Stevia rebaudiana: Phytochemical, pharmacological activities, and plant tissue culture (a mini-review)

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Abstract. Stevia rebaudiana is famous as a natural sweetener and potentially lowers blood sugar. The benefits of S. rebaudiana are numerous, and various pharmacological activities have been found, such as efforts to treat diseases such as diabetes, obesity, dental caries, hypoglycemia, and hypertension. The main sweet compounds in S. rebaudiana are rebaudioside A and stevioside, especially in the leaves. This review examines phytochemistry, pharmacological activity, micropropagation, and in-vitro culture modification of S. rebaudiana plants. Online and offline literature searches were conducted to compile the articles. PubMed (Medline), Web of Science, is used to retrieve online publications using the following search terms: S. rebaudiana, stevia herbs, traditional medicine, pharmacological activities, toxicity, secondary metabolite content, phytochemicals, and plant tissue culture of S. rebaudiana. This review is also a compilation of four previous reviews and the latest data from research journals on S. rebaudiana.

Keywords: stevioside, rebaudioside A, natural sweetener, lowering blood sugar, plant tissue culture

INTRODUCTION

The use of natural materials by the community is increasing. Many natural materials are used to improve health status and promote preventive and rehabilitative efforts.

One natural material often used is S. rebaudiana, natural sweetener with low calories. S. rebaudiana (Asteraceae family) from Paraguay. S. rebaudiana is a primary sweetener in leaf and stem tissue and is considered a sugar substitute [1]. S. rebaudiana is also one of the plants that have a high-potential sweetener and low calories [2]. Due to high demand, the large-scale cultivation of S. rebaudiana has been carried out in India [3]. Wild S. rebaudiana leaves contain 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside [3].

S. rebaudiana can become a major source of sweeteners in the future and can improve the quality of life for diabetic patients because it is low in calories. With the increasing incidence of diabetes, there is a need for natural non-calorie sweeteners with good taste and healthy properties. Besides the non-calorie sweetener properties, S. rebaudiana has many therapeutic values: antihyperglycemic, anticancer, and anti-hypersensitive. In some countries, S. rebaudiana is used as a natural control for diabetes [4],[5], and Japan has used S. rebaudiana as a sweetener in soft drinks and sweets [1].

Distribution and diversity of S. rebaudiana

S. rebaudiana is an endemic herb from Paraguay and Brazil's border with that country. The Stevia genus (Eupatorieae, Asteraceae) consists of about 150-200 herbaceous plants and bush species. It is a sweet herb from Paraguay, and for centuries, this herbal sweetener has been used by indigenous Guarani Indians to counteract the bitter taste of various plants, medicines, and drinks [6].

S. rebaudiana usually grow to a height of approximately 30 to 90 cm based on S. rebaudiana habitus. The leaves show a single, ovoid, pointed base, flat edge, 2-4 cm long, 1-3 cm wide, pinnate adventure, hairy, short stalk, green [7]. S. rebaudiana stems are oval-shaped and downy. S. rebaudiana has five white petals that will bloom throughout the year with a tubular crown. S. rebaudiana has fibrous roots divided into two parts, namely coarse fibrous roots and fine fibrous roots [8].
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*S. rebaudiana* does not like prolonged areas of waterlogging and heavy rain. *S. rebaudiana* plants naturally grow alongside swamps or in grassland communities up to 700 m above sea level, which are permanently moist but do not experience prolonged stagnant water. The suitable soil for this plant is the shallow water level, especially in fertile acid sand with pH 4-5. *S. rebaudiana* plants also grow well in neutral soils with a 6.5-7.5 [9].

In Indonesia, *S. rebaudiana* plants can grow in lowlands with an altitude of 250 m above sea level, but optimum growth is obtained in areas with altitudes of 800-2,000 m above sea level with optimum temperatures ranging from 20-30°C. In the lowlands, *S. rebaudiana* blooms faster so that leaf biomass production is low and dies if pruned too often [10].

**Propagation *S. rebaudiana***

Seeds, cuttings, and tissue culture can propagate *S. rebaudiana*. However, *S. rebaudiana* multiplication by using seeds is rarely done because the sprouts and seeds produced vary. Clonal propagation is usually done in small scale production, but it is not economical for large scale [2].

The most common *S. rebaudiana* propagation uses shoot stem cuttings, and side shoots cuttings. The seeds produced are more uniform than the seeds. The best results are obtained from shoot cuttings with 3-4 segments with a 7-10 cm length. Enlargement of cuttings can be done directly on the ground or using polybags and multi-tray. Of the two containers, the most efficient and economical is multi-tray.

Propagation of *S. rebaudiana* is preceded by breeding first. The nursery location should be in the middle of the *S. rebaudiana* planting plan to transport seedlings closer to the field. Stock plants planted in beds in the field are used as sources of supply of cuttings. Stock plants are the parent sources of cuttings considered superior (high leaf biomass production, slow flowering, high stevioside, and rebaudioside A content, and tolerant of pests and drought) [11],[12].

The cuttings used are shoots with 3-4 pairs of leaves and are not in the flowering phase or will flower, marked by decreasing leaf size. The lowest pair of leaves are removed, and the lower end of the cuttings is dipped in a paste containing a root-forming auxin (for example, Rootone F) before being planted in a multi-tray container, and the container must be placed in a tightly closed plastic lid.

Multi tray made of plastic material has as many as 128 (8 x 16) holes with 3 cm x 3 cm and 3 cm inside with a slightly smaller bottom size. The multi-tray is placed in a tightly closed transparent plastic lid to maintain the air humidity. The tray's cuttings are kept moist by spraying with small drops of water and drying the media. Roots begin to appear 3-5 days after planting. After two weeks, the lid is gradually opened at both halls ends. Around three weeks, cuttings have formed a good root with a shoot height of about 10 cm with 2-3 pairs of new leaves and ready to be planted in the field. They transport seedlings to the field because the tray can be held vertically without the seeds detached.

Seedlings taken root from multi trays are removed from the container and planted in the planting hole, and covered again with soil while the soil surface around the seedlings is pressed as needed so that the plant stands upright. Since the leaves and branches of the *S. rebaudiana* seed wilt quickly, planting should be done in the morning, afternoon, or cloudy. Planting when the sun is shining should be avoided. Watering is done immediately after planting the seeds to prevent the seeds from wilting and accelerating the soil's union with the soil. *S. rebaudiana* plants begin to form new leaves about one week after planting.

Maintenance is carried out in several ways, namely [13]:

a. Pruning plants
   This pruning aims to bring out many new shoots from pruned stems.

b. Irrigation
   Irrigation is done routinely every day unless there is rain, especially in the early stages of planting when plants are still sensitive to drought. *S. rebaudiana* plant water needs around 2.3 mm / plant/day.

c. Weed Control
   *S. rebaudiana* is very weak in competing with weeds, especially at the beginning of growth. Weeds only grow from planting holes around plants and in beds. Then weeding must be done routinely by the rate of weed growth. Weed growth will decrease considerably after the canopy of *S. rebaudiana* plants cover the soil surface.

d. Pest and disease control
   Pest and disease control is carried out if the attack level has exceeded the economic threshold. When *S. rebaudiana* leaves are to be harvested immediately, spraying of pesticides must be stopped no later than three weeks before harvest to avoid pesticide residues in the leaves.
Fertilization

*S. rebaudiana* plants require chemical fertilization, primarily N and K, sometimes requiring Mg, depending on land conditions. Visual symptoms of nutrient deficiencies in *S. rebaudiana* plants are N deficiency causing yellowing leaves, P deficiency of leaves will be dark green, and K deficiency of leaves will be chlorosis and pale spots.

**Benefits of *S. rebaudiana***

*S. rebaudiana* is widely used to treat diabetes, obesity, dental caries, hypoglycemia, and high blood pressure [14], [15], [16], [17]. This plant can inhibit certain bacteria's growth, helping its traditional treatment of wounds, immunomodulatory and natural sweetener [15], [18], [19], [20].

**Phytochemical Studies**

**Qualitative analysis of compounds in *S. rebaudiana***

Several studies of qualitative analysis of compounds in *S. rebaudiana* used the GC-MS and LC-MS instruments.

**Table 1.** Secondary metabolic content of *S. rebaudiana*

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid</td>
<td>Arginine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Histidine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glutamate acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspartic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proline</td>
<td></td>
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<tr>
<td></td>
<td>Tyrosine</td>
<td></td>
</tr>
<tr>
<td>Fatty acid</td>
<td>Palmitic acid</td>
<td>[23],[24]</td>
</tr>
<tr>
<td></td>
<td>Oleopalmitic acid</td>
<td></td>
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<tr>
<td></td>
<td>Stearic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oleic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linoleic acid</td>
<td></td>
</tr>
<tr>
<td>Phenolic Acid</td>
<td>Dicaffeoylquinic acid</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin 3-O-xylloside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apigenin-7-O-glucoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3,4-dimethoxinamite acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luteolin 7-O-rutinoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caffeic Acid</td>
<td></td>
</tr>
<tr>
<td>Sterol Fat</td>
<td>Pyrogallol</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>4-Methoxibenzoic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-Coumaric Acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-Methylcatechol</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Sinapic Acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cinnamic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Apigenin</td>
<td>[23],[27]</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td></td>
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<tr>
<td></td>
<td>Luteolin</td>
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### Kaempferol glycosides

<table>
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<tr>
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<th>Compound</th>
<th>References</th>
</tr>
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<tr>
<td>Flavonol</td>
<td>Kaempferol-3-O-arabino side</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin-3-O-β-D-arabino side Quercetin-3-O-β-D-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rhamnoside Quercetin-3-O-Glucoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin-3-O-rutinosida</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin-3-O-(4-O-trans-Caffeoyl)-α-L-rhamno-</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>pyranosyl-(1→6)-β-D-Galactopyranoside Kaempferol-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-O-rhamnoside Flavon Apigenin-4-O-β-D-glycosides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luteolin luteolin-7-O-β-D-glycosides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>kaempferol-3-O-rhamnoside flavan-3-ol</td>
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### Hydrocarbons

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<td>Decane</td>
</tr>
<tr>
<td></td>
<td>Dodecane</td>
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<tr>
<td></td>
<td>Tridecane</td>
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<tr>
<td></td>
<td>Hexadecane</td>
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<tr>
<td></td>
<td>Heptadecane</td>
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<tr>
<td></td>
<td>Octadecane</td>
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<tr>
<td></td>
<td>Nonadek</td>
</tr>
<tr>
<td></td>
<td>Heneicosane</td>
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<tr>
<td></td>
<td>Dotriacontan</td>
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### Aliphatic Alcohol

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyclodecanol</td>
</tr>
<tr>
<td></td>
<td>Hexadecane</td>
</tr>
<tr>
<td></td>
<td>iso-Heptadecanol</td>
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<td>Dotriacontan</td>
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### Diterpen Glycosides

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<thead>
<tr>
<th>Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stevioside</td>
</tr>
<tr>
<td></td>
<td>Steviol</td>
</tr>
<tr>
<td></td>
<td>Steviolbioside</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside A</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside B</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside C</td>
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<tr>
<td></td>
<td>Rebaudioside D</td>
</tr>
<tr>
<td></td>
<td>Dulcoside A</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside E</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside F</td>
</tr>
<tr>
<td></td>
<td>Rebaudioside M</td>
</tr>
<tr>
<td></td>
<td>Rubusoside</td>
</tr>
<tr>
<td></td>
<td>Dulcoside B</td>
</tr>
</tbody>
</table>

### Sesquiterpen

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-pinene</td>
</tr>
<tr>
<td></td>
<td>β-caryophyllene</td>
</tr>
<tr>
<td></td>
<td>Spathulenol</td>
</tr>
<tr>
<td></td>
<td>Caryophyllene oxide</td>
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</table>

### Alkaloids

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steviamine</td>
</tr>
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</table>

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**Fahrauk Faramayuda**

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**Figure 1.** Stevioside Structure

**Figure 2.** Rebaudioside A Structure

**Figure 3.** Rebaudioside B Structure

**Figure 4.** Rebaudioside C Structure
The main sweet compounds in *S. rebaudiana* are rebaudioside A and stevioside, especially in the leaves [32]. Other components found in small quantities include steviolbioside, rebaudioside B, C, D, E, F [33], and rubusoside [34]. Stevioside and rebaudioside A are the most exciting components of steviol glycosides because of their sweetness [34].

Quantitative analysis is commonly used to determine stevioside quantification and rebaudioside A from the plant material and nutrient samples [35]. HPLC is a simple, cost-effective, environmentally friendly, and adaptable method for simultaneous filtering and quantitative determination of glycoside dipped with other analytical techniques to extract and filtrate Rebaudioside A and Stevioside [36]. They extracted *S. rebaudiana* leaves using a microwave. A simple reversed-
Antimicrobial Activity

From methanol and ethanol extracts tested against gram-positive and gram-negative bacteria and their inhibition was seen. Methanol extracts from *S. rebaudiana* leaves showed a maximum inhibition zone (in mm) at a 100 mg/mL concentration compared to ethanol extract. Methanol extract at 100 mg/mL showed a maximum (123.33%) percentage inhibition relative to *Escherichia coli*, while ethanol leaf extract at 100 mg/mL showed a maximum percentage inhibition *Pseudomonas aeruginosa* (105.68%). Then, increasing the extract's concentration, the antibacterial activity against the pathogenic bacterial strains tested will also increase, which indicates that the antibacterial activity is concentration-dependent [42]: acetone extract, ethyl acetate extract, and chloroform extract. *S. rebaudiana* acetone extract had good inhibition against *Staphylococcus aureus* and *Bacillus subtilis*. Ethyl acetate *S. rebaudiana* extract is also very good at inhibiting the growth of *Vibrio cholerae* and *Trichophyton mentagrophytes* bacteria. [43].

Role of *S. rebaudiana* in dental caries

Stevioside and rebaudioside A are non-cariogenic sweeteners because they do not cause caries in experimental animals [44]. Stevioside can be a substitute for certain cariogenic compounds present in sucrose [16].

The role of *S. rebaudiana* in diarrhea

Diarrhea symptoms are increased water content and stool frequency caused by bacteria and viruses [45]. Previous studies reported that steviol efficiently inhibited the transmembrane conductance of cystic fibrosis (CFTR) [46]. In some countries, *S. rebaudiana* tea improves digestive organ function [47].

Effects on the skin

*S. rebaudiana* can treat skin problems such as acne, dermatitis, eczema and accelerate wound healing [47].

*S. rebaudiana* rebaudiana plant tissue culture

*S. rebaudiana* can be propagated in a generative and vegetative way. Vegetatively generally propagated by stem cuttings. Generative breeding is done by using seeds. However, this method is rarely done because getting the seeds is tricky and has a longer growth time and lower stevioside and rebaiside A content [48]. So tissue culture techniques can produce *S. rebaudiana* clonal seeds en masse, fast, and genetically identical, especially in the early stages of nursery [49]. Several goals are achieved by masteri
Stevia rebaudiana: Phytochemical, pharmacological activities, and plant tissue culture (a mini-review) (Fahruk Faramayuda)

Table 2. In Vitro Modification culture of S. rebaudiana

<table>
<thead>
<tr>
<th>Explant</th>
<th>Organ</th>
<th>Medium and Plant Growth Regulator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Callus granular and yellowish green</td>
<td>MS + 2 mg/L BA + 2 mg/L 2,4-D</td>
<td>[50]</td>
</tr>
<tr>
<td>Leaf</td>
<td>Shoots</td>
<td>MS + 0.3 mg/L NAA + 0.3 mg/L IBA</td>
<td>[50]</td>
</tr>
<tr>
<td>Leaf</td>
<td>Callus</td>
<td>MS + 1 mg /L 2,4-D + 1 mg/L NAA</td>
<td>[54]</td>
</tr>
<tr>
<td>Shoots</td>
<td>Root</td>
<td>MS + 2 mg/L IBA</td>
<td>[54]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BAP</td>
<td>[55]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BAP + 0.5 mg /L IAA</td>
<td>[55]</td>
</tr>
<tr>
<td>Peak, nodal, and leaf</td>
<td>Shoots</td>
<td>MS + 2 mg/L BA and 1 mg/L IAA</td>
<td>[56]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1.5 mg/L IBA and 0.5 mg/L Kinatin</td>
<td>[54]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 BAP mg/L + 0.05 NAA mg/L</td>
<td>[57]</td>
</tr>
<tr>
<td>Inter-nodal segment</td>
<td>Callus</td>
<td>MS + 3.0 mg/L 2,4-D</td>
<td>[53]</td>
</tr>
<tr>
<td>Bar segments</td>
<td>Shoots</td>
<td>MS + 0.5 mg/L BAP</td>
<td>[59]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BA + 0.05 mg/L NAA</td>
<td>[58]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BA + + 0.5 mg/L TDZ + 0.05 mg/L NAA</td>
<td>[58]</td>
</tr>
<tr>
<td>Leaf</td>
<td>Callus</td>
<td>MS + 0.75 mg/L NAA + 1 mg/L 2,4-D</td>
<td>[60]</td>
</tr>
<tr>
<td>Leaf</td>
<td>Callus</td>
<td>MS + 0.1 mg/L NAA + 2.0 mg/L BAP</td>
<td>[52]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BAP + 0.5 mg/L IBA</td>
<td>[61]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Shoots</td>
<td>MS + 1 mg/L BAP</td>
<td>[61]</td>
</tr>
<tr>
<td>Nodal</td>
<td>Root</td>
<td>MS + 0.25 or 0.5 mg/L IAA</td>
<td>[61]</td>
</tr>
<tr>
<td>Leaf</td>
<td>yellowish green callus</td>
<td>MS + (1.5 mg/L and 2.0 mg/L) NAA + 0.1 mg / L BA</td>
<td>[62]</td>
</tr>
</tbody>
</table>

culture, such as callus culture, which can produce secondary metabolites. In this approach, callus cultivation is directed to callus proliferation and how callus can be driven to produce higher metabolites [48].

Propagation of S. rebaudiana plants by tissue culture techniques is done using MS (Murashige and Skoog) media as a standard medium for tissue culture commonly used. MS media have high mineral salts and N compounds in NO₃⁻ and NH₄⁺. MS media can be used for any plant growth regulating agent commonly used in tissue culture as auxin. The role of auxin with relatively high levels causes callus growth. Giving hormones in tissue culture can be determined according to its purpose, such as inducing callus by administering auxin [50].

Many studies have developed the growth of S. rebaudiana plants using tissue culture techniques. In research on plant tissue culture using leaf parts to be developed with plant tissue culture methods.

Leaves taken from S. rebaudiana were planted on the media Murashige and Skoog. On the media, MS added growth regulators of 6-benzyladenine (BA) with different concentrations of 1.0, 2.0, and 3.0 mg/L and 2,4-dichlorophenoxyacetic acid (2,4-D) with different concentrations also between 0.5-4.0 mg/L or a combination of BA and 2,4-D with a concentration of 0.5-3.0 mg/L to induce callus growth. The results show that S. rebaudiana plants that grow well are on Murashige and Skoog media, which are given a combination of growth regulators 6-benzyladenine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D) with a concentration of 2.0 mg/L [51].

Leaf explants from S. rebaudiana plants and developed to grow callus. Planting was carried out on Murashige and Skoog media. This research investigates the effect of different combinations of auxin and cytokinin growth regulators and maintenance of S. rebaudiana under different media strengths from Murashige and Skoog media given to callus induction. Auxin growth regulators used are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthalene-acetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) with each concentration of 1 mg/L.
in combination with cytokinin growth regulators kinetin with a combination of concentrations of 0.1, 0.2 and 0.3 mg/L and then observed after 30 days of inoculation. The results show that callus development depends on the strength of the media MS, but there are no specific callus induction results between single strength and half media strength MS. The use of half the MS medium and the combination of growth regulators 2, 4-D at 1.0 mg/L and kinetin at 0.2 mg/L showed good initiation for callus and NAA at 0.1 mg/L combined with BAP at 2.0 mg/L is suitable for callus maintenance [52].

Callus from nodal explants and implantation on the media MS added growth regulators 2,4-D carried out concentrations at 2, 3, 4, and 5 mg/L. The results showed the highest callus found on MS media with growth regulator 3.0 mg/L 2,4-D [53]. The explants used were nodal explants using the media MS and added growth regulators 6-benzyladenine (BA), Kinetin, and naphthalene-acetic acid (NAA) singly or in combination aimed at making shoots [51]. Then there are explant nodal segments from buds proliferating to be subcultured for further induction of multiple shoots. The regenerated double shoots were cut, and each shoot was placed in MS media containing indole-3- acetic acid concentration(IAA), indole-3- butricacide (IBA), and naphthalene-acetic acid (NAA) are different for root induction. The results showed that the best response to growing shoots was MS enriched media with a combination of 1.5 mg/L BA and 0.5 mg/L kinetin. The best results were obtained from MS media enriched with 0.1 mg/L IAA for maximum root induction.

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