

Stevia Rebaudiana Bertoni's Economical Micropropagation and Evaluation of in Vitro Cultures to Enhance Steviol Glycosides

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Abstract

The leaves of *Stevia rebaudiana* Bertoni yield a significant quantity of zero-calorie sweetener that is 300 times sweeter than sucrose. The plant *S. rebaudiana* is incompatible with itself and only produces a few seeds that germinate slowly. The purpose of this study was to develop a cost-effective method for *S. rebaudiana* micropropagation. By dispensing with the establishing society medium in this review, the creation cost has diminished by roughly 34%. The proportion of stevioside to rebaudioside A, the primary steviol glycoside, determined the sensory characteristics. Using 0.02 mg/l NAA and 0.05 mg/l BAP, this study produced *S. rebaudiana* tissue with a rebaudioside A to stevioside ratio of 3.76 in a bioreactor. This plant tissue had a better flavor than the shoots that were grown in the field. Stevioside was more abundant than rebaudioside A in cell suspension, but there was no change in steviol glycosides. Moreover, the bristly root development conditions were additionally improved in this review, by utilizing A4, ATCC15834, and LBA9402 kinds of *Agrobacterium rhizogenes* for leaf and stem explants utilizing two change techniques including co-culture and infusion. The ATCC15834 strain and injection method produced the highest transformation rate (60%) in leaf tissue. In previous studies, this transformation rate was not observed. Stevioside and rebaudioside A were found in some hairy roots, and the amount of rebaudioside A was higher than that of stevioside in two of the roots.

The utilization of hereditary designing and biotechnology could impressively build the quality and amount of steviol glycosides and work on the flavor of *S. rebaudiana*.

Keywords: Rhizogenes; Glycoside; Micropropagation; Plant tissue

Introduction

Stevia rebaudiana Bertoni is a perennial shrub that belongs to the family Asteraceae. It produces steviol glycosides, which are a natural sweetener with no calories [1]. Steviol glycosides are 4–20% dry weight and approximately 300 times sweeter than sucrose. As a result, they might be a good alternative to sweeteners like sucrose, aspartame, and saccharin. Steviol glycosides are now widely used to replace sugar in many countries. This plant's most abundant sweeteners are Reb and Stv, respectively. A few examinations showed that the utilization of *S. rebaudiana* items doesn't adversely influence wellbeing.

According to the findings of a study conducted by Serio, one hectare of *S. rebaudiana* plants can yield anywhere from 1000 to 1200 kilograms of dried leaves containing 60–70 kilograms of Stv [2]. This yield is considered to be low in comparison to that of sugarcane or sugarbeet, but 70 kilograms of Stv, which is 300 times sweeter than sucrose, is equivalent to a yield of 21,000 kilograms of sucrose per hectare. *S. rebaudiana* is a self-incongruent plant that produces little seeds. The germination of its seeds is weak and the percentage of live seeds is low. As a result, from a native plant, micropropagation can quickly produce a large number of complete plants.

The culture and micropropagation of *S. rebaudiana* have been the subject of an increasing amount of research [3]. In any case, meager exploration has zeroed in on the economy in micropropagation. "Time" and "cost" are the most important factors that determine economic production in commercial production, which is regarded as the most significant issue. This study presents a monetary convention for *S. rebaudiana* micropropagation.

Cervical cancer is the fourth most common cancer among women worldwide, resulting in 570,000 new cases and 311,000 deaths. The occurrence of cervical malignant growth is diminishing a result of the expanded early identification rate with the treatment of pre-invasive infection, and human papillomavirus (HPV) inoculation. Be that as

it may, because of wellbeing disparities, almost 90% of the passings from cervical disease happen in low-and center pay nations since admittance to general wellbeing administrations is restricted [4]. In World Wellbeing Association Chief General declared a worldwide call for activity to dispense with cervical malignant growth. All nations must strive for an incidence rate of less than 4 per 100,000 women years in order to eradicate cervical cancer as a global public health issue. In order to accomplish this, high levels of coverage for HPV vaccination, precancerous lesions screening and treatment, and cancer management must be achieved and maintained for decades. When implemented as part of national programs with high coverage and in settings with resources for patient follow-up, additional diagnostic tests (colposcopy and pathology), and disease management, cytology-based screening has been used successfully to achieve these objectives.

When women with high-grade squamous cell cervical cytology visit a colposcopy clinic, they typically receive conventional treatment in two steps. A colposcopic examination and biopsy of the suspicious lesion are the first steps [5]. The patient is informed and advised in the second step, and the pathological reports, which may include additional diagnostic procedures or therapeutic options like the loop electrosurgical excision procedure (LEEP), are used to provide management. It has been reported that this method has a high loss-to-

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follow-up rate and a high level of patient anxiety.

A single visit is required for the "see-and-treat" approach for abnormal cervical cytology. During the same visit, colposcopy and diagnostic or therapeutic LEEP are performed. The benefits of this approach are a decrease in cost, a decrease in misfortune to follow-up, and a decrease in quiet tension contrasted and the traditional two-step approach. However, the "see-and-treat" strategy might result in excessive treatment [6]. A final cervical pathological report of CIN I/low-grade squamous intra-epithelial lesion (LSIL) or a normal result from LEEP specimens in high-grade squamous intra-epithelial lesion (HSIL) cervical cytology is considered overtreatment. The results of LEEP are postoperative draining and contamination. Additionally, a drawn out difficulty is a preterm birth, which has been accounted for to be 1.78 times more normal contrasted and no treatment.

Ebisch and co. published a meta-analysis that found that the "see-and-treat" approach overtreated 11.6% of women with HSIL cervical cytology and a colposcopic impression of a high-grade lesion. Overtreatment rates of 3.2–20% were found in subsequent studies [7]. The "see-and-treat" approach's overtreatment needs to be studied more thoroughly. The purpose of this study was to examine the factors that contribute to excessive treatment at a colposcopy clinic and the rate of overtreatment with the "see-and-treat" approach.

Segment scraps

Planning of plant materials

S. rebaudiana plant was acquired from the Farming Biotechnology Exploration Organization of Iran (ABRII). Solid apical shoots were chosen and managed to around 10 mm long. Tween 20, running tap water (10 minutes), a solution containing gentamicin antibiotic 50 mg/l and benomyl fungicide 1/1000 (15 minutes), ethanol (C₂H₅OH) 70% (45 seconds), and sodium hypochlorite (NaClO) 2.5% (8 minutes) were used to wash the explants, respectively [8]. The results related to micropropagation were obtained after the explants were washed three times with sterile double micropropagation. Momentarily, the consequences of root enlistment showed that the utilization of NAA, IBA, and 2,4-D for establishing and solidifying didn't create a huge contrast as far as root development, in contrast with control medicines without auxin (Information not shown). The results of the first experiment suggested that auxin might not be suitable for rooting.

Interventional cytology has since a long while back a deep rooted job at all pathology divisions in Sweden, yet with neighborhood variety in the quantity of techniques and cytology/histology example proportion [9]. The field has undergone a few subtle shifts over the years. Interventional cytology of the prostate is an early focal point of a method that is very seldom utilized today. In addition, core biopsies are preferred when a malignant tumor is suspected, resulting in a worldwide decline in interventional breast cytology in recent years. In Sweden, some interventional cytopathologists likewise use center needles, while this kind of testing is ordinarily performed by radiologists and some of the time specialists. Additionally, cytopathologists are increasingly utilizing non-diagnostic ultrasound guidance, albeit with significant regional variations.

As shown, the total number of cytological procedures has remained high at the Karolinska University Hospital in Solna over the past 25 years, and it has continued to rise at the University Hospital in Lund. In recent years, there have been more cytological samples taken in Lund as a result of both an increase in the total number of procedures and the centralization of some cytology from nearby hospitals.

Materials and Techniques

Concentrate on plan: A review study was led at the Workforce of Medication, Ramathibodi Clinic, Mahidol College, Bangkok, Thailand. After receiving approval from the COA of the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital, and Mahidol University MURA2020/839), patients who went to a colposcopy clinic served as the source of the cases. Women who underwent colposcopic examination and LEEP at the same visit and whose cervical cytology indicated high-grade squamous cytology, including atypical squamous cells, could not exclude HSIL (ASC-H), HSIL [10]. This study did not include women who were pregnant, younger than 25 years old, or had medical conditions that required pre-operative preparation (such as uncontrolled hypertension or were currently taking anticoagulants or antiplatelets). Colposcopic ally-directed biopsies were performed and managed in accordance with the pathological findings if there were visible gross lesions; As a result, these patients were left out. Women with a history of biopsy or colposcopic examination, as well as those with known histopathology, were also excluded. Gynecologic oncologists examined or supervised each case.

Measurement and collection: Data were taken from the medical records of patients. The current cervical cytological examination, the presence or absence of a transformation zone in the current cervical cytology, age, parity, menopausal status, age of first sexual encounter, number of lifetime sexual partners, method of contraception, and presenting symptoms were all collected. The colposcopic impression, adequacy, type of transformation zone, and percentage of lesion extension were all gathered. The LEEP histopathological reports were gathered. From LEEP specimens, overtreatment was defined as CIN1 or lower histological pathology. Inconveniences in something like fourteen days of techniques, like discharge and disease, were recorded.

The hypothesis of an infinite proportion of the population was used to estimate the sample size. A 5% error rate was set. 11.6% was the reference rate of overtreatment. With an alpha of 0.05 and a power of 80%, it was determined that 158 cases were required to reject the null hypothesis. To take into consideration information deficiency of 10%, an example size of 176 was required.

Clinical characteristics: The patients have been analyzed statistically using frequency and percentage for categorical data and mean and standard deviation (SD) or median and range for continuous data, depending on the situation [11]. Continuous data were analyzed with the appropriate Mann-Whitney U-test or Student's t-test in order to compare the characteristics of patients who received excessive treatment with those of patients who did not receive excessive treatment. The Chi-squared or Fisher's exact tests were used to compare categorical data. Co-factors with $p \leq 0.1$ were chosen for multi-variable strategic relapse investigation. $p < 0.05$ was considered to show importance. The odds ratio (OR) and the 95 percent confidence interval (CI) are shown as results. STATA Version 16 (StataCorp, College Station, TX, USA) was used for data analysis.

Results

Ladies with high-grade squamous cytology dealt with a 'see-and-treat' approach at the review colposcopy center were recognized. 220 cases were included in this study after 60 cases were excluded, as shown. the characteristics of the disease. Patients were approximately 38 years old on average. The majority of patients lacked symptoms, were premenopausal, and did not use contraception [12]. The majority of patients' cervical cytological profiles showed HSIL, with approximately

5% showing SCC and ASC-H. Colposcopic examination and final histopathological findings followed. One patient did not have a record of their colposcopic examination. 86.3% of colposcopic impressions for the remaining 219 patients were classified as high-grade lesions or cancer. From 220 ladies with high-grade squamous cytology going through LEEP, the eventual outcomes were typical for five patients and CIN1/LSIL for 20 patients. Therefore, the rate of overtreatment was 11.4%. From a positive margin, 31 percent of these patients required additional treatment. Draining and contamination were accounted for in three and nine patients, separately.

Discussion

The economic calculations involved in the production of *S. rebaudiana* are significant due to its industrial and commercial significance. 'Cultivating the 4–20 mm pieces of *S. rebaudiana* yielded the highest number of plants with a mortality rate of 12.5%. Time' and 'cost' are two key factors that decide financial creation. The time and location required to produce one plant using each of the micropropagation. Women with high-grade cytology and low-grade colposcopic impression were found to have a 29.3% overtreatment rate, according to a recent meta-analysis [13]. However, women with high-grade colposcopic impressions and cervical smears had an overtreatment rate of 11.6%. Likewise, found that women with high-grade cervical cytology had overtreatment rates of 22% for low-grade colposcopic impressions and 3.2% for high-grade colposcopic impressions, respectively. As a result, the two-step approach is suggested in these situations to avoid overtreatment.

In spite of the gamble of overtreatment, the 'see-and-treat' approach is straightforward, further develops consistence, diminishes patient uneasiness, is reasonable, and lessens the quantity of medical clinic visits. These advantages significantly outweigh the risk of overtreatment in women with high-risk abnormal cervical smear results like HSIL, especially in a setting with high volume and limited resources.

A strength of this study was the utilization of colposcopic discoveries, with colposcopy performed by or under the oversight of experienced gynecologic oncologists. Notwithstanding, this concentrate additionally had a few impediments. Right off the bat, the review configuration was review, which prompts the potential for determination inclination. Second, because the research was carried out in a single institute—a metropolitan teaching hospital—the findings may not apply to other settings [14]. Thirdly, because of restricted assets in Thailand, HPV testing has not been performed regularly for most patients in the beyond 12 years. As a result, information about HPV was not included in this study, especially for women with ASC-H. Finally, some cytological results could not be reviewed because the study hospital requires cervical cytological material to be kept for five years. Utilizing the aforementioned information with a carefully selected group of women who have high-grade cervical cytology for the "see-and-treat" approach has the potential to improve the quality of care, particularly for women who wish to maintain their fertility. In ladies who gave ASC-H cervical cytology, the consequences of the HPV test ought to be integrated into the choice. A two-step strategy is appropriate in the event that HPV testing is unavailable [15]. Additionally, the colposcopic impression should be taken into account. A two-step procedure would still be effective for colposcopy-diagnosed normal or low-grade lesions.

Conclusion

For some HPV genotypes, extended genotyping with reflex to

cytology has the same CIN3 sensitivity, specificity, and number of colposcopies per case as primary HPV screening with 16/18 genotyping and DS; Since HPV31/33/58/52 would not need to be triaged, cytology would require fewer tests than DS. HPV33/58/52 could also be triaged by cytology for guidelines committees aiming to reduce the number of colposcopy referrals and increase colposcopy efficiency. This strategy would be similar to DS if ASC-US/LSIL was used as the referral cutoff, but if >LSIL was used, the strategy would have higher specificity for a small decrease in sensitivity genotypes, this strategy is only marginally superior to extended genotyping with reflex to cytology, and it may be beneficial in settings where cytology is difficult to obtain, such as self-collection programs. Extended genotyping, whether it is reflexed to cytology or not, has the unique capability of adjusting clinical action thresholds to match the risk preferences of various national screening programs and simultaneously increases colposcopy efficiency.

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Conflict of Interest

None

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