CULTIVATION OF STEVIA
[STEVIA REBAUDIANA (BERT.) BERTONI]:
A COMPREHENSIVE REVIEW

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Stevia rebaudiana (Bert.) Bertoni is one of the 154 members of the genus Stevia. It is a sweet herb of Paraguay. The leaves of the shrub contain specific glycosides, which produce a sweet taste but have no caloric value. For centuries, this herbal sweetener has been used by native Guarani Indians to counteract the bitter taste of various plant-based medicines and beverages. Many countries have shown interest in its cultivation, and research activities have been initiated. Incorporation of this species in agricultural production systems, however, depends upon a thorough knowledge of the plant and its agronomic potential. The published literature on research and development of this crop is meager. The aim of this chapter is to describe the ecology, importance of the plant, and its production requirements, but major emphasis is given to the agronomic and management aspects of the plant to be grown as a crop. Further, this chapter represents an effort to compile the literature on S. rebaudiana and review the current status of understanding of the plant and its potential as an alternate source of cane sugar.

I. INTRODUCTION

Stevia rebaudiana (Bert.) Bertoni is one of the 154 members of the genus Stevia. It is a sweet herb of Paraguay, which contains natural noncaloric sweetener. It is of immense value due to its adaptability to wide climatic range, the high-sweet content, and its significant contribution to the welfare of human life. This offers a solution for complex diabetic problems and obesity in humans, being calorie free. The worldwide demand for high potency sweeteners, particularly natural sweeteners, is expected to increase in the years to come. This assumes center stage in the society, which is under the organic and natural food regime. Incorporation of this species in agricultural production systems, however, depends upon a thorough knowledge of the plant and its agronomic potential. The published literature on research and development of this crop is meager. The review of the literature in past emphasized on nutritional qualities and safety of stevioside, rather than Stevia cultivation (Felippe, 1977, 1978; Fletcher, 1955). Further, a review from China was recently done by Liu (1992).

The aim of this chapter is to describe the ecology and importance of the plant and its production requirements, but major emphasis is given to the agronomic and management aspects of the plant to be grown as a crop. Further, this chapter represents an effort to compile the literature on

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S. rebaudiana and review the current status of understanding of the plant and its potential as an alternate source of cane sugar.

II. AGRICULTURAL HISTORY

_Stevia rebaudiana_ is an endemic herb from Paraguay and the Brazilian border with that country (Felippe, 1977; Monteiro, 1982). The genus _Stevia_ (Eupatorieae, Asteraceae) [which consists of approximately 150–200 species of herbaceous, shrub and subshrub plants (Gentry, 1996)] is one of the most distinctive genera within the tribe Eupatorieae. Schmeling (1967) expressed that _Stevia_ is mainly found in Amambay, including the zone of San Pedro, Yhu, and near Jejui Guazu. It was used for many centuries as a sweetener. _Stevia_ first came to the attention of Europeans in the 1800s, yet it remained relatively obscure until it was planted and used in England during the sugar rationing of World War II. Many authors described the herb (Klages, 1951; Levy, 1984). History of _Stevia_ was dealt in detail by Machado and Dietrich (1981).

Although geographically widespread, this genus occurs exclusively in tropical and subtropical regions of the United States and Central and South America (Robinson and King, 1977). This has been historically used by the people of Paraguay as a sweetener and herbal remedy. Early reports indicated that _Stevia_ was known to the Spanish during the 16th century, but it remained in obscurity until it was again brought to the attention of Europeans in 1888 by M. S. Bertoni. Erstwhile _Eupatorium rebaudianum_ came to the attention of M. S. Bertoni in the year 1809, who studied it and renamed it as _Stevia_ in 1905 (Bertoni, 1899, 1905, 1918, 1927). Prior to any European discovery, it had long been known to the indigenous Guarani people native to that region. The leaves of this sweet herb, known to the Guarani Indians as Ka-a He-e, was used for centuries as a sweetener for bitter drinks such as maté (Soejarto et al., 1983). _Stevia_ is reported to be originated in South American gene center (Cerna, 2000).

During 1971, Japanese introduced _Stevia_ from Brazil (Crammer and Ikan, 1986) and conducted research to evaluate potential of _Stevia_. Today, Japan is a major grower and marketer for the sweetener and has approved it for use in many food products, including cereals, teas, and soft drinks. _Stevia_ has an ancient and venerable history in certain parts of the world. It is clear that this crop is native to valley of the Rio Monday in North Eastern Paraguay and is commonly found on the edges of marshland on acid infertile sand or muck soils of Paraguay. In Canada, _Stevia_ is being sold as an ingredient in tea but not as a sweetener (Borie, 2000). The task now is to convert _Stevia_ from its wild habitat to a modern crop well suited to different production environments along with efficient mechanized production.
III. AGRICULTURAL IMPACT AND USE

*Stevia* possesses numerous characteristics that make it a potentially valuable agricultural species (Tables I and II), although there are few reasons generally limiting its agronomic utility (Table III). Information on production of the 10 glycosides responsible for its sweetness in different plant parts is of great importance for both understanding the peculiarities of diterpenoid glycoside production and for adoption of mass scale production techniques. Studies conducted so far could suggest few management approaches for improving production requirements. This crop had made significant agricultural impact in countries such as Japan, China, Taiwan, Korea, Mexico, USA, Thailand, Malaysia, Indonesia, Australia, Tanzania, Canada, Abkhazia, Russia (Brandle and Rosa, 1992; Chen and Chang, 1978; Chu and Cheng, 1976; Donalisio et al., 1982; Dzyuba, 1998; Goenadi, 1983; Gvasaliya et al., 1990; Katayama et al., 1976; Lee et al., 1979; Lester, 1999; Saxena and Ming, 1988; Shock, 1982; Sumida, 1968), and efforts were initiated in India recently (Chalapathi, 1996).

Randi (1980) reviewed the potential uses of *Stevia* that produces sweet glycosides like stevioside, which may vary from 2 to 10% (Magalhaes, 2000), a noncaloric sweetener that does not ferment in the human body (Table II).

The leaves are used for sweetening, as is, or dried and pulverized, or soaked in water; the liquor is used for sweetening beverages. A Japanese firm is producing chewing gum from *Stevia*. Plants contain an aromatic resin, which has tonic action on digestive organs. It is also a source of gibberellin (Duke and deCellier, 1993). The herbage contains 0.12–0.16% essential oil, which is up to 0.43% in the inflorescence (Kinghorn and Soejarto, 1985).

### Table I

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Agronomically Important Characters of <em>Stevia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wide climatic adaptability</td>
</tr>
<tr>
<td>2</td>
<td>Perennial in nature (<a href="#">Andolfi et al., 2002</a>), unique regeneration capacity after frost injury (<a href="#">Singh and Kaul, 2005</a>)</td>
</tr>
<tr>
<td>3</td>
<td>Leaf is the economic part</td>
</tr>
<tr>
<td>4</td>
<td>Vegetative propagation is possible (<a href="#">Chalapathi et al., 1997b</a>)</td>
</tr>
<tr>
<td>5</td>
<td>3–4 harvests per year is possible (<a href="#">Donalisio et al., 1982</a>)</td>
</tr>
<tr>
<td>6</td>
<td>Intercropping is possible during the initial growing period</td>
</tr>
<tr>
<td>7</td>
<td>Easy propagation through seeds, stem cuttings, and division of roots (<a href="#">Singh and Kaul, 2005</a>)</td>
</tr>
</tbody>
</table>
Table II  
Product (Glycoside) Suitability Characters

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Improves cardiovascular functioning (Machado et al., 1986)</td>
</tr>
<tr>
<td>2</td>
<td>Effective in high blood pressure, obesity or chronic yeast infections (Elkins, 1997)</td>
</tr>
<tr>
<td>3</td>
<td>Diabetic safe (Kinghorn and Soejarto, 1985; Soejarto et al., 1983)</td>
</tr>
<tr>
<td>4</td>
<td>Antihuman rotavirus activity (Takahashi et al., 2001)</td>
</tr>
<tr>
<td>5</td>
<td>Calorie free—human physiology cannot metabolize the sweet glycosides contained in Stevia leaves, therefore, they are eliminated from the body with no caloric absorption (Elkins, 1997)</td>
</tr>
<tr>
<td>6</td>
<td>Improved overall gastrointestinal function (Alvarez, 1986)</td>
</tr>
<tr>
<td>7</td>
<td>Can be used in baking because its sweet glycosides do not break down when heated (Elkins, 1997)</td>
</tr>
<tr>
<td>8</td>
<td>Hypoglycemial action: Positive (Oviedo et al., 1970; Soejarto et al., 1983); Negative (Akashi and Yokoyama, 1975; Lee et al., 1979); Inconclusive (Boechk, 1986; Piheiro and Gasparini, 1981)</td>
</tr>
<tr>
<td>9</td>
<td>Stevia leaves also contain protein, fibers, carbohydrates, phosphorus, iron, calcium, potassium, sodium, magnesium, rutin (flavonoid), zinc, vitamin C, and vitamin A (Elkins, 1997)</td>
</tr>
<tr>
<td>10</td>
<td>Does not adversely affect blood sugar levels (Elkins, 1997)</td>
</tr>
<tr>
<td>11</td>
<td>Effective against microbes like Streptococcus mutans, Pseudomonas aeruginos, and Proteus vulgaris (Yabu et al., 1977)</td>
</tr>
<tr>
<td>12</td>
<td>50–400 times sweeter than white sugar (Elkins, 1997)</td>
</tr>
<tr>
<td>13</td>
<td>Nontoxic (Elkins, 1997)</td>
</tr>
<tr>
<td>14</td>
<td>Inhibits the formation of cavities and plaque (Elkins, 1997)</td>
</tr>
<tr>
<td>15</td>
<td>Contains no artificial ingredients (Elkins, 1997)</td>
</tr>
</tbody>
</table>

Table III  
Agronomically Challenging Characters

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Day length sensitivity/short day plant (Lester, 1999; Valio and Rocha, 1966)</td>
</tr>
<tr>
<td>2</td>
<td>Sensitive to water logging</td>
</tr>
<tr>
<td>3</td>
<td>Low to moderately resistant to drought (Jia, 1984)</td>
</tr>
<tr>
<td>4</td>
<td>Poor early growth (Borie, 2000)</td>
</tr>
<tr>
<td>5</td>
<td>Heavy weed competition at early stages (Andolfi et al., 2002)</td>
</tr>
<tr>
<td>6</td>
<td>Sensitivity to frost</td>
</tr>
<tr>
<td>7</td>
<td>Poor seed germination (Barathi, 2003; Carneiro et al., 1997; Duke, 1993; Shock, 1982)</td>
</tr>
<tr>
<td>8</td>
<td>Short period of germinantive power (Marcavillaca, 1985)</td>
</tr>
<tr>
<td>9</td>
<td>Poor tolerance to high soil pH (Shock, 1982)</td>
</tr>
<tr>
<td>10</td>
<td>Self incompatible (Chalapathi et al., 1997b)</td>
</tr>
<tr>
<td>11</td>
<td>Asynchronous seed maturity</td>
</tr>
</tbody>
</table>
IV. BOTANICAL DESCRIPTION

It is one of the 950 genera of the Asteraceae family (Lester, 1999; Soejarto et al., 1983). A systematic study of the North and Central American species of *Stevia* was done by Grashoff (1972). Although there are more than 200 species in the *Stevia* genus, Soejarto et al. (1983) had proved that *S. rebaudiana* gave the sweetest essence. It is a perennial herb with an extensive root system and brittle stems producing small, elliptic leaves (Shock, 1982).

Kingdom: Angiospermae  
Class: Dicotyledons  
Group: Monochlamydae  
Order: Asterales  
Family: Asteraceae  
Subfamily: Asteroideae  
Tribe: Eupatorieae (Cabrera et al., 1996)  
Genus: *Stevia*  
Species: *rebaudiana*

*Stevia* is normally described as perennial herb in its natural habitat in Paraguay, though under some environmental conditions and management situations it behaves as an annual or mixture of plants of both types. The cultivated plants reported to be more vigorous. It is also considered as a branched bushy shrub (Dwivedi, 1999). Since leaves are the principal sweet bearing parts of the plant, the proportion of leaf to whole plant, the leaf weight ratio is important. High ratios of leaf:stem are desirable in cultivated *Stevia* because of the low stevioside concentrations (<5 mg g⁻¹) in stem tissue. *Stevia* grows to about 50–60 cm tall (Brandle and Rosa, 1992; Lester, 1999), 100 cm (Shock, 1982), or up to 120 cm (Dwivedi, 1999).

A. GROWTH PATTERN

*Stevia* has a temperamental nature that is often reflected in its sluggish growth when the plants are first set out. After the first month, they pick up growth depending upon the prevailing weather conditions. Branching and tillering are also much more profuse (Shock, 1982). The growth pattern of *Stevia* can be divided into four stages: germination, grand growth period, flowering, and seed maturity. The first stage includes germination and establishment, the second vegetative growth, the third floral bud initiation to pollination and fertilization, and the fourth seed growth and filling. The duration of sowing to seed emergence is related to the temperature, and 24°C is considered optimal for seed germination (Goettemoeller and Ching, 1999). Other growth stages are discussed in detail under environmental versatility.
B. **PLANT MORPHOLOGICAL VARIATION**

Monteiro (1980) studied the morphological differences present in these plants but was unable to separate them into valid taxonomic varieties. There are reports of irregularity of the quantitative and qualitative production of the sweetening molecules from cultivated *S. rebaudiana* types also. The morphological differences between plants, so evident in present cultivation at Institute of Himalayan Bioresource Technology (IHBT), Palampur (Ramesh, unpublished data) as well as elsewhere in the world, are also linked to the natural reproductive biology of the species (*S. rebaudiana* belongs to the half-SIB species) (Tateo et al., 1998). The diversity of the aerial part of the cultivated plants (Tateo et al., 1998) as well as flowering behavior (Zaidan et al., 1980) was large, and they identified three photoperiod classes based on day length, which needs further research for suitability for cropping conditions in different parts of the world.

C. **ROOT SYSTEM**

The root is fibrous, filiform, and perennial, forming abundant stock (Schmeling, 1967) that is hardly ramified and does not deepen, distributing itself to the land surface; and is the only part that does not contain stevioside (Vargas, 1980; Zaidan et al., 1980). Sunk (as quoted by Taiariol, 2004) described that the fine roots congregate around the soil-surface and thicker roots in the deepest zones.

D. **STEM**

The stem is annual, subligneous, more or less pubescent, with tendency to decline, and more or less graft (Sakaguchi and Kan, 1982).

E. **LEAVES**

The first photosynthetic organs are formed after germination from the two cotyledons in the seed. They are rounded in shape. *Stevia* has an alternate leaf arrangement and herbaceous growth habit with flowers arranged in indeterminate heads. Leaves are small, lanceolate, oblong, serrate, and sweet (Dwivedi, 1999). For *Stevia*, the leaf area index (LAI) at 80 DAS was 4.83 (Fronza and Folegatti, 2003). Light or, more exactly, photosynthetically active radiation supplies plants with energy for photosynthate production. It is fairly obvious that the amount of intercepted light
principally depends on the leaf surface area of the crop, and is usually expressed as leaf area index.

F. Flowers

*Stevia* is self-incompatible (Chalapathi et al., 1997b; Miyagawa et al., 1986) and probably insect pollinated plant (Oddone, 1997). The flowers are small and white (Dwivedi, 1999) with a pale purple throat (Figs. 1, 2, 3, 4, 5, and 6). The pollen can be highly allergenic. The tiny white florets are perfect, borne in small corymbs of two to six florets. Corymbs are arranged in loose panicles (Goettemoeller and Ching, 1999). A plant takes more of a month in producing all its flowers (Taiariol, 2004).

G. Seeds

Shock (1982), Duke (1993), and Carneiro et al. (1997), had reported poor percentage of viable seeds in *Stevia*. Oddone (1997) considers “clear” seeds to be infertile. Seeds are contained in slender achenes, about 3 mm in length. Each achene has about 20 persistent pappus bristles. Reproduction in the

![Figure 1](image)  
**Figure 1** Corolla of single *Stevia* flower.
wild is mainly through seed, but seed viability is poor and highly variable (Lester, 1999). Seeds have very little endosperm and are dispersed in the wind via hairy pappus. A study undertaken to investigate the low seed germination of *Stevia* seeds through artificial pollination treatments as a means to increase seed germination revealed that some active manipulation of the blossoms is necessary to achieve pollination (Goettemoeller and Ching, 1999).

**H. SWEET GLYCOSIDE CONTENT IN PLANT PARTS**

There are 10 glycosides out of which stevioside and rebaudioside A are important. Details of the sweet glycosides are dealt in the Section VII. Plant organs contained different amounts of the sweet glycoside, stevioside, which declined in the following order; leaves, flowers, stems, seeds, and roots. Root was the only organ that does not contain steviosides. This made Metivier
and Viana (1979a) to hypothesize that stevioside may protect the aerial portions of the plant from herbivore predators. The sweetness in the leaves is two times higher than that in inflorescence (Dwivedi, 1999). The highest amount of steviosides was found in the upper young actively growing shoot sections, whereas lowest senescent shoot sections exhibited the lowest amount of such compounds. During ontogeny, a gradual increase in the stevioside concentration was observed in both mature Stevia leaves and stems, and this process lasted up to the budding phase and the onset of flowering (Bondarev et al., 2003b).

V. ENVIRONMENTAL VERSATILITY

The objective of this section is to describe and discuss briefly the relationships between selected environmental variables and the agronomic responses of Stevia. Stevia has been successfully grown apparently under variety of geographic locations around the world, although it originated in the highland regions of northeastern Paraguay that occur between 23 and 24° S latitude (Shock, 1982), and 54 and 56° E longitude (Alvarez, 1984; Bertonha
Figure 4  Single flower (Top view).

Figure 5  *Stevia* stamen.
et al., 1984; Monteiro, 1986). It is this extreme versatility that holds importance for this plant. Stevia is grown as a perennial crop in subtropical regions including parts of the United States, while grown as an annual crop in mid to high latitude regions (Goettemoeller and Ching, 1999). The results indicate that agronomic yield mainly depend on the genetic characters of the plant and the phenotypic expression, which ultimately is governed by the climatic and environmental factors (Ermakov and Kotechetov, 1996; Metivier and Viana, 1979a). Moreover, synthesis of terpenes is affected by them in many plants (Guenther, 1949; Krupski and Fischer, 1950; Langston and Leopold, 1954). Selected locations where Stevia is grown is presented in Table IV.

In association with most plants, the growth and flowering of Stevia are affected by radiation, day length, temperature, soil water, and by wind in exposed places. As early in 1976, the seasonal variation in stevioside content was studied by Chen et al. (1978). Tateo et al. (1999) had opined that environmental and agronomic factors have more influence on stevioside production than the growth habit. For Stevia crop, the ideal climate can be considered as semi-humid subtropical with temperatures ranging from –6 to 43°C with an average of 23°C (Brandle and Rosa, 1992). Research conducted at Egypt revealed that climate conditions such as temperature, length, and intensity of photoperiod greatly affected Stevia production and

Figure 6 Stevia stigma.
quality as evident from the remarkable increase in yield during the summer cuts than that during winter cuts (Allam et al., 2001).

Brandle and Rosa (1992) had reported comparable stevioside concentration at Delhi and Ontario to that found in Japan where long days are experienced during the growing season (Kinghorn and Soejarto, 1985) relative to the subtropical regions of the world, which might be due to cultivation under long days at Delhi and Canada. Under agro-climatic conditions at Palampur, stevioside content in leaf varied from 3.17 to 12% and from 1.54 to 3.85% in stem as estimated during the studies at IHBT, Palampur. Stevioside content in selected locations where Stevia is grown is presented in Table V.

### A. Geographic Distribution

Bertoni (1905) had described the distribution range of 22°30’–25°30’ S latitudes and from 55 to 57° W longitudes, while Sunk (1975) described it more precisely as 22–24° S and 55–56° W, respectively, within 200–700 m altitudinal zones. The native habitat of Stevia is at latitude of 25° S in a subtropical region in northeastern Paraguay between 500 and 1500 m above

### Table IV

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude(^a)</th>
<th>Longitude(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangalore, India (Chalapathi et al., 1997b)</td>
<td>12° N</td>
<td>77° E</td>
</tr>
<tr>
<td>Hosur, India (Barathi, 2003)</td>
<td>13° N</td>
<td>77° E</td>
</tr>
<tr>
<td>Sugar Crops Research Institute, ARC-Giza, Egypt</td>
<td>25° N</td>
<td>30° E</td>
</tr>
<tr>
<td>New Delhi, India</td>
<td>29° N</td>
<td>77° E</td>
</tr>
<tr>
<td>Uttarakhand, India</td>
<td>30° N</td>
<td>77° E</td>
</tr>
<tr>
<td>Palampur, India</td>
<td>32° N</td>
<td>76° E</td>
</tr>
<tr>
<td>California (Shock, 1982)</td>
<td>38° N</td>
<td></td>
</tr>
<tr>
<td>Western Georgia (Papunidze et al., 2002)</td>
<td>42° N</td>
<td>44° E</td>
</tr>
<tr>
<td>Pisa, Italy (Fronza and Folegatti, 2003)</td>
<td>43° N</td>
<td>11° E</td>
</tr>
<tr>
<td>Abkhazia (Eastern coast of black sea) (Gvasaliya et al., 1990)</td>
<td>45° N</td>
<td>70° E</td>
</tr>
<tr>
<td>Suwon, Korea (Lee et al., 1980)</td>
<td>37° 30’ N</td>
<td>127° E</td>
</tr>
<tr>
<td>Slovakia (Cerna, 2000)</td>
<td>49° N</td>
<td>20° E</td>
</tr>
<tr>
<td>Czech republic (Nepovim et al., 1998a)</td>
<td>50° N</td>
<td>15° E</td>
</tr>
<tr>
<td>Canada (Lovering and Reeleeder, 1996)</td>
<td>50–60° N</td>
<td>130° W</td>
</tr>
<tr>
<td>Moscow, Russia (Kornienko et al., 1995)</td>
<td>55° 45’ N</td>
<td>37° 42’ E</td>
</tr>
<tr>
<td>Indonesia (Goenadi, 1987)</td>
<td>0–5° S</td>
<td>110–120° E</td>
</tr>
<tr>
<td>Sukabumi, Indonesia (Basuki, 1990)</td>
<td>7° S</td>
<td>110° E</td>
</tr>
<tr>
<td>Brazil (Stefanini and Rodrigues, 1999)</td>
<td>25° S</td>
<td>50° W</td>
</tr>
<tr>
<td>Argentina (Cabanillas and Diaz, 1996)</td>
<td>30° S</td>
<td>60° W</td>
</tr>
</tbody>
</table>

\(^a\)Approximate and not the exact location where experiment was done.
Table V
Stevioside Content in Selected Locations Where Stevia is Grown

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Place</th>
<th>Stevioside content (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>San Piero a Grado, Pisa, Italy</td>
<td>6.49 (80 DAS)</td>
<td>Fronza and Folegatti (2003)</td>
</tr>
<tr>
<td>2</td>
<td>Academy of Sciences of Czech Republic, Flemingonam, Prague</td>
<td>5.2 (July)–6.5 (September)</td>
<td>Nepovim et al. (1998a)</td>
</tr>
<tr>
<td>3</td>
<td>Minga Guazu region of East Paraguay</td>
<td>4.36–9.89</td>
<td>Tateo et al. (1999)</td>
</tr>
<tr>
<td>4</td>
<td>Paraguay</td>
<td>10.23–13.46</td>
<td>Tateo et al. (1999)</td>
</tr>
<tr>
<td>5</td>
<td>Argentinian origin</td>
<td>5.16</td>
<td>Pryluka and Cernadasn (1985)</td>
</tr>
<tr>
<td>6</td>
<td>Brazilian origin</td>
<td>5.4</td>
<td>Pryluka and Cernadas (1985)</td>
</tr>
<tr>
<td>7</td>
<td>Palampur, India</td>
<td>8–10 (Average); 3.17–12 (Extreme range)</td>
<td>Based on present R&amp;D at the Institute IHBT, India</td>
</tr>
<tr>
<td>8</td>
<td>Place not known</td>
<td>6–20</td>
<td>Donalisio et al. (1982)</td>
</tr>
<tr>
<td>9</td>
<td>Bangalore, India</td>
<td>9.08</td>
<td>Ashwini (1996) and Chalapathi (1996)</td>
</tr>
<tr>
<td>10</td>
<td>Western Georgia</td>
<td>10</td>
<td>Papunidze et al. (2002)</td>
</tr>
</tbody>
</table>

msl, with an average annual temperature of 25°C and an average rainfall of about 1375 mm year\(^{-1}\) (Shock, 1982; Sumida, 1973). Kawatani et al. (1977) had surveyed Stevia plantation in southeast Asian countries and found taller plants near the tropic, but leaf productivity was less due to higher proportion of stems than leaves in these latitudes. This made him to conclude that subtropical latitudes are favorable for higher leaf recovery. European commission (1999) has opined that this is growing mostly at altitudes of 500–3000 m in semidry mountainous terrain. However, this perennial shrub has been grown at 60° N also, weathering the bitter chill of St Petersburg winters, but essentially it prefers tropical climate between 35 and 45° to either side of the equator (Midmore and Rank, 2002).

B. Day Length/Photoperiod

Stevia is highly sensitive to the day length and it requires 12–16 h of sunlight. This prompted many investigators to examine the effect of length of day and night and temperature variation on the cultivation and the resultant stevioside levels (Kudo, 1974; Metivier and Viana, 1979a; Mizukami et al., 1983; Valio and Rocha, 1966; Viana, 1981; Zaidan et al., 1980).
Day length variation had a profound influence on crop vegetative growth. This was confirmed by the studies conducted by Metivier and Viana (1979a). The results revealed that plants maintained under long day conditions were characterized by long internodes and a single main stem bearing large, horizontally held ovate leaves, whereas it was rosette up to bolting and thereafter internode length increased. Besides, this influenced flowering also.

Precise investigations on day length and time required for flowering were made by Kudo (1974), describing that flowering occurred within 46 days at 11 h day length, while it was extended to 96 days when photoperiod was extended for 12.5 h. Kudo and Koga (1977) found that under optimum day length conditions flowering started from 50 to 60 days after sowing and was also confirmed by Zaidan et al. (1980). However, Valio and Rocha (1966) opined that a photoperiod of 13–14 h might be necessary. The plant flowered in the 8, 10, 12, and 13 h photoperiod, though the highest percentage of flowering occurred in the 13 h photoperiod. This made researchers to believe that Stevia is an obligate short day plant (Lester, 1999) with a critical day length of about 13 h. Since glycoside synthesis is reduced at or just before flowering, delayed flowering with long days allowed more time for glycoside accumulation. Thus, Stevia production is best suited to a long day environment, where vegetative growth is longer and steviol glycoside yields will be higher. This was confirmed further by Metivier and Viana (1979a). They claimed that yield of sweetening compounds present in leaf tissue varied according to day length, as long days increased leaf area and leaf dry weight as compared to short days. Therefore, enhanced vegetative growth under long day conditions is not surprising as the high leaf:stem ratio may be a function of cultivation under long days (Brandle and Rosa, 1992). Apart from increment in leaf yield, 50% increase in stevioside concentration relative to short days was also observed (Metivier and Viana, 1979a).

In its native habitat, at 21–22° S latitude Stevia plants start flowering from January to March equivalent to July and September in the northern hemisphere. Subsequent flowerings occur in rapid succession as regrowth from the plant crown grows shorter each time until winter in July (Shock, 1982). If Stevia is grown at about 25°C, under continuous long day conditions (16-h photoperiod), it will remain in vegetative stage itself (Monteiro et al., 2001). Undoubtedly, short days promote flowering and so day length influences yield (Parsons, 2003) of aerial biomass.

Photoperiod requirements made researchers to conclude that cultivation in temperate areas with long summer days would be ideal for high stevioside yields but seed production would be difficult (Shock, 1982). In accordance with the above views, this is grown as a perennial crop in subtropical regions, where longer days favor leaf yield and stevioside contents (Goettemoeller and Ching, 1999). Flowering under short day conditions should occur
54–104 days following transplantation in southern hemisphere, depending on the day-length sensitivity of the cultivar (Lester, 1999).

It is worthwhile to mention that rooting of cutting is also dependent on day length, since Zubenko et al. (1991) had recorded better rooting and growth of cuttings made in April as compared to February due to increased day length and light intensity. If it is possible to screen a suitable genotype to suit day-length sensitivity, the plant’s total potential can be fully exploited.

C. TEMPERATURE

Temperature has been observed to influence on the availability of soil nutrients, germination, the growth of the plant and shoots, winter survival, photosynthesis, and respiration. It is rather difficult to delineate a particular process that is most affected by temperature. But it is commonly believed from the early work of Sumida (1980) (and as cited by Sakaguchi and Kan, 1982) that the optimal temperature range for the growth of Stevia is 15–30°C, though the plant can tolerate critical temperature of 0–2°C. However, Miyasaki (and as cited by Sakaguchi and Kan, 1982) showed the absolute limit as little as –3°C. Mizukami et al. (1983) had postulated that day night temperature variation is another determinant for stevioside production. They obtained best growth and stevioside yield under 25/20°C day/night regime. Not withstanding to these findings, Nepovim et al. (1998a) had concluded that temperature was not a decisive parameter for the stevioside content in the Stevia leaves, which is a controversy. Temperature influences yield (Parsons, 2003) either directly or through the diurnal variation as it plays a vital role in Stevia production as discussed by Barathi (2003), indicating that the maximum day temperature should not exceed 40°C and the night temperature should not fall below 10°C for favorable growth of Stevia.

A report made by Midmore and Rank (2002) on possibility of introducing Stevia in Australia indicated that vegetative growth is reduced when temperature is below 20°C.

In subtropical India, it has been successfully grown at a temperature regime of 28–39°C (Chalapathi et al., 1997b). Richard (2004) had stated that the average temperatures where Stevia is found growing ranges from –6 to 44°C and these areas are humid.

D. LIGHT

Studies conducted by Metivier and Viana (1979b) had indicated that to keep the plants in vegetative stage, light intensity of 0.089 cal cm\(^{-2}\) should be
maintained. *Stevia* is essentially a sun loving plant since the plant thrived in a warm, humid, and sunny climate (Jia, 1984). Under natural habitat it grows wild along with tall grasses under partial shade. Hence, the productivity is poor. The negative effect of shade was further confirmed by the observations made by Slamet and Tahardi (1988). They confirmed that shade reduced growth and rate of flowering. Further, about 60% reduction in light delayed the flowering, decreased plant biomass production, significantly decreased the percentage of flowering plants, and also reduced rate of flowering. Observations at IHBT, Palampur, India also confirmed that growth was reduced under partial shade.

VI. CULTIVATION

*Stevia* cultivation has been reported as early as 1970s (Mitsuhashi *et al*., 1975; Miyazaki *et al*., 1978). In the early phase of cultivation, *Stevia* crop exhibited much more vigor than in natural populations (Shock, 1982), a fact suggesting that with appropriate crop management practices we could expect luxuriant crop with fullest potential. Nowadays commercial cultivation is extended/attempted in Japan, southeast Asia, and United States (Fors, 1995; Sakaguchi and Kan, 1982), but it is being cultivated in some semitropical areas, humid Himalayan hilly regions, and humid hills of Assam in India (Dwivedi, 1999). As the plant does not survive winter climate, it is cultivated in Europe as a leaf crop under greenhouse conditions (European Commission, 1999). There have been studies on development of modern techniques of cultivation, propagation through tissue culture, and selection (Acuna *et al*., 1997; Akita *et al*., 1994; Ashwini, 1996; Ferreira and Handro, 1988a,b,c; Filho *et al*., 1992; Flachsland *et al*., 1996; Handro *et al*., 1993; Huang *et al*., 1995; Kornilova and Kashnikova, 1996; Nepovim and Vanek, 1998b; Patil *et al*., 1996; Sivaram and Mukundan, 2002; Tamura *et al*., 1984b). Considerations regarding the cultivation practices are discussed hereunder.

A. SEED GERMINATION, NURSERY, AND CROP ESTABLISHMENT

In general, seed germination is a problem in *Stevia* (Felippe and Lucas, 1971; Randi, 1980; Randi and Felippe, 1981; Rocha and Valio, 1972). Seeds sown in cold weather showed poor germination (Shock, 1982). Alvarez *et al.* (1994) had reported that it was impossible to sow the seeds immediately after harvest, and concluded that the seeds should be kept in sealed tight
containers in the refrigerator at 4°C, since it loses viability at room temperature. Further, studies indicated that germination was best at 25°C (Felippe and Randi, 1984; Randi and Felippe, 1981) and at this temperature, 63.2% of maximum germination (90.03%) occurred after 101.4 h (Takahashi et al., 1996). Cabanillas and Diaz (1996) had reported the performance of seeds under different temperature and light conditions at Argentina.

No viable seed treatment to enhance seed germination has been reported elsewhere. Because of its small size and the related bottlenecks in seed nutrition, it is a general practice to raise nurseries. It is propagated through either seeds or cuttings (Figs. 7 and 8). Seeds are germinated in the glasshouse in spring and the plants (usually 6–7 weeks old) are transplanted into the field (Lester, 1999). In the temperate latitudes, the production cycle for annual crops starts with the 6–7 weeks old plants grown from seed. Under Canadian conditions the initial establishment was very poor (Brandle et al., 1998). The seedlings raised from seeds are transplanted and the shoot is harvested after 4–5 months of growth (Dwivedi, 1999). Seeds were stored for 11 months at 4°C or at ambient temperature and humidity (Cabanillas and Diaz, 1999).

B. SPACING/CROP DENSITY

Crop density is a parameter decided by the crop spread above ground so as not to interfere with the development of the adjoining plants. However,
one should consider the root spread also. This is also dependent on the environment in which it is raised. Several authors tried different spacing (Angkapradip et al., 1986b; Barathi, 2003; Basuki, 1990; Carneiro et al., 1992; Chalapathi, 1996; Columbus, 1997; Donalisio et al., 1982; Katayama et al., 1976; Murayama et al., 1980).

Initial trials indicated that higher growth and yield, when low plant density was adopted (60 × 20 cm), while dry leaf yield was higher in denser planting (60 × 10 cm) (Murayama et al., 1980). In contrast, Lee et al. (1980) had reported that plant height, number of branches, and number of nodes were unaffected by planting density (50–70 cm between and 10–30 cm within rows), but dry leaf yield per plant decreased with increasing plant density. In accordance to the above, Donalisio et al. (1982) had recommended a plant population of 80,000–100,000 plants ha⁻¹.

Reduction in row-to-row spacing was also attempted. A spacing of 50 × 20 cm (Filho et al., 1997a) or 45 × 22.5 cm (Chalapathi, 1996) performed well but still narrow spacing of 25 × 25 cm was also tried by Angkapradipta et al. (1986b), however, this is not advisable considering the root spread of

Figure 8  *Stevia* nursery raised through seed.
the crop. Observations made at IHBT, Palampur indicated that, at 12 months after planting, the root spread was 30 cm on either side suggesting that for a multiple harvest crop the spacing should be higher than 30 cm on either side.

Basuki (1990) tried a very high density of 2 lakh plants ha\(^{-1}\) to manage weeds. However, this would result in poor crop growth due to intense light competition and the leaf:stem ratio will decline. Leaf yield was found to increase up to 1.1 lakh plants ha\(^{-1}\) for the first year of production (Brandle et al., 1998). Under Palampur conditions, 50,000 plants ha\(^{-1}\) were maintained at a spacing of 45 \(\times\) 45 cm (Singh and Kaul, 2005). The highest Stevia yield was obtained at 70 \(\times\) 25 cm spacing at Abkhazia (Gvasaliya et al., 1990). Therefore, it is advisable to carry out trials in each planting zone to establish adequate plant population density for that particular area.

C. Vegetative Propagation

Propagation of Stevia is usually by stem cuttings, which root easily but require high labor inputs. Poor seed germination is one of the factors limiting large-scale cultivation.

1. Method of Propagation

   a. Cuttings Gvasaliya et al. (1990) had reported that nearly 98–100% rooting was obtained, when current year’s cuttings were taken from leaf axils at Abkhazia. Rooting of cutting was best (96.7%) in cuttings from side shoots and from tops of the main shoot (92.3%). Further, cuttings from the top part of the main stem with four internodes generally gave the best results (Tirtoboma, 1988). However, the pair of leaves in the cutting as well as the season also act as determinants for the rooting percentage. Cuttings with four pairs of leaves rooted poorly, especially in February. In February, cuttings with two pairs of leaves rooted best and in April those with three pairs of leaves (Zubenko et al., 1991). Cuttings of 8 cm long were used by Carvalho et al. (1995).

   Use of 15 cm cutting gave significantly higher sprouting percentage with better shoot and root growth of sprouted cuttings over 7.5 cm cuttings (Chalapathi et al., 1999c, 2001), while direct planting in field was of limited success only (Chalapathi et al., 1999c).

   b. Rooting of Cuttings and Their Growth Pretreatment of cuttings with IBA, IAA, and its combination @ 1000 ppm caused callus injury due to higher concentration of growth regulators (Chalapathi et al., 1999c), while
paclobutrazol at 50 or 100 ppm was effective in inducing roots and sprout from stem cuttings (Chalapathi et al., 2001) under pot conditions. Plant growth and stevioside content in the leaves of the plants grown from stem tips were more uniform than in plants grown from seeds. Number of roots, above ground biomass and stevioside content were greater in the vegetative grown plants (Truong and Valicek, 1999).

c. Time of Planting

There is scant published information on this aspect. However, the optimal time of planting is primarily decided by the avoidance of climatic conditions, which militate its stand and establishment. Summer is always associated with dry weather and poor soil moisture conditions hindering crop establishment. Further, late autumn planting is associated with poor temperature and less time for plant development. Therefore, planting at the initiation of spring seems to be the best option. Plants are more productive when seedlings or rooted cuttings are set out as early as possible in the spring (Lee et al., 1979). Under northern hemisphere, planting is done during mid May (Brandle et al., 1998). Under the agro-climatic conditions of Palampur the ideal time of planting was observed to be during March–April so as to have two leaf harvests and one seed harvest in a crop (Ramesh, personal communication). Further, delayed planting during June–July resulted in poor leaf harvest as it entered flowering during September in Northern hemisphere, at Palampur, India.

Winter cereal growing is an established practice in many parts of the world. Therefore, possibilities of raising this crop along with winter cereal remain to be a challenge. Under practical considerations, several other factors and local farming situations determine the time of planting. In brief, raising nursery during winter under controlled environments offers a reliable solution so that plating can be taken up in the subsequent spring.

2. Method of Propagation on Sweet Glycosides Content

This is only a matter of leaf growth rather than for examining stevioside content in plants. Tamura et al. (1984a) had compared plants raised from seeds, cuttings, and stem tip culture and concluded that yield of sweetening compounds present in leaf tissue can vary according to method of propagation, while Nepovim et al. (1998a) had contradicted the former and stressed that the content of stevioside did not depend on the type of propagation. Since the crop is cross-pollinated, there must be variation in the advancing generations, thus, obtaining varying stevioside content. Variation in stevioside content in a population of Stevia was reported (Tateo et al., 1998). Therefore, plants developed from cuttings would be more uniform in growth with optimum stevioside concentration. This
suggested that vegetatively propagated material is the best propagule for higher stevioside productivity.

**D. NUTRIENT MANAGEMENT**

Nutrient requirements of this crop are low (Goenadi, 1987) to moderate since this crop is adaptable to poor quality soils in its natural habitat at Paraguay. When placed under commercial culture, for economic crops, manuring is necessary (Donalisio et al., 1982; Goenadi, 1985). Since leaf is the economic part of this crop, it is presumed that higher nutrient application may aid in higher yield. But only few works have been carried, mainly on nutritional aspects.

The visual symptoms of nutrient deficiency in *Stevia* were: N exhibiting yellowing of leaves, P as dark green leaves, and chlorotic and mottled leaves with K deficiency. Further, the secondary nutrients deficiencies were exhibited viz., apical necrosis, chlorosis and inverted “V” shaped necrosis, and small pale green leaves for Ca, Mg, and S, respectively (Utumi et al., 1999).

In tissue culture studies, it was found that changes in the composition of the nutrient medium may significantly modify the physiological processes (Sikach, 1998) and production of the steviol glycosides in *Stevia* tissues and exert in such a manner physiological regulation of this process (Bondarev et al., 1998).

1. Macronutrients

Results from Japan demonstrated that, at the time of maximum dry matter accumulation, *Stevia* consisted of 1.4% N, 0.3% P, and 2.4% K (Katayama et al., 1976). It is an established fact that nutrient application is better than no manuring and was also experimentally proved by Murayama et al. (1980) and Goenadi (1985), who obtained better growth rate and dry leaf yield than no manuring. This was further strengthened by Lee et al. (1980) who had recorded increase in leaf yield with moderate application of nitrogen, phosphorus, and potassium fertilizers in Korea.

Early studies with nitrogen nutrition by Kawatani et al. (1977) had indicated an increase in growth, stem thickness, and number of branches. Response to potassium was also obtained (Kawatani et al., 1980). The crop would require approximately 105 kg N, 23 kg P, and 180 kg K for a moderate biomass yield of 7500 kg ha$^{-1}$ under Canadian conditions (Brandle et al., 1998), thus suggesting the importance of fertilization. Deficiency of N, K, and Mg reduced vegetative growth in terms of leaf growth,
which ultimately reduced marketable part of the plant. However, Mg impaired root growth also to a greater extent. N, P, K, and S deficiencies decreased the shoot:root dry weight ratio, while it is reverse for Mg deficiency. Except Ca, all others decreased absorption of macronutrients (Utumi et al., 1999). This study suggests that a balanced use of fertilizers is an absolute necessity.

Besides improvement in growth, research conducted at Egypt showed a gradual and significant increase in fresh and dry leaves, stem, biomass yields, and total soluble carbohydrate as nitrogen fertilizer increased from 10 to 30 kg N. Dry leaves yield increased by 64 and 1.99% at the later dose as compared to lower dose (Allam et al., 2001).

In an Andosol with a pH of 4.5, N had no significant effect but P and K increased biomass production (Angkapradipita et al., 1986b). Increasing rate of N increased plant N content, whereas P and K did not do so in a latosol (Angkapradipita et al., 1986a).

If the nutritional requirements of the crop were established, it would suggest the need for fertilization either through organic means or inorganic means. This was attempted by Son et al. (1997) at Brazil. They concluded that shortly before or at flowering the production of 1 ton of dry leaves, demanded in kg: N-64.6, P-7.6, K-56.1, Ca-15.8, Mg-3.6, and S-3.6. In accordance with these findings, in a ratoon crop at Bangalore, growth and yield increased significantly with increasing rates of N, P, and K up to 40:20:30 kg ha⁻¹ with highest dry leaf yield. In India, responses were obtained in terms of nutrient uptake (Chalapathi et al., 1997a) for fertilization, growth and yield up to 60:30:45 kg NPK ha⁻¹ (Chalapathi et al., 1999b) at Bangalore.

Further, the nutritional demand for seed production is still higher than leaf production, which was reported to be, in kg, N-130, P-18.8, K-131.5, Ca-43.7, Mg-8.3, and S-9.7 (Son et al., 1997) for 1 ton.

2. Micronutrients

There appears to be poor requirement for the microelements. Since this crop prefers acid soils with low pH, this condition itself ensured adequate availability of micronutrients. However, even in acid soils response was noticed. The decreasing order of response of Stevia to microelements when sprayed in an acidic soil in terms of plant fresh weight was as follows: 0.1% Mn > 0.05% Mo > 0.02% Mo > 0.05% Zn > 0.1% B > 0.05% Mn > 0.02% Cu > 0.25% B > 0.2% Zn (Zhao, 1985). Experiments conducted in nutrient solutions indicated that Boron supplied at 10 ppm reduced growth, flowering, root weight, and caused leaf spotting also (Sheu et al., 1987). Filho et al. (1997a) had studied the micronutritional requirements of Stevia at Brazil. They concluded that shortly before or at flowering the production of 1 ton of
For seed production corresponding to 1 ton of dry leaves, the extraction of micronutrients, in g, was B-226, Cu-76, Fe-2550, Mn-457, and Zn-33.

Plants grown in nutrient solutions containing four concentrations of nutrients revealed following interactions before flowering. Mn, Fe, and Cu showed synergistic effects between N and P, P and Cu, and P and Fe; antagonistic effect between N and K, N and Zn, K and Mg, and K and S; and either synergistic or antagonistic interaction between Zn and B, and Mn and Mg (Lima and Malavolta, 1997).

3. Nutrient–Sweet Gycoside Relationship

There is a close association between nutrient supply and stevioside accumulation as evident from the studies all over the world. Though the requirements of micronutrients are lesser than macronutrients, experiments conducted in nutrient solutions indicated that Boron supplied at 5 ppm registered higher contents of stevioside and rebaudioside (Sheu et al., 1987). Among secondary nutrients, only severe Ca deficiency caused reduction in the glycoside concentration (Filho et al., 1997b). Besides, the role in growth and development, deficiencies of K, Ca, and S decreased the concentration of stevioside in the plant on dry weight basis while all deficiencies, except that of P, decreased the stevioside content in the plant (Utumi et al., 1999). Supporting these results, research at Egypt showed a gradual and significant increase in stevioside content as nitrogen fertilizer increased from 10 to 30 kg N to the tune of 1.99% at the higher dose (Allam et al., 2001).

E. Crop–Weed Competition and Weed Management

Stevia has a poor capacity to compete with weeds during the initial growth period and weeds are the principal competitors in limiting crop establishment and ultimately the yield. Furthermore, weeds make harvesting more difficult and increase weed seed build up in the soil. Cultural methods of weed control have always been important in the crop establishment process. Slow initial seedling growth rate (Shock, 1982) has been observed to accelerate weed competition. Weeds like Ageratum houstonianum, Borreria alata, Digitaria sp., Eleusine indica, Erechites valerifolia, Erigeron sumatrensis, Galinsoga parviflora, and Sida rhombifolia were reported to be present in Stevia culture (Basuki, 1990). For these reasons, weed management plays a vital role in good crop management practices. Some natural means of weed management, such as higher plant densities, have been attempted (Basuki, 1990). They demonstrated that high plant density
(2 lakh ha\(^{-1}\)) combined with black plastic mulch provided effective control of weeds. The crop requires weed control at the early stages. Notwithstanding this fact, work on weed management is lacking in literature.

Though there is a great deal of interest in organic cultivation, need for chemical weed management measures cannot be kept off. The choice of herbicide will depend upon the weed spectrum associated with the crop. There is a report that Stevia can tolerate trifluralin (Andolfi et al., 2002; Katayama, 1978). At Palampur, India, crop planted during June experienced severe weed competition due to poor crop establishment (Ramesh, personal communication). This was exacerbated due to heavy rains. There is no published evidence regarding safe herbicides for Stevia.

### F. Water Requirement

The knowledge of water requirement of crops in different growing phases elicits higher crop yield and rational use of water resource. In natural habitat, it occurs in areas where the sites are continuously moist but not subjected to prolonged inundation. Stevia usually occurs on locations with high level of underground water or with continually moistened soil. It does not require frequent irrigation, though it is susceptible to moisture stress (Shock, 1982). It indicated that the crop prefers moist soil. For economic crops of Stevia, irrigation is necessary (Donalisio et al., 1982). The plant has poor tolerance to pH, so it should not be grown with poor quality water (Shock, 1982). Plant growth was optimal at water content in soil of 43.0–47.6%. The average water requirement per day is 2.33 mm plant\(^{-1}\) (Goenadi, 1983). Therefore, to secure optimum water relations for Stevia plants is one of the factors closely connected with its cultivation (Cerna, 2000). It requires liberal watering after transplanting, and before and after harvesting of the leaves (Andolfi et al., 2002). The average crop evapotranspiration (Ete) was measured as 5.75 mm day\(^{-1}\), and water consumption was high during the entire cycle. Irrigation at 117% of Ete was 13% better than 100% Ete in terms of Stevia yield (Fronza and Folegatti, 2002a). Evapotranspiration during the cycle was divided in to 3 parts: 6.66 mm day\(^{-1}\) (0–25 days), 5.11 mm day\(^{-1}\) (26–50 days), and 5.49 mm day\(^{-1}\) (51–75 days) at Brazil (Fronza and Folegatti, 2002b).

The crop coefficient value (\(K_c\)) is the ratio between actual Ete to potential Ete. This could be used as a parameter to judge water requirements. Gonzalez (2000) had reported a crop coefficient value of 0.25 from 0 to 25 days, 0.56 from 26 to 50 days, and 0.85 from 51 to 80 days in Paraguay, whereas Fronza and Folegatti (2003) obtained 1.45, 1.14, and 1.16 at Italy for the said phases, respectively.
G. Soil Requirement

The occurrence of Stevia on acid, infertile, sandy, or muck soils with ample supply of water is consistent with observations of plant performance under cultivation (Shock, 1982). The plant can be grown in a wide range of soils but has poor tolerance to salinity and so it should not be grown in saline soils (Chalapathi et al., 1997b). This occurs on the edges of marshes or in grassland communities on soils with shallow water tables, the soils are typically infertile acid sands or mucks. Stevia will grow well on a wide range of soils given a consistent supply of moisture and adequate drainage. Stevia grows naturally on infertile, sandy acid soils with shallow water tables. This is normally in areas like the edge of mashes and grassland communities (Lester, 1999). But this can also grow in grasslands, scrub forests, and alpine areas (European Commission, 1999).

H. Harvest

The optimum time of harvest depends on the cultivar and growing season. Leaves are harvested about 4 months after planting by cutting the plants at about 5–10 cm above the soil level (Donalisio et al., 1982). This must however, the maximum crop biomass stage (Fig. 9), otherwise yield reduction is possible (Shuping and Shizhen, 1995). Since the crop is highly sensitive to low temperature, in cold areas, crop may be harvested before or at onset of winter (Columbus, 1997).

During flowering, stevioside dissipates from leaves (Bian, 1981; Hoyle, 1992), thus leaves should be harvested at the time of the flower (Figs. 10 and 11) emergence (Dwivedi, 1999) or before flowering (Barathi, 2003).

I. Growth Regulators

1. Foliar Application

The most effective preparation for increasing the concentration of stevioside in leaves was application of Humiforte (synthetic amino acids, N, P, K, and trace elements) in combination with aminol (amino acids and N). However, Maletran (lactic and anthranilic acids) gave the highest biomass of micropropagated plants under field conditions (Acuna et al., 1997). The best growth (root, stem, leaf, and whole plant fresh weight) was observed in the third harvest due to Gibberellic acid (GA₃) at 50 mg liter⁻¹ treatment.
No trends were observed in the 10 and 20 mg GA$_3$ liter$^{-1}$ treatments. However, the best overall growth was exhibited by the control (Stefanini and Rodrigues, 1999).

2. Tissue Culture

The combination of naphthalene acetic acid (NAA) and benzyl adenine (BA) @ 0.1–0.2 mg liter$^{-1}$ was found to induce shoot formation in *Stevia* explants. Further, the addition of GA to callus and suspension cultures resulted in a significant increase in their fresh weight (Bondarev *et al.*, [Figure 9](#) A field view of luxuriant *Stevia rebaudiana* at IHBT, Palampur, India.)
Whereas growth regulators depress the content of steviol glycosides, however, the ratio of glycosides remained the same (Bondarev et al., 2003a).

J. Seed Production

Seed yield up to 8.1 kg ha\(^{-1}\) is possible (Carneiro, 1990). However, the climatic requirements, of day length and temperature, are different for maximum vegetative production and for maximum flowering and seed production (Hoyle, 1992) since the crop is triggered to flowering under long day conditions. It is not the only determinant governing seed production but nutritional requirements are also higher. Seed production in the northern hemisphere would be best suited between 20 and 30° N latitude. The crop could be transplanted in February–March and seed collected in late summer. The test weight of *Stevia* seeds range between 0.15 and 0.30 g (Brandle et al., 1998) and 0.30 and 0.50 g under Palampur conditions (Ramesh, personal communication).

K. Correlation Studies

Several authors studied the yield dependence on various growth parameters as well as stevioside content (Brandle and Rosa, 1992; Buana, 1989; Buana and
Plant height and leaf number at second and fourth week after planting was positively correlated with *Stevia* biomass production at 30 DAT in a greenhouse experiment (Buana and Goenardi, 1985). In another study, plant height neither had any close relationship with production nor with leaf number or branch number in the first 4 weeks (Buana, 1989). A positive correlation...
between total soluble carbohydrate content and stevioside content was established by Nishiyama et al. (1991). Stevioside concentrations were uncorrelated to yield or leaf:stem ratio (Brandle and Rosa, 1992). Further, dry leaf yield was correlated with leaf size and thickness, content of rebaudioside A was correlated with rebaudioside C, and rebaudioside A to stevioside ratio was highly correlated with leaf thickness (Shyu et al., 1994).

The dry yield of Stevia was positively and significantly correlated with plant height, number of branches, leaves per plant, and dry matter accumulation. About 96.88% of the total variation in dry leaf yield was explained by a linear function of these four characters (Chalapathi et al., 1998). The number of branches, and yield of fresh and dry stem and leaf, was more variable than the number of leaf pairs, number of nodes before transplanting and at harvest, plant height at transplanting and leaf length at harvest. The characters most closely related to yield were fresh and dry weights of leaves and stems. Step-by-step regression showed that leaf dry weight/plant had the greatest effect on yield (Shu and Wang, 1988). Stevioside content is influenced by both leaf surface and number of roots; however, the former has greater influence on stevioside content than number of roots as evident from the correlation coefficient (Truong et al., 1999), since the chemical content of last fully expended leaf pairs was well correlated with plant nutrient status (Utumi et al., 1999).

L. Biotic Stresses

Earlier, diseases like powdery mildew (Erysiphe cichoracearum DC), Damping off (Rhizoctonia solani Kuehn.), and Stem rot (Sclerotium dephiniii Welch.) were reported by Thomas (2000). Two fungal diseases Septoria steviae and Sclerotinia sclerotiorum were reported in Stevia grown in Canada (Chang et al., 1997; Lovering and Reeleeder, 1996; Reeleeder, 1999). Occurrence of stem-rot disease was recorded by 0.1% in the crop field at Palampur, India (Megeji et al., 2005). Incidence of insects like aphids and white flies were observed in the experimental field at IHBT, Palampur, but these were below the threshold level. Similarly, attack of insects like aphids, mealy bugs, red spider mites, and whiteflies were reported by Thomas (2000).

M. Crop Productivity

Stevia is a semiperennial species, which can be maintained up to 5–6 years, with 2 or 3 harvests per year. Earlier, Bridel and Lavielle (1931a,b,c) and Metivier and Viana (1979a) reported a stevioside yield of 60–65 and 72 g kg$^{-1}$ dry leaf, respectively. In terms of economic biomass productivity, the dry leaf yield in the natural habitat, Paraguay, was between 1500 and 2500 kg ha$^{-1}$
under dry land conditions and around 4300 kg ha\(^{-1}\) with irrigation per year (Jordan Molero, 1984).

Leaf yields of 3000 kg ha\(^{-1}\) with a stevioside concentration of 105 mg g\(^{-1}\) equivalent to 66.2 ton ha\(^{-1}\) of sugar was obtained at Canada (Brandle and Rosa, 1992).

In Japan, 1 or 2 harvests per year is possible with a dry leaf yield of 3000 and 3500 kg ha\(^{-1}\) in the first year, 4000–4500 kg ha\(^{-1}\) in the second, 4000–6000 kg ha\(^{-1}\) in third, diminishing to 4000 kg ha\(^{-1}\) in the fourth year (Sunk, as quoted by Taiariol, 2004).

Under agro-climatic conditions of Palampur, first harvest is taken at 90–110 days after transplanting during June–July. Subsequently, second harvest is taken after 60–75 days of the first harvest in early September at the time of flower bud initiation. In case of late transplanted crop grown for single cut, harvesting is done after 3–4 months of transplanting and continues till flowering begins, because the maximum sweetener in the leaves is until the plant bears flowers. Perennial crop may continue up to 4 years, once planted, in the same field. Life span of the crop is reported to be 7–8 years and herb yield increases up to 4 years. Maximum amount of leaves are produced in the third or fourth year. Flowering of the plant should be avoided and pinching of the apical bud should be done to enhance bushy growth of the plant with side branches. In the first year, average fresh biomass yield of 15–20 ton ha\(^{-1}\) was obtained out of two harvests and increased in subsequent years up to 20–30 ton ha\(^{-1}\). An average dried leaf yield of 17, 20, 23, and 25 q ha\(^{-1}\) could be produced from this total biomass yield in the first, second, third, and fourth years, respectively (Singh and Kaul, 2005).

**VII. CHEMISTRY AND QUALITY**

The sweetness in *Stevia* is attributed to the presence of *ent*-kaurene diterpene glycosides, which are water soluble (Duke and deCellier, 1993; Lester, 1999) and 300 times as sweet as cane sugar (Metivier and Viana, 1979b). *Stevia* leaves accumulate a mixture of at least eight different glycosides derived from the tetracyclic diterpene steviol (Brandle *et al.*, 1998). The leaves contain stevioside, rebaudioside A, B, C, D, and E, dulcoside A, and steviolbioside. The sweetening potency (sucrose = 1) was 250–300, 350–450, 300–350, 50–120, 200–300, 250–300, 50–120, and 100–125, respectively (Cramer and Ikan, 1986). These products taste intensely sweet; for example, rebaudioside A has been shown to be up to 320 times sweeter than sucrose on a weight basis (Phillips, 1987). Stevioside is a white amorphous powder present in leaf and stem tissue, was first seriously considered as a sugar substitute in the early 1970’s (Kinghorn and Soejarto, 1985). The
sweetness in the leaves is two times higher than that of inflorescence (Dwivedi, 1999). Steviolbioside 2, rebaudioside A4, B5, C6, D7, E8, and F9, and dulcoside A10 are other compounds present but in lower concentration (Kennely, 2002; Starrat et al., 2002). This is an alternate to artificial sweeteners such as aspartame or sodium saccharin. There is no report of ill effect on human health in over 1500 years of continuous use by Paraguayans. In Japan (the biggest consumer market), there have been no reports of side effects. Reports on antifertility (Planas and Kuc, 1968) and its metabolic byproducts like steviol being highly mutagenic (but no confirmative reports are available for harmful effect on using this plant; Brandle and Rosa, 1992) leads to a controversy on safety concern of this plant in humans. The sweet compounds pass through the digestive process without chemically breaking down; making Stevia safe for those who need to control their blood sugar levels (Strauss, 1995). A more detailed discussion on biosynthesis, toxicity, metabolism, and nutritional implications of stevioside was reviewed by Geuns (2003), which contains 74 references. He concluded that most toxicity tests performed on stevioside have been negative and the use of purified stevioside as a food additive appears preferable from public safety point of view. The conclusion is that Stevia and stevioside are safe when used as a sweetener.

VIII. RESEARCH NEEDS

Good agricultural practices (GAP) of Stevia cultivation are the need of the hour. An integrated approach by a team of multidisciplinary scientists is required, leading to good manufacturing practices (GMP) of desired quality end product from this crop. Use of Stevia is intimately tied to two major sweet glycosides, stevioside and rebaudioside A, because of the prominence of these compounds in this plant. Therefore, research should be directed toward the improvement of stevioside and rebaudioside A through management and crop improvement strategies. Stevia gives a new direction for the farming community, businessmen, and also the researchers. The possible issues are enhancing the specific enzyme responsible for the production of these glycosides so that their yield gets enhanced. Quality of sweetness is also dependent on higher proportion of rebaudioside A to stevioside in the extracted composite powder.

In many countries, this is a crop of recent domestication. Therefore, agronomic considerations should be of high priority to utilize its maximum potential. Under subtemperate climate prevailing in mid hills of India and analogous regions of the world, growth of seedlings take longer time and vegetative propagation is restricted due to nonavailability of actively
growing shoots. This leads to delay in large-scale commercial plantation. Studies on production techniques and planting through rootstock are needed.

Water management component is considered to be critical, since the water resources are shrinking day by day. Integrated crop management comprising of weed, insect, disease, and nutrient management, should be inbuilt as a part of GAP. As a system study, the suitability of this crop in the traditional cropping systems is another determinant to avoid excess production. This complete packages of production technology will make the Stevia cultivation socially acceptable, cheaper, and economically viable.

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