MALE CONE DEVELOPMENT IN *PINUS RADIATA*

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ABSTRACT

Light microscopy and transmission electron microscopy were used to investigate the morphological, anatomical changes and the timing of these changes during male cone development of *Pinus radiata* growing in the central part of the North Island, New Zealand. The timing of developmental events, including the initiation of the male cone primordia, the onset of meiosis of pollen mother cells and the formation of pollen grains were recorded. Their relationship with environmental factors in comparison with pine species growing in the Northern Hemisphere was discussed.

Some significant morphological aspects of male cone buds, microsporophylls and structural/ultrastructural changes of microsporangia, tapetal cells and pollen mother cells during the meiotic processes in particular, were reported in the morphological and anatomical study.

In correlation with these structural/ultrastructrual changes, the soluble protein content, banding patterns of the total soluble protein, banding patterns of four isoenzymes during male cone development were studied by SDS-PAGE and isoelectric focusing techniques. Seven soluble protein species were detected by SDS-PAGE closely related to the different developmental stages of the male cone, and one of them with a molecular mass of 20.5 KD in particular was found to be a potential male cone tissue specific gene expression product. Acid phosphatase, esterase, malate dehydrogenase and peroxidase were studied during male cone development, using isoelectric focusing methodology. Variations in banding patterns of the enzyme activity and number of isoforms of each enzyme in relation to the different developmental stages of the male cone were revealed. A number of isoforms of these four isoenzymes were found to be unique to specific developmental stages.

A search for floral-specific genes controlling floral developmental events was attempted. MADS-box DNA sequences belonging to a homeotic gene family controlling floral development in higher plants are reported for the first time in the genus *Pinus* in this study.

The MADS box gene *AGAMOUS* from *Arabidopsis thaliana* was used as a probe to hybridise with genomic DNA of *P. radiata.* The tentative evidence of hybridisations was obtained in Southern blots, suggesting the possible existence of MADS box related DNA sequences in *P. radiata.* PCR technique was subsequently used to clone these sequences from genomic DNA of radiata pine to confirm the result obtained from Southern blot study . PCR with two degenerate primers targeted to highly conserved regions within the MADS- box resulted in the amplification of a 78 bp DNA sequence. These PCR amplified pine DNA sequences were subcloned in M13 and were sequenced by the dideoxy protocol. The analysis of these DNA sequence data and the amino acid sequences deduced from these DNA sequences showed that these DNA sequences can be divided into three groups, probably belonging to three MADS-box genes of *Pinus radiata.* Two DNA sequence groups are most likely to be the conserved regions of pine MADS-box genes, controlling the late steps of "floral" development which are homologous to class C genes determining the identity of male floral parts (stamens) and female parts (carpels) in angiosperms. One DNA sequence group is speculated to be the conserved region of pine MADS-box gene controlling the earlier steps of floral development, analogous to class B genes controlling petal and stamen development in angiosperms.

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ABBREVIATIONS

AGAMOUS gene	*AG*
Cetyltrimethyl ammonium bromide	CTAB
Dithiothreitol	DTT
(3-[4,5-Dimethylthiazol-2-yi]-2,5-diphenyltetrazolium bromide)	MTT
Dwarf shoot bud	DSB
Endoplasmic reticulum	ER
Ethanol	ETOH
FLORICAULA gene	*FLO*
Formalin-acetic-alcohol	FAA
Glacial acetic acid	HOAC
Isoelectric focusing	IEF
LEAFY gene	*LFY*
Long shoot lateral branch bud	LSLB
Long shoot terminal bud	LSTB
Methanol	MeOH
Nicotinamide adenine dinucleotide	NAD
N,N,N',N'-tetramethylethylenediamine	TEMED
Phenazine methosulfate	PMS
Pollen mother cells	PMCs
Pollen/male cone bud	PCB
Polyacrylamide gel electrophoresis	PAGE
Polymerase chain reaction	PCR
Rough endoplasmic reticulum	RER
Seed/female cone bud	SCB
Sodium dodecyl sulphate	SDS
Tertial butyl alcohol	TBA
Transmission electron microscopy	TEM
Trichloroacetic acid	TCA