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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1424.1430

Research Article
Comparative Evaluation of Nutritional and Mineral Composition Between Transgenic Sugarcane Overexpressing *SoSPS* 1 Gene and Non-transgenic Counterpart

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Abstract

Background and Objective: The high sucrose yield of transgenic sugarcane has been developed through the overexpression of gene for sucrose-phosphate synthase. Modification of the genome may result in alteration of biochemical profiles. This study was conducted to compare and evaluate the nutritional and mineral compositions between the transgenic and non-transgenic (NT) sugarcane counterpart. **Materials and Methods:** Four of transgenic lines with overexpressing *SoSPS* 1 gene and NT sugarcane were grown in greenhouse for 11 months. The nutritional and mineral compositions from leaves and stems were analyzed at the harvest. **Results:** Results revealed no significant differences in moisture, carbohydrates, crude fat and ash content between the transgenic lines and NT sugarcane. Protein and nitrogen contents were found to be significantly greater in steam of transgenic lines SP1 and SP3, including potassium content in both of the leaves and stems of transgenic lines. Although, the nutritional and mineral compositions were varied but their contents still within the range of Organization for Economic Co-operation and Development (OECD) reference values. **Conclusion:** The results indicated that the nutritional and mineral compositions are substantially equivalent between transgenic and NT sugarcane.

Key words: Transgenic sugarcane, sucrose phosphate synthase, nutritional and mineral, substantial equivalence

Citation: A Bagus Nur Sudrajat, Suherman and Bambang Sugiharto, 2020. Comparative evaluation of nutritional and mineral composition between transgenic sugarcane overexpressing *SoSPS* 1 gene and non-transgenic counterpart. Pak. J. Biol. Sci., 23: 1424-1430.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugarcane is an important crop across the world, from which 1800 million tons of sugar are produced each year. Hence, sugarcane is the source of 80% of the sugar used worldwide[1]. In Indonesia, nationwide sugar production does not meet demand. Over 50% of the current sugar demand in Indonesia is met through imports from other countries[2].

The method under consideration for increased sugar production is the development of a high sucrose sugarcane cultivar through cross-breeding and genetic engineering. It is well known that Sucrose-Phosphate Synthase (SPS) is a key enzyme for sucrose synthesis, thus determining sucrose content in plants[3]. The overexpression of SPS was found to increase SPS activity as well as the sucrose content in transgenic tomato fruits[4] and tobacco plants[5]. The *SoSPS* 1 gene for the photosynthetic SPS has been isolated from sugarcane[6] and overexpression of the gene has been observed to increase SPS activity, sucrose accumulation and biomass production in transgenic sugarcane[7].

Genetic engineering can solve nutrition problems through increasing the mineral content and yield of crops. This is because modification of the plant genome may result in alterations in gene expression and biochemical profiles, which may lead to changes in the nutritional composition of plants[8,9]. Therefore, transgenic plants produced through genetic engineering must be considered to be substantially equivalent compared with non-transgenic (NT) counterpart[9]. The substantial equivalence is used to indicate similarities in nutritional characteristics between transgenic and non-transgenic crops[10]. Compositional analyses of transgenic crops are important not only for the substantial equivalence, but also for conducting nutritional and toxicological comparison[11].

The Food and Agriculture Organization (FAO) and World Health Organization (WHO) mandated in 2000 that food derived from genetically-modified products must be evaluated for safety through a comparison with their conventional counterpart. The Organization for Economic Co-operation and Development (OECD) has reported that substantial equivalence requires the evaluation of food components derived from modern biotechnology. The evaluation deemed by substantial equivalence includes an analysis of macro- and micro-nutrient contents[12]. As part of the requirements of substantial equivalence, an analysis of the nutritional composition of a product must be conducted to determine whether the inclusion of modified genes in transgenic plants affects the nutritional value of the food[13,14].

This study was conducted to evaluate the nutritional and mineral composition of transgenic sugarcane which overexpressing the *SoSPS* 1 gene and its counterpart NT sugarcane. The results showed that the transgenic sugarcane lines have similarities to the non-transgenic counterpart in terms of nutritional and mineral composition.

MATERIALS AND METHODS

Study area: This research was conducted at the Laboratory of Molecular Biology and Biotechnology and greenhouse, Center for Development of Advanced Sciences and Technology (CDAST), University of Jember, Indonesia from July, 2018-May, 2019.

Plant materials: Four lines of transgenic sugarcane with overexpressing *SoSPS* 1 gene were used as plant materials[7]. The transgenic lines and the NT sugarcane counterpart were grown in pots containing 25 kg soil mixture of soil: sand: organic matter (50 : 25 : 25) for 11 months. The experiment was conducted with three replications. Fully-expanded leaves and stems were collected during harvest and used for nutritional and mineral composition analysis. The collected leaves were cut into pieces 2 cm in length and dried in oven incubator at 60°C for 72 h, then stored in 4°C for analysis. The harvested stems were crushed and resulted sugarcane juices were collected and stored in the freezer at -20°C. Separated bagasse was dried in the oven incubator at 60°C for 72 h and powdered with a warring blender.

Polymerase Chain Reaction (PCR) analysis: Genomic DNA was isolated from 2 g sugarcane leaves according to the method previously described[7]. PCR analysis was conducted to confirm the presence of the inserted transgene using the genomic DNA and a set of primers for the *npt* II gene[15]. The DNA resulted from PCR analysis was separated using 1% agarose gel electrophoresis and documented with GelDoc (Major Science, California, USA).

Proximate analysis

Moisture content: Moisture content in the freshly harvested leaves, stems and juice of sugarcane were determined using an automatic infrared moisture analyzer[16]. The weight of sugarcane leaves, stem and juice of 0.5 g each were placed on the moisture analyzer (Ohaus MB200, Melrose, USA) set to 105°C and allowed to warm for 20 min. Once tissues were dried to a constant weight as determined by programmed analysis parameters, the analysis was automatically terminated. Moisture content was recorded and presented as percent of the materials weight.

Protein content: Crude protein content in the leaves, bagasse and sugarcane juice were determined according to the Kjeldahl method. One g of each material was digested with 2.5 mL H_2SO_4 in the presence of 0.1 g selenium for 5 h at 375°C, then left to cool for 20 min at room temperature. Digested materials were distilled in a Kjeldahl distillation chamber (Buchi Kjelflex K-360, Flawil, Switzerland) with 50 mL of 30% NaOH and then evaporated ammonia was collected in 3% of boric acid solution. The ammonia content was measured by titration with 0.01 N HCl and the amount of titrated HCl was recorded to be used for the calculation for protein content. Crude protein content was calculated from nitrogen content multiplied by a factor of 6.25 N[17].

Crude fat content: The crude fat content in the leaves, bagasse and sugarcane juice were analyzed according to the Soxhlet extraction method[18]. Two g of each material was placed in an extraction tube containing 100 mL n-hexane. The tube was incubated at 100°C for 5 h, at which point the evaporate solvent was recovered using a reflux condenser. Extraction residue in the tube was dried in a hot-air oven for overnight at 105°C and then weighed. After drying the crude fat content was expressed as the weight of the residue in the extraction tube.

Ash content: Ash analysis was conducted by placing 2 g of leaves, bagasse, or sugarcane juice into a crucible muffle furnace and then weighed. The muffle furnaces were heated to 550°C for 8 h (Prep Ash® 340 Series Precisa, Dietikon, Swiss) and the resulting ash was cooled in a desiccator and then weighed. Ash content was calculated as the percent of loss in weight after heating[19].

Crude fiber content: Two g of dried sugarcane leaves or bagasse was sliced using Waring blender and digested using 50 mL of 1.25% H_2SO_4, then heated at 95°C for 30 min. Undigested material was filtered over a Buchner funnel and rinsed with hot water to remove the acidity. The residue was digested again using 50 mL of 1.25% NaOH, heated at 95°C for 30 min and rinsed with hot water. The residue was transferred into a crucible and dried in an oven at 105°C for overnight, then ashed in a muffle furnace at 550°C for 6 h (Prep Ash® 340 Series Precisa, Dietikon, Swiss). After cooling in the desiccator to room temperature, the residue was weighed. The crude fiber content was calculated as the percent loss in weight[17].

Total carbohydrate: Total carbohydrate content was calculated by difference, rather than through direct analysis. The constituents such as protein, crude fat, water, ash was determined individually, summed and subtracted from the total materials. The total carbohydrate was then calculated through the following equation[20]:

$$\text{Carbohydrate (\%): } 100-(\text{Protein (\%)}+\text{fat (\%)}+\text{ash (\%)})$$

Mineral content: Approximately 2 g of sugarcane leaves was weighed and ignited in a muffle furnace (Prep Ash® 340 Series Precisa, Swiss) at 550-600°C for 10 h. The ashed tissue was dissolved with 5 mL of 6 M HCl, filtered over a Buchner funnel and then diluted with distilled water until a volume of 50 mL was reached. Potassium (K), magnesium (Mg) and calcium (Ca), content was measured using an Atomic Absorption Spectrophotometer (AAS; ZA3300, Hitachi, Tokyo, Japan) according to the protocol established by Jiang *et al.*[21]. Phosphorus (P) content was determined by spectrophotometric methods. One mL of the extractant was added by 2.5 mL of the molybdate-vanadate reagent and then diluted by distillate water until 10 mL. After settling for 15 min the absorbance was measured with a spectrophotometer (Hitachi, Tokyo, Japan) at 400 nm.

Statistical analysis: All statistical analyses were performed in triplicate and represented as the mean±standard errors. Statistical significance in the data was calculated using an unpaired student t-test method with SPSS 22 software. A p-value of 0.05 was considered for determining statistical significance.

RESULTS

Detection of transgene by PCR: To confirm the authenticity of transgenic sugarcane lines, PCR analysis was performed using specific primers for *npt* II DNA rather than *SPS* gene since the interference of the endogenous gene. PCR analyses revealed that the corresponding *npt* II DNA, with molecular size approximately 550 bp was amplified in the leaf's genome DNA of the transgenic lines, but not in the NT sugarcane (Fig. 1). These results showed that the experiment used the transgenic sugarcane overexpressing of *SoSPS* 1 gene.

Proximate composition: A comparative evaluation of macro- and micro-nutrient contents between transgenic and non-transgenic plants is an important way by which the needs

Table 1: Proximate compositions in leaves of transgenic lines and NT sugarcane counterpart

Components (%)	NT	SP 1	SP 3	SP 7	SP 9	Reference range
Moisture	66.25±1.57	66.43±0.70	68.15±0.50	66.32±1.76	65.95±0.10	68.7-73.1
Protein	6.75±0.09	7.06±0.13	7.02±0.05	6.84±0.07	6.83±0.02	4.0-6.2
Carbohydrate	24.24±1.72	24.68±0.51	23.60±0.78	25.29±1.53	24.54±1.02	17.7-25.3
Fat	1.58±0.52	1.42±0.29	1.42±0.78	1.33±0.14	1.67±0.52	0.8-1.7
Ash	8.27±0.13	8.57±0.04	8.58±0.12	8.05±0.07	8.02±0.07	5.9-9.2
Fibre	40.83±0.76	42.17±0.76	42.15±0.41	41.93±0.93	41.53±0.50	30.9-36.3

Values are means±SD from three independent plants, there was no significant difference in proximate composition between transgenic lines and NT sugarcane, reference range is the range from the consensus document on compositional considerations for new varieties of sugarcane in OECD

Table 2: Proximate compositions in stems of transgenic lines and NT sugarcane

Components (%)	NT	SP 1	SP 3	SP 7	SP 9	Reference range
Moisture	78.85±0.45	80.12±1.08	79.60±1.51	78.32±1.13	79.87±1.09	60-65
Protein	0.17±0.02	0.24±0.02*	0.28±0.02*	0.19±0.02	0.20±0.03	0.5-0.6
Carbohydrate	19.87±0.86	18.07±1.34	18.63±1.41	20.08±0.18	18.44±1.13	19.98-48.7
Fat	0.58±0.14	0.67±0.29	0.58±0.14	0.58±0.14	0.67±0.29	0.07-1.7
Ash	0.91±0.14	0.91±0.14	0.91±0.14	0.83±0.14	0.82±0.14	1.0-3.9
Fibre	40.17±2.75	42.17±0.58	42.00±1.00	44.50±1.80	48.00±3.60	43.0-58.5

Values are means±SD from three independent plants, *denote significant different of protein content from NT sugarcane ($p \leq 0.05$), reference range is the range as described in legend of Table 1

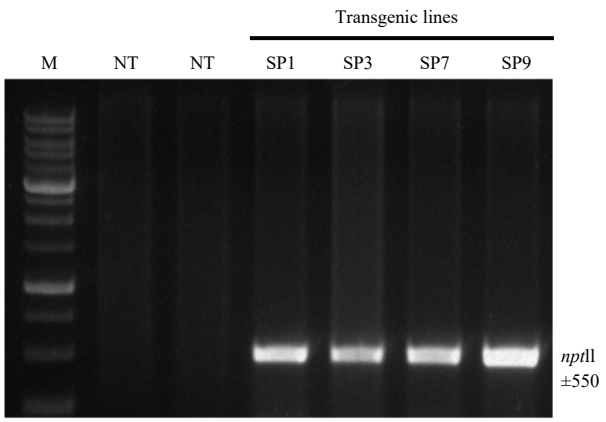

Fig. 1: PCR amplification of *npt* II gene from genomic DNA of transgenic lines and NT sugarcane

Genomic DNA was isolated from leaves of one-month grown sugarcane, PCR was conducted using the genomic DNA and a set of F1-R1 primers, the amplified DNA were separated in agarose gel electrophoresis (1%) and visualized with GelDoc. M: DNA molecular size marker (1 Kb DNA ladder), NT: Non-transgenic

of substantial equivalence should be analyzed. Upon measurements, there were no significant difference between the crude protein, carbohydrate, crude fat, ash and moisture contents of transgenic and NT sugarcane leaves (Table 1). The crude protein, carbohydrate, crude fat and ash contents were still within the range of OECD reference. Moreover, the fiber content in leaves was measured to be slightly increased in the transgenic lines when compared to the NT counterpart, likely due to the increase of sucrose content allocated to the sugarcane biomass.

Between transgenic and NT sugarcane, there was no significant difference in carbohydrate, crude fat, ash and moisture content in the stems (Table 2). However, the level of crude protein was found to be significantly higher in SP1 and SP3 in the stems of transgenic lines. The crude protein content of SP1 and SP3 lines were increased approximately by 1.5-fold compared to the level of NT sugarcane. The overexpression of *SoSPS* 1 gene driven by conservative promoter 35S has resulted in the increased protein content in stems. Generally, mostly proteins are located in leaves, therefore the overexpression of *SPS* gene was less visible in protein contents when compared to that of the stem. Moreover, the fiber content in stem of the transgenic lines was also found to be slightly higher when compared to that of NT sugarcane (Table 2).

The nutritional composition of sugarcane juice extracted from stems was also measured. There were no significant differences found in moisture, crude protein, carbohydrate, crude fat and ash content between transgenic lines and the NT counterpart (Table 3). The results showed that contents of proximate in sugarcane juice are in the range of OECD reference.

Mineral composition analysis: Mineral measurement revealed no significant differences in the N, P, Ca, or Mg content in leaves of transgenic lines and NT sugarcane. However, the K content was found to be significantly higher by 1.4-fold in the leaves of transgenic lines of SP1 and SP3 (Table 4).

The measurement of K content in the stem of SP1 and SP3 lines were significantly increased by 2.7-fold compared to the level of NT sugarcane. The increased levels of K content were

Table 3: Proximate compositions in juice extracted from stems of transgenic lines and NT sugarcane

Components (%)	NT	SP 1	SP 3	SP 7	SP 9	Reference range
Moisture	81.68±1.13	82.17±0.66	82.12±0.33	81.87±0.94	82.18±0.59	76.00-85
Protein	0.12±0.02	0.13±0.01	0.11±0.01	0.11±0.02	0.10±0.03	0.19-0.5
Carbohydrate	17.52±1.26	16.92±0.64	17.10±0.40	17.34±0.81	16.94±0.52	10.17-13.69
Fat	0.11±0.03	0.11±0.02	0.10±0.01	0.10±0.01	0.11±0.02	0.14-0.15
Ash	0.58±0.14	0.67±0.14	0.58±0.14	0.58±0.14	0.67±0.14	0.90-4.8

Values are means±SD from three independent plants, there was no significant difference between transgenic lines and NT sugarcane, reference range is the range as described in legend of Table 1

Table 4: Mineral contents in leave of transgenic lines and NT sugarcane

Components (%)	NT	SP 1	SP 3	SP 7	SP 9	Reference range
Nitrogen	1.20±0.04	1.25±0.04	1.25±0.03	1.20±0.03	1.20±0.02	1.60-1.9
Phosphorus	0.22±0.01	0.22±0.03	0.23±0.02	0.23±0.02	0.24±0.02	0.22-0.3
Potassium	1.46±0.06	1.97±0.18*	1.96±0.05*	1.61±0.13	1.59±0.05	1.20-2.3
Calcium	1.49±0.03	1.31±0.13	1.33±0.04	1.44±0.13	1.43±0.06	0.94-1.3
Magnesium	2.77±0.16	2.33±0.27	2.32±0.09	2.67±0.18	2.60±0.13	1.90-3.1

Values are means±SD from three independent plants, *denote significant different of potassium content from NT sugarcane ($p \leq 0.05$), reference range is the range as described in legend of Table 1

Table 5: Mineral content in stem of transgenic lines and NT sugarcane

Components (%)	NT	SP 1	SP 3	SP 7	SP 9	Reference range
Nitrogen	0.13±0.01	0.20±0.03*	0.20±0.02*	0.16±0.01	0.15±0.01	0.10-0.47
Phosphorus	0.93±0.16	0.86±0.10	0.89±0.04	0.96±0.24	0.89±0.14	0.70-0.98
Potassium	0.24±0.06	0.64±0.05*	0.66±0.06*	0.23±0.07	0.23±0.05	0.41-0.64
Calcium	0.29±0.05	0.32±0.07	0.31±0.04	0.26±0.02	0.25±0.02	0.16-0.28
Magnesium	0.40±0.02	0.41±0.02	0.42±0.02	0.42±0.08	0.38±0.02	0.40-0.60

Values are means±SD from three independent plants, *denote significant different of nitrogen and potassium content from NT sugarcane ($p \leq 0.05$), reference range is the range as described in legend of Table 1

found to be accompanied with higher N levels in the stem of transgenic lines SP1 and SP3 (Table 5). Interaction between N and K may an important aspect for higher biomass production of the transgenic sugarcane. In addition, higher N levels were found to be observably correlated with higher protein contents in the stems (Table 2). Although the N and K contents were found to have statistically-significant variations, all mineral contents were within the OECD reference range.

DISCUSSION

It is important to compare genetically modified plants with their traditional counterparts because product quality must be food-grade. In this study, the transgenic lines were evaluated for their substantial equivalent with the NT sugarcane counterpart. The results showed that although there were statistical differences in protein and mineral contents of the transgenic lines and NT sugarcane, but the variations were still found to be within the range of OECD reference[22]. Similar results were found in transgenic corn where protein, fat, fiber and fatty acids were found to be significantly higher[23]. It was also reported that the transgenic sugarcane overexpressing *SoSPS* 1 gene does not possess characteristics associated with allergenicity and toxicity[24]. These results indicated a substantial equivalent between transgenic lines and its NT counterpart.

Overexpression in the *SPS* gene has been found to result in increased sucrose and biomass production in transgenic sugarcane[7]. The higher of sucrose contents, the greater the activity of sucrose-degrading enzymes, whose activity is to produce hexoses. Subsequently, there is a greater allocation of the hexose to cellulose contents and plant growth[25]. Thus, the overexpression of SPS increased fiber content in leaves of SP1 and SP3 transgenic lines (Table 1 and 2). Similar results have also reported that overexpression of the *SPS* gene results in increased fiber content in transgenic cotton[5,26]. The increased fiber content in the transgenic lines were within the of OECD reference and that the fiber is not essential nutrition in the sugarcane industry.

Potassium (K) is a major macronutrient that contributes to an increase in cell turgor pressure during fiber elongation and plant growth. Studies on cotton fibers have shown that K modulates the fiber elongation rate[27,28]. The higher content of K (Table 4 and 5) may have resulted as a consequence of the increased fiber content (Table 1 and 2). Potassium content is involved in carbohydrate metabolism and that K deficiency leads to decrease the carbohydrate metabolism and plant growth. Photosynthesis and sugars have been shown to play an important role in regulating root mineral uptake[29,30]. The change of sugar levels regulates minerals absorption in plants. Thus, it is not surprisingly that K contents in transgenic lines were higher compared to NT sugarcane.

CONCLUSION

The comparison of nutritional and mineral composition in transgenic lines and NT sugarcane showed that there are similarities between them. Although there were variations in fiber, protein and mineral content, they were found to be within the range of OECD reference. The results of this study indicate that transgenic lines are compositionally equivalent to NT sugarcane.

SIGNIFICANCE STATEMENT

This study discovered that the transgenic sugarcane lines are compositionally equivalent to non-transgenic sugarcane counterpart. This proves that the inclusion of *SoSPS* 1 gene has no effect on the nutritional value of transgenic sugarcane. The finding can be beneficial to food safety assessment of the transgenic sugarcane. In addition, this study will help the researchers to uncover the critical areas for development and commercialization of transgenic sugarcane.

ACKNOWLEDGMENTS

This work was supported by the Indonesian Ministry of Research, Technology and Higher Education: grant number 1826/UN25.3.1/LT/2019 (PUSNAS).

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