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Acclimatization and early growth of tissue culture-derived *Stevia rebaudiana* at low altitude area in Bogor, Indonesia

Aklimatisasi dan pertumbuhan awal Stevia rebaudiana *asal kultur jaringan pada dataran rendah di Bogor, Indonesia*

Masna Maya SINTA*) & Dian Mutiara AMANAH

Pusat Penelitian Bioteknologi dan Bioindustri Indonesia, Jl. Taman Kencana No.1, Bogor 16128, Indonesia

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Abstrak

Aklimatisasi merupakan masa transisi sebelum kultur in vitro *ditanam di lingkungan* ex vitro*. Di daerah tropis, stevia seyogianya ditanam di dataran tinggi. Pengembangan klon stevia yang sesuai untuk dataran rendah di kawasan tropis sangat penting untuk memungkinkan penggunaan mekanisasi pada pertanaman stevia yang luas. Tujuan penelitian ini adalah menentukan pengaruh kondisi eksplan dan periode hardening terhadap daya hidup dan pertumbuhan pada tahap aklimatisasi dan pertumbuhan awal stevia klon BS 22 pada area terbuka di dataran rendah di wilayah tropis. Penelitian pertama dilangsungkan menggunakan umur tunas yang berbeda: 0, 1, 2, dan 3 minggu yang dikultur pada media padat sebagai sumber bahan eksplan. Penelitian kedua menggunakan satu buku stevia pada periode hardening dalam media cair selama 1, 4, 7 dan 10 hari. Aklimatisasi dilaksanakan dengan menanam eksplan dalam medium tumbuh campuran pada multi-tray dan diletakkan di dalam sungkup plastik tertutup selama 1 bulan. Tanaman yang berhasil hidup kemudian dipindah ke polibeg pada area terbuka dengan sinar matahari penuh. Pengamatan daya hidup dan pertumbuhan dilakukan pada akhir tahap aklimatisasi dan setelah 2 bulan di area terbuka. Hasil penelitian menunjukkan bahwa eksplan umur 1 minggu yang dikultur pada media padat mempunyai daya hidup tertinggi yakni 83%. Buku tunggal stevia yang dikultur dalam medium cair pada tahap hardening selama 4 hari meningkatkan daya hidup menjadi 97% selama aklimatisasi 1 bulan. Setelah aklimatisasi, tinggi tanaman secara rata-rata adalah 2,6 cm dengan 10,6 helai daun. Tanaman yang dipindah ke area terbuka tumbuh pesat dengan tinggi tanaman mencapai 12 cm dengan 30 helai daun dan daya hidup 63% setelah 2 bulan. Hal ini menunjukkan bahwa stevia klon BS 22 mungkin sesuai untuk dataran rendah di daerah tropis.*

*) Penulis korespondensi: mayasinta77@gmail.com

[Kata kunci: tanaman pemanis, eksplan tunas, tahap hardening, daya hidup, daerah tropis]

Abstract

Acclimatization is a transition period before *in vitro* culture will be planted in *ex vitro* environment. In the tropical region, stevia is should be planted at high altitude areas. The development of stevia clones suitable for low land area in the tropics is important to make it possible to apply mechanization in a large scale stevia plantation. The objective of the research was to determine the effect of explant conditions and hardening period on survival rate and growth during acclimatization stage and early growth of stevia clone B 22 in an open area at low altitude area in the tropics. The first experiment was conducted using different shoot ages: 0, 1, 2 and 3 weeks cultured on solid media as an explant material source. The second experiment was using a single node of stevia in different hardening periods in liquid media for 1, 4, 7 and 10 days. Acclimatization was carried out by planting the explants on a mixture growing medium in multi-trays and placed inside a closed plastic tunnel for 1 month. The survival rate and growth parameters were observed at the end of the acclimatization stage and after 2 months in the open area. The results show that 1-week explant age on solid media had the highest survival rate at 83%. Hardening single node of shoot in a liquid medium for 4 days increased the survival rate to 97% in 1-month acclimatization stage. After acclimatization, the plant height on average was 2.6 cm with 10.6 leaves. The survived plants planted in an open area grew rapidly to 12 cm in height with 30 leaves and survival rate 63% within 2 months. It indicated that stevia clone BS 22 may suitable for a low altitude area in the tropics.

[Key words: sweetener plant, shoot explant, hardening period, survival rate, tropical region]

Introduction

Stevia rebaudiana is a sweetener plant reported to have zero calorie, zero glycemic index, and with a sweetness level 200-300 times higher than sucrose. High sweetness of stevia is due to steviol glycosides in the leaves. Until now there are at least 30 different types of steviol glycosides, two of them are present in higher amounts namely stevioside and rebaudioside A (reb A) (Yadav & Guleria, 2012; Ceunen & Geuns, 2013; Prakash *et al*., 2014). Stevia with high stevioside content has a bitter aftertaste, while stevia with high reb A content has a low or no bitter aftertaste (Madan *et al.,* 2010; Rao *et al.,* 2012). Stevia with high reb A content is preferred in food and beverage industries.

In Indonesia likes other tropical countries, stevia must be planted at high altitude areas (Djajadi, 2014). High altitude areas in Indonesia is generally hilly so it is difficult to apply mechanization for a large-scale stevia plantation. In addition, the use of land at high altitude will compete with other horticultural crops. The tropical climate in Indonesia with an average photoperiod around 12 hours causes stevia to flower because stevia belongs to a short day plant with a critical day length of 13 hours (Ramesh *et al.,* 2006). Stevia is harvested just before flowering because the content of steviol glycosides in the leaves will drop drastically after flowering (Ramesh *et al*., 2006; Kumar *et al.,* 2014). In Indonesia stevia is harvested every 1-2 months for 2-4 years, while in subtropical areas stevia is harvested once or twice after 6-7 months.

Propagation of stevia is generally by cuttings (Djajadi, 2014). Propagation of stevia by cutting repeatedly for a long period of time will decrease plant viability and vigor. Seed propagation is not recommended because of low seed germination, the presence of self-incompatibility (Raina *et al*., 2013; Yadav *et al.,* 2014), and genetic segregation during meiosis. Propagation of stevia through *in vitro* shoots multiplication is a solution for stevia propagation. *In vitro* stevia propagation also restore the juvenile characteristic so the vigor and viability of the plants can be maintained.

Before planted in the field, *in vitro* stevia culture requires a transition condition (acclimatization) to *ex vitro* environments that are completely different (Hazarika, 2003). This is a critical period where a substantial number of stevia plantlets may die. The survival rates of stevia during acclimatization in some subtropical countries were between 50 to 94% (Sreedhar *et al*., 2008; Sadeak *et al*., 2009; Sairkar *et al*., 2009; Manjushaa & Sathyanarayana, 2010; Anbazhagan *et al.,* 2010; Verma *et al*., 2011; Hassanen & Khalil, 2013; Khalil *et al*., 2014; Luwanska *et al*., 2015). Acclimatization of stevia plantlets in Indonesian at a high altitude area was reported by Arlianti *et al.* (2013) with the highest survival rate only 50%. Acclimatization studies of stevia at a low altitude in Indonesia have not been conducted. The objective of this research was to study the influence of plantlet conditions and hardening period before acclimatization of stevia to improve plant viability and growth at a low altitude in the tropical area.

Materials and Methods

Explant source and culture conditions

The material used was stevia clone BS 22 that belongs to the collection of Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB). This clone was a derivative of BS clones containing 15% reb A and 5% stevioside of leaf dry weight (Sinta, 2018). The study was conducted from April to September 2018 in the Laboratory of Plant Cell Culture and Micropropagation, Bogor. Acclimatization was carried out at an IRIBB nursery in Ciomas, Bogor with an altitude of 260 meters above sea level (masl).

Explant propagation was done by cutting stevia plantlets into single nodes with two leaves, then planted on MS media (Murashige & Skoog, 1962) added with 3% sucrose and 3.5 g/L gelzan as a gelling agent. The media were placed in the glass jars (9 cm high and 5 cm in diameter), the media pH was adjusted to 5.7-5.8 before autoclaved at 121 °C with a pressure of 1 kg/cm^2 for 20 min. The cultures were placed in a light culture room under the fluorescent lamp, with the temperature at 26 °C and photoperiod of 12 hours for 3 weeks.

Effect of plantlet age as a material source

The 3 week-old stevia plantlets were cut into single nodes and cultured on solid MS media for 0, 1, 2 and 3 weeks as the treatments. The cultures were placed in a light culture room. After 0, 1, 2 and 3 weeks, the shoots were removed from the media and immersed in Benlate solution for 5 min. The shoots were then grown in a multi-tray with 128 holes. The growing media were a mixture of top soil: cocopeat: compost (3: 1: 1) (v/v/v). The multi-trays were placed in a closed transparent plastic tunnel 1 m x 3 m x 1 m. The plastic tunnel was tightly closed for 2 weeks and then opened gradually in the morning for 1-2 hours. After 1 month, survived stevia plants were moved to a 15 cm x 7.5 cm x 15 cm polybags with the same media composition and placed in an open area under full sunlight for 2 months.

The research was conducted in a randomized block design (RBD). Each treatment consisted of 10 shoots with 3 replications. Data on early growth (shoot height, number of leaves, number of shoots, number of roots and root length) of stevia shoots at different ages were observed before acclimatization. Plant survival rate and growth were observed after 1 month in the plastic tunnel and after 1 and 2 months in the open area.

Effect of hardening period

Hardening (pre-acclimatization) period is a transition period between *in vitro* culture and acclimatization to strengthen the condition of plantlets before acclimatization to increase their survival rate and growth. The plantlets of stevia clone BS 22 were cut into single nodes and cultured in liquid MS media for 1, 4, 7, and 10 days in glass jars. The jars were placed in a culture room at 26°C under fluorescent lights with 12 hours of irradiation.

This research was arranged in a randomized block design (RBD) with treatment was different hardening periods: 1, 4, 7, and 10 days. Each treatment consisted of 8 shoots and was repeated 6 times. Data on stevia growth (shoot height, leaf number, shoot number, root number, root length and stomata conditions) after hardening period were observed before the acclimatization stage. The stomatal condition was observed based on the presence or absence of closed stomata. Adaxial leaves were peeled thin, placed precisely on the glass object, and then observed with a microscope at 40x magnification.

After hardening, the shoots of stevia were washed from the sticking media with running water and soaked in Benlate® for 5 min. The shoots were then grown in a multi-tray with growing media and conditions were the same as the first experiment. After 1 month, the survived plants were transferred to a 15 cm x 7.5 cm x 15 cm polybags with the same media composition and placed in an open area under full sunlight. Plant survival rate and growth were observed after 1 month in the plastic tunnel and after 1 and 2 months in the open area.

Data analysis

Quantitative data, tables and graphs were processed using MS Office 2010. If there were zero numbers, the data were transformed by $\sqrt{x+0.5}$ before analyzed. All the data were analyzed using analysis of variance with SPSS 23. If there was any significant difference, the differences among treatment means would be determined by Duncan's multiple range test at P = 0.05.

Results and Discussion

Effect of plantlet age as a material source

Stevia grew very fast in *in vitro* culture. After 3 weeks, there were significant differences in the parameters of stevia growth in terms of height, node number, leaf number, root number, shoot number and root length (Table 1, Fig. 1). After 3 weeks, on average shoot height was 10.1 cm from 1.7 cm, node number was 6, leaf number was 19, root number was 7-8, root length was 4.6 cm, and average shoot number was 1.5 (Table 1). At the beginning of shoot growth, generally there would be two stevia shoots grown from axillary buds, however without plant growth regulators in the media, the shoots would not grow simultaneously.

All those shoots of stevia at different ages were planted in the multi-trays and placed inside the closed plastic tunnel for 1 month. The highest survival rate (83.3%) during acclimatization for 1 month was obtained in 1-week old shoots (Table 2). One-week-old shoot explants on average had 6 leaves, 2 cm in shoot height, 3 roots with 0.68 cm in length (Table 1). These small explants but already had short roots were suitable characteristics as explant sources for successful acclimatization of stevia. These results were in contrast to other plants such as kopyor coconut which requires certain plantlet high and root conditions to survive during acclimatization (Sumaryono & Riyadi, 2016), also on *Lippia rotundifolia* that require fully root during *in vitro* culture before acclimatization (Resende *et al*., 2015). Acclimatization is a transitional condition where plants initially growth in *in vitro* conditions with abundant nutrients and in controlled environments, must be able to adapt to *ex vitro* conditions with fluctuating environmental conditions.

Two months after being transferred into polybags under full sunlight, the survival rate of stevia has decreased to 63.3% from acclimated plantlet. This decrease may be because stevia was planted at a low altitude area. In the tropics, stevia

Table 1. Growth of stevia explants at different ages.

Tabel 1. Pertumbuhan eksplan stevia pada berbagai umur.

Explant age (weeks) *Umur eksplan (minggu)*	Shoot height *Tinggi tunas* (cm)	Node number *Jumlah buku*	Leaf number *Jumlah daun*	Shoot number *Jumlah tunas*	Root number *Jumlah akar*	Root length *Panjang akar* (cm)
0	1.73 c*)	1.00 d	1.93 d	1.00 c	0.00 c	0.00 d
1	2.03 c	1.88 c	6.28 c	1.80 a	3.38 b	0.68 c
2	6.71 b	4.88 b	13.25 b	1.45 b	8.35 a	2.35 b
3	10.09 a	6.08 a	18.95 a	1.50 b	7.60 a	4.64 a

*) Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

*) *Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada P = 0,05.*

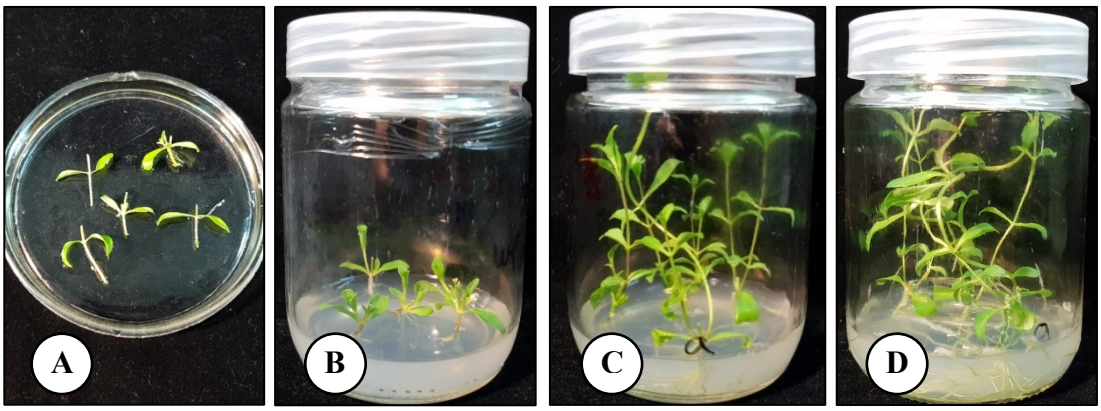

Figure 1. Shoots of stevia clone BS 22 used for acclimatization material after A) 0 week, B) 1 week, C) 2 weeks, and D) 3 weeks of culture.

Gambar 1. Tunas stevia klon BS 22 sebagai bahan aklimatisasi setelah kultur selama A) 0 minggu, B) 1 minggu, C) 2 minggu, dan D) 3 minggu.

grows well at a high altitude region. In Indonesia, stevia grows satisfactory at 700-1400 masl (Djajadi, 2014), while in Ethiopia, stevia grows well at an altitude of 1800-3000 masl (Mengesha *et al*., 2014).

The lowest survival rate of stevia during acclimatization was obtained from 3 weeks old shoots. All shoot died in the first month. Based on stevia morphology before acclimatization (Table 1), stevia shoots on this treatment already had the highest leaf number, height, root number and root length of all treatments. The shoots can reach 10 cm in height but lack of vigor so when planted on a multi-tray the stems were unable to support the plant to stand straight. In addition, long and many roots made the shoots hard to clean from the media and causing damages. Open wounds and the remaining media can attract microorganisms infecting the shoots. The 3-week old shoots of stevia had many leaves, in consequence, had high transpiration rates. Using a closed plastic tunnel during the acclimatization stage was intended to maintain high relative humidity in order to reduce the transpiration rate. Other means was by reduction of leaf area as carried out by Sumaryono *et al.* (2012) and Sinta *et al.* (2013) on the acclimatization of rubber plants.

Planting stevia directly after cutting shoots into single nodes (shoot age 0 weeks) also had a low survival rate (6.7%), almost all shoots died in the first month. The presence of open wounds on the stevia shoots made microorganisms easy to penetrate and infect the shoots causing the plant to die.

Stevia plants grew very fast after they were transferred to polybags placed in an open area without shading (Table 2). The growth of 1-week old shoot explants increased sharply after acclimatization period with average plant height from 3.7 cm to 21.1 cm and leaf number from 10.4 cm to 42.7 cm. The growth parameters of 2-week old shoot explants were similar to those of 1-week old shoot explants, except the survival rate was only 10% (Table 2).

Effect of hardening period

The highest survival rate of the first experiment was obtained at the treatment of using 1-week old shoots of stevia as material sources of acclimatization (Table 2). The second experiment was conducted based on the best results from the first experiment to determine the optimum hardening period. Hardening period was carried out in liquid media to simplify the handling of planting material, to minimize wounding roots and to ease in cleaning the shoots. The initial growth of stevia during hardening for 1 day to 10 days is shown in Table 3 and Figure 2.

The single nodes of stevia shoots were placed in glass jars containing liquid media. New shoots grew from axillary buds and reached 2.6 cm in height after 10 days. The average number of leaves also increased from 2 (1 day) to 7.8 (10 days). Shoot height, leaf number and shoot number had increased as longer of the hardening period. Roots started to emerge after 4 days in the hardening period.

Some stomata were closed after 7 and 10 days of the hardening periods (Table 3, Figure 3). On 1 and 4 days of hardening, all stomata were opened because all the leaves were soaked in liquid media. Stomata closure ability in plants during acclimatization is important, related to the transpiration process in plants. In very humid environments such as *in vitro* culture, stomata tend to open. In this condition, it is feared that some stomata unable to perform their function by losing their ability to close during acclimatization leading to plantlet death because of hydrolysis. Liquid cultures have known to increase the risk of hyperhydricity that characterized by thick stems, thick leaves and easily brittle, short internodes, and non-lignin stems (Kevers *et al.,* 2004). Plantlets with severe hyperhydricity symptoms frequently will lose the ability to close the stomata. However,

Table 2. Effect of explant age on survival rate, plant height and leaf number of stevia after acclimatization for 1 month and in open area for 2 months.

Tabel 2. Pengaruh umur eksplan terhadap daya hidup, tinggi tanaman dan jumlah daun stevia setelah 1 bulan aklimatisasi dan 2 bulan di ruang terbuka.

Explant age (weeks) *Umur eksplan (minggu)*	Survival rate *Daya hidup* (%)		Plant height *Tinggi tanaman* (cm)		Leaf number *Jumlah daun*	
	Acclimatization *Aklimatisasi*	2 months in open area *Di ruang terbuka 2 bulan*	Acclimatization *Aklimatisasi*	2 months in open area *Di ruang terbuka 2 bulan*	Acclimatization *Aklimatisasi*	2 months in open area *Di ruang terbuka 2 bulan*
0	6.7 b*)	0.0 b	2.1 c	0.0 b	8.5 b	0.0 c
1	83.3 a	63.3 a	3.7 b	21.1 a	10.4 a	42.7 a
2	13.3 b	10.0 b	5.5 a	21.7 a	11.5 a	33.5 b
3	0.0 b	0.0 b	0.0 d	0.0 b	0.0 c	0.0 c

*) Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

*) *Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada P = 0,05.*

Table 3. The initial growth of stevia during the hardening period.

Tabel 3. Pertumbuhan awal stevia selama periode hardening.

Hardening period (days) *Masa hardening (hari)*	Shoot height *Tinggi tunas (cm)*	Leaf number *Jumlah daun*	Shoot number *Jumlah tunas*	Root percentage *Persentase akar* (%)	Open stomata *Stomata terbuka*
1	1.2 b*)	2.0 c	1.0 c	0.0 c	+
4	1.5 b	2.3 c	1.7 b	44.4 b	+
7	2.3 a	6.7 b	2.0 a	81.5 a	-
10	2.6 a	7.8 a	1.9 a	59.3 ab	-

*) Means in the same column followed by the same letter(s) are not significantly different according to Duncan's multiple range test at P = 0.05.

*) *Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada P = 0,05.*

Notes: + = all stomata were open; - = some stomata were closed.
Keterangan:, + = semua stomata terbuka; - = beberapa stomata tertutup.

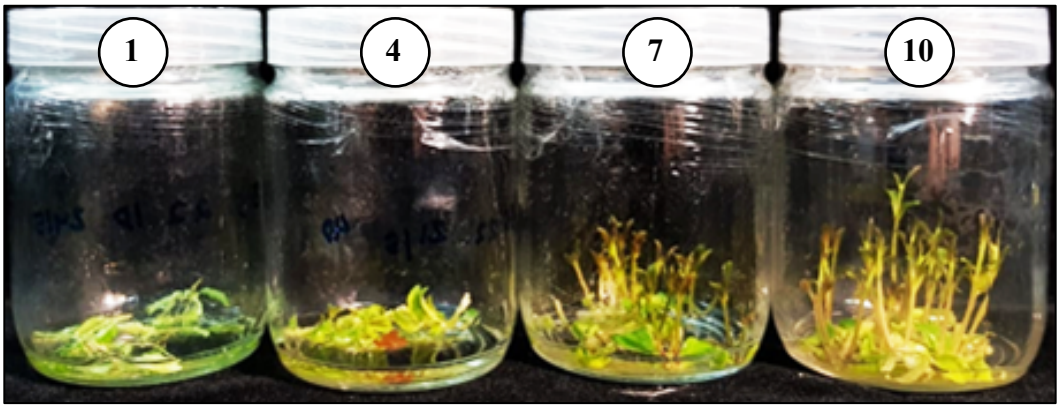

Figure 2. Stevia shoot growth after different hardening periods (1, 4, 7, 10 days).

Gambar 2. Pertumbuhan tunas stevia setelah masa hardening yang berbeda (1, 4, 7, 10 hari).

in this study there were only some stomata that were closed (Figure 3b).

The highest survival rate (97%) of stevia during 1-month acclimatization stage in a closed plastic tunnel was obtained in the 4 days hardening period, whereas the lowest survival rate (79%) was found in 10 days of hardening (Table 4). After 4 and 7 days of hardening the planting material used had grown with new leaves and some roots. This survival rate of stevia at 80-90% during acclimatization was higher than that reported in Indonesia by Arlianti *et al.* (2013) at 50 %.

One day hardening period provided an opportunity for stevia buds to cover open wounds, without attracting microorganisms during acclimatization as shown by high survival rate (91%) after 1 month. In term of planting material characteristics, stevia shoots in 1 day hardening period did not have any new shoots and roots yet.

The survival rate of the plants decreased after being transferred into the open area. The highest survival rate of stevia after 2 months planted in open area under full sunlight was found in 4 days hardening treatment with 62.5% survival rate (Table 5). After being transferred to polybags in an open area without shading, the plants grew rapidly on all hardening period treatments, therefore plant height and shoot number after 2 months were not significantly different among the treatments.

The survival rate of stevia in the field also depends on the clones. Research conducted by Sinta & Sumaryono (2019) on stevia planted on the high altitude area showed a decrease in stevia's survival rate by 0-30% depending on stevia clones. In this research, stevia clone BS 22 was used as explant, where BS 22 was a stevia mutant plants with high content of reb A and stevioside (Sinta, 2018). The decrease of plant survival rate after acclimatization period also occurred on *Hevea brasiliensis* plantlets due to physical factors during transferring the plants in to the open area (Sinta *et al*., 2013).

Hardening was carried out in liquid media to avoid acclimatization failure due to physical factors and to ease handling the plantlets compared to explants grown on solid media. In addition, cutting of plantlets into single nodes before acclimatization increases the numbers of planting material. In one culture bottle it can be obtained 25-30 stevia buds for acclimatization compared to only 4-6 explants on solid media.

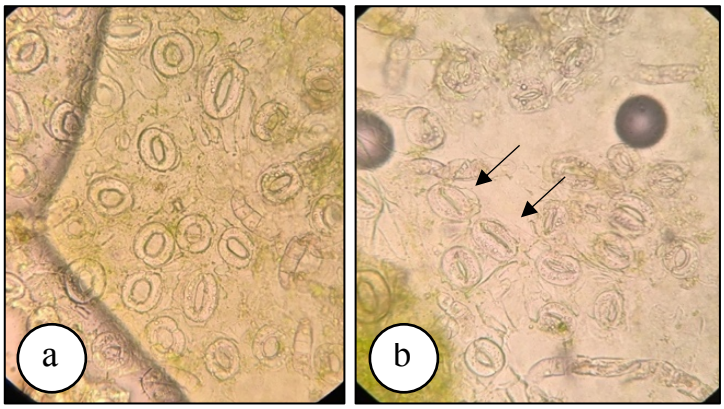

Figure 3. Stomata of stevia leaf during hardening period, a) all stomata were open, b) some stomata were closed (arrows).

Gambar 3. Stomata pada daun stevia saat hardening, a) semua stomata terlihat membuka, b) beberapa stomata tertutup (tanda panah).

Table 4. Survival rate and the growth of stevia after 1 month of acclimatization on multi-trays in a closed plastic tunnel.

Tabel 4. Daya hidup dan pertumbuhan stevia setelah aklimatisasi 1 bulan pada multi-tray di dalam sungkup plastik tertutup.

Hardening period (days) *Periode hardening (hari)*	Survival rate *Daya hidup* (%)	Plant height *Tinggi tanaman* (cm)	Leaf number *Jumlah daun*	Shoot number *Jumlah tunas*	Node number *Jumlah buku*
1	91.7 a*)	1.7 c	8.4 c	1.0 c	3.9 c
4	97.9 a	2.6 b	10.6 b	1.5 b	5.4 b
7	91.7 a	4.1 a	14.6 a	2.0 a	7.1 a
10	79.2 a	3.8 a	13.8 a	1.9 a	6.8 a

*) Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

*) *Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada P = 0,05.*

Table 5. Survival rate and the growth of stevia 2 months after transferring to polybags in the open area.
Tabel 5. Daya hidup dan pertumbuhan stevia 2 bulan setelah dipindah ke polibeg di ruang terbuka.

Hardening period (days) *Periode hardening (hari)*	Survival rate *Daya hidup* (%)	Plant height *Tinggi tanaman* (cm)	Leaf number *Jumlah daun*	Shoot number *Jumlah tunas*	Nodes number *Jumlah buku*
1	58.3 a*)	10.1 b	23.5 b	1.0 b	11.6 b
4	62.5 a	12.0 a	30.6 ab	1.1 b	15.2 ab
7	45.8 a	12.2 a	37.8 a	1.3 ab	19.0 a
10	20.8 b	11.0 ab	29.2 ab	1.6 a	14.9 ab

*) Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

*) *Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada P = 0,05.*

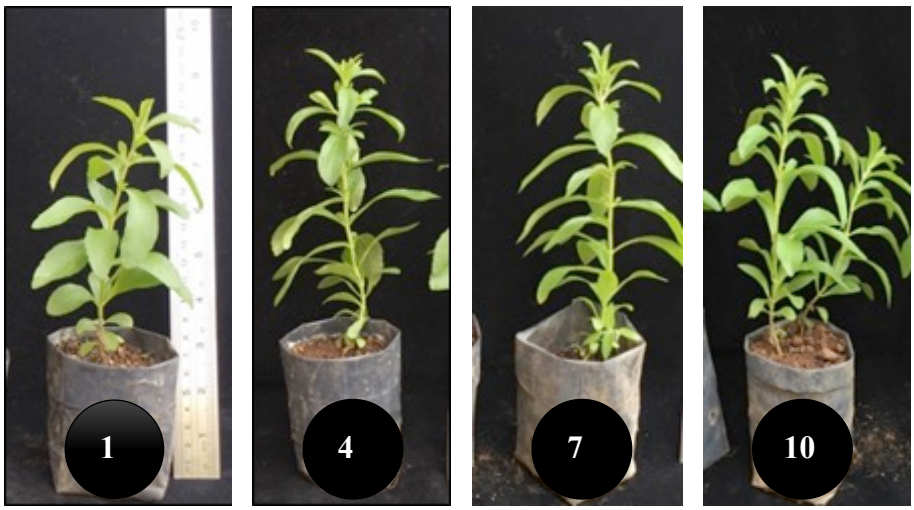

Figure 4. Growth of stevia after 1 month of acclimatization and 2 months in the open area.
Gambar 4. Pertumbuhan stevia setelah 1 bulan aklimatisasi dan 2 bulan di ruang terbuka

In Indonesia stevia generally starts flowering after 1-2 months of planting in the field. Stevia clone BS 22 started to form flowers after 3 months in the field. This was presumably because *in vitro* culture was able to restore the juvenile characteristics of the plants or this clone BS 22 may belong to late bloom varieties of stevia. Late bloom is considered a desirable trait of stevia planted in tropical regions because it will reduce harvesting time and cost as long as the productivity is comparable.

Stevia clone BS 22 has been acclimatized successfully in a closed plastic tunnel for 1 month with the survival rate at 83% to 97%. The survived plants were transferred to an open area under full sunlight and grew rapidly. All the experiments were conducted in Bogor, Indonesia where the altitude is 260 masl that is considered low land and not suitable for stevia growth in the tropical regions. This result indicates that stevia clone BS 22 may suitable for low altitude areas in tropical regions. Further growth of the stevia clone in the field at low altitude is necessary to confirm the assumption.

Conclusion

Acclimatization of stevia can be done using one-week old stevia explants grown on solid media with 83% survival rate. Hardening one node of the shoot in liquid media for 4 days increased the survival rate to 97%. Approximately 63% of acclimatized plants survived and grew rapidly when transferred into polybags in an open area within 2 months.

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