





The cumulative count of unique non-CS genomic blocks identified during the construction of a pan-genome with the sequential addition of genome assemblies.



Layout of the hidden Markov model (HMM) for filtering CNVb markers. The genome windows are classified into three classes: normal (NOR) with no CNV appearing, deletion (DEL), and duplication (DUP) during the identification of CNV blocks. The sequence of CNV status along the genome was regarded as the observations of the HMM (blue boxes in the figure). The hidden states (orange circles, "nor" for no CNV appearing, "del" for deletion, and "dup" for duplication) are regarded as the real CNV status of each genome window, which may be observed as different statuses at a specific probability (marked near the arrow from the orange circles to blue boxes). Following the hypothesis of the Markov chain, the probability of "real" CNV status transiting from one status to another was marked near the arrow between orange circles.



Workflow for CNVb marker scanning in wheat varieties based on ulcWGS. The process begins with the detection of CNV blocks in wheat accessions utilizing ulcWGS. Subsequently, identified CNV blocks of each accession are mapped against the CNVb marker library. If a CNVb shows significant overlap with a CNVb marker, the wheat accession is considered to carry that specific CNVb marker. The collective mapping of these CNV blocks to the CNVb markers enables the establishment of a CNVb fingerprint for each accession, which is a composite representation of the presence or absence of specific CNVb markers in the genome.



Fig S5 Count of CNVb markers on each chromosome.





400

600

800

1D

Distribution of CNVb markers across each chromosome. Blue blocks represent deletion block markers, while red blocks indicate duplication block markers. Stacked blocks denote distinct CNVb markers with overlapping regions.



The CNVb-deletion marker associates with the 1RS·1BL translocation. The "type 1" denoted the subtype 1RS·1BL translocation associated with CNVb.67.1 (chr1B: 0-239.3 Mb). The "type 2" denoted the subtype 1RS·7DL/7DS·1BL translocation associated with CNVb.67.2 (chr1B:0-236.7 Mb).



The CNVb.541 CNVb-deletion marker (chr3D: 592.1-616.0 Mb) corresponds to the introgression from *Thinopyrum ponticum* on chromosome 3D.



Comparative dot plot alignment of Zang1817 chromosome 2A and CS chromosome 2A (showing the first 30 Mb).



A paired comparison between Bima1 and Bima4. **a.** The pedigree diagram shows the breeding relationship between Bima1 and Bima4, originating from a cross between Biyumai and Mazhamai. **b.** The CNVb fingerprint of a paired comparison between Bima1 and Bima4. Each small square in the CNVb fingerprint denotes an individual CNVb marker.



Pairwise similarity distribution based on CNVb markers at ultra-low sequencing depth. Green represents the similarity between the same variety, while purple represents the similarity between different varieties.



Statistical power $(1-\beta)$ of the similarity density distributions for low-coverage sequencing datasets with different coverage.