CHROMOSOMAL ORGANIZATION OF TANDEM REPEATED DNA SEQUENCES IN PICEA ABIES


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Picea abies, tandem repeated DNA sequences, in situ hybridisation

In order to study the sequence composition and structure of the Norway spruce genome, different genomic libraries were made, sequenced and used for several different analyses. Four tandemly repeated sequences were selected from a randomly sheared total genomic DNA library (PAG004P22F, PAG003M02C, PAG004E03C, PAG006O16F) and used in FISH experiments together with an already characterised centromeric tandem repeat, PATR140. In situ hybridisation experiments were carried out in order to study the chromosomal organization of these highly repeated DNA sequences and to characterize the P. abies chromosome complement. Squashes made with colchicine-treated seminal roots were incubated, after DNA denaturation in 70% formamide, with the heat-denatured probes which were labelled with digoxigenin-11dUTP or biotin-16dUTP by polymerase chain reaction. The digoxigenin or biotin at the hybridisation sites was detected by using sheep antidigoxigenin-fluorescein or streptavidin-Cy3, respectively.

PAG004P22F repeats found nucleotide sequence homology at the centromeric region of nine out of twelve chromosome pairs and at the nucleolus organizing region of four pairs. PAG006O16F-related sequences were found at the centromeres of four pairs and the secondary constrictions of five out of six satellited pairs. Sequences related to PAG004E03C, PAG003M02C, probes showed centromeric localization in three chromosome pairs each that coincided with the three known locations for the patr140 probe. Each centromeric region could contain either different tandem repeats or only one kind of repeat. One chromosome pair did not show centromeric labelling after hybridisation with any probe. Two findings seem to be worth noting. In five out of six satellited chromosome pairs, the same tandem repeats are found both at centromeres and secondary constrictions. Surprisingly, and unlike what is usually observed, no tandem repeat is localized at the chromosome ends. Most chromosome pairs of the complement showed distinctive patterns of labelling after hybridisation with the probes. By combining the differences in labelling pattern and the morphology of the chromosomes, all pairs can be distinguished from each other.