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Review
Stevia rebaudiana (Bert.) BERTONI. A REVIEW

Revisión bibliográfica
Stevia rebaudiana (Bert.) Bertoni. Una revisión

Michel Martínez Cruz

ABSTRACT. Stevia (*Stevia rebaudiana* (Bert.) Bertoni) is a species of the genus Stevia, Asteraceae family native of tropical South America. This plant is considered medicinal, as several studies show that it may have beneficial effects on type II diabetes, it has glycosides with no calorie sweetening properties. Many of researchers on chemical and biological properties have been made in the recent past. Several countries have started commercial cultivation. The published literature on this crop is very sparse, so this work devotes an effort to compile the literature and review the status of research and development of cropping systems. Cuba is a country that has potential for Stevia cultivation, but, unlike other countries in the region where it is farmed, companies have been formed and have become true processes of industrialization. In our country has not yet has introduced the cultivation of Stevia production in agricultural scale neither industrial. Stevia for its therapeutic properties may contribute to human health, but also has other applications as cosmetic use, soil improver and supplement animal diets, among others.

Key words: diabetes, sweetener, glycosides

RESUMEN. La Stevia (*Stevia rebaudiana* (Bert.) Bertoni) es una especie del género Stevia de la familia de las Asteráceas nativa de la región tropical de Sudamérica. Es una planta considerada medicinal, pues varios estudios demuestran que puede tener efectos beneficiosos sobre la diabetes tipo II, ya que posee glicósidos con propiedades edulcorantes sin calorías. Muchas de las actividades de investigación sobre sus propiedades químicas y biológicas se han hecho en el pasado reciente. El objetivo de este trabajo fue compilar la literatura y revisar el estado actual de la investigación y el desarrollo de los sistemas de cultivo de esta especie. Cuba es un país que tiene potencial para el cultivo de Stevia, pero; a diferencia de otros países de la región en los cuales se cultiva esta especie, se han formado empresas y se han hecho verdaderos procesos de industrialización. En Cuba aún no se ha introducido el cultivo de la Stevia a escala de producción agrícola ni industrial. La Stevia por sus propiedades terapéuticas puede contribuir en la salud humana, pero también tiene otras aplicaciones como uso cosmetológico, mejorador de suelos, suplemento en dietas de animales, entre otros.

Palabras clave: diabetes, edulcorantes, glicósidos

INTRODUCTION

Stevia (*Stevia rebaudiana (Bert) Bertoni*) is a species of the genus *Stevia*, Asteraceae family, native to the tropical region of South America. It is still a wild plant in Paraguay, especially in the Department of Amambay, and the Argentine province of Misiones, but for several decades it has been grown due to its sweetening properties and negligible calorie content[A] (1, 2, 3).

The first commercial crop report in Paraguay was in 1964 (1, 4, 5). Since then, it has been introduced as a crop in a number of countries, such as Brazil, Korea, Mexico, United States, Indonesia, Tanzania and Canada since 1990 (6, 7, 8, 9, 10, 11, 12). At present, its production is focused on China and its main market is in Japan (13).

It is considered a medicinal plant, as several studies show that it may have beneficial effects on type II diabetes (14), since it has glycosides with non-calorie sweetening properties (15). Its sweetening power is 30 times greater than sugar and its extract reaches 200 to 300 times more[B]. Leaves have the highest content of stevioside and rebaudioside A, which are its main active ingredients (15).

Departamento de Genética y Mejoramiento Vegetal, Instituto Nacional de Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700.

✉ mmcruz@inca.edu.cu

[A] Blumenthal, M. "Perspectives of FDA'S new Stevia Policy, After four years, the agency lifts its ban-but only partially". Whole Foods Magazine, 1996, pp. 1-5.

[B] Ramírez, L. E. Informe agronómico sobre el cultivo de *Stevia rebaudiana*, la hierba dulce. Asociación Camino al Progreso. Poligrafiado, 2005.

S. rebaudiana extracts contain a high content of steviol diterpene glycosides. Stevioside and rebaudioside A are the main compounds responsible for sweetening that are usually accompanied by small amounts of other steviol glycosides (16).

At present, 41 % of sweeteners consumed in Japan are coming from *S. rebaudiana*. The sweetener obtained from this plant has beneficial effects on fat absorption and blood pressure regulation (17, 18), besides it is used to replace sugar for diabetic people, because it does not increase blood sugar levels; in contrast, some studies have demonstrated its hypo-glycemic property improving glucose tolerance.

In addition, this plant has antibiotic and anti-fungal properties, especially against bacteria such as *Entamoeba coli*, *Staphylococci aureus* and *Corynebacterium diphtheriae*, as well as the fungus *Candida albicans*, which frequently causes female vaginitis. It is also used in cosmetic preparations for skin spot and pimple treatments (17).

BOTANICAL DESCRIPTION

Stevia rebaudiana belongs to Asteraceae family and it is a perennial herb with pubescent, half-woody, erect stem; it does not have branches during its initial development, but it becomes multistem after its first growing cycle, reaching up to 20 stems in three to four years; It can grow until 90 cm in its natural habitat and in the tropics it may attain more than 100 cm high. It has a non-deepened thread-like taproot distributed near soil surface[C].

S. rebaudiana has somewhat pubescent elliptical, oval or lance-shaped leaves, opposite arranged in its early stages and alternate when plants reach their physiological maturity, prior to flowering[C].

It has small, whitish, hermaphrodite flowers with pentalobuled tubular corolla in small terminal or axillary chapters grouped in corymbose panicles[C].

It is a self-incompatible plant (protandry), so that pollination is entomophilous; it is said to be sporophytic and classified as compulsory apomictic[D]. The fruit is a clear (sterile) or dark (fertile) achene scattered by the wind (22). It is classified as a short-day plant, with a critical photoperiod of 12-13 hours depending on the ecotype. There are other species such as *Stevia eupotoria*, *S. obata*, *S. plummerae*, *S. salicifolia* and *S. serrata* (19).

ORIGIN AND DISTRIBUTION

Stevia rebaudiana (*Bert.*) *Bertoni* is a native plant from the subtropical jungle of Alto Parana, Southeast Paraguay. This plant was anciently used as a sweetener and medicine by aborigines (21). However, the genus *Stevia* consists of more than 240 species of native plants from South America, Central America and Mexico, with several species found as far away as Arizona, New Mexico and Texas. For centuries, Guarani tribes of Paraguay and Brazil used different Stevia species and mainly *Stevia rebaudiana*, which they called ka'a he'e or sweet grass[C].

It was first described in 1887 by the Swiss botanist Moisés Santiago Bertoni, who detailed its sweet taste. In 1900, the Paraguayan chemist Ovidio Rebaudi managed to isolate two active principles: one sweet and another bitter. Subsequently, these compounds were named stevioside and rebaudioside, which are 200-300 times sweeter than sucrose, heat stable and do not ferment[C].

Note that *S. rebaudiana* has over 144 varieties worldwide (20), highlighting Morita 2, besides, this species has many ecotypes; the variety Ariete is also widely cultivated at present because of its greater sweetening. Morita 2 variety was developed in Japan by Toyosigue Morita; its advantage is the higher dry leaf yields and better chemical content than the other varieties. In 1975, 28 different ecotypes were selected based primarily on their morphological characteristics, to determine that leaf stevioside content varied between 2,07 and 18,34 %[E] (21).

In 1980, a series of experiments were described to relate various plant characteristics with heritability in 22 varieties of *S. rebaudiana*. Then, 11 morphological features and six content characteristics were observed; out of these 17 features, only leaf dry weight showed a low correlation with heritability. The researcher concluded that morphological and content characteristics, especially those of active ingredients, have obvious selective effects (22).

[C] Guerrero, R. "Planta endulzante con mucho futuro". Diario La Prensa, Nicaragua, 2005.

[D] Monteiro, R. Taxonomía e biología da reproduçao de *Stevia rebaudiana* (Bert.) Bertoni. I Seminario Brasileiro sobre *Stevia rebaudiana* Bertoni. Inst. Instituto de Tecnología de Alimentos, Sao Paulo, Brasil, 1982.

[E] Gattoni, L. A. Caá-jhee: A Wild Shrub Native to Paraguay (*Stevia rebaudiana* Bert.). edit. Unpublished manuscript, Archives, Royal Botanic Gardens, Kew, Asuncion, 1945, 8 p.

The main worldwide Stevia producers are Japan, China, Korea, Taiwan, Thailand, Indonesia, Laos, Malaysia and Philippines; all these countries represent 95 % world production. It should be noted that Japan is the country with the largest amount of stevioside processing and extracting factories. In America, it is mainly grown in Paraguay, Brazil, Argentina, Colombia, Peru and very small crops in Ecuador (23).

Paraguay is one of the largest worldwide Stevia producers; approximately 1,500 hectares are devoted to this crop, generating direct employment to 10,000 people in the entire production chain. This country pretends to increase its sales to $10 million dollars per year, representing 10 % invoicing compared to Southeast Asian countries (100 million) (23).

PROPAGATION

Sexual

Stevia is sexually reproduced by achenes, showing high heterogeneity in the resulting population, mainly due to cross-pollination; much of its achenes are sterile, lightweight and easily scattered by the wind[F].

Seed collection is slow and difficult because of a non-uniform bloom, which affects seed maturity; they must be stored at low temperature and relative humidity conditions, preferably inside airtight containers in the dark; however, germination percentage is low, between 10 and 38 %[G] (11, 24).

Seedlings produced from seeds are performed in conventional seedbeds, similar to other vegetables, but with some recommendations and special practices, such as covering immediately after seeding with a thin cloth to prevent seeds from being carried away by wind. Considering all problems, achene propagation is useful for breeding, but not for cash crops (25, 26).

Asexual

Due to the high plant diversity obtained by seeds, agamic propagation is the best, because it keeps mother plant characteristics. This may be by shoots, cuttings and tissue culture[H].

Shoot reproduction can be used in small plantations, as its number is reduced; shoots are born at stem base or underground; then, small suckers appear, many of them with roots, which can be removed and planted in the definitive site[H].

Cutting propagation is the most convenient method for commercial scale; thus, it is important to have a mother plantation, which will provide the initial planting material[H].

In order to establish a mother plantation, plants should be selected according to the following desirable characteristics: vigor, rusticity and productivity. This management is similar to that of a commercial plantation, keeping plants in good nutritional and health status, mostly free of fungal diseases that may considerably affect plantation[F].

Once the mother plantation has been established for commercial or plant propagation, each cutting should be removed with five opposite, open leaves, 8 to 18 cm long. Cuttings with alternate leaves are not the most suitable for propagation, as plants are about to enter bloom, reducing its possibility of rooting and having young plants[F].

Cuttings should be planted in rooting beds of 1 m wide x 30-50 cm high; this activity must be done immediately to prevent future seedling desiccation[F]. Before planting, cutting apical portion is removed, as it usually gets rusty fast. Once cuttings are planted, irrigation is one of the most important cultural practices, as it will ensure a high percentage of rooting[F].

Tissue culture

Tissue culture is another vegetatively propagating method which allows more uniform plantations; besides, a quick clonal expansion is obtained[I] (15). *In vitro* or micropropagation is defined as any aseptic procedure that involves handling of plant organs, tissues or cells producing “clean” seedling populations, contrary to non-aseptic or conventional vegetative propagation (27).

[F] Melillo, P. ‘‘Agrotecnología para el cultivo de estevia o hierba dulce, in Fundamentos de Agrotecnología de Plantas Medicinales Iberoamericanas’’. En: eds. Martínez J. V., Yesed H. Y., y Cáceres A., edit. Convenio Andrés Bello, Santafé de Bogotá, 2000, 441 p.

[G] OMS. Métodos de control de calidad de Plantas Medicinales. Inst. OMS, Ginebra, 1992.

[H] Taiariol, D. R. Propagación vegetativa de *Stevia rebaudiana* bertoni. Tesis de Maestría, Facultad de Agronomía de la Universidad de Buenos Aires, Buenos Aires, Argentina, 1995.

[I] Carneiro, J. W. P. *Stevia rebaudiana* (Bert.) Bertoni, production of seed. Master Science Thesis, State University of Maringá, Brazil, 1990.

The benefits of micropropagation, compared with conventional systems, are the rapid increase of plant number, reduction of multiplication time, more amount of plants per area used, greater plant health control, easy transportation for the international exchange of materials and ability to rapidly multiply endangered species (18).

In vitro propagation consists of five stages: the initial or 0 stage to select a mother plant; initiation or I stage to establish the initial or primary culture; stage II for shoot multiplication; stage III for rooting or pre-transplanting to produce an autotrophic plant that survives under soil transplanting conditions, and stage IV for final transfer to the environment.

However, multiplication studies performed in Ecuador have varied traditional tissue culture stages as follows: stage 0: explant selection; stage I: initial crop introduction; stage II: *in vitro* multiplication; stage III: hydroponic autotrophic system (SAH) multiplication in coconut substrate; stage IV: final stage of rooting and acclimatization in multiplication trays (28).

a) Explant selection stage (Stage 0)

Selecting the best explant is very important, as this will determine the future propagation. Explants may have a diverse origin as leaf sections, micro-cuttings, roots, meristems, etc. In the case of Stevia, in Ecuador, it was introduced by using micro-cuttings with lateral and apical buds (29).

b) Initial crop introduction stage

Disinfection is carried out once the explant is selected, with successive solutions of liquid soap for ten minutes; then, it is passed through an iodine solution (Povidín 10 drops L^{-1}) for 15 minutes and finally through a Benlate solution (1 g L^{-1}). Explants are washed with sterile distilled water (ADE) and later they are carried to a laminar flow chamber, where commercial chlorine disinfection is performed at a concentration of 0,5 % active ingredient (30). Finally, it is introduced into the culture medium consisting of MS salts (31) supplemented with 20 g sucrose, 0,4 mg L^{-1} thiamine, 5 mg L^{-1} kinetin and 6 g washed agar; pH is adjusted to 5,6. For its introduction, it is important to individualize explants in pipes to decrease the risk of contamination (32).

c) *In vitro* multiplication stage

Once explants are adapted in the culture medium, multiplication is performed in type GA7 magenta boxes (Sigma) on MS medium supplemented in the same way as the previous phase, except that agar concentration is reduced from 4 to 4.5 % and pH is adjusted to 5,7-5,8 (32). Later on, in the laminar flow chamber, uninodal and apical segments are cut with scissors, then selected and seeded in different magenta boxes (33).

MULTIPLICATION OF AUTOTROPHIC SYSTEM

Hydroponic (SAH)

This system is based on seedling photo-autotrophic capacity, environmental factor management, micropropagation and hydroponics concepts. Seedlings have better quality because they are larger and acquire more adequate physiological functioning as well as uniform growth. This system allows greater number of plants in less time thus saving electricity, labor force, and materials, besides reducing transplanting stress. In addition, plants have excellent field adaptation[J].

In general, SAH use non-returnable containers, substrate and hydroponic solutions; also, one of the most important features is that no sucrose or growth regulators are added, obtaining real autotrophic plants with great adaptive ability to greenhouse conditions. Eventually the use of sucrose leads to develop pollution causing big economic losses. Thus, plants with high multiplication rates, highly adaptable vigorous stems and large leaves are attained, reducing mortality and pollulation[J].

Once the plants are acclimatized inside growth chamber, they are ready for the greenhouse or field.

CLIMATIC REQUIREMENTS

Stevia in its natural state grows in the semi-wet subtropical region of America, with rainfall ranging between 1400 and 1800 mm distributed throughout the year, temperatures from 24 to 28 ^{0}C and relative humidity of 75-85 %. This plant requires long days and high solar intensity (heliophany)[F].

[J] Benítez, J. y Navarrete, J. Aplicación del Sistema Autotrófico Hidropónico SHA (Técnica argentina) en variedades mejoradas del Ecuador, para la obtención de semilla prebálsica de papa [en línea]. 2002, [Consultado: 27 de noviembre de 2015], Disponible en: <http://nkxms1019hx1xmtstxk3k9sko.wpengine.netdna-cdn.com/wp-content/uploads/congreso%20ecuatoriano%201/j.benitez.doc>.

The optimal soils for Stevia crop are those with pH 6.5-7, low or no salinity, medium organic matter content, sandy loam to loamy texture, good permeability and drainage. This plant does not tolerate excess soil moisture or high organic matter content, mainly due to fungal problems that may cause great economic losses[F].

SOWING

Sowing is recommended at spacings of 20 cm between rows and 16 cm between plants, equivalent to a density of 180,000 plants per hectare (42). For a better plantation management, it is important to design roads 3 m wide every 100 m[F].

HARVEST

Stevia fresh leaves are the useful part for commercial purposes, which are taken when plants have a maximum of 5 % flower buds, making a cut at 6-8 cm from the soil and taking all fresh and healthy leaves available, leaving two to three pairs of leaves in the plant. The highest crop yield is achieved in the first three years and, if environmental and market conditions are favorable, up to four harvests are performed per year (28).

THE DRYING PROCESS

The final product quality depends on drying; leaves must be dried to the point of easy handling. Within the drying process, direct sunlight should be avoided, as this may change leaf chemical properties; if there are low solar intensity conditions and high relative humidity, the construction of rustic drying sheds or artificial drying is needed with a ventilation and heating system, which will help to have a uniform drying; the latter method is preferred[F] (8).

MAKING COMMERCIAL PREPARATIONS

Stevia market has been expanded to other fields, such as agricultural health and in the case of human consumption facing the growing demand for "light" products, it has diversified its use and presentations. At present, Stevia extracts are used as fertilizer in agriculture, with the aim of stimulating crop photosynthetic processes and obtaining a high fruit sugar concentration; also, by applying the extract with irrigation water, soil beneficial microorganism population (antagonists) is enriched and by applying finely powdered stem, it is possible to recover a contaminated soil through chemical fertilizers, so that it becomes fertile. Now, investigations are performed about feeding cattle, chickens and trouts with Stevia (1).

MAJOR DISEASES

This is a susceptible plant to pest and disease attacks; bibliography refers that its greatest phytopathological problems are caused by fungi and nematodes, whereas the most frequent pests are mites and biting, scraping and sucking insects (32, 34). These factors adversely affect yield and product quality; thus, a strict control must be done.

CAUSATIVE DISEASE AGENTS

The diseases found in Stevia crop are caused by fungal phytopathogenic group (34). Table I shows disease genera as well as a description of symptoms and affected organs.

Table I. Causal disease fungus genera in Stevia crop

Symptoms	Genera	Attacked organ
Wilting	*Fusarium* sp.	Root-Stem
	Rhizoctonia sp.	Root-Stem
	Sclerotium sp.	Root-Stem
Necrotic spots	*Septoria* sp.	Leaves
	Alternaria sp.	Leaf-Stem
Blackening	*Colletotrichum* sp.	Stem
	Phomopsis sp.	Stem
	Curvularia sp.	Stem
	Botryodiplodia sp.	Stem
	Phlyctaena sp.	Stem
Dark decay and abortion	*Aspergillus* sp.	Flowers
	Cladosporium sp.	Flowers

[K] Ramírez, L. E. Informe agronómico sobre el cultivo de *Stevia rebaudiana*, la hierba dulce. Inst. Asociación Camino al Progreso. Poligrafiado, 2005.

The first three groups of diseases (wilt, necrotic spots and blackening and canker) are those causing the highest production damage, considering that *Fusarium* sp, *Rhizoctonia sp* and *Sclerotium sp* fungi lead to plant death, thereby reducing plant population per unit area (34).

Necrotic spots have a negative influence on product quality, so that leaves are classified in a lower and less expensive category, because green leaves without spots are commercially preferred. Stem damage reduces plant size due to the progressive branch death (34).

The causative disease agents identified in Stevia plants belong to fungal group, since wilting damage caused by *Fusarium sp*, *Rhizoctonia sp* and *Sclerotium sp* as well as leaf spots caused by *Septoria sp* and *Alternaria sp* are the ones that could greatly reduce commercial leaf volume and quality (34).

So far, 11 phytonematode genera have been identified. The genus *Meloidogyne sp* is the greatest negatively influencing Stevia crop, due to its difficult control, which has damaged many horticultural species associated with this crop, causing its transfer to the species (34). To minimize pathogen inoculum source, it is not recommended to locate Stevia crops near oil-producing areas, because of the high affinity of these genera (34).

To reduce nematode effect, it is not recommended to establish Stevia crop in a previously cultivated soil with vegetables and to avoid seedling production in places used by family or commercial orchards. Another rule is not to transplant seedlings from infested areas (34).

Table II shows the main pests attacking Stevia crop.

Damage caused by insects of the orders *Coleoptera*, *Lepidoptera* and *Orthoptera* directly influence crop yield, because they eat leaves, so that leaf area is greatly reduced, resulting in a smaller commercial leaf harvest. Scraping and sucking pests (*Hemiptera*, *Homoptera*, *Acari* and *Thysanoptera*) injure indirectly, as they feed on cell juice or sap, decreasing plant growth by reducing available photoassimilates for metabolic processes (34).

Biting insects of *Diabrotica sp*, *Pseudoplusia includens*, *Spodoptera sp*. and *Schistocerca sp* cause direct damage to production due to their leaf consumption (34).

Therefore, crops should not be seeded near cucurbit, grass or legume (maize) plantations due to its polyphagous habit (34).

APPLICATIONS OF *Stevia rebaudiana*

Stevia is recommended by physicians for diabetics given its hypoglycemic properties (29); skin creams are also made for healing; there are some of the applications of Stevia (Table III).

Table II. List of mites and insects attacking Stevia

Scientific name	Order	Attacked organ
Tetranychus sp.	*Spacari*	Leaves
Agallia sp.	*Homoptera*	Leaves
Myzus persicae	*Homoptera*	Leaf-shoots
Diabrotica sp.	*Coleoptera*	Leaves
Dichelops furcatus	*Hemiptera*	Leaf-shoots
No identificado	*Hemiptera*	Leaf-shoots
Taylorilygus pallidus	*Hemiptera*	Leaf-shoots
Harmostes serratus	*Hemiptera*	Leaf-shoots
Proxys sp.	*Hemiptera*	Leaf-shoots
Trips tabaci	*Thysanoptera*	Leaf-shoots
Pseudoplusia includens	*Lepidoptera*	Leaves
Spodoptera sp.	*Lepidoptera*	Leaves
Schistocerca sp.	*Orthoptera*	Leaves
Gryllotalpa sp.	*Orthoptera*	Root-stalk
Pseudococcus sp.	*Homoptera*	Root

Taken from Orrego, 2001 (34).

Table III. Applications of Stevia in different fields of action

Fields of action	Applications
Pharmaceutical and nutraceutical (46)	Natural antioxidant (47) (48). Reduces glucose levels in the blood of (Non-insulin dependent) diabetics (16, 49). Reduces food anxiety and cravings for sweets or fats in the treatment of obesity (50, 51). Mild diuretic (helps lower uric acid levels). Beneficial for people with hypertension. Fights fatigue and depression. Improves gastrointestinal functions. Improves resistance to colds and flu. For the treatment of burns, wounds, eczema, seborrhea, psoriasis, dermatitis (52).
Human nutrition (53, 54)	Food sweetener: coffee, tea, gum, candy, etc. Sugar substitute in low-calorie drinks, sauces and pastries
Agricultural applications (9)	Revitalizes beneficial microorganisms and can restore soil fertility. Improves plant rooting, stimulating root growth. Purifies chemically contaminated soil and other chemicals. Increases plant resistance to pests and diseases. Improves crop health status and increases yield. Helps prevent fruit drop Prevents exhaustion by excessive fruiting and plant aging. Increases fruit sugar content and its flavor Increases vegetable content of vitamins, minerals and other nutrients. Improves postharvest product durability significantly by its antioxidant action
Livestock area (1)	Flavors fodder (for farm and domestic animals). As animal feeding to increase cattle, pig and poultry production. Stimulates appetite. Prevents diseases by reducing antibiotic use. Improves meat taste and quality (lower exudation and better conservation). Reduces the amount of broken eggs in laying hens. Prevents chicken gizzard erosion and ulceration (by stress and histamine overproduction).
Environmental applications (1)	Accelerates organic fertilizer (compost) production from organic wastes Reduces the concentration of nitrates, dioxins, soil fertilizers and pesticide residues
Cosmetic applications (1)	Complements cellulite treatments Helps prepare toothpastes and mouthwashes for oral hygiene Helps remove stains, softens wrinkles and makes beauty skin

STEVIA IN CUBA

In Cuba, this plant is poorly known, although environmental conditions are favorable for its production; besides considering the growing number of diabetics (so far more than 500 000), its use as a natural sweetener in patients with this disease would be a useful alternative; however, this crop is only reported at the Research Station of Medicinal Plants of San Antonio de los Baños, Mayabeque province, where a study was conducted on its agronomic performance and published at the Cuban Journal of Medicinal Plants in 2007 (23).

RESULTS OF THIS STUDY IN CUBA

At both sowing dates (December, 2000 and January, 2001), it was observed that seeds germinated five days later, although with low percentages: 40 % in December and 45 % in January; in addition, it was found that flowering occurs throughout the year in adult plants, with greater intensity from April to September.

It is noteworthy that this crop was put in tissue culture for *in vitro* propagation with successful results.

The total yield of fresh leaves for each planting date was 8,28 t ha^{-1} in February and 4,55 t ha^{-1} in March, indicating that the best seeding date was December and transplanting in February; in every case, plants showed a flowering-fruiting state.

Regarding total leaves, those from the useful part account for 50 % whereas total leaf yield of three harvests was 4,14 t ha^{-1} in February and 2,24 t ha^{-1} in March[G] (11, 24).

Within the first planting date, it was observed that plants reached a greater vegetative growth shown by a higher average number of branches (93 branches) compared with the value obtained in March: 56 branches per plant. Likewise, there was a dry weight: fresh weight relationship of 1:5, that is, 5 kg fresh leaves are required to obtain 1 kg dry leaves.

Alternaria sp. fungus was only observed in older leaves near the soil when rains started and a climatic change occurred; the species was also damaged at crop vegetative stage by *Agromyza* sp. (miner) and beetles, although the latter did not injure so much.

Quality indices showed the following results: humidity (10 %), total ashes (10 %), soluble substances in water (20 %) and soluble substances in ethanol at 70 % (25 %); according to World Health Organization (WHO) (30), these values are among established ranges as satisfactory by international standards.

It means there was not a favorable response from its seed propagation, although seedlings were made over the lower temperature months of the country. Literature points out that ideal temperatures for this crop should range between 20 and 25 °C; it also states its asexual reproduction or *in vitro* micropropagation is advised for its native regions, as it is allogamous with a high crossing rate; consequently, a great genetic variability makes its active ingredients highly variable (25).

Likewise, during its productive cycle, three cuttings are performed when the crop is at full sunlight, in non-clayey soil, with a density of 130 000 plants ha^{-1}, that is, spacing of 50 x 15 cm with frequent but light waterings, besides pruning 45 days after transplanting to favor branch emergence and accelerate crop cycle. It is also noted that yields and contents of active ingredients are highly variable; from 10 to 12 t of dry leaves per year are reached with plants from a selected clone in four to five cuts (32).

Therefore, according to literature[I] (26, 27), it is recommended to make a classical breeding program for obtaining plants with desirable characteristics or to establish a vegetative propagation system by tissue culture.

In Vitro PROPAGATION OF STEVIA (*Stevia rebaudiana*) IN CUBA. CASE STUDY

The study was conducted at the Biotechnological Laboratory from Genetics and Plant Breeding Department of the National Institute of Agricultural Sciences (INCA), located in San Jose de Las Lajas, Mayabeque province[D].

Cuttings from 5 to 7 cm long were seeded in pots of 14 cm top diameter containing a substrate mixture of organic material (filter cake) and compacted Red Ferralitic soil (1: 2 v/v ratio).

Shoots grew under semi-controlled conditions (metal frame, anti-aphid cloth walls and roof nylon) and irrigation was regularly applied (three times a week) by hose to keep adequate moisture.

Shoots from the field and controlled conditions (above mentioned) were cut four weeks later and divided into sections from 3 to 5 cm long with two buds and later disinfected following the scheme.

In the laminar flow cabinet, meristem apices of 0,5-0,7 mm long were extracted by using CARLZEISS stereo-microscope with increased mark (4X) and stem dissection until getting uninodal segments. Culture media pH was adjusted to 5.7 in all cases prior to adding the solidifying agent.

Sterilization was performed by autoclaving for 20 min at 121 ^{0}C with 1,5 atm of pressure. Subsequently, meristem apices were implanted in test tubes (25 X 150 mm) with 10 mL solid medium using MS basal medium (Murashige and Skoog, 1964) at 75 % supplemented with 100 mg L^{-1} Inositol + 2 mg L^{-1} 6-BAP (6-benzylaminopurine) + 30 g L^{-1} sucrose + AGAR 6,5 g L^{-1}, pH 5.7. Planting material was placed in growth chambers at temperatures of 25 ± 2 ^{0}C, relative humidity of 80-90 % and artificial light within a 16 h-light photoperiod, a light intensity of 18.75 µmol m^{-2} s^{-1}. A total of 10 explants were used per treatment. For shoot growth, the same culture medium was employed without applying plant hormones. Finally, Stevia plantlets were ready for acclimatization.

FINAL CONSIDERATIONS

Cuba has got potential for growing *Stevia rebaudiana (Bert.) Bertoni*; however, this crop has not yet been introduced to an agricultural or industrial production scale in our country, differently from other regional countries where this species is cultivated and enterprises are built as well as really good industrialization processes have been established.

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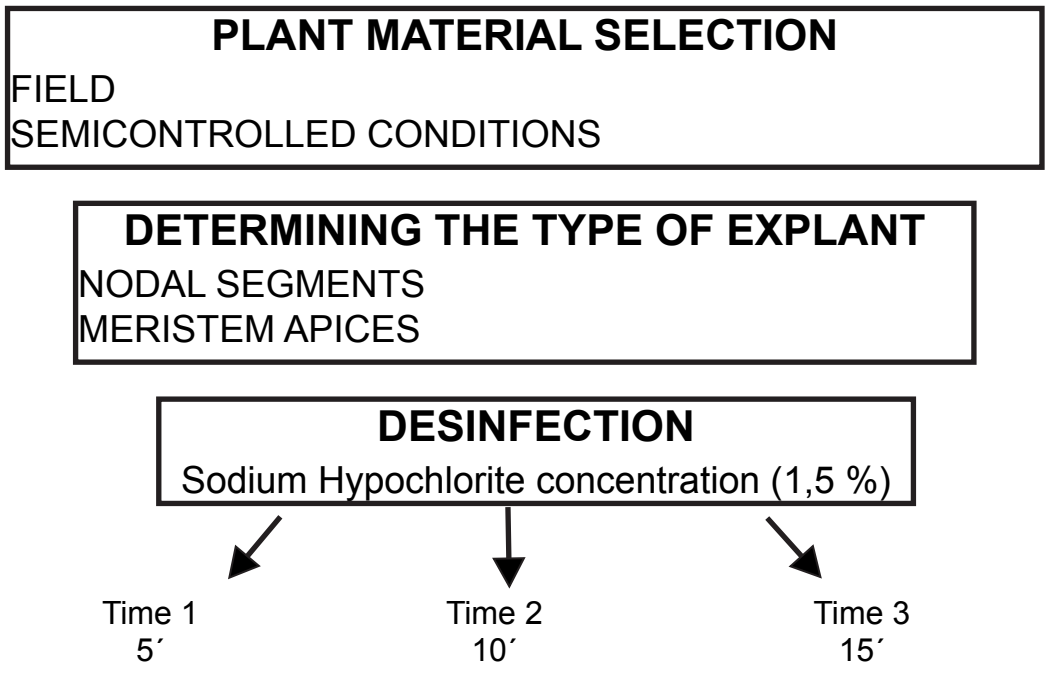

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