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# ANALYSIS OF GENETIC AND EPIGENETIC STRUCTURE AND VARIABILITY OF GRAPEVINE CENTROMERES THROUGH THE USE OF LONG READ SEQUENCING AND T2T ASSEMBLIES

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We produced assemblies of 3 European and Caucasian cultivated grapevine accessions, one European wild accession of the same species and one interspecific hybrid between North American wild species using PacBio HiFi and/or ONT long-reads and the Hifiasm software. A large fraction of the 19 grapevine chromosomes in each accession were T2T resolved and allowed us to perform a detailed analysis of the elusive structure of the grapevine centromeres. The comparison of up to 8 different centromeric haplotypes for the same chromosome has also allowed us to study the variability and the evolutionary history of these regions. The methylation analysis using ONT reads allowed us to study the epigenetic status of these regions despite their structural complexity and repetitiveness. The structure of the centromeres was reconstructed at fine resolution and revealed the presence of at least 10 families of tandem repeats, 6 of which are almost ubiquitous in the centromere of all chromosomes and 4 are rare, forming in some cases Megabase-scale arrays, intermixed with retrotransposons mainly belonging to the Athila and the chromovirus-domain containing Gypsy elements. All of these repeats formed highly ordered and symmetrical structures, frequently organised around an array of one of the six most common repeats. The grapevine centromeres appear to be densely DNA methylated, especially in the arrays of the most abundant tandem repeat. Extreme variability was observed in terms of structure and repeat composition both among

centromeres of different chromosomes as well as among centromeric haplotypes of different accessions. As high variation was observed among centromeres within cultivated accessions as between cultivated accessions and outgroup species, attesting a very high rate of evolution of these regions. None of the single repeat units formed an array that was always present in every centromere, not allowing us to conclude which is the minimal functional unit of the grapevine centromeres.