Asian Journal of Crop Science

ISSN 1994-7879

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Crop Science 1 (1): 63-65, 2009
ISSN 1994-7879

Identification of *Stevia rebaudiana* Bertoni Proteins by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

Ma Lei and Shi Yan
Dryland Technology Key Laboratory, Qingdao Agricultural University,
Qingdao, 266109, Shandong Province, China

Abstract: Four diverse genotypes of *Stevia rebaudiana* Bertoni were included for Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. Total proteins were analyzed through slab type SDS-PAGE. Based on SDS-PAGE, specific bands were suggested to be used for identifying *Stevia rebaudiana* Berton. This method has the advantages of simplicity, high sensitivity and good accuracy and the SDS-PAGE proved to be a powerful tool for differentiating *Stevia rebaudiana* Bertoni varieties.

Key words: Identification of variety, total protein, electrophoretogram

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni, Compositae), a perennial herb native to Paraguay, is known to accumulate sweet diterpene glucosides such as stevioside and rebaudioside A maiuly in the leaves. Stevioside, which is approximately 500 times as sweet as sucrose, is regarded as a valuable natural sweetening agent because of its relatively good taste and chemical stability (Yukiyoshi *et al*., 1984). Alternatives to sucrose serve a number of purposes. They are used to expand food and beverage choices for those who must or want to control caloric, carbohydrate, or sugar intake; assist weight control or reduction; aid in the management of diabetes; assist the control of dental caries; enhance the usability of pharmaceuticals and cosmetics; provide sweetness when sugar is not available; and assist the cost-effective use of limited resources (Cardello *et al*., 1999).

Two big problems can hardly be solved in *Stevia rebaudiana* Bertoni breeding and farming procedure, one is too long breeding period due to traditional breeding methods and the other one is stavioside content reduction due to impurity varieties (Tianhong and Yang, 1999; Motomu *et al*., 1994). Variety identification technique help to solve the above problems (Abdul *et al*., 2002).

Among biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm (Ahmad and Sliukard, 1992). Under the same growth conditions, leaf proteins can be used as genetic markers in four major areas: (1) analysis of genetic diversity within and between species, (2) plant domestication in relation to genetic resources conservation and breeding, (3) genome relationship and (4) as a tool in crop improvement. The SDS-PAGE is considered to be a practical and reliable method for species identification (Gepts, 1989).

One of the main aims of *Stevia rebaudiana* Bertoni cultivar identification is to use its specific genetic material for breeding programmes. The genetic distance between varieties can be assessed through morphological characterisation and genetic markers (Ahmad *et al*., 1996). The characterized material then helps the plant breeders to select the genotypes to be utilized in hybridization programme. The SDS-PAGE was implied to identify species of *Stevia rebaudiana* Bertoni which may

Corresponding Author: Shi Yan, Dryland Technology Key Laboratory, Qingdao Agricultural University, Qingdao, 266109, Shandong Province, China

be confused in the germplasm samples. In particular, the varieties which are confused and sometimes difficult to identify on the basis of morphological according to diagnostic techniques. Therefore, this situation suggested the need to have biochemical characterisation for identification of *Stevia rebaudiana* Bertoni species.

MATERIALS AND METHODS

The experiment was planted with two replications during Summer seasons of 2007 at Dryland Technology Key Laboratory of Shandong Province in Qingdao Agricultural University, Qingdao, China (36°N and 120° E).

Total protein were extracted from leaf of different diversities of *Stevia rebaudiana* Bertoni (No. 2 Shoutian from Japan, with high sugar, small leaf, low yield; No. 3 Xingnang bred by Jiangsu Yancheng Stevia Co. Ltd, with medium sugar, big leaf, high yield; No. 1 Qingtian bred by Qingdao Agriculture College, with high yield and disease-resistant; No. 2 Qingtian with significant difference from No. 1 Qingtian treated with Co-60 radiation, above species were planted in the same condition) with 100 mM Tris-Cl buffer (pH 8.0) containing 5 mM EDTA, 2% β-mercaptoethanol and 1 mM Phenyl Methyl Sulphonyl Fluoride (PMSF). The crude homogenate was kept for 45 min at 4°C and centrifuged at 11,000 rpm for 15 min. The extracted proteins were recovered as clear supernatant. Sample buffer (30 μL) was added to 30 μL of clear supernatant and mixed thoroughly in Eppendorf tube. The sample buffer (pH 6.8) contained the following final concentrations: 125 mM Tris, 20% glycerol, 10% β-mercaptoethanol, 0.01% Bromphenol Blue (BPB), 4% SDS. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to watch the movement of protein in the gel, the sample was boiled for 3 min before loading gel.

Total protein was analyzed through slab type SDS-PAGE using 5% upper gel and 15% lower gel. The SDS-PAGE of total protein was carried out in the discontinuous buffer system according to the method of Laemmili (1970) with a few modifications. The pH of lower gel was changed from 8.8 to 9.0 and the pH of upper gel was changed from 6.8 to 7.0 mL. In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. Electrophoresis was carried out at a constant voltage of 100 V for 9 h. The gels were stained for 3 h in 0.1% (w/v) coomassie brilliant blue R-250 followed by destaining in ethanol : water : glacial acetic acid (1 :17 :2). Protein bands were visnalized in a Tran illuminator under white light. Depending upon the presence or absence of polypeptide bands, differences between species was diagnosed for all possible pairs of protein types.

RESULTS AND DISCUSSION

The *Stevia rebaudiana* protein profiles were composed of approximately 25 bands. Most of the variation was observed in areas with mol in Fig. 1. The number of bands and position and definition of protein were different. Distinct quantitative variations were observed in the SDS-PAGE profile of total proteins of *Stevia rebaudiana*. The SDS-PAGE profile of total proteins of *Stevia rebaudiana* showed significant difference should be use for identification of variety.

This method has the advantages of simplicity, high sensitivity and good accuracy and this experiment proved that SDS-PAGE was a practical and reliable method for *Stevia rebaudiana* identification. It takes only 20 h from protein extraction to getting inspection reports and the SDS-PAGE is a fast and effective tool for differentiating *Stevia rebaudiana* Bertoni varieties based on the differences of protein components. Whereas a low level of inter-specific genetic diversity was observed

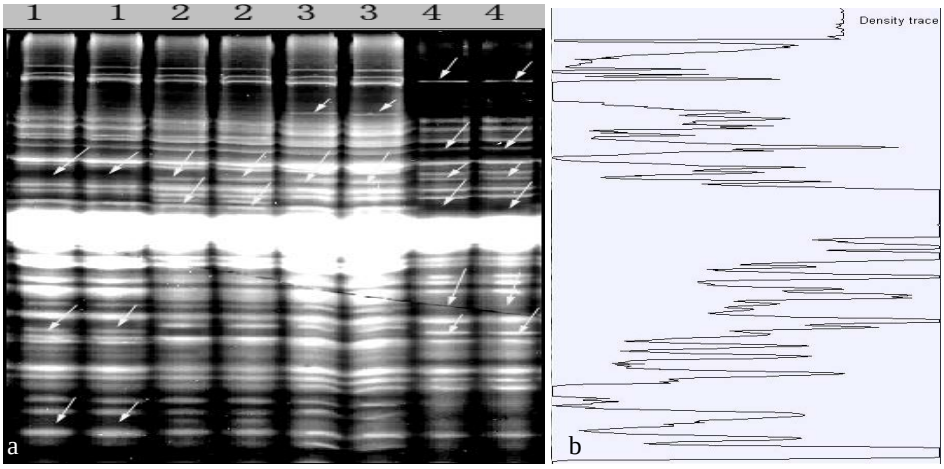

Fig. 1: (a) Variation in proteins of *Stevia rebaudiana*. The arrows indicate variation in different regions. 1: No. 2 Qingtian, 2: No. 1 Qingtian, 3: No. 2 Shoutian, 4: No. 3 Xingguang and (b) Fig. 1a was analyzed with the software of Quantity One and every peak responding a band and every peak corresponds with one band

for both the species and no clear differentiation was observed either for agronomic characteristics or their origin as various clusters consisted of mixed genotypes from different origins.

REFERENCES

Abdul, G., Z. Ahmad, Q. Afsari Sharif and M. Bashir, 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and V. *Radiata* (L.) R. Wilczek based on morphological traits and SDS-page. Euphytica, 123: 367-378.

Ahmad, F. and A.E. Slinkard, 1992. Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor. Applied Genet., 84: 688-692.

Ahmad, M., D.L. McNeil and A.G. Fautrier, 1996. Genetic relationships in *Lens* species and parentage determination of their interspecific hybrids using RAPD markers. Theor. Applied Genet., 92: 1091-1098.

Cardello, H.M.A.B., M.A.P.A. Da Silva and M.H. Damasio, 1999. Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Foods Hum. Nutr. (Formerly Qualitas Plantarum), 54: 119-130.

Gepts, P., 1989. Genetic Diversity of Seed Storage Proteins in Plants. In: Plant Population Genetics, Breeding and Genetic Resources, Brown, A.H.D., M.T. Clegg, A.L. Kahler and B.S. Weir (Eds.). Sinauer Associates Inc., Sunderland, Massachusetts, pp: 64-82.

Laemmili, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T_4. Nature, 227: 680-685.

Motomu, A., S. Takeo, K. Yoko and K. Michio, 1994. Mass propagation of shoots of *Stevia rebaudiana* using a large scale bioreactor. Plant Cell Reports, 13: 180-183.

Tianhong, C. and Z. Yang, 1999. Enrichment and separation of rebaudioside a from stevia glycosides by a novel adsorbent with pyridyl group. Sci. China ser. B, Chemis., 42: 277-282.

Yukiyoshi, T., N. Shigeharu, F. Hiroshi and T. Mamoru, 1984. Clonal propagation of *Stevia rebaudiana* bertoni by stem-tip culture. Plant Cell Reports, 10: 183-185.