Coffea Expansin Gene Family and Expansin Expression during Fruit Maturation

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SUMMARY

Expansins (EXP) are plant cell-wall loosing proteins involved in cell enlargement and developmental process such as organogenesis, seeds germination, cell wall dissolution and fruit ripening. Two families of EXP are known, α-expansin (EXPA), involved in the control of cell wall extension, and β-expansins (EXPB), the major allergens of grass pollen that also have cell wall-loosening activity. With the objective to study the role of EXP in coffee fruit maturation we selected EXP homologous sequences on the Brazilian Coffee Genome Project database. Full-length contigs were classified according the EXP family. Northern blots of pulp from fruits at the latest stages of maturation (22-32 weeks after flowering) showed increased transcription of one contig (*CaEXP1*). This higher transcription corresponds with a climacteric burst, during the expansion of pulp, with a decline in "cherry" fruits. The transcription of *CaEXP1* was also observed in roots, shoots, flower and flower buds, but not in leaves.

INTRODUCTION

Fruit softening is associated with cell wall disassembly mediated by the action of a complex of enzymes and proteins (Dotto et al., 2006). Expansins are a group of extracellular enzymes that directly modify mechanical properties of plant cell walls inducing cell wall extension and stress relaxation at acid pH condition (Li et al., 2002; McQueen-Manson et al., 1992). They act by causing a reversible disruption of hydrogen bonds between cellulose microfibrile and matrix polysaccharides, particularly xyloglucan, resulting in an irreversible elongation of plant cell walls. Localized expression of expansins is associated with the meristems and growth zones of the root and stems, as well as the formation of leaf primordia on shoot apical meristems (Reinhardt et al., 1998), expansins activity occurs in fruit softening (Brummell et al., 1999; Rose et al., 1997; Kalamaki et al., 2003; Yoo et al., 2003; Harrison et al., 2001; Obenland et al. 2003), abscission (Belfield et al., 2005), xylem formation (Gray-Mitsumune et al., 2004), seed germination (Chen and Bradford, 2000), penetration of pollen tubes through the stigma and style (Cosgrove et al., 1997; Pezzotti et al., 2002), formation of mycorrhizal associations with symbiotic fungi in root tissues (Balestrini et al., 2005), growth of parasitic plants (O'Malley and Lynn, 2000). Expansins represent a superfamily of plant proteins that are made of four families designated α-expansin (EXPA), β-expansin (EXPB), expansin-like α (EXLA) and expansin-like β (EXLB) (Kende et al., 2004). Members of the EXPA and EXPB families are known to have wall-loosening activity (Cho HT, Kende, 1997; Cosgrove et al., 1997; McQueen-Manson et al., 1992), whereas the other two families have been identified only from sequence homology, without protein function analysis (Lee et al., 2001; Li et al., 1992).

MATERIALS AND METHODS

In Silico Analysis

Contigs and singlets related with expansins were selected in the ESTs database of the Brazilian Coffee Genome Project (http://www.lge.ibi.unicamp.br/cafe/) through keyword search. After local Blast, all the sequences and contigs were clustering again using the Sequencher – Gene Codes program. Sequences were also analyzed by BlastX, BlastP at NCBI. Deduced amino-acid sequences were obtained using ORF finder at NCBI and aligned using Sequencher. ScanProsite (http://www.expasy.org) was used to verify the two domains specific for mature exp protein and to indicates an N-glycosilation linkage. The pollen allergen domain was verified for each using BlastP. The peptide signal region was observed using SignalP (http://www.cbs.dtu.dk/services/SignalP/). A phylogenetic tree was constructs (Mega 3.1 version), to verify the similarity degree within the coffee sequences and to determine their exp family (EXPA, EXPB, EXP-like-A and EXP-Like-B) according to *Arabidopsis thaliana* and *Oriza sativa* sequences from NCBI.

Total RNA was isolated from different tissues and pulp from fruits at different stages of maturation of *Coffea arabica* cv. IAPAR 59 according to Chang et al. (1993). One µg of total RNA was used to produce cDNA with Thermoscript^{TM} oligo DT System (Invitrogen) to amplify a Coffea exp gene. For Northern Blot analysis 10 ug of total RNA was transferred to nylon membranes and hybridized using UltraHyb solution.

RESULTS AND DISCUSSION

In Silico Analysis

Based on Brazilian Coffee Genome Project database 162 sequences from *EXP* were identified. After two rounds of clusterization, the sequences were saturated and aligned using the software Sequencher, forming 28 contigs. Contigs with ESTs from fruit libraries were listed in the Electronic Northern (Table 1).
The deduced amino-acid sequences were analyzed for the presence of expansin domains. A phylogenetic tree with heterologous EXP of different families showed that EXPA represents the major family in coffee genome. Contig 6 and 16 were classified as EXLB and contig 8 as an EXLA (Figure 1).

Table 1. Expansin coffee sequences including ESTs from fruit libraries.

Expansis	ESTs	Fruits and flowers	Leaves	Cells suspension	Flowers bud	Callus
CaEXPA1	28	4	5	13	1	2
CaEXPA2	11	1	X	1	4	3
CaEXPA3	8	2	4	X	1	1
CaEXPA4	8	3	X	1	3	3
CaEXPA5	51	2	3	3	X	2
CaEXPA6	4	1	1	X	X	X

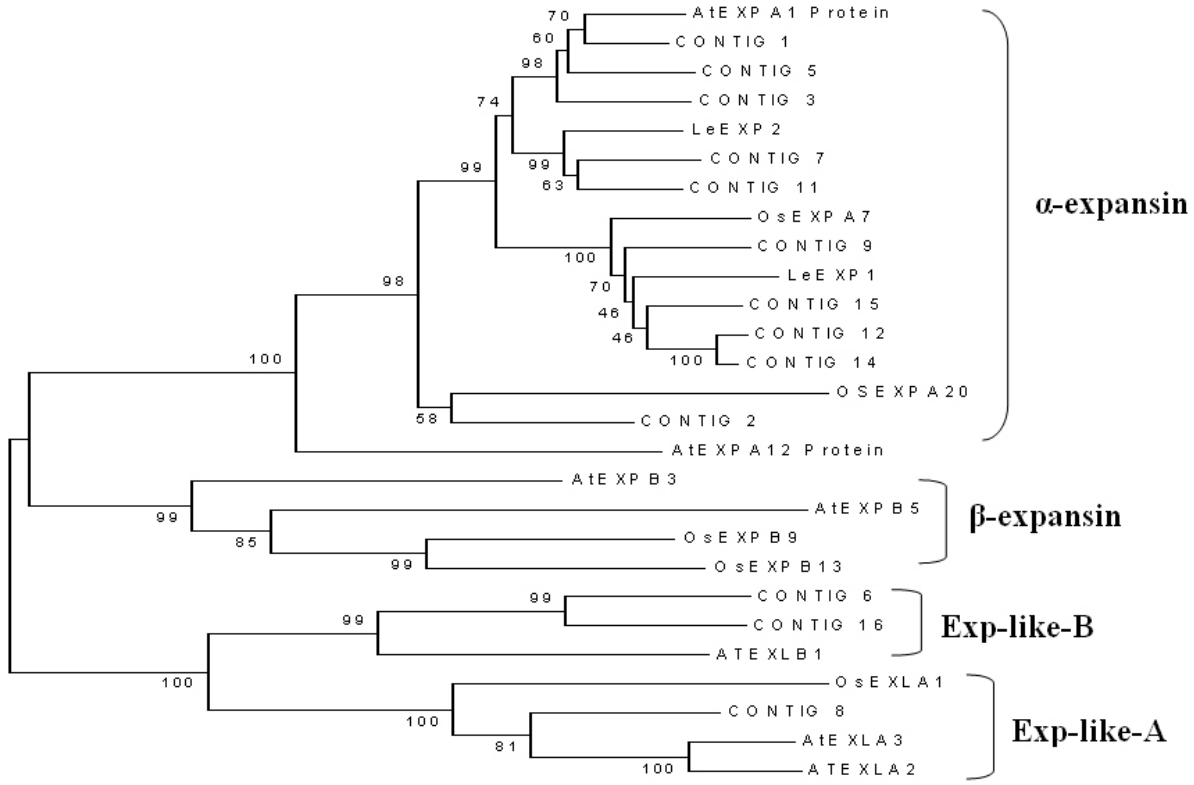

Figure 1. Phylogenetic tree of the expansin superfamily, including sequences (contigs) of *Coffea arabica* from the Brazilian Coffee Genome Project, *Arabidopsis thaliana* (At), *Oriza sativa* (Os) and *Lycopersicon esculentum* (Le) from the NCBI. The tree was constructed with MEGA 3.1 using neighbor joining and bootstrap values.

Gene Expression Analysis

The transcripts of coffee EXP, using *CaEXPA1* (Contig07) as a probe, were observed in different tissues (Figure 2A) and in the pulp at the latest stages of fruit maturation (Figure 2B). *CaEXP1* transcripts were detected mainly in flower buds, roots and during the stages of fruit pulp. Lower transcription was detected in flower and young shoots, but no transcripts were observed in leaves.

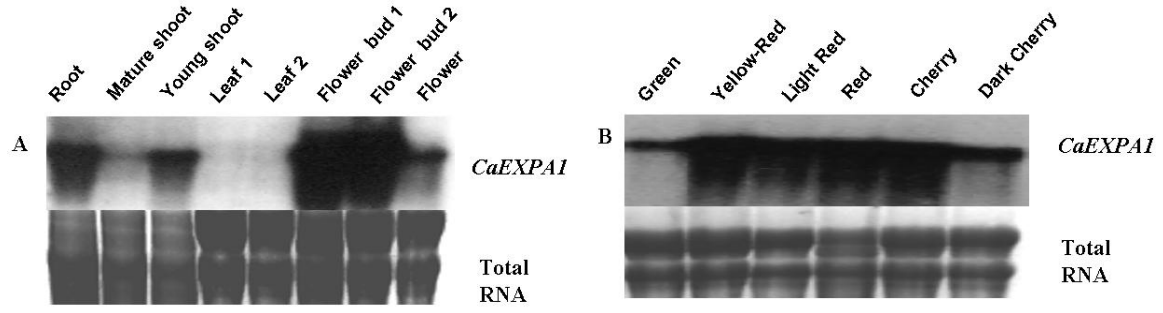

Figure 2. Northern Blot analysis of *C. arabica* total RNA hybridized with *CaEXPA1*. A) RNA from different coffee tissues. Leaf and flower bud samples were in duplicates. B) RNA from fruit pulp from different ripening stages. The total RNA used for the blot is showed below each sample in A and B.

REFERENCES

Balestrini R, Cosgrove DJ, Bonfante P(2005) Differential location of alpha-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus. *Planta* 220:889-899.

Belfield EJ, Ruperti B, Roberts JA, McQueen-Mason SJ (2005) Changes in expansin activity and gene expression during ethylene-promoted leaflet abscission in Sambucus nigra. *J Exp Bot* 56:817-823.

Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, 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.

Chang, S.; Puryear J.; Cairney, J. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol,11:113-116, 1993.

Chen F, Bradford KJ (2000) Expression of an expansin is associated with endosperm weakening during tomato seed germination. *Plant Physiol* 124:1265-1274.

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

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

Dotto MC, Martinez GA, Civello PM (2006) Expression of expansin genes in strawberry varieties with contrasting fruit firmness. Plant Physiol and Biochem 44: 301-307.

Gray-Mitsumune M, Mellerowicz EJ, Abe H, Schrader J, Winzell A, Sterky F, Blomqvist K, McQueen-Mason S, Teeri TT, Sundberg B (2004) Expansins abundant in secondary xylem belong to subgroup a of the alpha-expansin gene family. *Plant Physiol* 1135:1552-1564.

Harrison EP, McQueen-Mason SJ, Manning K(2001) Expression of six expansin genes in relation to extension activity in developing strawberry fruit. *J Exp Bot*, 52:1437-1446.

Kalamaki MS, Powell AL, Struijs K, Labavitch JM, Reid DS, Bennett AB (2003) Transgenic overexpression of expansin influence particle size distribution and improves viscosity of tomato juice and paste. *J Agric Food Chem* 51:7465-7471.

Kende H, Bradford K, Brummell D, Cho HT, Cosgrove DJ, Fleming A, Gehring C, Lee Y, Queen-Mason S, Rose J, Voesenek LA (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 CP, Ongaro V, Fleming A, Schipper O, Baldauf SL (2002) Plant expansin are a complex multigene family with an ancient evolutionary origin. *Plant Physiol* 128:854-864.

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

O'Malley RC, Lynn DG (2000) Expansin message regulation in parasitic angiosperms: marking time in development. *Plant Cell* 12:1455-1465.

Obenland DM, Crisosto CH, Rose JKC: Expansin protein levels decline with the development of mealiness in peaches. *Postharvest Biol Technol* 2003, 29:11-18.

Pezzotti M, Feron R, Mariani C (2002) Pollination modulates expression of the PPAL gene, a pistil-specific beta-expansin. *Plant Mol Biol* 49:187-197.

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 JK, Lee HH, Bennett AB (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc Natl Acad Sci* USA 94:5955-5960.

Yoo SD, Gao ZF, Cantini C, Loescher WH, van Nocker S (2003) Fruit ripening in sour cherry: changes in expression of genes encoding expansins and other cell-wall-modifying enzymes. *Am Soc Hortic Sci* 128:16-22.

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