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Bushiness in micropropagated
Zantedeschia: An investigation at the
environmental, physiological and
molecular levels

A thesis presented in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy

in

Plant Biology

at Massey University,

Palmerston North

Suzanne Maree D'Arth

2003

CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral Thesis entitled
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Abstract

The commercial *Zantedeschia* industry faced a potentially serious problem with the cultivar Florex Gold periodically producing 'bushy' plants with several short non-flowering shoots and squat tubers with multiple nodes. These plants, derived from tissue culture, resulted in a reduced financial return to the *Zantedeschia* industry.

A number of potentially causative factors were investigated in this project but no single factor emerged as the cause of the bushy syndrome. The selection of tubers with multiple eyes for initiation into tissue culture, or tubers themselves recently derived from tissue culture, may be a contributing factor to bushiness. A variable growing environment (screen house), shortened storage time (11 weeks) and plant position were also shown to accentuate individual bushy characteristics.

Endogenous cytokinin concentration and profile, determined by high performance liquid chromatography and radioimmunoassay, in tubers at three stages in the *Zantedeschia* life cycle were similar between bushy and non-bushy plants and cannot explain the bushy phenotype. Micropropagation of Florex Gold on Murashige and Skoog medium supplemented with low cytokinin concentration (4.4 μM 6-benzylaminopurine, 6-BAP) did not produce bushy plants or tubers. The use of elevated cytokinin during micropropagation did not produce plants with a range of bushy symptoms, although elevated thidiazuron (13.6 μM), but not 6-BAP, was shown to affect flower spathe length and plant shoot number. Florex Gold, when compared to a range of non-bushy *Zantedeschia* cultivars, displayed greater sensitivity to cytokinin during micropropagation assessed by a root length bioassay. Increased sensitivity to 6-BAP, during micropropagation may indicate a predisposition to bushiness.

Differences in the DNA methylation profile of bushy and non-bushy plants were found in DNA using DNA Amplified Fragment Length Polymorphism. However, the isolated sequences were not similar to any gene(s) currently listed in public databases.

Differential gene expression levels, determined by cDNA Amplified Fragment Length Polymorphism, found 60S and 18S ribosomal RNA down-regulated in bushy Florex Gold. These two genes may play a role in the expression of the bushy phenotype.

Changes in DNA methylation and differential gene expression patterns and the results from the micropropagation experiments, provide evidence that *Zantedeschia* Florex Gold tubers selected for commercial micropropagation should be deep and apically dominant with few nodes and buds, and not themselves recently derived from tissue culture.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
³ H-iPATA	³ H-isopentenyladenosine-trialcohol
³ H-ZRTA	³ H-zeatin riboside-trialcohol
5mdC	5-methyl-deoxycytidine
6-BAP	6-benzylaminopurine
¹⁴ C-AMP	¹⁴ C-adenosine-5'-monophosphate
ADP	adenosine 5'-diphosphate
AFLP	amplified fragment length polymorphism
AMP	adenosine 5'-monophosphate
AtIPT1 to 9	<i>Arabidopsis thaliana</i> isopentenyltransferases 1 to 9
ATP	adenosine 5'-triphosphate
B	amount of ³ H-ZRTA or ³ H-iPATA bound to the antibody in the presence of ZR or iPA
B ₀	amount of ³ H-ZRTA or ³ H-iPATA bound to the antibody in the absence of ZR or iPA
BLAST	basic local alignment search tool
BV	bed volume
C ₁₈	octadecyl silica
cDNA	complementary DNA
<i>cis</i> ZOG1	<i>cis</i> zeatin <i>O</i> -glucosyltransferase gene
<i>CKO</i>	cytokinin oxidase gene
<i>ckx1</i>	cytokinin oxidase gene
CPM	counts per minute
CPPU	<i>N</i> -phenyl- <i>N'</i> -(2-chloro-4-pyridyl)urea
<i>CRE1</i>	cytokinin response 1 gene
CTAB	cetyltrimethylammonium bromide
cZ	<i>cis</i> zeatin
cZ9G	<i>cis</i> zeatin 9 glucoside
cZNT	<i>cis</i> zeatin nucleotide
cZOG	<i>cis</i> zeatin- <i>O</i> -glucoside
cZR	<i>cis</i> zeatin riboside
cZROG	<i>cis</i> zeatin riboside- <i>O</i> -glucoside

d	day
DDRT-PCR	differential display reverse transcription PCR
DE52	DEAE cellulose 52
DMAPP	dimethylallyl pyrophosphate
DNA	deoxyribonucleic acid
df	degrees of freedom
DPU	diphenylurea
DZ	dihydrozeatin
DZ3G	dihydrozeatin-3-glucoside
DZ7G	dihydrozeatin-7-glucoside
DZ9A	dihydrozeatin-9-alanine (dihydrolupinic acid)
DZ9G	dihydrozeatin-9-glucoside
DZMP	dihydrozeatin riboside-5'-monophosphate
DZNT	dihydrozeatin nucleotide
DZOG	dihydrozeatin- <i>O</i> -glucoside
DZR	dihydrozeatin riboside
DZROG	dihydrozeatin riboside- <i>O</i> -glucoside
EDTA	ethylenediaminetetraacetate
EST	expressed sequence tag
EXO I	exonuclease I
FGC	Fast Growing Callus
FW	fresh weight
GA ₃	gibberellic acid
HPLC	high performance liquid chromatography
IBA	indole-3-butyric acid
iP	isopentenyladenine
iP7G	isopentenyladenine-7-glucoside
iP9G	isopentenyladenine-9-glucoside
iPA	isopentenyladenosine
iPDP	isopentenyladenosine-5'-diphosphate
iPMP	isopentenyladenosine-5'-monophosphate
iPNT	isopentenyladenine nucleotide
<i>ipt</i>	isopentenyltransferase gene
iPTP	isopentenyladenosine-5'-triphosphate
LSD	least significant difference

MET1	DNA methyltransferase gene
MPC	Magnetic Particle Concentrator
mRNA	messenger RNA
mT	<i>meta</i> topolin
NAA	naphthalene acetic acid
NCC	Nodular Compact Callus
OAcDZR	<i>O</i> -acetyldihydrozeatin
OAcZR	<i>O</i> -acetylzeatin
oT	<i>ortho</i> -topolin
oTR	<i>ortho</i> -topolin riboside
OXDZ	<i>O</i> -xylosyldihydrozeatin
OXZ	<i>O</i> -xylosylzeatin
PCR	polymerase chain reaction
PVPP	polyvinylpolypyrrolidone
RAPD	random amplified polymorphic DNA
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal RNA
SAP	shrimk alkaline phosphatase
SE	standard error of the mean
SSC	sodium chloride and sodium citrate buffer
T1	tuber one year from tissue culture
T2	tuber two years from tissue culture
TDZ	thidiazuron
TEA	triethylamine
TEMED	NNN'N'-tetramethylethylenediamine
TIF	Technology for Industry Fellowship
tRNA	transfer RNA
tZ	<i>trans</i> zeatin
tZR	<i>trans</i> zeatin riboside
UV	ultra violet
v/v	volume/volume
Z	zeatin
Z7G	zeatin-7-glucoside

Z9A	zeatin-9-alanine (lupinic acid)
Z9G	zeatin-9-glucoside
ZDP	zeatin riboside-5' diphosphate
ZMP	zeatin riboside-5' monophosphate
ZNT	zeatin nucleotide
ZOG	zeatin- <i>O</i> -glucoside
ZOG1	zeatin <i>O</i> -glucosyltransferase
ZOX1	zeatin <i>O</i> -xylosyltransferase
ZPT	zeatin riboside 5' triphosphate
ZR	zeatin riboside
ZROG	zeatin riboside- <i>O</i> -glucoside