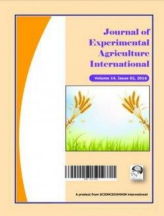

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Proteomic Analysis Reveals Differentially Accumulated Proteins in Banana Somaclonal Variants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KGL, LSF, AVS and TBC designed the study and performed the statistical analysis. Authors KGL and DEL wrote the first draft of the manuscript and managed the literature searches. Author LVP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To compare the protein profile of leaves from normal and giant banana plants to identify differentially accumulated proteins specific to each type.
Place and Duration of Study: Central Molecular Biology Lab (LCBM), between April and November of 2017.
Methodology: Protein samples were analyzed in triplicate by two-dimensional gel electrophoresis. The gels were stained with Coomassie Blue G250 and evaluated by the Image Master 2D Platinum

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5.0 software. Differentially abundant proteins were treated with trypsin enzyme, and aliquots of each hydrolysate sample were analyzed by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometer. The molecular mass profile of each sample was subjected to comparative analysis using the MASCOT program and the NCBI database.
Results: Of the 36 differentially abundant proteins that were analyzed, 21 were identified as being related to root metabolism, photosynthesis, protein translation, and carbon and nitrogen fixation. Notably, glutamine synthetase was more abundant in the leaves of the giant banana plants.
Conclusion: This pioneering work used proteomic analysis to identify differentially abundant proteins in the leaves of normal and giant banana plants. Glutamine synthetase was revealed as a potential molecular marker that could be used to screen out plants of undesired variants. These results highlight some of the biochemical alterations that occur in banana trees with gigantism, providing a basis for future research on micropropagation programs and biofactories.

Keywords: Banana; somaclonal variation; bidimensional electrophoresis; mass spectrometry; proteomic profile.

1. INTRODUCTION

Banana (*Musa* spp.) is one of the world's most important economic and social crops, with cultivation occurring in more than 125 countries. Globally, banana ranks as one of the most popular fruits, with 106.5 million tons produced per year [1,2]. Brazil cultivates more than 474 thousand hectares of banana plants and produces seven million tons of the fruit, placing it among the five largest producers in the world [3].

Conventional field propagation relies on transplanting banana suckers that have been severed from existing plants. Plant production of banana suckers is seasonally dependent, limiting the amount of available planting material [4]. Conventional propagation also risks spreading pathogenic agents from the field plant donors.

In Brazil, micropropagation has increasingly been used to meet demand [5]. Micropropagation ensures the production of plants that are free of pests and disease, preventing dissemination of potential pathogens [6]. Nevertheless, *in vitro* propagation can lead to the occurrence of somaclonal variations, caused either by genetic or epigenetic modifications [7]. In Cavendish banana cultivars, for example, the occurrence of off-types in tissue-cultured plantlets can range from 6 to 38% [4].

In the banana cultivar Prata Anã (AAB), the most frequent somaclonal variation is characterized by plant overgrowth and the production of bunches with low economical value, known as giant plants [8].

Since these morphological characteristics can only be identified in adult variant plants, molecular markers are needed to facilitate early detection.

Guimarães et al. [9] worked with somaclonal variants of gigantism in the Prata Anã cultivar and concluded that markers for simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD) were unable to detect variants. Chromosome counting was similarly ineffective. In other research, RAPD primers were used to identify a variegated plant in a micropropagated banana cultivar Grand Naine (AAA). However, other variations such as giant plants were grouped as normal plants [10].

Proteins play a central role in biological processes, and proteomic differential analysis can be used to determine which proteins are affected by genetic variation during plant growth and development [11]. The identification of up- and down-regulated proteins related to gigantism could provide useful information on the biochemical processes related to this type of somaclonal variation. These proteins could be used as molecular markers for the early identification of undesired somaclonal variants so that these off-type plants could be excluded from the field.

Recent research has used proteomic profiles to evaluate banana protein expression under various conditions. In the case of the banana cultivar Grand Naine, it was possible to correlate the induction of somatic embryogenesis with increases in a stress-related cationic peroxidase and plant growth hormone-related proteins such as indole-3-pyruvate monooxygenase and adenylate isopentenyl transferase [12].

Many researchers have used the proteomic approach to identify and track changes in proteins involved in growth mechanisms. Two-dimensional gel electrophoresis (2-DE) and mass spectrometry have been used to identify proteins at different ripening and climacteric stages in bananas and other fruits, such as papaya and apple [13,14]. One study compared banana pulp at pre-climacteric and climacteric stages [15]. Chitinases were the most abundant type of protein in unripe banana while three heat shock proteins and isoflavone reductase were abundant at the climacteric stage [15].

This study identified differently abundant proteins on banana leaves with and without the giant plant somaclonal variation using 2-DE and matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) tandem mass spectrometry.

2. MATERIALS AND METHODS

2.1 Plant Material

Foliage leaves from both normal and giant plants of *Musa* spp. cultivar Prata Anã (AAB) were provided by a private property located in Lavras, MG, Brazil (Fig. 1).

All plants were 18 months old. The giant plants were selected according to morphological parameters, such as lanceolate and more upright leaves, larger size, and thinner pseudostems. All samples were obtained from the same clone established through an *in vitro* propagation protocol conducted by BIOCELL Ltda (Sete Lagoas, MG, Brazil).

2.2 Banana Leaf Protein Extraction

Total protein extracts were prepared from the leaves of both normal and giant plants. Triplicate preparations of 200 mg each were ground to a fine powder with a mortar and pestle in combination with liquid nitrogen, following the method described by Gallardo et al. [16]. Approximately 150 mg of powder was used for protein extraction at 4°C in 660 µL of a buffer containing: 7 M urea, 2 M thiourea, 4% (w / v) CHAPS, 18 mM Tris-HCl, 14 mM Trizma base, a protease inhibitor cocktail (GE Healthcare), 53 units mL^{-1} DNase I (Sigma), 4.9 Kunitz units mL^{-1} RNase A (Sigma), and 0.2% (v / v) Triton X-100. After ten minutes, 14 mM dithiothreitol (DTT) was added. The protein extracts were stirred for 20 minutes and then centrifuged (35,000 g for ten minutes) at 4°C. The supernatant was submitted to a second centrifugation as above. The final supernatant, corresponding to the total protein extract, was stored at -20°C.

Protein concentrations were measured according to the Bradford procedure (1976) [17] by applying the GeneQuant unit (GE-Healthcare) and using bovine serum albumin as a standard. The extracted protein samples were loaded onto a sodium dodecyl sulfate (SDS)-polyacrylamide gel and dyed with Coomassie G-250 blue.

Fig. 1. Normal and giant banana plants. The arrow highlights the giant plant

2.3 Protein Separation by 2-DE

The Multiphor II (GE Healthcare) system was used for 2-DE following the manufacturer's instructions. Protein samples of 250 mg each were loaded onto 18 cm immobilized pH gradient (IPG) strips (linear pH gradient of 3–10; GE Healthcare). The Multiphor II (GE Healthcare) was used under the following conditions: a total of 55,000 Voltage-hours at 15°C; gradient 150 V for one minute; gradient 150 V for three hours; gradient 500 V for three hours; gradient 3500 V for five hours; gradient 3500 V for 12 hours; maximum current setting of 50 µA per strip. Next, for the first-dimension isoelectric focusing step, the IPG strips were equilibrated for 20 minutes on a buffer (100 mM Tris–HCl pH 8.8, 6 M urea, 2% SDS, 30% glycerol, and 0.2 mg / mL bromophenol blue) containing 5 mg / mL DTT (to reduce the proteins). Then, the strips were equilibrated for another 20 minutes in a similar buffer that contained 25 mg / mL of iodoacetamide (to alkylate proteins) instead of DTT. The second dimension SDS-polyacrylamide gel electrophoresis (PAGE) was performed at 15°C with 12% resolving gels (GE Healthcare) using the Multiphor II apparatus (GE Healthcare) under the following conditions: Step-n-hold 600 V for 30 minutes and 600 V for 2 hours (programmed as step and hold) or until the bromophenol blue dye front had run off the gel. The low and high molecular weight protein markers (SDS Calibration Kit) for SDS electrophoresis (GE Healthcare) were used for the molecular mass determinations. At least three replicate gels were run for each sample.

Following electrophoresis, the gels were stained with Coomassie blue G-250 solution for 48 hours and then stored in an ammonium sulfate 20% (p / v) solution, according to Neuhoff, Arold, Taube & Ehrhardt [18].

2.4 Image Analysis

The stained two-dimensional gels were digitalized at high resolution using a scanner (ImageScanner, GE-Healthcare) and processed with the Ulmax MagicScan 4.6 software. Image analysis was performed using the ImageMaster 2D Platinum 5.0 (GE-Healthcare) software. To evaluate the differences in protein abundance across different 2-DE gels, the normalized protein spot volume (spot area multiplied by its intensity, as measured by optical density) was applied as a parameter. The relative spot volume (% Vol) represents the ratio of a given spot volume ($\mathrm{Vol_s}$) to the sum of all spot volumes detected in the gel with *n* spots ($\%\mathrm{Vol} = \frac{\mathrm{Vol}}{\sum_{S=1}^{n}(\mathrm{Vol_s})} \mathrm{x}\ 100$). Protein spots with significant and reproducible changes were considered as being differentially expressed proteins. The normalized volumes of the spots from replicate gels were subjected to a student's ANOVA test (*P = .05*) and only statistically significant data, with a difference of at least 1.2 X between treatments was considered.

2.5 In-gel Protein Digestion

Spots with differential expression were excised, and the gel fragments were washed in 25 mM ammonium bicarbonate and 50% acetonitrile until completely de-stained. After drying, gel fragments were placed in 50 µL protease solution [20 ng/µL sequence grade modified trypsin (Promega Biosciences, CA, USA) in 25 mM ammonium bicarbonate] on ice for 30 min. Excess protease solution was then removed and replaced with 20 µL 25 mM ammonium bicarbonate. Digestion was performed at 58°C for 30 min. Peptide extraction was performed twice for 15 min with 30 µL 50% acetonitrile and 5% formic acid. Trypsin digests were then concentrated in a SpeedVac (Savant, USA) to a volume of about 10 µL and desalted using a Zip-Tip (C18 resin, P10; Millipore Corporation, Bedford, MA, USA).

2.6 Protein Identification by MALDI-TOF/ TOF

The spectrometric analyses were performed by matrix-assisted laser desorption-time of flight-tandem mass spectrometry (MALDI-TOF-TOF MS) (Autoflex III Smartbeam, Bruker Daltonics, Germany). The analysis was performed by applying 0.5 µL of sample and 0.5 µL of matrix α-cyano-4-hydroxycinnamic acid (CHCA) in a Maldi Target Plate (MTP) *AnchorChip* 800/384.

The spectra generated by mass spectrometry (MS) and tandem mass spectrometry (MS/MS) were obtained in the positive mode. The operation mode for acquisition was linear, with a laser repetition rate of 50 Hz, ion source voltage 1 at 20 kV, ion source voltage 2 at 18.3 kV, ion source lens voltage 6.75 kV and a shot number of 200. The collision-induced dissociation (CID) was used to fragment trypsin peptides in the peptide mass fingerprinting (PMF).

The mass spectrometry results were obtained as the ratio mass/charge for each sample using the software FlexControl 3.0. The analyses of the acquired datasets were performed with the aid of FlexAnalysis 3.0 and Biotools 3.0 software. The apparatus was calibrated by external standards (*Protein Calibration Standard* I and IV - Bruker Daltonics).

The molecular mass profiles of the samples obtained by MS and MS/MS were subject to comparative analysis using the software MASCOT to compare the masses of the observed peptides with a database and consequently identify the most probable proteins in the sample. The database used in the analysis was NCBI (http://www.ncbi.nlm.nih.gov/).

3. RESULTS AND DISCUSSION

Plant tissues contain several substances that can interfere with protein extraction [19] and impact proteomic analysis [20], such as phenolic compounds, proteolytic and oxidative enzymes, terpenes, pigments, organic acids, inhibitor ions, and carbohydrates.

In our study, the protein extraction process resulted in 18 mg / ml of proteins. Protein integrity was verified on SDS-PAGE gels that generated protein profiles with well-defined bands (Fig.2.), demonstrating that our methods extracted the proteins similarly and efficiently.

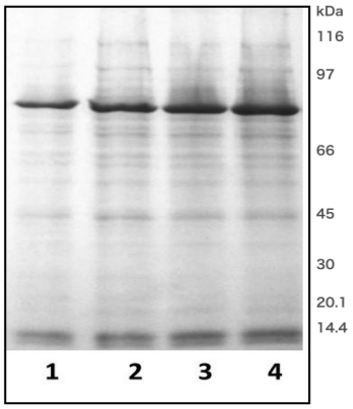

Fig. 2. Protein profile of leaf proteins detectedon SDS-PAGE gels. Protein samples were loaded as follows: 7 µg of protein in well 1; 10 µg in well 2; 15 µg in well 3, and 20 µg in well 4

To analyze the quality of the 2-DE gel results, protein extracts were first separated under isoelectric focalization at a 3–10 pH range. The 2-DE gel presented a large number of individualized proteins (spots) without any horizontal or vertical smearing (Fig. 3), indicating the absence or limited number of potential interfering compounds, such as lipids and carbohydrates.

Using the ImageMaster 2D Platinum 5.0 software (ANOVA, $P = .05$), 224 spots were detected on the normal banana samples, and 267 spots were detected on the giant plant samples. Out of all these spots, 36 represented percent volume differences (% volume = volume occupied by each spot on the gel), with protein abundance varying from 1.2 X to 7.3 X. In this subgroup, 31 spots were more abundant on the normal banana plant, while five were more abundant on the giant banana plant.

MALDI-TOF/TOF tandem mass spectrometry enabled the identification of 21 proteins from the 36 spots excised from the gel (Table 1).

These proteins were related to energy, photosynthesis, translation, and carbon and nitrogen fixation (Fig. 4).

In the samples from the giant banana plants, a large amount of glutamine synthetase (GS) protein was observed. GS is responsible for catalyzing glutamine formation by condensing molecules of glutamate and ammonia [21] with the concomitant hydrolysis of adenosine triphosphate (ATP). GS plays a crucial role in the assimilation and re-assimilation of ammonia derived from a wide variety of metabolic processes during plant growth and development, including photorespiration and nitrogen transport [22]. The amide moiety of glutamine is used to synthesize essential metabolites, such as amino acids, nucleic acids, and amino sugars [23]. Optimal functioning of the metabolic pathways for nitrogen and carbon are critical in plant growth and biomass accumulation [24].

Biochemical studies have identified two GS isoenzymes: cytosolic GS (GS1) and chloroplastic GS (GS2). GS2 is abundantly expressed in the leaf mesophyll, while GS1 is less frequently expressed in the leaves and more concentrated in the phloem [25].

Previous studies have evaluated GS1 expression in tobacco leaves. Overexpression of GS1 led to 70% higher shoots and 100% greater root dry weight as well as a 50% increase in leaf area [26]. Other research presented a large-growth, light-dependent phenotype characterized by increases of fresh weight, dry weight, and soluble leaf proteins. These findings

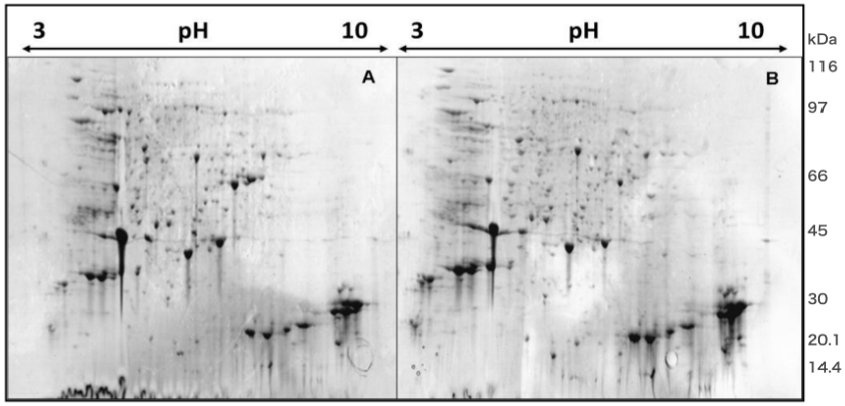

Fig. 3. 2-DE gels for both normal banana leaf (A) and giant plant leaf (B) samples

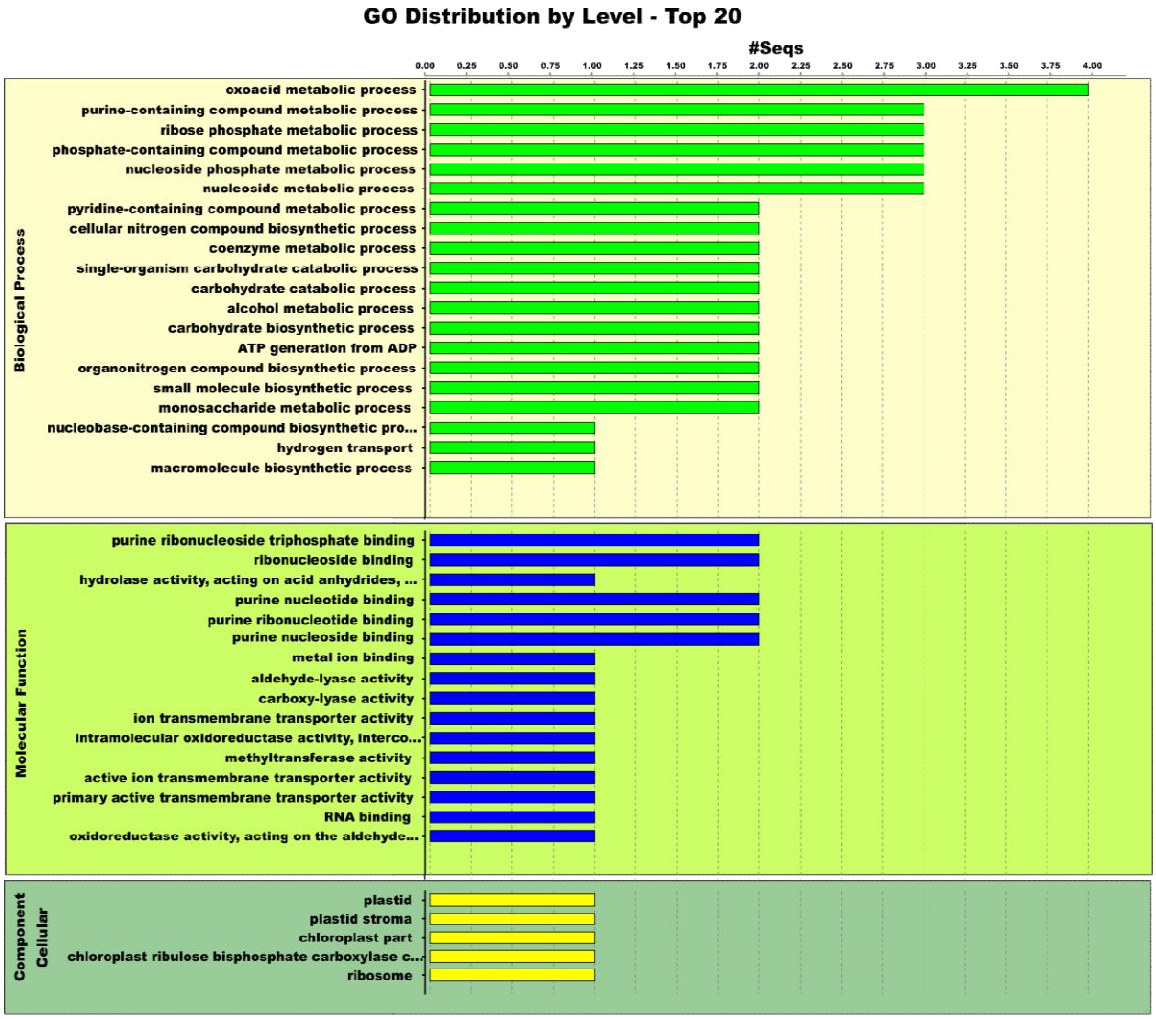

Fig. 4. The Blast2GO results are summarized into three main GO categories: biological process (BP), cellular component (CC), and molecular function (MF). Only the top ten GO terms for each main function category are shown (green: BP; blue: MF; yellow: CC)

demonstrate the importance of this enzyme to the photosynthesis-photorespiration ratio (via ammonia assimilation), as noted by the lower levels of free ammonia, the increase in the ratio of serine to glycine (3.5 times), the higher intermediate photorespiratory levels, and the increases in the levels of glutamine and carbon dioxide [27].

Recent research has correlated higher plant biomass and better growth phenotypes of transgenic *Arabidopsis thaliana* [28] and transgenic *Nicotiana tabacum* [29] with the improved efficiency of nitrogen assimilation due to overexpression of the GS gene.

This data suggests that a greater abundance of GS might be related to the giant banana plant's increased growth. Nevertheless, more studies should be conducted to confirm that GS can be used as a molecular marker for the early identification of giant banana plants.

In our study, several proteins were identified as having higher concentrations in the normal banana plants compared to the giant plants. The normal plants had higher protein concentrations of gliceraldehyde-3-phosphate dehydrogenase (GAPDH), fructose 1,6-biphosphate aldolase (ALDO), triose-phosphate isomerase (TPI), synthase ATP sub-unities, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) sub-unities proteins, THF-dependent enzyme (T-protein), and Elongation factor Tu (EF-Tu).

The RuBisCo protein is an important Calvin cycle enzyme with a carboxylase that catalyzes the incorporation of carbon dioxide into organic forms. Other Calvin cycle enzymes were identified as being abundant in the normal banana plants, including GAPDH, ALDO, and TPI (Fig. 5).

The three latter (GAPDH, ALDO and TPI) proteins also act as glycolytic enzymes in the cytosol [30]. Therefore, these proteins participate both in the metabolic paths to obtain energy through the oxidation of sugars and in the conversion of carbon dioxide to triose-phosphate, which can be converted into glucose, starch, and sucrose. By playing an important role in the synthesis of triose-phosphate, these proteins participate in the creation of compounds that will either be used for the generation of energy or as construction blocks in growing plants.

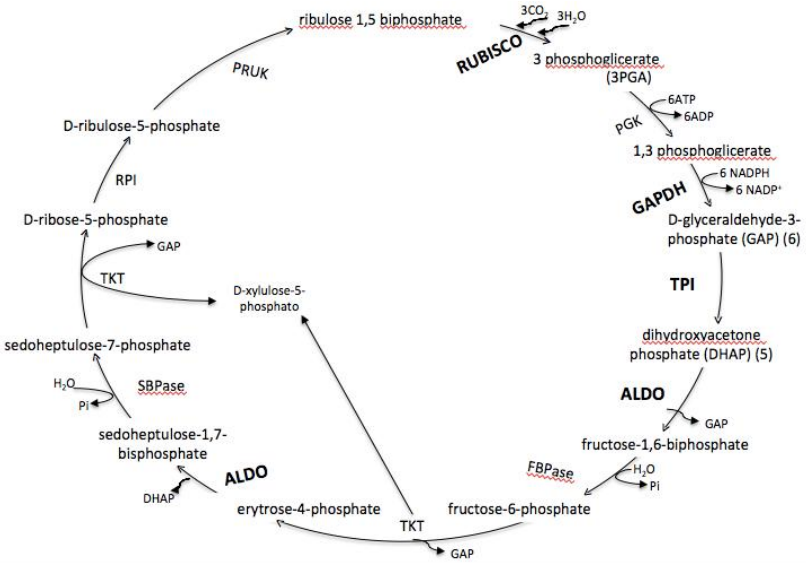

Fig. 5. The Calvin cycle. Abundant proteins in normal banana plants are shown in bold letters. Enzymes encoded by non-differentially expressed genes are shown with normal letters. Abbreviations for enzymes: ALDO: aldolase (fructose-bisphosphate aldolase); FBPase: fructose-1,6-bisphosphatase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PGK: phospho-glycerate kinase; PRUK: phosphoribulokinase; RPE, ribulosephosphate 3-epimerase; RPI: ribose 5-phosphate isomerase; Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase; SBPase: sedoheptulose-1,7-bisphosphatase; TKT: transketolase; TPI: triose-phosphate isomerase

Table 1. Significantly changed protein abundance in banana normal and 'giant Plant'. Proteins identified after analysis in MALDI-ToF/ToF, followed by analysis in the MASCOT program. Mean of protein abundance (% Vol) * in normal banana and "giant plant", with a quantitative increase from 1.2 to 7.3 times and a significant result in the *t-student test* (*P=.05*)

Spot no.	Accession no.	(% Vol)*		Variation of normalised volume	Protein	Score	Peptide sequence
		Normal	Giant plant				
1556	XP_009393673.1	0,09± 0,01	0,158 0,08	1.7X[2]	Glutamine synthase	146	IIAEYIWIGGSGLDLR HETADINTFLWGVANR
1231	NP_084803.1	0,6 ± 0,30	0,21 ± 0,19	3.0X[1]	ATP synthase CF1/alfa	281	ADEISNIIR LIESPAPGIISR ASSVAQVVTTFR IAQIPVSEGYLGR IVNTGTVLQVGDGIAR
1237	NP_084803.1	0,3 ± 0,14	0,17 ± 0,11	2.2X[1]	ATP synthase CF1/alfa	72	EAYPGDVFYLHSR IVNTGTVLQVGDGIAR
1323	NP_084803.1	0,2 ± 0,16	0,17 ± 0,08	1.6X[1]	ATP synthase CF1/alfa	140	IAQIPVSEGYLGR EAYPGDVFYLHSR IVNTGTVLQVGDGIAR
1649	XP_021609330.1	0,3 ± 0,04	0,22 ± 0,08	1.7X[1]	Fructose 1,6 bisphosphate aldolase	102	YAAVSQDNGLVPIVEPEILLDGEHGIDR LASIGLENTEANR
1664	AAV70659.1	0,36± 0,05	0,30 ± 0,04	1.2X[1]	Gliceraldehyde-3-phosphate dehydrogenase	181	VPTVDVSVVDLTVR GILGYVEEDLVSTDFVGDSR
1843	gi|22796842|	1,2 ± 0,15	0,21 ± 0,08	6.0X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	91	VALEACVKAR DNGLLLHIHR
1847	gi|209417537|	1,0 ± 0,07	0,13 ± 0,03	7.3X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase.	101	NHGMHFR IMTLGFVBLLR
1863	gi|4538610|	2,8 ± 0,33	0,94 ± 0,12	3.0X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	123	VALEACVKAR DNGLLLHIHR EMTLGFVDLLR WSPELAAACEVWK
2005	gi|131974|	0,3 ± 0,09	0,12 ± 0,01	2.9X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	56	DNGLLLHIHR

Spot no.	Accession no.	(% Vol)*		Variation of normalised volume	Protein	Score	Peptide sequence
		Normal	Giant plant				
2086	gi|30421728|	0,36±0,04	0,17 ± 0,02	2.1X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	71	VALEACVKAR DNGLLLHIHR
2237	gi|156143103|	0,7 ± 0,12	0,16 ± 0,10	4.3X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	90	TFQGPPHGIQVER LIYYTPDYETKNTDILAAFR
2284	gi|17224634|	8,7 ± 0,40	3,40 ± 0,30	2.6X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	157	LNYYTPDYEVKDTDILAAFR TFQGPPHGIQVER
2324	gi|304011|	4,1 ± 0,50	1,64 ± 0,69	2.5X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	97	VALEACVKAR EMTLGFVDLLR
2327	gi|18026902|	0,4 ± 0,21	0,18 ± 0,07	2.3X[1]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	66	EMTLGFVDLLR
2489	gi|209417537|	1,1 ± 0,22	2,00 ± 0,57	1.8X[2]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	146	EMTLGFVDLLR EIKFEFEPVDKIDK
2589	gi|172246334|	1,7 ± 0,96	3,03 ± 0,20	1.8X[2]	Ribulose-l,5- bisphosphate carboxylase/oxygenase	130	LNYYTPDYEVKDTDILAAFR
1987	XP_009418696.1	0,5 ± 0,12	0,35 ± 0,05	1.5X[1]	Triose phosphate isomerase	88	VIACIGEKLEER VATPQQAQEVHLAVR
1800	ABF69972.1	0,3 ± 0,05	0,22 ± 0,08	1,7X[1]	T-protein	178	GGAVDWHIHDER VRDDHIYLVVNAGCR IYFGEFCTLDIHGSHCFLTR
3872	XP_010105219.1	0,2 ± 0,06	0,12 ± 0,02	1.7X[1]	Elongation factor Tu (EF-Tu)	71	QDQVDDEELLQLVELEVR GITINTATVEYETESR

[1] *Corresponds to greater abundance of proteins in normal banana.*
[2] *Corresponds to the greater abundance of proteins in the banana 'giant plant'*

Like the previously discussed enzymes, the ATP synthase CF1 alpha chain is a key enzyme in plant metabolism and performs an important role in multiple cellular processes [30,31].

When kept at their ideal levels, these enzymes play a fundamental role in maintaining cellular homeostasis, preserving metabolism, and, consequently, promoting normal growth of the banana tree and its fruit. A decreased amount of these enzymes in giant banana plants might compromise metabolism.

Our results also showed that normal banana plants had higher concentrations of the T-protein. The T-protein and three other enzymes (pyridoxal phosphate-dependent enzyme or P-protein, NAD^+ dependent enzyme or L-protein, and lipoic acid-containing protein or H-protein) make up the glycine cleavage multi-enzyme system (GCS). This system is essential for photorespiration and for one-carbon metabolism [32] and directly affects the biosynthesis of nucleic acids, proteins, and lignin [33,34].

GCS also combines with serine hydroxymethyltransferase (SHM), a mitochondrial enzyme, to convert glycine to serine. The combined GCS/SHM reaction represents the mitochondrial portion of the photorespiration cycle, which occurs in all C3 plant photosynthesis tissues, and converts the 2-phosphoglycolate generated by the RuBisCo enzyme to the 3-phosphoglycerate Calvin cycle metabolite [35,36].

Other studies have demonstrated that GCS suppression can be lethal to plants [32,37]. However, if the *Arabidopsis* T-protein is reduced by only five percent, there is no significant alteration to plant growth and photosynthesis, as well as no change in the photorespiratory carbon flux. An increase in *Arabidopsis* T-protein expression neither increased GCS activity nor altered any other metabolic process. Thus, it had no effect on plant growth and photosynthesis [32].

The present data provide no insight on the effect of decreased T-protein expression in giant banana plant leaves. We believe that this finding should be assessed in future studies, since the decreased T-protein levels converge with lower concentrations of RuBisCo, GAPDH, ALDO, and TPI.

In our results, one last protein, EF-Tu, was shown to have higher concentrations in normal plants. EF-Tu proteins belong to either the translacional GTPase group or the G-Protein group. EF-Tu proteins are multifunctional and well preserved [38]. They play a fundamental role in the elongation phase of protein synthesis in bacteria and organelles, including mitochondria and plant plastids [39,40]. EF-Tu genes are positively regulated during plant abiotic stress [40]. In plants under temperature stress, EF-Tu protein precursors protect synthase citrate, malate dehydrogenase, and RuBisCo proteins from thermal aggregation and inactivation, avoiding cellular respiration and photosynthesis restrictions [41,42].

The current study found larger concentrations of EF-Tu proteins in normal banana plants, indicating their importance in preserving metabolic enzymes and ensuring balance of cell activities, such as protein synthesis and energy generation.

4. CONCLUSION

In conclusion, comparative proteomic analysis has allowed us to identify energy metabolite proteins and stress-responsive proteins from normal and giant banana plants.

Proteins related to photosynthesis, energy production, and translation was identified as differentially expressed in normal plants.

In giant plants, GS was the only abundant protein revealed by our analysis, and it could be a potential molecular marker for early identification of variant plants. Our results also highlight some of the biochemical alterations that occur in banana trees with gigantism, providing a basis for future research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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