**Supplementary materials**

**Supplementary Table 1.** Chromosome 3AL-specific PCR primers developed from the predicted genes to locate the breakage point of line D2532-1-2.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer name | Primer sequences | Predicted genes | Chromosome location | PCR  Product |
| 3AL-1 | F: ATTCTTCAGGGTGCCTAGATGC  R: GTGTTCACCGCGTTAGTCTC | TraesCS3A01G511400 | 730.93Mb | + |
| 3AL-2 | F: CTTGGGCTTCACATCAGCAG  R: GCAGCTCTAATGGAAATCCTG | TraesCS3A01G511600 | 731.00Mb | + |
| 3AL-3 | F: CTGGAAAACTGCACTATCAGCC  R: GCTCATTAGGCGACACAACG | TraesCS3A01G514300 | 733.04Mb | + |
| 3AL-4 | F: GTTGCACGCATGTCACACAC  R: CTCATGTGGCTGAAGAGGTCC | TraesCS3A01G518000 | 735.47Mb | + |
| 3AL-5 | F: ACCGACCCATTCCTGTTCAC  R: ACTCAGTCATGGTGAAACTCCG | TraesCS3A01G518100 | 735.48Mb | + |
| 3AL-6 | F: ACATCAACGCCCACTTCATC  R: TGAGGGAATGGAACTTGAAGC | TraesCS3A01G519700 | 737.18Mb | + |
| 3AL-7 | F: GACGAGTTCAAGTATGAGGCG  R: GCGTCCACTTAGTACATCTTGC | TraesCS3A01G519800 | 737.20Mb | + |
| 3AL-8 | F: AAGTCGCATAAAGATGACTGGC  R: AACATCCACACCTTCATTGCTG | TraesCS3A01G523400 | 738.90Mb | + |
| 3AL-9 | F: GCTATAACAGCCTCCTGACTGC  R: GGGGAGAGTCATGGGTATTG | TraesCS3A01G523500 | 738.92Mb | + |
| 3AL-10 | F: CCATCAAGAACAGCCCCAAC  R: ACCAACTCAGCTACTCCACCTC | TraesCS3A01G527300 | 741.75Mb | + |
| 3AL-11 | F: GCTCTGCTTCTATCAGCTCCAG  R: GAAGGACGACATGAAGAGC | TraesCS3A01G529700 | 743.10Mb | + |
| 3AL-12 | F: TACATATGCGGGCTTCCTGTC  R: GCCTGAGTCATGGCATATGGT | TraesCS3A01G531900 | 744.66Mb | + |
| 3AL-13 | F: TGCCTGACCTCGTAGTCTGC  R: AACAAGATGCACCCTGTCGTC | TraesCS3A01G532500 | 745.51Mb | + |
| 3AL-14 | F: CCCTCTTGTACCCTGCAGTTG  R: GAGGACCGTAGCGACTATGACC | TraesCS3A01G535400 | 746.94Mb | - |
| 3AL-15 | F: CATGTTTCGACAGACGGACG  R: ACCTGGGCTATCTGCAACAAG | TraesCS3A01G537100 | 749.18Mb | - |

**Supplementary Figure Captions**

**Supplementary Fig. 1.** PCR profiling of markers TNAC1099 (**a**), TNAC1207 (**b**), TNAC1240 (**c**) and CINAU1063 (**d**).

The arrows indicate the specific amplification of chromosome 2Vb.

**Supplementary Fig. 2.** Physical location of *D. breviaristatum* 2Vb chromosome specific markers.

The left diagram shows the physical location of PLUG markers on wheat chromosome 2D based on the comparison of IWGS genome reference 1.0, with the red lines indicating the predicted centromeric regions by centromeric specific repeats. The middle karyotype diagram shows the FISH hybridization signals of Oligo-pSc119.2 (green), Oligo-pTa535 (red) and Oligo-(CAA)7 (blue). The “+” represent amplification, while “-” represents no amplification of 2Vb specific bands. The chromosomes at the bottom of the table showed the 2Vb deleted chromosomes by FISH with probes Oligo-pSc119.2 (green) and Oligo-pTa535 (red).

**Supplementary Fig. 3.** Physical map of chromosome 2Vb-specific markers in four regions based on PCR amplification using 2Vb deletion lines.

**Supplementary Fig. 4.** The comparison of physical map of PLUG markers and CINAU markers on wheat chromosome 2A, 2B, 2D and 2Vb based on the comparison of IWGS reference genome v1.0, with the red lines indicating the predicted centromeric regions by centromeric specific repeats.

**Supplementary Fig. 5.** FISH of line D2532-1-2 (a) and the physical map (b) of the translocation chromosomes in comparison with those of 3A and 2VbS chromosomes. Bar showed 10μm.

The arrows show the putative breakage points on chromosomes 3A and 2VbS. The red color on chromosome 3A indicated the predicted centromeric regions by revealed by location of CCS1 sequences by searching for the reference genome sequences v1.0 at <https://urgi.versailles.inra.fr/blast/>.