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**Genetic diversity and flowering
in *Clianthus* and New Zealand *Sophora*
(Fabaceae)**

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of the requirements for the degree of

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Abstract

Clianthus and New Zealand *Sophora* species are woody legumes endemic to New Zealand, with high ornamental value and biodiversity significance. Research was conducted to address the fact that little is known about the details of their developmental characteristics, genetic structure and relatedness of the wild populations, and their molecular mechanism of flowering.

Genetic diversity and relatedness of all remaining wild populations of *Clianthus* and samples of all New Zealand *Sophora* species were investigated using ISSR and AFLP markers. Genetic relationships were established for *Sophora* species, *Clianthus* wild populations and cultivars, and most individuals in each of the wild *Clianthus* populations. The molecular evidence did not support the recent separation on morphological grounds of the two *Clianthus* species, *C. maximus* and *C. puniceus*.

Postharvest treatments were tested to extend vase life of the short-lived cut *Clianthus maximus* and *Sophora tetraptera* flowers. Appropriately treated *Clianthus* cut flowers lasted 10-12 days in the vase, with over 80% of flowers opening. Similar postharvest treatments did not improve the vase performance of cut *Sophora* flowers.

Detailed calendars of vegetative and reproductive growth, and of floral ontogeny were developed for *Clianthus* and *Sophora*. Contrasting behaviours of both vegetative and reproductive growth were observed between these two legumes. A long period of summer-autumn dormancy of vegetative and reproductive growth in *Sophora*, and mass abortion of initiated *Clianthus* inflorescences during most of the year were observed. Unusual floral ontogeny processes, with precocious carpel initiation and delayed petal development, were observed in both species.

An efficient two-step quantitative real-time RT-PCR protocol for detailed gene expression analysis of large numbers of samples was developed using SYBR Green DNA dye and a LightCycler instrument. The consistency of this protocol was optimised with regards to sample and template preparation, primer design, and determination of appropriate internal controls for gene expression quantification. Differences of gene expression in the range of 5-7 orders were effectively detected.

Putative partial homologues of *LEAFY*, *APETALA1*, *PISTILLATA*, and *AGAMOUS* were isolated from both *Clianthus* and *Sophora*. Detailed temporal and spatial expression of each floral identity gene was investigated using quantitative real-time RT-PCR. The expression patterns, together with the sequence similarity, showed that these new isolated gene fragments were most probably *LEAFY*, *APETALA1*, *PISTILLATA*, and *AGAMOUS* homologues in *Clianthus* and *Sophora*, and that the ABC model of floral development is generally applicable to both species. However, there were important variations in temporal expression patterns compared to those of herbaceous species. A bimodal expression pattern of *LEAFY* and *APETALA1* homologues was observed in *Sophora*, but not in *Clianthus*, coincident with their contrasting patterns of floral initiation and development.

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Abbreviations

AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
BLAST	basic local alignment search tool
c.	approximately
cDNA	complementary DNA
cm	centimeter
cpDNA	chloroplast DNA
DTT	dithiothreitol
g	gram
h	hour
ISSR	inter-simple sequence repeats
ITS	internal transcribed spacer
km	kilometer
l	litre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
mRNA	messenger RNA
ng	nanogram
nmol	nanomole
nr DNA	nuclear DNA
<i>P</i>	probability
PCR	polymerase chain reaction
RAPD	randomly amplified polymorphic DNA
RT	reverse transcription
s	second
SE	standard error
STS	silver thiosulfate
v/v	volume/volume
w/v	weight/volume
µg	microgram
µl	microlitre
°C	degrees Celsius