

Satellite DNA in *Paphiopedilum* subgenus *Parvisepalum* as revealed by high-throughput sequencing and fluorescent in situ hybridization

ABSTRACT

Background: Satellite DNA is a rapidly diverging, largely repetitive DNA component of many eukaryotic genomes. Here we analyse the evolutionary dynamics of a satellite DNA repeat in the genomes of a group of Asian subtropical lady slipper orchids (*Paphiopedilum* subgenus *Parvisepalum* and representative species in the other subgenera/sections across the genus). A new satellite repeat in *Paphiopedilum* subgenus *Parvisepalum*, SatA, was identified and characterized using the RepeatExplorer pipeline in HiSeq Illumina reads from *P. armeniacum* ($2n = 26$). Reconstructed monomers were used to design a satellite-specific fluorescent in situ hybridization (FISH) probe. The data were also analysed within a phylogenetic framework built using the internal transcribed spacer (ITS) sequences of 45S nuclear ribosomal DNA. **Results:** SatA comprises c. 14.5% of the *P. armeniacum* genome and is specific to subgenus *Parvisepalum*. It is composed of four primary monomers that range from 230 to 359 bp and contains multiple inverted repeat regions with hairpinloop motifs. A new karyotype of *P. vietnamense* ($2n = 28$) is presented and shows that the chromosome number in subgenus *Parvisepalum* is not conserved at $2n = 26$, as previously reported. The physical locations of SatA sequences were visualised on the chromosomes of all seven *Paphiopedilum* species of subgenus *Parvisepalum* ($2n = 26–28$), together with the 5S and 45S rDNA loci using FISH. The SatA repeats were predominantly localised in the centromeric, peri-centromeric and sub-telocentric chromosome regions, but the exact distribution pattern was species-specific. **Conclusions:** We conclude that the newly discovered, highly abundant and rapidly evolving satellite sequence SatA is specific to *Paphiopedilum* subgenus *Parvisepalum*. SatA and rDNA chromosomal distributions are characteristic of species, and comparisons between species reveal that the distribution patterns generate a strong phylogenetic signal. We also conclude that the ancestral chromosome number of subgenus *Parvisepalum* and indeed of all *Paphiopedilum* could be either $2n = 26$ or 28, if *P. vietnamense* is sister to all species in the subgenus as suggested by the ITS data.

Keyword: Fish; Fluorescent in situ hybridization; Karyotype; *Paphiopedilum*; Satellite DNA