

**Jan M.C. GEUNS**

**Proceedings of the 5th Stevia symposium,  
organised by EUSTAS 2011**

# **Stevia: Break-Through in Europe**

**KULeuven, 28 – 29 June 2011**

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# CHAPTER 1

## Understanding the biosynthesis of steviol glycosides, a convergence of genomics and biochemistry

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### ABSTRACT

Steviol glycosides are found in high concentrations in the leaves of the Paraguayan perennial herb *Stevia rebaudiana* and their intense sweetness, as well as high concentration in *Stevia* leaf tissue, has made them the subject of research interest for over 100 years. Steviol glycosides are diterpenoids whose biosynthetic pathways share four steps in common with gibberellic acid formation. The convergence of genomics and plant biochemistry has led to the rapid elucidation of the genes coding for the various enzymes in the biosynthetic pathway. Functional characterization of the enzymes coded for by those genes has resulted in the characterization of three glucosyltransferases, two terpene cyclases, and two cytochrome P<sub>450</sub>s from the pathway. The P<sub>450</sub> enzyme, ent-kaurene 13-hydroxylase (CYP72A60) which is the enzyme that catalyzes the first committed step in the synthesis of the steviol, will be discussed.

**KEYWORDS:** steviol, P<sub>450</sub>, kaurenoic acid, *Stevia rebaudiana*

## CHAPTER 2

### Light, day length and nodal position: how do they contribute to steviol glycosides biosynthesis in *Stevia rebaudiana* leaves?

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#### Keywords:

Terpene cyclase; Cytochrome P<sub>450</sub>; Glycosyltransferases; Photoperiodic response; Nodal position; Real-time quantitative PCR; Steviol glycosides; *Stevia rebaudiana*

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**Abbreviations:** ANOVA: analysis of variance; bp(s): base pair(s); Dul A: dulcoside A; *ent*-CPP: *ent*-copalyl pyrophosphate; *ent*-CPS: *ent*-copalyl pyrophosphate synthase; *ent*-KAH: *ent*-kaurenoic acid 13-hydroxylase; *ent*-KAO: *ent*-kaurenoic acid oxidase; *ent*-KO: *ent*-kaurene oxidase; *ent*-KS: *ent*-kaurene synthase; LD: long day; Reb A: rebaudioside A; Reb C: rebaudioside C; ROS: reactive oxygen species; RP-HPLC: reversed-phase high performance liquid chromatography; RT-q PCR: real-time quantitative polymerase chain reaction; SD: short day; ST: stevioside; SVgly: steviol glycoside; UGT: uridine diphosphate-dependent glycosyltransferase.

## CHAPTER 3

### **Accumulation pattern of steviol glycosides in *Stevia rebaudiana* Bertoni and its stimulation by red-light**

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#### **ABSTRACT**

The leaves of the Paraguayan herb *Stevia rebaudiana* Bertoni contain at least 30 steviol glycosides (SVglys), of which ST, Reb A, Reb C and Dul A accumulate the most. The accumulation of these SVglys occurs within different plant parts and is dependent on numerous environmental, agronomic and physiological factors. It is generally known that the greatest content of SVglys is reached when

## CHAPTER 4

### Evaluation of *Stevia rebaudiana* genotypes

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#### ABSTRACT

In the course of field experiments with *Stevia rebaudiana*, a vigorously growing genotype, named 'Gawi', adapted to European temperate zone climate conditions was selected at the University of Bonn. However, its stevioside-to-rebaudioside A-ratio was unsatisfactory. Therefore, alternative genotypes were collected. For the evaluations of vegetative performance and sweetener yield and quality, expressed as content of rebaudioside A, eight plants per genotype were cultivated in containers. They were kept under open field conditions and protected by two types of foil houses (FEP and PE), respectively, in order to support genotypes unsuitable to the local climate.

In terms of vegetative growth, the local selection Gawi out-yielded the other genotypes tested. However, with regard to sweetener quality, in 2009, it was out-performed by two genotypes, C and F, providing 140 % and 102 % greater contents of rebaudioside A, respectively. In 2010, lower temperatures and global solar radiation impaired over-all performance and modified ranking and profiles of the genotypes. Cultivation in foil houses had varying effects in 2009. Under lower temperatures in 2010, it resulted in improved biomass production and sweetener content. The transparency for PAR and UV radiation of the foil material was of minor importance.

**KEYWORDS:** *Stevia rebaudiana* genotypes, evaluation, performance, leaf dry weight, yield of rebaudioside A, profiles



## CHAPTER 5

### ***In vitro* conservation and cryopreservation of banana: a model for Stevia?**

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**KEYWORDS:** Banana, *Stevia*, active and base collection, *in vitro* conservation, medium-term storage, cryo-preservation, tissue-culture, gene-bank

#### **ABSTRACT**

The Laboratory of Tropical Crop Improvement of the KULeuven hosts the largest worldwide collection of banana (*Musa* spp.) comprising over 1300 edible and wild bananas, introduced from 52 different sources. Managed by the International Transit Center of Bioversity International, the gene-bank plays a key role on a global level bringing the assurance that a large proportion of the crop known gene-pool is conserved in perpetuity. The collection is also recognized as the most extensive source of healthy germplasm that is easily and freely accessible to users involved in banana research, conservation and breeding.

Banana, a perennial crop and vegetatively propagated, is traditionally conserved in field collections. Since this type of collection is easily affected by adverse environmental conditions, Bioversity established the global banana collection *in vitro*. Germplasm is maintained as tissue-culture plants under minimal growth conditions, allowing storage for a period of one year on average. Although this mode of preservation has proven its value, stored materials require regular monitoring in order to ensure their viability, health and genetic integrity. The maintenance of such a large active collection is thus labour intensive. Hence, to ensure the secure and cost-effective long-term preservation of the collection, a base collection using cryopreservation is being established. Freeze-preservation protocols utilizing the droplet vitrification were developed and are successfully applied to the different genotypes in the collection. To date, 850 accessions are stored in liquid nitrogen (-196°C) and a black-box safety duplicate is maintained off-site.

Based on the expertise built in banana, researchers at the Laboratory of Tropical Crop Improvement have been involved in developing conservation protocols for