

UNIVERSITI TEKNOLOGI MARA

**EFFECT OF ACUTE GAMMA
IRRADIATION TOWARDS
MORPHOLOGICAL
CHARACTERISTICS, GENETIC
VARIATIONS AND STEVIOSIDE
COMPOUND OF *Stevia rebaudiana***

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ABSTRACT

Stevia rebaudiana is a short-day plant that would generate flower early under a constant day length less than 12 hours in Malaysia. The main problem of stevia in Malaysia is its photoperiod sensitive trait that could stop producing more leaf biomass and resulting in low content of steviol glycosides compound. Cultivation of stevia in Malaysia is a challenge due to the lack of suitable variety that suit the local condition. Therefore, development of new stevia variety is needed. The method applied was acute gamma irradiation with six doses (50 Gy, 100 Gy, 250 Gy, 325 Gy, 400 Gy and 500 Gy) to investigate the effect of radiation on stevia seeds. The qualitative and quantitative analysis were carried out to determine the changes of morphological traits of irradiated stevia. The LD₅₀ value was at 182 Gy indicated that 50% of irradiated stevia seedlings would die if exposed to more than 182 Gy. The number of days for seed to germinate varied in all treatments and none of the seedlings survived at 400 Gy and 500 Gy of irradiation. The plant height recorded was highest in treatment 50 Gy (142.0cm±1.98). Meanwhile, for other parameters such as the number of leaves, the number of branches were decreased as the dose increases. The parameter mentioned on 250 Gy and 325 Gy obtained decreased in numbers as the doses were exceed the LD₅₀. The findings of this study suggest that doses of radiation range below than 325 Gy were recommended for the improvement of stevia by acute gamma irradiation. Next, study on genetic variation and steviol glycosides content were carried out to evaluate the effect of gamma irradiation on the stevia plant. The study on molecular analysis was performed using PCR based ISSR marker to assess the genetic variation of the irradiated stevia by using ten ISSR primers. Based on the observation, ISSR molecular analysis generated 81.48% of genetic polymorphism and showed 18.52% on monomorphism. It showed that IS12, IS90 and IS52/1 primers were found to be 100% polymorphic. Meanwhile for IS20 and IS21 primers were 100% monomorphic. For the steviol glycoside analysis on the stevioside content of the irradiated stevia was quantified by Brix and HPLC analysis. The results showed that there is an increment on treatment 250 Gy which is 7% and 4.26% with the highest stevioside compound present in the leaf compared to other treatment respectively. We can conclude that the content of the stevioside affected by the different doses of gamma irradiation. Hence this study contributed to the development of stevia mutants using acute gamma irradiation and DNA polymorphism revealed by the PCR based ISSR can be used as the molecular marker for identifying alterations and mutation in gamma treated plants. In conclusion, different observations on morphological characteristics and stevioside compound content of irradiated stevia are due to the gamma mutation occurs randomly. On the other hand, PCR based ISSR offers a reliable molecular marker for the detection on genetic variations in gamma irradiated stevia plant.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Stevia rebaudiana Bertoni is a herbaceous perennial plant native to Paraguay that belongs to the Asteraceae family (South America) and it is well-known plant for its sweet taste. It 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. Stevia is a woody shrub that can reach 80 cm in height when it is fully matured. Moreover, the stevia genus comprises of at least 110 species but there may be as many as 300 (Goyal et al., 2010). At present, more than 200 species of stevia are present around the world, but *Stevia rebaudiana* is the only one with a sweet taste (Shivanna et al., 2013; Ahmad et al., 2020). In Malaysia, stevia cultivation dates back to the early 1970's (Tan et al., 2008). The earliest work done in Malaysia was conducted under Malaysian Agriculture Research and Development Institute (MARDI). The most beneficial part of this shrub is the leaves and it contains a high level of steviol glycoside, stevioside and rebaudioside A. Only 18 species out of 110 that were examined for sweetness possessed this characteristic (Goyal et al., 2010). Compare to normal sugar or sucrose, stevia taste 250-300 times sweeter and is considered as a good replacement for sugar, particularly for diabetics (Ashwell, 2015; Ali Zafar et al., 2022). Stevia is unique because it produces steviol glycosides that give the leaves a sweet taste (Halimatun and Zarina, 2022; Emily, 2022). According to Yadav et al. (2011) stevioside was viewed as a sugar substitute with the intention of commercialization in the early 1970s. Steviol glycosides, which are found in stevia leaves several times sweeter than sugar. They can be used as an alternative to artificial sweeteners for people who lead healthy lifestyles. The leaves also contain other compounds such as phenols, flavonoids, vitamins and alkaloids (Angelini et al., 2018). These properties make *Stevia rebaudiana* as a foremost species towards the development of functional foods characterized by a low caloric contribution and potent antioxidant properties appropriate for diabetics, dieters and health-conscious people.