In Silico Characterization and Transcription Analysis of Two Alpha-Expansins Isoforms in *Coffea arabica* L.

I.G.F. BUDZINSKI[1], L.F.P. PEREIRA[2], T. SERA[1], L.G.E. VIEIRA[1]

[1]Plant Biotechnology Laboratory, Agronomic Institute of Paraná (IAPAR), Londrina, PR, Brazil
[2]Embrapa – Café, Brasília, DF, Brazil

SUMMARY

The development and maturation of coffee fruit reflects on grain size, cup quality and consequently better prices for the coffee produced. It is characterized by intense cell division, elongation and softening of cell walls in different fruit tissues. Those processes are related with the action of different proteins, including expansins. The objective of this work was to identify α-expansins genes expressed during coffee fruit development and maturation. Through *in silico* analysis in the Brazilian Coffee Genome Project database we selected two α-expansins genes present in fruit cDNA libraries. We observed that both isoforms (*CaEXPA1* and *CaEXPA2*) have signal peptide and specific N- terminal and C-terminal domains for expansins proteins. To study gene expression, we collected tissues (root, plagiotropic shoots, leaf, bud flower and flower) from *C. arabica* cv. IAPAR-59 and fruits from both cv. IAPAR-59 and cv. IAPAR-59 Graúdo (a genotype with larger fruit/grain size). Fruits were monthly collected after flowering. The transcripts of coffee *CaEXPA*1 and *CaEXPA*2 were observed at different coffee tissues and at different fruit ripening stages, indicating spatial and temporal differences on both isoforms transcription pattern. *CaEXPA*1 was mainly expressed during the early stages of fruit development and at the end of the fruit ripening period. On the other hand, *CaEXPA2* was only expressed in the last steps of fruit ripening.

INTRODUCTION

Coffee fruit growth is an asynchronous process, resulting in the presence of fruits in different stages of ripening in the same plant. The presence of green fruits and over-ripened fruits in the same batch of grains changes the acidity, the bitterness and consequently the quality of the product (Pereira et al., 2005). The early stages of coffee fruit development and ripening are characterized by intense cell division, perisperm development, elongation and softening of cell walls. Those processes are related with the action of different proteins, including expansins, pectin methylesterases, xyloglucanases. Expansins are plant cell-wall loosening proteins that induce cell wall extension and stress relaxation at acidic pH condition (McQueen-Manson et al., 1992). These proteins play roles in a diverse range of developmental process including fruit development and ripening (Rose et al., 1997; Brummell et al., 1999; Vidhu et al., 2005; Dotto et al., 2006; Vidhu et al., 2007; Ishimaru et al., 2007). Expansins represent a protein superfamily, that are formed by four families designated α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB) (Kende et al., 2004). Members of the EXPA and EXPB families are known to have wall-loosening activity (Cho and Kende, 1997; Cosgrove et al., 1997), whereas the other two families have been identified only from sequence homology, but protein function analysis has not been reported (Lee et al., 2001; Li et al., 2002).

MATERIALS AND METHODS

Through analyses on the Brazilian Coffee Genome Project database (http://www.lge.ibi.unicamp.br/cafe/), we selected two α-expansin isoforms named *CaEXPA*1 and *CaEXP*2, both containing sequences from fruit cDNA libraries. Predicted amino acid sequences were obtained using ORF finder at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). ScanProsite (http://www.expasy.org) was used to verify the two domains specific for mature expansin protein. The pollen allergen domain was verified for each isoform using BlastP and the signal peptide region was identified using SignalP (http://www.cbs.dtu.dk/services/SignalP/). To gene expression analysis, fruits were monthly collected, after flowering, from *Coffea arabica* cv. IAPAR-59 and *C. arabica* cv. IAPAR-59 Graúdo, at the experimental station of the Agronomic Institute of Paraná (IAPAR, Londrina, BR). We also collected tissues (root, plagiotropic shoots, leaf, bud flower and flower) from cv. IAPAR-59. Total RNA was isolated from different tissues and fruits at different stages of maturation from cv. IAPAR 59 and cv. IAPAR 59 Graúdo, according to Chang et al. One μg of total RNA was used to produce cDNA with Thermoscript^{TM} oligo DT System (Invitrogen) to amplify the two α-expansin genes, which were used as probes for transcript analysis. For Northern Blot analysis 15 ug of total RNA was transferred to nylon membranes and hybridized using UltraHyb solution. The specificity of each probe was tested through dot-blot analysis (date not shown).

RESULTS AND DISCUSSION

Through *in silico* analysis we observed that both *CaEXPA*1 and *CaEXPA*2 isoforms have a signal peptide, ranging from 21 to 30 aminoacids. They have the specific domains for expansins proteins, an N- terminal distantly related to the catalytic domain of glycoside hydrolase (GH-45) and a C-terminal domain distantly related to group-2 grass pollen allergens. *CaEXPA*1 and *CaEXPA*2 presented high identity (from 80% to 86% and from 79% to 85%, respectively) with expansins from other plants according to BlastX analysis. Northern blot from different tissues of coffee showed for *CaEXPA*1, transcripts in roots and bud flowers (Figure 1A) and for *CaEXPA*2 transcripts were observed in shoots (Figure 1B).

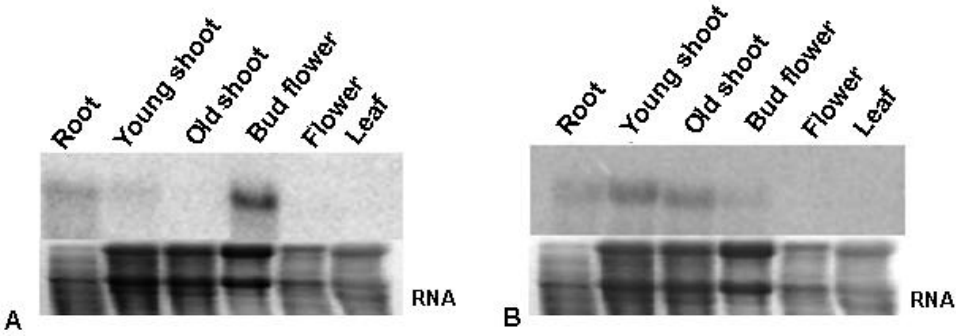

Figure 1. Northern blot analysis of two *C. arabica* α-expansin genes. Total RNA from root, young shoot, old shoot, bud flower and flower from *C. arabica* cv. IAPAR-59. A – Hybridization using *CaEXPA*1 as probe. B – Hybridization using *CaEXPA*2 as probe.

*CaEXPA*1 was similarly transcribed in both genotypes (Figure 2). Transcripts were detected during the early stages of fruit development and in the last steps of fruit ripening. For cv. IAPAR-59, increased transcripts were observed in November (60 days after maturation – DAF), a period of rapid expansion of the fruit and perisperm development (Figure 2). Interestingly, in the genotype cv. IAPAR-59 Graúdo, we also observed transcripts at 60 DAF; however, higher accumulation of transcripts was detected at 90 DAF (Figure 2). This

expression pattern of *CaEXPA*1 in cv. IAPAR-59 Graúdo probably indicates that in this genotype fruit expansion and perisperm development continues until 90 DAF, a longer period of time when compared to cv. IAPAR-59. Increased transcripts were also observed in April (210 DAF) for both cultivars. These results probably suggest the participation of *CaEXPA*1 in the fruit development and ripening process.

Out	Nov	Dec	Jan	Feb	Mar	April
Fruit initial expansion (Perisperm elongation)			Endosperm growth		Fruit maturation	

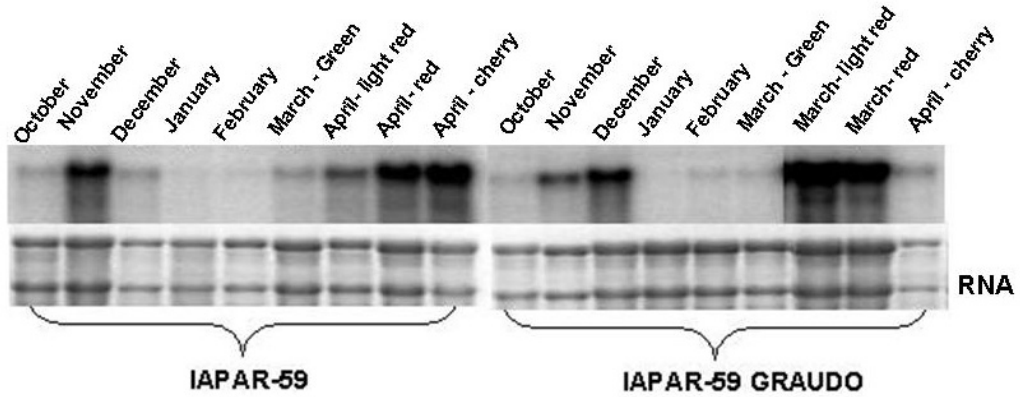

Figure 2. Northern blot of *CaEXPA1* during fruit development. Total RNA from the whole fruit of *C. arabica* cv. IAPAR-59 and cv.IAPAR-59 Graúdo in different stages of fruit development and ripening. Fruits were monthly collected after flowering: October (30 DAF), November (60 DAF), December (90 DAF), January (120 DAF), February (150 DAF), March (green - light red - red, 180 DAF) and April (light red - red and cherry, 210 DAF).

Out	Nov	Dec	Jan	Feb	Mar	April
Fruit initial expansion (Perisperm elongation)			Endosperm elongation		Fruit maturation	

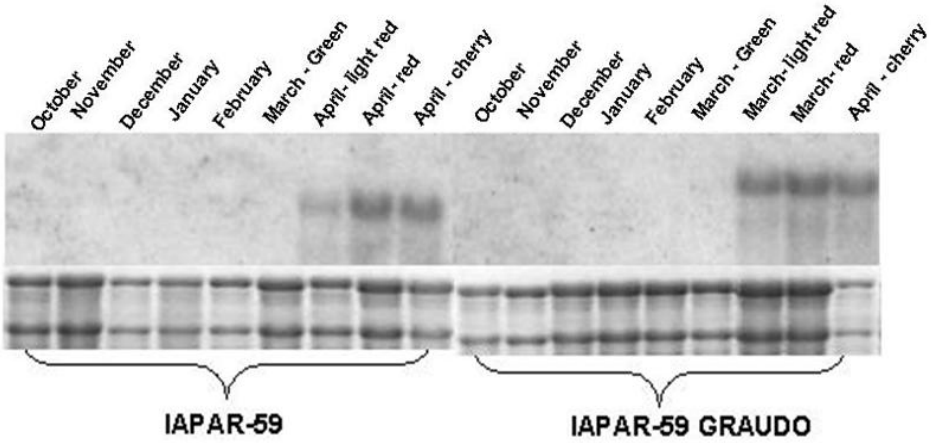

Figure 3. Northenr blot analysis of *CaEXPA2* during fruit development. Total RNA from the whole fruit of *C. arabica* cv. IAPAR-59 and cv.IAPAR-59 Graúdo in different stages of fruit development and ripening. Fruits were monthly collected after flowering: October (30 DAF), November (60 DAF), December (90 DAF), January (120 DAF), February (150 DAF), March (green - light red - red, 180 DAF) and April (light red - red and cherry, 210 DAF).

On the other hand, the isoform *CaEXPA2* (Figure 3), showed specific expression during the later stages of fruit ripening for cv. IAPAR-59 and cv. IAPAR-59 Graúdo, suggesting the involvement of this isoform in the process which involves the cell wall disassembly of pericarp. In climacteric fruit, as the case of *C. arabica*, fruit softening is found to be ethylene-dependent overall, which may imply that CaEXPA2 is probably regulated by ethylene. Some expansins are expressed in several organs and tissues type, whereas others shows very tight specificity in their spatial and temporal expression patterns (Rose et al., 1997; Cho and Kende, 1997), indicating that regulation of cell wall extensibility could be controlled at least in part by differential regulation of the different expansin genes (Reinhardt et al., 1998).

The findings presented in this work provided basic information about the participation of expansin in coffee fruit development and maturation and can be used for more detailed experiments involving coffee fruit. Our group is currently studying the pattern of transcription of *CaEXPA*1 and *CaEXPA*2 in each fruit tissue (pericarp, perisperm and endosperm), looking in detail for the spatial and temporal characterization of those genes.

ACKNOWLEDGMENTS

This research was supported by the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP&D-Café).

REFERENCES

Brummell D.A., Harpster M.H., Civello P.M., Palys J.M., Bennett A.B., Dunsmuir P. (1999) Modification of expansin protein abundance in tomato fruit alters softenbing and cell wall polymer metabolism during ripening. Plant Cell 2:2203-2216.

Cho H.T., Kende H. (1997) Expression of expansin genes is correlated with growth in deepwater rice. Plant Cell 1997, 9:1661-1671

Cosgrove D.J., Bedinger P., Durachko D.M. (1997) Group I allergens of grass pollen as cell wall-loosening agents. Proc Natl Acad Sci USA 94:6559-6564.

Dotto M.C., Martinez G.A., Civello P.M. (2006) Expression of expansin genes in strawberry varieties with contrasting fruit firmness. Plant Physiology and Biochemistry 44: 301-307.

Ishimaru M., Smith D.L., Gross K.C., Kobayashi S. (2007) Expression of three expansin genes during development and maturation of Kyoho grape berries. Journal of Plant Physiology 164: 1675-1682.

Kende H., Bradford K., Brummell D., Cho H.T., Cosgrove D.J., Fleming A., Gehring C., Lee Y., Queen-Mason S., Rose J., Voesenek L.A. (2004) Nomenclature for members of the expansin superfamily of genes and proteins. Plant Mol Biol 55:311-314.

Lee Y., Choi D., Kende H. (2001) Expansins: ever-expanding numbers and functions. *Curr* Opin Plant Biol 4:527-532.

Li Y., Darley C.P., Ongaro V., Fleming A., Schipper O., Baldauf S.L. (2002) Plant expansin are a complex multigene family with an ancient evolutionary origin. *Plant* Physiol 128:854-864.

McQueen-Manson S., Durachko D.M., Cosgrove D.J. (1992) Two endogenous proteins that induce cell wall expansion in plants. Plant Cell 4:1425-1433.

Pereira L.F.P., Galvão R.M., Kobayashi A.K., Cação S.M.B., Vieira L.G.E. (2005) Ethylene production and acc oxidase gene expression during fruit ripening of *Coffea araica* L. Brazilian Journal of Plant Physiology, v. 17: 283 – 289.

Reinhardt D, Witter F, Mandel T, Kuhlemeier C. (1998) Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. *Plant Cell* 10:1427-1437.

Rose J.K., Lee H.H., Bennett A.B. (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proceedings of the National Academy of Science 94:5955-5960.

Vidhu A.S., Chourasia A., Pravendra N. Softening in mango (*Mangifera indica* cv. Dashehari) is correlated with the expression of an early ethylene responsive, ripening related expansin gene, MiExpA1 (2005) Postharvest Biology and Technology 38: 223-230.

Vidhu A.S., Aniruddha S.P., Pravendra N. (2007) Multiple forms of α-expansin genes are expressed during banana fruit ripening and development. Postharvest Biology and Technology 45: 184-192.