Seasonal changes of non-structural carbohydrates related to the growth and development of gentians

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

Doctor of Philosophy

in Plant Science

at

Massey University

Palmerston North

New Zealand

Yuguo Wang

2014

Abstract

The growth and development of perennial gentians (*Gentiana* L.) for cut flowers are seasonally controlled. It was hypothesized that in these plants the availability of carbohydrates is a limiting factor influencing the development of crown buds, winter survival, spring re-growth, and their development to flowering; this in turn influences the yield and quality of flowering shoots. By focussing on the seasonal changes of non-structural carbohydrates (NSCs) in various organs, and the effect of differential carbohydrate supply on their growth and development, the current thesis aimed to understand the physiological function of NSCs and their potential influence on the commercial production of flowering shoots.

In addition to sucrose, fructose and glucose, the unique carbohydrates, gentianose, gentiobiose and L-bornesitol were found in the gentian hybrids investigated. Gentianose was the main storage carbohydrate, accounting for 50% - 70% total NSCs in storage roots year round, likely playing similar roles as starch and/or fructan do in other plant species. The concentration of these NSCs in various organs fluctuated with seasonal changes, reflecting periods of storage and utilization of NSCs, i.e. the transition between sink and source, in the growth and development of plants during an annual growth cycle.

When harvesting flowering shoots, commercial growers of gentian leave a set number of leaves to maintain carbohydrate levels; however, optimization of leaf number/area has never been assessed in terms of carbohydrate reserves. Utilizing varying levels of defoliation, a positive correlation was determined between the resulting concentration of NSCs in crowns (crown buds, rhizomes and storage roots) and both the number and size of new crown buds, supporting the hypothesis that the availability of carbohydrates is a limiting factor influencing the development of crown buds. Experiments *in vitro* both verified and extended the level of understanding, indicating that the morphogenesis of crown buds, including form, dormancy, and differentiation, was influenced by multiple factors including, carbohydrates, photoperiod and ethylene.

Reserves of NSCs in crowns in autumn were positively correlated with both winter survival and spring re-growth. Consistent with these results, the dynamics of NSC concentration and the activity of relevant glycoside hydrolases (GHs) indicated that a large amount of gentianose, up to 106 mg g^{-1} fresh weight (FW) accumulated in storage roots

before winter was subsequently hydrolysed and remobilized to the crown buds to impart cold tolerance in winter and be used during their subsequent re-growth in spring.

Substantial gentiobiose, up to 38 mg g^{-1} FW, accumulated in stem tissue of the shoots before anthesis commenced, while gentianose increased by up to 26 mg g^{-1} FW in petals before floret opening. These two forms of carbohydrates were considered likely candidates facilitating the fast development of florets. During the development and senescence of florets, the activity of relevant GHs controlling the hydrolysis of gentianose, gentiobiose, and sucrose, was associated with increase of pressure potential driving the opening of floret buds. The stage-specific and dramatic changes of accumulation, conversion and mobilization of NSCs in association with the activity of relevant enzymes, also provided a useful experimental model system to study the metabolism and physiological function of these unique NSCs in gentians.

The current study has provided increased understanding of the roles of specific NSCs, particularly gentianose and gentiobiose, in the growth and development of gentians. The thesis also offers a framework of information to improve the balance between carbohydrate storage in underground crowns and harvest of flowering shoots, and therefore benefits the sustainability of yield and quality of flowering shoots in commercial production.

Acknowledgements

First and foremost, I express my deepest gratitude to my main supervisor Dr Keith Funnell for his wonderful guidance by providing invaluable suggestions, support, challenges and encouragement throughout my study. Your persistence and patience in offering editorial commentary on my thesis from the start to submission of this thesis are extremely appreciated. Without your great support, this thesis would not have been possible. I express my sincere gratitude to Dr David Woolley for his insightful suggestions and constructive feedback, yet friendly guidance on the experimental plans and thesis writing, particularly your supervision when Keith was away. I express my sincere gratitude to Mr Ed Morgan for his invaluable suggestions and support in the research programme and thesis editing, particular your expert advice and help with regard the *in vitro* experiments. I express my sincere gratitude to Dr Jocelyn Eason for her invaluable support and guidance in the research and thesis editing, particularly your assistance in developing the methodology for carbohydrate analysis and detecting enzyme activity. I feel privileged to have had excellent supervision from my four supervisors. Your inspiration for getting this thesis started, along with your expert advice for research and thesis writing, guarantee the accomplishment of my thesis.

I would like to thank the staff at Massey's Institute of Agriculture and Environment and the Plant Growth Unit, especially Chris Rawlingson, James Slater and Kay Sinclair for their technical and instrument support. I would also like to thank many staff at The New Zealand Institute for Plant & Food Research, especially Dr David Lewis for his help with using the HPLC instrumentation, Dr Erin O'Donoghue for her help with using the HPLC and the protein analysis, Maree Debenham for her help with tissue culture techniques, Sheryl Somerfield for her help with the starch measurements, Lyn Watson, Philip West and Jun Zhou for their help with the protein analysis and gel electrophoresis, as well as Steve Arathoon for his lots of help. I am very grateful to Dr Xiongzhao He and Mr Duncan Hedderley for their Statistical help and Dr Nick Gould for his help with aphid stylectomy. Your time and commitment are most appreciated. I am also thankful to other staff and postgraduate students for their help and providing a friendly environment throughout my study.

Acknowledgements

Financial support received for the research from The New Zealand Institute for Plant & Food Research Limited, via the New Zealand Foundation for Research, Science and Technology (contract C02X0702), Plant & Food Research CORE funding: 12058 – "Fashionable Plants for the Ornamentals Industry", Turners & Growers Research Grant, Helen E Akers PhD Scholarship, and Massey University Doctoral Hardship Bursary, is appreciated very much.

I am very grateful to my wife Hongxia Liu and son Xingan Wang for their understanding, support and patience. I am also thankful to my parents, sisters and brothers for their unconditional support throughout my study.

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List of Symbols and Abbreviations

ANCOVA	Analysis of covariance
BM	Basic medium
C	Concentration of osmolality
CAZy	the Carbohydrate-Active Enzyme
CBNC	Number of crown buds per cluster
CN	Cluster number of crown buds
DCB	Diameter of crown buds
DTT	Dithiothreitol
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative light scattering detector
FW	Fresh weight
GDD	Growing-degree-days
GH(s)	Glycoside hydrolase(s)
GLM	General linear model
h	Hour
HPLC	High performance liquid chromatography
i	van 't Hoff factor
IAA	indole-3-acetic acid
LCB	Length of crown buds
MS	Murashige & Skoog
MWCO	Molecular weight cut off
NAA	1-naphthaleneacetic acid
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NBT	Nitroblue tetrazolium
NMR	Nuclear magnetic resonance
NSC(s)	Non-structural carbohydrate(s)
NZ	New Zealand
PAGE	Polyacrylamide gel electrophoresis
PPFD	Photosynthetic photon flux density
PVPP	polyvinylpolypyrrolidone
R	Gas constant
SN	Shoot number
SSP	Proportion of crown buds sprouting in Spring
T	Thermodynamic (absolute) temperature
TCBN	Total number of crown buds per plant
TDZ	Thidiazuron
TNC	Total non-structural carbohydrates
WSP	Proportion of plants surviving winter
Ψ	Water potential
Ψ_m	Matric potential
Ψ_p	Pressure potential
Ψ_s	Osmotic potential