Ethephon-induced shoot production in two cultivars of gentian cultured *in vitro*: Anatomical, morphological and physiological aspects associated with endogenous balance of auxin and cytokinin

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Marzieh Keshavarzi

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Dedication

This thesis is dedicated to my parents for being strong and supportive and to my love Mohsen.

Abstract

For gentian as an export-orientated ornamental crop in NZ, the research within this thesis was motivated by the industry goal of producing highly branched propagules, in order to make high quality pot plants. As part of the *in vitro* propagation system, application of ethephon as a plant growth regulator (PGR), increased the formation of shoots from nodal explants of the cultivar 'Little Pinkie' but not in 'Showtime Diva'. While only two shoots were expected to arise from a metamer of gentian, a maximum of 20 shoots developed from explants of 'Little Pinkie', at 40 $mg.L^{-1}$ ethephon. Therefore, the research undertaken within this thesis focussed on investigating the mechanism by which shoot formation in explants of gentian was influenced. Main objectives were to investigate the origin of high shoot numbers, the effect of ethephon on shoot number and the underlying mechanisms behind the ethephon's effect. As a results of these studies it was proposed that a possible underlying mechanism was the effect of ethephon on auxin/cytokinin balance.

Both light and scanning electron microscopy were utilised to identify the type of buds that shoots originated from in both cultivars of gentian. Shoots arising at the node of 'Little Pinkie' typically had a combination of axillary, co-lateral and adventitious origins. Number of adventitious shoots increased in the presence of ethephon as well as with PAT inhibitor 1-n-Naphthylphthalamic acid (NPA) or PGR (cytokinin). Therefore, it was suggested that morphological mechanism for stimulation of shoot formation was similar for all types of buds. However, 'Showtime Diva' did not produce multiple shoots beyond those derived from pre-existing axillary buds, irrespective of the presence or absence of ethephon as a stimulator for shoot formation.

A range of experiments using laser ethylene detector ETD-300 carried out to quantify ethylene concentration in the headspace of culture vessels released from ethephon in the medium. Ethylene was supplied either as gaseous ethylene, at a concentration of 60 $nL.L^{-1}$ or released from ethephon incorporated into the media, but only direct absorption of ethephon from the medium was effective to increase shoot formation.

The unique characteristic of meristematic activity at nodes of 'Little Pinkie', was likely derived from cells differing in their sensitivity to the endogenous balance of phytohormones and, in particular, a low ratio of auxin: cytokinin. Therefore, low ratio of auxin: cytokinin was the hypothesized mechanism for increased shoot formation following the application of ethephon, NPA or cytokinin. Increased shoot formation in 'Little Pinkie' as a result of either

ethephon or NPA applied through the culture medium, provided evidence to support the hypothesised inhibitory effect of ethephon on polar auxin transport (PAT). However, while basipetal transport of radiolabelled auxin (^{14}C-IAA) was inhibited by NPA, ethephon unexpectedly did not affect it. Rather than the direct inhibition on PAT, ethylene is suggested to have reduced auxin synthesis and/or changed its metabolism. In doing so, the reduction in endogenous concentration of auxin by ethephon or NPA, as well as any increase in endogenous content of cytokinin, could be responsible for the hypothesised reduction in the ratio of auxin: cytokinin and increased shoot formation.

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تقدیم به کسانی که مرا با عشق به این دنیا آوردند،

از خواسته‌های خود گذشتند تا من به خواسته‌هایم برسم،

برایم با عشق کتاب و دفتر میخریدند، تا بیاموزم آنچه را که فکر میکردند خود نمیدانند.

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دوستتان دارم و

قلبم همیشه با شماست...

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List of Symbols and Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
$AgNO_3$	Silver nitrate
AVG	Aminoethoxyvinylglycine
BA	N6-benzyladenine
BAP	2-hydroxybenxylamino purine
Bp	Bud primordia
C	Cortex
$CoCl_2$	Cobalt chloride
CPAS	3-cyclopropyl-1-enyl-propanoic acid sodium
CRD	Completely Randomised Design
C_{Tm}	Concentration of ethylene at the time of measurement
Dpm	disintegrations per minute
ETD-300	ethylene detector
Ethephon	[2-chloroethyl] phosphoric acid
FW	Fresh weight
GA3	Gibberellic acid
$g.L^{-1}$	gram per litre
GC	Gas chromatography
HPLC	High pressure liquid chromatography
RP-HPLC	Reverse phase high pressure liquid chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
kPa	Kilopascal

$L.h^{-1}$	Liter per hour
LS	Linsmaier and Skoog vitamins
LSD	Least significant difference
$mg.L^{-1}$	milligram per litre
mL	Millilitre
Mm	Millimetre
mM	Millimolar
µM	Micromolar
MS	Murashige Skoog salts
NAA	1-naphthaleneacetic acid
$nL.h^{-1}$	nanoliter per hour
$nL.L^{-1}$	nanoliter per liter
NPA	1-n-Naphthylphthalamic acid
NZ	New Zealand
ODS	Octadecyl silica
P	Pith
Ph	Phloem
PAT	Polar auxin transport
PATI	Polar auxin transport inhibitors
PBZ	Paclobutrazol
PFR	The NZ Institute for Plant & Food Research Ltd.
PGRs	Plant growth regulators
Ppb	part per billion
PPFD	Photosynthetic photon flux density

Pptv	part per trillion volume
Ps	Primary stem
REML	Restricted Maximum Likelihood
R_L	Rate of leakage
R_{MR}	rate of release from medium
RP-HPLC	Reverse phase high pressure liquid chromatography
SAM	S-adenosylmethionine
SEM	Scanning electron microscopy
SLs	Strigolactones
Ss	Secondary shoots
Ssvc	Secondary shoot vascular cylinder
STS	Silver thiosulfate
TDZ	Thidiazuron
TEA	trimethylamine
TIBA	2,3,5 triiodobenzoic acid
Vc	Vascular cylinder
$\mu mol.m^2.s^{-1}$	Micro molar per square meter per second
°C	Degree centigrade
^{14}C-IAA	2-^{14}C-indole-3-acetic acid;
1-MCP	1-methylcyclopropene
1º	Primary
2º	Secondary
3º	Tertiary
2,4-D	2,4-dichlorophenoxyacetic acid

List of publications

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