

**Sample**

**Development of genic simple sequence markers for *Stevia rebaudiana* Bertoni using transcriptome data.**

**Afiq Azmi Azrul-Murad**1, Christina Seok Yien Yong1,\*, Yoeng Leh Tan1, Nurul Izza Ab Ghani1,2

1Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

2Laboratory of Sustainable Animal Production and Biodiversity, Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, UPM, Serdang, Selangor 43400, Malaysia

***\*Correspondence***

*Email: chrisyong@upm.edu.my; Telephone: +6016-8973221*

**Abstract**

*Stevia rebaudiana* is a high potential agricultural crop that yields natural compounds known as diterpenoid steviol glycosides (SGs), which have gained acceptance in recent years as a healthier alternative to substitute sucrose and other artificial sweeteners. Amid the escalating demand for Stevia, particularly in the food industry, the need to produce high-yield Stevia varieties via molecular breeding is highly desired. In this study, we investigated an in-house leaf and stem tissues transcriptome dataset consisting of 103,890 *de novo* assembled contigs using bioinformatic approaches. A total of 8,789 genic-SSR markers in 8,065 contigs were discovered. Pure trinucleotide genic-SSRs (52.66%) were the predominant repeats, followed by pure di- (35.32%), hexa- (6.48%), penta- (3.84%) and tetra-nucleotides (1.69%) in this dataset. Pathway analysis on 1,617 annotated contigs containing single pure genic-SSR further mapped them to 530 proteins in KEGG database; where most of them were mapped to the metabolic pathway, secondary metabolite biosynthesis pathway, and antibiotics biosynthesis pathway. A total of 20 genic-SSR markers were developed, in which 17 were successfully amplified in three different Stevia varieties, with three polymorphic markers found. Identification of SSRs from transcriptome data is proven to be an effective approach to develop novel SSR markers linked to functional genes for non-model plants. Genic-SSRs associated with genes of agronomic importance discovered in this study may contribute towards developing genetic markers for selective or molecular breeding of Stevia in the future.

**Keywords**: *Microsatellite*; *Molecular marker*; *Secondary biosynthesis pathway*; *Pure repeats*; *Diterpenoid steviol glycosides*