

Sugar Metabolism during Coffee Fruit Development

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SUMMARY

In this study, we investigated more particularly the sucrose synthase (Susy: EC2.4.1.13) at the biochemical and molecular levels during the development of coffee fruits. In addition, feeding experiments with ¹⁴C-sucrose and incubation of fruits with ¹⁴CO₂ were carried out. Our results suggest that Susy is the main enzyme responsible for sucrose metabolism in coffee fruits. In the pulp and endosperm of fruits at the last stage of maturation, a peak of Susy activity was observed and correlates with an increase of sucrose. At the molecular level, we cloned two cDNAs encoding different Susy isoforms. We also showed that both Susy-encoding genes were expressed in coffee fruits, with differences regarding their spatial and temporal expression. The ¹⁴C-experiments showed that sugars are not only transported from the leaves to the fruits, but also there is an intense communication among the tissues composing the fruits.

RÉSUMÉ

Dans cette étude, nous avons analysé plus particulièrement la saccharose synthase (Susy: EC2.4.1.13) au niveau biochimique et moléculaire durant le développement des fruits de caféier. De plus, des expériences de nutrition utilisant du ¹⁴C -saccharose et d'incubation de fruits avec du ¹⁴CO₂ ont aussi été réalisées. Nos résultats suggèrent que la Susy est la principale enzyme responsable du métabolisme du saccharose dans les fruits de caféier. Dans la pulpe et l'endosperme de fruits au dernier stade de maturation, un pic de l'activité Susy est observé et se superpose avec l'augmentation de la quantité de saccharose mesurée. Au niveau moléculaire, nous avons cloné deux ADNc codant pour des isoformes différentes de Susy. Nous avons aussi montré ces deux gènes de Susy s'expriment dans les fruits de caféier, avec toutefois des différences spatiales et temporelles concernant leur expression. Les expériences de marquage au ¹⁴C ont montré que les sucres ne sont pas seulement transportés des feuilles vers les fruits, mais qu'il y a aussi des communications intenses entre les tissus qui composent les fruits.

INTRODUCTION

In green coffee beans, the carbohydrate fraction represents about half of the dry weight, and participates in the extensive chemical changes associated to coffee roasting (Bradbury, 2001). Sucrose is considered as the major precursor of coffee flavor and aroma, because it is rapidly degraded during the roasting. Despite of that, little is known about sugar metabolism in coffee, particularly considering that coffee fruits take more than 30 weeks to reach maturation. Therefore, the aim of the present work was to increase our knowledge about sugar metabolism

in coffee, mainly to understand the sink-source relationships occurring between the different tissues present during coffee fruit development.

MATERIAL AND METHODS

Plant Material

Fruits of *Coffea arabica* cv IAPAR 59 were harvested from plants in the field (Sept. 2002/ April 2003) every 4 weeks from flowering until complete maturation. Fruit tissues (perisperm, endosperm, pulp) were separated and used independently to extract total RNA (Rogers et al., 1999a) or were subjected to sugars and enzyme activity analyses. In addition, leaves or fruits of branches bearing fruits were exposed to $^{14}\text{CO}_2$ (Carneiro et al., 1999 – see scheme Figure 2) for 4 h and then collected 24 h later. Also detached fruits were fed with ^{14}C -sucrose and collected after 24 h (Vitória and Mazzafera, 1999).

Sugar determinations and enzyme analyses

Ethanol extracts were used to determine soluble sugars by HPLC with pulse amperometric detection or by colorimetry (Buysse and Merckx, 1993; Van Handel, 1968). Susy activity and protein concentrations were determined according to Craig et al. (1999) and Bradford (1976), respectively. Susy activity was measured for sucrose synthesis. Ethanol extracts were also obtained in the ^{14}C experiments for determination of total radioactivity. For the distribution of radioactivity in the sugars, they were separated in HPLC using a radioactivity monitor.

Nucleic acid manipulation

The partial SUS1 cDNA sequence is available in GeneBank under accession number AJ575256. For the expression analyses, northern blots were carried out using 15 μg of total RNA (Rogers et al., 1999a). Filters were hybridized independently with SUS1 and SUS2 partial cDNA fragments labeled with ^{32}P .

RESULTS

Fruit growth, sugar concentrations and ^{14}C distribution

Figure 1A shows the tissues growth during fruit development. In all tissues analyzed, reducing sugars (RS, mainly fructose and glucose) were in higher amount than sucrose (Figure 1B and C), except in the last stages of the endosperm development (205 and 234 DAF). The amount of RS was particularly important in the perisperm at 90 DAF, when the endosperm was too small to be separated. After 90 DAF, the amount of RS in the perisperm decreased rapidly, concomitantly with the endosperm growth. In this tissue, RS were not detectable from 120 DAF to 234 DAF. Sucrose accumulated gradually up to 6% at the time the maturation was completed. RS were in low concentration along the pulp development but increased rapidly in the latest stages of maturation, particularly when the fruit shifts green to red, between 205 and 234 DAF.

Branches bearing fruits at approximately 120 DAF were incubated with $^{14}\text{CO}_2$. The results showed that in addition to the leaves, $^{14}\text{CO}_2$ is actively assimilated in green fruits due to the photosynthesis. Photoassimilates (sucrose) are actively exchanged in all fruit tissues (Figure 2A). The highest accumulation in the perisperm shows its importance as a transfer tissue. Fruits fed with ^{14}C -sucrose showed that indeed there is a transport from the pulp to the endosperm (Figure 2B). Already in the pulp a large fraction of the sucrose was converted to

reducing sugars (Figure 2C), what is in agreement with the data shown in Figure 1. The same was observed with the $^{14}\text{CO}_2$ incubation fruits (data not shown).

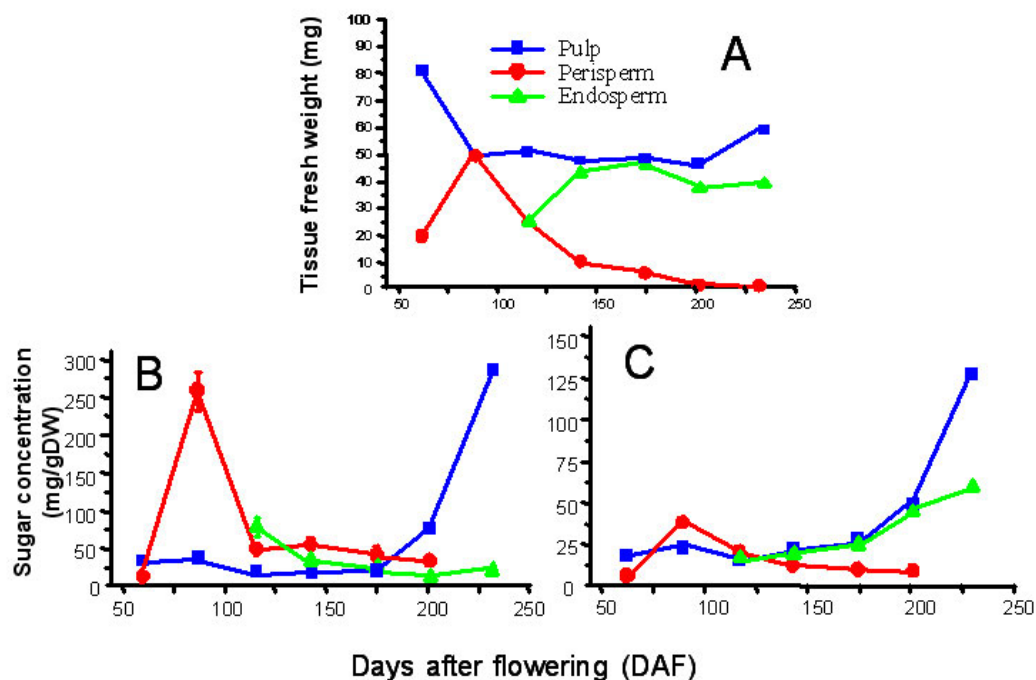


Figure 1. Fruits aroxtth (A), reducing sugars (B) and sucrose (C).

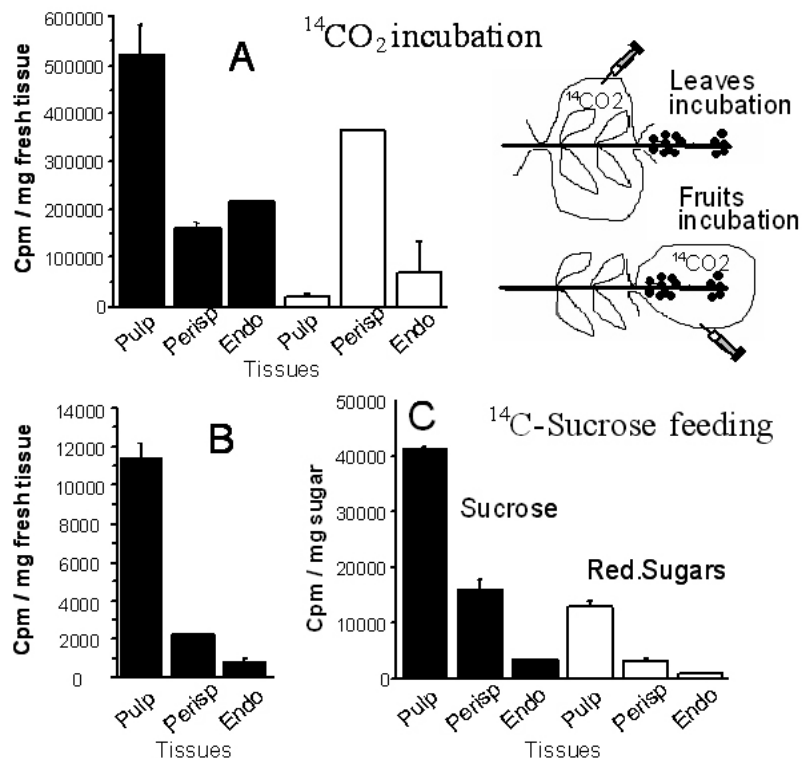


Figure 2. Distribution of radioactivity in fruits of branches incubated with $^{14}\text{CO}_2$ (A-see scheme), in fruits fed with ^{14}C -sucrose (B) and reducing sugars (white boxes) in these fruits (C).

Isolation of Susy cDNA sequences

A partial Susy cDNA (SUS1) was cloned using degenerated primers in RT-PCR experiments with total RNA from coffee fruits. Its deduced protein shows 97% of similarity with the SUS2 protein from *S. tuberosum* (P49039). By Southern-blotting we showed that SUS1 was a member of a small gene family containing at least two genes (data not shown). Its corresponding full-length cDNA from *C. arabica* and equivalent gene from *C. canephora* were recently cloned and are under sequencing. Another Susy-encoding partial cDNA sequence (named SUS2) was also identified from the Brazilian Coffee Genome Project and presents only 59% of identity with SUS1 at the nucleic level and 74% at the protein level.

Susy activity during fruit growth

In all tissues analyzed, Susy always appeared to be more active than acid invertases (data not shown). However, because sugar analyses showed that there was no sucrose accumulation during the major part of coffee bean development, for example in the perisperm, we presumed that Susy functioned probably as a sucrose-degrading enzyme *in vivo*. The increase of Susy activity in the latter stages (205-234 DAF) of pulp and endosperm development was simultaneously accompanied by the increase of sucrose content in these tissues, supporting the conclusion the enzyme functions in the sucrose-synthesis sense that at these stages (Figure 3).

Analysis of Susy gene expression

The expression of Susy-encoding genes was checked in the fruit tissues using the SUS1 and SUS2 partial cDNA as probes (Figures 3 and 4). SUS1 mRNAs were detected in the perisperm at 90 DAF and no further observed. It was also observed at 125 DAF with a peak at 150 DAF in the endosperm, and with two expression peaks (at 60 and 150 DAF) in the pulp. In addition, SUS2 gene appeared to be expressed in the last stages of pulp development (200-230 DAF), overlapping the peak of Susy activity detected in this tissue (Figure 4).

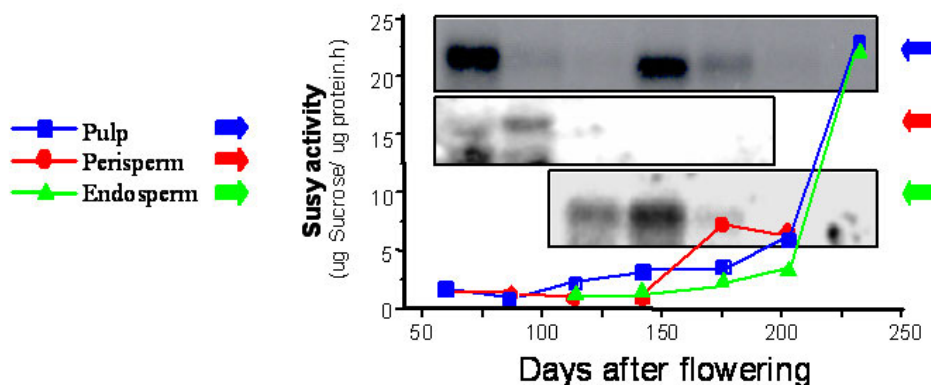


Figure 3. Susy activities in separated tissues of coffee fruits. SUS1 gene expression (Northern-blot) is also presented.

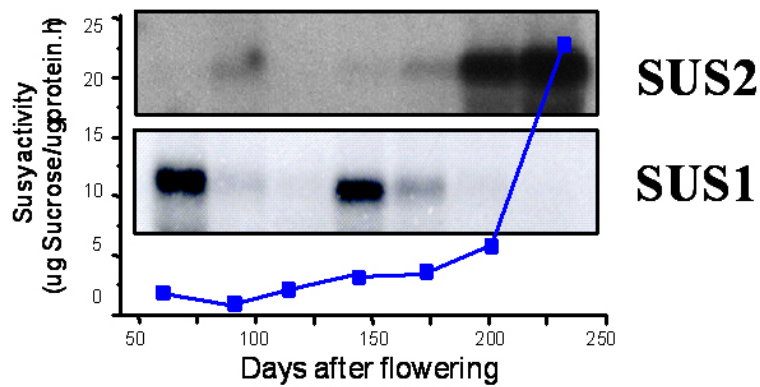


Figure 4. Expression of SUS2 genes and Susy activity (from Figure 3) in coffee pulp under development.

DISCUSSION

Concentrations of RS and sucrose measured in fruit tissues confirmed previous information obtained on coffee (Rogers et al., 1999b). The data on ^{14}C also showed that the pulp plays an important role in fixing CO_2 and that the perisperm seems to behave as a passage tissue. Therefore, the decrease of RS sugars in the perisperm may also be due to a translocation to the endosperm. Indeed, incubating fruits with ^{14}C -fructose showed a prompt transfer to the endosperm (data not shown).

From our results, we deduced that the transient accumulation of RS in the perisperm and endosperm probably is a consequence of the sucrose-degrading function of Susy rather than invertases. This was supported by the fact that no expression of invertase-encoding genes was detected in all these tissues (data not shown), whereas expression of Susy-encoding genes was observed in all tissues tested. This suggests that at least two Susy isoforms are implicated in sucrose metabolism in coffee fruits. In that sense, we proposed that the SUS1 functions mainly as a sucrose-degrading enzyme both in the perisperm and endosperm and that SUS2 should control the final concentration of sucrose found both in the pulp and in mature coffee beans.

ACKNOWLEDGEMENTS

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REFERENCES

- Bradbury A.G.W (2001) In: Clarke R.J. and Vitzthum O.G. (Eds) *Coffee: Recent Developments*, Blackwell Science, Oxford, pp. 1-17.
- Bradford M.M. (1976) *Anal. Biochem.* 2: 248-254.
- Buysse J., Merckx R. (1993) *J. Exp. Bot.* 44: 1627-1629.
- Carneiro R.G., Ferraz L.C.C.B., Mazzafera P. (1999) *J. Nematology* 31: 348-355.
- Craig J., Barrat P., Tatge H., Dejardin A., Barber L., Wang T., Hedley C., Martin C., Smith A.M. (1999) *Plant J.* 17: 350-362.
- Rogers W.J., Michaux S., Bastin M., Bucheli P. (1999b) *Plant Sci.* 149: 115-123.
- Van Handel E. (1968) *Anal. Biochem.* 22: 280-283.
- Vitória A.P., Mazzafera, P. (1999) *J. Agric. Food Chem.* 47: 1851-1855.