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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
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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-borneositol 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
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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.
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Vertical lines = ± SE

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<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>BM</td>
<td>Basic medium</td>
</tr>
<tr>
<td>C</td>
<td>Concentration of osmolality</td>
</tr>
<tr>
<td>CAZy</td>
<td>the Carbohydrate-Active Enzyme</td>
</tr>
<tr>
<td>CBNC</td>
<td>Number of crown buds per cluster</td>
</tr>
<tr>
<td>CN</td>
<td>Cluster number of crown buds</td>
</tr>
<tr>
<td>DCB</td>
<td>Diameter of crown buds</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
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<tr>
<td>DW</td>
<td>Dry weight</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELSD</td>
<td>Evaporative light scattering detector</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh weight</td>
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<tr>
<td>GDD</td>
<td>Growing-degree-days</td>
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<td>Glycoside hydrolase(s)</td>
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<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>i</td>
<td>van ‘t Hoff factor</td>
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<tr>
<td>IAA</td>
<td>indole-3-acetic acid</td>
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<tr>
<td>LCB</td>
<td>Length of crown buds</td>
</tr>
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<td>MS</td>
<td>Murashige &amp; Skoog</td>
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<td>MWCO</td>
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<td>NZ</td>
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<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
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<tr>
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<tr>
<td>PVPP</td>
<td>polyvinylpolypyrrolidone</td>
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<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>SN</td>
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</tr>
<tr>
<td>SSP</td>
<td>Proportion of crown buds sprouting in Spring</td>
</tr>
<tr>
<td>T</td>
<td>Thermodynamic (absolute) temperature</td>
</tr>
<tr>
<td>TCBN</td>
<td>Total number of crown buds per plant</td>
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