Morphological, physiological, and molecular studies on the effect of shoot architecture on phase change and floral transition in *Eucalyptus occidentalis* and *Metrosideros excelsa*

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Abstract

Shoot morphogenesis in *Eucalyptus occidentalis* and *Metrosideros excelsa* was analysed at the morphological, physiological and molecular levels to understand the regulation of phase change and the floral transition. Study of the regulation of these developmental plant processes is limited in woody species due to their long juvenile phase.

Six ecotypes of *E. occidentalis* were grown to two predetermined architectures (free branching or single stem). Free branching plants of ecotype 13648 displayed adult shoot phenology (lanceolate leaves) earlier than single stem counterparts. In addition, changes in leaf morphology in free branching plants were accompanied with changes in leaf anatomy and gas exchange signifying that in *E. occidentalis* complexity of shoot architecture had a significant effect on rate of phase change. Flowering was observed in all but one ecotype irrespective of architecture demonstrating that vegetative phase change and floral transition are temporally uncoupled in this species.

To understand the floral transition at the molecular level in *E. occidentalis*, partial homologues of the inflorescence meristem identity gene *TERMINAL FLOWER1* and floral meristem identity genes *LEAFY* and *APETALA1* were isolated. The expression patterns of these meristem identity genes during development of free branching and single stem plants were analysed by quantitative real-time PCR. Increased levels of expression of *EOLFY* and *EOAP1* (relative to *α-TUBULIN)* were displayed at more proximal nodes in free branching plants than in single stem plants. Elevated floral meristem identity gene expression levels correlated with flower initiation.

Further, effects of architecture and environment on gene expression were monitored in *E. occidentalis*. The overriding effect of shoot architecture on the floral transition was observed under warm long day and ambient environments. Elevated levels of *EOLFY* and *EOAP1* were correlated with floral bud score and *EOAP1* was found to be a reliable marker of floral transition in *E. occidentalis*. Low levels of *EOTFL1* expression were detected in buds irrespective of their position on the plant leading to the suggestion that this might have contributed to the precocious flowering observed in this species.

In contrast to *E. occidentalis*, *M. excelsa* attained adult shoot phenology (pubescent leaves) faster when grown as single stem plants than as free branching plants. It appears that growth as height is required for vegetative phase change in this species. However, floral transition occurred only once single stem plants were allowed to branch. Vegetative phase change and the transition to flowering seem to be coordinated in this species with the former being a pre-requisite for the latter.

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ANOVA	analysis of variance
bp	base pair
cDNA	complementary deoxyribonucleic acid
CSIRO	Australian Commonwealth Scientific and Research Organization
DEPC	diethyl pyrocarbonate
EDTA	ethylene diamine tetracetic acid
FMI	floral meristem identity
JPEG	joint photographic experts group
Kb	kilo base
LED	light emitting diode
LMA	leaf mass per unit area
ORF	open reading frame
Pa	Pascal
PCR	polymerase chain reaction
rpm	revolutions per minute
SE	standard error
SDS	sodium dodecyl sulphate
Tris-HCl	tris (hydroxymethyl) aminomethane hydrochloride
UV	ultraviolet