

SUMMARY

Nucleolar dominance (ND) describes an epigenetic uniparental silencing of 35S rRNA genes in plant hybrids and allopolyploids. Despite many years of research on ND, the mechanisms of this phenomenon are still poorly understood. Thus, this study primarily focuses on the molecular mechanisms underlying the selection of 35S rRNA genes set for repression in *Brachypodium hybridum*. This allotetraploid grass with a genome composition DDSS (D-subgenome from *B. distachyon*; S-subgenome from *B. stacei*) presents a valuable model for ND studies due to its compact genome and low content of repetitive sequences. The research aimed at population-level analysis of 35S rDNA loci in different *B. hybridum* accessions.

By employing the FISH technique, it was shown that most of the studied *B. hybridum* genotypes possess the sum of 35S rDNA loci as expected based on their number in its putative progenitors. Using Southern hybridisation and bioinformatic analysis, the contributions of rDNA homoeologues across different *B. hybridum* genotypes were evaluated, and the gradual elimination of the S-subgenome rDNA loci was shown in most studied genotypes. The expression patterns of 35S rDNA homoeologues at different developmental stages were examined through RT-qPCR with genome-specific primers and RT-CAPS. Expression data demonstrated that ND is a genotype-, generation-, tissue- and individual-specific phenomenon. Furthermore, the methylation status of rRNA genes was assessed in selected *B. hybridum* genotypes using Southern hybridisation on gDNA subjected to restriction with methylation-sensitive enzyme, showing an essential role of DNA methylation in ND maintenance.

The findings underline that ND in *B. hybridum* is developmentally regulated in some but not all genotypes. Moreover, it was shown that ND is independent of maternal effect. In corroboration with previous ND studies on *B. hybridum*, this study expands our knowledge of ND mechanisms and advances our understanding of the intricate interactions among gene expression, epigenetics, and population diversity. It also demonstrates the significance of *B. hybridum* as a model for deciphering the enigmatic phenomenon of ND.