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REVIEW

Stevia rebaudiana: Reporting data from the sweetener crop for researchers and smallholder farming assistants

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ABSTRACT. In science, technology and assistance to smallholder farming or large crop acreages of *Stevia rebaudiana* is necessary to report data using information components to facilitate investors to understand the local field conditions to make assertive economic decisions for long periods. *Stevia rebaudiana* produces consistent natural sweeteners with safe performance as reported in a multitude of scientific publications. The leaf chemicals components are suitable to humans with healthy disorders affected by the consumption of sugarcane or many other industrial sweeteners. Otherwise, aspects related to the agriculture of sweeteners production still require additional discussion to improve qualitative and quantitative traits necessary for cultivating cost-effective crops. The current text highlighted some agriculture decisions necessary to make the crop more profitable to investors as researchers, agriculture influencers, farmer assistants and consultants of companies looking for alternatives within agro-ecological systems.

Keywords: sweetener; seeds; bedding plants; crop production.

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Introduction

Stevia rebaudiana (Bert) Bertoni is a semi-perennial plant classified in the Kingdom Plantae, Subkingdom Tracheobionta, Super division Spermatophyta, Division Magnoliophyta, Class Magnoliopsida, Subclass Asteridae, Group Monoclamidae, Order Asterales, Family Asteraceae, Subfamily Asteroideae, Super tribe Helianthodae, Tribe Eupatorieae and Genus Stevia (Yadav, Singh, Dhyani, & Ahuja, 2011). The first element of its scientific name (Smith Jr., 2014) is followed by the epithet rebaudiana, which provides the name of the species Stevia rebaudiana. Historically, the Stevia rebaudiana (Bert) Bertoni has human name in parenthesis because it indicates the botanist (Moises Bertoni) who first published the specific epithet rebaudiana honoring the chemist Ovidio Rebaudi. After the parenthesis (Smith Jr., 2014), we have the same surname (Bertoni), who later transferred the species into the current genus Stevia, unlike Eupatorium when it was first classified. Scientific reports with only the plant terminology "Stevia" suggest many species of the genus Stevia. Many popular names will not be part of this introduction (Carneiro, 2015) because the Guarani name (Ka´a he´ē) enumerates many of the common names. Stevia rebaudiana has been highlighted as a natural sweetener producer because it has a multitude of sweet steviol glycosides with emphasis on the rebaudioside-A for replacing sugarcane products that cannot be ingested by diabetic or overweight patients.

The possibility of spontaneous establishment of the *Stevia rebaudiana* as weed outside its origin center which is the Amambai Hills (22°38' S, 55°53' W and altitude above 511 m) is incipient. At the Iguatemi Research Farm (23°21' S, 52°04' W and altitude above 550 m), we did not observe any occurrence of this species as spontaneous plants over 40 years cropping field plots or cultivated fields for on-farm observations. The biological traits of *Stevia rebaudiana* are similar to those of many horticultural crops cultivated for leaf production. Thus, the sequence of inputs for establishing profitable and sustainable crops requires the availability of seed lots with high genetic, physiological and sanitary qualitative traits, and the production of vigorous bedding plants to supply crop growth during many growing periods, seasons, or both.

Next, reports must notify readers about the quality of the soil for transplanting bedding plants, the status of the water sources for irrigation purposes, the application of chemical or organic fertilizers to promote

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uniform growth and development. Furthermore, cultivars with rebaudioside-A levels are suitable for eliminating the aftertaste of commercial products and capable of multiplying plants using commercial seeds as soon as possible. When cultivars are not available, the characteristics of the landrace population can be reported because they suggest biodiversity in breeding programs (Brandle & Rosa, 1992). Authors must be committed to follow the stages of plant development (Carneiro, 2007; Le Bihan, Cosson, Rolin, & Levraud, 2020), regardless the objective of the investigation, either for leaf production, steviol glycoside content, seed science and technology, sustainable crop production, or all of them.

Currently, the sweetener market of *Stevia rebaudiana* has supplied genotypes from companies that have developed many cultivars with qualitative standards of steviol glycosides as the rebaudioside-A. However, agricultural protocols have not yet been reported as they are necessary. Most of the time, readers are not aware of the origin of the leaves from which the sweetener contents have been investigated. Moreover, cultivars multiplied by seeds are still incipient (Parris, Shock, & Qian, 2016) as well as cultivars suitable for plant growth under lower latitudes in both hemispheres. We must consider that the flowering of *Stevia rebaudiana* under photoperiod less than 13h can last for two months or even more. Thus, the information of only the pre-flowering period is not precise for making agricultural decisions under field conditions, where leaves are harvested for steviol-glycoside analyses.

Next, most crops are distributed above 40° N under a Mediterranean climate (Clemente, Angelini, Ascrizzi, & Tavarini, 2021; Fronza & Folegatti, 2003) or not (Le Bihan et al., 2021), where have also increased the number of scientific and technical publications. However, genotypes adapted to mid or lower latitudes have not yet been investigated in depth, despite the many attempts with available cultivars. The introduction of cultivars has been the most affordable method to start crops in countries that have not yet achieved a complete system of plant breeding and crop production. Concepts attached to the systems of plant production must be highlighted.

Seed production

First, seed production requires understanding some aspects of the crop physiology because *Stevia rebaudiana* is a short day C_3 plant that requires around 13 hours of light to start the flowering period.

Years ago, Carneiro (2015) reported two concepts that affect the seed production: one about the growing seasons and the other about the growing periods. The first concept considers that under a photoperiod higher than 13:00 hours, *Stevia rebaudiana* maintain vegetative growth. Francis (1970) is one tutorial guide necessary for researchers and scholars who are introducing not only *Stevia rebaudiana* in new latitudes, but also many other plants requiring short days for seed production under field conditions. In latitudes and altitudes without snowing episodes, we can consider a growing season with 365 days. Two growing periods were suggested by Fronza and Folegatti (2003) for higher latitudes in Italy (approximately 43° N, 10° E, and an altitude of 5 m), but in Maringá, Paraná State, Brazil (23°21' S, 52°04' W, and an altitude of 542 m) the crop establishment from November 1 to 12, can provide two profitable growing periods (the first and the second) when the cultivar has plants with tolerance to photoperiods affordable for leaf production. Moreover, new cultivars that support leaf production must support at least a third growing period without developing any flowering stages. In tropical climates, the usual two growing periods have intense rainfall and high temperatures, and these environmental conditions are not affordable for seed production under field conditions in south latitudes, or even for harvesting leaves without cost-effective leaf-dryer systems.

Currently, the alternative is the production of seeds under glass- or plastic-house conditions where water must be supplied to the plants by drip irrigation. Seed quality is significantly affected when water is sprayed on mature or maturing seeds (Carneiro, 1990). These facts explain the harvesting of seed lots with lower quantitative estimates. Under flowering conditions, the crop has individual plants with flower buds, flowers under anthesis, pollinated flowers, seeds under maturation, and mature seeds. Consequently, we can see along the flowering stages the presence of mature seeds that can be harvested by handy workers.

Furthermore, an important decision to produce high-quality seeds is to provide beehives with stingless bees to supply flowers with effective pollinators and avoid high levels of unfertilized flowers that produce fruits with yellow color classified as inert material in the seed purity test (Carneiro, 2014a). In Maringá, Paraná State, Brazil, we provided honey bees (*Apis mellifera* L.) under glasshouse conditions without any episode with handy-workers accidents. The flowering architecture of *Stevia rebaudiana* indicates the differentiation of corymbs of capitula in the first two growing seasons of plant development (Carneiro, 1990; Carneiro, 2007) or cymes of capitula (Brandle, Starratt, & Gizen, 1998; Carneiro, 2007) in the next crop growing season.

Thereafter, the flowering system is modified in a similar way to other agricultural plants, and basic plants have to be replaced by foundation plants. In seed production systems, basic plants are technical term as well as foundation or genetic plants. Thus, the information of only the pre-flowering period is not precise for making agricultural decisions under field conditions, where leaves are harvested for steviol-glycoside analyses.

Currently, the occurrence of lower percentages of seed germination is not the objective of plant research. In the growing season of 2019/2020 at the Iguatemi Research Farm (23°21' S, 52°04' W and an altitude of 542 m), we achieved 87% germination using a plastic box and germitest paper Cell-065. In the last seven years, we changed the recommended germitest paper (Brasil, 2009) to sterile medical care cotton because it maintains seedborne diseases around the germinating fruit-like seeds (Carneiro, personal communication). This method increases the quality of the seed germination data. Thus, estimates higher than 80% have been the common figure in our seed production systems, where there are artificial control of photoperiod and water. Every place chosen to cultivate *Stevia rebaudiana* must have the driest period of the year identified and selected as the best moment for seed production. In Maringá, Paraná State, Brazil, despite the climate Cfa, August has most of time the weather with mild temperatures, low levels of humidity in the air, and scarce rains. The literature is still incipient regarding the effects of air humidity on the seed quality of *Stevia rebaudiana*. The flowering stage develops a unique ecosystem on the *Stevia rebaudiana* canopy, where many species of aphid insects feed on flowers, seeds, and leaves where they multiply. White cochineal and white flies were common in some of the experimental plots. Studies aimed to understanding these micro ecosystems does not yet exist but they are important to agriculture investors.

Seed testing

The rules for seed testing (Brasil, 2009; International Seed Testing Association [ISTA], 2017) have protocols for purity and seed germination tests for most cultivated plants, and literature in scientific journals or technical reports have the specific literature for *Stevia rebaudiana* (Carneiro, 2014a; 2014b). The quantity of high-quality seeds required for the germination test of horticultural plants is four replications of 100 seeds, eight replications of 50 seeds, or 16 replications of 25 seeds (400 seeds). Unfortunately, these figures were reduced to only 200 seeds when investigative methods were applied to many crops. In the case of *Stevia rebaudiana*, we have to consider two factors. The achene is a small dry fruit with a pappus ready for seed dispersion (Angelini, Martini, Passera, & Tavarini, 2018), but the pappus can be eliminated when the aim is not investigating the seed quality; otherwise, the pappus must be part of the fruit because the mechanical abrasion of the fruit can damage the seeds, affect the seed quality and consequently the interpretation of the results. The number of replications must be determined under laboratory conditions. The number above six replications permits exploratory data analysis when the aim of the experiment is to understand the responses from plant populations.

Light, water and temperature have to be controlled during the germination test. However, researchers must pay attention to the quality of LED light during germination tests because commercial white and red LED lights can still deliver photons in the region of 730 nm (Carneiro, personal communication). Most of the time, the percentage of seed germination does not reach high standards, but this fact should not be misunderstood as primary seed dormancy because this physiological characteristic is not present in the seeds of *Stevia rebaudiana*. However, this could be the case of secondary dormancy (Popinigis, 1977, page 72, Table 15, free for download, and with original English references), but this fact in *Stevia rebaudiana* still requires indepth investigation. This careful protocol can be applied to many other species of the Asteraceae family, where spontaneous plants also develop dry fruit production.

One trait significant in seed production is the seed size, which still has to be characterized by companies developing cultivars of *Stevia rebaudiana*. Moreover, only a few research stations or universities have equipment for grading *Stevia rebaudiana* seeds based on their size, despite the fact that this technology is available worldwide from specific grading machinery companies. Furthermore, ten replications of 100 seeds can be recommended for research and development because they allow the researcher to apply box-and-whisker plots (Carneiro, 2015). This graphical representation of seed production and technology highlights the presence of variability and asymmetric distribution of data, and it is reader-friendly to understand datasets (Krzywinski & Altman, 2014).

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Site description

In research and development, the materials and methods section must have the first paragraph describing the location where the experiment or the crop field was established. In Maringá at 23°21' S, 52°04' W, and an altitude of approximately 542 m, Paraná State, Brazil, we have been cropping a plant population of Stevia rebaudiana for 43 years. Here, we have described the climate Cfa following the Köppen classification; however, under some weather conditions, we can have temperatures randomly ranging from 5 to 30°C and relative humidity from 53 to 100% as we found from August 1 to September 30, 2018. Furthermore, in some winter days, frost temperatures below zero degrees Celsius for two or three days are common. Leaves of Stevia rebaudiana at the flowering stage can successfully support these ice-like conditions, unlike when the regrowing stems have less than ten vegetative nodes $(V_{4,10.0})$. Thus, the darkness of leaf plants indicates cells destroyed by ice formation (Carneiro, personal communication), and agriculture investors lose a significant number of days in the growing period, despite the fact that mild frost does not kill the plants, but it can certainly affects seed quality under field conditions. This information also helps agronomists, plant biologists, and biochemists to be more assertive about the effects of weather on steviol glycoside contents. Next, the report of average temperatures must be avoided because at higher latitudes, the autumn and spring seasons have a range between the minimum and the maximum temperatures large enough to induce cropping mistakes. In agriculture, the best decision is to report the weather conditions during the growing periods. Next, the soil classification and the chemical analysis of macro- and micronutrients have to be described as the last paragraph to facilitate the next one to report the methodology. Please, see the literature about bedding plant production as an example (Carneiro, Muniz, & Guedes, 1997).

In bedding plant production, the electrical conductivity of the growing medium must be reported in μ S. cm⁻¹ or mS. cm⁻¹. Some soil laboratories may not yet have electrical conductivity devices, but the cost is affordable to professional budget of researcher or farming assistant, and the methodology to prepare the sample is available in the literature on bedding plant production (Landis & Dumroese, 2006; Tomé Jr., 1997).

Production of bedding plants

Bedding plants of *Stevia rebaudiana* can be produced by sowing pure seeds or by rooting stem cuttings when the uppermost nodes from every lateral stem is sharply cut by scalpel at the angle of 45 degrees above the sixth node. This technique induces the growth of uniform bedding plants, improves water absorption, but as soon as possible they have to be immersed into wet agriculture vermiculite (Carneiro, personal communication). Organic growing medium in this propagation system have always be avoided. The rooting of these cuts happens under spray irrigation system controlled by timer that favor oxygen levels in the growing medium. Uniformity or not in the circumference from these internodes still requires investigation. Experiments under plastic- or glass-house conditions have to be replicate using horticulture trays randomly distributed in the space because of shadows. Styrofoam horticulture trays is highly recommended because of the size of the cells 35 mL, the robustness construction and the long life-span of the product contrast with black plastic tubes usually lost by handy workers under field conditions.

Bedding plants are significantly affected by the container volume where they are growing. Thus, the choice and description of the containers are always necessary. The market has a multitude of trademarks, types, shapes, and container sizes, but Styrofoam trays are the best choice for transplanting management under field conditions. They have a uniform volume, are not lost in the field during handy labor, and have a long working lifespan in farm systems. The volumes of individual containers must be reported in mL. In agricultural systems that use seed germination to raise bedding plants, the number of nodes has to be reported (Carneiro, 2007), unlike the linear size of these bedding plants at the end of the experiment or at transplanting under field conditions. In cell trays, some overgrown bedding plants can etiolate because they compete for light under containers of small volumes (Carneiro et al., 1997).

The growing medium in which seeds germinate depends on the type of bedding plant production. The use of seeds to produce bedding plants allows many local sources of growing medium to be amended with animal manure. Laying hen manure (Carneiro et al., 1997) or goat manure (Carneiro, unpublished data) are the best options, although both still require further investigation. Next, there is a new option with organic fertilizer such as Super Magro that may be sprayed on the leaves. How to prepare the solution of "super magro" is available in the literature (CTA-ZM, 2000). Dosages of Super Magro and the stages of development for the application on *Stevia rebaudiana* crops still require in-depth investigation. In contrast, the rooting of cuttings

requires non-organic growing media such as vermiculite or perlite. In Maringá, Paraná State, Brazil, we experienced only vermiculite (Carneiro, personal communication). In both cases, the literature is still incipient about products to amend growing media to cultivate *Stevia rebaudiana*.

Next, the application of concepts based on agro-ecology methodologies that study the relationship between humans, plants, animals, and environmental conditions is the approach of researchers committed to cost-effective technologies for smallholder farmers around the world. Agro-ecology is an integrated approach that simultaneously applies ecological and social concepts and principles to the design and the management of sustainable agriculture and food systems (FAO, 2022). In the next 50 years, agricultural designs have to be changed because the environmental conditions do not support the current model where the soil has been lost by the intense use of heavy machinery, reduced sources of potable water because of the high levels of different contaminants, reduced biodiversity of soil microorganisms or its loss in some countries, and the application of political decisions that will enlarge the gap between the wealthy and poverty.

Furthermore, efficient micro-organisms as fungi, bacteria and yeast to produce bio-inputs in agriculture have increased as inoculants, soil conditioners, bio-fertilizers, nematicide and bio-fungicides (Vidal, Saldanha, & Verissimo, 2020). Their long-term action in the environment is favorable to microbial antagonists, promotes plant resistance to phyto-pathogen (De Medeiros; Silva, & Pascholati, 2018), increase the plant rooting and synthesis of secondary plant metabolic. In Brazil, the application of efficient microorganism in agriculture system is highlighted because of the use of *Bradyrhizobium* in soybean crops where the absorbed biological nitrogen reduces or eliminate the application of chemical nitrogen formulation, in corn crops inoculated with *Azospirillum brasilense* which promotes the plant growth (Barbosa et al., 2022a), and *Trichoderma* applied as bio-control and plant growth of many crops (Barbosa et al., 2022b). These bio-products must be expanded to many other crops as the *Stevia rebaudiana* because the National Program of Bio-Inputs according to the Decree n°10375/2020 (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2020).

Moreover, The researches which evaluate different species of *Trichoderma*, *Bacillus*, *Paecilomyces*, *Pochonia*, *Bradyrhizobium*, *Azospirillum*, and arbuscular mycorrhizal fungi have achieved the biodiversity of conidial saprophytic fungi from the Amazonian forest (Magalhães, Carvalho, Souza, & Pereira, 2017) as *Brachysporiella* sp., *Pseudobotrytis terrestris*, *Gonytrichum* sp., *Ellisembia* sp. and yeast *Pichia* sp. which promoted the rooting of eucalyptus clones (Mendes, Bonaldo, & Maciel, 2022) and passion fruit (Trento et al., 2021) from which was detected phenolic compounds after inoculation with arbuscular mycorrhizal fungi (AMFs) *Glomus etunicatum*, *Glomus intraradices*, and mixed inoculation (*Glomus clarum* and *Gigaspora margarita*). In experiments with the absence of phosphate fertilizers the contents of total phenolic compounds, the above ground dry biomass and the number of leaves were higher in the treatments inoculated with AMFs (Riter Netto, Freitas, Martins, Carvalho, & Vitorazi Filho, 2014). Thus, the investigation of *Stevia rebaudiana* responses to this new paradigm of plant production is a new approach for bedding plant production and sweeteners concentration in agro-ecological production systems.

Furthermore, homeopathy has a huge potential to improve or modify plant responses in the current agriculture and environmental designs. Agriculture researchers working with homeopathy have found unprecedented effects from seed treatment (Zanco, Boff, Werner, & Boff, 2021; Zanco; Boff, & Boff, 2013), enzymes activation (Marques, Belato, Kagami, Bonato, & Mourão, 2013; Oliveira, Maia, Alencar, Schwan-Estrada, & Bonato, 2013), pathogen control (Faedo, Verdi, Pinto, Kretszchmar, & Boff, 2022; Júnior, Correoso, Copacheski, Boff, & Boff, 2021; Souza Gonçalves, Boff, & Araújo, 2021), plant nutrition (Souza Gonçalvez, Boff, & Mora, 2018), soil and water restoration (Zanco, 2022). In *Stevia rebaudiana*, researching about homeopathic effects on seed germination and foliar area was verified with the means of foliar area larger than the control when *Kali carbonica* (100 cH) and *Calcarea carbonica* (200 cH) and highest seedling vigor with *Arnica montana* 12 cH (Zanco 2022). Additional experiments have been designed using other active ingredients and dilution from 13 to 200 cH to investigate seed quality, seedling vigor, levels of variability within population of bedding plants and consequently plant growth.

The transplanting period must be ended with the application of grasses mulching (Carneiro, 1990), which protects the soil against erosion, improves soil aeration and root penetration, and buffers the environment with earthworm-friendly organisms. Records from the mesocosms under earthworm conditions had lower concentrations of carbon and larger water-stable macro-aggregates than those of the control (Coq, Barthès, Oliver, Rabary, & Blanchart, 2007). Remember that irrigation is necessary to cultivate cost-effective crops. In many countries, systems of *Stevia rebaudiana* production have protected the soil with plastic film, which is

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expensive, fragile to weather conditions, and a soil contaminant that can reduce water uniformity along the soil profile. Otherwise, organic mulching is a source of nutrients for cropping *Stevia rebaudiana*, it maintains the effective microorganism population, and avoids sand grains on leaves that can be harvested to prepare organic blend tea. The choice of grass to apply mulching depends on the C:N ratio, lignin, and polyphenol content, which are the major determinants of organic matter decomposition, nutrient release, and soil fertility (Palm, Giller, Mafongoya, & Swift, 2001). *Panicum maximum* Jacq stems have a longer lifespan under tropical field conditions than other grasses of the genus *Brachiaria* sp., but *Oryza sativa* L. stem leftover can also be an alternative in South East Asia.

Data analysis

An assertive decision before starting an investigation in agriculture using spontaneous weeds, sweeteners, or medicinal plants is to apply exploratory data analysis (EDA) (Krzywinski & Altman, 2014; Spitzer, Wildenhain, Rappsilber, & Tyers, 2014; Maher, Markey, & Ebert-May, 2013; Waltenburg & McLauchlan, 2012; Hossa, Carneiro, Guedes, & Braccini, 2017). We recommend searching the web using the following words: *Nature methods, statistics*" because these articles are free to download. Finally, the coefficient of correlation is efficient as size effect test (Statistic Solutions, access on April 10, 2021) and must be indicated by numbers with levels of significance, instead of colors. The analysis of steviol glycosides from experiments under field conditions or pot plants under plastic houses has to follow the same statistical design describing crop yield and traits, and samples collected from each replication should be expressed by g plant-1. Triplicates under biochemistry laboratory conditions to represent treatments under pot or field conditions do not consider the variation verified under field experimental designs if not all replications from every treatment would not be evaluated. Finally, mixed models must be part of the analysis when seed lots, which are random independent variables, are also the motive of the investigation (Pereira, Janeiro, Guedes, & Carneiro, 2019), or even weather conditions, because the rainfall and temperature under some field conditions are under massive weather changes.

Plant breeding

Many companies have dedicated their financial efforts to supplying the sweetener market with varieties containing rebaudioside-A. In Brazil, two accessions, CE7 and CE14, presented the best results for the production of rebaudioside-A (Francisco, Pereira, Machado, Kanis, & Deschamps, 2018), and field responses at 25°23′ S, 49°07′ W, and an altitude of 920m indicated a ratio of 0.60 when the rebaudioside-A was compared with the stevioside (Gomes et al., 2018). These responses were similar to those reported by Andrade et al. (2021) but lower than those above 0.80 (Andrade et al., 2021). However, the search for genotypes multiplied by seeds in conjunction with a high content of sweeteners is imperative because leaf production still requires cost-effective robustness in agricultural system design.

Improvement of *Stevia rebaudiana* through plant selection is possible (Brandle & Rosa, 1992). Along with the training of personnel to produce high seed quality and bedding plants, a population containing approximately one thousand plants cultivated under local environmental conditions can be scanned under laboratory conditions for the presence of rebaudioside-A, thus eliminating the after-taste of commercial products. However, we consider imperative that bedding plants have to be produced under conditions above 13h. Many years ago, in Maringá, Paraná State, Brazil, we (Carneiro and assistants, personal communication) visualized the presence of this photoperiodic gene (Válio & Rocha, 1977) in the population multiplied in the Iguatemi Research Farm. In contrast, the majority of varieties have been maintained under the photoperiod limit of 13 hours before flowering. This requirement for sunlight has been reducing the range of latitude intervals for cropping *Stevia rebaudiana*. The effect of this natural photoperiod is limiting for leaf production because the number of growing periods in both hemispheres is reduced to a maximum of three or four, and consequently the harvesting time must be less than one week. Peasant farmers have difficulty following the calendar, and the crop initiates the flowering period when the sweetener content is reduced by the sink strength.

Cropping two or three cultivars simultaneously and cultivars requiring different photoperiods to extend the vegetative stages may optimize the cost of harvesting and drying leaves. Furthermore, latitudes above 30° may expose the crops to the risk of frost damage within the plant population and reduce the lifespan of crop development in established crops. Investors and consultants have difficulty managing these characteristics because the literature is poor when describing available cultivars under protection of copyright laws.

Finally, the seven gene domains for photoperiod detected in *Stevia rebaudiana* (Razali, Samsulrizal, & Zainuddin, 2020) can start the development of many cultivars adapted to different latitudes around the world.

Conclusion

Despite the efforts to understand the presence of steviol-glycosides in populations of *Stevia rebaudiana*, a shortage of investments in agriculture traits and cost-effective systems of leaf production still persists in the agriculture literature. A new paradigm of plant production is still necessary to elaborate cost-effective system of leaf production.

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