Molecular Biology Reports (2022) 49:1973–1984
https://doi.org/10.1007/s11033-021-07011-7

ORIGINAL ARTICLE

The *SAUR* gene family in coffee: genome-wide identification and gene expression analysis during somatic embryogenesis

Fabiana Couto Zanin[1] · Natália Chagas Freitas[1] · Renan Terassi Pinto[1] · Wesley Pires Flausino Máximo[1] · Leandro Eugenio Cardamone Diniz[2] · Luciano Vilela Paiva[1]

Received: 14 June 2021 / Accepted: 24 November 2021 / Published online: 16 January 2022

Abstract

Background *Small auxin-up RNA* (*SAUR*) genes form a wide family supposedly involved in different physiological and developmental processes in plants such as leaf senescence, auxin signaling and transport, hypocotyl development and tolerance to abiotic stresses. The transcription of *SAUR* genes is quickly induced by auxins, a group of phytohormones of major importance on embryo development. To better understand the distribution and expression profile of such still not explored family in *Coffea* sp., especially during the development of somatic embryogenesis (SE), *SAUR* members were characterized in silico using the available *Coffea canephora* genome data and analyzed for gene expression by RT-qPCR in *C. arabica* embryogenic samples.

Methods and results Over *C. canephora* genome 31 *CcSAURs* were distributed by 11 chromosomes. Out of these 31 gene members, 5 *SAURs* were selected for gene expression analysis in *C. arabica* embryogenic materials. *CaSAUR12* and *CaSAUR18* were the members highly expressed through almost all plant materials. The other genes had more expression in at least one of the developing embryo stages or plantlets. The *CaSAUR12* was the only member to exhibit an increased expression in both non-embryogenic calli and the developing embryo stages.

Conclusion The identification of SAUR family on *C. canephora* genome followed by the analysis of gene expression profile across coffee somatic embryogenesis process on *C. arabica* represents a further additional step towards a better comprehension of molecular components acting on SE. Along with new research about this gene family such knowledge may support studies about clonal propagation methods via somatic embryogenesis to help the scientific community towards improvements into coffee crop.

Keyword Small auxin-up RNA. Genomic structure. Phylogenetic relationship. RT-qPCR. *Coffea* sp. Somatic embryogenesis

✉ Luciano Vilela Paiva
luciano@ufla.br

Fabiana Couto Zanin
fabi.czanin@hotmail.com

Natália Chagas Freitas
natalia_biotec@hotmail.com

Renan Terassi Pinto
renantpinto@gmail.com

Wesley Pires Flausino Máximo
wesleypfm@gmail.com

Leandro Eugenio Cardamone Diniz
leandro.diniz@embrapa.br

[1] Central Laboratory of Molecular Biology, Department of Chemistry, Federal University of Lavras (UFLA), Lavras, MG 37200000, Brazil

[2] Brazilian Agricultural Research Corporation (Embrapa Tabuleiros Costeiros), Aracaju, SE 49025040, Brazil

Introduction

Somatic embryogenesis (SE) is a morphogenetic pathway in which a plant can produce embryos across the asexual reproduction mechanism based especially on the totipotency capacity of its cells, that is, certain living cells are able to generate entire and new functional organisms under the right circumstances and conditions [1]. Such technique in coffee has been widely studied since 1970 [2] and it is currently considered a powerful biotechnological tool for crop improvement in diverse plant species.

Currently, the most employed methods for propagation of coffee plants have been either by seeds or grafting. In the first, the material purity reduces over time based on genetic variability of seeds, whilst in the grafting approach the production is tough of scaling up and lacks efficient protocols available for horticultural propagation [3]. So, an efficient SE protocol may be useful in producing a large number of elite coffee clones given high proliferation and regeneration capacity of embryogenic cells what makes SE a promising biotechnological tool to benefit greatly the coffee culture propagation sector.

Although many established protocols described in literature are genotype-dependent, a few global characteristics are very similar among species as the initial stimuli triggered by plant hormones [4]. The right balance between plant growth regulators (PGRs) applied exogenously and internal factors may induce plant tissue-specific cells to reprogram into totipotent undifferentiated cells capable of producing new embryos, but it is known that auxins play an important role in the SE initiation process, as factors influencing embryogenesis have a role on modulating this hormone signaling and biosynthesis [5]. It is supposed that exogenous auxins may trigger the synthesis of endogenous auxins and help to transport polar auxins in responsive tissues, which would establish an essential auxin gradient for the embryo bilateral symmetry initiation [6].

Two major phases are considered crucial in SE: the induction and embryo development stage. In the first, somatic cells undergo a reorganization of its physiological and molecular state in addition to changes in gene expression patterns to acquire embryogenic competence, which will be expressed later during the second SE phase [1]. Little knowledge is available concerning the molecular regulations taking place throughout the SE in *Coffea* sp., but some genes have already been associated to this morphogenetic process. Differential gene expression of the homologous genes *BABYBOOM* (*BBM*) and *Somatic embryogenesis receptor-kinase* (*SERK*) was detected in embryogenic cell suspensions of *C. arabica*, what shows their potential to become molecular markers for cells with higher embryogenic capacity [7–9]. Some other gene families have been related to the activity maintenance of transport, signaling, synthesis and homeostasis of auxin, such as *Auxin efflux PINFORMD* (*PIN*), *Auxin influx AUXIN RESISTANT 1* (*AUX1*)*/LIKE AUX1* (*LAX*), *Auxin responsive factors* (*ARF*), *AUX/IAA*, *Gretchen Hagen3* (*GH3*) and *Small auxin up RNA* (*SAUR*) [10, 11].

The *SAUR* genes have been associated with early response to auxins and were firstly identified in soybean [12], and from that, explored in other plant species, including tomato and potato [13], rice [14], apple [15] and arabidopsis [16]. This family has expanded in number of members along with plant evolution [17] and the genomic structure of *SAUR* family members shows some similar features: majority of *SAUR* genes is intronless, contain one or more auxin-responsive elements (AuxREs) and a downstream destabilizing (*DST*) conserved element in the 3' untranslated region (3' UTR) consisting of three conserved elements separated by non-conserved bases supposedly responsive to gene instability. Another family characteristic is the presence of a specific conserved domain (CDD superfamily cl03633) with still unknown function [14, 16]. Although most of the members have not yet functional analysis, due to the large size of this family and relatively recent exploration, some members were already pointed out on influencing hormones signaling transduction, such as auxins and abscisic acid, to modulate, for example, adventitious root development, stomatal movement and hypocotyl gravitropism and phototropism [18–20].

Notwithstanding the little knowledge about the function of the *SAUR* family in coffee plants, a few reports have correlated the expression of some these genes with plantlet development, regulation of ethylene levels, hypocotyl elongation, cell expansion regulation, and auxin synthesis and transport [15, 21–23]. Taking the scarce information about this gene family in coffee into account, without overlooking their participation in auxin response, our work aimed to identify *SAUR* members in the *Coffea canephora* genome (the annotated genome of *Coffea* sp. publicly available) focusing on better understand the roles of the family, but also its relation with somatic embryogenesis and plantlet development in *Coffea arabica* materials, the most planted, commercialized, and exported species of coffee by Brazil.

Materials and methods

In silico analysis of SAUR members in *Coffea canephora* genome and phylogenetic relationship

The SAUR members of *C. canephora* were obtained from a protein comparison between sequences available on the Coffee Genome Hub v1.0 (http://coffee-genome.org/) [24] against known SAUR proteins of *Solanum lycopersicum* [13], *Arabidopsis thaliana* [16], and *Oryza sativa* [14]

available on public databases. The data were then checked for the presence of CDD superfamily conservative domain (cl03633), a characteristic SAUR family domain. The resulting *C. canephora* sequences were used to perform multiple sequence alignments using ClustalW [25] along proteins from other species. The distribution of *SAUR* genes across *C. canephora* chromosomes was made with the tool "Locus search" also available on Coffee Genome Hub.

In order to investigate the phylogenetic correlation of *CcSAUR* genes (Supplementary data S1), a maximum likelihood tree was constructed with SAUR protein sequences found in arabdopsis, rice, tomato and coffee (Supplementary data S2) by using the pieces of software MEGA X and IQ-Tree [26–28] with a bootstrap of 1,000 replicates. Subcellular localization prediction of each CcSAUR was performed using three different softwares: CELLO v2.5 (http://cello.life.nctu.edu.tw/) [29], Plant-mSubP (http://bioinfo.usu.edu/Plant-mSubP/) [30] and DeepLoc (http://www.cbs.dtu.dk/services/DeepLoc/index.php) [31] (Supplementary Table S3). The theoretical molecular weight and isoelectric point (pI) were predicted with ProtParam tool (https://web.expasy.org/protparam/) [32].

Plant material and in vitro culture conditions

Embryogenic calli (EC) and non-embryogenic calli (NEC) (Fig. 1a) were induced in vitro from leaves of *C. arabica* cv. Catuaí Amarelo IAC 62 matrix plants grown under greenhouse conditions. The leaf explants were incubated following the Teixeira et al. protocol [33]. Embryogenic cell suspensions (ECS) (Fig. 1b) were obtained by transferring yellow proembryogenic calli to Erlenmeyer flasks containing liquid multiplication medium T3 [34] at inoculum density of 10 g callus L^{-1} [35]. Flasks were maintained in the dark under constant agitation at 100 rpm in a growth room at 25 °C, and the medium was replaced every 15 days. The cell suspensions were cultivated for three months. After multiplication, the EC cells were submitted to differentiation with the regenerated embryos cultured in RR medium [36] at inoculum density of 1.0 g L^{-1}. Maturation and germination of somatic embryos (Fig. 1c and d) were accomplished following the method described by Teixeira et al. [33] in MGM medium. The plantlets (Fig. 1e) were maintained in MS medium for growth.

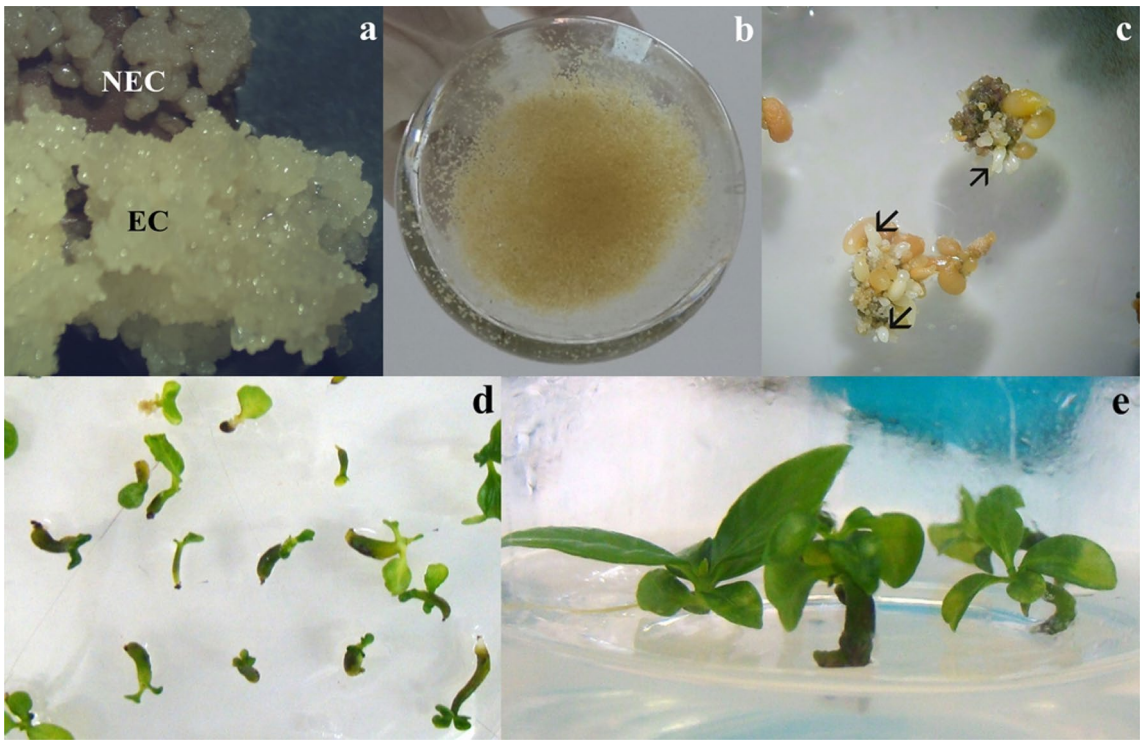

Fig. 1 Visual aspect of the somatic embryogenesis process in *C. arabica* L. **a** Embryogenic (EC) and non-embryogenic calli (NEC); **b** Embryogenic cell suspensions with 3-months culture time; embryos at the stages **c** torpedo; **d** cotyledonary; and **e** plantlets

Expression analysis of *CcSAUR* genes by RT-qPCR in *C. arabica* embryogenic material

Total RNA extraction of EC and ECS *C. arabica* samples was performed using the Kit NucleoSpin® (Macherey Nagel, Düren, DE), while the extractions of NEC and embryos in the torpedo, cotyledonary and plantlet stages were performed using ConcertTM Plant RNA Reagent (Invitrogen, Carlsbad, CA, US), both following the manufacturer's instructions. RNA integrity was verified by electrophoresis in agarose gel through ethidium bromide staining. Synthesis of cDNA was performed with the Kit High-Capacity cDNA Reverse Transcription (Applied Biosystems, Foster City, CA, US) from 1,000 ng of RNA, as recommended.

Selection of *C. canephora* homolog *SAUR* genes in *C. arabica* (*CaSAUR5*, *CaSAUR12, CaSAUR13, CaSAUR18* and *CaSAUR20*) for gene expression was based on previous in silico analyses from RNA-Seq dataset of *C. arabica* transcriptome of embryogenic materials (unpublished data). The RT-qPCR assays were performed with the equipment ABI PRISM 7500 Real-Time PCR (Applied Biosystems, Foster City, CA, US) at a total volume of 10 µL containing 10 ng of cDNA, 1X SYBR® Green Master mix (Applied Biosystems, Foster City, CA, US) and 0.4 µM of each primer (Supplementary Table S1). All RT-qPCR assays were carried out in technical and biological triplicates. The gene expression data were analyzed as determined in Pfaffl [37] with the reference genes 24S and PP2A described by Freitas et al. [38] and gene expression plots were constructed through the graphing and statistical analysis software SigmaPlot v.11.0 (Systat Software, San Jose, CA, US).

Statistical data analysis

RT-qPCR data were submitted to the analysis of variance (ANOVA) through the Sisvar software (DES/UFLA, Lavras, BR) [39] for statistical analysis. Mean values were compared by the Scott-Knott test at 5% significance.

Results

In silico identification of *SAUR* genes on *C. canephora* genome

We found 31 amino acid sequences of putative *C. canephora* SAUR members and some basic information about them is described on Table 1. The distribution of the 31 *CcSAURs* is more concentrated on chromosome 2 (10 members), while the chromosomes 1, 4, 5, 7 and 9 had just 1 member each. Chromosome 3 contained no *SAUR* gene (Fig. 2; Supplementary Table S2); the ORF lengths varied from 165 to 831 bp encoding proteins ranging in size from 54 to 276 amino acids; predicted molecular weight varied of 5943.01 to 31,364.89 and theoretical pI of 4.60 to 10.96. The most common predicted subcellular localization was at the nucleus, followed by mitochondria localized proteins.

Besides the basic features and genomic distribution, we performed an analysis to elucidate the gene structure of each *SAUR* putative member on *C. canephora*. Almost 84% (26 out of 31) of the genes have only one exon (Fig. 3), four only two exons and only one gene have three exons. According to the current annotation on the *C. canephora* genome, it was possible to identify UTR sequences for ten of these genes.

Also, we constructed a phylogenetic tree with *C. canephora* amino acid sequences and 71 AtSAURs, 99 SlSAURs and 56 OsSAURs. In general, *C. canephora* members were grouped together with those from other species. The only exception was a concise group of eight coffee members: CcSAUR1, CcSAUR8, CcSAUR9, CcSAUR22, CcSAUR23, CcSAUR28, CcSAUR29 and CcSAUR30. This group is close to a clade containing only *A. thaliana* SAURs, with 13 members. See Fig. 4

Gene expression analysis by RT-qPCR

The gene expression analysis of some *CaSAURs* was performed in the somatic embryogenic materials and in plantlets under development using quantitative real time PCR. The *CaSAUR5* gene had no expression in the EC and ECS materials and the higher expressions were found in torpedo, cotyledonary and plantlets samples (Fig. 5a). For the *CaSAUR12* there was no statistical difference between its expression profile observed for NEC, torpedo and cotyledonary embryos, and plantlets samples. The lowest expression of this gene was observed in ECS (Fig. 5b). *CaSAUR13* had its highest expression (42 fold) in the cotyledonary embryos, and the lowest in the ECS (~ onefold) (Fig. 5c). Expression profile of *CaSAUR18* in mostly samples was significantly similar, but its expression in NEC and ECS was statistically low, achieving values of twofold and onefold, respectively (Fig. 5d).

Finally, *CaSAUR20* showed the highest expression value in cotyledonary embryos (approximately 67 fold), which was also the most relevant expression of a *CaSAUR* gene among all samples under analysis. Its expression profile in NEC, torpedo, and plantlet ranged of 18 to 28 fold and in EC and ECS just a basal expression was detected (Fig. 5e).

Discussion

SAUR gene family on *C. canephora*

A smaller number of *SAUR* genes was found on *C. canephora* in comparison to other species, such as *Solanum*

Table 1 Description of putative *C. canephora* SAUR members basic features

Name	Locus ID[a]	ORF lenght (bp)	Protein (aa)	Molecular Weight (Da)	pI[b]	Predicted subcellular localization[c]
CcSAUR1	Cc00_g22640	480	88	9987.68	8.66	N/A
CcSAUR2	Cc00_g26580	321	107	12,126.86	7.05	Mitochondrial
CcSAUR3	Cc00_g29740	303	100	11,885.56	7.99	Nuclear
CcSAUR4	Cc01_g10550	432	143	15,993.29	6.75	Cytoplasmic
CcSAUR5	Cc02_g16700	297	98	11,301.01	7.91	N/A
CcSAUR6	Cc02_g16710	321	106	11,652.44	9.10	Mitochondrial
CcSAUR7	Cc02_g16720	300	99	11,115.68	6.90	N/A
CcSAUR8	Cc02_g16730	318	105	11,949.77	8.53	N/A
CcSAUR9	Cc02_g16740	309	102	11,481.36	7.88	N/A
CcSAUR10	Cc02_g16750	285	94	10,610.35	7.03	N/A
CcSAUR11	Cc02_g16760	291	96	10,663.36	8.52	N/A
CcSAUR12	Cc02_g16790	318	105	11,937.86	8.51	Mitochondrial
CcSAUR13	Cc02_g24230	537	178	20,374.67	10.8	Mitochondrial
CcSAUR14	Cc02_g40000	549	182	20,306.60	9.33	Chloroplast
CcSAUR15	Cc04_g00010	483	160	17,926.48	6.30	Nuclear
CcSAUR16	Cc05_g16250	426	141	15,994.96	8.56	Nuclear
CcSAUR17	Cc06_g04040	831	276	31,364.89	9.75	Nuclear
CcSAUR18	Cc06_g06020	387	128	14,235.40	8.39	Cell membrane
CcSAUR19	Cc06_g12640	498	165	18,447.58	5.09	Nuclear
CcSAUR20	Cc06_g12650	366	121	13,551.41	5.15	Nuclear
CcSAUR21	Cc07_g19210	309	102	11,962.58	6.86	Cytoplasm
CcSAUR22	Cc08_g08380	309	102	11,419.22	5.77	Cell membrane
CcSAUR23	Cc08_g08390	468	155	17,690.51	9.28	N/A
CcSAUR24	Cc08_g12980	552	183	20,330.43	10.43	Mitochondrial
CcSAUR25	Cc09_g10510	312	103	11,647.46	5.83	N/A
CcSAUR26	Cc10_g01860	318	105	12,013.90	6.73	N/A
CcSAUR27	Cc10_g01880	447	148	17,010.70	9.47	Mitochondrial
CcSAUR28	Cc11_g04780	165	54	5943.01	10.96	Mitochondrial
CcSAUR29	Cc11_g04790	438	145	16,402.06	9.10	Plastid
CcSAUR30	Cc11_g04800	312	102	11,401.04	4.60	Cell membrane
CcSAUR31	Cc11_g17330	480	159	17,726.39	8.89	Nuclear

[a]Locus ID – a database in the Coffee Genome Hub

[b]*pI* – proteins isoelectric point

[c]Predicted subcellular localization equal on at least two out of the three software used

lycopersicum (99 *SAURs*) [13], *A. thaliana* (82 *SAURs* of which 2 are pseudogenes) [16], *Oriza sativa* (58 *SAURs* with also 2 pseudogenes) [14] and *Malus domestica* (80 *SAURs)* [15]. The *SAUR* gene family expanded greatly throughout plant evolution, with variation in gene number that can reach ten times of magnitude from bryophyta to flowering plants [17], probably due to the effect of these genes on transducing hormone signals for plant development which led to their retention after whole genome duplication events, or even gene duplications.

The small number of *C. canephora SAURs* could be in reason of a decreased duplication rate of these genes on this species. This would be in agreement with the protein distribution on the phylogenetic tree, in which only one cluster with exclusive CcSAUR proteins was formed, while many SAUR proteins from *A.thaliana*, *S. lycopersicum* and *O. sativa* were allocated together on exclusive groups (Fig. 4). A similar finding was reported for moso bamboo (*Phyllostachys edulis*) [40]. The *SAUR* genes on *C. canephora* were unequally distributed among chromosomes, with predominant location on chromosome 2. This could be explained by the larger size of this chromosome [24], however, a similar finding was already previously described for *S. lycopersicum*, in which the chromosome 1 has 31 *SAUR* members and others, *i.e.* chromosome 2 and 8, have only two members [13]. Despite the chromosomal distribution, the observed

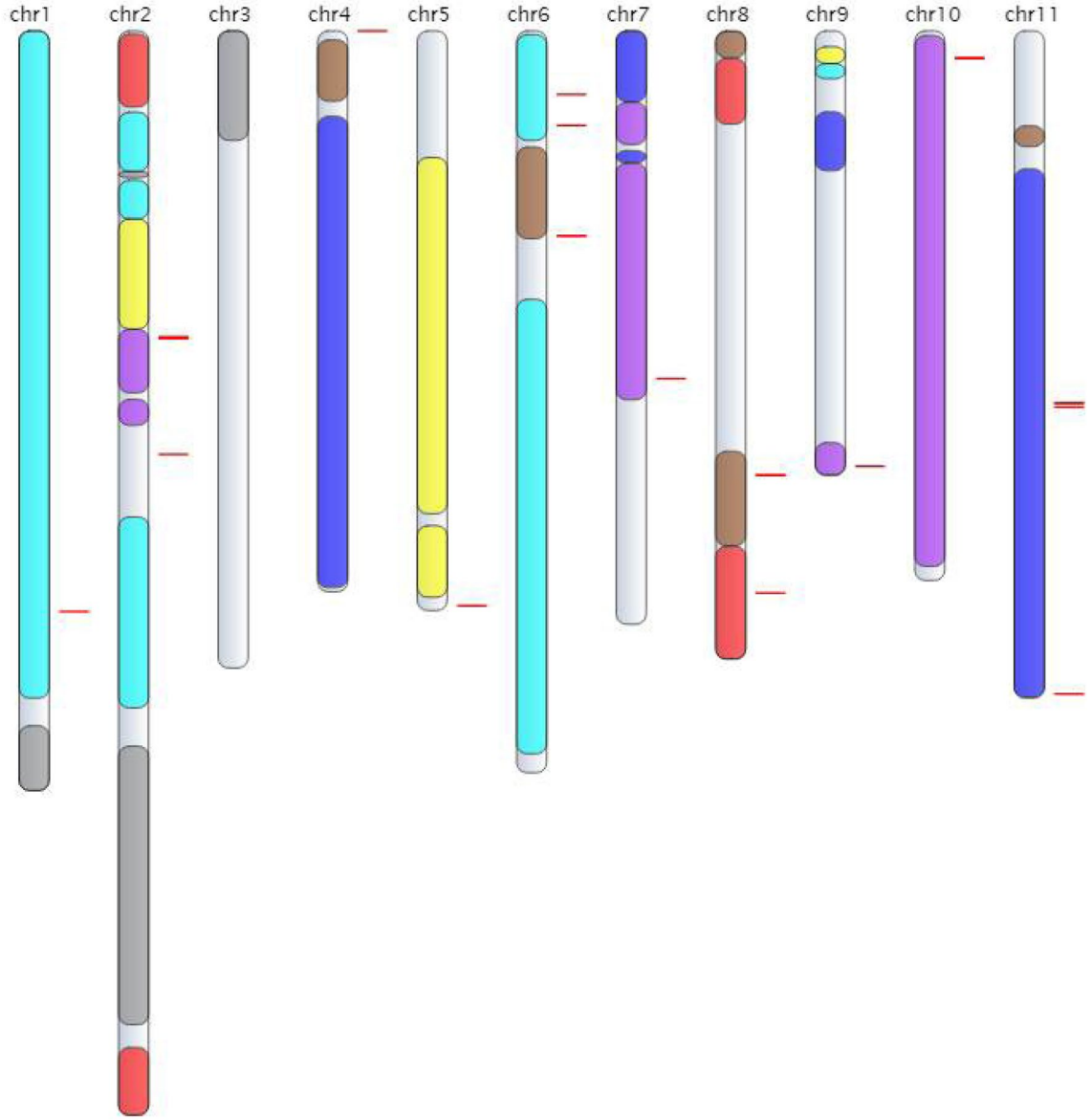

Fig. 2 Distribution of *CcSAUR* genes on 11 chromosomes of the *Coffea canephora*. Ancestral blocks correspond to the 7 core eudicot chromosomes (Denoeud et al., 2014). The blocks blue, red, violet, brown, grey, light-blue and yellow represent the ancestral blocks G1, G2, G3, G4, G5, G6 and G7, respectively. Red lines represent the position of *CcSAUR* genes on chromosomes (Supplementary Table S1). Identification of *CcSAURs* distribution was performed using the tool "Locus search" available on Coffee Genome Hub with "Locus ID" of *CcSAUR* genes as input data

pattern of a high rate of clustered *SAUR* members on genome regions (80.8% of the genes are clustered together) described on the same report [13] indicates that the lower number of *SAUR* members on *C. canephora* could be explained by lack of duplication of these genes, as they are not arranged in genomic clusters. However, the *C. canephora* genome is in its first version of annotation and about 35% of its assembled content (Mb) is unmapped [24], therefore, due to the relative small size and gene structure (mostly just one exon) of *SAURs*, some of the members of this gene family might not be annotated yet. Therefore, we provided a list of genomic locations where other putative *SAUR* genes might be found, based on Blastn analysis against whole chromosomes, and further confirmed, in despite of the current genome annotation (Supplementary Table S4).

The identified CcSAURs have the expected protein size for this family (about 60 to 180 amino acids) [41] and they were divided into seven distinguished groups on a phylogenetic tree (Fig. 4), with CcSAURs present in all of them. Again, a common pattern of forming clusters due to usually

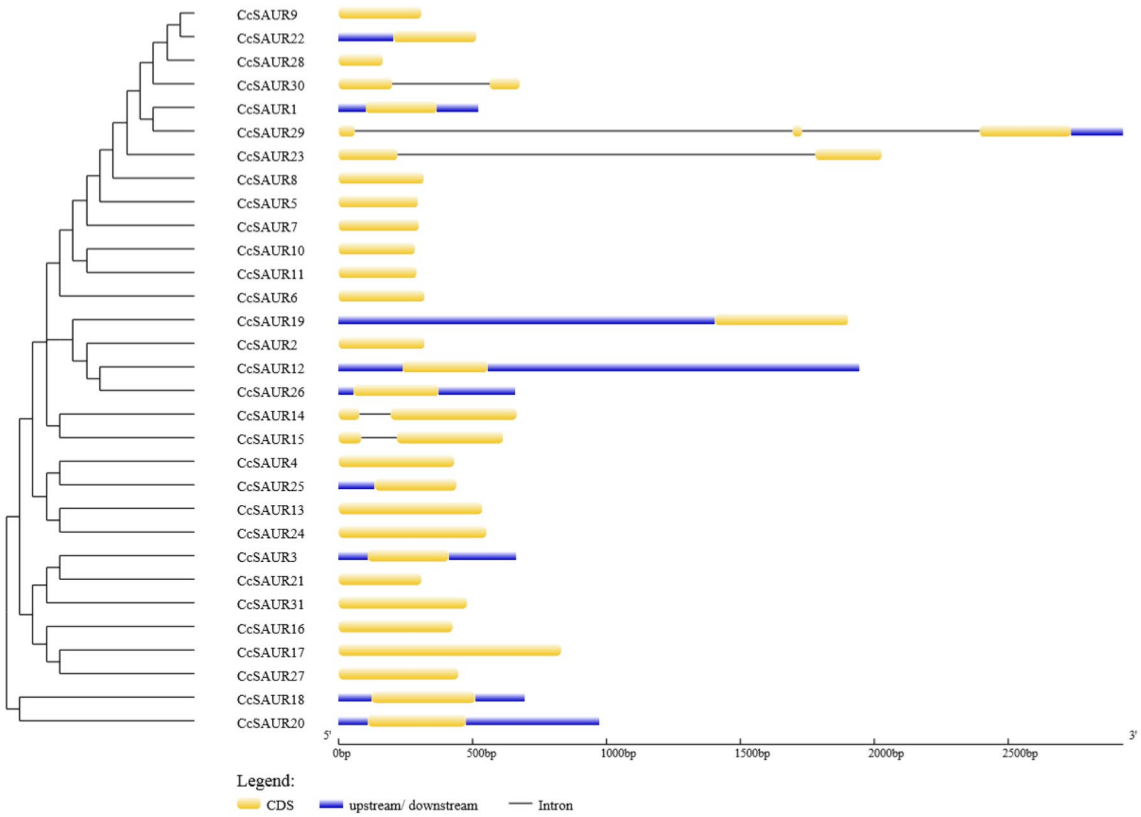

Fig. 3 Phylogenetic relationship and genomic structure of *C. canephora* putative *SAUR* genes. Exons are represented by yellow ellipses, introns by black lines and upstream (5')/downstream (3') untranslated regions (UTRs) by blue rectangles

high level of tandem and segmental duplications [41] is not observed for *C. canephora* members. On the group 1, we highlight the unique cluster of nine CcSAURs which is composed by similar SAUR members but with different genomic locations (Table 1). A further study may clarify whether this family didn't expand as usual on *C. canephora* or a larger number of members are masked by gene annotation miss-prediction.

Expression analysis

Although the SAUR family has been identified in many other plant species, the majority of its members have still unknown function. This family exhibits a high similarity between their members, making more difficult to generate specific *SAUR* mutant organisms for loss-of-function studies. The few SAURs with function already characterized seems to be involved mostly with auxin-mediated development that includes expansion and cell elongation [21, 42, 43]. Recent findings, however, expand the influence of these proteins to transduction of signals from ABA to modulate cell expansion and ion homeostasis [47] and mediate drought stress adaptation [48]. Other studies have shown two SAUR subfamilies in arabidopsis acting as cell expansion positive regulators, where these proteins are associated with plasma membrane and apparently regulate the action of cell expansion-promoting H + ATPases [42, 43]. There is a hypothesis of auxin-mediated cellular expansion in which susceptible cells would activate the proton pump present at the plasma membrane upon auxin exposure. As a result, a reduction at the apoplastic pH would cause a modification at the cell wall by the activity of specifically proteins, leading to a plasma membrane hyperpolarization and, lastly the uptake of water and solutes into the cell [44]. Beyond this, the interaction of SAUR proteins with H^+ATPases can influence stomatal movement [20] and adventitious root development [18].

Spartz et al. [21] demonstrated that the Arabidopsis *SAUR19* gene inhibits the action of PP2C phosphatases by preventing the Thr-97 amino acid dephosphorylation from C-terminal autoinhibitory domain in the H^+ ATPases, which would lead to the cellular expansion regulation. More recently, the same hypothesis was tested in tomato, wherein the *AtSAUR19* overexpression in tomato conferred the same phenotype in plants. These studies suggest that the SAUR-PP2C.D regulation is a conserved mechanism of cellular expansion [23]. The expression profile of *CaSAUR5*, a gene 54% similar to *AtSAUR19* at sequence level, is likely an indicative of the inhibition of PP2C phosphatases since

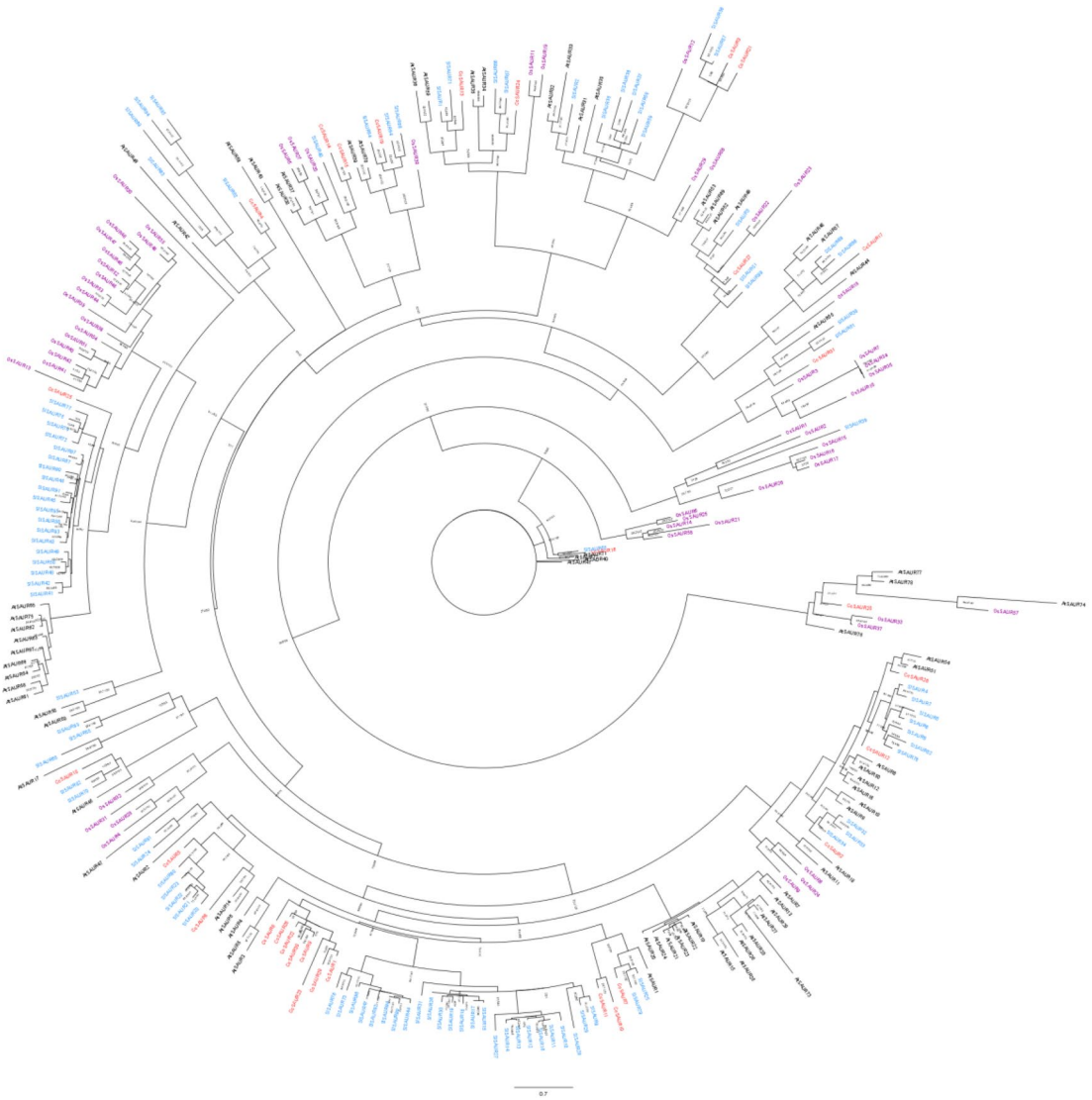

Fig. 4 Phylogenetic tree analysis of SAUR proteins found in different species. The tree was constructed using IQ-Tree software, applying Maximum Likelihood method with 1,000 bootstrap replicates and using Jones-Taylor-Thornton (JTT) model with empirical AA frequencies from the data and Gamma distributed rates for SAUR proteins of *Coffea canephora* (Cc), *Arabidopsis thaliana* (At), *Solanum lycopersicum* (Sl) and *Oryza sativa* (Os). The SAUR family proteins in *C. canephora* are marked in red, *A. thaliana* in black, *S. lycopersicum* in blue and *O. sativa* in purple; for each node, the following parameters are displayed: SH-aLRT support (%) / ultrafast bootstrap support (%)

CaSAUR5 is more expressed in embryogenic tissues with evident cellular elongation and expansion, as observed in torpedo and cotyledonary embryos, but also in plantlets under development. Non-embryogenic calli are more vacuolated and present larger cells than embryogenic tissues in early stages, what would explain the higher expression of *CaSAUR5* in NEC compared to EC or ECS, corroborating the expression profile in growing cells and tissues [21].

Although auxins usually induce the expression of *SAUR* genes, it is still unclear how it occurs. Generally, EC possesses more auxin synthesized by the tryptophan pathway [45], but the *CaSAUR5* expression was low in this sample. Since the expression of *SAUR* genes could be triggered by other plant hormones, it is possible that the H^+ ATPase activation through the PP2C.D inhibition might be related to other metabolic routes.

The *CaSAUR13* gene presented 63% of similarity with the previously characterized *AtSAUR36*. This gene is reported to be involved with foliar senescence and integration of auxin and giberelin signaling to regulate biological functions involved with maintenance of apical hook on etiolated plantlets, hypocotyl elongation under light and fertility [44, 46]. The *SAUR36* knockout in arabdopsis lines displayed a phenotype with 83% more leaf area, suggesting

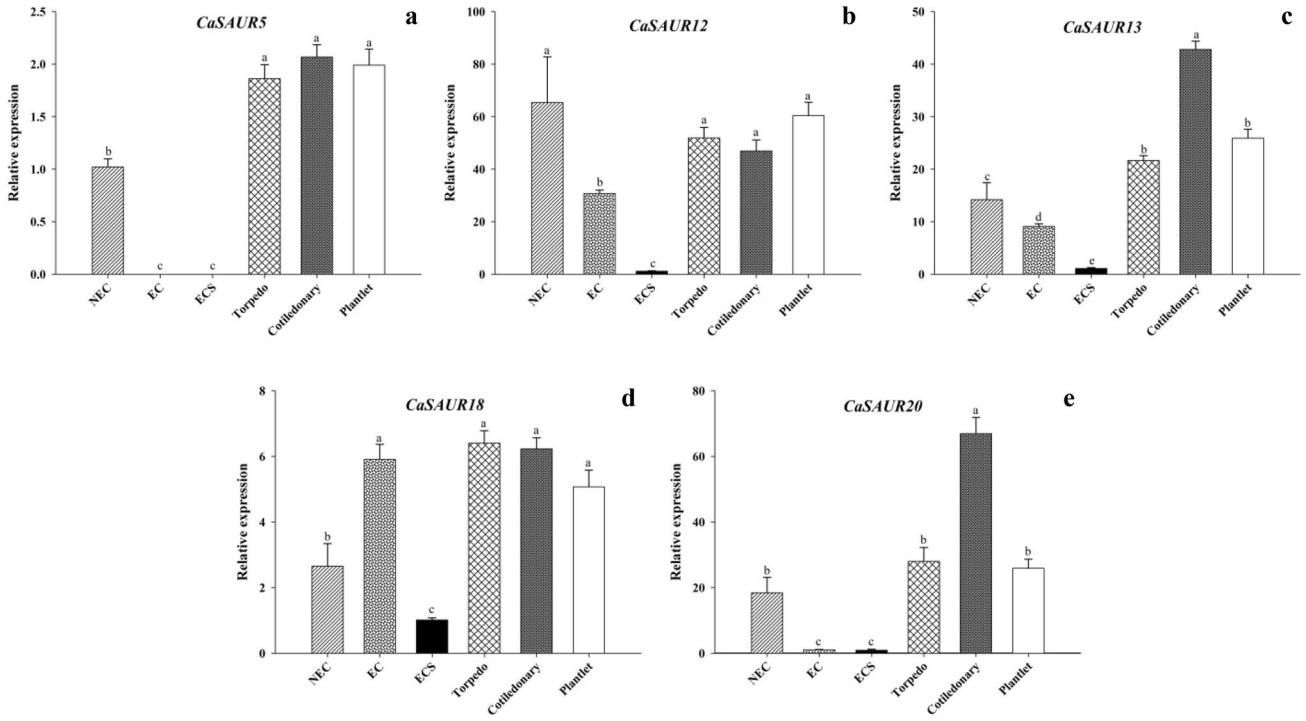

Fig. 5 Expression profile of **a** *CaSAUR5*, **b** *CaSAUR12*, **c** *CaSAUR13*, **d** *CaSAUR18* and **e** *CaSAUR20* genes in in vivo samples related to somatic embryogenesis of *C. arabica* through RT-qPCR. Data were normalized to 24S and PP2A reference genes and error bars represent ± s.d. (n = 3). Statistical data analysis was performed by ANOVA followed by comparisons using the Scott-Knott test at 5% of significance ($p < 0.05$)

this gene might be involved to the inhibition of cellular expansion [46]. Additionally, Stamm and Kumar [44] demonstrated that 4 days old plantlets exhibited a high expression on the apical meristem and in distinct regions of roots from transgenic lines expressing GUS under activity of the *AtSAUR36* promoter. The observed pattern would be consistent with tissues at high levels of cell division and/or elongation, similar with the observed on embryos at the cotyledon, torpedo and plantlets stage. Recently, new *SAUR* members (*SAUR41* subfamily) were characterized in *A. thaliana* as positively influencing cell expansion, activated by abscisic acid [47] and, in this study, CcSAUR16 is found as the most similar (protein level, Fig. 4) to these members, which point to further possibilities of exploring SAUR influence on cell expansion during coffee somatic embryogenesis.

In the present study we also identified the *CaSAUR20* gene which exhibited 46% of similarity with the *SAUR76* of arabidopsis involved in ethylene- and auxin-related pathways. The *AtSAUR76* is involved in cell elongation downstream to auxin response and its expression is regulated by ethylene through changes in the endogenous auxin transport and/or biosynthesis [49]. The mutants overexpressing *AtSAUR76* produced plantlets with higher number of meristematic cells and smaller leaf area, what not delete a chance this gene is influencing positively the root meristematic activity but interfering negatively on leaf development.

Another study analyzed the *SAUR76-78* subfamily in arabidopsis. These genes were identified based on their putative association with ETR_2 and ETR_4 ethylene receptors. Transgenic plants overexpressing the *SAURs 76*, *77* and *78* developed larger leaves than the wild-type lineage as a result of cell growth and expansion, once the number of cells showed little difference. The analyses also showed a reduction on the ethylene response of seedlings, suggesting these genes could affect the signaling of subfamily II of ethylene receptors by direct interaction and, at the same time, promote plant development and growth through auxin responses regulation [22].

Auxin and ethylene are two very important plant hormone regulators and their interactions are being even further studied at physiological and molecular levels. It has already been reported that auxins might stimulate ethylene biosynthesis and that ethylene could reduce the endogenous auxin concentration by IAA conjugation and/or decarboxylation [50]. In our study the highest *CaSAUR20* expression was recorded in cotyledonary embryos. Such behavior could be possibly related to the biosynthesis of auxin-induced ethylene, triggering the gene expression since high concentrations of ethylene inhibit coffee somatic embryogenesis. The

same way that high ethylene concentration inhibits somatic embryogenesis in coffee, this hormone is also known by its importance in different aspects of plant development such as plantlet growth and adventitious root formation, what would explain the high expression of the *CaSAUR20* in different developmental stages of embryos. It is also possible that the CaSAUR20 interacts with PP2C phosphatases promoting cellular expansion through H^+ ATPase, since more than one SAUR protein in arabidopsis has been reported to interact with clade D PP2Cs [21].

The *CcSAUR12* and *CcSAUR18* genes presented similarity only with non-characterized *SAURs* in other species (98% similarity with *AtSAUR51* and 79% with *SlSAUR71*, respectively). The *CaSAUR12* had high expressions in almost all samples, going up to more than 60 fold in both NEC and plantlet samples. This profile leads us to believe that somehow this gene is involved with cellular growth and/expansion induced by auxin. *CaSAUR18* presented lower expression levels than *CaSAUR12, CaSAUR13* and *CaSAUR20*, but also showed high expressions in EC, torpedo and cotyledonary embryos, and plantlets, what indicates this gene could be supposedly related to a variety of functions during plant development.

Concisely, most of the *SAUR* genes analyzed in this work had higher expression profiles in samples with intense cellular growth and expansion unlike the other two samples with more cellular divisions (EC and ECS), which is in agreement with the main well-known function of SAUR proteins, that is, the indirect regulation of H^+ ATPases mediated cellular acid growth. Nonetheless, such expression profile suits the dynamics of auxin-mediated coffee embryo development, a process already shown to be dependent of different auxin conjugates and auxin transport during differentiation step [51]. The only exception regarding this pattern is for the gene *CaSAUR18*, which is expressed in embryogenic calli at a similar proportion that in developing embryos, suggesting a possible involvement of the protein encoded by this gene also in cellular division mediated by auxin signaling.

Conclusion

The in silico identification of SAUR family in *C. canephora* genome followed by analysis of its gene expression profile across coffee somatic embryogenesis in *C. arabica* represents a further additional step towards a better comprehension of molecular components acting on somatic embryogenesis. Our work opens unexplored doors regarding this gene family in coffee and through additional research about how these genes could interfere within somatic embryogenesis morphogenetic pathway, the scientific coffee community will be supported by tools helpful for the development of efficient clonal propagation methods via somatic embryogenesis to improve, in the future, the quality and production of coffee worldwide.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-07011-7.

Acknowledgements We would like to thank the National Council for Scientific and Technological Development (CNPq) from Ministry of Science and Technology, Brazil, and the Foundation of Support Research of the State of Minas Gerais (FAPEMIG) for the financial support provided to this study. The Coordination for the Improvement of Higher Education Personnel (CAPES) from Ministry of Education, for the scholarships awarded to the authors. We also thank the Federal University of Lavras (UFLA) for providing the infrastructure and equipment to accomplish this research, including real time PCR machine, computers and servers for bioinformatics analyses, which is supported by Ministry of Science and Technology and Ministry of Education.

Authors' contributions All authors contributed to the study conception and design. Leandro Eugenio Cardamone Diniz and Luciano Vilela Paiva get funding acquisition for the financial support from funding agencies. Fabiana Couto Zanin and Natália Chagas Freitas conducted in vitro culture experiments and gene expression analyses through RT-qPCR. Wesley Pires Flausino Máximo and Renan Terassi Pinto carried out the studies about the phylogenetic relationship, genomic structure and the statistical analysis for RT-qPCR data. Fabiana Couto Zanin, Natália Chagas Freitas, Renan Terassi Pinto, and Wesley Pires Flausino Máximo contributed to writing the manuscript. All authors contributed equally to revising the manuscript.

Funding FAPEMIG, CNPq, and CAPES. Conselho Nacional de Desenvolvimento Científico e Tecnológico,Coordenação de Aperfeiçoamento de Pessoal de Nível Superior,Fundação de Amparo à Pesquisa do Estado de Minas Gerais

Data availability All data for gene and amino acid sequences referred to species used into the *SAUR* family genomic structure relationship and phylogenetic tree are available on the Supplementary material (Supplementary data S1 and S2, respectively). Embryogenic and non-embryogenic calli herein used were obtained under in vitro culture conditions previously described in the materials and methods. The primer sequences for amplification of the *CaSAUR* genes are also available at the Supplementary material (Supplementary Table S1). In case of any information required for reproduction of the experimental data is not present elsewhere within publication content, please directly contact the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval Not applicable.

Consent to participate All authors are consent about their participation at the manuscript.

Consent for publication All authors are consent about publishing this manuscript.

References

1. Fehér A, Pasternak TP, Dudits D (2003) Transition of somatic plant cells to an embryogenic state. Plant Cell Tiss Org 74:201–228. https://doi.org/10.1023/A:1024033216561
2. Staritsky G (1970) Embryoid formation in callus tissues of coffee. Acta Bot Neerl 19:509–514. https://doi.org/10.1111/j.1438-8677.1970.tb00677.x
3. Etienne H, Breton D, Breitler J-C, Bertrand B, Déchamp E, Awada R, Marraccini P, Léran S, Alpizar E, Campa C, Courtel P, Georget F, Ducos J-P (2018) Coffee somatic embryogenesis: how did research, experience gained and innovations promote the commercial propagation of elite clones from the two cultivated species? Front Plant Sci 9:1630. https://doi.org/10.3389/fpls.2018.01630
4. Campos NA, Panis B, Carpentier SC (2017) Somatic embryogenesis in coffee: the evolution of biotechnology and the integration of omics technologies offer great opportunities. Front Plant Sci. https://doi.org/10.3389/fpls.2017.01460
5. Ikeuchi M, Favero DS, Sakamoto Y, Iwase A, Coleman D, Rymen B, Sugimoto K (2019) Molecular mechanisms of plant regeneration. Annu Rev Plant Biol 70:377–406. https://doi.org/10.1146/annurev-arplant-050718-100434
6. Wójcik AM, Wójcikowska B, Gaj MD (2020) Current perspectives on the auxin-mediated genetic network that controls the induction of somatic embryogenesis in plants. Int J Mol Sci 21:1333. https://doi.org/10.3390/ijms21041333
7. Silva AT, Barduche D, do Livramento KG, Ligterink W, Paiva LV, (2014) Characterization of a putative *Serk-Like* Ortholog in embryogenic cell suspension cultures of *Coffea arabica* L. Plant Mol Biol Rep 32:176–184. https://doi.org/10.1007/s11105-013-0632-x
8. Silva AT, Barduche D, do Livramento KG, Paiva LV, (2015) A putative *BABY BOOM-like* gene (*CaBBM*) is expressed in embryogenic calli and embryogenic cell suspension culture of *Coffea arabica* L. In Vitro Cell Dev-Pl 51:93–101. https://doi.org/10.1007/s11627-014-9643-z
9. Torres LF, Diniz LEC, Do Livramento KG, Freire LL, Paiva LV (2015) Gene expression and morphological characterization of cell suspensions of Coffea arabica L. cv. Catiguá MG2 in different cultivation stages. Acta Physiol Plant. https://doi.org/10.1007/s11738-015-1924-6
10. Su YH, Liu YB, Zhang XS (2011) Auxin-cytokinin interaction regulates meristem development. Mol Plant 4:616–625. https://doi.org/10.1093/mp/ssr007
11. Pinto RT, Freitas NC, Máximo WPF, Cardoso TB, Prudente DO, Paiva LV (2019) Genome-wide analysis, transcription factor network approach and gene expression profile of *GH3* genes over early somatic embryogenesis in *Coffea* spp. BMC Genomics 20:812. https://doi.org/10.1186/s12864-019-6176-1
12. McClure BA, Guilfoyle T (1989) Rapid redistribution of auxin-regulated RNAs during gravitropism. Science 243:91–93. https://doi.org/10.1126/science.11540631
13. Wu J, Liu S, He Y, Guan X, Zhu X, Cheng L, Wang J, Lu G (2012) Genome-wide analysis of SAUR gene family in Solanaceae species. Gene 509:38–50. https://doi.org/10.1016/j.gene.2012.08.002
14. Jain M, Tyagi AK, Khurana JP (2006) Genome-wide analysis, evolutionary expansion, and expression of early auxin-responsive *SAUR* gene family in rice (*Oryza sativa*). Genomics 88:360–371. https://doi.org/10.1016/j.ygeno.2006.04.008
15. Wang P, Lu S, Xie M, Wu M, Ding S, Khaliq A, Ma Z, Mao J, Chen B (2020) Identification and expression analysis of the small auxin-up RNA (SAUR) gene family in apple by inducing of auxin. Gene 750:144725. https://doi.org/10.1016/j.gene.2020.144725
16. Park JE, Kim YS, Yoon HK, Park CM (2007) Functional characterization of a *small auxin-up RNA* gene in apical hook development in *Arabidopsis*. Plant Sci 172:150–157. https://doi.org/10.1016/j.plantsci.2006.08.005
17. Zhang H, Yu Z, Yao X, Chen J, Chen X, Zhou H, Lou Y, Ming F, Jin Y (2021) Genome-wide identification and characterization of small auxin-up RNA (SAUR) gene family in plants: evolution and expression profiles during normal growth and stress response. BMC Plant Biol 21:4. https://doi.org/10.1186/s12870-020-02781-x
18. Yin H, Li M, Lv M, Hepworth SR, Li D, Ma C, Li J, Wang SM (2020) SAUR15 promotes lateral and adventitious root development via activating H+-ATPases and auxin biosynthesis. Plant Physiol 184:837–851. https://doi.org/10.1104/pp.19.01250
19. Wang X, Yu R, Wang J, Lin Z, Han X, Deng Z, Fan L, He H, Deng XW, Chen H (2020) The Asymmetric expression of SAUR genes mediated by ARF7/19 promotes the gravitropism and phototropism of plant hypocotyls. Cell Rep 31:107529. https://doi.org/10.1016/j.celrep.2020.107529
20. Wong JH, Klejchová M, Snipes SA, Nagpal P, Bak G, Wang B, Dunlap S, Park MY, Kunkel EN, Trinidad B, Reed JW (2021) SAUR proteins and PP2C. D phosphatases regulate H+-ATPases and K+ channels to control stomatal movements. Plant Physiol 185:256–273. https://doi.org/10.1093/plphys/kiaa023
21. Spartz AK, Ren H, Park MY, Grandt KN, Lee SH, Murphy AS, Sussman MR, Overvoorde PJ, Gray WM (2014) SAUR inhibition of PP2C-D phosphatases activates plasma membrane H+-ATPases to promote cell expansion in *Arabidopsis*. Plant Cell 26:2129–2142. https://doi.org/10.1105/tpc.114.126037
22. Li ZG, Chen HW, Li QT, Tao JJ, Bian XH, Ma B, Zhang WK, Chen SY, Zhang JS (2015) Three SAUR proteins SAUR76, SAUR77 and SAUR78 promote plant growth in *Arabidopsis*. Sci Rep. https://doi.org/10.1038/srep12477
23. Spartz AK, Lor VS, Ren H, Olszewski NE, Miller ND, Wu G, Spalding EP, Gray WM (2017) Constitutive expression of Arabidopsis *SMALL AUXIN UP RNA19* (*SAUR19*) in tomato confers auxin-independent hypocotyl elongation. Plant Physiol 173:1453–1462. https://doi.org/10.1104/pp.16.01514
24. Denoeud F, et al. (2014) The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 345:1181–1184. DOI: https://doi.org/10.1126/science.1255274 Coffee Genome Hub (http://coffee-genome.org/). Cited 10 March 2020.
25. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673
26. Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection foraccurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285
27. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096
28. Nguyen LT, Schmidt HA, Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300
29. Yu CS, Chen YC, Lu CH, Hwang JK (2006) Prediction of protein subcellular localization. Proteins 64:643–651. https://doi.org/10.1002/prot.21018
30. Sahu SS, Loaiza CD, Kaundal R (2020) Plant-mSubP: a computational framework for the prediction of single- and multi-target protein subcellular localization using integrated machine-learning approaches. AoB Plants. https://doi.org/10.1093/aobpla/plz068

31. Armenteros JJA, Sønderby CK, Sønderby SK, Nielsen H, Winther O (2017) DeepLoc: prediction of protein subcellular localization using deep learning. Bioinformatics 33:3387–3395. https://doi.org/10.1093/bioinformatics/btx431
32. Gasteiger E, Hoogland C, Gattiker A, Duvaud Se, Wilkins MR, Appel RD, Bairoch A (2005) Protein Identification and Analysis Tools on the ExPASy Server, In: Walker JM (ed) The Proteomics Protocols Handbook, Humana Press, Totowa Doi: https://doi.org/10.1385/1-59259-890-0:571
33. Teixeira JBJ, Junqueira CS, Pereira AJPC, Mello RIS, Silva APD, Mundim DA (2004) Multiplicação clonal de café (Coffea arábica L.) via embriogênese somática. Embrapa Recursos Genéticos e Biotecnologia. https://ainfo.cnptia.embrapa.br/digital/bitstream/CENARGEN/24709/1/doc121.pdf. Cited 1 June 2018
34. Boxtel JV, Berthouly M (1996) High frequency somatic embryogenesis from coffee leaves. Plant Cell Tiss Org 44:7–17. https://doi.org/10.1007/BF00045907
35. Zamarripa A, Ducos JP, Bollon H, Dufour M, Pétiard V (1991) Production d'embryons somatiques de caféier en milieu liquide: effets densité d'inoculation et renouvellement du milieu. Café, Cacao, Thé 35:233–244
36. Maciel ALR, Rodrigues FA, Pasqual M, Carvalho CHS (2016) Large-scale, high-efficiency production of coffee somatic embryos. Crop Breed Appl Biot 16:102–107. https://doi.org/10.1590/1984-70332016v16n2a16
37. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45. https://doi.org/10.1093/nar/29.9.e45
38. Freitas NC, Barreto HG, Fernandes-Brum CN, Moreira RO, Chalfun A, Paiva LV (2017) Validation of reference genes for qPCR analysis of *Coffea arabica* L. somatic embryogenesis-related tissues. Plant Cell Tiss Org 128:663–678. https://doi.org/10.1007/s11240-016-1147-6
39. Ferreira DF (2014) Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. Ciênc Agrotec 38:109–112. https://doi.org/10.1590/S1413-70542014000200001
40. Bai QS, Hou D, Li L, Cheng Z, Ge W, Liu J, Li X, Mu S, Gao J (2017) Genome-wide analysis and expression characteristics of small auxin-up RNA (*SAUR*) genes in moso bamboo (*Phyllostachys edulis*). Genome 60:325–336. https://doi.org/10.1139/gen-2016-0097
41. Stortenbeker N, Bemer M (2019) The SAUR gene family: the plant's toolbox for adaptation for adaptation of growth and development. J Exp Bot 70:17–27. https://doi.org/10.1093/jxb/ery332
42. Chae K, Isaacs CG, Reeves PH, Maloney GS, Muday GK, Nagpal P, Reed JW (2012) *Arabidopsis SMALL AUXIN UP RNA63* promotes hypocotyl and stamen filament elongation. Plant J 71:684–697. https://doi.org/10.1111/j.1365-313X.2012.05024.x
43. Spartz AK, Lee SH, Wenger JP, Gonzalez N, Itoh H, Inzé D, Peer WA, Murphy AS, Overvoorde PJ, gray WM, (2012) The *SAUR19* subfamily of *SMALL AUXIN UP RNA* genes promote cell expansion. Plant J 70:978–990. https://doi.org/10.1111/j.1365-313X.2012.04946.x
44. Stamm P, Kumar PP (2013) Auxin and gibberellin responsive Arabidopsis *SMALL AUXIN UP RNA36* regulates hypocotyl elongation in the light. Plant Cell Rep 32:759–769. https://doi.org/10.1007/s00299-013-1406-5
45. Michalczuk L, Cooke TJ, Cohen JD (1992) Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry 31:1097–1103. https://doi.org/10.1016/0031-9422(92)80241-6
46. Hou K, Wu W, Gan SS (2013) *SAUR36*, a SMALL AUXIN UP RNA gene, is involved in the promotion of leaf senescence in Arabidopsis. Plant Physiol 161:1002–1009. https://doi.org/10.1104/pp.112.212787
47. Qiu T, Qi M, Ding X, Zheng Y, Zhou T, Chen Y, Han N, Zhu M, Bian H, Wang J (2020) The SAUR41 subfamily of SMALL AUXIN UP RNA genes is abscisic acid inducible to modulate cell expansion and salt tolerance in Arabidopsis thaliana seedlings. Ann Bot 125:805–819. https://doi.org/10.1093/aob/mcz160
48. He Y, Liu Y, Li M, Lamin-Samu AT, Yang D, Yu X, Izhar M, Jan I, Ali M, Lu G (2021) The Arabidopsis SMALL AUXIN UP RNA32 protein regulates ABA-mediated responses to drought stress. Front Plant Sci 12:259. https://doi.org/10.3389/fpls.2021.625493
49. Markakis MN, Boron AK, Van Loock B, Saini K, Cirera S, Verbelen J-P, Vissenberg K (2013) Characterization of a Small Auxin-Up RNA (SAUR)-Like Gene Involved in *Arabidopsis thaliana* Development. PLoS ONE. https://doi.org/10.1371/journal.pone.0082596
50. Sagee O, Riov J, Goren R (1990) Ethylene-enhanced catabolism of [C]indole-3-acetic acid to indole-3-carboxylic acid in citrus leaf tissues. Plant Physiol 92:54–60. https://doi.org/10.1104/pp.92.1.54
51. Márquez-López RE, Pérez-Hernández C, Ku-González Á, Galaz-Ávalos RM, Loyola-Vargas VM (2018) Localization and transport of indole-3-acetic acid during somatic embryogenesis in *Coffea canephora*. Protoplasma 255:695–708. https://doi.org/10.1007/s00709-017-1181-1