Table S1. The accession numbers of the *Zymoseptoria tritici* IPO323 genome

Chromosome	Accession number
Chr1	NC_018218.1
Chr2	NC_018217.1
Chr3	NC_018216.1
Chr4	NC_018215.1
Chr5	NC_018214.1
Chr6	NC_018213.1
Chr7	NC_018212.1
Chr8	NC_018211.1
Chr9	NC_018210.1
Chr10	NC_018209.1
Chr11	NC_018208.1
Chr12	NC_018207.1
Chr13	NC_018206.1
Chr14	NC_018205.1
Chr15	NC_018204.1
Chr16	NC_018203.1
Chr17	NC_018202.1
Chr18	NC_018201.1
Chr19	NC_018200.1
Chr20	NC_018199.1
Chr21	NC_018198.1

Table S2. Information on the genome assembly of *Zymoseptoria tritici* strains

Strains	Genome size (Mbp)	Read depth (x)	# of Contigs	Contig L50 (Mbp)
IPO323 §	39.7	NA	21 chromosomes	NA
STIR04A26b ¶	32.6	NA	1154	0.09
STIR04A48b ¶	31.8	NA	940	0.11
WAI320	36.1	66.3	2226	0.07
WAI321	37	51.7	2049	0.08
WAI322	37.7	62.4	2227	0.08
WAI323	37.1	50	2295	0.07
WAI324	37.2	55.8	2486	0.07
WAI326	37	61.4	2446	0.06
WAI327	37.8	64.3	2131	0.08
WAI328	37.1	62.7	2614	0.06
WAI329	35.9	37.8	2473	0.06
WAI55	37.4	107.3	2279	0.06
WAI56	43.4	152.6	4033	0.06
WAI147	37.2	152.7	2226	0.06
WAI332	36.8	165.2	2183	0.08
ST99CH_9G4c	34.5	55.3	1648	0.08
ST99CH9B8b	34.5	56.3	1878	0.07
ST99CH1A5	34.7	41	1532	0.08
ST99CH1E4	34.8	46.1	1587	0.07
ST99CH3B8	34.1	31.7	1419	0.09
ST99CH3C4	34.9	28.9	1621	0.08
ST99CH3D1	34.7	28.4	1467	0.08
ST99CH3D7	33.1	30.7	1318	0.1
ST99CH3F5	34.8	29.1	1646	0.07

§ IPO323 was sequenced and assembled by (Goodwin et al 2011).

¶ STIR04A26b and STIR04A48b were sequenced and assembled by (Stukenbrock et al 2011).

Table S3. Genomic information of 43 species in the Dothideomycetes class, whose protein genes were used to search for homologs in intergenic regions in *Zymoseptoria tritici*.

Species in the Dothideomycetes class	Number of proteins	Additional sources for annotation
Acidomyces richmondensis	21515	EST
Alternaria alternata	29053	
Ascochyta rabiei	10660	
Aureobasidium melanogenum	10768	EST
Aureobasidium namibiae	20523	EST
Aureobasidium pullulans	18671	RNA-seq
Aureobasidium subglaciale	21592	EST
Baudoinia panamericana	21019	EST
Bipolaris maydis	39114	
Bipolaris oryzae	24156	EST
Bipolaris sorokiniana	24545	EST
Bipolaris victoriae	25782	EST
Bipolaris zeicola	25959	EST
Cenococcum geophilum	14833	RNA-seq
Clohesyomyces aquaticus	15811	EST
Coniosporium apollinis	18634	
Diplodia corticola	21739	
Diplodia seriata	17855	RNA-seq
Dothistroma septosporum	12640	EST
Epicoccum nigrum	12088	
Glonium stellatum	14279	RNA-seq
Hortaea werneckii	15756	
Lepidopterella palustris CBS 459.81	13861	EST
Leptosphaeria maculans	25298	
Macrophomina phaseolina	14297	
Mycosphaerella eumusae	12668	RNA-seq
Neofusicoccum parvum	21867	
Paraphaeosphaeria sporulosa	29508	EST
Parastagonospora nodorum	33283	
Pseudocercospora fijiensis	26184	EST
Pseudocercospora musae	13169	RNA-seq
Pyrenochaeta sp. DS3sAY3a	14990	EST
Pyrenophora teres	23834	EST
Pyrenophora tritici-repentis	24634	
Rachicladosporium antarcticum	18783	
Rachicladosporium sp. CCFEE 5018	18892	
Setosphaeria turcica	23459	EST
Sphaerulina musiva	20987	EST
Stagonospora sp. SRC1lsM3a	14425	EST
Stemphylium lycopersici	9145	
Verruconis gallopava	22768	
Zymoseptoria brevis	10625	RNA-seq
Zymoseptoria tritici	46566	EST

A

```
                                exon                                      intron
MYCGRDRAFT_35527_3 AGTGGAGTCATGGAACGCCGGTAAGAAAATGAAGTGCAGAAAGGTTGCGTGGCGCGAGATTGTCTCGGACGCCGGGCCTT
NC_018213_849513   AGCGATCGATAGAACTGCGGGT...CG.C......A....TG.CC.......TC......C..................
                       intergenic          TSD                          IIHE
                                                                                                exon
MYCGRDRAFT_35527_3 CGTCCA-TTCCACACTGTCTACGACATCCGCTAACCATATGCGCTTTTCTTTCTAGGTGGAGCATTCGATCGCGGA
NC_018213_849513   ......C..TTTG..C...............G...T.G.A......G...GA...GTCCTGTGGAAACAGATGTA
                                                                          TSD        intergenic
```

B

```
                                exon                                      intron
MYCGRDRAFT_85807_1 AGGAATACGCCAATGACACTGTAAGAAAAATCGAAGGGTAGAACGCCTGTAGCTGTCCGAGATTGTCTCTCGGGCTATGC
NC_018212_2513358  ATCAAGGCCCTTCTGCGAATGT.....----....C.AC..........C.............................
                       intergenic          TSD                          IIHE
                                                                                                exon
MYCGRDRAFT_85807_1 CAGCAGGTCTCAGTCTGCCCTTATGTCC-GTCCGAACTATTGCTAACCCTTCTA-CCTTCTGTTTTAGGTTCTCGGCGGC
NC_018212_2513358  ........TA............G.AC..T..T................T....GT.............GTCAATTTGCCG
                                                                                        TSD  intergenic

MYCGRDRAFT_85807_1 TGCTCTGT
NC_018212_2513358  GGAAGACG
```

C

```
                                exon                                      intron
MYCGRDRAFT_85156_2 ACCGCGGGCATCTTCTGCACGTAAGAAAACCGAAGGGCAGAACGCCTGCAGCTGTCCGAGA--TTGTCTCTCGGGCTATG
NC_018208_1042270  TCTGCCAAGATGAATACGGCGTA...G....A..C.........................GA................
                       intergenic          TSD                          IIHE
                                                                                                exon
MYCGRDRAFT_85156_2 CCAGCAGGTCTCAGTCTGCCCTTATACCCTGTCCGAACTATTGCTGACCCTTCTGCCCTTCTGTTTTAGGTACCCAGCCG
NC_018208_1042270  ..................G.....C.........A.G.C......A.T.......T.........G...GTAGCGGTGTG
                                                                                        TSD  intergenic

MYCGRDRAFT_85156_2 AATTCATGA
NC_018208_1042270  CCGGGTACA
```

Figure S1. Sequences alignments of introns and their intergenic homologs. In each alignment, the top sequence shows the intron and adjacent exon sequences, while the bottom sequence shows intergenic intron homolog and flanking intergenic sequences. For each intron, the annotated gene name together with the intron number is shown. While for each intergenic intron homolog, the accession number of the chromosome together with the sequence position is shown. Putative target-site duplications (TSDs) are in red.

```
39491i2a
                    ----   ++++++++++++++++++++
                10 X 82      11122222333445556666
                 1 X 9095   51890257813625478123 5
WAI55               TCAT[+]TGCCTACTGTGCGTAGAGTTC
WAI326              ....[+].....................
WAI56               ....[+].....................
WAI147              ....[+].....G..T....CG.GTGGT
WAI332              ....[+].....G..T....CG.GTGGT
WAI320              ....[+].A...............A...
WAI329              ....[+].....................
WAI328              ....[+].....G..T....CG.GTGGT
WAI324              ....[+].A...............A...
WAI323              ....[+].....G..T....CG.GTGGT
WAI327              CT.C[+].....................
WAI322              CT.C[+].....................
WAI321              ....[+].....G..T....CG.GTGGT
IPO323              C...[-]..TGC.GCAGAAA.G.GTGGT
ST99CH9G4c          ....[-]..TGC.GCAGAAA.G.GTGGT
ST99CH9B8b          ....[+]CA.............T.....
ST99CH3D1           ...C[+]........A........A...
ST99CH3B8           .T.C[+].....................
ST99CH1A5           C..C[+].....................
ST99CH3F5           ....[+]CA.............T.....
ST99CH3C4           CT.C[+].....................
ST99CH1E4           ..T.[-]..TGC.GCAGAAA.G.GTGGT
ST99CH3D7           CT.C[+].....................
STIR04A48b          ....[+].A...............A...
STIR04A26b          ....[-]..TGC.GCAGAAA.G.GTGGT


39671i6
                    ----------------------------   +++
                10 X 199987777666555554444333221      1
                 1 X 05219873082064321054109808718   581
WAI55               AATTCAGCTAATCATTCACGAGTAATATC[+]TTC
WAI326              ..............................[+]...
WAI56               ..............................[+]...
WAI147              ..............................[+]...
WAI332              ..............................[+]...
WAI320              ..............................[+]...
WAI329              ..............................[+]...
WAI328              ..............................[+]...
WAI324              ..............................[+]...
WAI323              ..............................[+]...
WAI327              ..............................[+]...
WAI322              ..............................[+]...
WAI321              ..............................[+]...
IPO323              ..............................[+]...
ST99CH9G4c          GGACTGAGCGGAGG---GTAGACGGCGCT[-]CCT
ST99CH9B8b          ..............................[+]...
ST99CH3D1           ..............................[+]...
ST99CH3B8           GGA.TGA......................[+]...
ST99CH1A5           ..............................[+]...
ST99CH3F5           ..............................[+]...
ST99CH3C4           ..............................[+]...
ST99CH1E4           GGACTGAGCGGAGG---GTAGACGGCGCT[-]CCT
ST99CH3D7           ..............................[+]...
STIR04A48b          GGACTGAGCGGAGG---GTAGACGGCGCT[-]CCT
STIR04A26b          GGACTGAGCGGAGG---GTAGACGGCGCT[-]CCT

41247i4
                    ----   ++++++++
                10 X 1        23344
                 1 X 1852   57891814
WAI55               TTTG[+]TATTAATT
WAI326              ....[+]........
WAI56               ....[+]........
WAI147              ....[+]........
WAI332              ....[+]........
WAI320              GGGA[-]GGCCGGGG
WAI329              ....[+]........
WAI328              ....[+]........
WAI324              ....[+]........
WAI323              GGGA[-]GGCCGGGG
WAI327              GGGA[-]GGCCGGGG
WAI322              ....[+]........
WAI321              GGGA[-]GGCCGGGG
IPO323              ....[+]........
ST99CH9G4c          ....[+]........
ST99CH9B8b          GGGA[-]GGCCGGGG
ST99CH3D1           ....[+]........
ST99CH3B8           GGGA[-]GGCCGGGG
ST99CH1A5           GGGA[-]GGCCGGGG
ST99CH3F5           ....[+]........
ST99CH3C4           ....[+]........
ST99CH1E4           ....[+]........
ST99CH3D7           ....[+]........
STIR04A48b          GGGA[-]GGCCGGGG
STIR04A26b          GGGA[-]GGCCGGGG
```

Figure S2. Sequence alignments flanking intron insertion sites from the 20 IPAP loci. Only informative sites (nucleotide differences flanking each intron site) from ±100 nt are shown with the nucleotide coordinates at the top. Dots indicate identities relative to the top sequence, whereas letters represent nucleotide differences. Intron presence is indicated by [+], while intron absence is shown as [-]. The strains are ordered following the phylogenetic tree in Figure S3.

```
48390i1
                    --------   +++++++++
                10 X 9987654    133455568
                 1 X 53622096   489425755
WAI55               AAACCCCC[-]AAGTCCTCA
WAI326              TCG.A..A[+].TC......
WAI56               TCG.A..A[+].TC......
WAI147              TCG.A..A[+].TC......
WAI332              ........[-].........
WAI320              TCGAA..A[+]...C.....
WAI329              ........[-].........
WAI328              TCG.A..A[+].TC......
WAI324              ........[-]C........
WAI323              TCGAA..A[+].........
WAI327              ........[-].........
WAI321              TCGAA..A[+].........
IPO323              TCGAA..A[+].........
ST99CH9G4c          TCGAA..A[+]....TA.T.
ST99CH9B8b          ....A..A[+]....TA.T.
ST99CH3D1           TCGAA...[-].TC......
ST99CH3B8           TCGAA...[-]........C
ST99CH1A5           TCGAAAAA[+]....TA.T.
ST99CH3F5           TCG.....[-]...C.....
ST99CH3C4           TCGAAAAA[+]....TA.T.
ST99CH1E4           TCGAAAAA[+]....TA.T.
ST99CH3D7           .......A[+]......C..
STIR04A48b          TCGAA...[-].........
STIR04A26b          TCG.A...[-].........
```

```
60105i2a
                     ---   +
                10 X 611   1
                 1 X 210   6
WAI55                CAT[+]A
WAI326               G..[+].
WAI56                G..[+].
WAI147               ...[+].
WAI332               ...[+].
WAI320               G..[+].
WAI329               ...[+].
WAI328               ...[+].
WAI324               G..[+].
WAI323               ...[+].
WAI327               ...[+].
WAI322               ...[+].
IPO323               .TC[-]C
ST99CH3D1            ...[+].
ST99CH1A5            .TC[-]C
ST99CH3F5            ...[+].
ST99CH3C4            .TC[-].
ST99CH3D7            ...[+].
STIR04A26b           ...[+].
```

```
65466i6
                    ------------   ++++++++++++++
                10 X 188887652         111222333589
                 1 X 094108036843   278235239345061
WAI55               ACCCAGACGCGG[-]AGAGAGAGTGCTCTC
WAI326              ............[-]...............
WAI56               ............[-]...............
WAI147              CTTTCAGGCAAA[+]GCGAGAGACAGCTCT
WAI332              CTTTCAGGCAAA[+]GCGAGAGACAGCTCT
WAI320              ............[-]...............
WAI329              CTTTCAGGCAAA[+]GCGAGAGACAGCTCT
WAI328              ............[-]...............
WAI324              ............[-]...............
WAI323              ..A...G.....[-]...............
WAI327              ..A...G.....[-]...............
WAI322              ..A...G.....[-]...............
IPO323              CTTTCAGGCA..[+]GCGAGAGACAGCTCT
ST99CH9G4c          ............[-]...............
ST99CH9B8b          CTTTCAGGCA..[+]GCGAGAGACAGCTCT
ST99CH3D1           CTTTCAGGCA..[+]GCGAGAGACAGCTCT
ST99CH3B8           ..A...G.....[-]...............
ST99CH1A5           ............[-]...............
ST99CH3F5           ..A...G.....[-]...............
ST99CH3C4           ............[-]...............
ST99CH1E4           CTTTCAGGCA..[+]GCGAGAGACAGCTCT
ST99CH3D7           ..A...G.....[-]...............
STIR04A48b          ..A...G.....[-]...............
STIR04A26b          ..A...G.....[-]...............
```

Figure S2. Continued

```
70308i3
                    --------   ++++++++++++
                10 X 7542221     33336788999
                 1 X 52385291   306790817013
WAI55               CCAACTCA[+]ATGTGACCAGTG
WAI326              .TCTTCA.[-]GC..........
WAI56               ........[+]............
WAI147              .TCTTCA.[-]GC..........
WAI332              ........[+]............
WAI320              .TCTTCA.[-]GC..........
WAI329              .TCTTCA.[-]GC..........
WAI328              ........[+]............
WAI324              ........[+]............
WAI323              .TCTTCA.[-]GC..........
WAI327              .TCTTCA.[-]GC..........
WAI322              .TCTTCA.[-]GC..........
WAI321              .TCTTCA.[-]GC..........
IPO323              ...TTCAG[+].C.CC..AG...
ST99CH9G4c          .TCTTCA.[-]GC..........
ST99CH9B8b          ...TTCAG[+].C.CCC......
ST99CH3D1           .TCTTCA.[-]GC..........
ST99CH3B8           ...TTCAG[+].C..........
ST99CH1A5           ...TTCA.[-]GC..........
ST99CH3F5           A..TTCAG[+].CC...TAGTCA
ST99CH3C4           .TCTTCA.[-]GC...C......
ST99CH1E4           ...TTCA.[-]GC...C......
ST99CH3D7           ...TTCA.[-]GC..........
STIR04A48b          ...TTCA.[-]GC.....AG...
STIR04A26b          .TCTTCA.[-]GC..........


70396_i1_2a
               100 X 00000000001   11   12
                10 X 88531111100   23   71
                 1 X 92546321041   73   31
WAI55               ACCCAGAGTCT[+]TC[-]TC
WAI326              ...........[+]..[-]..
WAI56               ...........[+]..[-]..
WAI147              ...........[+]..[-]..
WAI332              ...........[+]..[-]..
WAI320              .TTTTATCGTC[-]CG[+]..
WAI329              ...........[+]..[-]..
WAI328              ...........[+]..[-]..
WAI324              ...........[+]..[-]..
WAI323              .TTTTATCGTC[-]CG[+]C.
WAI327              .TTTTATCGTC[-]CG[+]C.
WAI322              .TTTTATCGTC[-]CG[+]C.
IPO323              ...........[+]..[-]..
ST99CH9G4c          .TTTTATCGTC[-]CG[+]C.
ST99CH9B8b          .TTTTATCGTC[-]CG[+]C.
ST99CH3D1           .TTTTATCGTC[-]CG[+]C.
ST99CH3B8           .TTTTATCGTC[-]CG[+]C.
ST99CH1A5           G..........[+]..[-].T
ST99CH3F5           .T.........[+]..[-]..
ST99CH3C4           .T.........[+]..[-]..
ST99CH1E4           ..TTTATCGTC[-]CG[+]..
ST99CH3D7           ...........[+]..[-]..
STIR04A48b          ...........[+]..[-]..
STIR04A26b          ...........[+]..[-]..

71401i3
                    -----------------   ++++++
                10 X 88755332222111        4569
                 1 X 90430213210431851   127253
WAI55               TCAACGCTTCACTGTTA[+]GCTAGG
WAI326              .................[+]......
WAI56               .................[+]......
WAI147              .................[+]......
WAI332              .................[+]......
WAI320              .................[+]......
WAI329              .................[+]......
WAI328              .................[+]......
WAI324              .................[+]......
WAI323              .................[+]......
WAI327              .................[+]......
WAI322              .................[+]......
WAI321              .................[+]......
IPO323              .................[+]......
ST99CH9G4c          .....T...........[+]......
ST99CH9B8b          ...TGTTCAGCTCACCT[-]CA....
ST99CH3D1           CTGTGT.CAGCT.ACCT[-]CAATTA
ST99CH3B8           .................[+]......
ST99CH1A5           .................[+]......
ST99CH3F5           .................[+]......
ST99CH3C4           .................[+]......
ST99CH1E4           .................[+]......
ST99CH3D7           .................[+]......
STIR04A48b          .................[+]......
STIR04A26b          .................[+]......
```

Figure S2. Continued

```
72460i2
                      -------   +
                 10 X 766532
                  1 X 8601343[+]9
WAI55                 TACCACG[+]T
WAI326                .......[+].
WAI56                 .......[+].
WAI147                .......[+].
WAI332                .......[+].
WAI320                GGATTTA[-]C
WAI329                .......[+].
WAI328                .......[+].
WAI324                .......[+].
WAI323                .......[+].
WAI327                .......[+].
WAI322                .......[+].
WAI321                .......[+].
IPO323                .......[+].
ST99CH                .......[+].
ST99CH3D1             .......[+].
ST99CH3B8             .......[+].
ST99CH1A5             .......[+].
ST99CH3F5             .......[+].
ST99CH3C4             .......[+].
ST99CH1E4             .......[+].
ST99CH3D7             .......[+].
STIR04A48b            .......[-].
STIR04A26b            GGATTTA[-]C


77797i3
                      --------------   +++
                10 X 9663222111
                 1 X 34147528637654   369
WAI55                 TCCACCCTGACAGT[-]GCA
WAI326                ..............[-]...
WAI56                 ..............[-]...
WAI147                ..............[-]...
WAI332                ..............[-]...
WAI320                ..............[-]...
WAI329                ..............[-]...
WAI328                ..............[-]...
WAI324                ..............[-]...
WAI323                ..............[-]...
WAI327                ..............[-]...
WAI322                ..............[-]...
IPO323                GTTGAATCCCTTCG[+]CGC
ST99CH9G4c            GTTGAATCCCTTCG[+]CGC
ST99CH9B8b            ..............[-]...
ST99CH3D1             ..............[-]...
ST99CH3B8             ..............[-]...
ST99CH1A5             ..............[-]...
ST99CH3F5             ..............[-]...
ST99CH3C4             GTTGAATCCCTTCG[+]CGC
ST99CH1E4             ..............[-]...
ST99CH3D7             G.............[-]...
STIR04A48b            .TTGAATCCC....[-]...
STIR04A26b            .TTGAATCCC....[-]...

77869i3
                      -------------   +++
                 10 X 8875554321100   249
                  1 X 9076307207152   201
WAI55                 TTCATGCGGTTTC[-]CCC
WAI326                CCTGCTTAACCGG[-]GTG
WAI56                 CCTGCTTAACCGG[-]GTG
WAI147                CCTGCTTAACCGG[-]GTG
WAI332                CCTGCTTAACCGG[-]GTG
WAI320                CCTGCTTAACCGG[-]GTG
WAI329                .............[+]...
WAI328                .............[+]...
WAI324                CCTGCTTAACCGG[-]GTG
WAI323                CCTGCTTAACCGG[-]GTG
WAI327                CCTGCTTAACCGG[-]GTG
WAI322                CCTGCTTAACCGG[-]GTG
WAI321                CCTGCTTAACCGG[-]GTG
IPO323                CCTGCTT..... [+]....
ST99CH9G4c            .............[+]...
ST99CH9B8b            .............[+]...
ST99CH3D1             CCTGCTT......[+]...
ST99CH3B8             .............[+]...
ST99CH1A5             CCTGCTTAACCGG[-]GTG
ST99CH3F5             .............[+]...
ST99CH3C4             .............[+]...
ST99CH1E4             .............[+]...
ST99CH3D7             .............[+]...
STIR04A48b            .............[+]...
STIR04A26b            CCTGCTTAACCGG[-]GTG
```

Figure S2. Continued

```
84542i2

                    --------------------    ++++++++++++
                 10 X 997655433200      13346899
                  1 X 412074830493   12696926136
WAI55                CCTCCCATCCGA[-]TCTATGGACTC
WAI326               .............[-]...........
WAI56                ATCAGTCCTTAC[+]AGAGCAAGGCG
WAI147               .............[-]...........
WAI332               .............[-]...........
WAI320               .............[-]...........
WAI329               .............[-]...........
WAI328               .............[-]...........
WAI324               ......T..T..[-]...GCAAG...
WAI323               ATCAGTCCTTAC[+]AGAGCAAGGCG
WAI327               .............[-]...........
WAI322               ATCAGTCCTTAC[+]AGAGCAAGGCG
WAI321               ATCAGTCCTTAC[+]AGAGCAAGGCG
IPO323               ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH               ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH               ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH3D1            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH3B8            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH1A5            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH3F5            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH3C4            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH1E4            ATCAGTCCTTAC[+]AGAGCAAGGCG
ST99CH3D7            .............[-]...........
STIR04A48b           ATCAGTCCTTAC[+]AGAGCAAGGCG
STIR04A26b           ATCAGTCCTTAC[+]AGAGCAAGGCG


84625i1
                    ---------------   +++++
                 10 X 998887777654331    1399
                  1 X 718529630749710   35801
WAI55                CTCTCCTTCGTTAGT[+]CGCTC
WAI326               ...............[+].....
WAI56                ...............[+].....
WAI147               ...............[+].....
WAI332               ...............[+].....
WAI320               ............G..[+].....
WAI329               ...............[+].....
WAI328               ............G..[+].....
WAI324               ...............[+].....
WAI323               ........TA.CG.G[-]TA...
WAI327               ...............[+].....
WAI322               ...............[+].....
WAI321               ........TA.CG.G[-]TA...
IPO323               ...............[+].....
ST99CH9G4c           .............A.[+].....
ST99CH9B8b           TC.........C...[+].....
ST99CH3D1            T.TG.TC...CC.A.[+]..T..
ST99CH3B8            .............G[-]TA...
ST99CH1A5            T.TGTT.CT...G.G[-]TA...
ST99CH3F5            .............G[-]TA.CA
ST99CH3C4            ...............[+].....
ST99CH1E4            TC.GTTC...CC.A.[+].....
ST99CH3D7            .............G[-]TA.CA
STIR04A48b           T.TG.TC...CC.A.[-].....
STIR04A26b           T.TG.TC...CCG.G[-]TA.CA

102064i6
                    --------------------------   ++++++++++++++++
                 10 X 99988887777666655554321         111234778899
                  1 X 6108632874398608210942074   3456589102083537
WAI55                CCGGCTGATTTTTTATGTTAGCGGC[+]ATCTAGCTTCCCACAG
WAI326               ..........................[+]................
WAI56                ..........................[+]................
WAI147               ..........................[+]................
WAI332               ..........................[+]................
WAI320               ..........................[+]................
WAI329               ..........................[+]................
WAI328               ..........................[+]................
WAI324               ..........................[+]................
WAI323               GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAG.TTTTGA
WAI327               GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAG.TTTTGA
WAI322               ..........................[+]................
WAI321               ..........................[+]................
IPO323               ..........................[+]................
ST99CH9G4c           ..........................[+]................
ST99CH9B8b           ..........................[+]................
ST99CH3D1            GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAG.TTTTGA
ST99CH3B8            ..........................[+]................
ST99CH1A5            GTACTCCGAACCACGCTCCCATATG[-]GAGCGATAG.TTTTGA
ST99CH3F5            GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAG.TTTTGA
ST99CH3C4            ..........................[+]................
ST99CH1E4            GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAGATTTTGA
ST99CH3D7            GTACTCCGAACCACGCTCCCATATG[-]GAGCGATAG.TTTTGA
STIR04A48b           ..........................[+]................
STIR04A26b           GT.CTCCGAACCACGCTCCC.TATG[-]GAGCGATAG.TTTTGA
```

Figure S2. Continued

```
103686i9

                 10 X 888665544    499
                  1 X 875301065   2314
WAI55                 CGATACTCC[+]TATT
WAI326                .........[-]....
WAI56                 .........[-]....
WAI147                TTGCCTAAT[+]CC..
WAI332                .........[-]....
WAI320                .........[-]....
WAI329                TTGCCTAAT[+]C.CA
WAI328                TTGCCTAAT[+]C.CA
WAI324                .........[-]....
WAI323                TTGCCTAAT[+]C.CA
WAI327                .........[-]....
WAI322                .........[-]....
WAI321                TTGCCTAAT[+]CC..
IPO323                TTGCCTAAT[+]C...
ST99CH9G4c            .........[-]....
ST99CH9B8b            TTGCCTAAT[-]....
ST99CH3D1             .........[-]....
ST99CH3B8             TTGCCTAAT[-]..CA
ST99CH1A5             .........[-]....
ST99CH3F5             .........[-]..CA
ST99CH3C4             .........[-]....
ST99CH3D7             .........[-]....
STIR04A48b            .........[-]....
STIR04A26b            .........[-]....


105101i3
                      __________   ++++
                 10 X 1999888752    588
                  1 X 0921732347   1256
WAI55                 AACGAATCGT[+]ACCT
WAI326                ..........[+].T..
WAI56                 ..........[+].T..
WAI147                ..........[+].T..
WAI332                ..........[+].T..
WAI320                ..........[+]....
WAI329                ..........[+].T..
WAI328                ..........[+]....
WAI324                ..........[+].T..
WAI323                ..........[+]....
WAI327                ..........[+]....
WAI322                ..........[+]....
WAI321                ..........[+]....
IPO323                ..........[+]..G.
ST99CH9G4c            .C....C.AC[-]CT..
ST99CH9B8b            .C....C.AC[-]CT..
ST99CH3D1             TCTCGC.T..[+]..G.
ST99CH3B8             TCTCGC.T..[+]....
ST99CH1A5             .C....C.AC[-]CT..
ST99CH3F5             ..........[+].T..
ST99CH3C4             .C....C.AC[-]C.G.
ST99CH1E4             TCTCGC.T..[+]..G.
ST99CH3D7             TCTCGC.T..[+].T..
STIR04A48b            TCTCGC.T..[+].T.C
STIR04A26b            ..........[+]..G.


111698i2_i4
                100 X 0000000   1111111111111111   11111122222222222222
                 10 X 1236899   0111123344555666   77888901122234455666
                  1 X 8100469   9258973925147069   69235765817892847013
WAI55                 ATGCCAT[-]ACTTTTCTCAGTATTA[+]CTCCTTTCTCTCGCCCTGCG
WAI326                .......[-]................[+].....................
WAI56                 .......[-]................[+].....................
WAI147                .......[-]................[+].....................
WAI332                .......[-]................[+].....................
WAI320                .......[-]................[+].....................
WAI329                .......[-]................[+].....................
WAI328                .......[-]................[+].....................
WAI324                .......[-]................[+].....................
WAI323                C......[+]TTCG..AA.C.CTCCT[-]TCGAA..ACGCTC.T.AAT.
WAI327                C......[+]TTCG..AA.C.CTCCT[-]TCGAA..ACGCTC.T.AAT.
WAI322                .......[-]................[+].....................
IPO323                C......[-]................[+].....................
ST99CH9G4c            C......[-]......G.T.......[+].....................
ST99CH9B8b            CCTGACG[-]CTA.CCA.TGACTC.T[+]T...CCC..T.T.T.TAT.C
ST99CH3D1             .......[-]................[+].....................
ST99CH3B8             .......[-]................[+].....................
ST99CH1A5             C.....C[+]TTCG..AA.C.CTCCT[-]TCGAA..ACGCTC.T.AAT.
ST99CH3F5             .......[-]................[+].....................
ST99CH3C4             C.....C[+]TTCG..AA.C.CTCCT[-]TCGAA..ACGCTC.T.AAT.
ST99CH1E4             .......[-]................[+].....................
ST99CH3D7             C......[-]......G.T.......[+].....................
STIR04A48b            .......[-]................[+].....................
STIR04A26b            C......[-]......G.T.......[+].....................
```

Figure S2. Continued

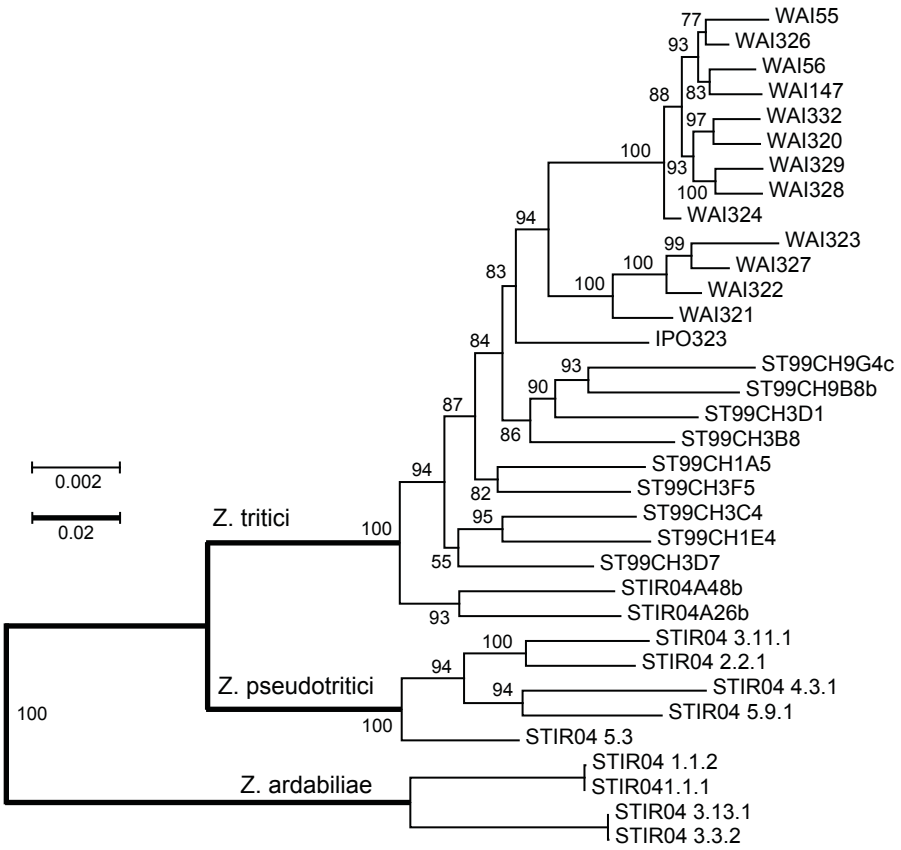

Figure S3. Phylogenetic relationship of the *Z. tritici, Z. pseudotritici* and *Z. ardabiliae* strains based on the concatenated nucleotide sequences of 1829 commonly-present single-copy protein genes.

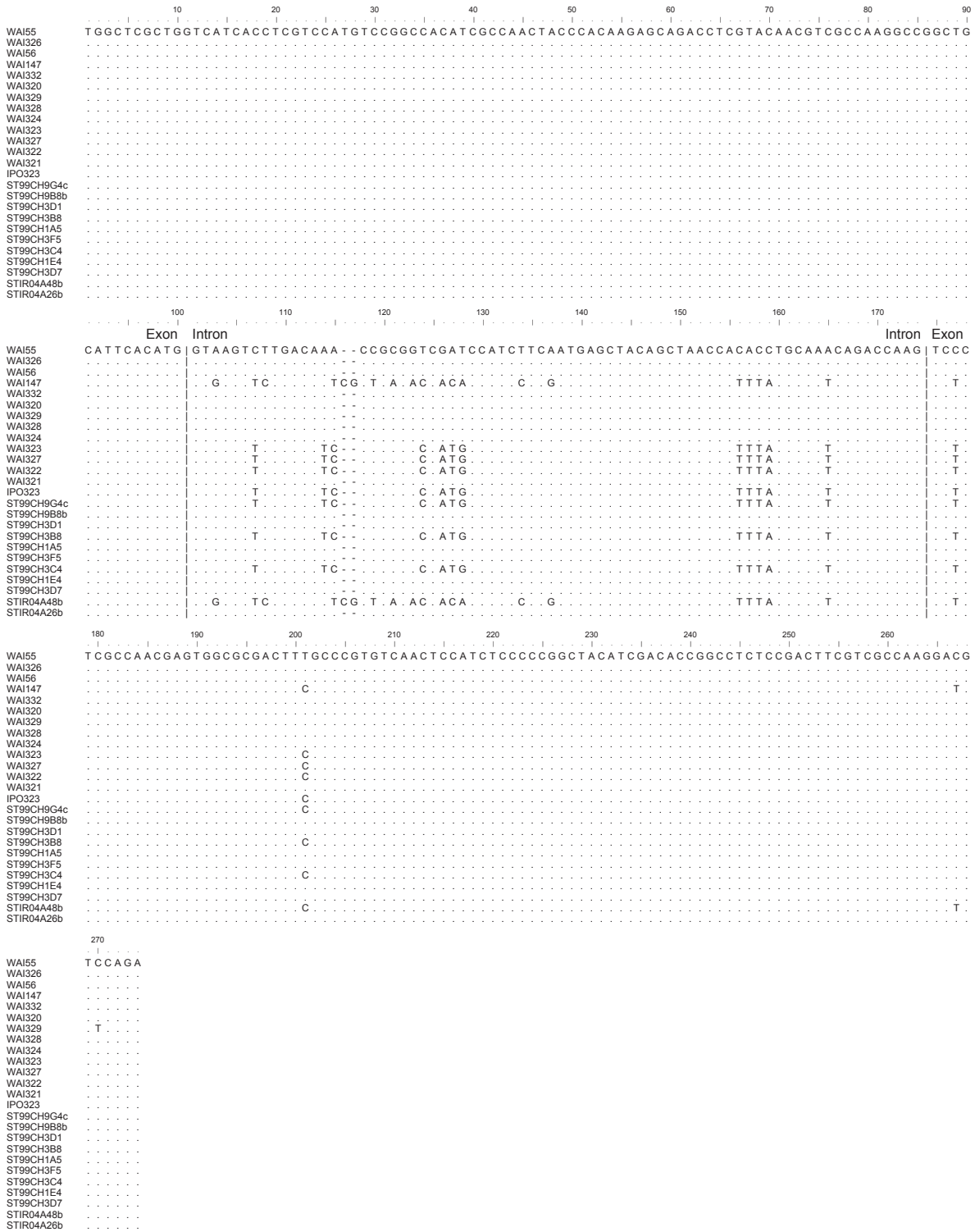

Figure S4. An example (the ID64923i1 intron) illustrating intron exchange at a locus of fixed introns. Strain names are ordered following the phylogenetic tree in Figure S3.

```
77869i3
                10 X 1100   2
                 1 X 7152   2
WAI55                TTTC[+]C
WAI326               CCGG[-]G
WAI56                CCGG[-]G
WAI147               CCGG[-]G
WAI332               CCGG[-]G
WAI320               CCGG[-]G
WAI329               ....[+].
WAI328               ....[+].
WAI324               CCGG[-]G
WAI323               CCGG[-]G
WAI327               CCGG[-]G
WAI322               CCGG[-]G
WAI321               CCGG[-]G
IPO323               ....[+].
ST99CH9G4c           ....[+].
ST99CH9B8b           ....[+].
ST99CH3D1            ....[+].
ST99CH3B8            ....[+].
ST99CH1A5            CCGG[-]G
ST99CH3F5            ....[+].
ST99CH3C4            ....[+].
ST99CH1E4            ....[+].
ST99CH3D7            ....[+].
STIR04A48b           ....[+].
STIR04A26b           CCGG[-]G
STIR043.11.1         ....[+].
STIR042.2.1          ....[+].
STIR044.3.1          .CGG[-].
STIR045.9.1          CC..[+].
STIR045.3            .CGG[-].
STIR041.1.2          ....[+].
STIR041.1.1          ....[+].
STIR043.13.1         ....[+].
STIR043.3.2          ....[+].
```

Figure S5. Examples of introgression at IPAP loci. Strain names are ordered following the phylogenetic tree in Figure S3.

```
102064i6

                 10 X 4321        11123
                  1 X 42074   345658910
WAI55                 GCGGC[+]ATCTAGCTT
WAI326                .....[+].........
WAI56                 .....[+].........
WAI147                .....[+].........
WAI332                .....[+].........
WAI320                .....[+].........
WAI329                .....[+].........
WAI328                .....[+].........
WAI324                .....[+].........
WAI323                .TATG[-]GAGCGATAG
WAI327                .TATG[-]GAGCGATAG
WAI322                .....[+].........
WAI321                .....[+].........
IPO323                .....[+].........
ST99CH9G4c            .....[+].........
ST99CH9B8b            .....[+].........
ST99CH3D1             .TATG[-]GAGCGATAG
ST99CH3B8             .....[+].........
ST99CH1A5             ATATG[-]GAGCGATAG
ST99CH3F5             .TATG[-]GAGCGATAG
ST99CH3C4             .....[+].........
ST99CH1E4             .TATG[-]GAGCGATAG
ST99CH3D7             ATATG[-]GAGCGATAG
STIR04A48b            .....[+].........
STIR04A26b            .TATG[-]GAGCGATAG
STIR043.11.1          .....[+]........C
STIR042.2.1           .....[+]........C
STIR044.3.1           .....[+]........C
STIR045.9.1           .....[+]........C
STIR045.3             .....[+]........C
STIR041.1.2           ATATT[-]GAG..ATAG
STIR041.1.1           ATATT[-]GAG..ATAG
STIR043.13.1          ATATT[-]GAG..ATAG
STIR043.3.2           ATATT[-]GAG..ATAG
```

Figure S5. Continued.

```
111698i2
                  10 X 2   3344
                   1 X 4   4703
WAI55                 T[-]ACTT
WAI326                .[-]....
WAI56                 .[-]....
WAI147                .[-]....
WAI332                .[-]....
WAI320                .[-]....
WAI329                .[-]....
WAI328                .[-]....
WAI324                .[-]....
WAI323                .[+]TTCG
WAI327                .[+]TTCG
WAI322                .[-]....
IPO323                .[-]....
ST99CH9G4c            .[-]....
ST99CH9B8b            G[-]CTA.
ST99CH3D1             .[-]....
ST99CH3B8             .[-]....
ST99CH1A5             C[+]TTCG
ST99CH3F5             .[-]....
ST99CH3C4             C[+]TTCG
ST99CH1E4             .[-]....
ST99CH3D7             .[-]....
STIR04A48b            .[-]....
STIR04A26b            .[-]....
STIR043.11.1          .[-]..CG
STIR042.2.1           C[+]TTCG
STIR044.3.1           C[+]T.CG
STIR045.9.1           C[+]TTCG
STIR045.3             .[-]..CG
STIR041.1.2           G[-]GTA.
STIR041.1.1           G[-]GTA.
STIR043.13.1          G[-]GTA.
STIR043.3.2           G[-]GTA.
```

Figure S5. Continued.

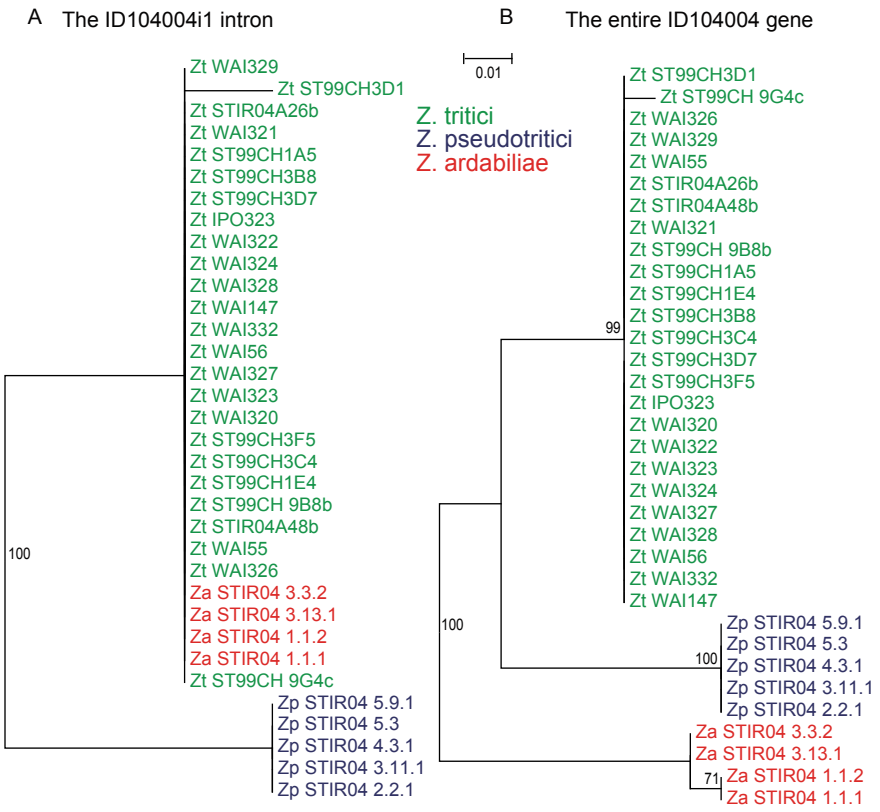

Figure S6. Intron introgression between *Z. tritici* and *Z. ardabiliae* at a locus of fixed introns. The phylogenetic tree on the left is based on sequences of the ID 104004i1 intron (i.e., the first intron in the ID104004 gene), while the phylogenetic tree on the right is based on the entire coding sequences of the ID104004 gene. Bootstrap values when > 50% are shown.