Galactinol Synthase Gene Expression in *Coffea Arabica* L. under Water Stress

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SUMMARY

Galactinol synthase (GolS) catalyzes the formation of galactinol, which will produce raffinose oligosaccharides (RFOs). Expression of GolS during water deficit stress has been reported, probable due to the function of RFOs as osmoprotectant. Three galactinol synthase isoforms (*CaGolS1, 2* and *3*) were identified from the Coffee Genome Brazilian Project database (http://www.lge.ibi.unicamp.br/cafe/). The isoforms have similar length to others *GolS* genes. Northern blot analysis showed an increased in *CaGolS1* transcripts in coffee plants under water shortage when compared to plants in well-watered conditions. Transcripts of *CaGolS1* were specifically found on leaves and not in other tissues.

INTRODUCTION

Galactinol synthase (GolS) is the key enzyme in the biosynthesis of oligosaccharides from raffinose (RFO) family (Keller and Pharr, 1996). It plays a regulatory role in carbon partioning, acts directly in many processes of plant physiological development and in plant responses to abiotic stresses. Galactinol is formed from UDP-galactose and *myo*-inositol by the action of galactinol synthase (EC 2.4.1.123; GolS), which belongs to glycosil transferase 8 family (Campbell et al., 1997). Increased transcription of *Galactinol synthase* gene during water deficit has been reported. In C*ucumis melo*, it was observed that GolS activates the RFO metabolism in plants submitted to drought stresses. It was also demonstrated that *CmGolS1* transcription occurs in mature leaves and seeds during plant development, while *CmGolS2* trasncription was observed only in mature leaves (Volk et al., 2003). In *Arabidopsis thaliana*, from a family of seven *GolS* genes, three of them were stress responsive. *AtGolS1* and *2* were induced by drought and high-salinity stress, while *AtGolS3* was induced by low temperatures. These results showed that GolS plays a key role in the accumulation of galactinol and raffinose under abiotic stress conditions, conferring drought-stress tolerance to plants, since galactinol and raffinose may function as osmoprotectants (Taji et al., 2002). In *Ajuga reptans,* a model plant used to study the regulation of RFO metabolism, two distinct *GolS*, *ArGolS1* and *ArGolS2* were identified (Sprenger and Keller, 2000). The objective of this study was to increase the knowledge on the transcription expression of *GolS* genes in coffee plants under drought stress.

MATERIALS AND METHODS

Three galactinol synthase isoforms (*CaGolS1, 2* and 3) were identified from the Coffee Genome Brazilian Project database (http://www.lge.ibi.unicamp.br/cafe/). The isoforms

presented integrity and similarity to *GolS* gene, which allowed the primer design. The contigs formed by different tissues of coffee stressed plants were analyzed using bioinformatic programs such as BlastP and BlastX. Also, it was used the TargetP 1.1 Server program to detect signal peptides, and the program PSORT – *Prediction of Protein Localization Sites* version 6.4 for prediction and localization of proteins. Six month-old plants of *Coffea arabica* cv. IAPAR-59, cultivated in 1L pots in greenhouse, were submitted to a 5-day period without water, being evaluated by total water potential, osmotic potential and photosynthetic rates. Total RNA from different tissues was extracted based on protocol (Chang et al., 1993) for Northern blot analysis to detect the *Galactinol synthase* transcripts in leaves submitted to drought stress, as well as in different tissues under well-watered conditions (Figure 1).

RESULTS AND DISCUSSION

From 91 sequences found into the Brazilian Coffee Genome Project database (http://www.lge.ibi.unicamp.br/cafe/) three full-length galactinol synthase isoforms were identified (Table 1). CaGolS1, CaGolS2 and CaGolS3 isoforms have 1005, 1026 and 1017 bases pairs (bp), coding for proteins of 314, 341 and 338 amino-acids (aa), respectively. All the three isoforms present the glycosyl transferase domain pfam01501.

Table 1. Eletronic Northern of *Coffea arabica GolS* contigs.

Gene	Total ESTs	Leaves	Callus	Root	Cells suspension	Branch	Walter stress	Fruits	Plantlets
CaGolS1	49	32	X	1	5	1	10	X	X
CaGolS2	15	4	1	X	2	1	3	3	1
CaGolS3	6	2	X	X	X	X	4	X	X

When submitted to water deficit, the plants showed no symptom during the first two days without watering. A progressive decreased in water and osmotic potential was observed from the third day until the end of the treatment (Figure 1). Photosynthesis rates were stable until the second day of stress, falling quickly after that. Lost of turgor from the leaves after the second day was also observed.

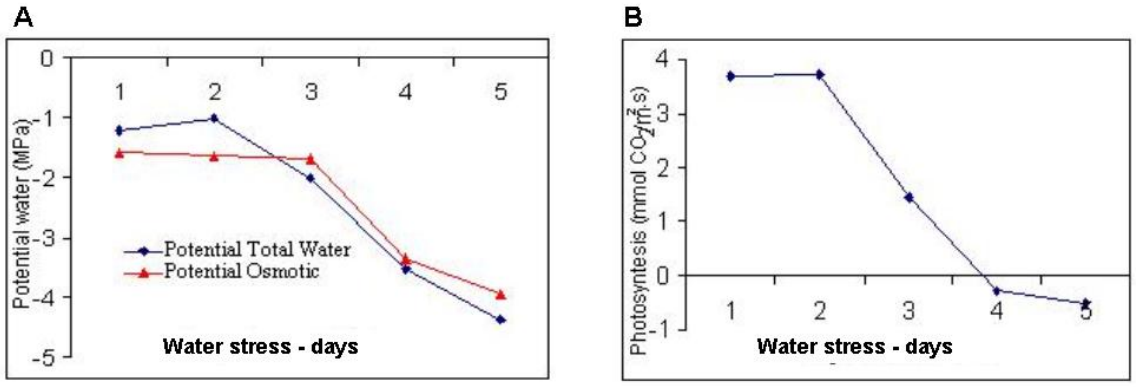

Figure 1. A - Total water and osmotic potential during water stress. B –Photosynthesis rate during water stress.

From the third day under water shortage (leaf water potential –2,02 MPa), coffee plants presented increased *CaGolS1* expression compared to plants in well-watered conditions (–1,2 MPa) (Figure 2). Similarly to found in *Cucumis melo* (Volk et al., 2002) and *Arabidopsis thaliana* (Taji et al., 2002; Liu et al., 1998), the *GolS* gene in *C. arabica* is involved in the

plant response to water deficit.Northern blot analyses showed that transcription of *CaGolS1* was tissue specific for mature and young leaves, and were not observed in roots, branches, floral buds, red fruit pulp and ripe seeds of *Coffea arabica* cv. IAPAR-59 grown under field conditions (Figure 2).

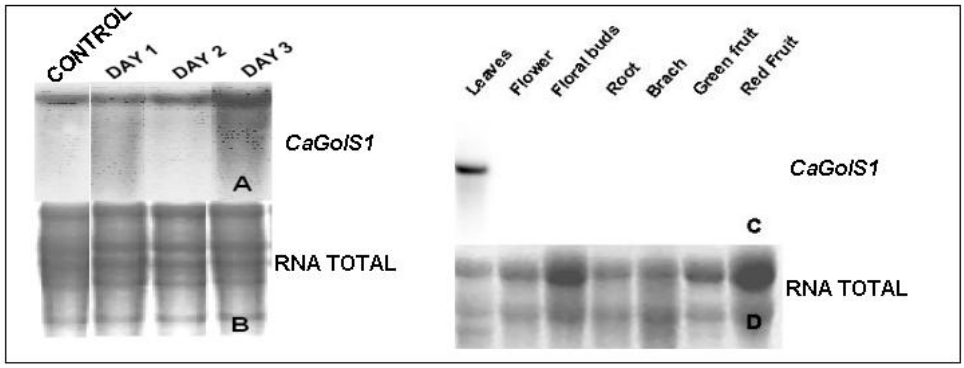

Figura 2. Northern blot analysis of total *C. arabica* RNA hybridized with CaGolS1. A) RNA from leaves of plants growing under different water deficit stress. B) RNA from different coffee tissue as described. Total RNA loading control is represented under the blots.

REFERENCES

Campbell, J. A., Davies, G. J., Bulone, V., Henrissat, B. A classification of nucleotide-diphospho-sugar glycosyltransferase based on amino acid sequence similarities. Biochem J., 326: 929-942, 1997.

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

Keller, F., Pharr, D. M. Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Zamski E, Schaffer AA., editors. Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships. New York: Marcel Dekker; p. 157-183, 1996.

Liu J-J, Krenz Dc, Glavez Af, De Lumen BO. Galactinol synthase (GS): increased enzyme activity and levels of mRNA due to cold and desiccation. Plant Sci.;134:11-20, 1998.

Sprenger, N., Keller, F. Allocation of raffinose family oligosaccharides to transport and storage pools in *Ajuga reptans*: the roles of two distinct galactinol synthases. Plant J; p. 21:249-258, 2000.

Taji, T. Ohsumi, et al. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. Plant J. p. 29: 417-426, 2002.

Volk, G. M., Haritatos, E.E., Turgeon, R. *Galactinol synthase* gene expression in melon. J Am Soc Hortic Sci. v. 128, p. 8-15, 2003.

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