Open Access Library Journal
2021, Volume 8, e8164
ISSN Online: 2333-9721
ISSN Print: 2333-9705

A Review of Research Results on *Stevia rebaudiana* Bertoni in Yucatan Mexico from 2010 to 2017

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How to cite this paper: Ramírez-Jaramillo, G., Lozano-Contreras, M.G., Moguel-Ordoñez, Y.B., Aviles-Baeza, W.I. and Ramírez-Silva, J.H. (2021) A Review of Research Results on *Stevia rebaudiana* Bertoni in Yucatan Mexico from 2010 to 2017. *Open Access Library Journal*, **8**: e8164.
https://doi.org/10.4236/oalib.1108164

Received: November 8, 2021
Accepted: December 18, 2021
Published: December 21, 2021

Abstract

This paper integrates information on research studies carried out, in *Stevia rebaudiana* Bertoni, by the Southeast Regional Research Center (CIRSE) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) in Mexico. *Stevia* is an ancient plant of Paraguayan origin with great potential to produce natural sweeteners. There is a constant demand at the national and international levels for this crop so new varieties adapted to different environments are required in order to reduce production costs, avoid dependence from abroad, improve yields and increase the acreage. The Mexican CIRSE has documented a series of innovations since 2010 in *stevia* such as: 1) studies of the productive potential, 2) production of seedlings with biological rooters, 3) population densities, 4) pre-emergence control of weeds, 5) fertilization doses, 6) materials better adapted to Mexican environment conditions and 7) evaluation of main *stevia* glycosides such as *Stevioside* and *Rebaudioside-A* among the most important. The main scientific findings are reported in this paper with the hope that stevia producers use it as a guide to improving their stevia production crop.

Subject Areas

Agricultural Engineering

Keywords

Innovations, Sweetener, *Ka᾽a Heʼë* (Sweet Herb), Tropic of Mexico

1. Introduction

Stevia is a native plant from the Amambay mountain range located between southern Brazil and northern Paraguay [1] [2], This is a region where the original *Guaraní* people are located and the plant is known as *Ka᾿a He᾿ë* (sweet herb) which has been used for centuries, for the Guarani tribes of Paraguay and Brazil as sweetener to counteract the bitter taste of medicines and in yerba mate tea. It is also used as medicinal infusions to treat stomach acidity, glycemic regulation and hypertension [3] [4] [5] [6] [7]. It is a species of the *Asteraceae* family (formerly *Compositae*); and it is a perennial herb that can reach 100 cm high [8].

Japan was the first country to accept the use of *Stevia* and it is being used for more than 40 years [9] as dried leaves and using the active and pure *steviosides* to replace cyclamate and saccharin in more than 40% of the sweetener market [10]. The commercial blockade during the Second World War and the high international price of sugar forced the Japanese to look for new sweetener alternatives [11]. They discovered the active ingredients and their attributes when chemically analyzing the *Stevia;* since then, approximately 50% of the sweetener market has been substituted by *Stevia* [12]. The commercial exploitation of *Stevia* has increased significantly since 1970, when Japanese researchers developed a series of protocols for the extraction and purification of the sweetening compounds in the plant [13] [14].

Despite the fact that stevia began to be produced by Japan in 1970, in Mexico its cultivation started in 2010 with an increasing interest by local farmers. According to data from the Agrifood and Fisheries Information Service (SIAP) stevia was officially reported by the Mexican government in 2012 [15].

Stevia was first introduced to Mexico in 2010 through the National Institute of Forestry, Agricultural and livestock Research (INIFAP) in order to study its adaptability. The best areas found with good production potential were the states of Chiapas, Yucatán, Quintana Roo, Campeche, Veracruz and Nayarit according to CIRSE [15].

Stevia cultivation is an innovative and very profitable alternative [16]. However, in Mexico, at the beginning of its development the average yields obtained were not economically feasible for farmers [16]. In 2010, agronomic research was practically negligible so technological information was highly needed [17].

In order to face the problem of lack of information the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) launched different investigation strategies related to: studies on productive potential, optimal population densities, production under irrigation conditions, weed control, nutritional requirements, soil fertilization, content of Stevíoside and Rebaudíosido-A in different materials and profitability analysis under Yucatán conditions in México [17].

During these last nine years, specific results have emerged such as: Development and Technological Diffusion of Stevia under Irrigation Conditions, Production of Stevia seedlings by using biological rooters, Genetic improvement to produce clonal varieties, weed control in early stages and economic profitability by integrating all the above mentioned technologies.

Even though the existing research results, developed throughout these nine years in Mexico, it is still important to keep searching for new technological innovations capable to incentivize farmers to produce stevia with more high profitability. This paper documents the technological innovations generated by the Southeast Regional Research Center of INIFAP in Mexico in order to share an important research background for future research programs.

2. Materials and Methods

This work was carried out at the Southeast Regional Research Center (CIRSE) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) with the headquarters located in the City of Mérida, Yucatán, Mexico.

A documentary research was carried out in order to select, compile, organize, interpret and analyze information of the main projects that have been conducted in the state of Yucatan Mexico; and the main technological innovations are to be mentioned.

This kind of research has become, in a short space of time, in technique which can include different matters from manual to mechanical processes [18]. Following are mentioned the most important characteristics of the documentary research:

- It is common to all types of research with a theoretical or referential foundation, being natural or social sciences
- It obtains data from reviewed documents of different areas
- It organizes the collected data consistently
- It allows to rediscover or reinterpret different aspects of a subject
- It helps to identify gaps, omissions or misrepresentations from reference sources
- It suggests new perspectives and/or analytical theories based on the information obtained
- It requires the ability for synthesis, deduction and analysis
- It provides strength to the researcher's conclusions

For documentary research, in a strategic sense, there are two types of essential sources: The Primary Research sources and the Secondary ones. For this work the following sources of information were considered:

Primary research sources gave us the first-hand, original and relevant information. For this case, the projects related to the cultivation of *Stevia* and their corresponding reports from 2010 to 2021 were considered as Primary sources [18].

Secondary research sources were considered all information gathered from other sources which were submitted to a process of scrutiny, restructuring, analysis, and criticism. The secondary sources would be other previous biographies or history books that expose at least part of the theme under study. Printed or digital documents from conferences, symposia or publicity information were considered Secondary sources [18].

Based on the information from the primary and secondary sources, basic information of projects that were carried out from 2010 to 2021 at CIRSE and their main contributions are presented in Table 1. Details of each projct will be described later on.

3. Results and Discussion

Projects Description

Project 1. Validation, development and technological dissemination in Stevia (*Stevia rebaudiana* Bertoni) under irrigation conditions in south-southeast Mexico.

Subproject 1.1: Determination of the productive potential of *Stevia rebaudiana* Bertoni in Mexico.

Contribution 1.1.1. There is a continuous demand for stevia at national and international level but due to the scarcity of raw material the Mexican government, industrials, private and social sectors are highly interested in its cultivation. To do so, better decision-making is needed and the best potential areas are to be selected.

Table 1. Projects of *Stevia rebaudiana* Bertoni which were carried out from 2010 to 2017 in CIRSE-INIFAP.

Project Names	Type of Project and Researcher in charge	Period	Funds ($) in Mexican pesos	Results
Validation, Development and Technology Dissemination of Stevia (*Stevia rebaudiana* Bertoni) under Irrigation Conditions in South-Southeast Mexico.	Basic and Applied Research Ramírez Jaramillo Genovevo	2010 to 2012	1,200,000.00	1) Pre-emergent Weed Control 2) Population density to reduce costs 3) Determination of glycoside content in leaves 4) Potential areas for stevia cultivation in Mexico 5) Technological Validation in four states of South-Southeast Mexico
Research, Development, Promotion and Technology Transfer for the use of biofertilizers in different agro-ecological environments.	Applied research Lozano Contreras Mónica Guadalupe	2010 to 2012	250,000.00	1) Biological rooters for stevia seedlings
Stevia Seedling Production	Applied research Ramírez Jaramillo Genovevo	2012 to 2014	300,000.00	1) Protocol for stevia seedlings production
Genetic breedings of stevia (*Stevia rebaudiana* Bertoni) to obtain Mexican clonal varieties	Applied research Ramírez Jaramillo Genovevo	2015 to 2017	300,000.00	1) Lot of clonal varieties
Characterization of genetic materials of *Stevia rebaudiana* Bertoni.	Applied research Ramírez Jaramillo Genovevo	2017	200,000.00	1) Characterization of an outstanding material

INIFAP has databases in * .shp format about soils, climate types, precipitation, temperature and altitude and the agroecological requirements of the crop. This information was used to reclassify the areas in suboptimal and optimal conditions using the **Arc/View 3.3 software.** In Mexico there are more than 3 million hectares with optimal condition and more than 2 million with suboptimal ones with a geographical distribution according to **Figure 1**. It is feasible to increase the surface and the productivity of stevia cultivation mainly in the Pacific, Gulf and in the Yucatan Peninsula [19] [20] [21] [22] [23].

More than 13 states of the Mexican Republic were identified with optimal conditions and more than 14 states showed to be in a suboptimal environment to produce stevia under irrigation. The key factors to classify high potential areas were: altitude, temperature and type of soil.

Subproject 1.2. Weed control to establish *Stevia rebaudiana* Bertoni, in Yucatan, Mexico.

Contribution 1.2.1: The high labor demand for weed control represents important increases in the production costs. Profitability is reduced when there is a lack of hand laborers. This study aimed to evaluate other alternatives for weed control to reduce the dependence on labor trying to avoid the contamination of the harvested leaves.

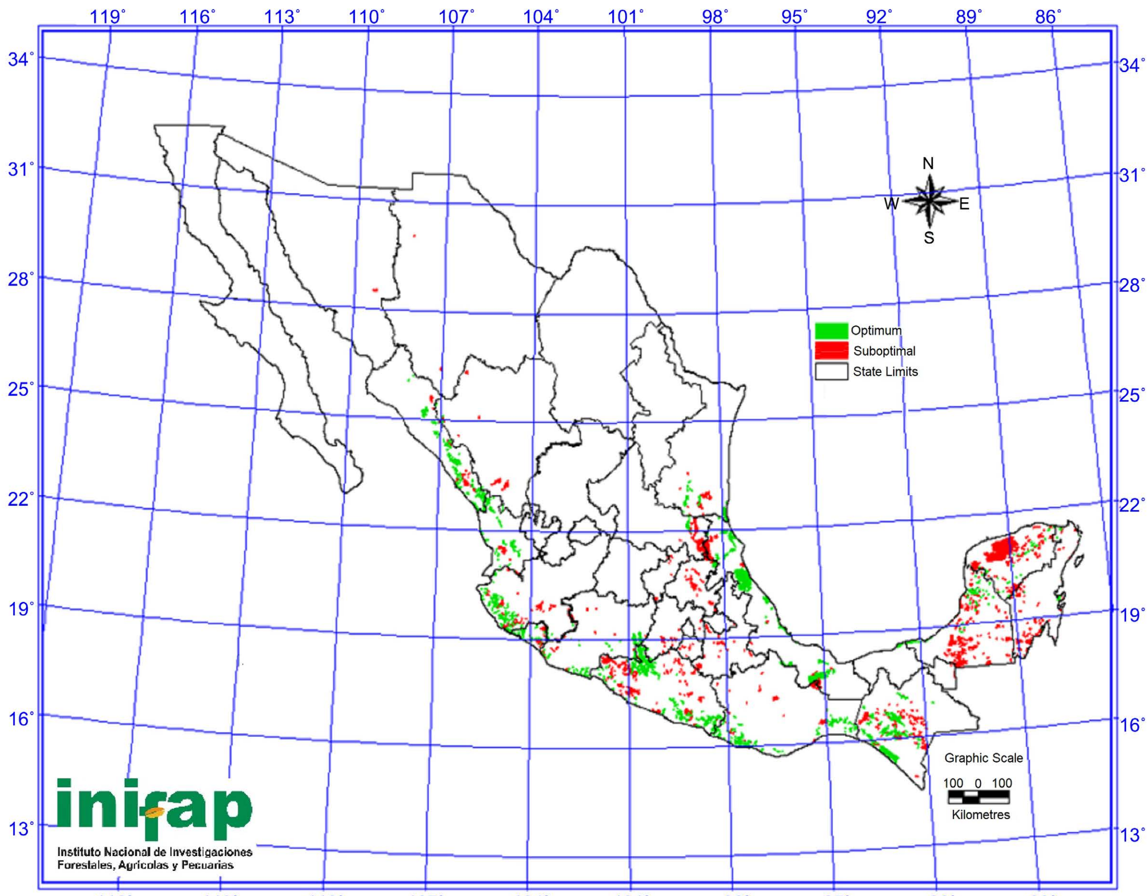

Figure 1. Geographical distribution of potential areas for stevia cultivation in Mexico.

The *Trifuralin* herbicide and their effect on weed control were evaluated at three different doses at first stage of stevia production in a red deep *Luvisol* and a stony *rendzic Leptosol* in Muna and Mocochá, Yucatan Mexico.

The weed cover was recorded 18 and 35 days after the application (DAA) of Trifluralin in both localities (**Table 2**). High Variation Coefficients (VC) were found ranging from 120% to 186%. However, when analyzing the numerical values it was evident that the herbicide was highly effective to control weeds in both localities.

In Mocochá, weeds were reduced from 43.76% (T1) to 93.50% (T2 and T3) at 18 DAA and from 62.7% (T1) to 88.72% (T2) and 91.75% (T3) at 35 DAA. In Muna the weed reduction was more drastic, even with the lower dose of Trifluralin. This was probably due to the fact that the deep red soil favored a more homogeneous dispersion of the herbicide on the surface. Weed cover was reduced above 95% in Muna.

Subproject 1.3. Population density for the establishment of *Stevia rebaudiana* Bertoni in Yucatan, Mexico

Contribution 1.3.1: At the beginning when stevia was introduced in Mexico, a high plant density of 100 to 200 thousand seedlings per hectare was recommended. However, high densities favored the incidence of fungal diseases and the production cost increased due to high seedling prices in the market (2.00 to 5.00 Mexican pesos per seedling).

With the new technology, INIFAP proposed to farmers, a new population density ranging from 58,000 to 60,000 plants per hectare, planting the seedlings, in triangle position, at 40 cm distance each other on beds of 1-meter-wide and 50 meters long.

Four crop lines on each bed are recommended to be irrigated with two irrigation strips and droppers every 25 to 30 centimeters. Transplanting 250 plants per row and 1000 plants in the 4 rows producers can reduce the population density, the production cost diminishes by up to 33.8% and the Benefit/Cost ratio ranges from 2.27 to 2.90 [12] [24] (**Figure 2**).

Table 2. Weed cover (%) and herbicide effect (% of reduction) in a stevia crop. Application of Trifluralin in two localities of Yucatan, Mexico.

	Mocochá				Muna			
Treatments	**18 DAA**	**% of Red.**	**35 DAA**	**% of Red.**	**18 DAA**	**% of Red.**	**35 DAA**	**% of Red.**
T0 (Control) (0 ml of c.m./l)	7.70 NS	-------	24.75 NS	-----	7.88 NS	------	46.25 NS	------
T1 (5 ml of c.m./l)	4.33	43.76	9.21	62.78	0.06	99.23	2.25	95.13
T2 (7.5 ml of c.m./l)	0.50	93.50	2.79	88.72	0.15	98.09	1.00	97.83
T3 (10 ml de c.m./l)	0.50	93.50	0.75	91.75	0.07	99.11	1.27	97.25
VC (%)	122.41		121.92	-----	186.53	-----	130.68	------

c.m./l = Commercial Material per liter of water. NS = No Significant Differences. DAA = Days After Application.

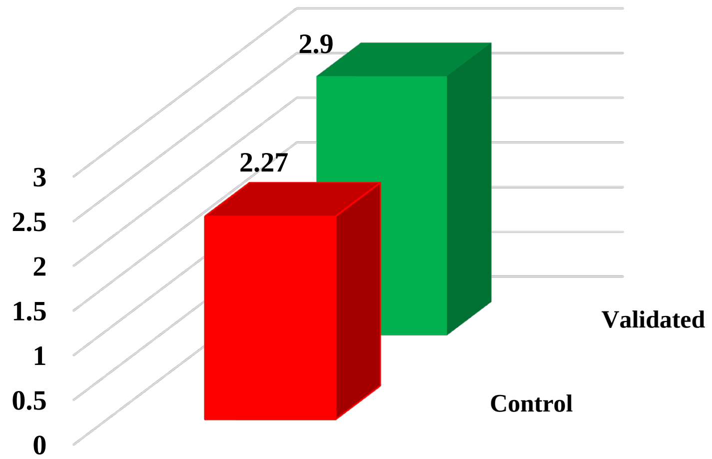

Figure 2. Benefit cost ratio (B/C) with low (60,000 plants·ha^{-1}) and high (100,000 plants·ha^{-1}) population densities.

Subproject 1.4. Validation of technology on *Stevia rebaudiana* (Bertoni), in the South-Southeast of Mexico under irrigation conditions.

Contribution 1.4.1: At international level there are outstanding countries such as Colombia and Peru with proper technologies to grow stevia and Peru with high yields of 7.0 to 8.0 t·ha^{-1} of dry leaves under irrigation conditions while Paraguay does not exceed 3 or 4 t·ha^{-1} under irrigation and 1.0 or 2.0 t·ha^{-1} under rainforest.

In order to promote new technologies to private companies, interested on stevia cultivation and industrialization, it was basic to validate the existing INIFAP technology in plots of cooperating companies. The validation was carried out in the South-Southeast region of Mexico in 5 plots scattered in the states of Yucatán (2), Quintana Roo (1), Chiapas (1) and Veracruz (1). There are private companies in those states highly interested to cultivate, industrialize and commercialize stevia [12] [25]-[30].

The results have shown that stevia is well adapted to the Yucatan climate and soils conditions. In 2011, yield at first harvest was 3.54 t·ha^{-1} in stony soils (*Leptosols*) of Baca and 4.23 t·ha^{-1} in the *Luvisols* of Tizimín both localities in the state of Yucatán, Mexico.

The plant height in Tizimín was 48 cm while in Baca 46 cm; the canopy diameter in Tizimín was 46 cm in Tizimín and 41 cm in Baca. So based on the validation results it can be concluded that Yucatán has good production potential for stevia crop.

Subproject 1.5. Content of *Stevioside* and *Rebaudioside A*, in three materials of *Stevia rebaudiana* Bertoni cultivated in the South-Southeast of Mexico

Contribution 1.5.1: This work aimed to evaluate the content of *Stevioside* and *Rebaudióside* A in leaves of Stevia diverse genetic materials established in South-Southeast Mexico.

Four genetic materials from Paraguay were evaluated in the state of Quintana

Roo, Mexico: three creoles (creol 1, Paraguayan Creol and the SM1) and one genetic improved material named Morita II.

Morita II was evaluated in the states of Yucatán, Quintana Roo, Veracruz and Chiapas. Approximately 1 kg of leaves were cut 3 months after planting and the content of Stevioside and Rebaudioside A were quantified using a liquid chromatography (HPLC).

There were found statistical differences (P < 0.05) in the content of sweeteners between the studied materials. The concentration of *Stevioside* for Creol 1, Paraguayan Creol, SM1 and Morita II were 7.15c ± 0.70, 5.09b ± 0.04, 5.03b ± 0.10 and 1.92c ± 0.43 grams per each 100 grams of dry leaves; and the concentrations of *Rebaudioside* A were 2.93b ± 0.27, 2.42b ± 0.08, 1.72a ± 0.09 and 10.12a ± 2.11grams per 100 g respectively. No significant statistical differences (P > 0.05) were found between the Glycoside content of Morita II established in the different states.

The *Stevioside* was the glycoside with higher concentration in all materials with the exception of Morita II (Table 3). This material showed different behavior as compared to the other materials since it presented a higher concentration of Rebaudioside A. This is an advantage due to the fact that *Rebaudioside* A is less bitter and sweeter than *Stevioside*. Morita II is a material with great potential for the sweetener industry [21] [22] [31].

Project 2. Research, Development, Promotion and Technological Transfer for the use of Bio-fertilizers in Different Agroecological Environments.

Subproject 2.1. Biological rooters for the production of stevia seedlings.

Contribution 2.1.1. Conventionally, to favor the rooting of stevia cuttings, commercial products based on hormones (auxins) are applied such as *in-dolbutyric* and *naphthalene* acetic acids. Another better option is the application of beneficial microorganisms, such as M*ycorrhizae* and bacteria such as *Azospirillum* or *Bacillus*, which act as biological rooters.

Beneficial microorganisms improve plant development and nutrition and increase crop tolerance to biotic or abiotic stress. The interest in the use of *Mycorrhizae* and bacteria is due to a positive symbiosis effect, since M*ycorrhiza* increases the absorption of nutrients when water absorption is improved and at

Table 3. *Esteviosíde* and *Rebaudioside A* content in four stevia materials.

Material	***Estevioside***	***Rebaudioside A***
	g/100 g of dry leaves Average ± Standard deviation	
Creol	7.15a ± 0.70	2.93b ± 0.27
Paraguayan Creol	5.09b ± 0.04	2.42b ± 0.08
SM1	5.03b ± 0.10	1.72b ± 0.09
Morita II	1.92c ± 0.43	10.12a ± 2.11

[a,b,c]different letters in the same column indicate significant statistical differences.

the same time avoids pathogens whilst bacteria produce growth stimulating phytohormones such as *in-doleacetic acid*, *indole butyric acid* and biologically fixes of gaseous nitrogen in a natural way.

To improve inoculation in the stevia cuttings, 15 grams of Mycorrhiza *Rhizophagus intraradices* or the mixture of *Bacillus sp*. and *Azospirillum brasilienses* should be mixed and stirred in 1 litter of water until having a homogeneous solution. The cuttings need 1 minute to be soaked in the solution before transplanting. Prolonged exposure in the solution can cause damage and failure of the rooting [32].

Project 3. Production of Stevia Seedlings

Subproject 3.1. The protocol to produce stevia seedlings

Contribution 3.1.1. A proper rooting of stevia is needed during plant production for future transplantation in the field. So, special care must be taken when handling the cuttings in the production trays. It is basic to remove some pairs of leaves and keep at least two internodes and three to four pairs of leaves. The proper length proposed is of approximately 8 centimeters after eliminating the lower parts of the cuttings. These are the parts that first enter into a negative oxidation process avoiding an optimal rooting. It is important that the plants, after being inoculated with biofertilizers (Mycorrhiza *Rhizophagus intraradices* or the mixture of *Bacillus* sp. and *Azospirillum brasilienses*), are placed on the trays (200 cavities) in a time no longer than 4 hours to avoid dehydration. The optimal depth of planting should be between 2.5 to 3.0 cm (**Figure 3**). It is important to compact, by pressing a little, the commercial rooting substrate in the trays and leaves should not be in contact with the substrate due to fungal diseases [21] [33].

Project 4. Genetic Improvement of Stevia (*Stevia rebaudiana* Bertoni) to Obtain Mexican Clonal Varieties

Subproject 4.1. Clonal Varieties in Yucatán

Contribution 4.1.1. Ten stevia materials with good phenotypic characteristics were studied, produced and selected since 2012. Just two out of the ten materials showed outstanding agronomic behaviour.

Project 5. Project: Characterization of Genetic Materials of *Stevia rebaudiana* Bertoni.

Subproject 5.1. Characterization of Two Clonal Varieties

Contribution 5.1.1. The characterization of a new clonal variety named in maya terms *Ch'ujuk* (Sweety) [34] [35] was obtained. The main characteristics are mentioned below.

Plant Height. *Ch'ujuk* is a clonal variety with an intermediate cycle (80 to 100 days), semi-decumbent plant with 10% to 70% of the stems in contact with the ground, average height of 60 cm as compared to the control Morita II with 45 cm (**Figure 4**). It is resistant to lodging since it is a multi-stem plant with abundant roots in relation to Morita II with a single stem, scarce root development and more susceptible to lodging and soil fungi deseases [36].

Figure 3. A 25 days old seedling ready to be transplanted.

Figure 4. The *Ch'ujuk* clonal variety is 20 cm higher than Morita II.

Stem type and characteristics. It has above eight primary stems while Morita II only has three. Regarding secondary stems, the *Ch'ujuk* clone is highly branched (Figure 5) as compared to Morita II with little branching stems. The stem diameter is 15.9 mm against 8.39 for Morita II; although the internode length is a little bit greater in Morita II (8 cm) than that of the clone (7 cm) [36].

Leaf type and characteristics. An important factor in the yield of the "stevia" is the leaves. The average length of the leaves of *Ch'ujuk* is 6 cm against the 4.5 cm of Morita II. In the case of the width, Ch'ujuk showed to have a 3 cm wide leaves and Morita II just 1.5 cm and the petioles are sessile in both varieties. The margins of the blades, with 12 teeth, are crenate in *Ch'ujuk* while Morita II has serrate margins with 18 teeth. All leaves are in opposite position through the stems in all cases [36].

Inflorescence color. The color of the inflorescence is white [36].

Crop cycle of the Clonal Variety Ch'ujuk. The clonal variety has a short cycle (80 to 100 days) while Morita II has an intermediate one (100 to 130 days).

Figure 5. *Ch'ujuk* variety presents 6 cm long leaves with multi-stems and abundant roots.

This is an advantage of *Ch'ujuk* over Morita II since it can be harvested up to six times per year [36].

Sanitary Behavior. The *Ch'ujuk* clone has a moderate resistance to the fungi: *Rhizoctonia solani*, *Fusarium* sp and *Sclerotium rolfsii* and Morita II is moderately susceptible to *Alternaria* sp., *Septoria* sp. and *Cercospora* sp. [36].

Concentration of Glycosides. The concentration of the main glycosides measured in the clonal variety was 6.78 g of *Stevioside* per 100 g of dry leaves, 7.46 g of *Rebaudioside-A* per 100 g of dry leaves and 2.14 g of *Rebaudioside-C* per 100 g of dry leaves whilst for Morita II the numbers were 3.97, 14.5 and 2.24 g per 100 g of dry leaves of *Stevioside*, *Rebaudioside-A* and *Rebaudiósido-C* respectively [36].

Yield Performance. The clonal variety showed a potential yield of 9.67 t·ha^{-1} of dry leaves per year representing 0.73 t·ha^{-1} of *Rebaudioside-A*, 0.65 t·ha^{-1} of *Stevioside* and 0.20 t·ha^{-1} of *Rebaudioside-C* [36].

4. Conclusions

The Mexican CIRSE-INIFAP has technological innovations able to impact stevia production in Mexico. Potential zones have been defined where the largest areas are located in the states of the Pacific from Nayarit to Chiapas.

The technology referred to population density (plants per hectare) can balance the profitability in favor of the producers besides the advantage of having a clonal variety named *Ch'ujuk* well adapted to the agroclimatic conditions of the country including the tropics. The clonal variety *Ch'ujuk* is a good alternative for cultivation since it has shown better agronomic behavior and yield potential than the control Morita II.

In addition, the clonal variety *Ch'ujuk* presented an adequate balance between *Stevioside* and *Rebaudioside-A* making it attractive for the sweetener industry and for direct consumption for health purposes.

The yield of dry leaves obtained with the clon was 1.7 times higher (9.67

t·ha^{-1}) than that of the Morita II (3.5 t·ha^{-1}). This new variety is recommended for the states of the Yucatan Peninsula in Mexico such as: Campeche, Quintana Roo and Yucatán; although, there are other states, located in the tropics, with great potential like: Nayarit, Michoacán, Jalisco, Guerrero, Oaxaca, Chiapas, Veracruz and Tabasco.

Acknowledgements

We thank the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), for financing this work as part of the project called: **Caracterización de mate-riales geneticos de *Stevia rebaudiana* Bertoni**. (**Characterization of Genetic Materials of *Stevia rebaudiana* Bertoni**). **Project number** "15233034015".

Conflicts of Interest

The authors declare no conflicts of interest.

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