

UNIVERSITI TEKNOLOGI MARA

**GENERATING F₁ HYBRIDS OF
STEVIA (*Stevia rebaudiana* BERTONI)
USING TRIGONA-ASSISTED
POLLINATION TECHNIQUE**

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ABSTRACT

Stevia was first introduced into Malaysia in 1970s; however, to date there is no variety that is practical or suitable for local production under our tropical environmental conditions. Hybridization by manual crossing is extremely laborious and difficult. It is further aggravated by its very poor seed germination. Utilization of stingless bees in assisted-pollination contributed towards a significant breakthrough in overcoming barriers in hybridization for stevia breeding work. The main objective of the study is to generate a new hybrid local stevia variety. Six of ten accessions were selected as parents namely MS0012, K1, Bangi, MS023, Rawang and Taman Pertanian. Parents differed significantly in terms of plant height (13.5 cm – 28.0 cm), days to flowering (4 weeks – 17 weeks) and number of branches (1 - 7). *Trigona*-assisted pollination generated 389 seeds which comprised 76.1 percent black seeds. Five of 27 potential hybrids were selected based on their overall seedling vigour and a number of characteristics namely leaf size, number of branches, and height. However, only two potential hybrids survived due to acclimatization problems. For maternity test, five ISSR markers were used, and only two primers (IS57 and IS85) were found to be polymorphic. Molecular marker analysis confirmed that two hybrids were putative. Only F₁ hybrid 2 survived acclimatization and was evaluated. F₁ hybrid 2 was unlike its male parent K1, since it was significantly different in terms of its profuse branching habit. The study proved that the new technique of hybridization using *Trigona*-assisted pollination was able to produce successful F₁ hybrid for stevia breeding work.

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

In the search for alternative sweeteners to replace sugar for daily usage, *Stevia rebaudiana* Bertoni has been utilized in many forms of products. First discovered by a Swiss botanist, Moises Santiago Bertoni, this plant is utilized ever since. This plant is also known by many kinds of names such as sweet leaf, sugar leaf and many more, but it is more commonly known as stevia. Originated from Paraguay, this herbaceous plant is a well-known plant for its sweet taste. The main source for its sweetness is the leaf and it contains a high level of steviol glycosides, stevioside and rebaudioside-A. Compare to normal sugar or sucrose, stevia tastes 300 times sweeter and is considered as a good replacement for sugar, particularly for diabetics.

Based on Guleria *et al.*, (2104), stevia showed the highest net return per hecter compare to other medicinal and aromatic plants (safed musli, aloe vera and lemon grass). Nordiana *et al.*, (2016) investigated the cost of stevia production between sugarcane which came into conclusion that stevia production able to generate higher profit compared to sugarcane. Rise in demand for low calories food products due to health awareness, boost the demand for stevia (Paula *et al.*, 2016). With high demand for alternative sweeteners has increased which such demand able to increase the economic value of stevia in the world.

Stevia was introduced in Malaysia in the 1970s when the plant was first propagated and planted. The earliest work done in Malaysia was conducted by Tan *et al.*, (2008) under Malaysian Agriculture Research and Development Institute (MARDI). In the study, stevia accessions were collected and propagated for selection under Malaysia condition. However, from the sixty-nine accessions, only five were suitable and recommended as planting materials for Malaysia condition. From the finding, they concluded that the main problem for stevia in Malaysia is photoperiod sensitive. From all of the plants domesticated in Malaysia, stevia in the earliest to flower as compared to its origin. This contributes to low in leaf yield and plant growth. Harvesting of stevia needs to be carried out before flowering because the level of the sweetness decreases as soon as the plant begin to flower. Even though there