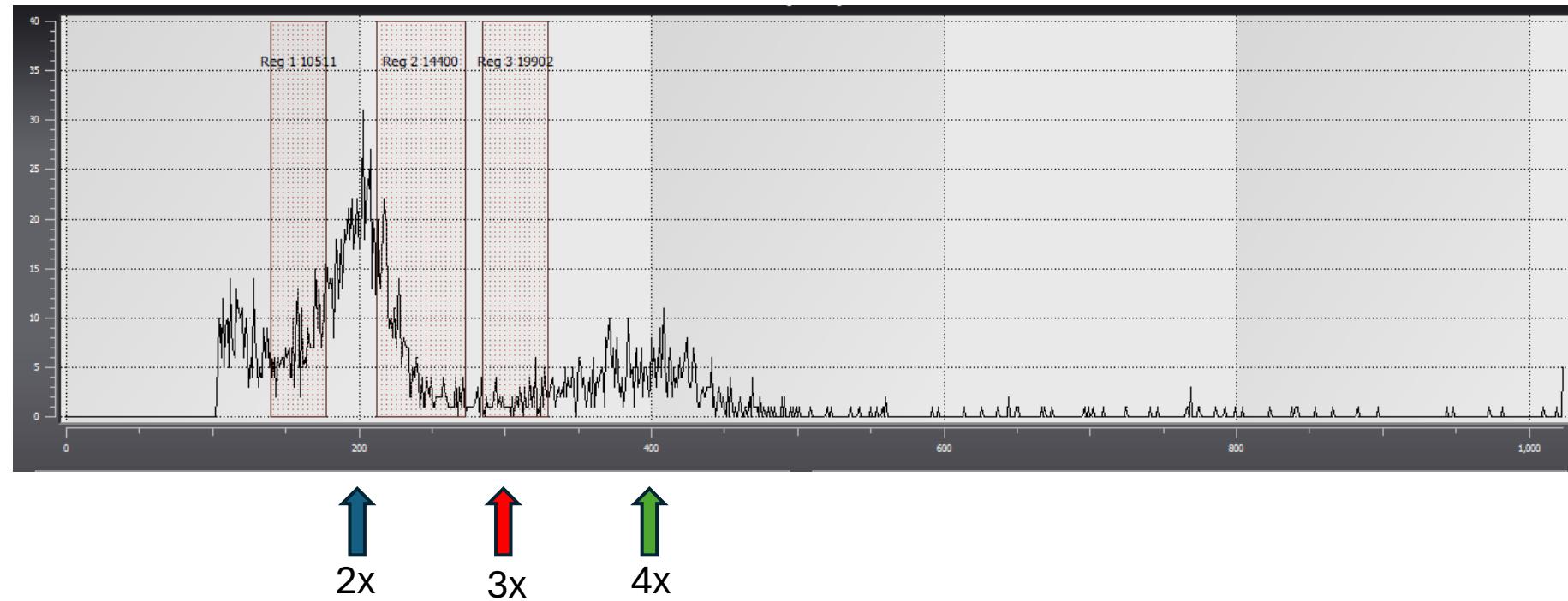
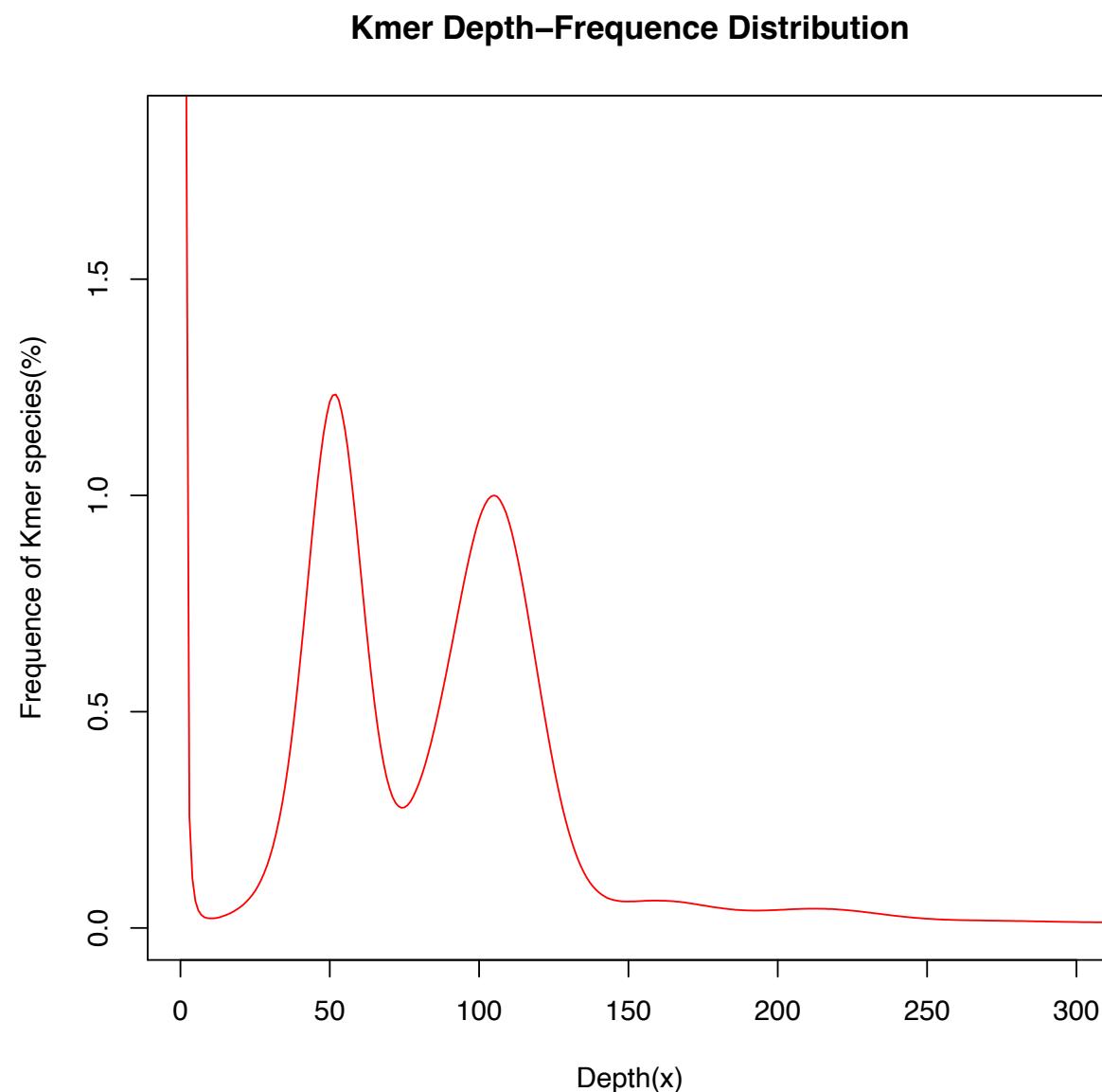


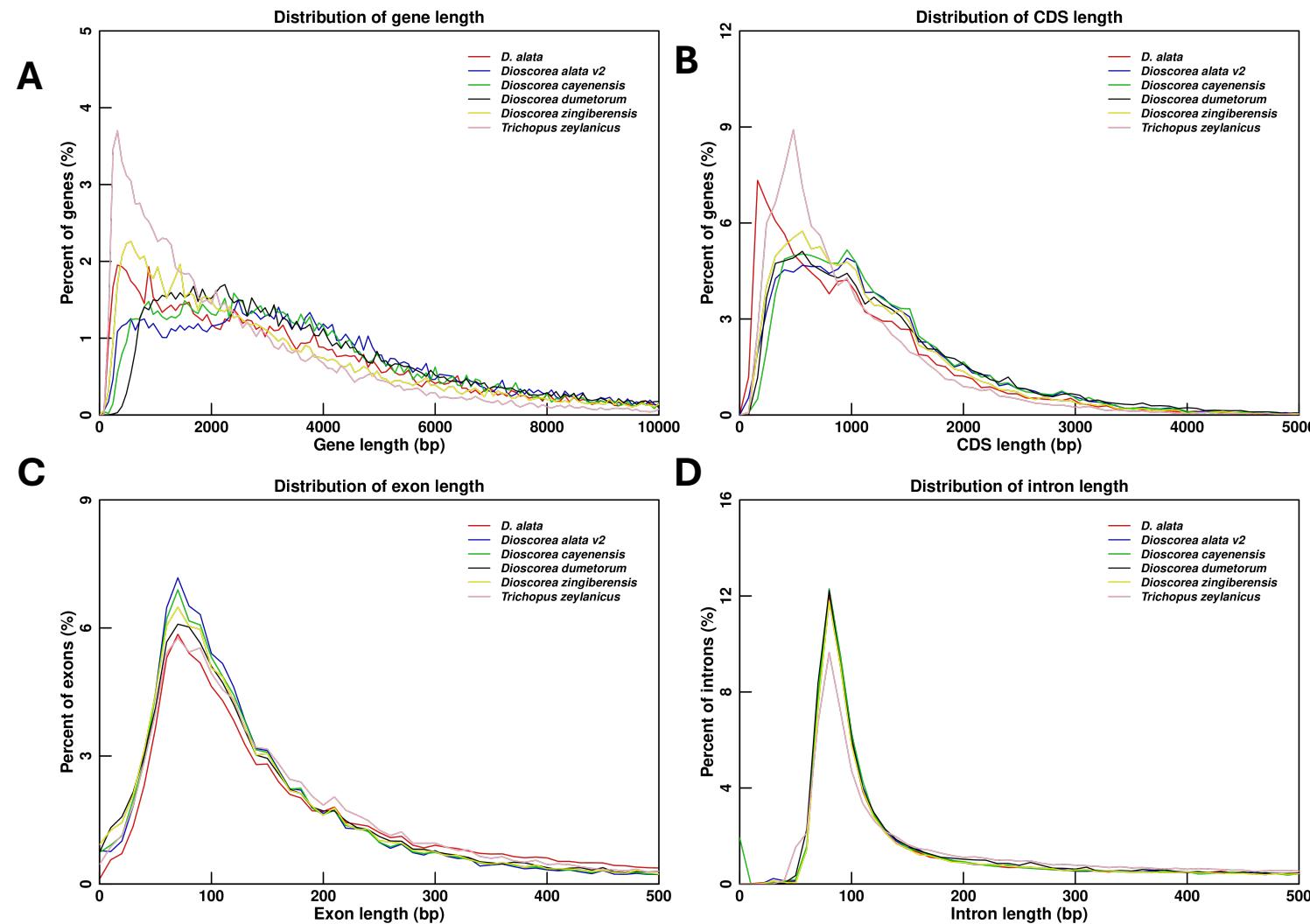
**Supplementary Figure 1:** Flow cytometry-based determination of the ploidy level in ‘Kabusa’. The ploidy level was assessed by comparing the position of the main peak with the references indicated by the arrows. According to the results, ‘Kabusa’ is identified as a diploid cultivar (2X).



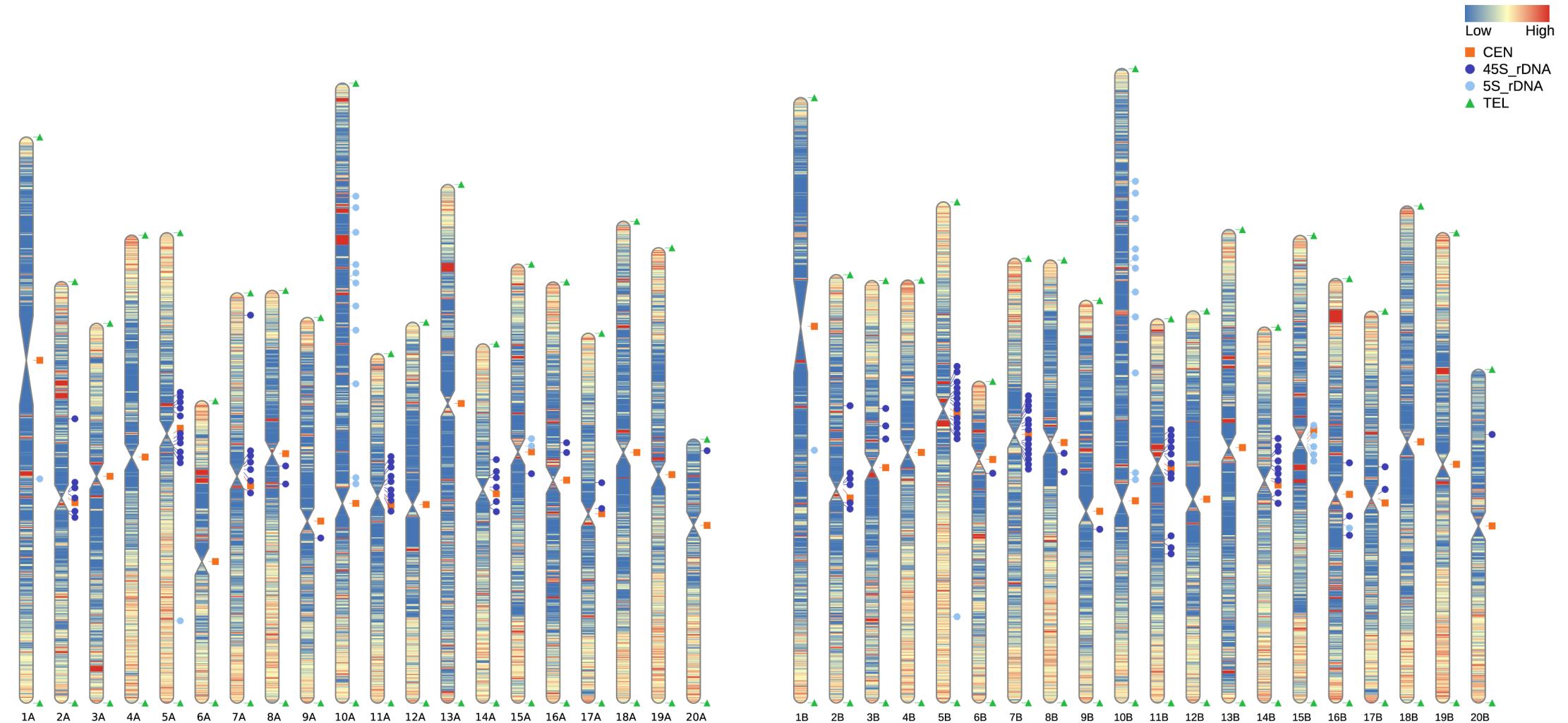
**Supplementary Figure 2:** k-mer analysis for estimation of the ‘Kabusa’ genome size. The genome size was estimated to approximately 494.13 Mb.



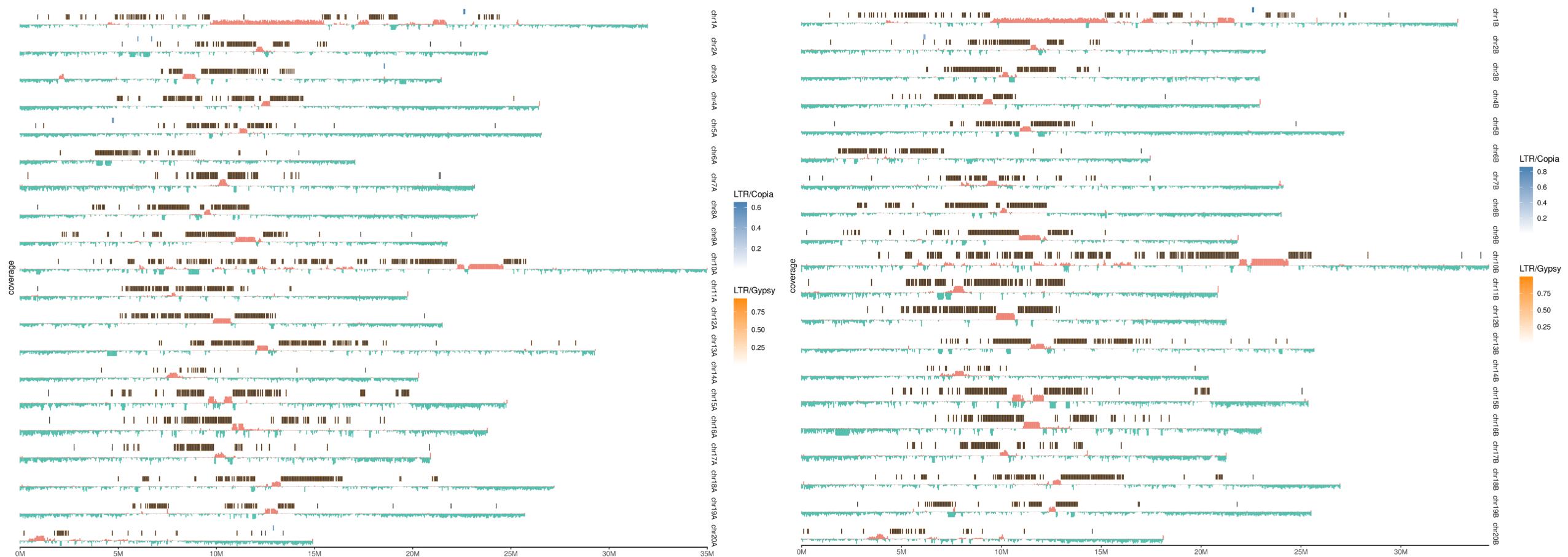
**Supplementary Figure 3:** Distribution of the length of gene and gene components in the 'Kabusa' genome assembly compared to related species. **A.** Gene length distribution. **B.** Coding sequence (CDS) length distribution. **C.** Exon length distribution. **D.** Intron length distribution.



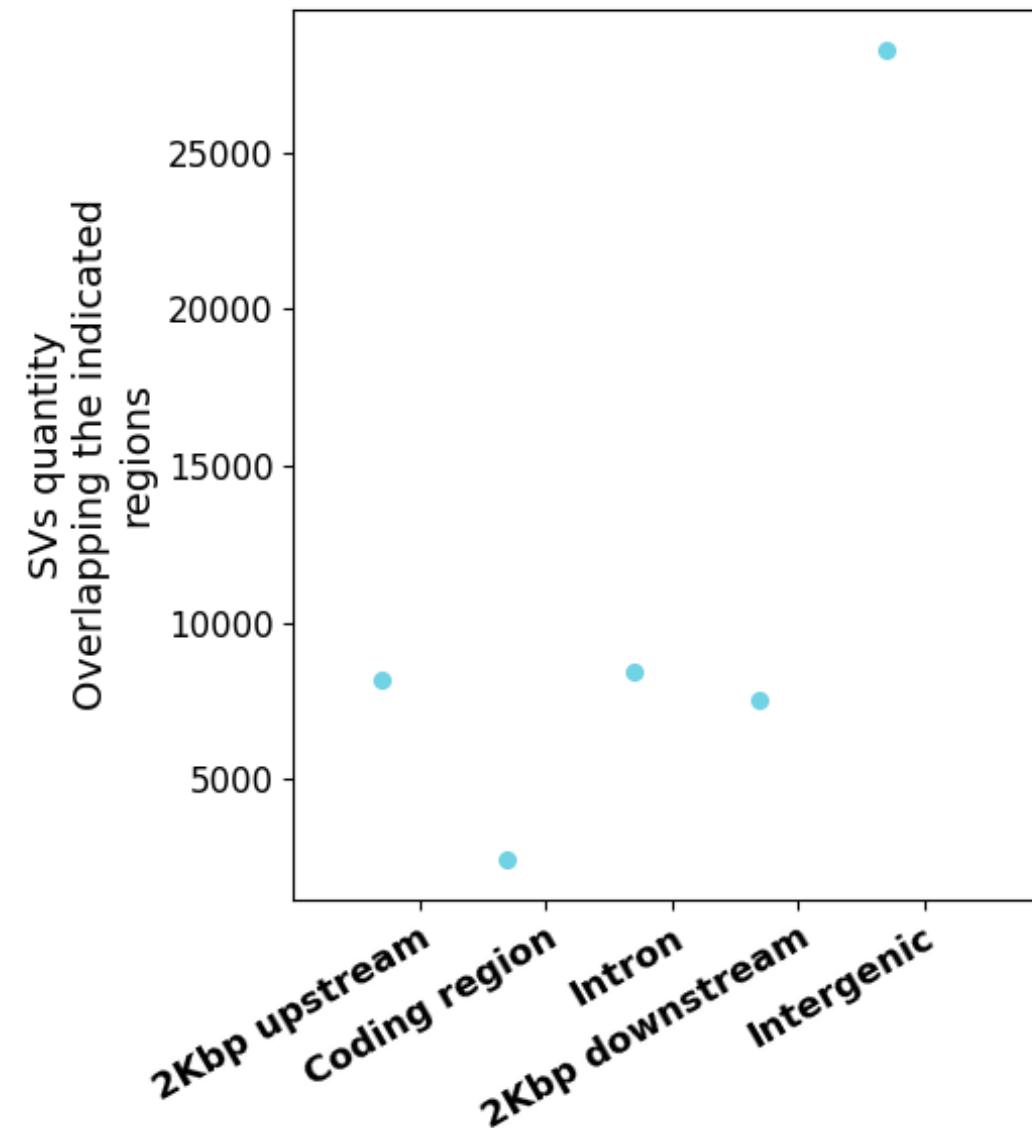
**Supplementary Figure 4:** Centromere and telomere detection map in the two haplotype genome assemblies of 'Kabusa'. Orange squares and green triangles represent centromere and telomeres within the assembled genome. Blue indicates low gene density; red indicates high gene density. Deep blue and light blue circles represent the distribution along the chromosomes of 45S\_rDNA and 5S\_rDNA, respectively.



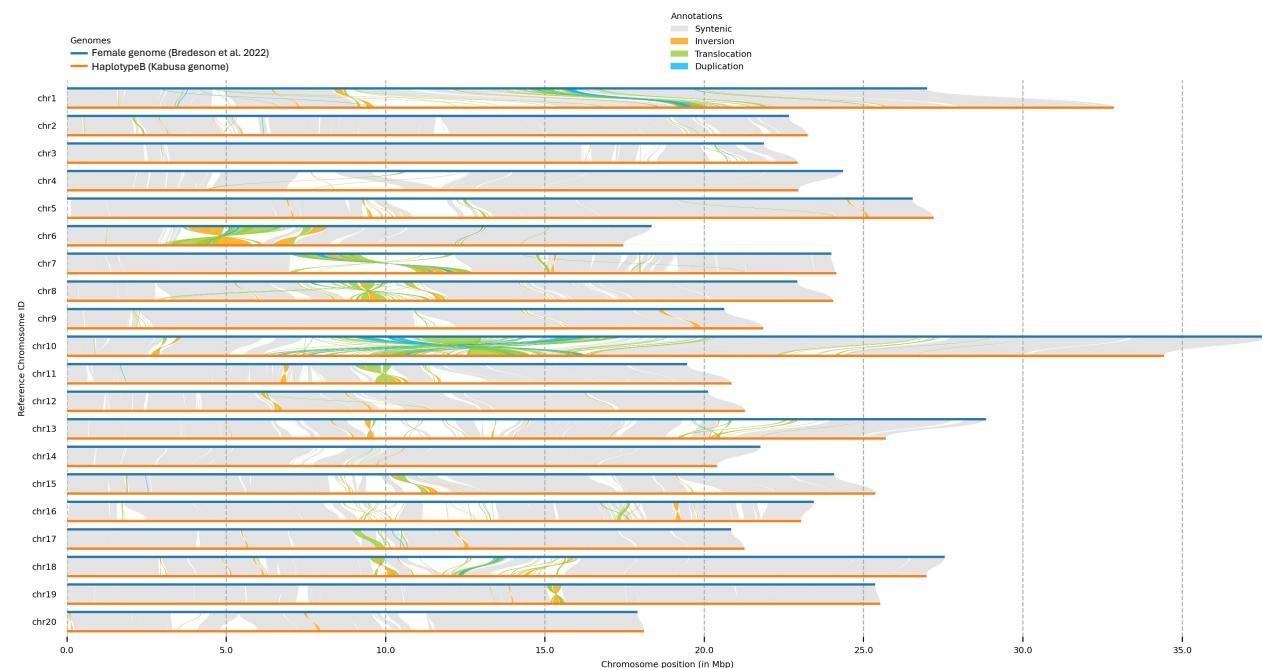
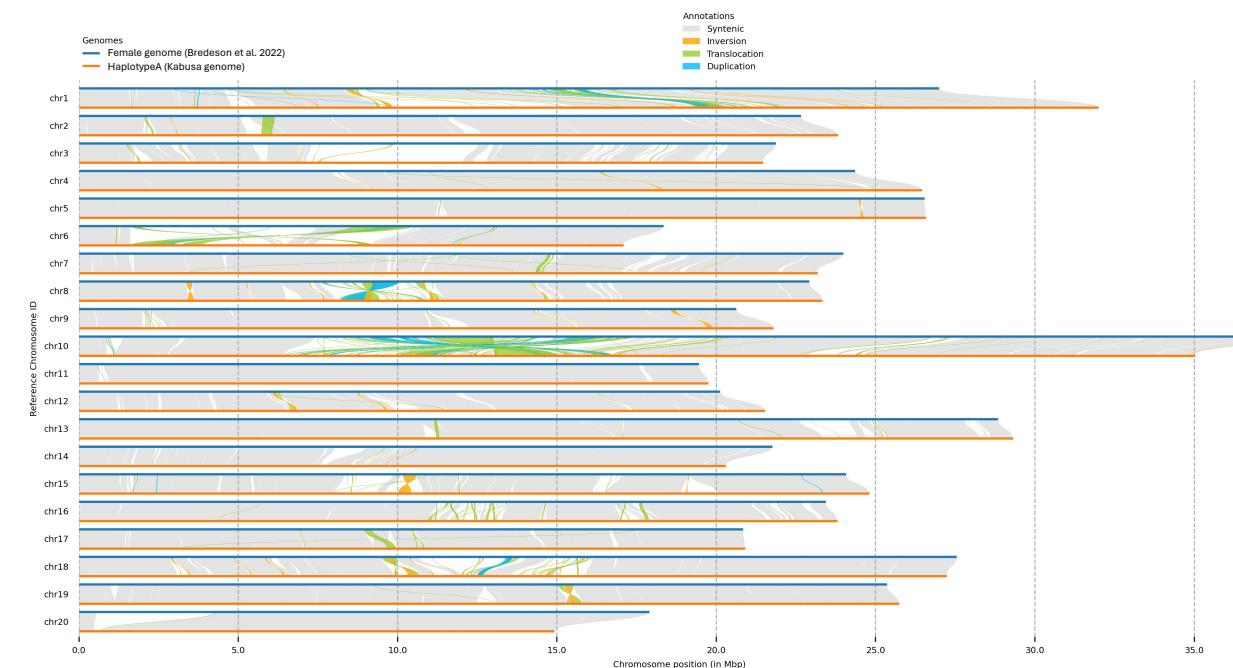
**Supplementary Figure 5:** Centromere detection in the two haplotype genome assemblies of 'Kabusa'. The first layer representing LTR/Copia density (blue color), the second layer representing LTR/Gypsy density (orange color), the third layer representing tandem repeat density (ruby red color) and the fourth layer representing the gene density (turquoise color) along the chromosomes.



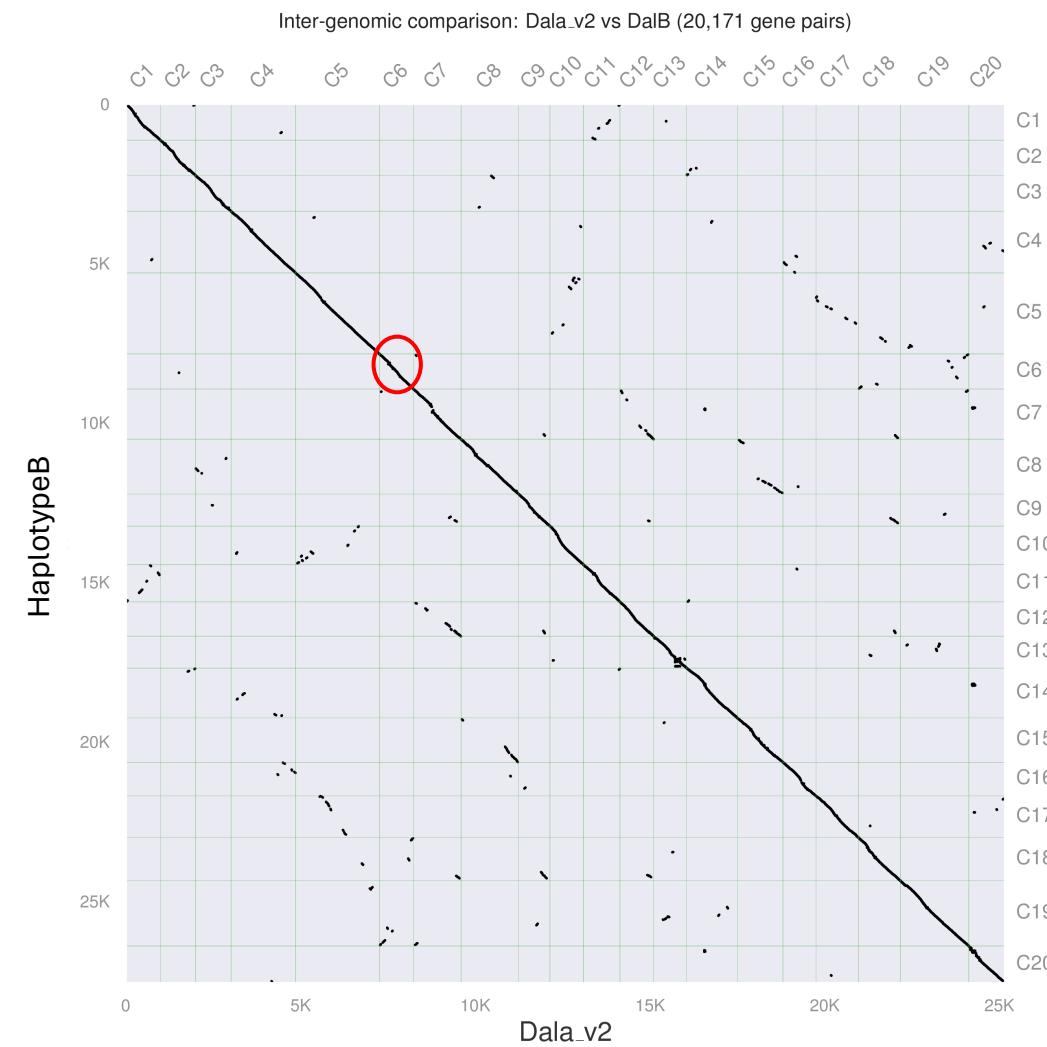
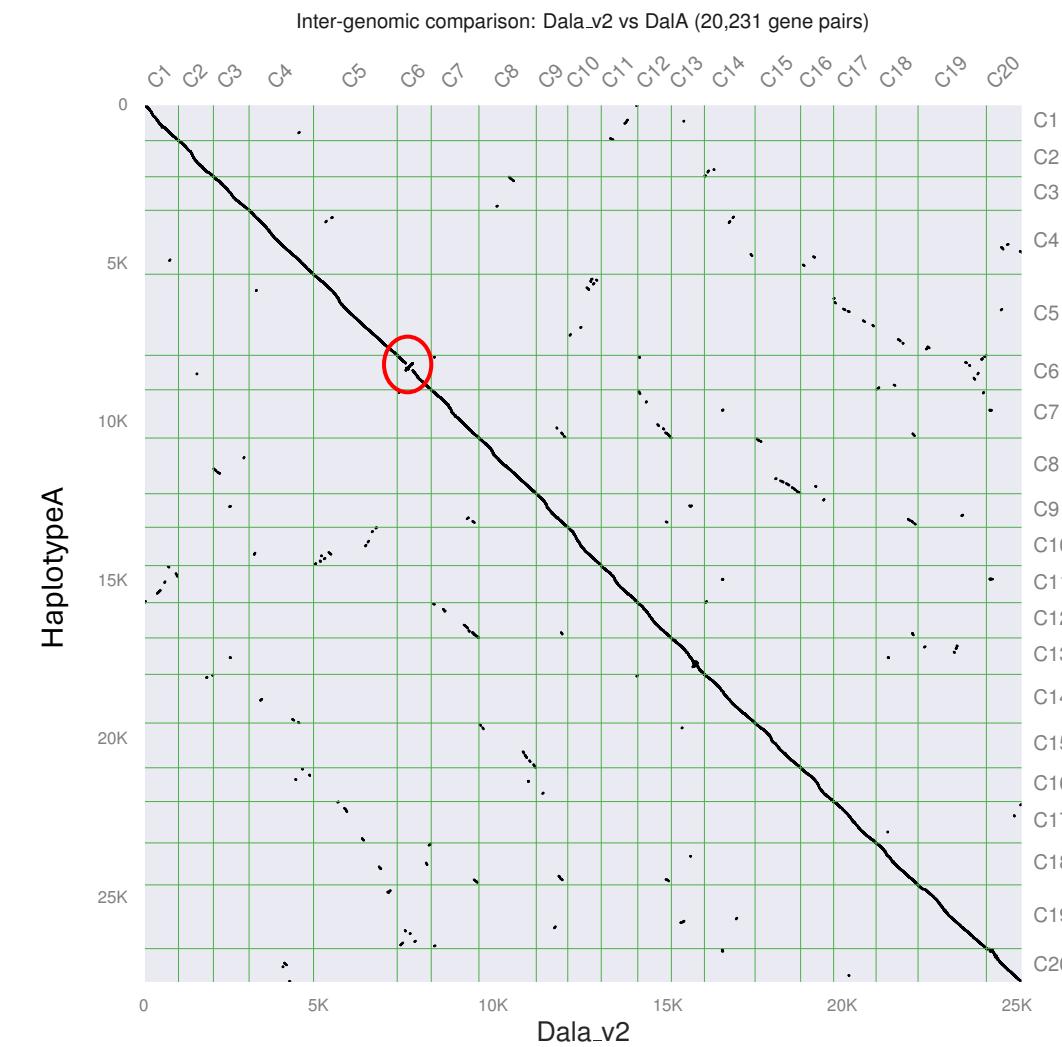
**Supplementary Figure 6:** Quantity of structural variations between the two haplotype assemblies of the 'Kabusa' genome mapped to different genomic regions



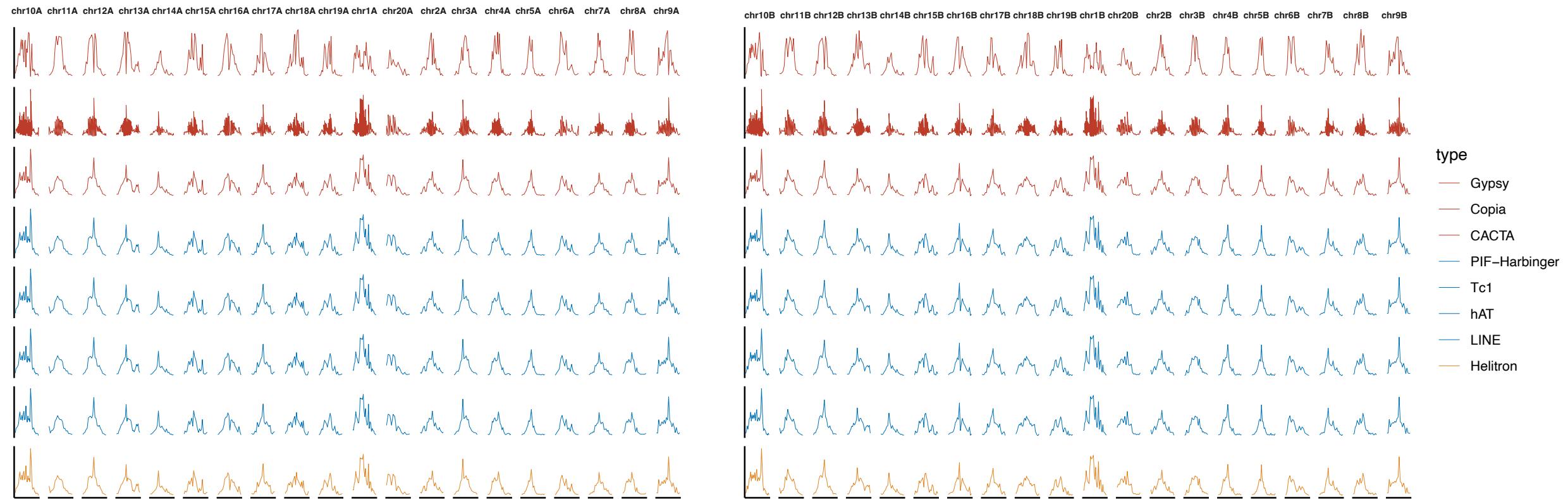
**Supplementary Figure 7:** Collinearity analysis between the HaplotypeA, HaplotypeB of the 'Kabusa' genome and the female genome assembly from Bredeson et al. (2022).



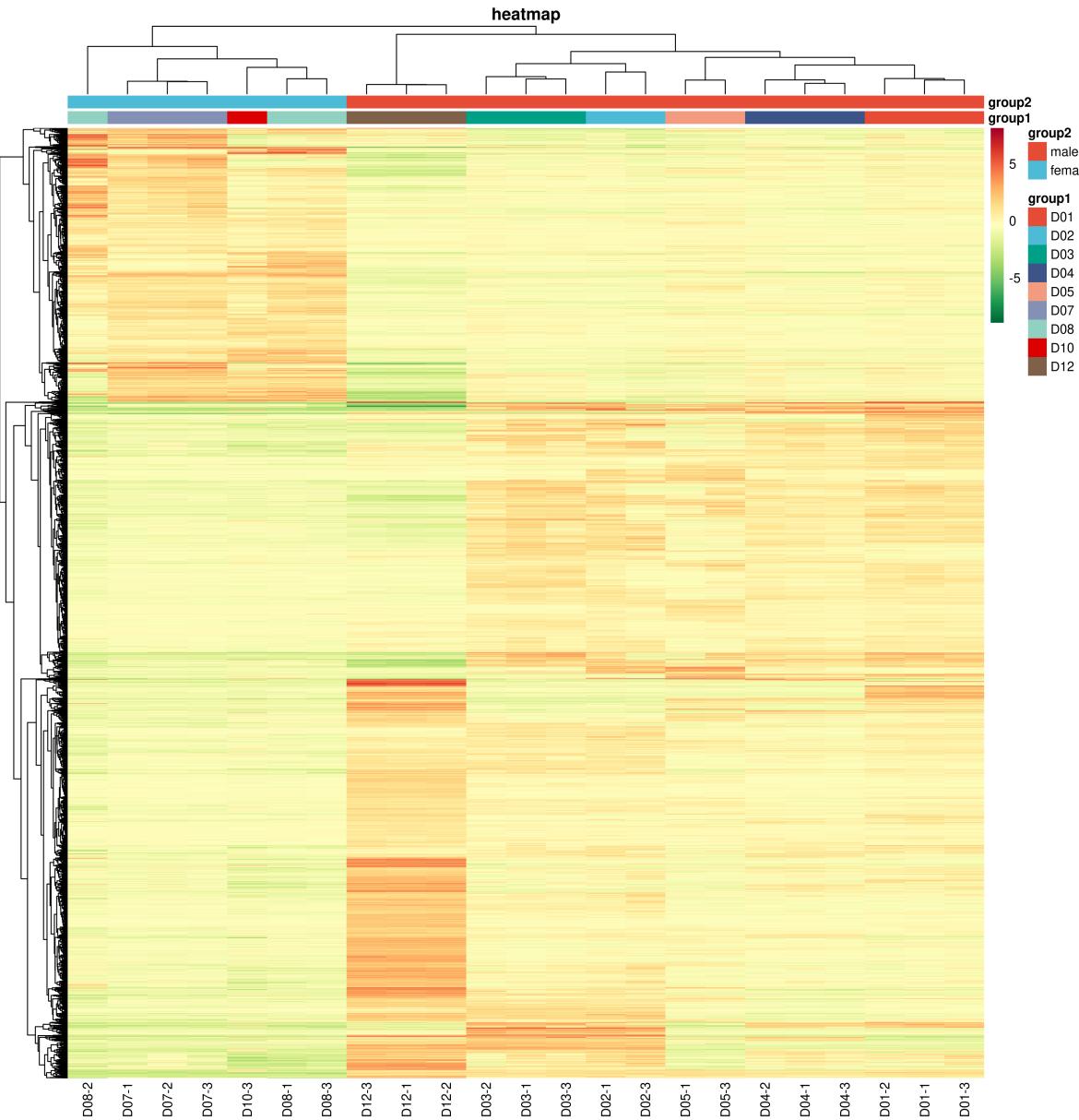
**Supplementary Figure 8:** Intergenomic comparison of the HaplotypeA, HaplotypeB of the 'Kabusa' genome and the female genome assembly from Bredeson et al. (2022). Red circle shows the location of the pericentric inversion in DalaChr6A vs DalaChr6F but absent in DalaChr6B vs DalaChr6F.



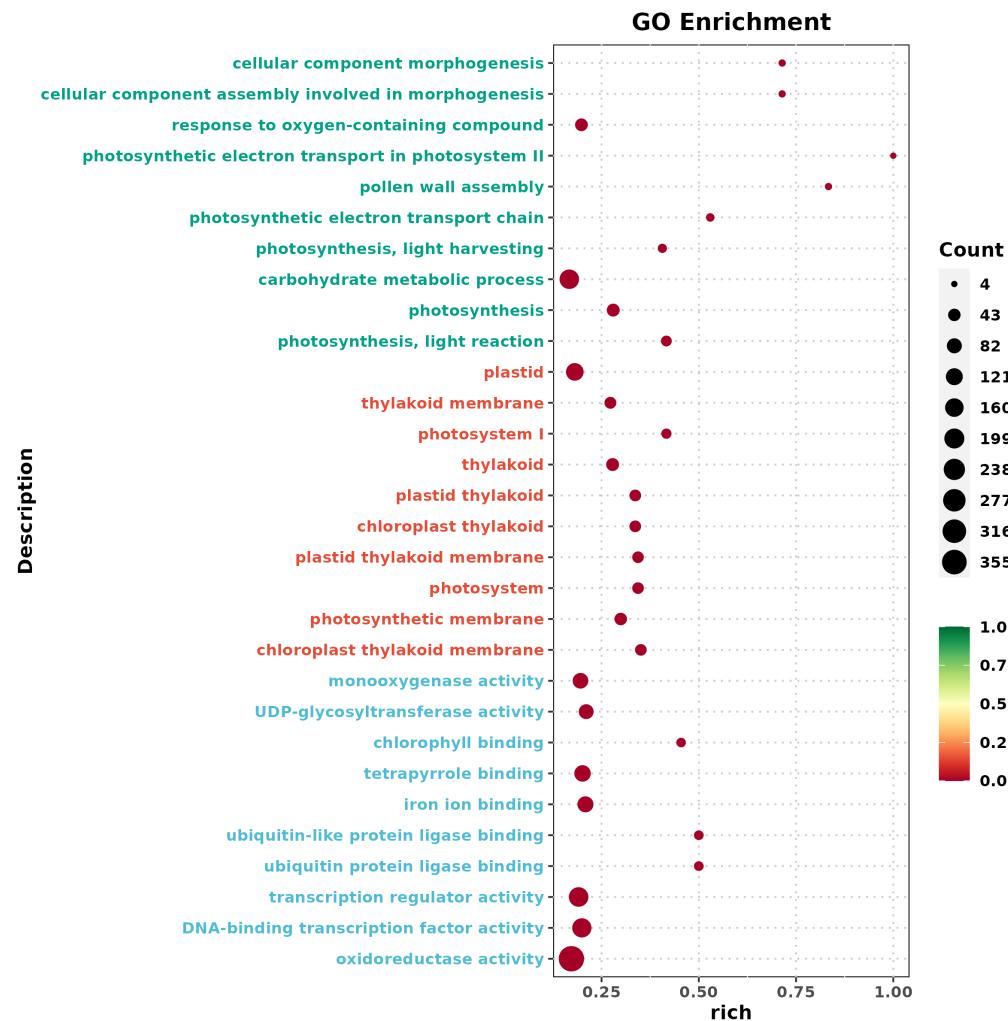
**Supplementary Figure 9:** Distribution of transposable elements in the two haplotype assemblies of the ‘Kabusa’ genome



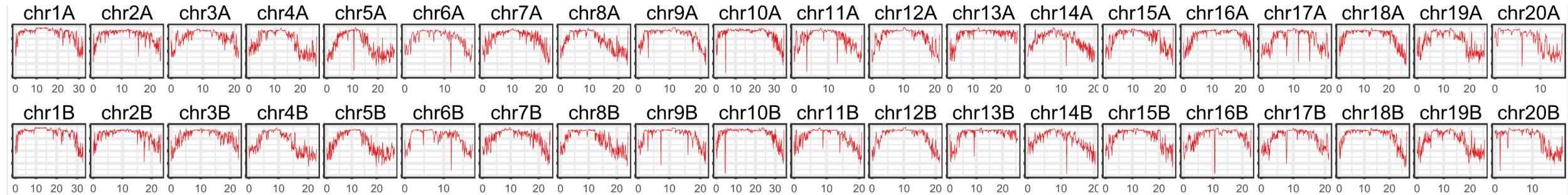
**Supplementary Figure 10:** Gene expression profiling and heatmap clustering of male and female offspring from the cross 'Kabusa' x '74F'.



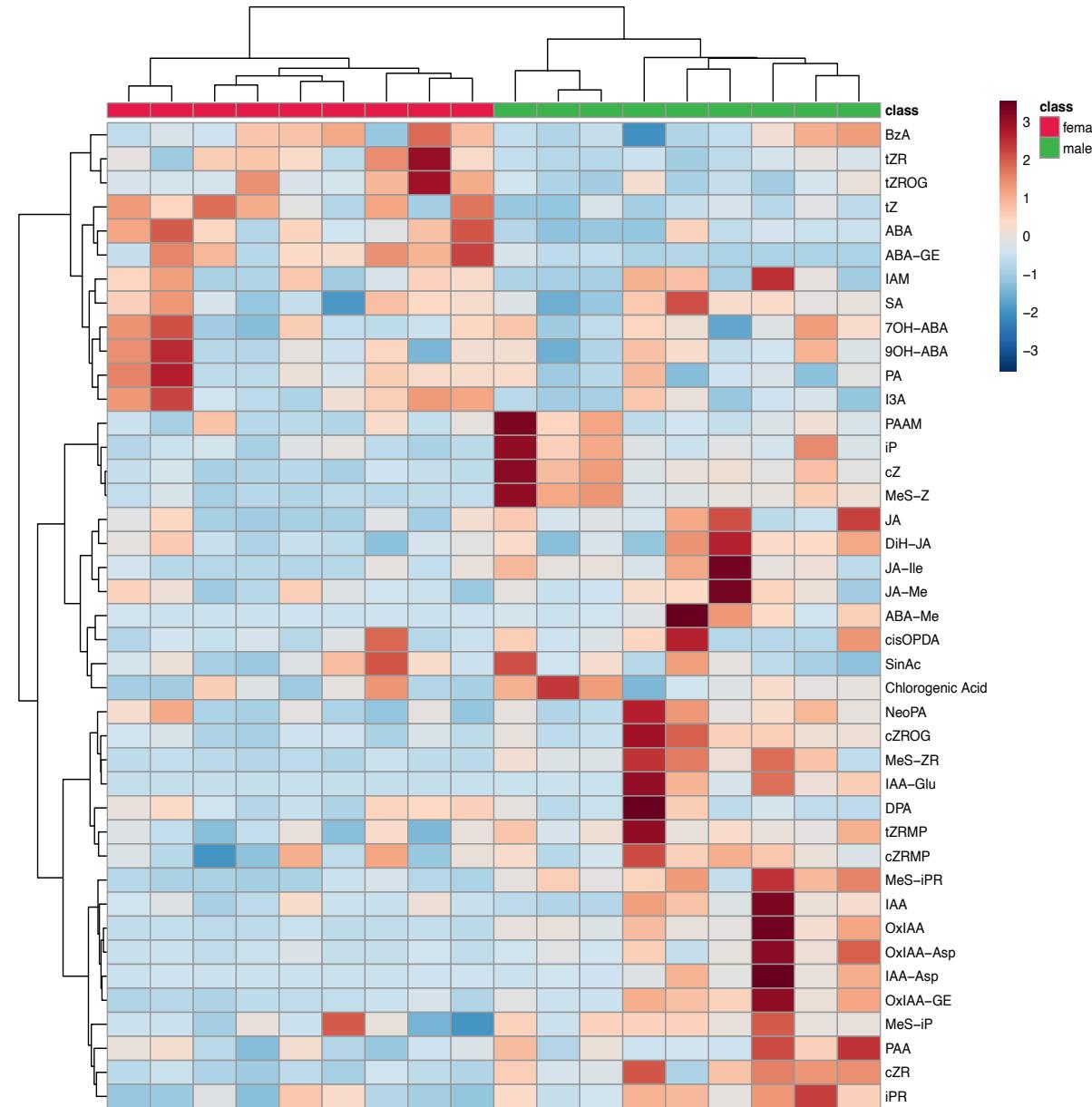
**Supplementary Figure 11:** Gene ontology enrichment analysis of the 6917 sex-biased expressed genes detected between male and female groups in *Dioscorea alata*



**Supplementary Figure 12:** Patterns of methylation level along homologous chromosomes in the two haplotype assemblies of the ‘Kabusa’ genome. Plot was generated using a window of 100 kb.



**Supplementary Figure 13:** Heatmap clustering of the male and female offspring based on the hormone quantity. 41 compounds representing five classes of hormones (Cytokinin, ABA, JA, Auxin, Phenolics) were quantified in flowers of three male and three female offspring (3 biological replicates for each material) from the cross ‘Kabusa’ x ‘74F’.



**Supplementary Figure 14:** Alignment of coding sequence and protein sequence from Y-linked COI1 gene (*DalaChr6AG083880*) and its homolog X-linked (*DalaChr6BG080900*).

|                  |  |      |
|------------------|--|------|
| DalaChr6AG083880 | CGCTCTCCAAGCGCTGGAAGGACTCCATCCCTCTCGCCCTCCCTCTACTCCCCGTTGGCGTCTTCGATGGCCTCCAC            | 160  |
| DalaChr6BG080900 | CGCTCTCCAAGCGCTGGAAGGACTCCATCCCTCTCGCCCTCCCTCTACTCCCCGTTGGCGTCTTCGATGGCCTCCAC            | 160  |
| DalaChr6AG083880 | CGCGTTGACGCCGATGCCGCATCTCCACATGGTCTCCCTCCGCCCTTGAGGCCCTGAGGAGCTCGTCATCTACTGTCCTTTC       | 240  |
| DalaChr6BG080900 | CGCGTTGACGCCGATGCCGCATCTCCACATGGTCTCCCTCCGCCCTTGAGGCCCTGAGGAGCTCGTCATCTACTGTCCTTTC       | 240  |
| DalaChr6AG083880 | TCCGCTTCTCTTCTCGCCTCTGGCTCTCCCTCCGGAGCCATTCCCTCCGCCCTTGAGGCCCTGAGGAGCTCGTCATCTACTGACCCGC | 320  |
| DalaChr6BG080900 | TCCGCTTCTCTTCTCGCCTCTGGCTCTCCCTCCGGAGCCATTCCCTCCGCCCTTGAGGCCCTGAGGAGCTCGTCATCTACTGACCCGC | 320  |
| DalaChr6AG083880 | CGAAAAGCCGGCTCCCTGCCGCTTGGACGCCATTGGATCTGCCAAGAATCTGGAGGATCTCAAGCTCTGGGGGTTTCTC          | 400  |
| DalaChr6BG080900 | CGAAAAGCCGGCTCCCTGCCGCTTGGACGCCATTGGATCTGCCAAGAATCTGGAGGATCTCAAGCTCTGGGGGTTTCTC          | 400  |
| DalaChr6AG083880 | TGACGAAATCCCCAACTGGGGTGTCTTCAGCGCTTGAGGGTTTGGAGATTGTTGGGGCGCCGTAGGGATACTGCG              | 480  |
| DalaChr6BG080900 | TGACGAAATCCCCAACTGGGGTGTCTTCAGCGCTTGAGGGTTTGGAGATTGTTGGGGCGCCGTAGGGATACTGCG              | 480  |
| DalaChr6AG083880 | ATCAAGGATGTTGTGAGGGCTGCCCTAAACTGTGGAGTTGGCATTGCTGGTTGCGATGGTGTGAGCTCCGTTCCAT             | 560  |
| DalaChr6BG080900 | ATCAAGGATGTTGTGAGGGCTGCCCTAAACTGTGGAGTTGGCATTGCTGGTTGCGATGGTGTGAGCTCCGTTCCAT             | 560  |
| DalaChr6AG083880 | TGAATTTCGATTGCTGGAGAAAATGCAGGGTGGATTCCCTGGGTTCTGCTAATTCTCCCTTCATCTCTCTTCCCAAAGC          | 640  |
| DalaChr6BG080900 | TGAATTAGATTGCTGGAGAAAATGCAGGGTGGATTCCCTGGGTTCTGCTAATTCTCCCTTCATCTCTCTTCCCAAAGC           | 640  |
| DalaChr6AG083880 | TTGTGATTCTCGAGTTGCAAGGTTAGTTGATCATGTTAATGAGAGCCATTCCCTCCGCCCTCATCTATTGCTAAA              | 720  |
| DalaChr6BG080900 | TTATGATTCTCGAGTTGCAAGGTTGAT-----GAGC-----TTGAG-----GTTGCTT-----CTT-----CTT-----          | 689  |
| DalaChr6AG083880 | ACTTCAGGCAAGTGTAGTGTCTACAAAGGGATGTGGAAAGCTGCCCTGATTTGGAGTATTGTCATCAGAGGTGTGCA            | 800  |
| DalaChr6BG080900 | --ATCAAG-----GTAGTGTCTACAAAGGGATGTGGAAAGCTGCCCTGATTTGGAGTATTGTCATCAGAGGTGTGCA            | 761  |
| DalaChr6AG083880 | ATGGAGTTGGAAATGCAATAAGCTCTGTGCTCCAATCTGGAGTGGAGCTGAGGATCTGCTTATGAAGATTGAATTCACTG         | 880  |
| DalaChr6BG080900 | ATGGAGTTGGAAATGCAATAAGCTCTGTGCTCCAATCTGGAGTGGAGCTGAGGATCTGCTTATGAAGATTGAATTCACTG         | 841  |
| DalaChr6AG083880 | GAGATCTAGACACACTTCACCCATTCTCAAAATTGACCTGTTGATTCTCAACATCATCAGAAGTTATGCAAGTT               | 960  |
| DalaChr6BG080900 | GAGATCTAGACACACTTCACCCATTCTCAAAATTGACCTGTTGATTCTCAACATCATCAGAAGTTATGCAAGTT               | 921  |
| DalaChr6AG083880 | GGAAATCCACGGGCCTATGTTGCGGCTTGGCCAGAAGAACAGCCTTAAATGGATTCCAGATTCTGAGTATTCTTG              | 1040 |
| DalaChr6BG080900 | GGAAATCCACGGGCCTATGTTGCGGCTTGGCCAGAAGAACAGCCTTAAATGGATTCCAGATTCTGAGTATTCTTG              | 1001 |
| DalaChr6AG083880 | CTTGGAAAGGGTCTTGTTCGGTCAAGGTCACCGGTTGAATCCAATGCAAAAGCTGAACACTCTTGAGTCATTGGTTAAGT         | 1120 |
| DalaChr6BG080900 | TTTGGAAAGGGTCTTGTTCGGTCAAGGTCACCGGTTGAATCCAATGCAAAAGCTGAACACTCTTGAGTCATTGGTTAAGT         | 1081 |
| DalaChr6AG083880 | ACAGTGTCAATCTGCAGAAGCTGGTATTAGAATCTCAAGATGCGAAACTGCCATGAGACTCGAGATGAATTCTTTAAG           | 1200 |
| DalaChr6BG080900 | ACAGTGTCAATCTGCAGAAGCTGGTATTAGAATCTCAAGATGCGAAACTGCCATGAGACTCGAGATGAATTCTTTAAG           | 1161 |
| DalaChr6AG083880 | CAGATATGCAAAATTCAAGCATAATGAACTCATACATAGTTCACATCGAATAA                                    | 1251 |
| DalaChr6BG080900 | CAGATATGCAAAATTCAAGCATAATGAACTCATACATAGTTCACATCGAATAA                                    | 1212 |

|                  |  |     |
|------------------|--|-----|
| DalaChr6AG083880 | MNSVPEGVQHQHILSQMNARDIASCACVSKRWKDSIPFLPSLYFPRGVFDGLPDRVADAAlSHMVSSALRLEELVIVCPF   | 80  |
| DalaChr6BG080900 | MNSVPEGVQHQHILSQMNARDIASCACVSKRWKDSIPFLPSLYFPRGVFDGLPDRVADAAlSHMVSSALRLEELVIVCPF   | 80  |
| DalaChr6AG083880 | SASLLASWLSLRSHSLRRLELRMDCTAEKPAPCRDLAIGSAKLNEDLKLWGVSLTKSPNWGVILQRLRVLIEVGAARVDTA  | 160 |
| DalaChr6BG080900 | SASLLASWLSLRSHSLRRLELRMDCTAEKPAPCRDLAIGSAKLNEDLKLWGVSLTKSPNWGVILQRLRVLIEVGAARVDTA  | 160 |
| DalaChr6AG083880 | IKDVVQACPCKLVELALLGCDGCVSIELDCLEKCRFLGSAANSVHLSSPKVILELQGFSWIVHNERHSLRRLSIAK       | 240 |
| DalaChr6BG080900 | IKDVVQACPCKLVELALLGCDGCVSIELDCLEKCRFLGSAANSVHLSSPKVILELQGFLDFVVFVFLSGSVYKVVDVG     | 240 |
| DalaChr6AG083880 | TSGKCSVYKVDTGKLPDLEYLSIRGVQWSWNATSSVILQSGSEVKHLLMKIEFTCDLDTLQPFQOIDLVDFNNHQNQKLGF  | 320 |
| DalaChr6BG080900 | KLPDLEYLSIRGVQWSWNATSSVILQSGSEVKHLLMKIEFTCDLDTLQPFQOIDLVDFNNHQNQKLCKFEIHGAMFAALCQK | 320 |
| DalaChr6AG083880 | PIIHGAMFAALCQKNSLKNLDSRFSIPCLEEVIVTVRSPNPMQKINTLESLKVYSVNLQKLIVIRISKMRNCHETADEFFK  | 400 |
| DalaChr6BG080900 | NSLKNLDSRFSIPCLEEVIVLVMVRSPLNPMQKINTLESLKVYSVNLQKLIVIRISKMRNCHETADEFFKQICKFKHMHNIV | 400 |
| DalaChr6AG083880 | QTCFKFHMHNIVHIE 416  |     |
| DalaChr6BG080900 | HIC----- 403   |     |