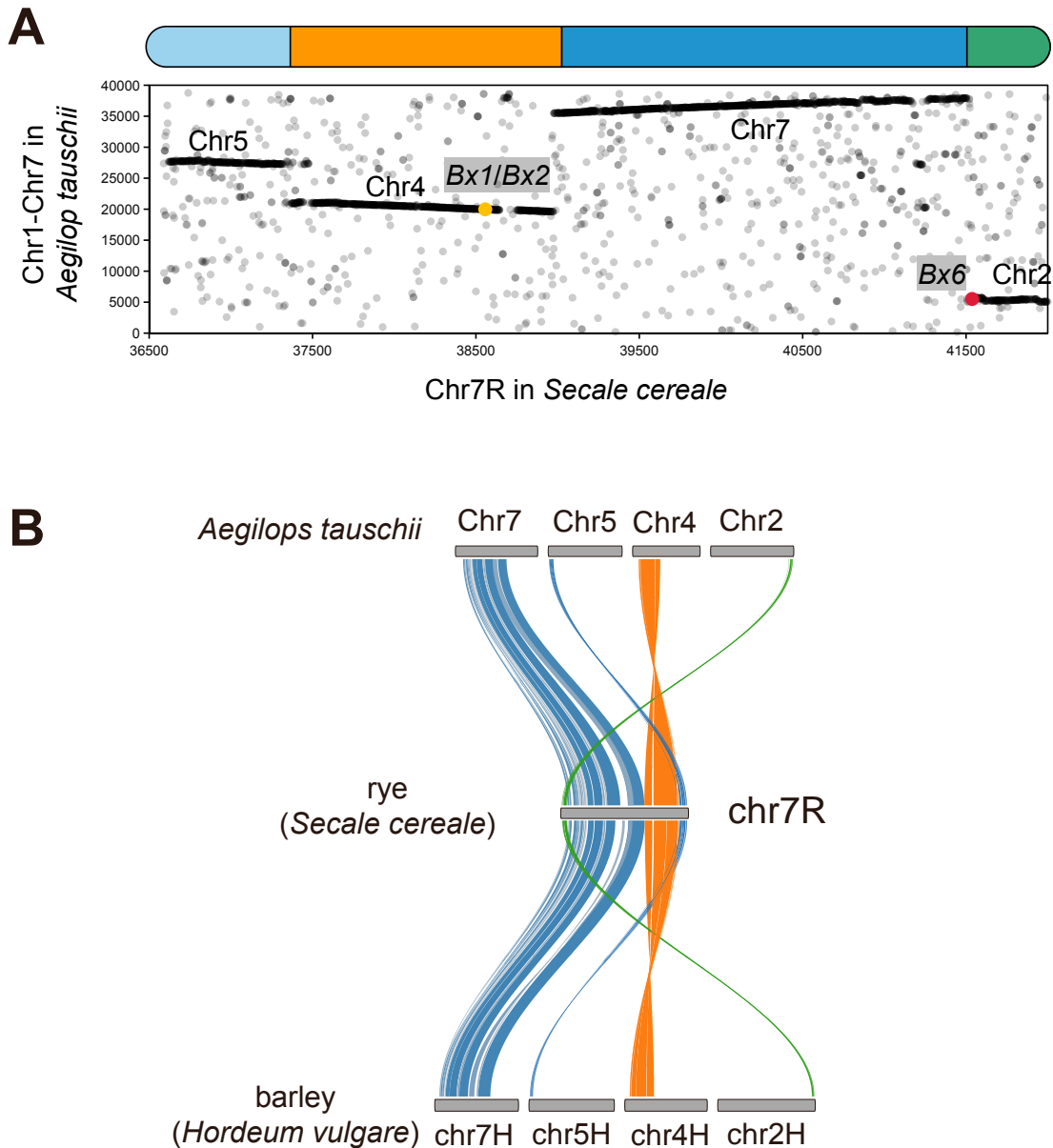
Supplemental Figure 9 Selection pressure on Bx genes in *Echinochloa* and Triticeae. In the box plots, the horizontal line shows the median value, and the whiskers show the 25% and 75% quartile values of ω . B-copy, Bx genes; N-copy, native homologs of Bx genes.



Supplemental Figure 10 Negative relationship between selection indicator ω values and gene expression or response dominance in *E. crus-galli*.



Supplemental Figure 11 Genomic synteny among Bx cluster elements (gene and intergenic regions) in grass genomes based on nucleotide sequences. **(A)** the synteny plots between Bx clusters from *E. haploclada* and other grass genomes (*E. crus-galli* CH, *D. oligosanthos*, *Z. mays*, *S. cereale* and *T. aestivum* A). The gray shadows indicate the positions of Bx genes in *E. haploclada* (from left to right: Bx3, Bx4, Bx5, Bx8, Bx1 and Bx2). Red and blue colors represent consistent and opposite orientations between two collinear genetic elements, respectively. **(B)** Synteny plots of three randomly selected evolutionarily conserved blocks between *E. haploclada* and *Z. mays*. The genomic region information is shown near the axis.



Supplemental Figure 12 Genomic synteny between chromosome 7R in rye (*S. cereale*) and other Triticeae genomes. **(A)** Chromosome 7R in rye is syntenic to chromosomes 5, 4, 7 and 2 in *Triticum/Aegilops*. *Bx1* and *Bx2* are located on chromosome 4 and *Bx6* on chromosome 2 in *Triticum/Aegilops*, while they all are on chromosome 7R in *Secale*. **(B)** Synteny among *A. tauschii*, *S. cereale* and *H. vulgare* genomes. Considering *Hordeum* is the outgroup genus of *Secale* and *Aegilops*, the chromosomal fusions of 7R in rye are *Secale*-specific.

Supplemental Table 1. A list of plant genomes used in this study

| Lineage | Sub-family | Species | Ploid | Assembly level | Version | Genome Abbr. |
|------------------------|--------------------------|-----------------------------------|------------|----------------|------------------|------------------------|
| Outgroup | Bromeliaceae | <i>Ananas comosus</i> | 2 | chromosome | F153 | Acom |
| Pharoideae | Pharoideae | <i>Pharus latifolius</i> | 2 | chromosome | 60161 | Plat |
| BOP | Bambusoideae | <i>Olyra latifolia</i> | 2 | scaffold | / | Olat |
| | | <i>Bonia amplexicaulis</i> | 6 | scaffold | / | Bamp |
| | Oryzoideae | <i>Leersia perrieri</i> | 2 | chromosome | GCA_000325765.3 | Lper |
| | | <i>Oryza barthii</i> | 2 | chromosome | GCA_000182155.2 | Obar |
| | | <i>Oryza brachyantha</i> | 2 | chromosome | GCA_000231095.2 | Obra |
| | | <i>Oryza glaberrima</i> | 2 | chromosome | GCA_000576495.1 | Ogla |
| | | <i>Oryza punctata</i> | 2 | chromosome | GCA_000573905.1 | Opun |
| | | <i>Oryza rufipogon</i> | 2 | chromosome | GCA_000817225.1 | Oruf |
| | | <i>Oryza sativa</i> | 2 | chromosome | v7.0 | Osat |
| | <i>Zizania latifolia</i> | 2 | scaffold | v01 | Zlat | |
| | Pooideae | <i>Aegilops tauschii</i> | 2 | chromosome | v4.0 | Atau |
| | | <i>Brachypodium distachyon</i> | 2 | chromosome | v3.1 | Bdis |
| | | <i>Brachypodium stacei</i> | 2 | chromosome | v1.1 | Bsta |
| | | <i>Hordeum vulgare</i> | 2 | chromosome | / | Hvul |
| | | <i>Triticum aestivum</i> | 6 | chromosome | iwgsc_refseqv1.0 | Taes_A; Taes_B; Taes_D |
| | | <i>Triticum dicoccoides</i> | 4 | chromosome | WEWSeq_v.1.0 | Tdic_A; Tdic_B |
| <i>Triticum urartu</i> | | 2 | chromosome | Tu | Tura | |
| <i>Secale cereale</i> | 2 | chromosome | Weining | Scer | | |
| PACMAD | Panicoideae | <i>Alloteropsis semialata</i> | 2 | scaffold | AUS1_V1.0 | Asem |
| | | <i>Cenchrus americanus</i> | 2 | chromosome | Camericanus.prot | Came |
| | | <i>Cenchrus purpureus</i> | 4 | chromosome | GWHAORA00000000 | Cpur_A; Cpur_B |
| | | <i>Dichantherium oligosanthes</i> | 2 | scaffold | ASM163321v2 | Doli |
| | | <i>Digitaria exilis</i> | 4 | chromosome | CM05836 | Dexi_A; Dexi_B |
| | | <i>Miscanthus sinensis</i> | 4 | chromosome | v7.1 | Msin_A; Msin_B |
| | | <i>Panicum hallii</i> | 2 | chromosome | v3.1 | Phal |
| | | <i>Setaria italica</i> | 2 | chromosome | v2.2 | Sita |
| | | <i>Setaria viridis</i> | 2 | chromosome | v2.1 | Svir |

| | | | | | |
|---------------|-------------------------------|---|------------|-----------------|---------------------------|
| | <i>Sorghum bicolor</i> | 2 | chromosome | v3.1.1 | Sbic |
| | <i>Zea mays</i> | 2 | chromosome | PH207_v1.1 | Zmay |
| | <i>Echinochloa haploclada</i> | 2 | chromosome | v1 | Ehap |
| | <i>Echinochloa oryzicola</i> | 4 | chromosome | v2 | Eory_AT; Eory_BT |
| | <i>Echinochloa crus-galli</i> | 6 | chromosome | v3 | Ecru_AH; Ecru_BH; Ecru_CH |
| | <i>Echibochloa colona</i> | 6 | chromosome | v1 | Ecol_DL; Ecol_EL; Ecol_FL |
| Chloridoideae | <i>Cleistogenes songorica</i> | 4 | chromosome | GWHANUQ00000000 | Cson_A; Cson_B |
| | <i>Eragrostis curvula</i> | 2 | scaffold | / | Ecur |
| | <i>Eragrostis tef</i> | 4 | chromosome | v3.1 | Etef_A; Etef_B |
| | <i>Eragrostis nindensis</i> | 4 | contig | v2.1 | Enin |
| | <i>Oropetium thomaeum</i> | 2 | contig | Othomaeum | Otho |
| | <i>Zoysia japonica</i> | 4 | scaffold | Zjaponica_r1.1 | Zjap |

Supplemental Table 2. Core Bx genes (*Bx1* to *Bx6* and *Bx8*) and corresponding native homologs in grass genomes

| Species | Genome | Bx | Bx copy | Chromosome | Native analogues | |
|------------------------|--------|------------|-----------------------|------------|-----------------------|--------------------|
| <i>A. tauschii</i> | | <i>Bx1</i> | AET4Gv20530100 | 4D | AET5Gv21022100 | |
| | | | | | AET5Gv21022200 | |
| | | | | | AET5Gv21021300 | |
| <i>T. urartu</i> | | <i>Bx1</i> | TuG1812G0400002465.01 | Tu4 | TuG1812G0500004630.01 | |
| | | | | | TuG1812G0500004636.01 | |
| | | | | | TuG1812G0500004637.01 | |
| | | | | | TuG1812G0500004639.01 | |
| <i>T. dicoccoides</i> | A | <i>Bx1</i> | TRIDC4AG013300 | 4A | TRIDC5AG063330 | |
| | | | | | TRIDC5AG063270 | |
| <i>T. dicoccoides</i> | B | <i>Bx1</i> | TRIDC4BG037100 | 4B | TRIDC5BG067890 | |
| | | | | | | TRIDC5BG067900 |
| | | | | | | TRIDC5BG067910 |
| <i>T. aestivum</i> | A | <i>Bx1</i> | TraesCS4A01G097400 | chr4A | TraesCS5A01G441100 | |
| | | | | | | TraesCS5A01G441300 |
| | | | | | | TraesCS5A01G440800 |
| | | | | | | TraesCS5A01G440900 |
| <i>T. aestivum</i> | B | <i>Bx1</i> | TraesCS4B01G207100 | chr4B | TraesCS5B01G445100 | |
| | | | | | | TraesCS5B01G445200 |
| | | | | | | TraesCS5B01G444500 |
| | | | | | | TraesCS5B01G444600 |
| <i>T. aestivum</i> | D | <i>Bx1</i> | TraesCS4D01G207900 | chr4D | TraesCS5D01G448400 | |
| | | | | | | TraesCS5D01G448500 |
| | | | | | | TraesCS5D01G448200 |
| | | | | | | TraesCS5D01G447800 |
| | | | | | | TraesCS5D01G447900 |
| <i>S. cereale</i> | | <i>Bx1</i> | ScWN7R01G203400 | 7R | ScWN5R01G490200 | |
| | | | | | ScWN5R01G489600 | |
| | | | | | ScWN5R01G489700 | |
| | | | | | ScWN5R01G489500 | |
| | | | | | ScWN5R01G506300 | |
| | | | | | ScWN5R01G490300 | |
| | | | | | ScWN5R01G492000 | |
| <i>Z. mays</i> | | <i>Bx1</i> | Zm00008a014942 | chr04 | Zm00008a005484 | |
| <i>D. oligosanthos</i> | | <i>Bx1</i> | OEL13077.1 | KV783575.1 | | |
| <i>E. haploclada</i> | | <i>Bx1</i> | eh_chr4.2680 | eh_chr4 | eh_chr1.483 | |
| <i>E. oryzicola</i> | AT | <i>Bx1</i> | AT04.1672 | AT04 | | |
| <i>E. oryzicola</i> | BT | <i>Bx1</i> | BT04.2196 | BT04 | BT01.27 | |
| | | | | | | BT01.28 |
| <i>E. crus-galli</i> | AH | <i>Bx1</i> | AH04.2215 | AH04 | AH01.218 | |
| <i>E. crus-galli</i> | BH | <i>Bx1</i> | BH04.2292 | BH04 | BH01.528 | |
| | | | BH04.2293 | | | |
| <i>E. crus-galli</i> | CH | <i>Bx1</i> | CH04.2618 | CH04 | CH01.385 | |
| | | | | | CH01.383 | |

| | | | | | |
|------------------------|-----|------------|-----------------------|------------|------------|
| <i>E. colona</i> | DL1 | <i>Bx1</i> | DL04.2268 | DL04 | DL01.551 |
| <i>E. colona</i> | DL2 | <i>Bx1</i> | DL04.2169 | DL04 | DL01.551 |
| <i>E. colona</i> | EL | <i>Bx1</i> | EL04.1881 | EL04 | EL01.455 |
| | | | | | EL01.456 |
| <i>E. colona</i> | FL | <i>Bx1</i> | FL04.1773 | FL04 | FL01.461 |
| | | | | | FL01.462 |
| <i>A. tauschii</i> | | <i>Bx2</i> | AET4Gv20530000 | 4D | |
| <i>T. urartu</i> | | <i>Bx2</i> | TuG1812G0400002466.01 | Tu4 | |
| <i>T. aestivum</i> | A | <i>Bx2</i> | TraesCS4A01G097500 | chr4A | |
| <i>T. aestivum</i> | B | <i>Bx2</i> | TraesCS4B01G207000 | chr4B | |
| <i>T. aestivum</i> | D | <i>Bx2</i> | TraesCS4D01G207800 | chr4D | |
| <i>S. cereale</i> | | <i>Bx2</i> | ScWN7R01G203500 | 7R | |
| <i>Z. mays</i> | | <i>Bx2</i> | Zm00008a014943 | chr04 | |
| <i>D. oligosanthos</i> | | <i>Bx2</i> | OEL13078.1 | KV783575.1 | OEL15381.1 |
| <i>E. haploclada</i> | | <i>Bx2</i> | eh_chr4.2681 | eh_chr4 | |
| <i>E. oryzicola</i> | AT | <i>Bx2</i> | | | AT08.48 |
| | | | | | AT08.49 |
| <i>E. oryzicola</i> | BT | <i>Bx2</i> | BT04.2195 | BT04 | BT08.44 |
| <i>E. crus-galli</i> | AH | <i>Bx2</i> | AH04.2216 | AH04 | |
| <i>E. crus-galli</i> | BH | <i>Bx2</i> | BH04.2291 | BH04 | |
| <i>E. crus-galli</i> | CH | <i>Bx2</i> | CH04.2619 | CH04 | |
| <i>E. colona</i> | DL1 | <i>Bx2</i> | DL04.2269 | DL04 | |
| <i>E. colona</i> | DL2 | <i>Bx2</i> | DL04.2172 | DL04 | |
| <i>E. colona</i> | EL | <i>Bx2</i> | EL04.1882 | EL04 | |
| <i>E. colona</i> | FL | <i>Bx2</i> | FL04.1774 | FL04 | |
| <i>T. urartu</i> | | <i>Bx3</i> | TuG1812G0500000122.01 | Tu5 | |
| <i>T. dicoccoides</i> | A | <i>Bx3</i> | TRIDC5AG001550 | 5A | |
| <i>T. dicoccoides</i> | B | <i>Bx3</i> | TRIDC5BG001410 | 5B | |
| <i>T. aestivum</i> | A | <i>Bx3</i> | TraesCS5A01G008900 | chr5A | |
| <i>T. aestivum</i> | B | <i>Bx3</i> | TraesCS5B01G007200 | chr5B | |
| <i>T. aestivum</i> | D | <i>Bx3</i> | TraesCS5D01G014300 | chr5D | |
| <i>S. cereale</i> | | <i>Bx3</i> | ScWN2R01G577700 | 2R | |
| <i>Z. mays</i> | | <i>Bx3</i> | Zm00008a014937 | chr04 | |
| <i>D. oligosanthos</i> | | <i>Bx3</i> | OEL13081.1 | KV783575.1 | |
| <i>E. haploclada</i> | | <i>Bx3</i> | eh_chr4.2676 | eh_chr4 | |
| <i>E. oryzicola</i> | AT | <i>Bx3</i> | AT04.1669 | AT04 | |
| <i>E. oryzicola</i> | BT | <i>Bx3</i> | BT04.2201 | BT04 | |
| <i>E. crus-galli</i> | AH | <i>Bx3</i> | AH04.2211 | AH04 | |
| <i>E. crus-galli</i> | BH | <i>Bx3</i> | BH04.2297 | BH04 | |
| <i>E. crus-galli</i> | CH | <i>Bx3</i> | CH04.2613 | CH04 | |
| <i>E. colona</i> | DL1 | <i>Bx3</i> | DL04.2265 | DL04 | |
| <i>E. colona</i> | DL2 | <i>Bx3</i> | DL04.2165 | DL04 | |
| <i>E. colona</i> | EL | <i>Bx3</i> | EL04.1877 | EL04 | |
| <i>E. colona</i> | FL | <i>Bx3</i> | FL04.1772 | FL04 | |
| <i>A. tauschii</i> | | <i>Bx4</i> | AET5Gv20025200 | 5D | |
| <i>T. urartu</i> | | <i>Bx4</i> | TuG1812G0500000121.01 | Tu5 | |
| <i>T. dicoccoides</i> | A | <i>Bx4</i> | TRIDC5AG001540 | 5A | |

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|------------------------|-----|------------|-----------------------|------------|-----------------------|
| <i>T. dicoccoides</i> | B | <i>Bx4</i> | TRIDC5BG001400 | 5B | |
| <i>T. aestivum</i> | A | <i>Bx4</i> | TraesCS5A01G008800 | chr5A | |
| <i>T. aestivum</i> | B | <i>Bx4</i> | TraesCS5B01G007100 | chr5B | |
| <i>T. aestivum</i> | D | <i>Bx4</i> | TraesCS5D01G014200 | chr5D | |
| <i>S. cereale</i> | | <i>Bx4</i> | ScWN2R01G577600 | 2R | |
| <i>Z. mays</i> | | <i>Bx4</i> | Zm00008a014938 | chr04 | |
| <i>D. oligosanthos</i> | | <i>Bx4</i> | OEL13080.1 | KV783575.1 | |
| <i>E. haploclada</i> | | <i>Bx4</i> | eh_chr4.2677 | eh_chr4 | |
| <i>E. oryzicola</i> | AT | <i>Bx4</i> | AT04.1670 | AT04 | |
| <i>E. oryzicola</i> | BT | <i>Bx4</i> | BT04.2199 | BT04 | |
| | | | BT04.2200 | BT04 | |
| <i>E. crus-galli</i> | AH | <i>Bx4</i> | AH04.2212 | AH04 | |
| <i>E. crus-galli</i> | BH | <i>Bx4</i> | BH04.2296 | BH04 | |
| <i>E. crus-galli</i> | CH | <i>Bx4</i> | CH04.2614 | CH04 | |
| <i>E. colona</i> | DL1 | <i>Bx4</i> | DL04.2266 | DL04 | |
| <i>E. colona</i> | DL2 | <i>Bx4</i> | DL04.2166 | DL04 | |
| <i>E. colona</i> | EL | <i>Bx4</i> | EL04.1878 | EL04 | |
| <i>A. tauschii</i> | | <i>Bx5</i> | AET5Gv20025100 | 5D | AET2Gv21022600 |
| <i>T. urartu</i> | | <i>Bx5</i> | TuG1812G0500000120.01 | Tu5 | TuG1812G0200005151.01 |
| <i>T. dicoccoides</i> | A | <i>Bx5</i> | TRIDC5AG001530 | 5A | TRIDC2AG065830 |
| <i>T. dicoccoides</i> | B | <i>Bx5</i> | TRIDC5BG001390 | 5B | |
| | | | TRIDC5BG004710 | 5B | |
| <i>T. aestivum</i> | A | <i>Bx5</i> | TraesCS5A01G008700 | chr5A | |
| <i>T. aestivum</i> | B | <i>Bx5</i> | TraesCS5B01G007000 | chr5B | TraesCS2B01G485100 |
| | | | TraesCS5B01G029900 | chr5B | |
| | | | TraesCS5B01G030000 | chr5B | |
| <i>T. aestivum</i> | D | <i>Bx5</i> | TraesCS5D01G014100 | chr5D | TraesCS2D01G464200 |
| <i>S. cereale</i> | | <i>Bx5</i> | ScWN2R01G577500 | 2R | |
| <i>Z. mays</i> | | <i>Bx5</i> | Zm00008a014940 | chr04 | |
| <i>E. haploclada</i> | | <i>Bx5</i> | eh_chr4.2678 | eh_chr4 | eh_chr8.27 |
| <i>E. oryzicola</i> | BT | <i>Bx5</i> | BT04.2198 | BT04 | |
| <i>E. crus-galli</i> | BH | <i>Bx5</i> | BH04.2295 | BH04 | |
| <i>E. crus-galli</i> | CH | <i>Bx5</i> | CH04.2615 | CH04 | |
| <i>E. colona</i> | DL2 | <i>Bx5</i> | DL04.2167 | DL04 | |
| <i>E. colona</i> | FL | <i>Bx5</i> | | | FL02.2320 |
| <i>A. tauschii</i> | | <i>Bx8</i> | AET7Gv20307400 | 7D | AET7Gv20836200 |
| <i>T. urartu</i> | | <i>Bx8</i> | TuG1812G0700001239.01 | Tu7 | TuG1812G0700003832.01 |
| <i>T. dicoccoides</i> | A | <i>Bx8</i> | TRIDC7AG014040 | 7A | |
| <i>T. dicoccoides</i> | B | <i>Bx8</i> | TRIDC1BG074840 | 1B | |
| <i>T. aestivum</i> | A | <i>Bx8</i> | | | TraesCS7A01G344300 |
| <i>T. aestivum</i> | B | <i>Bx8</i> | TraesCS7B01G016800 | chr7B | TraesCS7B01G238800 |
| <i>T. aestivum</i> | D | <i>Bx8</i> | TraesCS7D01G116700 | chr7D | |
| <i>S. cereale</i> | | <i>Bx8</i> | ScWN4R01G395500 | 4R | |
| <i>Z. mays</i> | | <i>Bx8</i> | Zm00008a014941 | chr04 | |
| <i>D. oligosanthos</i> | | <i>Bx8</i> | OEL13079.1 | KV783575.1 | |
| <i>E. haploclada</i> | | <i>Bx8</i> | eh_chr4.2679 | eh_chr4 | |
| <i>E. oryzicola</i> | AT | <i>Bx8</i> | AT04.1671 | AT04 | |

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|------------------------|-----|------------|-----------------------|---------|--------------------|
| <i>E. oryzipicola</i> | BT | <i>Bx8</i> | BT04.2197 | BT04 | |
| <i>E. crus-galli</i> | AH | <i>Bx8</i> | AH04.2214 | AH04 | |
| <i>E. crus-galli</i> | BH | <i>Bx8</i> | BH04.2294 | BH04 | |
| <i>E. crus-galli</i> | CH | <i>Bx8</i> | CH04.2616 | CH04 | |
| <i>E. colona</i> | DL1 | <i>Bx8</i> | DL04.2267 | DL04 | |
| <i>E. colona</i> | DL2 | <i>Bx8</i> | DL04.2168 | DL04 | |
| <i>E. colona</i> | EL | <i>Bx8</i> | EL04.1879 | EL04 | |
| <i>A. tauschii</i> | | <i>Bx6</i> | AET2Gv20088500 | 2D | AET1Gv20212300 |
| <i>T. urartu</i> | | <i>Bx6</i> | TuG1812G0200000785.01 | Tu2 | |
| <i>T. dicoccoides</i> | A | <i>Bx6</i> | | | TRIDC1AG012200 |
| <i>T. dicoccoides</i> | B | <i>Bx6</i> | TRIDC2BG006360 | 2B | TRIDC1BG014960 |
| <i>T. aestivum</i> | A | <i>Bx6</i> | TraesCS2A01G051700 | chr2A | TraesCS1A01G085800 |
| <i>T. aestivum</i> | B | <i>Bx6</i> | TraesCS2B01G066000 | chr2B | TraesCS1B01G103700 |
| <i>T. aestivum</i> | D | <i>Bx6</i> | | | TraesCS1D01G087300 |
| <i>S. cereale</i> | | <i>Bx6</i> | ScWN7R01G511600 | 7R | ScWN1R01G109200 |
| | | | ScWN7R01G511000 | | |
| <i>Z. mays</i> | | <i>Bx6</i> | Zm00008a014884 | chr04 | Zm00008a033413 |
| <i>D. oligosanthos</i> | | <i>Bx6</i> | | | OEL32741.1 |
| <i>E. haploclada</i> | | <i>Bx6</i> | eh_chr9.1288 | eh_chr9 | eh_chr6.2252 |
| <i>E. oryzipicola</i> | AT | <i>Bx6</i> | AT09.1105 | AT09 | AT06.700 |
| <i>E. oryzipicola</i> | BT | <i>Bx6</i> | BT09.1964 | BT09 | BT06.802 |
| | | | | | BT06.803 |
| <i>E. crus-galli</i> | AH | <i>Bx6</i> | AH09.1073 | AH09 | AH06.747 |
| <i>E. crus-galli</i> | BH | <i>Bx6</i> | | | BH06.788 |
| | | | | | BH06.790 |
| <i>E. crus-galli</i> | CH | <i>Bx6</i> | CH09.1289 | CH09 | CH06.821 |
| | | | | | CH06.823 |
| <i>E. colona</i> | DL1 | <i>Bx6</i> | | | DL06.2287 |
| | | | | | DL06.2288 |
| <i>E. colona</i> | DL2 | <i>Bx6</i> | DL09.1179 | DL09 | DL06.2287 |
| | | | | | DL06.2288 |
| <i>E. colona</i> | EL | <i>Bx6</i> | EL09.1058 | EL09 | EL06.1910 |
| | | | EL09.1059 | | EL06.1911 |
| | | | | | EL06.1913 |
| <i>E. colona</i> | FL | <i>Bx6</i> | FL09.1015 | FL09 | FL06.479 |
| | | | | | FL06.480 |
| <i>D. exilis</i> | A | <i>Bx6</i> | Dexi7A01G0007160 | 7A | Dexi4A01G0007650 |
| | | | | | Dexi4A01G0007670 |
| <i>D. exilis</i> | B | <i>Bx6</i> | Dexi7B01G0008100 | 7B | Dexi4B01G0007960 |
| | | | | | Dexi4B01G0007980 |
| | | | | | Dexi4B01G0007990 |
| <i>S. italica</i> | | <i>Bx6</i> | Seita.7G107200 | 4 | Seita.4G083700 |
| <i>S. viridis</i> | | <i>Bx6</i> | Sevir.7G114500 | 4 | Sevir.4G082900 |

Supplemental Table 3. Topology tests of two hypothesis on transfer of Bx genes in Triticeae from Panicoideae

| Datasets | Alignment with MAFFT | | | Alignment with MAFFT and Gblocks trimming | | |
|-------------------|----------------------|---------------|---------------|-------------------------------------------|---------------|---------------|
| Topologies tested | PO vs Non-PO | | | PO vs Non-PO | | |
| | AU | RELL | SH | AU | RELL | SH |
| <i>Bx1</i> | 1.0000/4.98e-34 | 1.0000/0.0000 | 1.0000/0.0000 | 0.9999/5.41e-5 | 1.0000/0.0000 | 1.0000/0.0000 |
| <i>Bx2</i> | 1.0000/9.72e-9 | 1.0000/0.0000 | 1.0000/0.0000 | 0.9996/0.0004 | 1.0000/0.0000 | 1.0000/0.0000 |
| <i>Bx3/4/5</i> | 1.0000/1.43e-5 | 1.0000/0.0000 | 1.0000/0.0000 | 0.9981/0.0019 | 1.0000/0.0000 | 1.0000/0.0000 |
| <i>Bx8</i> | 1.0000/2.58e-44 | 1.0000/0.0000 | 1.0000/0.0000 | 1.0000/5.75e-5 | 1.0000/0.0000 | 1.0000/0.0000 |
| <i>Bx6</i> | 1.0000/1.18e-6 | 1.0000/0.0000 | 1.0000/0.0000 | 1.0000/4.4e-61 | 1.0000/0.0000 | 1.0000/0.0000 |

PO, Panicoideae origin; non-PO, non-Panicoideae origin; AU, approximately unbiased test; REll, resampling estimated log-likelihood method; SH, Shimodaira-Hasegawa test.