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Preliminary evaluation and selection of stevia under Malaysian conditions

(Penilaian dan pemilihan awal aksesi stevia dalam keadaan Malaysia)

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Key words: stevia, accessions, field evaluation, leaf yield, ratooning

Abstract

Accessions of stevia (*Stevia rebaudiana* Bertoni) were introduced from Russia, Paraguay, Canada, USA and Japan. After vegetative propagation, they were field planted for observations on their leaf yield over 11 harvests in the period of one year. Of the 67 accessions, six with high rates of success in vegetative propagation (thus, yielding more plants) were selected. All of these originated from Canada, except for one from Russia. The selected accessions were evaluated in two field trials, over seven and six harvests. The highest yielding accessions were MSR 028, MR 012 and MR 007. MSR 028 topped 10 t/ha in cumulative fresh leaf yields, which is equivalent to 2.8 t/ha dry leaf yield and similar to what has been reported in Canada where stevia is planted as an annual. Thus, ratooning may be able to overcome the problem of early harvests (after 1 month) necessitated by the short daylength conditions in Malaysia.

Introduction

Stevia (Stevia rebaudiana Bertoni) is a herbaceous plant originating from Paraguay. The plant, especially the leaves, produces diterpene glycosides, primarily stevioside and rebaudioside, which have a sweetening effect reportedly 40–250 times sweeter than sucrose (Brandle 2004). For this reason, interest in this plant has picked up in recent years because of its potential as a non-calorific natural sweetener – a boon to the health food industry, particularly targeted to individuals with an obesity problem as well as diabetics.

Interest in this plant has also arisen in Malaysia as a result of the sugar shortage not too long ago. Except in the extreme northern part of the Peninsula, the generally wet climate of Malaysia does not favour sugarcane cultivation, and the country has been largely dependent on sugar imports to satisfy local demand. Malaysians are known for their 'sweet tooth', consuming a lot of sugar not only in their drinks and beverages but also in their snacks, such as traditional cakes. This unhealthy habit may be partially responsible for the alarming rise in diabetic cases – from 0.65% of the population in 1960 to 16–18% in 1998 (Mustaffa 1998).

Apart from a doctoral thesis in 1990 (Wong 1990), very little research has been carried out on stevia in the country. With the resurgence of interest, in 2004, several introductions were made from various sources which were subsequently evaluated under local conditions. This paper reports preliminary results of these evaluations,

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culminating with the shortlisting of a number of promising accessions.

Materials and Methods

Single plot observations

Through a collaborative project with a private company, Stevian Biotechnology Corporation Sdn. Bhd., accessions of stevia were introduced from Russia, Paraguay, Canada, USA and Japan. The accessions were multiplied vegetatively through cuttings in a greenhouse used as the nursery. When they reached a height of 5 cm, the plants were transferred to the field at MARDI Headquarters Station, Serdang.

In the field, the various accessions including one originally studied by Wong (1990), were planted in 1.2 m wide ridges, spaced 24 cm apart, with a plant spacing of 20 cm. Basal fertilizer using organic fertilizer (processed chicken dung) at 1 t/ha was applied between ploughing and building the beds. After the first ratoon, 0.5 t/ha of organic fertilizer was applied. The next fertilizer application was after the third ratoon, using 0.5 t/ha of organic fertilizer and 0.5 t/ha of a compound NPK fertilizer (10:10:10), and thereafter after every 1½ months as in *Table 1*. The fertilizers were applied between the plant rows (Muhammad Ghawas et al. 2007). Manual weeding was carried out twice per month.

Owing to early bud emergence under the short daylength conditions in Malaysia (*Plate 1*), the plants were harvested at one month after field-planting in July 2005. Harvesting was carried out by cutting back the plants at about 8–10 cm from the soil surface. Thereafter, the plants were harvested approximately every month over a period of one year from the time of field-planting (until May 2006), totalling 11 harvests. Owing to the variable numbers of plants from each accession, the final cumulative yield (in t/ha) of each accession was calculated based on a population of 166,667 plants/ha.

Table 1. Frequency of fertilizer applications and rates in the evaluation trials on stevia accessions

Ratoon	Months after field planting	Fertilizer rate (t/ha)	
		Organic	NPK
1st	3.0	0.5	–
2nd	4.5	–	–
3rd	6.0	0.5	0.5
4th	7.5	–	–
5th	9.0	0.5	–
6th	10.5	–	–
7th	12.0	1.0	1.0

Plate 1. Early flowering in the field due to local short-day conditions

Selection of promising accessions were based on two criteria:

- Group A comprised accessions which had a high rate of success in vegetative propagation, yielding more plants.
- Group B comprised accessions which had a high computed cumulative yield per hectare.

Replicated trials

All the replicated trials adopted the same agronomic and cultural practices as before.

Trial 1 The six accessions in Group A were evaluated in a replicated trial (following a Randomized Complete Block Design or RCBD) over seven harvests from November 2005 to July 2006. Two checks were included in the trial, namely MSR 031 (an accession of Russian origin with moderate yield, of interest to Stevian Biotechnology Corporation Sdn. Bhd.)

and MSR 069, the 'Malaysian' accession. Sample size per plot in each of the three replications was 30 plants.

Trial 2 The above trial was duplicated with the six accessions in Group A in a replicated trial (RCBD) over six harvests from February to July 2006. No check variety was used in Trial 2. Every plot in each of the four replications accommodated 45 sample plants as well as border rows. Trial 2 was conducted to confirm the yield performance of the accessions over a different growing period.

Results and discussion

General botanical description

Stevia is a perennial herbaceous plant, with simple, opposite leaves with rather short petioles (*Plate 2*). Leaf shape can range from oblanceolate (MSR 007) to ovate (MSR 012) to spatulate (MSR 028) (*Plate 3*). The leaf margin is generally dentate although may be scalloped as in MR 028.

The plants are determinate, i.e. will stop growing once they bloom as the flowers are borne at the tips of the branches or stems. Flower heads consist of five white florets each.

Single plot observations

A total of 68 accessions resulted from the seed batches introduced as well as one 'Malaysian' accession, left over from the research carried out by Wong (1990) (*Table 2*). Two of the total of 69 accessions failed to be propagated and were dropped from the field evaluation. The number of plants propagated from each accession ranged from 2 to 87.

There was an increasing trend in yield over the 11 harvests as shown by the yields per harvest, averaged over the 67 accessions (*Figure 1*). There appears to be a dip in yields at the sixth and ninth harvests.

Plate 2. ***Stevia rebaudiana*** *plant*

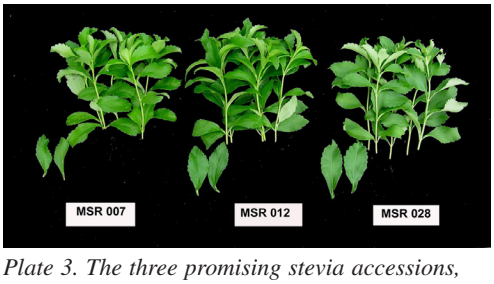

Plate 3. The three promising stevia accessions, MSR 007, MSR 012 and MSR 028

Table 2. Number of stevia accessions derived from seeds of different origins

Accession numbers	No. of seedling accessions	Surviving accessions	Country of origin
MSR 001–MSR 028	28	27	Canada
MSR 029–MSR 054	26	25	Russia
MSR 055–MSR 057	3	3	Paraguay
MSR 058–MSR 060	3	3	USA
MSR 061–MSR 068	8	8	Japan
MSR 069	1	1	'Malaysia'

Data from those accessions with the most number of plants (Group A) as well as those with the highest extrapolated yields from the 11 harvests over a period of one year (Group B) are presented in *Table 3*. In Group A, accessions MSR 012 and MSR 029 were higher yielding than the check, MSR 069 (67.4 t/ha). Except for MSR 029 which came from Russia, these accessions originated from Canada. All the nine accessions in Group B outyielded MSR 069 by 223–300%, i.e. about 2¼ to 3 times. The countries of origin of these accessions were Canada, Russia and USA.

Replicated trials

The six accessions in Group A were observed to have wider leaves. Mean yields of these six accessions over the seven harvests in Trial 1 are presented in *Figure 2*. There was a peak in yields at the sixth harvest, followed by a decline at the seventh harvest. This peaking in yield did not seem to be associated with the monthly rainfall pattern. All six accessions produced cumulative yields not significantly different from the two checks, MSR 031 and MSR 069. The highest cumulative yield obtained by accession MSR 007 at 10.4 t/ha was 56% and 78% higher than MSR 031 and MSR 069, respectively. This is followed closely by MSR 028 at 10.3 t/ha (*Table 4*).

In Trial 2, the trend in increasing yields followed by yield decline was repeated (*Figure. 3*), with the peak at the fifth harvest rather than the sixth. Again, the peak was not associated directly with the rainfall pattern. The best performing accession after six harvests was MSR 028 at 10.8 t/ha, a yield which was significantly higher than all the other accessions except MSR 012 (*Table 4*).

Based on the results from Trials 1 and 2, it may be concluded that the most promising accessions in Group A are MSR 028, MR 012 and MR 007 (*Plate 3*). It

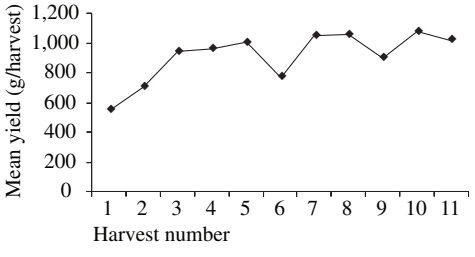

Figure 1. Yield trend (averaged over 67 accessions) of stevia over 11 harvests in one year

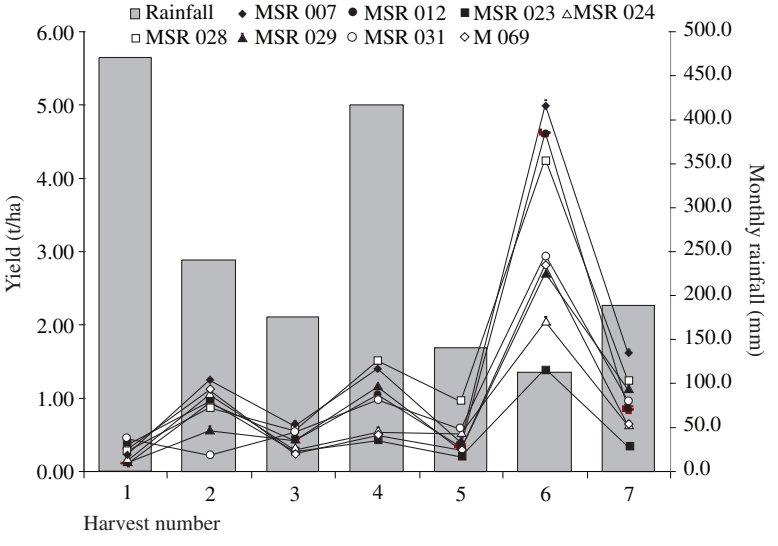

Figure 2. Yield trends of six selected stevia accessions compared to two checks (MSR 031 and MSR 069) over seven harvests in Trial 1

Table 3. Stevia accessions which are promising in terms of number of plants or cumulative yields compared with the check MSR 069

Accession	Origin	Plants/ plot	Harvest number											Total (g)	Cumulative yield (t/ha)
			1	2	3	4	5	6	7	8	9	10	11		
Group A															
MSR 007	Canada	87	2500	3800	3380	2800	*	3000	5620	4620	2170	3410	3720	35020	67.22
MSR 012	Canada	76	2760	2910	2020	4120	*	4550	4940	4940	3100	5560	3920	38820	85.30
MSR 023	Canada	66	1910	2050	1980	2060	3500	2100	1700	1700	1800	1650	1780	22230	56.25
MSR 024	Canada	63	1100	1600	*	1760	2670	2050	2350	2350	2400	2550	2660	21490	56.97
MSR 028	Canada	79	1950	2200	3400	3380	4700	2150	1680	1680	2000	2850	3560	29550	62.47
MSR 029	Russia	79	2200	6200	5200	5500	5300	5600	2960	2960	2490	1750	1520	41680	88.11
Group B															
MSR 005	Canada	5	280	400	420	320	320	320	380	380	310	760	1000	4890	163.33
MSR 013	Canada	18	800	800	1720	2780	2260	1440	1550	1550	1400	1580	1520	17400	161.43
MSR 017	Canada	17	400	450	1300	2380	2340	1360	1760	1760	1750	800	3400	17700	173.88
MSR 025	Canada	18	900	1020	1700	1880	1460	1600	2280	2280	2200	2100	1800	19220	178.32
MSR 050	Russia	14	510	970	1540	1500	1500	1150	1240	1240	1150	1700	1100	13600	162.23
MSR 051	Russia	14	500	930	1520	1300	1680	1200	1260	1260	1200	1100	680	12630	150.66
MSR 058	USA	15	460	980	1700	1400	1280	800	2000	2000	1690	1480	720	14510	161.54
MSR 059	USA	10	220	480	1520	890	900	790	1600	1600	720	1280	660	10660	178.02
MSR 060	USA	14	600	850	1100	1100	1300	600	2400	3000	1760	2220	2020	16950	202.19
MSR 069	'Malaysia'	58	2500	2850	*	*	*	1650	2700	2700	3660	4500	2860	23420	67.43

*No harvest because plant growth too little

is also interesting to note that at the peak harvest, the yield was almost half the cumulative yield after sixth or seventh harvests. This may have implications on the decision of whether or not to continue maintaining and harvesting the ratoon crops past the peak yields.

It has been estimated in Canada that a dry yield of 2.2 t/ha of dried leaves is necessary to make stevia cultivation profitable (Columbus 1997). With a conversion rate of roughly 28% from fresh to dry, this is equivalent to a fresh yield of 7.88 t/ha. The practice in Canada is to handle the crop as an annual and completely harvest it in one go when the plants reach about 40–60 cm tall after about 4 months. This is followed by replanting. However, in Malaysia because of short daylength conditions, the plants have to be harvested much earlier before they flower, i.e. at about one month after field-planting. However, by managing stevia as a ratooning crop, the data from our trials show that yields exceeding the break-even yield set by Canada were achieved after 6–7 months.

Table 4. Cumulative yields over 7 and 6 harvests of selected stevia accessions in Group A

Accession	Cumulative yield (t/ha)	Peak yield (t/ha)
At 6th harvest		
MSR 007	10.44a	5.00
MSR 028	10.33ab	4.26
MSR 012	8.60ab	4.63
MSR 029	6.81ab	2.70
MSR 031 (Check 1)	6.71ab	2.96
MSR 024	5.80ab	2.04
MSR 023	5.42b	1.41
MSR 069 (Check 2)	5.85ab	2.81
At 5th harvest		
MSR 028	10.76a	4.26
MSR 012	7.07ab	3.42
MSR 024	5.41b	2.21
MSR 007	5.15b	2.13
MSR 029	4.52b	2.14
MSR 023	3.84b	1.37

Values with the same letter are not significantly different (p <0.05)

Using the above-mentioned conversion rate, 10 t/ha fresh yield is equivalent to 2.8 t/ha dry yield. This is higher than the yields reported in China (1.3–1.4 t/ha), equivalent to Canadian yields (2.85 t/ha), but lower than yields in USA (3.6 t/ha), Russia (1.4–5.5 t/ha) and India (4 t/ha) (Midmore and Rank 2002). In order to improve stevia yields in Malaysia, it is obviously necessary to select for less photosensitive genotypes to ensure that the plants do not flower so early which necessitates an equally early harvest, leaving insufficient time for the crop to develop more biomass.

It will also be necessary to study the performance of the nine accessions in Group B which show high yield potential but were marred by their lower success rate in vegetative propagation. With the improving techniques in vegetative propagation since

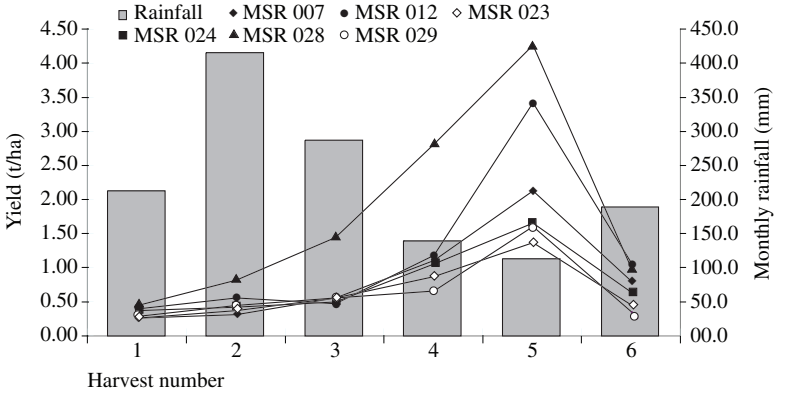

Figure 3. Yield trends of six selected stevia accessions over six harvests in Trial 2

the time of their introductions, this problem with multiplication rate may be overcome.

Acknowledgement
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Abstrak

Aksesi stevia (*Stevia rebaudiana* Bertoni) telah dibawa masuk dari Rusia, Paraguay, Kanada, Amerika Syarikat dan Jepun. Selepas pembiakan tampang, aksesi tersebut ditanam di ladang untuk pemerhatian hasil daun selama 11 penuai dalam tempoh setahun. Antara 67 aksesi, enam yang menunjukkan kadar pembiakan tampang yang tinggi (dengan menghasilkan lebih anak pokok) dipilih. Semuanya berasal dari Kanada, kecuali satu aksesi dari Rusia. Aksesi terpilih dinilai dalam dua uji kaji ladang masing-masing selama tujuh dan enam penuaian. Aksesi yang paling berpotensi dari segi hasil terdiri daripada MSR 028, MR 012 dan MR 007. MSR 028 dapat melebihi hasil segar kumulatif sebanyak 10 t/ha dalam kedua-dua uji kaji. Hasil ini bersamaan dengan 2.8 t/ha hasil daun kering dan tidak berbeza daripada apa yang dilaporkan di Kanada apabila stevia ditanam sebagai tanaman semusim. Ini bermakna, melalui cara ratun, mungkin masalah menuai awal (selepas satu bulan) yang terpaksa dilakukan kerana keadaan tempoh siang yang pendek di Malaysia dapat diatasi.

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