

Proteomic analysis reveals differentially accumulated proteins in banana somaclonal variants.

ABSTRACT

Aims. Although banana micropropagation has increasingly been employed in the past years it presents undesired somaclonal variation occurrence, among them, gigantism. Banana with gigantism causes great losses to producers, due to not only to the presence of taller trees but also to bunch anomaly. The aim of this work was to compare the protein profile of leaves from both normal and 'giant plants'.

Place and Duration of Study. The experiments were carried out in the *Central Molecular Biology Lab (LCBM)*, between April and November of 2017.

Methodology. Protein samples (triplicate) were analyzed by two-dimensional gel electrophoresis. The gels stained with Coomassie Blue G250 were evaluated by the ImageMaster 2D Platinum 5.0 program. The differentially abundant proteins were treated with the trypsin enzyme and aliquots of each hydrolysate sample were analyzed by mass spectrometry. Sample molecular mass profile obtained through MALDI-TOF-TOF MS was subjected to comparative analysis using the MASCOT program and NCBI database.

Results. Of the 36 differentially abundant proteins, 21 were identified and related to root metabolism, photosynthesis, carbon and nitrogen fixing, as well as protein translation. Glutamine synthase, which was more abundant in the leaves of 'giant plant', was highlighted among the identified proteins.

Conclusion. This is a pioneering work, in which proteomic analysis was applied to identify differentially abundant proteins on leaves of both normal banana and 'giant plants'. Glutamine synthase could be a potential molecular marker applied for early identification of variant plants. Moreover, such results contribute to clarify biochemical alterations that occur to banana trees with gigantism, hence serving as a basis for future research regarding micropropagation program and biofactories.

Keywords: banana; somaclonal variation; bidimensional eletrophoresis; mass spectrometry, proteomic profile

1. INTRODUCTION

Banana cultivation is one of the most important economical and social crops, with more than 125 producing countries. Banana is considered as the number one world fruit rank, with a 106.5 million ton production per year [1, 2]. Brazil produces more than 474 thousand hectares and produces seven million tons, which places it among the five largest world producers [3].

In conventional field propagation, the production of suckers is highly season dependent and, hence, availability of planting material in a given season is often a limiting factor [4]. Furthermore, material coming from the field could bring along pathogen agents.

In order to meet the demand, micropropagation in Brazil has been increasingly used lately [5]. Micropropagation ensures the production of pest and disease free plants, hence, reducing pathogen dissemination [6]. Nevertheless, *in vitro* multiplication could lead to the occurrence of somaclonal variation, of either genetic or epigenetic modification [7]. The example could be banana trees, in which the occurrence of off-types from tissue cultured plantlets ranged from 6 to 38 % in Cavendish cultivars [4].

In 'Prata-anã' (AAB) banana, the most frequent somaclonal variation is characterized by plant overgrowth and the production of bunches with low economical value, known as 'Giant plant' [6].

Since morphological characters are only identified in variant adult plants, in order to achieve early diagnosis, efforts have been made to find markers through molecular and cytogenetic techniques.

Guimarães et al [9] worked with somaclonal variants (gigantism) in the Prata-anã cultivar and concluded that the SSR (Simple Sequence Repeats) and RAPD (randomly amplified polymorphic) markers, plus chromosomal counting, did not enable a variant individual diagnosis. In another research, which also applied RAPD primers in micropropagated banana cv Grand naine (AAA) it was possible to identify a "variegated plant". However, other variations such as 'Giant plant' were grouped as normal plants [10].

Proteins have a central role on biological processes and proteomic differential analysis allows the determination of proteins affected by genetic variation during the growth and development of plants [11]. The identification of up- and down-regulated gigantism-related protein could provide information on the biochemical steps relevant, which originate from somaclonal variation and they can be used as a molecular markers in order to identify early somaclonal variants. This could be useful in avoiding take off-types plants to the field.

Some research works have applied proteomic profile evaluation in banana under various situations. Recently, in the case of bananas cv. Grand Naine, it was possible to relate the somatic embryogenesis induction to a higher stress-related cationic peroxidase and plant growth hormone-related proteins as indole-3-pyruvate monooxygenase and adenylate isopentenyltransferase [12].

Aimed at identifying and tracking changes in key-proteins involved in the growth mechanisms, many researches have used proteomic approach, by employing 2-DE and mass spectrometry to identify total proteins at different ripening and climacteric stages in bananas and other fruits, such as papaya and apple [13, 14]. In one research work banana pulp at pre-climacteric and climacteric stages were compared [15]. Chitinases were the most abundant types of proteins in unripe banana and three heat shock proteins and isoflavone reductase were abundant at the climacteric stage [15].

The objective of this work was to identify differently abundant proteins on banana leaves

without and with gigant somaclonal variation through two-dimensional gel electrophoresis and mass spectrometry (MALDI-Tof/Tof).

2. MATERIALS E METHODS

2.1 Plant Material

Foliage leaves *Musa* spp. cultivar Prata Anã (AAB), from normal and 'giant plant' were provided by a private property located in Lavras – MG, Brazil (Figure 1).



Figure 1. Normal plants and 'giant plant'. The arrow highlights the 'giant plant'.

All plants were 18 month-old. The 'giant plants' were selected according to morphological parameters such as, lanceolate and more upright leaves, larger size and thinner pseudostem. All samples were obtained from the same clone through *in vitro* propagation

2.2 Banana leaf protein extraction

Total protein extracts prepared from leaves, (200 mg) in triplicates, were ground to a very fine powder in a mortar and pestle using liquid nitrogen, following the method described by Gallardo et al [16]. Approximately 150 mg powder were used for protein extraction at 4°C in 660 μ L of buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 18 mM Tris-HCl, 14 mM Trizma base, 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 10 min, 14 mM dithiothreitol (DTT) was added and the protein extracts were stirred for 20 min and centrifuged (35,000g for 10 min) at 4°C. The supernatant was submitted to a second centrifugation as above. The final supernatant corresponded to the total protein extract and stored at -20°C.

Protein concentrations were then measured according to Bradford procedure (1976) [17], by applying the GeneQuant unit (GE-Healthcare) and bovine serum albumin as standard. The extracted total protein samples were submitted to SDS-polyacrylamide gel and dyed with Coomassie G-250 blue.

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2.3 Protein separation by two-dimensional gel electrophoresis

Multiphor II (GE Healthcare) system was used for two-dimensional gel electrophoresis following manufacturer's instructions. Two hundred and fifty micrograms of protein were loaded onto 18 cm IPG strips (linear pH gradient of 3–10; GE Healthcare). The Multiphor II (GE Healthcare) was applied under the following conditions: a total of 55,000Vh at 15°C; Gradient 150V for 1 minute; Gradient 150V for 3h; Gradient 500V for 3h; Gradient 3500V for 5h; Gradient 3500V for 12h; maximum current setting of 50 μ A per strip. Next, IEF, IPG strip were equilibrated for 20 min on a buffer (100 mM Tris–HCl pH 8.8, 6M urea, 2% SDS, 30% glycerol, and 0.2 mg/mL bromophenolblue) containing 5 mg/mL DTT (to reduce proteins). Then, another 20 min equilibration in the same buffer, but containing 25 mg/mL iodoacetamide (to alkylate proteins) instead of DTT, was conducted. The second dimension SDS-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 600V for 30 minutes; 600V for 2h (program as “step and hold”), or until the bromophenol blue dye front had run off the gel. The low and high molecular weight protein marker (SDS Calibration Kit) for SDS electrophoresis (GE Healthcare) was used for molecular mass determinations. At least three replicate gels were run for each sample.

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

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2.4 Image analysis

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

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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 Biotoools 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 have various interfering substances, such as phenolic compounds, proteolitics and oxidative enzymes, terpenes, pigments, organic acids, inhibitor ions and carbohydrates, which can jeopardize protein extraction [19] and drastically impact the results of a wide number of proteomics analyses [20].

In our work, the protein extraction resulted in 18mg/ml proteins. Protein integrity was verified on SDS-PAGE gel, which resulted in a protein profile with well-defined bands (Figure 2), thus, demonstrating that the methods extract proteins similarly and efficiently.

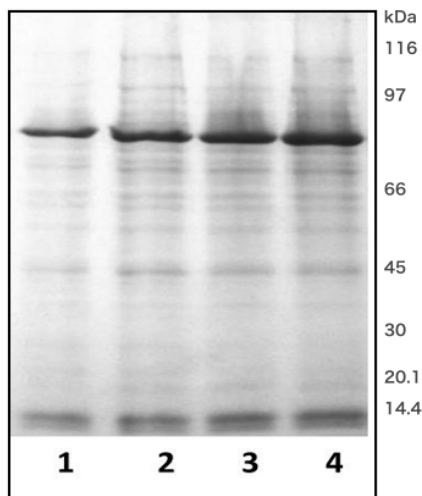


Figure 2 – Protein profile of leaf samples detected on SDS-PAGE gels Protein profile of proteins extracted in gel SDS-PAGE 12,5%. 7 µg of total protein were applied in wells 1; 10 µg in well 2; 15 µg in well 3 and 20 µg in well 4.

In order to analyze 2-DE gel quality, protein extracts were first separated under isoelectric focalization at a 3-10 pH range. 2-DE gel presented a large number of individualized proteins (*spots*), without either horizontal or vertical smear (Figure 3), thus, revealing the absence or small number of interferers such as lipids and carbohydrates.

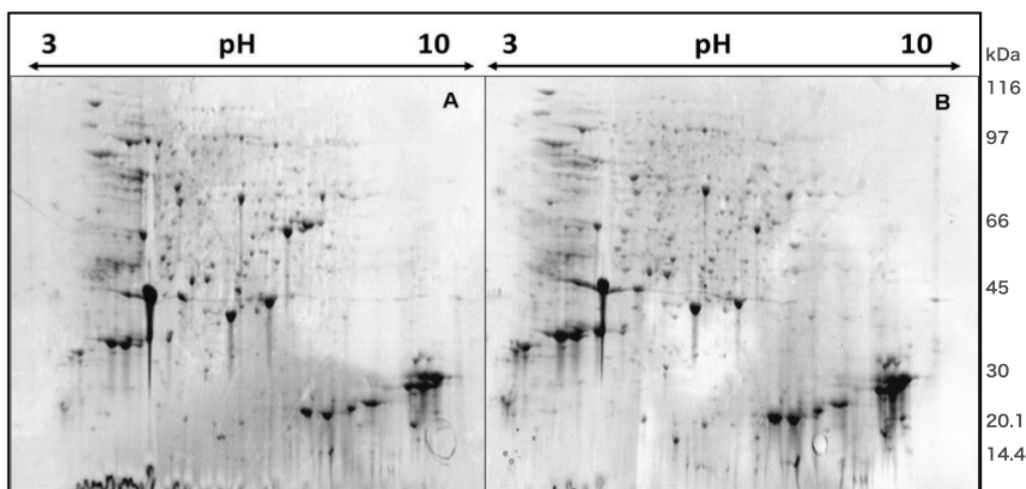


Figure 3 – Two-dimensional gels from both normal banana leaf (A) and 'giant plant' (B) leaf samples.

By using the software ImageMaster 2D Platinum (v.5.0) (ANOVA, $P = .05$) 224 *spots* were detected on normal banana samples and 267 *spots* on 'giant plant' samples. Of these, 36 presented volume percentage differences (% Volume - volume occupied by each *spot* on the

201 gel) among themselves, with protein abundance varying from 1.2X to 7.3X. From these, 31
202 *spots* were more abundant on the normal banana and 5 presented greater protein abundance
203 on the 'giant plant'.

204 Mass spectrometer analysis system MALDI-ToF/ToF enabled the identification of 21
205 *spots* from the 36 protein *spots* excised from the gel (Table 1), all being related to the energy,
206 photosynthesis, carbon and nitrogen fixation as well as translation (Figure 4).

207

208 **Table 1** - Significantly changed protein abundance in banana normal and 'giant Plant'. Proteins identified after analysis in MALDI-ToF/ToF, followed by
 209 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
 210 and a significant result in the *t-student test* ($P=0.05$).

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Spot no.	Accession no.	(% Vol)*		Variation of normalised volume	Protein	Score	Peptide sequence
		Normal	Giant plant				
1556	XP_009393673.1	0,09 $\square$ 0,01	0,158 $\square$ 0,08	1.7X ²	Glutamine synthase	146	IIAEYIWIGGSGGLDLR HETADINTFLWGVANR
1231	NP_084803.1	0,64 $\square$ 0,30	0,21 $\square$ 0,19	3.0X ¹	ATP synthase CF1/alfa	281	ADEISNIIR LIESPAPGIISR ASSVAQVVTTFR IAQIPVSEGYLGR IVNTGTVLQVGDGIAR
1237	NP_084803.1	0,37 $\square$ 0,14	0,17 $\square$ 0,11	2.2X ¹	ATP synthase CF1/alfa	72	EAYPGDVFYHLHSR IVNTGTVLQVGDGIAR
1323	NP_084803.1	0,28 $\square$ 0,16	0,17 $\square$ 0,08	1.6X ¹	ATP synthase CF1/alfa	140	IAQIPVSEGYLGR EAYPGDVFYHLHSR IVNTGTVLQVGDGIAR
1649	XP_021609330.1	0,38 $\square$ 0,04	0,22 $\square$ 0,08	1.7X ¹	Fructose 1,6 bisphosphate aldolase	102	YAAVSQDNGLVPIVEPEILLDGEHGIDR LASIGLENTEANR
1664	AAV70659.1	0,36 $\square$ 0,05	0,30 $\square$ 0,04	1.2X ¹	Gliceraldehyde-3-phosphate dehydrogenase	181	VPTVDVSVVDLTVR GILGYVEEDLVSTDFVGDSR
1843	gi 22796842	1,27 $\square$ 0,15	0,21 $\square$ 0,08	6.0X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	91	VALEACVKAR DNGLLLHIHR
1847	gi 209417537	1,00 $\square$ 0,07	0,137 $\square$ 0,03	7.3X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase.	101	NHGMHFR IMTLGFVBLLR
1863	gi 4538610	2,82 $\square$ 0,33	0,94 $\square$ 0,12	3.0X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	123	VALEACVKAR DNGLLLHIHR EMTLGFVDLLR WSPELAAACEVWK
2005	gi 131974	0,35 $\square$ 0,09	0,12 $\square$ 0,01	2.9X ¹	Ribulose-1,5- bisphosphate	56	DNGLLLHIHR

					carboxylase/oxygenase		
2086	gi 30421728	0,36 □ 0,04	0,17 □ 0,02	2.1X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	71	VALEACVKAR DNGLLLHIHR
2237	gi 156143103	0,70 □ 0,12	0,16 □ 0,10	4.3X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	90	TFQGPPHGIQVER LIYYTPDYETKNTDILAAFR
2284	gi 17224634	8,72 □ 0,40	3,40 □ 0,30	2.6X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	157	LNYYTPDYEVKDTDILAAFR TFQGPPHGIQVER
2324	gi 304011	4,14 □ 0,50	1,64 □ 0,69	2.5X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	97	VALEACVKAR EMTLGFVDLLR
2327	gi 18026902	0,42 □ 0,21	0,18 □ 0,07	2.3X ¹	Ribulose-1,5- bisphosphate carboxylase/oxygenase	66	EMTLGFVDLLR
2489	gi 209417537	1,12 □ 0,22	2,00 □ 0,57	1.8X ²	Ribulose-1,5- bisphosphate carboxylase/oxygenase	146	EMTLGFVDLLR EIKFEFEPVDKIDK
2589	gi 172246334	1,72 □ 0,96	3,03 □ 0,20	1.8X ²	Ribulose-1,5- bisphosphate carboxylase/oxygenase	130	LNYYTPDYEVKDTDILAAFR
1987	XP_009418696.1	0,54 □ 0,12	0,35 □ 0,05	1.5X ¹	Triose phosphate isomerase	88	VIACIGEKLEER VATPQQAAQEVHLAVR
1800	ABF69972.1	0,38 □ 0,05	0,22 □ 0,08	1,7X ¹	T-protein	178	GGAVDWHIHDER VRDDHIYLVVNAGCR IYFGEFCTLDIHGSHCFLTR
3872	XP_010105219.1	0,21 □ 0,06	0,12 □ 0,02	1.7X ¹	Elongation factor Tu (EF-Tu)	71	QDQVDDEELLQLVELEVR GITINTATVEYETESR

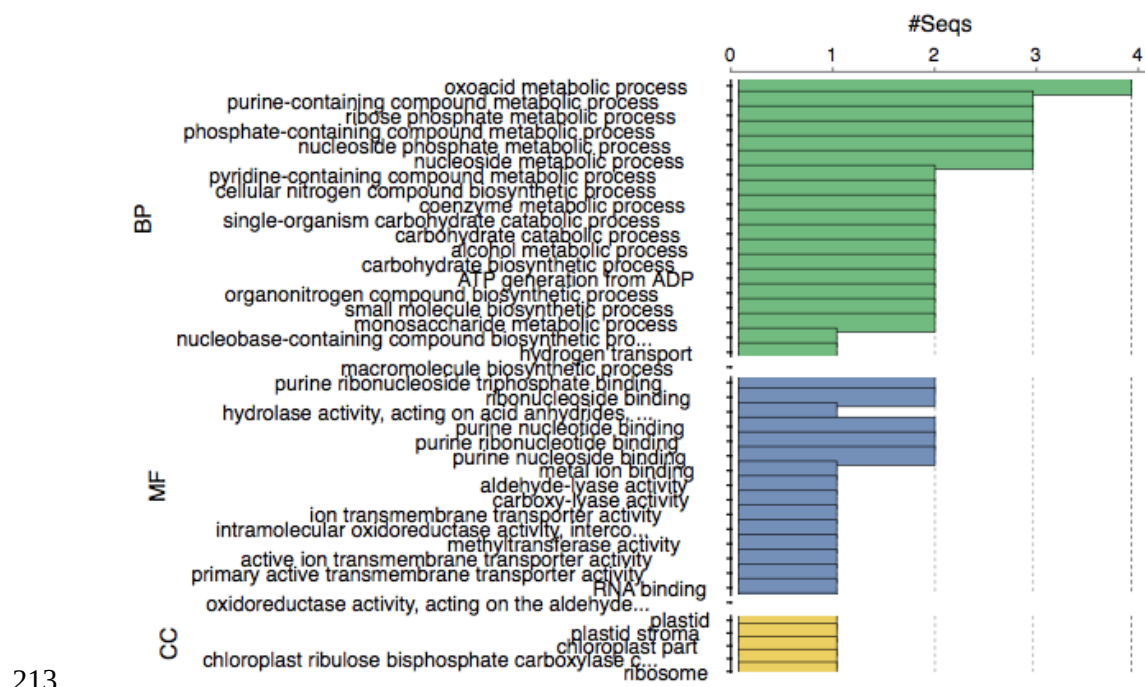


Figure 4. Functional category distribution of identified proteins with known functions.
***BP: Biological process; MF: Molecular function; CC: cellular component.**

In 'giant plants', a larger amount of glutamine synthase protein (GS) was noted, which is responsible for catalyzing glutamine (GLU) formation, hence, incorporating a glutamate ammonium molecule [21] with concomitant hydrolysis of 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 compounds breakdown [22]. The amide moiety of glutamine is utilized as the donor of amino residue to synthesize a number of essential metabolites such as amino acids, nucleic acids, and amino sugars [23]. The coordination and optimal functioning of the metabolic pathways for nitrogen and carbon assimilation in plants are critical in determining plant growth and, ultimately, biomass accumulation [24].

Biochemical studies demonstrate the presence of two GS isoenzymes; a cytosolic (GS1) and a chloroplastic one (GS2). The GS2 expression is abundant on the leaf mesophyll whereas GS1 is less frequent on leaves and more concentrated in phloem [25].

GS cytosolic (GS1) isoenzyme overexpression was evaluated in tobacco leaves. In such tissues overexpression GS1 had 70% higher shoot and 100% greater root dry weight as well as 50% more leaf area [26]. Other research work, presented a large-growth, light-dependent phenotype as evidenced by increases on fresh weight, dry weight and soluble proteins of leaves. These findings show the importance of this enzyme as a key component on the photosynthesis-photorespiration relation (via ammonium assimilation), as noted by lower free ammonium levels, increase on the Ser/Gly ratio (3,5 times), higher intermediate photorespiratory levels and GLU and CO₂ level increase [27].

Recent research works related higher plant biomass and better growth phenotype of transgenic *Arabidopsis thaliana* [28] and transgenic *Nicotiana tabacum* [29] with the improved efficiency of nitrogen assimilation, due to overexpressed GS gene.

Therefore, we suggest that a greater abundance of this protein might be related to the 'Giant banana plant' growth. Nevertheless, more studies should be conducted in order to assure the use of these proteins (and/or gene) as molecular marker on the early identification of 'giant plants'.

Higher concentrations of glyceraldehyde-3-phosphate dehydrogenase, fructose 1,6-biphosphate aldolase, triose-phosphate isomerase, synthase ATP sub-unities, **Rubisco** sub-unities proteins, T- protein and Elongation factor Tu (EF-Tu) was observed in normal plants, as compared to giant plants.

Rubisco protein is an important Calvin cycle enzyme, which consists of a carboxylase that catalyzes the incorporation of CO₂ in organic form. Besides **Rubisco**, there are also glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fructose 1,6-bisfosfato aldolase (ALDO) and triose-phosphate isomerase (TPI) proteins that participate in the Calvin Cycle (Figure 5).

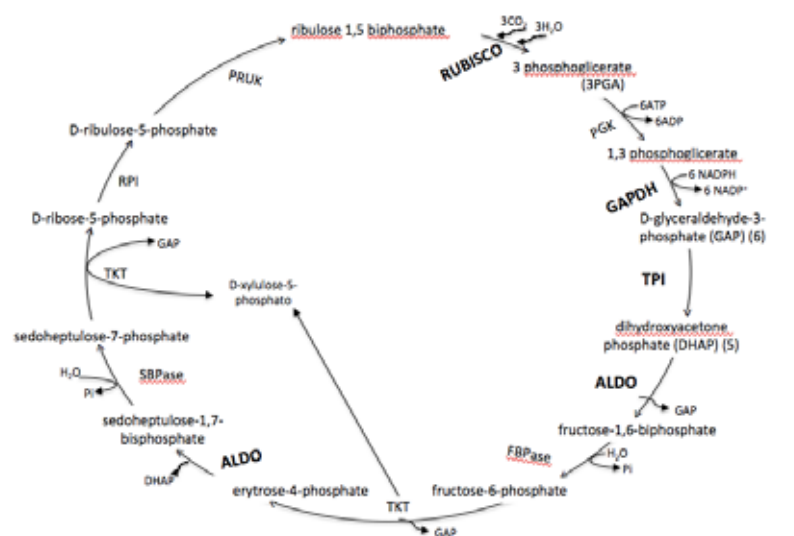


Figure 5. Calvin cycle. Abundant protein in the normal plants are shown in bold letters. Enzymes encoded by nondifferentially expressed genes are shown with normal letters. Abbreviations for enzymes: ALDO: aldolase (fructose-bisphosphate aldolase); FBPase: fructose-1,6-bisphosphatase; GAPDH: glyceraldehyde-3-phosphate 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.

Furthermore, in cytosol, the three latter ones are **enzymes of glycolysis** [30]. Therefore, these proteins participate both in the metabolic paths to obtain energy through oxidation of sugars and in the CO₂ conversion into triose-phosphate. In other words, these enzymes play an import role on the triose-phosphate synthesis, which might be converted into glucose, starch

267 and sucrose that will be used either for the generation of energy or serve as “construction
268 blocks” in growing plants.

269 In agreement with the previously described enzymes, "ATP synthase CF1 alpha chain"
270 is a key enzyme for the energy metabolism of the plant kingdom and performs an important role
271 on the multiple cellular processes [30, 31].

272 Therefore, when kept at their ideal levels, these enzymes have a fundamental role on
273 the cellular homeostasis maintenance, thus, preserving metabolism and consequently keeping
274 banana tree and fruit growth normal. A lesser amount of these enzymes might compromise this
275 metabolism in 'giant plants'.

276 THF-dependent enzyme (T-protein), along three other enzymes (pyridoxalphosphate-
277 dependent enzyme/P-protein, NAD⁺ dependent enzyme/L-protein and lipoic acid-containing/ H-
278 protein) comprises the glycine cleavage multi-enzyme system (GCS). This is essential for the
279 photorespiration and for one-carbon metabolism [32], which directly affects the biosynthesis of
280 nucleic acids, proteins, lignin and many others [33, 34].

281 Moreover, GCS combines with SER hydroxymethyltransferase (SHM). That is a second
282 mitochondrial enzyme, on the conversion of glycine to Serine. The combined GCS/SHM
283 reaction represents the mitochondrial portion of the photorespiration, which occurs in all C3
284 plant photosynthesis tissues, and converts the 2-phosphoglycolate generated by the Rubisco
285 enzyme on the 3-phosphoglycerate Calvin Cycle metabolite [35, 36].

286 Other works demonstrate that the exclusion of GCS activities on plants could be lethal
287 [32, 37]. However, if only a 5% T-protein reduction on Arabidopsis leaves occurs, there will be
288 no significant alterations on plant growth and photosynthesis, as well as no change on the
289 photorespiratory carbon flux. The T-protein expression increase on Arabidopsis leaves neither
290 increased GCS activity nor altered other metabolic process, thus, it had no **affect** on plant
291 growth and photosynthesis [32].

292 Based on these present data, we found no inference related to the effects caused by a
293 lesser T-protein abundance in banana 'Giant Plant' leaves. We believe that this finding must be
294 better assessed in future studies, since it converges with low concentrations of either Rubisco,
295 glyceraldehyde-3-phosphate dehydrogenase, fructose 1,6-bi-phosphate aldolase and triose-
296 phosphate isomerase.

297 EF-Tu proteins, which belong to either GTPases translacional or G-Proteins groups, are
298 well preserved and multifunctional [38]. These proteins play a fundamental role on the
299 elongation phase of protein synthesis in bacteria and organelles, including mitochondria and
300 plastids in plants [39, 40]. EF-Tu genes are positively regulated in abiotic stress in plants [41].
301 EF-Tu protein precursors knowingly avoid aggregation and inactivation of synthase citrate,
302 **malate dehydrogenase** and Rubisco proteins in plants under temperature stress, hence,
303 avoiding cellular respiration and photosynthesis restrictions [42, 43].

304 In the current work, larger concentrations of EF-Tu proteins were found in normal
305 banana trees, which shows their importance in preserving metabolic enzymes and thus
306 assuring cell activity balance, such as protein synthesis and energy generation.

4. CONCLUSIONS

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

Photosynthesis-related proteins, energy production and translation process were identified as differentially expressed proteins in normal plant.

In 'giant plants', our analysis revealed one abundant protein, Glutamine synthase, responsible for catalyzing glutamine formation. Glutamine synthase could be a potential molecular marker applied for early identification of variant plants. Moreover, such results contribute to clarify biochemical alterations that occur to banana trees with gigantism, hence serving as a basis for future research.

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