Biochemical and genomic analysis of sucrose metabolism during coffee (Coffea arabica) fruit development

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Abstract

Sucrose metabolism and the role of sucrose synthase were investigated in the fruit tissues (pericarp, perisperm, and endosperm) of Coffea arabica during development. Acid invertase, sucrose phosphate synthase, and sucrose synthase activities were monitored and compared with the levels of sucrose and reducing sugars. Among these enzymes, sucrose synthase showed the highest activities during the last stage of endosperm and pericarp development and this activity paralleled closely the accumulation of sucrose in these tissues at this stage. Carbon partitioning in fruits was studied by pulse–chase experiments with ¹⁴C-sugars and revealed high rates of sucrose turnover in perisperm and endosperm tissues. Additional feeding experiments with ¹⁴CO₂ showed that leaf photosynthesis contributed more to seed development than the pericarp in terms of photosynthate supply to the endosperm. Sugar analysis, feeding experiments, and histological studies indicated that the perisperm plays an important role in this downloading process. It was observed that the perisperm presents a transient accumulation of starch which is degraded as the seed develops. Two full-length cDNAs (CaSUS1 and CaSUS2) and the complete gene sequence of the latter were also isolated. They encode sucrose synthase isoforms that are phylogenetically distinct, indicating their involvement in different physiological functions during cherry development. Contrasting expression patterns were observed for CaSUS1 and CaSUS2 in perisperm, endosperm, and pericarp tissues: CaSUS1 mRNAs accumulated mainly during the early development of perisperm and endosperm, as well as during pericarp growing phases, whereas those of CaSUS2 paralleled sucrose synthase activity in the last weeks of pericarp and endosperm development. Taken together, these results indicate that sucrose synthase plays an important role in sugar metabolism during sucrose accumulation in the coffee fruit.

Key words: Coffea arabica, endosperm, gene expression, sucrose synthase, sugar partitioning.

Introduction

Coffee is a very important crop with more than seven millions tons of green beans produced every year on about 11 millions hectares worldwide. After oil, coffee ranks second on international trade exchanges, being responsible for several million jobs in producer and consumer
countries. The two main species cultivated throughout the tropical world are *Coffea arabica* (2n=4x=44), which grows in highlands and represents approximately 70% of world production, and *Coffea canephora* (2n=2x=22), which grows in lowlands and represents the remaining 30%. Production involving other species, such as *Coffea liberica*, is incipient and consumption limited to local and restricted markets (Söndahl and Lauritis, 1992).

In terms of cup quality, *C. arabica* (Arabica) is appreciated to a greater extent by consumers due to its lesser bitterness and better flavour compared with *C. canephora* (Robusta). What exactly determines cup quality is a complex phenomenon that is far from being understood. Sucrose is one of the compounds in the raw coffee bean that has been implicated as an important precursor of coffee flavour and aroma because it degrades rapidly during roasting, forming anhydro-sugars (such as 1,6 anhydro-glucose) and other compounds like glyoxal (De Maria et al., 1994). Such molecules are then able to react principally with amino acids (Maillard reaction) forming aliphatic acids, hydroxymethyl furfural and other furans, and pyrazine. These compounds are all considered to be essential contributors to coffee flavour, either as volatile (Grosch, 2001) or non-volatile (Homma, 2001) components. The preference for Arabica coffees seems to be related in part to differences in sucrose content (Casal et al., 2000), which range from 5.1% to 9.4% of dry matter in harvested coffee beans of this species, whereas for Robusta these values are always lower, usually ranging from 4% to 7% of dry matter (Ky et al., 2001; Campa et al., 2004).

Despite the importance of sucrose as a precursor of coffee beverage quality, nothing is known about its distribution in tissues nor the role of key enzymes of its metabolism, such as invertases (EC 3.2.1.26, β-fructosidase, β-fructofuranosidase), sucrose phosphate synthase (SPS: EC 2.4.1.14), and sucrose synthase (SUS: EC 2.4.1.13). Invertases, which catalyse the irreversible hydrolysis of sucrose to glucose and fructose, are involved in various aspects of the plant life cycle and the response of the plant to environmental stimuli (Roisch and González, 2004). By contrast, SPS (UDP-glucose: δ-fructose 6-phosphate 2-glucosyl-transferase) appears to function mainly in the direction of sucrose synthesis (UDP-glucose+ fructose-P→sucrose-P+UDP) while SUS (UDP-glucose: δ-fructose 2-glucosyl-transferase) catalyses a reversible reaction (UDP-glucose+fructose→sucrose+UDP). Both enzymes are thought to play a major role in sucrose partitioning for energy purposes as well as in metabolic, structural, and storage functions of plant cells (see review by Sturm and Tang, 1999). Sucrose cleavage activity of SUS is also linked to cell wall biosynthesis by providing UDP-glucose for the cellulose synthase complex (Amor et al., 1995) and substrates for starch synthesis in sink organs (see review by Herbers and Sonnewald, 1998).

The present study was conducted to understand sucrose metabolism and its transport during coffee fruit development. Concentrations of reducing sugars and sucrose, and activities of sucrose-metabolizing enzymes, were monitored in the pericarp, perisperm, and endosperm throughout fruit development. To analyse sugar partitioning between these tissues, histological studies and feeding experiments using 14C-labelled sucrose, fructose, and CO₂ were carried out. In addition, two distinct SUS-encoding cDNAs were isolated (designated *CaSUS1* and *CaSUS2*) from mRNA of coffee endosperm. The molecular structure of these sequences and relationships between sucrose/reducing sugar contents, activities of sucrose-metabolizing enzymes, and expression of SUS genes are presented and discussed.

### Materials and methods

#### Plant materials

Fruits and tissues were harvested from 15-year-old plants of *Coffea arabica* cv. IAPAR 59 cultivated under field conditions. Fruits were collected between 13:00 h and 16:00 h, every 4 weeks from flowering (end of September 2002) up to complete maturation (mid-May 2003). After collection, tissues were immediately frozen in liquid nitrogen and stored at −80°C before being analysed. Fruit tissues (perisperm, endosperm, and pericarp) were separated and used independently to extract total RNA or were analysed for sugars and enzyme activity. The same material was used for the 14CO₂ and 14C feeding experiments.

#### Sugar determination, enzymatic analysis and western blotting

Fruits tissues were freeze-dried, ground in a mortar and pestle, and extracted with 80% ethanol in a Polytron homogenizer using 1 ml per 300 mg of tissue. Extraction proceeded for 30 min at 75°C in capped tubes and the supernatant was obtained after centrifugation. The extraction was carried out three times with the same volume of ethanol, and the combined supernatants were used for the analysis of sugars. Total soluble sugars (Dubois et al., 1956), sucrose (Van Handel, 1968), and reducing sugars (Somogyi, 1952) were determined in the extracts. For analysis of enzymes the tissues were extracted with 100 mM HEPES, pH 7.0, containing 2 mM MgCl₂, 10 mM 2-mercaptoethanol, and 2% (w/v) ascorbic acid. The supernatant recovered by centrifugation (27 000 g for 20 min) was desalted on PD10 minicolumns (Amersham Biosciences) and the protein content determined with a ready-to-use Bradford (1976) reagent (Bio-Rad). Acid invertase (AI) was assayed by incubating an aliquot of the desalted extract containing 60 μg of protein with 25 mM sucrose in 50 mM citrate-phosphate buffer pH 3.5, at 37°C, for 1 h (modified from Yelle et al., 1991). SPA was assayed by incubating 60 μg of protein with 25 mM uridine 5′-diphosphoglucose (UDPG), 25 mM fructose-6-phosphate, 30 mM glucose-6-phosphate, 20 mM phenyl-β-glucoside in 50 mM K-phosphate buffer, pH 7.5. The assay was incubated at 37°C for 1 h and stopped by the addition of 30% (w/v) KOH and boiling for 10 min. Sucrose phosphate content was determined according to Van Handel (1968). SUS activity was assayed in the direction of sucrose synthesis in a reaction containing 50–60 μg of protein, 25 mM UDPG, 25 mM α-fructose, 50 mM 2-(N-morpholino)ethanesulphonic acid hydrate (MES), at pH 6. The amount of protein, substrate concentrations, and pH used in this assay were defined in preliminary tests. After 60 min of incubation at 30°C the reaction was stopped by the addition of 30% (w/v) KOH and boiling for 10 min. Sucrose content was...
determined according to Van Handel (1968). Extracts obtained for enzyme analysis were used in western blot experiments. The proteins were separated by 10% (w/v) polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes using a Mini protein electrophoresis apparatus (Bio-Rad). The membranes were probed with a polyclonal antibody towards SUS from *Psisum sativum* using the protocol described by Dèjardin *et al.* (1997). The membranes were developed using an anti-rabbit secondary antibody conjugated with alkaline phosphatase.

**14C-feeding experiments**

Three pulse-chase experiments were carried out to study sucrrose metabolism in coffee fruits. In the first experiment, incubation with $^{14}$CO$_2$ was carried out with fruits at 120±20 DAF with the perisperm, endosperm, and pericarp representing respectively 20%, 28%, and 52% of the fruit fresh weight, or with leaves. Two Eppendorf$^\text{™}$ tubes were left inside the plastic bags, one containing a 1 M HCl solution and the other an aqueous solution carrying 10 M$\text{dpm}$ NaH$^{14}$CO$_3$ (50–62 mCi mmol$^{-1}$, Amersham Biosciences). To which a few drops of 3 M HCl solution were applied and the fruits left inside the plastic bags, one containing a 1 M HCl solution, the ethanolic extracts were reduced in volume in a SpeedVac (Savant) and the sugars separated for 20 h and the sugars revealed with aniline reagent developed for 20 h and the sugars revealed with aniline reagent (Walkey and Tillman, 1977). The spots were cut from the chromatograms were probed with a polyclonal antibody towards SUS from *Coffeea arabica* (Phoenix). The sections were mounted on glass slides, heated to 65 °C, followed by staining for 3 min in 0.05% (w/v) toluidine blue in 0.1 M acetate buffer, pH 4.7, and washing in running water for 5 min (O’Brien *et al.*, 1964). Sections were tested for starch with Lugol’s iodine solutions according to Johansen (1940). The sections were observed and photographed with a light microscope (Olympus, model BX51).

**Histological studies**

Fruits were collected at 40, 60–75, and 205–234 DAF and fixed in neutral formalin buffer (10% (v/v) formal, 0.4% (w/v) NaH$_2$PO$_4$, H$_2$O, 0.65% (w/v) anhydrous Na$_2$HPO$_4$). Dehydration was carried out by treating segments sequentially with 30%, 50%, and 70% (v/v) ethanol for 12 h at each concentration. The material was then treated with a plastic resin (Historesin, Leica) according to the protocol of Gerrits and Smid (1983). The polymerized resin blocks were then glued onto wooden blocks with plastic adhesive and 12 μm-thick sections cut on a rotary microtome (American Optical M 820, Phoenix). The sections were mounted on glass slides, heated to 65 °C, followed by staining for 3 min in 0.05% (v/v) toluidine blue in 0.1 M acetate buffer, pH 4.7, and washing in running water for 5 min (O’Brien *et al.*, 1964). Sections were tested for starch with Lugol’s iodine solutions according to Johansen (1940). The sections were observed and photographed with a light microscope (Olympus, model BX51).

**cDNA isolation and gene cloning**

Cloning of SUS cDNA sequences was facilitated by the use of EST available from the Brazilian Coffee Genome Project (http://www.lge.ibi.ucamp.br/cafet). The CaSUS1 cDNA was isolated from endosperm of fruits at 147 DAF. Total RNA was extracted as described previously (Rogers *et al.*, 1999a) and 500 μg were treated with Oligotex dT beads (Qiagen) to purify 2 μg of mRNA which was reverse-transcribed according to the protocol defined in the Marathom$^\text{™}$ cDNA amplification kit (BD Biosciences Clontech). Subsequently, a 3′ RACE PCR reaction was performed using the AP1 primer 5′-CCA TCT TAA TAC GAC TCA CTA TAG GGC-3′ from the Kit and the UPIC13MI1 SUS primer 5′-TAT ACT CGT TTT TTC CGT TAC TCT TTT TT-3′ deduced from a cluster formed by the collection of 250 SUS-encoding ESTs, found within the Brazilian Coffee Genome Project. PCR was performed using a PTC-100 Thermocycler (MJ Research) with Advantage2 Taq DNA polymerase according to the supplier (BD Biosciences Clontech) under the following conditions: initial denaturation 94°C, 1 min; followed by 35 cycles of 94°C, 30 s; 52°C, 30 s; and 72°C, 6 min, and a final extension step of 72°C, 6 min. A PCR fragment of about 3 kb was obtained, ligated in pTOP2.1 (Invitrogen) and amplified in *E. coli* TOP10 cells (Invitrogen). A recombinant plasmid was selected, purified using the Qiafilter extraction Kit (Qiagen) and double-strand sequenced with universal and internal primers. The CaSUS2 cDNA was isolated by the same 3′ RACE PCR reaction as described for CaSUS1 cDNA isolation, except that mRNAs were from endosperm of fruits at 234 DAF. The UPIC4MI1 5′-GAA AGC GCT AGA GAA CTC TTG ATC GAG TA-3′ primer used was deduced from a SUS-encoding contig formed by the compilation of 12 ESTs that did not cluster with the CaSUS1 cDNA. PCR was performed as follows: initial denaturation 94°C, 1 min, and 35 cycles of 94°C, 30 s; 52°C, 30 s; and 72°C, 6 min, with a final extension step of 72°C, 6 min. A PCR fragment of about 3 kb was obtained, ligated in pTOP2.1 (Invitrogen) and amplified in *E. coli* TOP10 cells (Invitrogen). A recombinant plasmid was selected, purified using the Qiafilter extraction Kit (Qiagen) and double-strand sequenced with universal and internal primers. The CaSUS2 cDNA was isolated by the same 3′ RACE PCR reaction as described for CaSUS1 cDNA isolation, except that mRNAs were from endosperm of fruits at 234 DAF. The UPIC4MI1 5′-GAA AGC GCT AGA GAA CTC TTG ATC GAG TA-3′ primer used was deduced from a SUS-encoding contig formed by the compilation of 12 ESTs that did not cluster with the CaSUS1 cDNA. PCR was performed as follows: initial denaturation 94°C, 1 min, and 35 cycles of 94°C, 30 s; 68°C, 6 min, with a final extension step of 72°C, 6 min. The PCR amplified fragment was treated as previously described. The CaSUS2 gene was amplified from genomic DNA (10 ng) of *C. arabica* cv. IAPAR 59 using the primers UPIC4MI1 and REVCA5 5′-GGG AGA CTC GCG CGG AGA CCA GAC ATC T-3′ according to the protocol from its corresponding cDNA. PCR conditions used for the CaSUS2 cDNA amplification were as described for CaSUS1, except that 40 cycles were performed. The fragment obtained was cloned in pTOPO2.1 (Invitrogen) and double-strand sequenced.

**Probe preparation**

The internal probe (probe A: 667 bp) of CaSUS1 was amplified by PCR using this cDNA as a template, the specific primers SUS10 5′-GGT ATC CTT AGA CCG GTG-3′ and SUS11 5′-GGA TCA AAA ACA TCA ATG CC-3′, and the Advantage2 Taq DNA polymerase under the following conditions: initial denaturation step 94°C, 1 min;
followed by 35 cycles of 94 °C, 1 min; 50 °C, 1 min; 72 °C, 3 min, with a final extension of 72 °C, 6 min. The 3′ probe (probe B: 544 bp) of *CaSUS1* was amplified under the same conditions except that the primers UPC5C3 5′-AGC GAG CTC CTT GCC AA-3′ and REV5C5 5′-CTT ATT ACA AAA TGA CAT TTG A-3′ were used. The *CaSUS2* probe (probe C: 589 bp) localized in the 3′ region of the *CaSUS2* cDNA was amplified with the primers UCP6 5′-ACT CTG CAA TGG TAA A-3′ and REV7C7 as described above, using this cDNA as template. All these probes were purified by ethanol precipitation in presence of 10% (v/v) NaAc pH 5.2, resuspended in water and quantified. Then 50 ng was labelled by random-priming with 50 Ci of [α-32P]dCTP (Amersham Biosciences) according to Sambrook *et al.* (1989).

Northern and Southern-blot analysis

Total RNA (15 µg) was denatured in 12.55 M formamide, 2.2 M formaldehyde, and 20 mM 3-(N-morpholino)-propanesulphonic acid (MOPS) buffer, pH 7.0 (also containing 5 mM Na-acetate and 0.1 mM EDTA) at 65 °C for 5 min and fractionated on a 1.2% (w/v) agarose gel containing 2.2 M formaldehyde in MOPS buffer (Rogers *et al.*, 1999a). Hybridization with UltrahybTM buffer (Ambion) and washing steps were performed according to the manufacturer’s recommendations. To ensure that equal amounts of total RNA were loaded, gels stained with ethidium bromide were performed for each northern blot analysis. RNA blots were prepared in duplicate and probed independently with probe A (*CaSUS1*) and C (*CaSUS2*). Genomic DNA was extracted from fresh coffee leaves as described previously (Marraccini *et al.*, 2001). For Southern-blot analysis, 10 µg were digested with restriction endonucleases DrdI, EcoRI and HindIII independently, separated on a 0.8% (w/v) agarose gel and finally transferred to Hybond N+ membranes (Amersham Biosciences). Hybridization and washings were carried out as described before (Marraccini *et al.*, 2001).

Multiple alignments and phylogenetic analysis

Phylogenetic analyses were conducted using MEGA version 3.0 (Kumar *et al.*, 2004). Multiple alignments of SUS proteins were obtained by CLUSTALW program (Thompson *et al.*, 1994) followed by manual adjustment. A phylogenetic tree was inferred by the Neighbor-Joining (NJ) method with Poisson distance. Bootstrap analysis was carried out (5000 trials) to assess support for individual nodes.

**Results**

**Coffee fruit growth**

Under the field conditions used in this study, fruits of *C. arabica* cv. IAPAR 59 completed their maturation within eight to nine months. Since fruits did not grow during the first two months following anthesis and fecundation (Fig. 2A), producing cherries of very small size (<2 mm), tissue separation was not possible at this stage, which explains the absence of data for sugars (Fig. 2C, D) and enzymatic assays (Fig. 3A, C) at 30 d after flowering (DAF). Between 60 and 90 DAF, a rapid expansion of the perisperm occurred, followed by the development of the endosperm which became detectable at 75 DAF and easily separable from the perisperm at 118 DAF, when perisperm and endosperm were present in equal proportions (Fig. 2B). Afterwards, the perisperm gradually disappeared up to the formation of a thin tissue known as the silver skin membrane, which surrounds the endosperm at the mature stage (Fig. 1C), making its analysis difficult between 205 and 234 DAF. On the other hand, the endosperm turned from a liquid (147 DAF) to the solid state (176 DAF), before dehydration during the month up to harvest (between 205 and 234 DAF). Over the same period, the observed increase of the cherry fresh weight appeared to be related exclusively to the increase in mass of the pericarp (Fig. 2B).

**Accumulation of sugars in coffee fruits**

Sugar content was measured in each tissue during fruit development. Reducing sugars (glucose and fructose) accumulated during the perisperm expansion phase up to 260 mg g⁻¹ dry weight (DW) at 89 DAF (Fig. 2C). An accumulation of sucrose was also observed over this period, albeit to lesser extent (32 mg g⁻¹ DW) in this tissue (Fig. 2D). After 89 DAF, both reducing sugars and sucrose...
decreased steadily in the perisperm as this tissue disappeared, but the reducing sugars/sucrose ratio always remained greater than 1. In the endosperm, the reducing sugars/sucrose ratio was greater than 1 during the two first developmental stages analysed (118 and 147 DAF). Then, during the following stages, the amount of reducing sugars gradually decreased to become almost undetectable in the mature endosperm (Fig. 2C), whereas sucrose accumulated close to 6% of the DW (Fig. 2D). In the pericarp, the reducing sugars and sucrose presented a sudden accumulation after 176 DAF, reaching, respectively, 280 and 180 mg g\(^{-1}\) of the DW at maturation (Fig. 2C, D).

**Enzymatic activities during coffee fruit development**

In order to evaluate the importance of sucrose-metabolizing enzymes during coffee fruit development, AI, SPS, and SUS activities were measured *in vitro* in protein extracts prepared from isolated pericarp (60–234 DAF), endosperm (118–234 DAF), and perisperm (60–205 DAF) tissues (Fig. 3A–C).

AI activity in the perisperm reached a maximum (5.52 μg reducing sugars h\(^{-1}\) μg\(^{-1}\) protein) at 60 DAF (Fig. 3A), therefore preceding the peak of reducing sugars detected at 89 DAF in this tissue (Fig. 2C). Thereafter, AI activity decreased with development becoming undetectable between 89 and 176 DAF. In the pericarp, AI showed two peaks of similar values (near 4 μg reducing sugars h\(^{-1}\) μg\(^{-1}\) protein) at 118 and 205 DAF without concomitant changes of reducing sugar content. Whatever the developmental stage analysed, no AI activity was detected in the endosperm. SPS activity remained low in the perisperm between 60 and 118 DAF, but was maximal (5.16 μg sucrose h\(^{-1}\) μg\(^{-1}\) protein) at 147 DAF and decreased slightly afterwards. In the pericarp, SPS activity was almost negligible during early development, but reached a maximum (6.98 μg sucrose h\(^{-1}\) μg\(^{-1}\) protein) at 205 DAF and decreased up to maturation. As for the perisperm, maximal SPS activity in the endosperm was observed at 147 DAF, and then decreased gradually towards maturation.

SUS activity was low in the perisperm from 60 to 118 DAF, but increased between 147 and 205 DAF. Activities showed similar patterns in both the pericarp and endosperm, with a small but continuous increase between 60 (pericarp) or 120 (endosperm) and 205 DAF, followed by a sudden increase at harvest time. In addition, activities at 234 DAF presented a similar range (near 23 μg sucrose h\(^{-1}\) μg\(^{-1}\) protein) and paralleled the sucrose accumulation observed in these tissues (Fig. 2D), as well as being relatively constant over the day (data not shown).

A western blot analysis of endosperm proteins was also carried out using antibodies against the major SUS isoform of pea teguments, probably corresponding to the product of the *PsSUS1* (GB accession number AJ012080) gene (C Rochat, personal communication). Under semi-denaturing
electrophoresis conditions, a single SUS isoform was recognized in protein extracts obtained at 118 and 147 DAF (Fig. 3D). A weaker signal was also observed at 176 DAF, but no antibody cross reaction was detected near maturation, at 205 and 234 DAF.

Analysis of 14C distribution in coffee fruits

To analyse sugar metabolism and transfer in coffee cherries, pulse–chase experiments were performed by incubating leaves or green (±120 DAF) fruits with 14CO2 and measuring the distribution of radioactivity in the different fruit tissues after 24 h (Fig. 4A). When 14CO2 incubations were carried out with fruits, most of the radioactivity remained in the pericarp, but a significant proportion was detected in the perisperm and endosperm. When 14CO2 was supplied to leaves, low radioactivity was found in the pericarp, whereas the perisperm accumulated the major proportion of radioactivity. In both experiments, the large accumulation of radioactivity detected in the perisperm reveals the importance of this tissue in photosynthetic translocation within coffee cherries. The distribution of radioactivity was also determined in fruits after 4 h of the incubation with 14CO2 and a similar situation was observed, although less radioactivity was detected in the fruit tissues (data not shown).

Feeding with 14C-sucrose and 14C-fructose was carried out by applying the labelled compounds to a cut made at the peduncle insertion in the fruit (Fig. 4B–D). In the pericarp, high levels of radioactivity (total and specific) were found in the form of sucrose when labelled sucrose was fed, and as reducing sugar (mainly fructose) when feeding was performed with labelled fructose. Although this experiment did not allow a clear conclusion to be drawn as to the participation of the pericarp in the assimilation process, it did provide information on sugar metabolism in each tissue. For example, the significant accumulation of radioactivity in the perisperm indicates a role for this tissue in the transfer of sugars between the pericarp and the endosperm (Fig. 4B). Besides the transport of labelled sucrose and fructose from the pericarp to perisperm and endosperm, these sugars were readily metabolized to reducing sugars and sucrose, respectively (Fig. 4C, D), indicating the simultaneous synthesis and degradation of sucrose within these tissues. When 14C-sucrose was supplied, the perisperm presented a higher specific radioactivity in the form of sucrose and reducing sugars than for the same sugars found in the endosperm (Fig. 4C). The opposite was observed when 14C-fructose was fed, with the highest sucrose specific radioactivity being found in the endosperm, indicating that active conversion of reducing sugars to sucrose occurred in
Fig. 4. Analysis of sucrose transport and metabolism in coffee cherries by $^{14}$C pulse–chase experiments. (A) Fruits at 120±20 DAF (dotted bars) or leaves (grey bars) were exposed to $^{14}$CO$_2$ for 24 h and total radioactivity was measured in pericarp (pc), perisperm (ps), and endosperm (en) tissues. A schematic representation of this experiment is also given (right panel of graph A). For feeding experiments (B–D), labelled compounds were applied to a cut made at the peduncle insertion of fruits at 120±20 DAF as shown in the schematic representation given in the right panel of graph B. Fruits were then kept illuminated for 24 h and dissected to measure the radioactivity in each tissue. (B) $^{14}$C-sucrose (black bars) or $^{14}$C-fructose (white bars) were administered and their distribution was estimated by measuring the total radioactivity. After $^{14}$C-sucrose (C) or $^{14}$C-fructose (D) feedings, the distribution of the radioactivity in sucrose (black bars) and reducing sugars (white bars) was evaluated in pericarp, perisperm and endosperm tissues. (E) Fruits at different developmental stages were detached and incubated with $^{14}$CO$_2$ to determine the distribution of radioactivity in each tissue.
this tissue. Such conversions also occurred in the pericarp where the same specific radioactivity was found in sucrose and reducing sugars when $^{14}$C-fructose was used in the feeding experiment.

In a third feeding experiment, fruits at different developmental stages were detached and incubated with $^{14}$CO$_2$ (Fig. 4E). These data show that even during its disappearance with fruit growth, the perisperm retains its role as a transit tissue. Fruit photosynthesis also sharply decreases with the maturation of coffee fruits, as suggested by the gradual decrease of radioactivity in all tissues, mainly in the pericarp.

**Histological study of coffee fruits**

Tissue organization and evolution was analysed by histological sections of fruits at different developmental stages (Fig. 5A–D). At 40 DAF, the perisperm constituted the main tissue of the fruit and the endosperm was not apparent (Fig. 5A). The latter began to develop internally to the perisperm between 60 and 75 DAF (Fig. 5B). At the mature stage (205–234 DAF), the endosperm filled the entire inner space of the locule while the perisperm was reduced to a few cell layers surrounding the endosperm (Fig. 5C). Throughout development the perisperm always appeared tightly connected to the endocarp by the funicule (pedicel). At the funicule/perisperm boundary, a symplastic continuity was observed with xylem and phloem vascular tissues entering directly in contact with the perisperm. These vascular tissues were not observed within the perisperm or endosperm tissues. Also, no vascular connection was observed between the pericarp and the perisperm. The endocarp is a thick cellulosic tissue (Fig. 1C) which is present from the beginning of fruit formation and seems to provoke the isolation of the perisperm. Therefore, if some vascular connection between pericarp and perisperm truly exists, it could only be through the peduncle. Whatever the developmental stage, the perisperm surface facing the endocarp had an epiderm formed by a cuticle-like layer.

![Histological analysis of coffee fruits at different stages of ripening.](https://example.com/fig5.png)
(Fig. 5A–C). It could also be observed that perisperm cells in contact with endosperm cells lose their shape, indicating that they are undergoing important changes supposedly ending in cell death (see black arrows in Fig. 5B and C), since at the end of fruit development the perisperm is reduced to a few cell layers (Fig. 5C). Lugol’s iodine staining also showed that young perisperm tissue (60–75 DAF) contained starch granules that were no longer observed in the regions close to the growing endosperm (Fig. 5D).

Analysis of CaSUS1 and CaSUS2 cDNA sequences

The CaSUS1 cDNA is 2979 bp long containing a 188 bp 5’ untranslated region, a 293 bp 3’ untranslated region including a putative poly(A) addition signal site (TAATA) located 18 bases upstream of a polyA tail of 77 adenosine residues. It also has a single open reading frame coding for a protein of 806 amino acids with a theoretical molecular mass of 92.5 kDa and an estimated isoelectric point (pI) of 6.70. This protein contains a typical SUS motif (pfam00862) in its first 554 amino acid residues and a glycosyl transferase motif (pfam00534) in its C-terminal part (amino acids residues 565–727), confirming that it belongs to the SUS family. At the amino acid level, it shows high homology (95% with 89% of identity) with SUS deduced from the Sus3-65 and Sus4-16 genes of Solanum tuberosum (Fu and Park, 1995). In comparison with other SUS proteins, the CaSUS1 isoform contains a putative phosphorylation site LTRVHSLR (amino acid residues 6–13), with Ser-11 being the probable site of this modification, and two hydrophobic domains of 21 amino acids (motif I: 269–289; motif II 675–695) that may be involved in the binding of this protein to membranes.

The CaSUS2 full-length cDNA is 2889 bp long and contains 31 bp of 5’ untranslated region, 377 bp of 3’ untranslated sequence, a poly A tail of 45 adenosine residues and an open reading frame coding for a protein of 811 amino acids. The predicted molecular mass of CaSUS2 protein is 92.8 kDa with an estimated pI of 6.57. This protein shows high similarity to the SUS2 isoform from potato (AAO67719), CitSUSA from Citrus unshiu (BAA88904) and CppS2 from the resurrection plant Craterostigma plantagineum (Hochst.) (CAB38022) with 92%, 91%, and 90% similarity, respectively. The N-terminal putative phosphorylation site of CaSUS2 differed from the CaSUS1 sequence, but retained the Ser-11 amino acid residue. By comparison, these two coffee SUS isoforms showed only 68% of identity and 82% of similarity, therefore demonstrating that they are phylogenetically distant.

Isolation of the SUS2 gene from C. arabica

The genomic sequence of C. arabica for the CaSUS2 gene was amplified by a PCR-reaction using primers localized at the extremities of its corresponding cDNA. Sequence analysis revealed that this gene contains 15 exons interrupted by 14 introns, all of them bordered by the 5’-GT/3’-AG consensus. Its structure was strictly identical to that observed for the group of dicot SUSA genes characterized by the split of exons 6 and 12 (Komatsu et al., 2002). Nucleic sequences of the CaSUS2 gene in common with the CaSUS2 cDNA revealed that this gene truly encodes for the cloned cDNA.

Southern blot analysis

The structure and complexity of CaSUS1 and CaSUS2 genes in the C. arabica genome was examined by Southern blot analysis conducted using the probes A and B of CaSUS1 cDNA and the probe C of CaSUS2 cDNA (Fig. 6). In all cases, the hybridization patterns obtained were
different, indicating that the probes were gene-specific under the stringent conditions used. For the internal probe of CaSUS1, single bands were observed with Dral (5.2 kb) and HindIII (5.8 kb) digestions, whereas three bands (2.6, 0.45, and 0.3 kb) were detected with the EcoRI digestion (Fig. 6A). These results were those expected from a comparison of the position of the probe with the restriction map of the CcSUS1 gene from C. canephora (Leroy et al., 2005). When using the CaSUS1 distal probe (Fig. 6B), the Dral fragment at 5.2 kb and the EcoRI fragment at 2.6 kb were conserved. This is to be expected considering that no restriction sites for these enzymes are supposed to exist in the CaSUS1 gene. For the HindIII digestion, three bands were observed, a strong one at 1.5 kb and two others at 2.8 kb and 3.0 kb since only one HindIII restriction site is supposed to exist in the SUS1 gene of C. canephora.

For CaSUS2, hybridization revealed a fragment of 6.4 kb when the Arabica genome was digested with HindIII (Fig. 6C). From the CaSUS2 gene sequence and HindIII digestion, at least two bands should be expected: one around 400 bp and another greater than 560 bp. With EcoRI digestion, three signals were observed: one at 2.7 kb and two others at 1.3 (faint band) and 1.1 kb. For the Dral analysis, three bands were also revealed at 3.2 kb for the higher and 1.2 (faint) and 0.9 kb for the smaller. For these two digestions, only two bands should be expected as the probe overlapped a genomic region with only one EcoRI and Dral restriction site.

**Expression of SUS genes in individual tissues of coffee fruits**

Cherries were collected regularly between 30 DAF to 234 DAF. To analyse the expression of CaSUS1 and CaSUS2 genes, total RNA was extracted from entire fruits as well as individually from the pericarp (60–234 DAF), perisperm (60–176 DAF), and endosperm (118–234 DAF). CaSUS1 transcripts of approximately 2.9 kb were strongly detected in the pericarp, with peaks at 60 and 147 DAF (Fig. 7B), and also at immature stages of perisperm (60–89 DAF) and endosperm (118–147 DAF) development (Fig. 7C, D). CaSUS1 gene expression was not observed in the later stages of perisperm (118–176 DAF), pericarp and endosperm development (205–234 DAF). As expected, the CaSUS2 mRNA was also approximately 2.9 kb. CaSUS2 transcript levels were maximal at later stages of pericarp (205–234 DAF) and endosperm (234 DAF) development (Fig. 7B, D). Weak CaSUS2 expression was also observed in the early stages (60 and 89 DAF) of perisperm development (Fig. 7C), as well as at 176 DAF. For both experiments, the hybridization pattern of entire fruits with CaSUS1 and CaSUS2 probes coincided quite well with those detected individually for each tissue (Fig. 7A).

Because the perisperm tissue was reduced to a thin membrane (silver skin) surrounding the endosperm (bean)

![Fig. 7. Expression of CaSUS1 and CaSUS2 genes during coffee fruit development.](https://academic.oup.com/jxb/article-abstract/57/12/3243/449464)
Discussion

Sugar partitioning during coffee fruit development

In higher plants, sugar metabolism has been shown to be essential for the control of seed development, mainly through the regulation of the source/sink process (Herbers and Sonnewald, 1998). For example, the high ratio of hexoses (H) to sucrose (S) observed in embryos of Vicia faba characterize the phase of intensive cell division (Weber et al., 1998). The transition of the pre-storage phase to the maturation (storage) phase, when cell elongation and differentiation occur, is characterized by a clear switch in carbohydrate state, from a high to low H/S ratio. In sink organs of most plant species, these changes are controlled by the sucrose-cleaving enzymes like invertase, which has several isoforms differing in their biochemical properties and cellular localization, and SUS (Sturm and Tang, 1999). While invertase only functions in the direction of sucrose hydrolysis, SUS is a reversible enzyme capable of degrading and synthesizing sucrose. In this study, high H/S ratios coincided with the expansion phases of fruit tissues, as in the perisperm at 89 DAF, the endosperm between 118–147 DAF, and the pericarp between 205–234 DAF (Fig. 2). In the perisperm, the transition from a high to a low H/S ratio also corresponded to its gradual disappearance and to the beginning of endosperm growth. The same was observed in the endosperm where transition from a high to low H/S ratio coincided with the end of volume increase and the beginning of the storage phase (Rogers et al., 1999a).

In the perisperm, the highest concentration of reducing sugars was observed at 89 DAF (Fig. 2C) while maximal AI activity was found at 60 DAF (Fig. 3A). No particular variations of SUS activity were observed at this period. However, in part, these reducing sugars could come from starch degradation, as indicated by the histological study of young (60–75 DAF, Fig. 5D) perisperm (see below), but not apparently from the cleavage of sucrose imported from the leaves or pericarp. Maximal SPS activity in the perisperm and endosperm tissues was detected at 147 DAF, when the perisperm was in decline and the rapid growth phase of the endosperm terminated. Despite the generally held involvement of SPS in sucrose resynthesis, activities were not accompanied by sucrose accumulation in these tissues. However, they did overlap temporally with the synthesis of 11S storage proteins (Rogers et al., 1999a), the beginning of polysaccharide accumulation (Redgwell et al., 2003), and \( \alpha \)-galactosidase activity (Marraccini et al., 2005), essential for galactomannan deposition in endosperm cell walls. Therefore, SPS activity detected here appeared to be well co-ordinated with the differentiation of the endosperm into storage tissue, as reported during cotyledon development of Vicia faba (Weber et al., 1996). Other evidence of a lack of relationship between sugar concentration and enzymatic activities was obtained with the pericarp (Fig. 3A), where maximal AI activity observed at 118 DAF was not followed by reducing sugar accumulation, as well as with the perisperm at 176 and 205 DAF.
(Fig. 3C) where no changes of reducing sugar and/or sucrose contents paralleled SUS activity. A similar but inverse situation was observed in the endosperm, where the level of reducing sugars at 118 DAF was not accompanied by a significant increase of either AI and SUS activities. The degradation and (re)synthesis cycle of sucrose as well as the existence of rapid sugar transfers between the perisperm and endosperm tissues, rather than measurements of enzymatic activities per se, could explain these results (Nguyen-Quoc and Foyer, 2001). Such a complex interaction among tissues is also supported by the pulse–chase experiments with $^{14}$C-fructose and $^{14}$C-sucrose and the specific radioactivity of sucrose and reducing sugars found in the different tissues. In addition, the action of other sucrose-cleaving enzymes, for instance, neutral invertases (Hubbard et al., 1991) and the presence of enzymatic activities in tissues and compartments that are difficult to investigate, cannot be excluded. This might be the case of immature (0–60 DAF) perisperm where maximal AI activity was measured at 60 DAF (Fig. 3A) or with the endosperm which can be identified a few days after anthesis (De Castro and Marraccini, 2006), but can only be easily separated from the perisperm after 118 DAF. Finally, the presence of extracellular (apoplastically localized) invertase, facilitating sucrose transfers out of maternal tissues (Weber et al., 1997; Nguyen-Quoc and Foyer, 2001), could have been missed during tissue isolation.

By contrast, SUS activities detected in the pericarp and endosperm tissues between 176 and 234 DAF (Fig. 2D) were paralleled closely by sucrose accumulation in these tissues (Fig. 3C). In addition, the large accumulation of reducing sugars occurring in the pericarp at 205 and 234 DAF (Fig. 2C), also reported by Marín-López et al. (2003), might arise from the relatively high AI activity measured in this tissue (Fig. 3A). It could also result from the degradation of complex polysaccharides since transcripts of polygalacturonase (EC 3.2.1.15), that parallel those of ACC (1-aminocyclopropane-1-carboxylic acid) oxidase, were reported for late stages of pericarp maturation (Cação et al., 2003; Pereira et al., 2005).

The C partitioning and sugar exchanges existing between the perisperm, pericarp, and endosperm tissues of fruits was investigated by pulse–chase experiments with $^{14}$C-labelled compounds (Fig. 4). In the experiment with $^{14}$C-O$_2$, incubation of leaves yielded more radioactivity in the pericarp than fruits exposed to $^{14}$CO$_2$. This indicates that the pericarp retains most of the carbon assimilated in loco, while the carbon consumed by the endosperm comes predominantly from the leaves. Vaast et al. (2005) estimated that photosynthesis by the pericarp may account for approximately 30% of the total carbon allocated in the fruit. Perhaps this might be due in part to a lack of vascular connections between the pericarp and the other fruit tissues, as mentioned above. In this study, it is also shown that the pericarp contribution to CO$_2$ assimilation decreased gradually with maturation, since less radioactivity was found in the tissues of mature (reddish) fruits exposed to $^{14}$CO$_2$ than in younger ones (Fig. 4E). Independently of the pathways used to transport sucrose between the different tissues of coffee fruits, these experiments highlight the existence of intensive exchange of sugars between fruit compartments which occur mainly through simultaneous biosynthetic and catabolic processes of sucrose metabolism.

Coffee fruit histology: role of the perisperm tissue during bean development

Coffee fruits have a quite peculiar tissue organization and development (De Castro and Marraccini, 2006). In *C. arabica*, the perisperm arises from the nucellus and constitutes the predominant tissue occupying the inner space (locule) of fruits until 90–100 DAF. This locule space is delimited by the endocarp (also called the parchment layer; Fig. 1C), which is a hard and cellulose-rich tissue present from the early stages of fruit development. As the fruit grows, this space is rapidly occupied by the endosperm while the perisperm, at this point referred to as the silver skin, becomes a thin tissue reduced to few cell layers surrounding the endosperm.

For the first time, the histological study presented here clearly reveals the connections existing between these tissues. This identified the presence of vascular tissues originating in the central endocarp region and entering directly into contact with the perisperm exclusively by the funicule extremity (Fig. 5A). This situation persists during further fruit development, even when the perisperm is reduced to the silver skin. The epiderm of the perisperm facing the endocarp also contains cell layers showing a modified cell wall structure and probably undergoing death. At the moment, it is not known whether this particular differentiation would favour or impede solute exchanges. Whatever the situation, the continuity observed between vascular tissues of the funicule and the perisperm indicates the symplastic unloading of photosynthates from the sieve tube (Patrick and Offler, 1995) to the perisperm and subsequent diffusion within this tissue.

At early and late developmental stages, a cuticle-like layer was also detected on the surface of the perisperm facing the endocarp, but not on the perisperm side facing the endosperm. By analogy with the structure and function of other plant seeds, this cuticle should form an impermeable barrier to apoplastic transfers by isolating a sector of the seed apoplast from the perisperm and preventing solute delivery by passive (diffusion) mechanisms (see review by Patrick and Offler, 1995). Finally, the large number of plasmodesmata present in endosperm cells (see review by De Castro and Marraccini, 2006) argues for simple diffusion or symplastic transfer of solutes in this tissue.

Histological studies also revealed the presence of starch granules in the perisperm (60–75 DAF; Fig. 5D), but not in the cells closer to the endosperm, suggesting starch
hydrolysis in this particular region. This disappearance of starch could also explain the peak of reducing sugars detected in the perisperm at 89 DAF. When transferred into the endosperm, reducing sugars could provide the energy necessary for cell division and elongation and function as precursors for the synthesis of storage protein and polysaccharides. Previous observations also indicate the function of the perisperm as a tissue capable of providing the endosperm with additional precursors, such as organic acids for the synthesis of chlorogenic acids (Rogers et al., 1999b).

The investigation also shows that the role of the perisperm is not limited to transient accumulation of biochemical precursors. Even when reduced to a thin membrane at 205 DAF, the results presented here clearly demonstrate that the perisperm retained the capacity to transcribe genes, such as CaSUS2 for example (Fig. 7E), and to retain SUS enzymatic activities (Fig. 3C). Taken together with the results of the pulse–chase experiments, these data clearly demonstrate the important function of the perisperm tissue in bean development.

Two isoforms of sucrose synthase are expressed in coffee fruits

The coincidence of SUS activities and sucrose accumulation observed in pericarp and endosperm tissues between 176 and 234 DAF (Figs 2D, 3C) led to the performance of an in-depth study of the molecular characteristics of SUS. The use of EST sequence data available from the Brazilian Coffee Genome Project allowed the isolation of two SUS-encoding cDNAs of similar size, but differing in the
primary amino acid sequence of the proteins they encode. The deduced amino acid sequence of the CaSUS1 cDNA showed a high degree of similarity with SUS of the Dicot SUS1 group (Fig. 9) frequently identified in sink organs of other plant species, as with SUS3, SUS4 from potato tubers (Fu and Park, 1995), and SUS1 from pea seed coat (Dégardin et al., 1997) and citrus fruit (Komatsu et al., 2002). By contrast, the deduced amino acid sequence of CaSUS2 cDNA presented high sequence similarity with SUS of the dicot SUSA group, such as SUSA from C. unshiu (Komatsu et al., 2002). This protein also showed relatively high identity (82%) and homology (90%) to the SS2 protein coded by the CpSS2 (AJ132000) gene from the resurrection plant Craterostigma plantagineum (Kleines et al., 1999).

At the amino acid level, the SUS1 and SUS2 proteins shared only 68% of identity and 82% of similarity, therefore agreeing with the existence of two differentially expressed and non-allelic loci for this enzyme in coffee. The phylogenetic organization of coffee sucrose synthases into at least two distinct groups was reinforced by the comparison of SUS-encoding genes (Fig. 9). Indeed, the structure of the previously reported CcSUS1 gene of C. canephora (Leroy et al., 2005) clearly differed from the SUS2 gene of C. arabica described here, mainly by the split of the exons 5 and 11 observed in the latter, but not in the former. In this regard, the organization of the coffee CcSUS1 gene is similar to that of the AtSUS1 and CitSUS1 genes, respectively, from A. thaliana and C. unshiu, whereas that of the CaSUS2 appeared to be close to the genes belonging to the dicot SUSA group (Komatsu et al., 2002; Baud et al., 2004).

At the nucleic acid level, CaSUS1 cDNA from C. arabica (this study) presented 18 differences over the 2421 bp of the protein coding sequence of the CcSUS1 gene from C. canephora (Leroy et al., 2005). Fifteen of them were silent and did not modify the final amino acid sequence of the SUS1 protein; two others were considered as neutral (F532 and R663) in CaSUS1 changed, respectively, by Y and K amino acid residues in CcSUS1) and only one led to a radical amino acid change (G259 in CaSUS1 changed E in CcSUS1). None of these changes affected the N-terminal phosphorylation site, the putative hydrophobic domains, or the theoretical pl of the proteins and 5′ and 3′ untranslated regions, suggesting that these sequences were highly conserved during evolution.

In comparison with the CcSUS1 gene sequence published for C. canephora (Leroy et al., 2005), the Southern blot analysis performed with the distal probe of the CaSUS1 revealed additional bands (Fig. 6B, lines E, H). The same was observed when using the CaSUS2 probe overlapping the 3′ untranslated region of this cDNA (Fig. 6C). Because C. arabica is amphidiploid resulting from a natural cross between diploid species C. eugenoides and C. canephora (Lashermes et al., 1999), these patterns could characterize sequence divergence in the 3′ regions of independent SUS1 and SUS2 genes coming from each parent. Alternatively, they could correspond to hybridization of the probe with more distant SUS-encoding genes. Whatever the possibility, the simple patterns obtained suggest that CaSUS1 and CaSUS2 genes were probably unique in the Arabica genome. Sequence alignment of full-length CaSUS1 cDNA with a partial SUS1 cDNA (AJ575256) previously amplified by RT-PCR using degenerated primers deduced from conserved SUS domains (Marraccini et al., 2003), revealed eight differences. Interestingly, all of them affected the third base of codons, without modifying the amino acid composition of the deduced SUS1 protein. Since both CaSUS1 cDNA sequences were amplified from the same cultivar (IAPAR 59) of C. arabica and verified by double-strand sequencing, this suggests that they originated from different alleles of the CaSUS1 gene.

CaSUS1 and CaSUS2 genes are differentially expressed and may assume different functions

Transcripts of CaSUS1 were abundant in the pericarp at 60 and 147 DAF. They were also high at the immature stage of perisperm and endosperm development and decreased towards the ripening stage. By contrast, mRNA levels of CaSUS2 were undetectable in young endosperm and barely detectable in young pericarp and perisperm, but increased towards the ripening of pericarp and endosperm tissues. The high CaSUS2 gene expression observed at 205 and 234 DAF in the pericarp and at 234 DAF in the endosperm overlapped perfectly the peaks of SUS activity and sucrose concentration measured at the latest developmental stages of these tissues. The differential expression of CaSUS1 and CaSUS2 genes presented here showed great similarities, respectively, with the expression profiles of CitSUS1 and CitSUSA genes of C. unshiu (Komatsu et al., 2002). In this species, the CitSUS1 gene was expressed early in the development of peel and edible tissues, whereas CitSUSA was expressed in the latest developmental stages of these tissues (Komatsu et al., 2002). In addition, sucrose accumulation and SUS activity coincided quite well with the CitSUSA expression profile and to a lesser extend that of CitoSUS1. In coffee, mRNA levels of CaSUS1 detected in the pericarp and in the early stages of perisperm and endosperm development were not accompanied by SUS activity or particular alterations of sucrose or reducing sugar contents. Even when SUS enzymatic assays were tested in the direction of sucrose degradation, no SUS activity was detected at the time of CaSUS1 gene expression (data not shown). However, western blot analysis using antibodies raised against the SUS1 isoform of pea teguments (Dégardin et al., 1997) clearly identified a SUS protein in the endosperm at 118–147 DAF (Fig. 3D), that probably corresponds to the CaSUS1 protein. This is also supported by the phylogenetic dendrogram, where both proteins clustered in the Dicot A group (Fig. 9) and by the perfect
overlapping of the western blot and CaSUS1 gene expression in the endosperm (Fig. 7D). The absence of SUS activity coincident with CaSUS1 gene expression could be explained by the necessity of post-translational modifications as shown in soybean, where the binding of ENOD40 peptide A to SUS was required to activate sucrose-cleaving activity at the early stage of root nodele organogenesis (Röhrig et al., 2004).

At the phylogenetic level, the CaSUS2 isoform was classified in the USA group of plant SUS (Fig. 9) which also contains the proteins coded by the AtSUS2 and AtSUS3 genes of Arabidopsis thaliana and CpSUS2 from the resurrection plant C. plantagineum (Komatsu et al., 2002; Baud et al., 2004). In the former species, the AtSUS2 gene was considered as a marker of seed maturation since its maximal expression was detected in seeds at 12 DAF and decreased towards maturation. By contrast, AtSUS3 transcripts were not detected before 12 DAF, but increased steadily after this time and also paralleled the sucrose accumulation observed during the late-maturation phase (Baud et al., 2002). In addition, AtSUS3 expression was up-regulated under dehydration, such as in leaves submitted to water deprivation (Baud et al., 2004). This could explain its high expression in the late-maturation phase of seed development where intense desiccation was reported (Baud et al., 2002). Interestingly, the CaSUS2 isoform also had great similarity with the SUS protein of CpSUS2 gene from C. plantagineum, whose expression increased in roots submitted to water depletion (Kleines et al., 1999). Although effects of water stress on SUS gene expression were not studied in the present investigation, the endosperm dehydration observed during the late stages of endosperm development (De Castro et al., 2005) or in roots of field-grown coffee plants (Fig. 8), could explain the high levels of CaSUS2 transcripts detected in these tissues.

Whatever the situation, the high CaSUS2 gene expression in pericarp and endosperm reported here argue strongly for an important role of this gene in sucrose accumulation in these tissues. This should be confirmed by comparing SUS activities and CaSUS2 gene expression in endosperms of Arabica (this study) and Robusta species that differ in their sucrose content (Ky et al., 2001; Campa et al., 2004). Such experiments are underway. In addition, the importance of CaSUS2 in cup quality through a control of sucrose metabolism is being verified by growing coffee trees under shade and also by reducing the number of fruits on the tree. In both cases it is known that cup quality is improved.

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References


De María CAB, Trugo LC, Moreira RFA, Werneck CC. 1995. Sink-and vascular-associated sucrose metabolism is being verified by growing coffee trees under shade and also by reducing the number of fruits on the tree. In both cases it is known that cup quality is improved.

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References


