Soluble Sugars, Enzymatic Activities and Gene Expression during Development of Coffee Fruits Submitted to Shade Condition

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SUMMARY

Effects of shade (50% light) condition on development and sugar metabolism was analyzed in fruits of *Coffea arabica* L. var. IAPAR 59. Fresh weight measurements of separated tissues (pericarp, perisperm and endosperm) showed that the increased size observed in beans of shaded plants was correlated with high perisperm development. Reducing sugar contents in the pericarp and endosperm of fruits from shaded plants appeared higher than in the same tissues of control ("full-sun") plants. On the contrary, sucrose content was slightly lower in shade than in control beans. At the enzymatic level, sucrose synthase activities detected in the latest stages (197-231 DAF) of perisperm development from shaded plants were higher than from of control, confirming the importance of this tissue in coffee fruit development. At the molecular level, the *CaSUS1* and *CaSUS2* gene expression was also analyzed in the endosperm from control and shaded fruits. Expression level of *CaSUS2* was higher in mature beans (260 DAF) of shaded plants than in control plants (231 DAF). These results showed that light condition affect sucrose metabolism of coffee beans.

INTRODUCTION

In coffee, many factors are known to influence "coffee cup quality", like the species or varieties cultivated, the plant age, harvest period and the post-harvest processes applied to obtain dried beans (Leroy et al., 2006). Shade either provided naturally or artificially was also reported to give high coffee quality probably by lowering tree stress, favoring slow ripening of cherries and adequate bean filling (Vaast et al., 2006). Near infrared reflectance spectrophotometry (NIRS) analyses of coffee beans showed positive correlations between fat matter content and altitude and shaded conditions (Decazy et al., 2003). In addition, inverse correlation was often observed between sucrose and caffeine, trigonelline and chlorogenic acids levels (Vaast et al., 2006). In a previous study, we analyzed sucrose metabolism during fruit development of *Coffea arabica* L. var. IAPAR 59 at the biochemical and molecular levels (Geromel et al., in press). It was shown that sucrose synthase (SUS: EC 2.4.1.13) had the highest activities during the last stage of endosperm and pericarp development that paralleled the accumulation of sucrose in these tissues. Pulse-chase experiments with ^{14}C-fructose and ^{14}C-sucrose also indicated the existence of intensive exchange of sugars between fruit compartments occurring mainly through simultaneous biosynthetic and catabolic processes of sucrose (Geromel et al., in press). Because sucrose is important precursor of

coffee flavor and aroma (Homma, 2001; Grosch, 2001), the objectives of the present work were to study the effects of shade on sugar metabolism.

MATERIALS AND METHODS

Plant material

Fruits were harvested from plants of *Coffea arabica* L. var. IAPAR 59 cultivated in field conditions (Agronomic Institute of Paraná State, Londrina-Paraná, Brazil). Plants were cultivated under control ("full-sun", FS: photosynthetic photon flux density of 2250 $\mu Em^{-2} s^{-1}$ at noon in summer season) or shaded (SH) conditions. In the latter case, light was artificially reduced using a net allowing 50% of the photosynthetic photon flux density.

Coffee sampling and processing

Cherries (100 gr) were randomly collected every four weeks from the flowering (09-2003) up to the complete maturation (05-2004) from 10 individual field-grown plants For sugar quantification and enzymatic analyses, cherries were immediately frozen in liquid nitrogen and stored at –80 °C.

Sugar content and enzymatic activities

Sugar contents and enzymatic activities were determined as described before (Geromel et al., in press).

Gene expression

RNA extraction, Northern-blot analysis and probe labeling were as described before (Geromel et al., in press).

RESULTS

Characteristics of fruit growth

In our field conditions, fruits from plants of *C. arabica* L. var. IAPAR 59 grown under FS and SH condition completed their maturation in around 231 DAF ± 30 and 260 DAF ± 30 respectively. Tissue fresh weights (FW) were determined separately for perisperm and endosperm at regular stages of coffee cherry development. Perisperm expansion was rapid after 60 DAF, with higher FW in SH compared to FS condition at 87 DAF (Figure 1A). The endosperm only became easily detachable from the perisperm at 120 DAF and reached FW of 0.47 ± 0.03 gr and 0.51 ± 0.07 gr at the mature stage of FS and SH conditions, respectively at the 231 DAF and 260 DAF (Figure 1B).

Reducing sugar and sucrose contents during coffee cherry development

Reducing sugar (RS: mainly glucose and fructose) contents were measured in separated tissues of fruits grown in FS and SH conditions (Figure 2A-B). The main difference between FS and SH conditions concerned the maintenance of higher hexose content ("sink") in the perisperm of SH fruits (Figure 2A and B). In the pericarp, RS were low up to 175 DAF and increased rapidly after this time to reach 404 mg g^{-1} DW and 326 mg g^{-1} DW respectively in SH and FS conditions. Sucrose was also accumulated during latest stages of endosperm

development, reaching 72.5 and 61.2 mg g^{-1} DW, respectively at 231 DAF in FS and 260 DAF in SH conditions (data not shown).

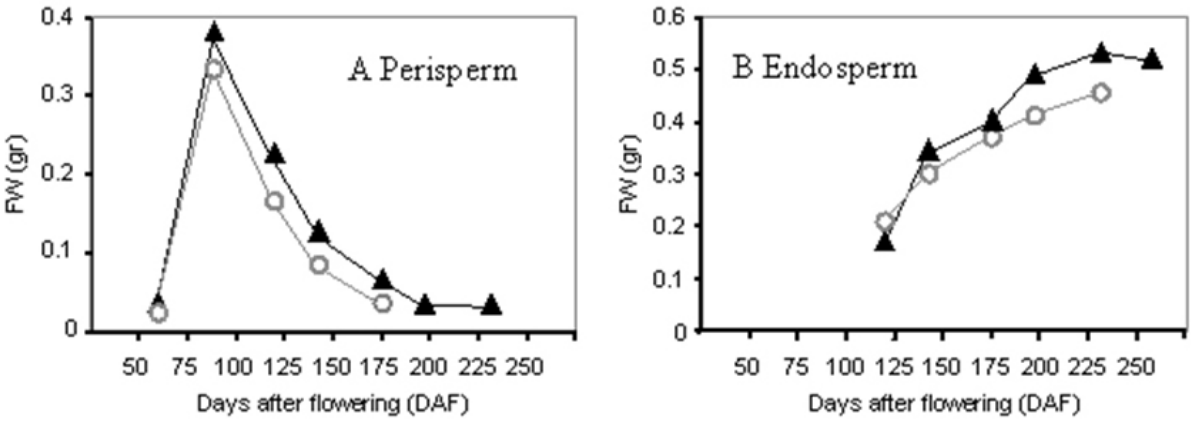

Figure 1. Evolution of tissue fresh weights during ripening of *C. arabica* L. var. IAPAR 59 fruits grown under "full sun" (open circle) and shade (closed triangle) conditions. Fresh weights are given in grams for perisperm (A) and endosperm (B) separated tissues.

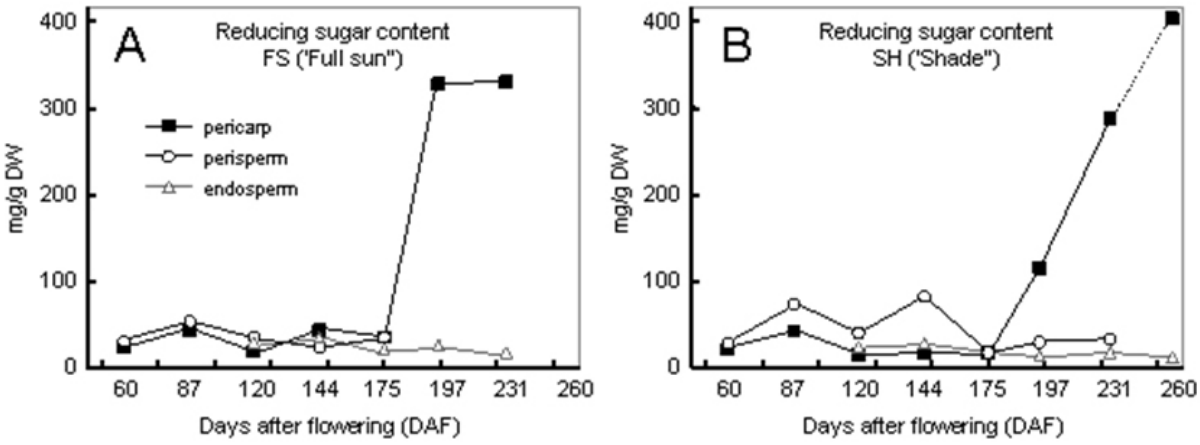

Figure 2. Evolution of reducing sugar contents in separated tissues from fruits of *C. arabica* L. var. IAPAR 59 grown under FS (A) or SH (B) conditions. Data are given mg.g^{-1} of dry weight (DW) in pericarp (black square), perisperm (open circle) and endosperm (open triangle) tissues.

Enzymatic activities during coffee fruit development

Sucrose synthase (SUS) activity was monitored in pericarp, perisperm and endosperm tissues separated from FS and SH cherries (Figure 3A-C). In the pericarp, SUS activities showed similar patterns in FS and SH conditions, with a continuous increase from 60 to 197 DAF where a peak was detected (Figure 3A). A regular increase of SUS activity was observed in the perisperm of FS cherries, particularly between 120 and 175 DAF (Figure 3B). SUS activity in SH perisperm was higher than in FS, maximal at 231 DAF and declined at the mature stage (260 DAF). In FS endosperm, SUS activities reached a peak at 175 DAF and decreased towards the harvest (Figure 3C). The profile differed in SH endosperm where maximal SUS activity was observed at the mature stage (260 DAF).

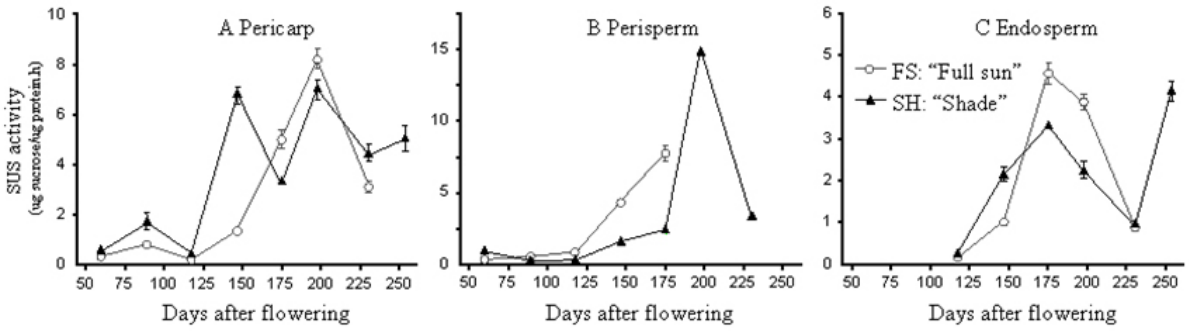

Figure 3. Profiles of SUS (sucrose synthase) activity during coffee fruit development. Activity was assayed in the sense of sucrose synthesis (µg sucrose. hr^{-1} μg^{-1} protein) in pericarp (A), perisperm (B) and endosperm (C) tissues separated from fruits grown in full-sun (FS: open circle) or shade (SH: black triangle).

Expression of SUS-encoding genes

CaSUS1 and *CaSUS2* gene expression was monitored during the maturation of endosperm that developed under FS and SH conditions. SUS expression profiles in FS endosperm for the maturation period 2003-2004 reported here where similar to those obtained in endosperm of fruits harvested from plants growing at FS condition in 2002-2003 (Geromel et al., in press). However, SH plants showed an early expression of *CaSUS1* in the endosperm, as well an increase on expression of *CaSUS2* during the late stages of development, compared with expression patterns observed in endosperm of FS plants.

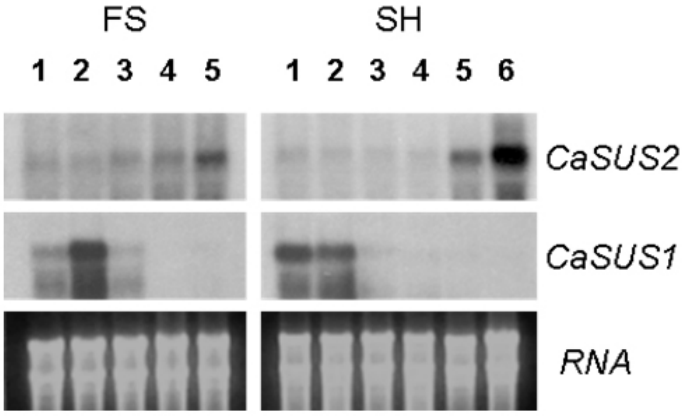

Figure 4. Expression of *CaSUS1* and *CaSUS2* genes in developing endosperm from fruits grown in full-sun (FS) or shade (SH). Total RNA (15 µg) isolated from endosperm at regular developmental stages (lane 1, 120 DAF; lane 2, 144 DAF, lane 3, 175 DAF; lane 4, 197 DAF; lane 5, 231 DAF and lane 6, 260 DAF) was separated in a formaldehyde-agarose gel and transferred onto a nylon membrane. Probes used correspond to *CaSUS1* and *CaSUS2* cDNA sequences (Geromel et al., in press). RNAs stained by ethidium bromide (bottom) were used to monitor the equal loading of samples.

CONCLUSION AND DISCUSSION

In coffee, the first tissue to develop soon after the fecundation is the perisperm and its volume defines the locule space that will be further occupied by the endosperm (bean) (De Castro and Marraccini, 2006). Here, we showed that the one month delay of the endosperm (bean)

development observed in SH condition was a consequence of the longest persistence of the perisperm tissue. In addition, high development of the perisperm tissue during early developmental stages in shade condition could also explain the increase in size of SH beans (Geromel et al., in preparation). We also reported higher reducing sugar content in pericarp of SH than in FS condition, a situation that was also observed in mature (dried) coffee beans (Geromel et al., in preparation). As reported in other works (Vaast et al., 2006; Decazy et al., 2003), lower sucrose content was observed in SH than in FS mature beans. Even limited, these data reinforce the idea that intensive sugar exchanges exist between the pericarp and endosperm (Geromel, in press) and that the slow down ripening process of shaded berries favors a complete filling of coffee beans (Vaast et al., 2006).

The decrease on light intensity affected SUS activities in coffee fruits. For example, SUS activity in the latest stages of perisperm development was significantly higher in SH than in FS condition. Even reduced to a thin membrane (silver skin) surrounding the endosperm at this time (De Castro and Marraccini, 2006), these observations confirmed the important function of this tissue in bean development process (Geromel, in press; Rogers et al., 1999). In a previous work, the CaSUS2 isoform of SUS was proposed to be the main enzyme responsible of sucrose accumulation because its expression coincided with the peak of SUS enzymatic activity and sucrose accumulation observed during the latest stages of endosperm development (Geromel, in press). On the other hand, the CaSUS1 isoform of SUS was proposed to function as a sucrose-degrading enzyme since its expression (by Northern-blot) and detection (by Western-blot) was never accompanied by sucrose accumulation (Geromel, in press). The situation appeared quite different here where SUS activities appeared lower than those measured in 2002-2003 and did not showed a continuous increase during latest stages of endosperm development. Since no acid invertase activity exists in endosperm (Geromel, in press; Geromel et al., in preparation), higher sucrose-degrading activity in SH than in FS mature endosperm could explain high RS and low sucrose contents in the former. At the molecular level, *CaSUS1* expression detected in young stages (120-175 DAF) of SH and FS endosperm correlates quite well with SUS activity in this tissue. High *CaSUS2* expression in SH endosperm at 260 DAF could also explain the SUS peak observed at the same time in this tissue. However, *CaSUS2* expression in FS endosperm at 231 DAF was not followed by SUS activity. This could be a consequence of the biannual bearing pattern commonly reported in sun-grown coffee plants (Vaast et al., 2006) since FS-grown plants analyzed here effectively presented high and low productivity in 2002-2003 and 2003-2004 respectively (data not shown). Another explanation could be that plants suffered of water limitation for several weeks in 2003-2004, mainly during endosperm expansion and storage phase (Geromel et al., in preparation). Finally, the existence of post-translation modification of SUS protein (Winter and Huber, 2000) could also explain the asynchrony between the transcription of SUS-encoding genes and SUS activities measured in the endosperm.

Altogether, these results showed that environmental (i.e. lighting) conditions modified sugar metabolism in developing coffee beans both at gene expression and enzymatic levels. Rapid degradation and (re)synthesis of sucrose between pericarp, perisperm and endosperm tissues as well as transfers of C-compounds between these tissues also explain the lack of clear relationships between sugar contents and enzymatic activities (Geromel et al., in press). Howeve

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