

EXPANDING THE KARYOTYPE OF SLASH PINE AS A PRELUDE TO PHYSICAL MAPPING

PINE

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Abstract:--Cytological exploration of the pine genome has been ongoing for more than a century. For the first seventy years we knew little more than chromosome number for pines. Constancy in chromosome number throughout the genus coupled with uniformity in size and morphology between chromosomes within species has given cytologists few practical means by which to distinguish individual chromosomes in *Pinus* (Sax and Sax 1933). Several Karyotypes were published between 1950 and 1970 based in constriction patterns in chromosomes in haploid tissues but were not reproducible in metaphase spreads from diploid tissues making them of limited utility for routine karyotyping. The task of karyotyping became even more formidable when pines proved recalcitrant to standard chromosome banding procedures like giemsa, DAPI and CMA (Pedrick 1970; Borzan and Papes 1973).

The advent of fluorescent *in situ* hybridization, or FISH, in the last decade has finally made the pine genome visually accessible in the microscope. In the FISH protocol, labeled probes are hybridized to spread metaphase chromosomes which are affixed to glass microscope slides. Sites of probe hybridization on the chromosomes are detected in an epifluorescence microscope after application of a fluorophore conjugated to an antibody specific to the label on the probe. The microscope image is one of brightly lit bands of color against a dark background that is reminiscent of lights on a Christmas tree.

In 1995, Doudrick et. al. published the first FISH Karyotype for pine using metaphase spreads from slash pine. These authors also discussed the potential utility of FISH for physically mapping the many genetic linkage groups that have been identified for slash pine. Implementing physical mapping using this karyotype is problematic because it requires two ribosomal probes and banding by two different fluorochromes for diagnostic karyotyping. This necessitates stripping and reprobng which is labor intensive and time consuming and also limiting in terms of the number of new probes that can be placed on the existing karyotype. Reported below are recent expansions to this karyotype that could accelerate routine karyotyping and thus serve as a prelude to physical mapping in this species.

In the original karyotype, Doudrick et. al. used a heterologous probe from sugar beet to locate 5s rDNA sites. Three chromosomal locations were defined by this probe. In the present study, a homologous probe specific for the 5s rDNA coding region was generated and labeled by PCR using primers P1 and P2 from Brown and Carlson (1997). Using this probe, an additional 15 sites were defined, a 5-fold increase in available bands for karyotyping and a 2-3fold increase in the number of chromosomes with detectable 5s rDNA sites.

Experiments in which the Telomere Repeat Sequence, or TRS, from *Arabidopsis thaliana* (Richards and Ausubel 1988) was used as a probe, generated bands on all slash pine chromosomes. In addition to hybridization at the telomere, there are an additional 36 interstitial and centromeric bands defined by this probe with a range of 2-6 bands per chromosome.

By comparison, there are a total of 46 bands in the existing karyotype excluding the 6 major constrictions which are seen as negative bands or cleared regions. Of these 46 bands, 16 are

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synonymous leaving a total of 30 unique bands. The addition of 15 more 5s rDNA bands and 36 telomeric bands would more than double the number bands detectable in slash pine.

The number of bands detected by the telomere probe make it a likely candidate for stand alone karyotyping. Use of a single probe for routine karyotyping would greatly facilitate physical mapping. More experiments are planned to assess the accuracy of this probe when used singly for karyotyping and the feasibility of automated karyotyping in slash pine based on telomeric band profiles.

Keywords: *Pinus elliottii* var. *elliottii*, karyotype, fluorescent *in situ* hybridization, ribosomal DNA, telomere DNA

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