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GENETIC STUDIES OF STEVIOSIDE CONTENT AND OTHER AGRONOMIC CHARACTERS AND THEIR RESPONSES TO CLONING IN STEVIA REBAUDIANA BERT.

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GENETIC STUDIES OF STEVIOSIDE CONTENT AND OTHER AGRONOMIC CHARACTERS AND THEIR RESPONSES TO CLONING IN *STEVIA REBAUDIANA* BERT.

By

WONG LOCK JAM

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I would like to dedicate

this thesis to Maggie for giving me

the best years of her life

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September 1991

Supervisor: Prof. Dr. Yap Thoo Chai

Faculty: Agriculture

Stevia rebaudiana Bert. produces the sweetener stevioside, which is 300 times sweeter than cane sugar. However, the mean stevioside content of commercial cultivars was generally low and among the better cultivars the average stevioside content was in the region of seven per cent. Studies were set up to investigate the genetic system of stevioside content and other traits, the effects of cloning and the relationship of stevioside content to the other traits. Three segregating populations were studied, whereupon 18 lines were selected for studying the effects of cloning.

The mean stevioside contents of JPK(1), JPK(2) and OKA populations were 5.0%, 4.7% and 5.8% respectively. Plant heights for the three respective populations were 45.1 cm, 41.9 cm and 42.0 cm. The mean LAI for JPK(1) and OKA were 1.7 and 1.0 respectively. Dry leaf and dry stem weight were 6.3 g and 17.6 g per plant for JPK(1), 7.2 g and 12.6 g for JPK(2), and 4.9 g and 16.1 g for OKA.

The clonal studies revealed highly significant differences between lines for plant height, branch number, leaf area, leaf weight, stem weight, stevioside content and stevioside weight. However, cloning has no effect on the above traits.

In the 7 x 7 diallel analysis, effects due to genotypes were highly significant for stevioside content, stevioside weight, plant height, leaf and stem weights. Heterobeltiosis was also detected in the same traits, but was more prominent for both stevioside content and stevioside weight. For the former, 19.1% of the crosses showed heterobeltiosis, whereas for the latter it was 9.5%. Analyses for GCA and SCA effects showed additive genes were more important than non-additive genes for stevioside content and stevioside weight. Non-additive genetic effects were important for plant height and leaf weight. For stem weight, both additive and non-additive

effects were important. JPK 24, JPK 48 and OKA 10 had the highest GCA estimates for both stevioside content and stevioside weight. The same lines also had very high stevioside contents, 11.5%, 9.8% and 8.6%, respectively, and stevioside weights, with 69, 44 and 54 mg per plant, respectively. The W_r/V_r analysis showed gene action for stevioside content among the six parents, but excluding OKA 43, was partial dominance. OKA 43 exhibited non-allelic interaction for this trait in its crosses. For stevioside weight the gene action was also partial dominance. The non-stevioside traits of plant height, leaf and stem weights had gene action varying from complete dominance to over-dominance.

It was concluded that further improvement for both stevioside content and stevioside weight could be simultaneously achieved, since both traits were highly correlated, by intercrossing among JPK 24, JPK 48 and OKA 10 followed by selection among the progenies and the genotypes of selected lines can then be fixed by cloning.

Abstrak tesis dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi syarat ijazah Doktor Falsafah

KAJIAN GENETIK KANDUNGAN STEVIOSIDE DAN CIRI-CIRI AGRONOMI LAIN DAN TINDAKBALAS KEPADA KLONING DI DALAM *STEVIA REBAUDIANA* BERT.

Oleh

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Stevia rebaudiana Bert. menghasilkan satu pemanis, stevioside, yang 300 ganda lebih manis jika dibandingkan dengan gula tebu. Walau bagaimanapun, purata kandungan stevioside bagi kultivar komersial pada keseluruhannya adalah rendah, dan bagi kultivar yang baik adalah dalam lingkungan tujuh peratus. Kajian telah dijalankan untuk menyiasat sistem genetik yang mempengaruhi kandungan stevioside, kesan pembiakan klon, dan perhubungan antara kadar stevioside dengan ciri-ciri lain. Tiga populasi pengasingan telah dikaji, di mana 18 titisan terpilih untuk pengkajian kesan pembiakan klon.

Min kandungan stevioside bagi populasi JPK(1), JPK(2) dan OKA adalah 5.%, 4.7% dan 5.8%. Ketinggian pokok bagi ketiga-ketiga populasi tersebut adalah 45.1 cm, 41.9 cm dan 42.0 cm dan bilangan ranting masing-masing adalah 34.3, 18.9 dan 39.8 sepokok. Min LAI (indeks luas permukaan daun) bagi JPK dan OKA adalah 1.7 dan 1.0. Berat daun kering dan berat dahan bagi sepokok adalah 6.3 g dan 17.6 g bagi populasi JPK(1), 7.2 g dan 12.6 g bagi populasi JPK(2), 4.9 g dan 16.1 g bagi populasi OKA. Perkaitan kadar stevioside adalah ketara dengan ketinggian pokok, bilangan ranting, luas permukaan daun dan berat daun bagi populasi JPK(1), tetapi untuk populasi OKA perkaitan kadar stevioside hanya dengan luas permukaan daun. Manakala bagi populasi JPK(2), perkaitan yang ketara hanya dengan berat dahan sahaja.

Pembiakan klon tidak mempengaruhi ketinggian pokok, bilangan ranting, luas permukaan daun, berat daun, berat dahan, kandungan stevioside dan berat stevioside. Perbezaan antara titisan adalah ketara bagi setiap ciri-ciri yang dicatitkan.

Bagi analisis kacukan separuh dialel 7 x 7, perbezaan antara genotip adalah berkesan bagi kandungan stevioside, berat stevioside, ketinggian pokok, berat daun dan berat dahan. Heterobeltiosis juga adalah ketara

tentang ciri-ciri tersebut, khususnya bagi kandungan stevioside dan berat stevioside. Analisis untuk kesan GCA (general combining ability) dan SCA (specific combining ability) terhadap tujuh titisan menunjukkan bahawa gen aditif adalah lebih berkesan jika dibandingkan dengan gen bukan aditif bagi kandungan stevioside dan berat stevioside. Gen bukan aditif adalah berkesan bagi ketinggian pokok dan berat daun. Kedua-dua gen aditif dan bukan aditif adalah berkesan bagi berat dahan. Ketiga-tiga klon induk JPK 24, JPK 48 dan OKA 10 mempunyai GCA yang paling tinggi bagi kandungan stevioside dan berat stevioside, juga mempunyai min kandungan stevioside yang paling tinggi, iaitu 11.5%, 9.8% dan 8.6%, dan min berat stevioside yang paling tinggi, iaitu 69, 44 dan 54 mg setiap satu. Analisis W_r/V_r menunjukkan pergerakan gen separuh dominan bagi kandungan stevioside antara enam daripada sejumlah tujuh klon induk. OKA 43 melalui titisan kacukan mempamerkan interaksi bukan alel. Jenis pergerakan gen untuk berat stevioside adalah separuh dominan. Untuk ciri-ciri bukan stevioside seperti ketinggian pokok, berat daun dan berat dahan, jenis pergerakan gen adalah dari dominan penuh ke `over-dominance'

Kesimpulannya, kemajuan lanjutan bagi kedua-dua kandungan stevioside dan berat stevioside boleh dicapai

dengan kacukan antara JPK 24, JPK 48 dan OKA 10 diikuti dengan pemilihan dan pembiakan titisan terpilih dilakukan dengan melalui cara pembiakan klon.

CHAPTER I

INTRODUCTION

In the exploitation of plants for the production of natural sweeteners, *Stevia rebaudiana* Bert. stands out as an important crop because of its intrinsic value as a producer of the sweetener stevioside. The latter is about 300 times sweeter than cane sugar, i.e. 3.5 g of stevioside would have the equivalent sweetness of 1 kg of cane sugar. Since stevioside has negligible carbohydrate value and the amount to be consumed each time is very small, it is aptly dubbed `sugarless sugar'. Although it tastes like sugar, stevioside is different from sugar in that it does not contribute to health problems such as diabetics, tooth decay, obesity and heart related diseases.

Stevia is an introduced crop in Malaysia and there are at least three reasons for interest in its cultivation in this country. Firstly, the tropical environment of this country, characterized by high annual rainfall with hardly any distinct agricultural drought and perpetual sunshine throughout the year, is capable of sustaining continuous cropping all the year round. Secondly, this crop was first brought into Malaysia by

Japanese entrepreneurs with the main purpose of producing *Stevia* leaves cheaply for the stevioside factories in Japan as it is not economical to do so in their own country. Thirdly, *Stevia* cultivation has potential in our country because of its ready market for sweeteners. Malaysia imports more than one million tonnes of sugar annually, incurring a loss in foreign exchange exceeding 600 million ringgit per annum (Kementerian Pertanian, 1984; 1985; and 1986). Local production of cane sugar could only meet 20 per cent of the total demand. Furthermore, it is not economically feasible for any expansion in sugar-cane hectareage since agroclimatically suitable land is not readily available.

Unfortunately, the interest in *Stevia* failed to sustain its cultivation on a commercial scale. The main reason for the failure was the unavailability of widely acceptable *Stevia* cultivars with good adaptation and high stevioside content. *Stevia* leaves produced by local farmers could not be marketed. When the Japanese entrepreneurs first introduced *Stevia* cultivation in Malaysia, each company brought their own cultivars from Japan. The main reason for doing so was the high variability in stevioside contents among the cultivars. The stevioside contents of their cultivars were later found to vary from 4% to 7% (Wong and Lim, 1980). Believing theirs were superior, *Stevia* cultivars were

regarded as company secret. As such, one company would not buy *Stevia* leaves produced by another. Instead, each company would send *Stevia* leaves back to their parent companies in Japan.

In order to assist *Stevia* cultivation in this country, the development of locally adapted genotypes with high stevioside content is essential. Hence, studies have been set up to investigate the genetic system affecting the trait on stevioside content; the effect of selection and cloning on it; and its relationship to selected characters of the plant. Hopefully, information and cultivars generated from the studies conducted would be useful for planning our *Stevia* breeding programme in Malaysia.

CHAPTER II

LITERATURE REVIEW

The review of literature is organised under two main sections, the first section is on the development and cultivation of *Stevia rebaudiana* as it is a new crop (refer Plate 1), and the second is on the diallel mating systems because of its relevance to the research herein conducted.

Stevia rebaudiana Bertoni. - A New Crop

Stevia rebaudiana Bertoni. is a small shrub found mainly in the Amambai Highlands and the high northern plateau of Paraguay (Crosby and Wingard, 1979; Gosling, 1901). Hence, the plant is native to Paraguay (Shock, 1982; Soejarto *et al.*, 1983). It has been used in Paraguay and Brazil as a sweetener for a long time and is well known for sweetening the Paraguayan mate' tea (Inglett, 1981; Crammer and Ikan, 1986).