**Supplementary Materials**

Supplementary *Figure S1*

Examples of chromosome number observation for F1 hybrids between *T. turgidum* and *T. monococcum*. (a) a cell of AS2231-2/CItr17652 F1; (b) a cell of AS2305/PI355517 F1; (c) a cell of AS2637/ CItr13961 F1; (d) a cell of AS2380/ CItr13963 F1.

Supplementary*Figure S2*

Examples of chromosome number observation for synthetic amphiploids. (a) Syn-TAM-3 (2n=42); (b) Syn-TAM-24 (2n=42); (c) Syn-TAM-33 (2n=42); (d) Syn-TAM-37 (2n=42).

Supplementary *Figure S3*

Plant morphology of synthetic amphiploid Syn-TAM-3(center) and its parents *T. turgidum* ssp. *durum* AS2637 (left) and *T. monococcum* ssp. *monococcum* CItr13961 (right).

Supplementary*Figure S4*

Chromosome pairing in pollen mother cells at meiotic metaphase I in amphiploids. (a) Syn-TAM-3 with 21 bivalents; (b) Syn-TAM-24 with 19 bivalents, 1 trivalent and 1 univalent; (c) Syn-TAM-33 with 17 bivalents, 1 trivalent, 1 univalent and 1 pentavalent; (d) Syn-TAM-37 with 19 bivalents, 1 trivalent and 1 univalent. The univalent, trivalent and pentavalent chromosome pairing is indicated by a short arrow, long arrow, and arrowhead, respectively.

Supplementary *Table S1*

The 156 cross combinations with crossability percentages over 5%.

Supplementary *Table S2*

The means of crossability percentages for 60 *T. turgidum* lines from five subspecies with *T. monococcum* ssp. *monococcum.*

Supplementary *Table S3*

The means of crossability percentages for 83 *T. monococcum* ssp. *monococum* accessions with *T. turgidum* lines.

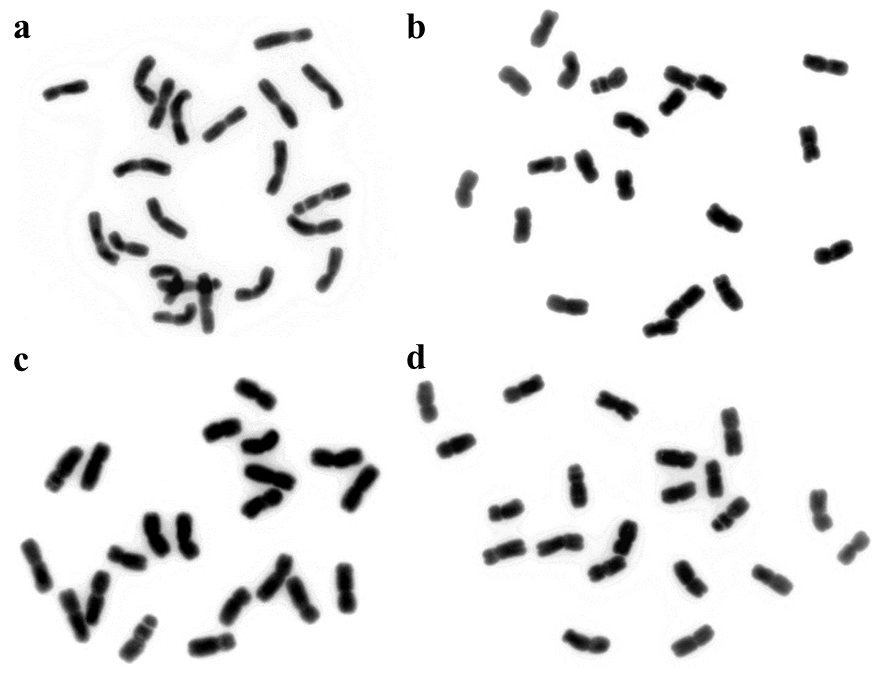
Supplementary *Table S4*

Selfed seeds of treated F1 plants of *T. turgidum* with *T. monococcum.*

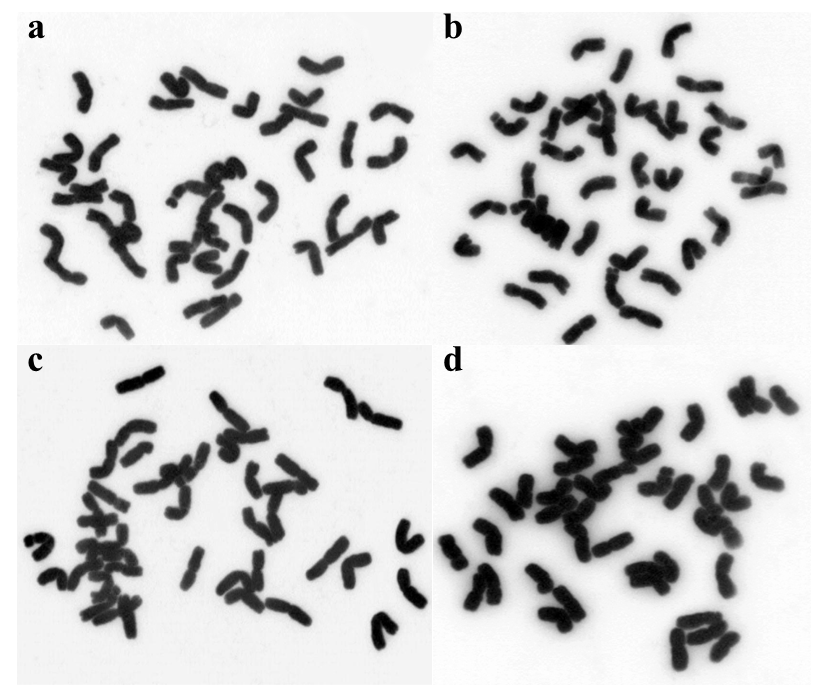
Supplementary *Table S5*

Stripe rust resistance and *Glu-A1mx* alleles of amphiploids and their parents.

***Figure S1*** Examples of chromosome number observation for F1 hybrids between *T. turgidum* and *T. monococcum*. (a) a cell of AS2231-2/CItr17652 F1; (b) a cell of AS2305/PI355517 F1; (c) a cell of AS2637/ CItr13961 F1; (d) a cell of AS2380/ CItr13963 F1.



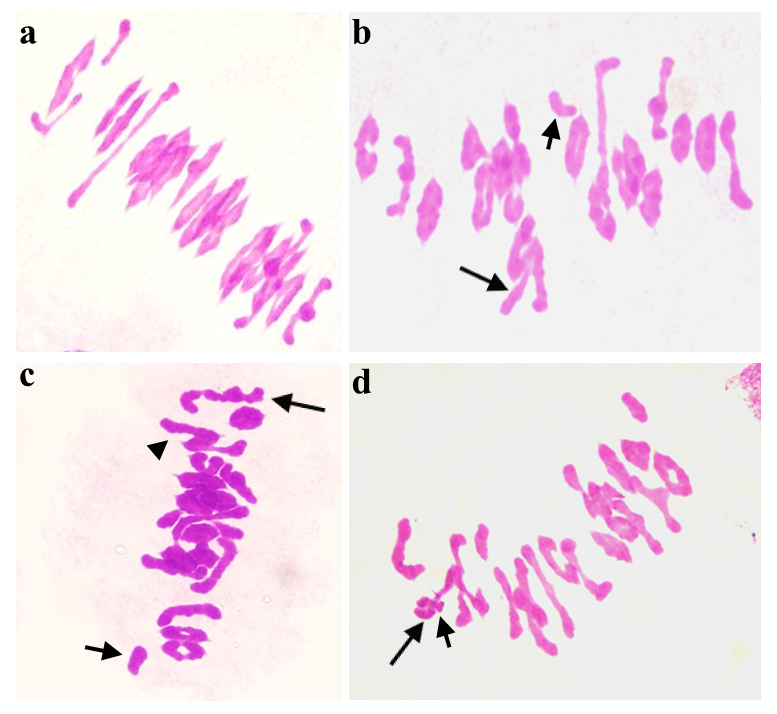
***Figure S2*** Examples of chromosome number observation for synthetic amphiploids. (a) Syn-TAM-3 (2n=42); (b) Syn-TAM-24 (2n=42); (c) Syn-TAM-33 (2n=42); (d) Syn-TAM-37 (2n=42).

******

***Figure S3*** Plant morphology of synthetic amphiploid Syn-TAM-3(center) and its parents *T. turgidum* ssp. *durum* AS2637 (left) and *T. monococcum* ssp. *monococcum* CItr13961 (right).



***Figure S4***Chromosome pairing in pollen mother cells at meiotic metaphase I in amphiploids. (a) Syn-TAM-3 with 21 bivalents; (b) Syn-TAM-24 with 19 bivalents, 1 trivalent and 1 univalent; (c) Syn-TAM-33 with 17 bivalents, 1 trivalent, 1 univalent and 1 pentavalent; (d) Syn-TAM-37 with 19 bivalents, 1 trivalent and 1 univalent. The univalent, trivalent and pentavalent chromosome pairing is indicated by a short arrow, long arrow, and arrowhead, respectively.

****

***Table S1*** The 156 cross combinations with crossability percentages over 5%

|  |
| --- |
| ssp. *turgidum* × *T. monococcum* (81 combinations) |
| AS2231-2 × CItr17652 (66.67%); AS2231-2 × PI518452 (33.33%); AS2236-2 × PI330551 (19.23%); AS2239 × CItr13962 (19.15%); AS2239 × CItr17652 (26.32%); AS2239 × PI221416 (32.35%); AS2239 × PI330551 (40.32%); AS2239 × PI355529 (17.65%); AS2239 × PI518452 (5.56%); AS2240 × PI272535 (14.29%); AS2240 × PI290509 (8.33%); AS2255 × CItr13961 (52.38%); AS2285 × CItr17652 (57.14%); AS2285 × PI290509 (20%); AS2291 × CItr13962 (15.85%); AS2291 × CItr17653 (47.14%); AS2291 × PI264935 (10%); AS2291 × PI265008 (22.22%); AS2295 × CItr13961 (30%); AS2295 × CItr13963 (43.96%); AS2295 × PI272535 (22.5%); AS2295 × PI352484 (54.55%); AS2295 × PI352486 (64.29%); AS2295 × PI355517 (45%); AS2295 × PI355543 (27.27%); AS2295 × PI377670 (27.27%); AS2296 × PI10474 (50%); AS2296 × PI266844 (43%); AS2296 × PI355519 (25%); AS2296 × PI427927 (6.25%); AS2298 × PI191096 (42.86%); AS2299 × CItr13963 (11.9%); AS2299 × PI355538 (7.14%); AS2305 × CItr13962 (16.67%); AS2305 × CItr13963 (17.5%); AS2305 × PI355517 (60.71%); AS2305 × PI355519 (14.71%); AS2305 × PI362610 (22.92%); AS2305 × PI428167 (8.33%); AS2305 × PI428173 (12.5%); AS2305 × PI428175 (36.54%); AS2308 × CItr13963 (10%); AS2308 × PI355517 (59.09%); AS2308 × PI560726 (8.33%); AS2308 × PI560727 (27.27%); AS2310 × CItr13961 (47.69%); AS2310 × PI167634 (26.67%); AS2310 × PI355517 (37.5%); AS2310 × PI355519 (39.29%); AS2310 × PI362610 (22.73%); AS2310 × PI428173 (16.67%); AS2310 × PI542473 (35%); AS2312 × PI168805 (13.16%); AS2312 × PI355519 (43.33%); AS2312 × PI362610 (63.64%); AS2313 × PI168805 (83.33%); AS2313 × PI272535 (12.5%); AS2313 × PI355519 (6.58%); AS2326 × CItr13963 (6.82%); AS2326 × PI191096 (56.25%); AS2326 × PI272535 (33.33%); AS2326 × PI355519 (31.03%); AS2326 × PI427959 (31.25%); AS2334 × PI191096 (89.29%); AS2334 × PI355519 (46.71%); AS2334 × PI355521 (29.17%); AS2378 × PI167634 (50%); AS2380 × CItr13961 (33.33%); AS2380 × CItr13963 (63.33%); AS2380 × CItr17653 (60.42%); AS2380 × PI168805 (31.25%); AS2380 × PI237659 (25%); AS2380 × PI295058 (43.75%); AS2380 × PI428161 (55.56%); AS2381 × CItr13961 (18.18%); AS2381 × PI167634 (40%); AS2381 × PI168805 (45.45%); AS2381 × PI190945 (8.33%); AS2381 × PI191381 (33.33%); AS2382 × PI355519 (13.89%); AS2382 × PI427959 (13.89%) |
| ssp. *dicoccon* × *T. monococcum* (59 combinations) |
| PI113963 × PI192063 (15.38%); PI113963 × PI307984 (10.71%); PI154582 × PI307984 (6.67%); PI191781 × CItr17657 (27.27%); PI191781 × PI190940 (20.21%); PI191781 × PI191098 (25.37%); PI191781 × PI266844 (37.5%); PI191781 × PI272535 ( 9.29%); PI221401 × CItr13961 (13.33%); PI221401 × PI191098 (25%); PI221401 × PI266844 (10%); PI221401 × PI272535 (32.95%); PI221403 × CItr13962 (7.76%); PI221403 × CItr17652 (21.43%); PI221403 × CItr17657 (11.76%); PI221403 × PI190946 (42.11%); PI221403 × PI289599 (18%); PI221403 × PI355543 (21.88%); PI221403 × PI377668 (11.9%); PI221403 × PI377671 (5.26%); PI221403 × PI428154 (6.67%); PI221403 × PI503874 (7.5%); PI221403 × PI584654 (26%); PI306533 × CItr17662 (16.67%); PI306533 × PI10474 (11.36%); PI352331 × PI10474 (13.04%); PI352331 × PI428175 (12.5%); PI352331 × PI584654 (25%); PI352335 × PI272535 (20.59%); PI352358 × PI272535 (25%); PI352359 × CItr17652 (23.68%); PI352359 × CItr17659 (5.88%); PI352359 × CItr17662 (30%); PI352359 × PI167589 (21.88%); PI352359 × PI190942 (10.53%); PI352359 × PI377671 (9.21%); PI352359 × PI560726 (15.38%); PI352369 × PI221416 (20.15%); PI352369 × PI355519 (20%); PI352369 × PI428161 (27.5%); PI355465 × CItr13962 (6.06%); PI355465 × PI266844 (5.56%); PI355465 × PI272535 (23.53%); PI355477 × CItr17658 (11.11%); PI355490 × PI221416 (23.91%); PI355490 × PI355519 (25.51%); PI355497 × PI289599 (15.12%); PI355507 × CItr13963 (19.12%); PI355527 × PI191098 (10.71%); PI355527 × PI584654 (20%); PI377655 × CItr17653 (78.79%); PI377655 × PI377671 (80%); PI377655 × PI560727 (27.27%); PI434998 × PI428158 (20%); PI94666 × PI355543 (6.67%); PI94670 × CItr17662 ( 25%); PI94670 × PI191098 (15.38%); PI94670 × PI503874 (13.64%); PI94675 × PI272535 (10.53%) |
| ssp. *turanicum* × *T. monococcum* (9 combinations) |
| PI184526 × CItr13962 (11.11%); PI211691 × PI190940 (7.69%); PI211691 × PI355519 (18.09%); PI306665 × CItr13961 (13.33%); PI306665 × PI355519 (46.3%); PI306665 × PI427959 (7.69%); PI306665 × PI428158 (25%); PI352514 × PI221416 (14%); PI352514 × PI518452 (22.73%) |
| ssp. *durum* × *T. monococcum* (5 combinations) |
| AS2262 × CItr13961 (44.66%); AS904 × PI190942 (44.44%); AS904 × PI191096 (18.33%); AS904 × PI355519 (45%); AS2637 × CItr13961 (26.92%) |
| ssp. *persicum* × *T. monococcum* (2 combinations) |
| AS2268 × CItr13962 (12.5%); AS2268 × CItr13963 (46.74%) |

***Table S2*** Crossability of 60 *T. turgidum* lines from five subspecies with *T. monococcum* ssp. *monococcum*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *T. turgidum* ssp. | No. of cross combinations | No. of florets pollinated | No. of kernels | Crossability percentage |
| *persicum* | 2 | 140 | 49 | 35.00% |
| *durum* | 8 | 514 | 157 | 30.54% |
| *turgidum* | 130 | 4844 | 1129 | 23.31% |
| *turanicum* | 13 | 504 | 76 | 15.08% |
| *dicoccon* | 111 | 4808 | 572 | 11.90% |
| Total | 264 | 10810 | 1983 | 18.34% |

***Table S3*** The crossability of 83 *T. monococcum* ssp. *monococum* accessions with *T. turgidum* lines

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *T. monococcum* Genotypes | No. of cross combinations | No. of florets pollinated | No. of kernels | Crossability percentage(%) |
| PI352486 | 1 | 14 | 9 | 64.29% |
| PI352484 | 1 | 22 | 12 | 54.55% |
| PI355517 | 4 | 114 | 57 | 50% |
| CItr17653 | 5 | 250 | 114 | 45.6% |
| PI190942 | 1 | 18 | 8 | 44.44% |
| PI190946 | 1 | 38 | 16 | 42.11% |
| PI191096 | 5 | 176 | 67 | 38.07% |
| PI428161 | 2 | 58 | 21 | 36.21% |
| PI542473 | 1 | 20 | 7 | 35% |
| CItr13961 | 10 | 758 | 262 | 34.56% |
| PI330551 | 2 | 88 | 30 | 34.09% |
| PI168805 | 5 | 122 | 41 | 33.61% |
| PI362610 | 3 | 92 | 30 | 32.61% |
| CItr13963 | 9 | 686 | 222 | 32.36% |
| PI355521 | 1 | 24 | 7 | 29.167% |
| PI355519 | 14 | 892 | 245 | 27.47% |
| PI428175 | 2 | 84 | 23 | 27.38% |
| PI560727 | 2 | 88 | 24 | 27.27% |
| PI377670 | 1 | 22 | 6 | 27.27% |
| PI167634 | 4 | 122 | 32 | 26.23% |
| PI 272535 | 1 | 20 | 5 | 25% |
| PI377671 | 3 | 144 | 33 | 22.92% |
| PI265008 | 1 | 36 | 8 | 22.22% |
| CItr17652 | 7 | 294 | 65 | 22.11% |
| PI167589 | 1 | 32 | 7 | 21.88% |
| PI191381 | 2 | 56 | 12 | 21.43% |
| PI221416 | 5 | 330 | 67 | 20.3% |
| PI355529 | 1 | 68 | 12 | 17.65% |
| PI295058 | 2 | 40 | 7 | 17.5% |
| PI190940 | 2 | 120 | 21 | 17.5% |
| PI191098 | 6 | 304 | 47 | 15.46% |
| PI290509 | 2 | 54 | 8 | 14.81% |
| PI272535 | 14 | 596 | 88 | 14.77% |
| CItr17657 | 3 | 110 | 16 | 14.55% |
| CItr17662 | 5 | 152 | 22 | 14.47% |
| PI266844 | 11 | 436 | 63 | 14.45% |
| PI428158 | 3 | 70 | 10 | 14.29% |
| PI355543 | 4 | 112 | 15 | 13.39% |
| PI584654 | 5 | 198 | 26 | 13.13% |
| PI560726 | 2 | 50 | 6 | 12% |
| PI377668 | 1 | 42 | 5 | 11.9% |
| PI10474 | 6 | 204 | 24 | 11.76% |
| PI190942 | 1 | 38 | 4 | 10.53% |
| PI264935 | 1 | 20 | 2 | 10% |
| PI237659 | 2 | 50 | 5 | 10% |
| CItr13962 | 11 | 712 | 68 | 9.55% |
| PI518452 | 6 | 254 | 18 | 7.09% |
| PI289599 | 7 | 318 | 22 | 6.92% |
| PI428154 | 1 | 30 | 2 | 6.67% |
| PI427927 | 1 | 32 | 2 | 6.25% |
| PI192063 | 3 | 64 | 4 | 6.25% |
| PI190945 | 2 | 98 | 6 | 6.12% |
| PI428173 | 4 | 120 | 7 | 5.83% |
| PI307984 | 3 | 92 | 5 | 5.43% |
| PI503874 | 4 | 116 | 6 | 5.17% |
| PI428167 | 2 | 40 | 2 | 5% |
| PI427959 | 1 | 20 | 1 | 5% |
| PI355538 | 2 | 46 | 2 | 4.35% |
| CItr17658 | 2 | 46 | 2 | 4.35% |
| PI289605 | 1 | 24 | 1 | 4.17% |
| PI427959 | 16 | 486 | 18 | 3.7% |
| PI190915 | 1 | 28 | 1 | 3.57% |
| PI272558 | 1 | 32 | 1 | 3.13% |
| PI277136 | 2 | 48 | 1 | 2.08% |
| CItr17659 | 3 | 138 | 2 | 1.45% |
| PI435000 | 4 | 118 | 1 | 0.85% |
| PI538721 | 3 | 82 | 0 | 0 |
| PI435001 | 2 | 72 | 0 | 0 |
| PI428172 | 1 | 28 | 0 | 0 |
| PI428166 | 1 | 38 | 0 | 0 |
| PI428165 | 1 | 50 | 0 | 0 |
| PI428152 | 1 | 22 | 0 | 0 |
| PI428150 | 1 | 54 | 0 | 0 |
| PI428149 | 2 | 42 | 0 | 0 |
| PI418587 | 1 | 24 | 0 | 0 |
| PI362616 | 1 | 32 | 0 | 0 |
| PI345186 | 1 | 48 | 0 | 0 |
| PI323437 | 1 | 22 | 0 | 0 |
| PI306542 | 1 | 28 | 0 | 0 |
| PI286068 | 1 | 38 | 0 | 0 |
| PI167591 | 2 | 50 | 0 | 0 |
| PI119435 | 1 | 32 | 0 | 0 |
| CItr17654 | 1 | 22 | 0 | 0 |
| Total | 264 | 10810 | 1983 | 18.34 |

***Table S4*** Production of selfed seed from colchicine treated *T. turgidum* × *T. monococcum* hybrid F1 plants

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Combinations | Germination rate of F1 seeds (%)a | No. F1 Plants colchicine treated | No. S1 seeds | Germination rate of S1 seeds (%)b |
| AS2310 × CItr13961 | 100(18) | 2 | 153 | 100(10) |
| AS2305 × PI355517 | 100(13) | 2 | 128 | 100(10) |
| PI221401 × PI191098 | 100(6) | 2 | 104 | 50(10) |
| AS2295 × PI355517 | 77.8(9) | 2 | 95 | 100(10) |
| AS2380 × CItr13963 | 100(22) | 2 | 91 | 100(10) |
| AS904 × PI190942 | 100(8) | 2 | 91 | 80(15) |
| AS2295 × PI352486 | 90(9) | 2 | 70 | 90(10) |
| PI355465 × CItr13962 | 100(4) | 2 | 68 | 100(10) |
| AS2637 × CItr13961 | 100(10) | 2 | 66 | 100(10) |
| PI154582 × PI307984 | 100(2) | 1 | 65 | 50(10) |
| AS2305 × CItr13962 | 100(7) | 2 | 58 | 100(10) |
| AS2305 × PI289605 | 100(1) | 1 | 56 | 100(10) |
| PI221401 × PI272535 | 100(10) | 4 | 50 | 90(10) |
| PI377655 × CItr17653 | 80(10) | 2 | 50 | 100(10) |
| AS2231-2 × CItr17652 | 100(10) | 2 | 48 | 100(10) |
| PI221403 × PI266844 | 100(1) | 1 | 48 | 100(10) |
| AS2268 × CItr13962c | 100(6) | 2 | 45 | 100(10)c |
| AS2305 × PI428175 | 100(9) | 2 | 44 | 100(10) |
| AS2313 × PI168805 | 90(20) | 2 | 44 | 100(10) |
| AS2285 × CItr17652 | 100(10) | 2 | 42 | 100(10) |
| AS2381 × PI190945 | 50(6) | 4 | 40 | 50(10) |
| AS2305 × PI428167 | 100(2) | 1 | 36 | 70(10) |
| AS2310 × PI428173 | 100(4) | 2 | 36 | 100(10) |
| PI184526 × CItr13962 | 83(6) | 2 | 34 | 100(10) |
| PI221403 × PI190946d | 100(10) | 4 | 33 | 0(10)d |
| PI352358 × PI272535 | 80(5) | 4 | 33 | 80(10) |
| PI94670 × PI518452 | 100(1) | 1 | 32 | 70(10) |
| AS2299 × CItr13963 | 60(5) | 2 | 31 | 100(10) |
| AS2312 × PI362610 | 100(14) | 2 | 31 | 100(10) |
| AS2382 × PI355519 | 100(8) | 2 | 29 | 100(10) |
| PI221401 × CItr13961 | 50(4) | 2 | 29 | 30(10) |
| AS2231-2 × PI518452 | 100(10) | 2 | 28 | 90(10) |
| PI221403 × CItr13962 | 100(4) | 2 | 27 | 90(10) |
| AS2310 × PI362610 | 80(5) | 4 | 24 | 100(10) |
| PI306533 × CItr17662 | 87.5(8) | 2 | 24 | 90(10) |
| AS2381 × PI167634c | 87.5(8) | 2 | 23 | 90(10)c |
| AS2295 × PI377670 | 100(6) | 2 | 22 | 100(10) |
| AS2380 × CItr17653 | 100(11) | 2 | 22 | 14.29(7) |
| AS2240 × PI290509d | 100(4) | 2 | 21 | 0(10)d |
| AS2334 × PI355521 | 100(7) | 5 | 20 | 40(10) |
| PI113963 × PI307984d | 100(3) | 2 | 17 | 0(10)d |
| PI352335 × PI272535 | 28.6(7) | 2 | 16 | 50(10) |
| PI377655 × PI560727 | 60(10) | 2 | 16 | 60(10) |
| AS2295 × PI272535 | 100(9) | 2 | 15 | 60(10) |
| AS2382 × PI427959 | 100(3) | 5 | 15 | 80(10) |
| PI355527 × CItr13962c | 33(3) | 1 | 15 | 50(10)c |
| AS2291 × CItr13962 | 100(9) | 5 | 14 | 100(10) |
| AS2268 × CItr13963 | 100(10) | 4 | 13 | 70(10) |
| AS2299 × PI355538 | 100(2) | 2 | 12 | 80(10) |
| PI352359 × PI560726 | 100(4) | 2 | 12 | 88.89(9) |
| PI355477 × PI266844 | 100(1) | 1 | 12 | 100(10) |
| AS2308 × PI355517d | 100(13) | 2 | 10 | 0(10)d |
| AS2380 × PI168805 | 100(5) | 2 | 10 | 100(10) |
| PI113963 × PI192063d | 100(4) | 2 | 10 | 0(10)d |
| PI352359 × CItr17652 | 100(5) | 4 | 10 | 60(10) |
| AS2296 × PI10474 | 100(5) | 2 | 9 | 44.44(9) |
| PI94670 × PI503874 | 100(3) | 1 | 9 | 66.67(9) |
| AS2291 × PI265008 | 100(7) | 3 | 7 | 100(7) |
| AS2308 × CItr13963 | 100(5) | 2 | 7 | 85.71(7) |
| PI191781 × PI191098 | 86(14) | 5 | 6 | 40(6) |
| PI221403 × PI584654d | 100(13) | 2 | 6 | 0(6)d |
| PI94670 × PI191098 | 75(4) | 1 | 6 | 66.67(6) |
| AS2291 × PI427959 | 100(1) | 5 | 5 | 60(5) |
| AS2239 × PI355529c | 100(12) | 5 | 3 | 33.33(3)c |
| AS2310 × PI355517 | 88.9(9) | 1 | 3 | 100(3) |
| AS2255 × CItr13961d | 100(10) | 5 | 2 | 0(2)d |
| PI221403 × PI428154c | 100(2) | 2 | 2 | 50(2)c |
| AS2291 × CItr17653 | 100(10) | 5 | 1 | 100(1) |
| AS2305 × CItr13963c | 100(8) | 3 | 1 | 100(1)c |
| AS2310 × PI167634c | 100(10) | 4 | 1 | 100(1)c |
| AS2236-2 × PI330551 | 40(5) | 1 | 0 | - |
| AS2239 × CItr13962 | 100(13) | 5 | 0 | - |
| AS2239 × CItr17652 | 70(10) | 2 | 0 | - |
| AS2239 × PI221416 | 100(5) | 2 | 0 | - |
| AS2239 × PI330551 | 100(12) | 5 | 0 | - |
| AS2239 × PI435000 | 100(1) | 1 | 0 | - |
| AS2239 × PI518452 | 100(2) | 2 | 0 | - |
| AS2240 × PI272535 | 100(2) | 2 | 0 | - |
| AS2255 × PI277136 | 100(1) | 1 | 0 | - |
| AS2262 × CItr13961 | 77.8(9) | 2 | 0 | - |
| AS2285 × PI290509 | 100(6) | 2 | 0 | - |
| AS2291 × PI264935 | 100(2) | 2 | 0 | - |
| AS2295 × CItr13961 | 100(9) | 2 | 0 | - |
| AS2295 × CItr13963 | 100(10) | 2 | 0 | - |
| AS2295 × PI352484 | 100(12) | 2 | 0 | - |
| AS2295 × PI355543 | 100(6) | 2 | 0 | - |
| AS2296 × PI191096 | 100(1) | 1 | 0 | - |
| AS2296 × PI266844 | 80(5) | 2 | 0 | - |
| AS2296 × PI355519 | 100(5) | 2 | 0 | - |
| AS2298 × PI191096 | 100(5) | 3 | 0 | - |
| AS2298 × PI355519 | 100(2) | 1 | 0 | - |
| AS2305 × PI355519 | 100(5) | 4 | 0 | - |
| AS2305 × PI362610 | 100(11) | 2 | 0 | - |
| AS2305 × PI428173 | 100(3) | 2 | 0 | - |
| AS2308 × PI560726 | 100(2) | 2 | 0 | - |
| AS2308 × PI560727 | 100(6) | 2 | 0 | - |
| AS2310 × PI355519 | 100(5) | 3 | 0 | - |
| AS2310 × PI542473 | 100(5) | 2 | 0 | - |
| AS2312 × PI168805 | 75(4) | 2 | 0 | - |
| AS2312 × PI355519 | 100(5) | 3 | 0 | - |
| AS2313 × PI272535 | 100(2) | 3 | 0 | - |
| AS2313 × PI355519 | 100(5) | 1 | 0 | - |
| AS2313 × PI427959 | 100(1) | 1 | 0 | - |
| AS2326 × CItr13963 | 100(3) | 2 | 0 | - |
| AS2326 × PI168805 | 100(1) | 1 | 0 | - |
| AS2326 × PI190915 | 100(1) | 1 | 0 | - |
| AS2326 × PI191096 | 100(5) | 3 | 0 | - |
| AS2326 × PI272535 | 100(12) | 2 | 0 | - |
| AS2326 × PI355519 | 40(5) | 3 | 0 | - |
| AS2326 × PI427959 | 100(5) | 3 | 0 | - |
| AS2334 × PI191096 | 100(5) | 3 | 0 | - |
| AS2334 × PI355519 | 94.12(17) | 5 | 0 | - |
| AS2378 × PI167634 | 70(8) | 2 | 0 | - |
| AS2380 × CItr13961 | 100(16) | 4 | 0 | - |
| AS2380 × PI237659 | 80(5) | 2 | 0 | - |
| AS2380 × PI428161 | 55.6(9) | 2 | 0 | - |
| AS2381 × CItr13961 | 100(8) | 2 | 0 | - |
| AS2381 × PI168805 | 100(10) | 2 | 0 | - |
| AS2381 × PI191381 | 91.67(12) | 3 | 0 | - |
| AS2382 × CItr13961 | 100(2) | 2 | 0 | - |
| AS2382 × PI266844 | 100(1) | 1 | 0 | - |
| AS904 × PI191096 | 50(4) | 2 | 0 | - |
| AS904 × PI355519 | 100(10) | 3 | 0 | - |
| PI113963 × CItr17652 | 50(4) | 2 | 0 | - |
| PI191781 × CItr17657 | 100(12) | 2 | 0 | - |
| PI191781 × PI190940 | 20(5) | 1 | 0 | - |
| PI191781 × PI266844 | 100(5) | 3 | 0 | - |
| PI191781 × PI272535 | 100(13) | 2 | 0 | - |
| PI211691 × PI190940 | 100(2) | 2 | 0 | - |
| PI211691 × PI355519 | 25(4) | 1 | 0 | - |
| PI221401 × PI266844 | 66.67(3) | 2 | 0 | - |
| PI221403 × CItr17652 | 100(5) | 2 | 0 | - |
| PI221403 × CItr17657 | 100(4) | 1 | 0 | - |
| PI221403 × PI272558 | 100(1) | 1 | 0 | - |
| PI221403 × PI289599 | 100(9) | 2 | 0 | - |
| PI221403 × PI355543 | 80(5) | 2 | 0 | - |
| PI221403 × PI377668 | 100(5) | 2 | 0 | - |
| PI221403 × PI377671 | 100(2) | 1 | 0 | - |
| PI221403 × PI503874 | 100(3) | 2 | 0 | - |
| PI306665 × CItr13961 | 50(4) | 2 | 0 | - |
| PI306665 × PI355519 | 80(5) | 2 | 0 | - |
| PI306665 × PI428158 | 60(5) | 2 | 0 | - |
| PI352331 × PI10474 | 66.7(6) | 2 | 0 | - |
| PI352331 × PI428175 | 100(4) | 2 | 0 | - |
| PI352331 × PI584654 | 100(5) | 3 | 0 | - |
| PI352359 × CItr17659 | 50(2) | 2 | 0 | - |
| PI352359 × CItr17662 | 80(5) | 2 | 0 | - |
| PI352359 × PI167589 | 100(5) | 2 | 0 | - |
| PI352359 × PI377671 | 20(5) | 2 | 0 | - |
| PI352369 × PI221416 | 80(5) | 2 | 0 | - |
| PI352369 × PI355519 | 100(5) | 2 | 0 | - |
| PI352369 × PI428161 | 100(5) | 2 | 0 | - |
| PI352514 × PI518452 | 40(5) | 2 | 0 | - |
| PI355465 × PI272535 | 40(5) | 2 | 0 | - |
| PI355477 × CItr17658 | 100(2) | 2 | 0 | - |
| PI355490 × PI355519 | 20(5) | 1 | 0 | - |
| PI355507 × CItr13963 | 88.9(9) | 4 | 0 | - |
| PI355527 × PI191098 | 100(3) | 2 | 0 | - |
| PI355527 × PI584654 | 100(5) | 2 | 0 | - |
| PI434998 × PI428158 | 100(4) | 2 | 0 | - |
| PI94666 × PI355543 | 100(2) | 1 | 0 | - |
| PI94670 × CItr17662 | 100(5) | 2 | 0 | - |
| PI94675 × PI272535 | 75(4) | 2 | 0 | - |

a The number of F1 hybrid seeds of *T. turgidum* with *T. monococcum* used for germination are indicated in brackets

b The number of S1 seeds of synthetic amphiploids used for germination are indicated in brackets

c Selfed S2 seeds were not obtained from the S1 plants

d S1 seeds failed to germinate.

***Table S5*** Stripe rust resistance and *Glu-A1mx* alleles of amphiploids and their parents

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Amphiploid code | Cross combinations | Seedling stage ITs | | |  | Adult stage ITs | | |  | *Glu-A1mx* alleles | |
| *T. turgidum* | *T. monococcum* | amphiploids |  | *T. turgidum* | *T. monococcum* | amphiploids |  | *T. monococcum*a | amphiploidsb |
| Syn-TAM-1 | AS2231-2 × CItr17652 | 4 | 7 | 3 |  | 3 | 2 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-2 | AS2231-2 × PI518452 | 4 | 2 | 2 |  | 3 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-3 | AS2637 × CItr13961 | 1 | 6 | 3 |  | 2 | 2 | 1 |  | *Glu-A1m-c* | *Glu-A1m-c* |
| Syn-TAM-4 | AS2285 × CItr17652 | 4 | 7 | 3 |  | 6 | 2 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-5 | AS2291 × CItr13962 | 5 | 6 | 3 |  | 6 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-6 | AS2291 × CItr17653 | 5 | 6 | 3 |  | 6 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-7 | AS2291 × PI265008 | 5 | 6 | 3 |  | 6 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-8 | PI94670 × PI503874 | 9 | 4 | 9 |  | 9 | 2 | 9 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-9 | PI94670 × PI518452 | 9 | 2 | 9 |  | 9 | 2 | 9 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-10 | PI154582 × PI307984 | 6 | 3 | 4 |  | 9 | 2 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-11 | PI191781 × PI191098 | 4 | 3 | 3 |  | 3 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-12 | PI221401 × CItr13961 | 6 | 6 | 6 |  | 3 | 2 | 3 |  | *Glu-A1m-c* | *Glu-A1m-c* |
| Syn-TAM-13 | PI221401 × PI191098 | 6 | 3 | 3 |  | 3 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-14 | PI221401 × PI272535 | 6 | 3 | 4 |  | 3 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-15 | PI221403 × CItr13962 | 6 | 6 | 6 |  | 6 | 2 | 6 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-16 | PI221403 × PI266844 | 6 | 4 | 4 |  | 6 | 1 | 4 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-17 | PI306533 × CItr17662 | 6 | 7 | 7 |  | 9 | 1 | 3 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-18 | PI352335 × PI272535 | 2 | 3 | 2 |  | 2 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-19 | PI352359 × PI560726 | 9 | 6 | 9 |  | 9 | 2 | 6 |  | *Glu-A1m-f* | N/A |
| Syn-TAM-20 | PI355465 × CItr13962 | 2 | 6 | 4 |  | 2 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-21 | PI355477 × PI266844 | 4 | 4 | 3 |  | 8 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-22 | PI377655 × CItr17653 | 6 | 6 | 6 |  | 6 | 2 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-23 | PI377655 × PI560727 | 6 | 1 | 9 |  | 6 | 2 | 7 |  | *Glu-A1m-f* | N/A |
| Syn-TAM-24 | PI184526 × CItr13962 | 4 | 6 | 4 |  | 2 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-25 | AS2295 × PI272535 | 2 | 3 | 2 |  | 2 | 1 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-26 | AS2295 × PI352486 | 2 | 2 | 2 |  | 2 | 1 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-27 | AS2295 × PI355517 | 2 | 2 | 1 |  | 2 | 2 | 1 |  | *Glu-A1m-b* | *Glu-A1m-b* |
| Syn-TAM-28 | AS2295 × PI377670 | 2 | 2 | 1 |  | 2 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-29 | AS2299 × CItr13963 | 4 | 3 | 2 |  | 4 | 2 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-30 | AS2299 × PI355538 | 4 | 3 | 2 |  | 4 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-31 | AS2305 × CItr13962 | 4 | 7 | 2 |  | 4 | 2 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-32 | AS2305 × PI289605 | 4 | 1 | 2 |  | 4 | 1 | 1 |  | *Glu-A1m-e* | N/A |
| Syn-TAM-33 | AS2305 × PI355517 | 4 | 2 | 3 |  | 4 | 2 | 1 |  | *Glu-A1m-b* | *Glu-A1m-b* |
| Syn-TAM-34 | AS2305 × PI428167 | 4 | 1 | 3 |  | 4 | 1 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-35 | AS2305 × PI428175 | 4 | 2 | 1 |  | 4 | 2 | 1 |  | *Glu-A1m-c* | N/A |
| Syn-TAM-36 | AS2308 × CItr13963 | 2 | 3 | 3 |  | 2 | 2 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-37 | AS2310 × CItr13961 | 4 | 6 | 2 |  | 4 | 2 | 2 |  | *Glu-A1m-c* | *Glu-A1m-c* |
| Syn-TAM-38 | AS2310 × PI355517 | 4 | 2 | 3 |  | 4 | 2 | 2 |  | *Glu-A1m-b* | *Glu-A1m-b* |
| Syn-TAM-39 | AS2310 × PI362610 | 4 | 2 | 2 |  | 4 | 1 | 2 |  | *Glu-A1m-e* | N/A |
| Syn-TAM-40 | AS2310 × PI428173 | 4 | 1 | 4 |  | 4 | 1 | 2 |  | *Glu-A1m-c* | N/A |
| Syn-TAM-41 | AS2312 × PI362610 | 4 | 2 | 2 |  | 4 | 1 | 1 |  | *Glu-A1m-e* | N/A |
| Syn-TAM-42 | AS2313 × PI168805 | 4 | 3 | 2 |  | 4 | 1 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-43 | AS2380 × CItr13963 | 4 | 3 | 2 |  | 4 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-44 | AS2380 × CItr17653 | 4 | 6 | 2 |  | 4 | 2 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-45 | AS2380 × PI168805 | 4 | 3 | 3 |  | 4 | 1 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-46 | AS2382 × PI355519 | 4 | 2 | 3 |  | 4 | 1 | 1 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-47 | PI94670 × PI191098 | 9 | 3 | 1 |  | 9 | 1 | 2 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-48 | PI352359 × CItr17652 | 9 | 7 | 8 |  | 9 | 2 | 6 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-49 | AS2296 × PI10474 | 2 | 1 | 2 |  | 2 | 1 | 2 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-50 | AS2382 × PI427959 | 4 | 1 | 3 |  | 4 | 1 | 2 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-51 | AS2381 × PI190945 | 4 | 3 | 6 |  | 4 | 2 | 2 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-52 | AS2268 × CItr13963 | 4 | 3 | 3 |  | 4 | 2 | 2 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-53 | AS904 × PI190942 | 9 | 1 | 7 |  | 6 | 2 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-54 | AS2291 × PI427959 | 5 | 1 | 2 |  | 6 | 1 | 2 |  | *Glu-A1m-d* | N/A |
| Syn-TAM-55 | PI352358 × PI272535 | 4 | 3 | 3 |  | 6 | 1 | 1 |  | *Glu-A1m-d* | *Glu-A1m-d* |
| Syn-TAM-56 | AS2334 × PI355521 | 4 | 6 | 3 |  | 4 | 2 | 1 |  | *Glu-A1m-h* | *Glu-A1m-h* |

aAccording to Li et al. (2016); bN/A: we failed to differentiate the *Glu-A1mx*from the *Glu-A1x* alleles in the amphiploid background.