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RELATIVE EXPRESSION OF GENES CHIA1, SGF14C AND CHS8* IN SOYBEAN SEED COATS[1]

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SUMMARY - The aim of this study was to evaluate the relative expression of three candidate genes, CHIA1, SGF14c and CHS8 * possibly involved in seed quality, in contrasting seed coats from four soybean genotypes. Two genotypes with yellow seed coats, BMX Potência RR and CD 202, and two genotypes with black seed coats, TP and IAC were studied to determine the relative gene expression through the qPCR technique, in seven stages of seed coat development for all four genotypes, at 25, 30, 35, 40, 45, 50 and 55 days after anthesis. The CHIA1 and SGF14c genes showed higher expression in cultivar CD 202; the former in the final stages of seed coat development, at 55 days after anthesis, the latter gene at earlier stages, specifically at 25 days after anthesis. The CHS8* gene showed higher expression in CD 202 seed coats at 50 days after anthesis. All three genes expressed at higher levels on genotypes of yellow seed coats, and are considered relevant to new areas of research based on the expression of genes related to seed quality.

Keywords: *Glycine max*. Protection structure. Candidate genes. Seed quality.

EXPRESSÃO RELATIVA DOS GENES CHIA1, SGF14C E CHS8* EM TEGUMENTOS DE SEMENTES DE SOJA

RESUMO - O objetivo do trabalho foi avaliar a expressão relativa de três genes candidatos, CHIA1, SGF14c e CHS8*, possivelmente envolvidos com a qualidade das sementes, em tegumentos contrastantes de quatro genótipos de soja. Foram utilizados dois genótipos de tegumentos amarelos, BMX Potência RR e CD 202, e dois de tegumentos pretos, TP e IAC. A expressão relativa dos genes foi avaliada pela técnica qPCR, em sete fases de desenvolvimento dos tegumentos dos quatro genótipos, aos 25, 30, 35, 40, 45, 50 e 55 dias após a antese. Os genes CHIA1 e SGF14c apresentaram maior expressão na cultivar de soja CD 202. O primeiro, nas fases finais de desenvolvimento dos tegumentos, aos 55 dias após a antese, e o segundo gene, nas fases iniciais, mais precisamente aos 25 dias após a antese. O gene CHS8* apresentou maior expressão nos tegumentos da cultivar aos 50 dias após a antese. Todos os três genes estudados têm maior expressão nos genótipos de tegumentos amarelos, sendo os mesmos importantes para novas frentes de pesquisas com base na expressão de genes relacionados à qualidade das sementes.

Palavras-chave: *Glycine max*. Estrutura de proteção. Genes candidatos. Qualidade de sementes.

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INTRODUCTION

The quality of soybean seeds is related to seed coat intrinsic characteristics, in which more fragile seeds result from seed coats more susceptible to mechanical damage, thus rendering the seeds prone to deterioration. Research data on this subject has found that, in general, black seed coats are more resistant than yellow seed coats, thus providing higher seed quality on physiological, physical and sanitary traits (CAPELETI et al., 2005; MERTZ et al., 2009; WEBB et al., 1995).

Coupled with the current knowledge on seed technology, research based on molecular biology techniques can contribute to the generation of new information regarding the role of seed coats, which reflects on the quality of the seed produced.

Following this concept, Mertz et al. (2010) evaluated the differential expression of gene fragments by the cDNA-AFLP technique on soybean genotypes with contrasting seed coat coloration. The authors identified proteins related to seed quality in black-coated genotypes, probably involved with the seed structural integrity (MOÏSE et al., 2005), cell wall regeneration (YANG et al., 2008), defense against pathogens, abiotic stresses, cell division and transcription factors (LI et al., 2005). Other research work in agreement with these comments include Capeleti et al. (2005), Mertz et al. (2009) and Webb et al. (1995).

However, it is worth noting that thousands of other genes are expressed in soybean seed coats and need to be properly identified. This is justified primarily to generate information for soybean breeding programs, since the identification of genes of interest within specific genotypes could be the basis for their selection for future crossings, seeking the transfer of such traits to new soybean cultivars. This is crucially important when it comes to genes related to biotic and abiotic stresses, which can compromise the quality of the seeds produced. Among these genes of interest are CHIA1, SGF14c and CHS8.

The CHIA1 gene encodes for the chitinase enzyme that belongs to group I, according to its primary structure, characterized by the presence of chitin-binding domain in the N-terminal region, rich in highly conserved cysteines. This group of enzymes catalyze the hydrolysis of beta-1, 4-N-acetylglucosamine present in chitin. This polysaccharide is the major cell wall component of fungi and the exoskeletons of arthropods, being absent in plants (KASPRZEWSKA, 2003). Therefore, the presence of this group of enzymes in plant tissues is important because it contributes to the defense against pathogens, which is dependent on their level of expression in the plant tissue (KERN et al., 2010).

SGF 14-3-3 proteins are encoded by a multigene family involved in signaling pathways that regulate plant development, and also provide protection against biotic and abiotic stresses such as drought tolerance (LI; DHAUBHADEL, 2011; XU; SHI, 2006). According to some studies, this multigene family has been mentioned to perform important functions such as gene regulation, protein synthesis and structure, degrees of control on the primary metabolism, as well as with the proton pump bound to the plasma membrane, chromatin remodeling; and hormone metabolism (PAUL et al., 2008; SCHOONHEIM et al., 2009).

The chalcone synthase (CHS) present in all plants, is encoded by a multigene family and is a key enzyme in the biosynthesis of flavonoids (TUTEJA; VODKIN, 2008), being able to show different expression patterns. Besides being normally expressed during plant development, the CHS genes are also expressed under stress conditions such as the incidence of UV rays, bacterial infection and fungi or insect attack. Also, these genes act in plants as signaling molecules - microorganism interactions, in the synthesis of pigments and phytoalexins, also having antioxidant functions attractive to pollinators (DAO et al., 2011).

This study aimed to assess the relative expression of three candidate genes possibly involved on seed quality, CHIA1, SGF14c and CHS8* on contrasting seed coats from four soybean genotypes, by the qPCR technique in different stages of development.

MATERIAL AND METHODS

The first stage of the experiment consisted on the seed multiplication of four soybean genotypes, CD 202 (conventional) and BMX Potência RR (GM) cultivars with yellow seed coats, and TP and IAC lines, with black seed coats. Seed multiplication was carried on in a greenhouse located in the Capão do Leão city, Rio Grande do Sul state, Brazil, during the 2012/2013 harvest season.

During anthesis flowers were marked to ensure that all sampled seeds were at the same stage of development. Seven legume harvests were collected every five days (25, 30, 35, 40, 45, 50 and 55 days after anthesis) for each of the four genotypes.

Immediately after collection the seeds, yet within the legumes, were submerged in liquid nitrogen to facilitate the seed coat separation with the aid of sterilized blades, taking care to keep the plant tissue free of impurities. Once separated from the seeds, seed coats from each genotype were stored in Ultrafreezer at - 80°C until processing for RNA extraction.

RNA was extracted in a single day for all treatments (seed coat sampling times for each genotype) using the reagent *Concert Plant RNA Reagent* (Invitrogen™). After extraction, RNA samples were treated to DNase and had their purity and integrity measured by analysis of absorbance (260/280 nm) and electrophoresis in 1% agarose gel. RNA extrac-

tion and cDNA synthesis were performed using three biological replicates, each replicate consisting of a seed coat mixture from seeds collected at each stage of development.

Single-stranded cDNAs were synthesized by reverse transcription from 2µg of total RNA in a final volume of 20µL, using the *SuperScript III*® enzyme (Invitrogen™), according to manufacturer instructions. To assess cDNA quality, a semi-quantitative PCR was performed using *Master Mix Go Taq,* cDNA from each sample, water and β-actin. The purity and integrity of the cDNA were also measured to ensure the quality of the material used.

Five normalizing genes ACT11, SKIP16, UKN1, UKN2 and ß ACTIN were preliminarily tested. To evaluate their stability, eight random cDNA samples were used, choosing for normalizing genes ACT11 and SKIP16, which showed less variation of expression among samples.

For the design of primers a search was carried out for ESTs sequences from corresponding proteins to candidate genes listed on Table 1, together with the NCBI (National Center for Biotechnology Information) database. Primers were designed with the aid of Vector NTI Advance 11.0 (Invitrogen™) Program.

Table 1. Ratio of target genes, access and primer sequences used for qPCR.

Gene/Access		Primer sequence
CHIA1	Ss[1]	5' CCTTCTTGGGGCCACAGCAGAA 3'
(AF202731)	As	5' GGCTCTGGCAACCTTCTCCACAGTA 3'
SGF14c	Ss	5' CGAACGCTATGAAGAAATGGTGGAAGCA 3'
(HM004361.1)	As	5' GAAAGAATCCTCCACGAAGCCCTACG 3'
CHS8*	Ss	5' TCATTGATAGCATATGGATACCGAAGTTGATGGG 3'
(AY237728.1)	As	5' CCACCGTCTACTACGACTGTTTGTTAGTTTG 3'

[1] Ss: sense; AS: antisense

To set conditions for the reaction, tests were conducted to establish the annealing temperature, using the temperature gradient between 50°C and 60°C for candidate genes and normalizing genes, as well as to determine the optimal final concentration of each primer. To determine the latter, analyses for different concentrations (100, 200 and 400 nm) were used and the recommendations for use of the commercial *Master Mix*. The same cDNA sample was used for all tests, to allow for the final comparison of results.

The quality of the amplified product was verified by dissociation curves at the end of qPCR, given the progressive increase of the reaction temperature. Thus, calculation of the fluorescence emission proceeded by evaluating the analytical specificity of the primers by denaturing the generated PCR product.

Serial dilutions, i.e. 1:3; 1:30; 1:300; and 1:3000 were performed to establish the *Slope*; and to calculate individual gene efficiency through the equation $E = | 10^{(-1/slope)} | -1$ (ZHAO; FERNALD, 2005) (Table 2).

The real time quantitative gene expression analysis of target genes was performed by the Light-Cycler 480 Instrument II (96) equipment (Roche Applied Science®), using SYBR® Green.

The relative expression of the three candidate genes was calculated based on the amplification efficiency (E) and of the PCR cycle, considering the increase in fluorescence above the baseline signal (PFAFFL, 2001). After obtaining the values for gene relative expression, these were normalized using the values observed for the check treatment, considering the 25-day post anthesis development phase of genotype BMX Potência RR.

The results from gene expression were subjected to analysis of variance and when interactions between factors were detected, genotype comparisons within each seed coat developmental phase and for each genotype across the different phases were performed. Treatment means were compared by the Tukey test at 5% likelihood level using the Winstat version 2.0 statistical program (MACHADO; CONCEIÇÃO, 2003).

RESULTS AND DISCUSSION

The qualitative results for amplification presence or absence indicated an optimum temperature of 60°C. Based on this determination, the following cycling parameters were established: pre-incubation (1x 95° C for 5 minutes); amplification (45x 95° C for 10 seconds, 60° C for 20 seconds, 72° C for 20 seconds); Melting curve (1x 95° C for 5 seconds, 50° C for 1 min, 98°C continuous - Eight acquisitions / degree); and cooling (1x 40°C for 10 seconds). The optimum final concentration for each primer was considered to be of 200 nM.

The slope values and the efficiency of amplification of primers for each gene are shown on Table 2. All values were close to those accepted by *Applied Biosystems* ®, which endorses a Slope value of -3.2.

Regarding gene expression, the analysis of variance indicated an interaction between contrasting genotypes and stages of soybean seed coat development (days after anthesis) for the three target genes under evaluation (Table 3).

Table 2. Values for Slope and primer efficiency of amplification.

Gene	Slope (S)	Efficiency (E)
CHIA1	-2,992	1,15886
SGF14	-3,164	1,07041
CHS8*	-3,339	0,99292

Table 3. Summary of the analysis of variance for the relative expression of genes CHIA1, SGF14c and CHS8* present in the seed coats of four contrasting soybean genotypes.

Source of varation	D.F.	Mean Square		
		CHIA1	SGF14c	CHS8*
Genotypes (F1)	3	165,776[1]	116,175[1]	88,671[1]
Seed coat sampling dates (F2)	6	242,331[1]	24,607[1]	37,649[1]
Int. F1xF2	18	225,679[1]	46,641[1]	44,654[1]
Treatments	27	222,723[1]	49,471[1]	47,988[1]
Residuals	28	0,103	3,239	0,819

D.F. – Degrees of Freedom. [1] Significant at 1% likelihood by the *F* test.

The results concerning the expression of CHIA1 gene (Figure 1) pointed to differences among genotypes and stages of seed coat development.

CHIA1 expression at DAA (days after anthesis) 25 was relatively low in all soybean genotypes, with genotypes BMX Potência RR and TP showing the highest values. However, this difference was not observed at 30, 35, 40 and 45 DAA. At 50 DAA genotype TP exhibited a very high gene expression in relation to the rest, which did not differ between them.

The highest level of CHIA1 seed coat expression was observed at DAA 55 in genotype CD 202, followed by IAC and TP and BMX Potência RR, with no differences between the latter two genotypes.

Given the results observed in Figure 1, we determined that the CHIA1 gene has peak expression levels at later stages of seed coat development, when compared to the initial and intermediate stages of seed formation, in agreement with findings by Gijzen et al. (2001). According to these researchers, the gene encoding for the chitinase enzyme present in soybean seed coats has higher expression at the final stages of seed maturation, being associated to tissue senescence and response to infection by pathogens.

Induction of chitinase is often coordinated with the expression of enzymes β-1, 3- specific glucanases and other pathogenesis-related proteins (PR proteins) in response to pathogen attack, as well as in response to elicitors and abiotic factors (PULLA et al., 2011). In addition, other defensive processes can be included, such as oxidative burst, phytoalexin accumulation and cell wall lignification and stiffening (DURRANT; DONG, 2004).

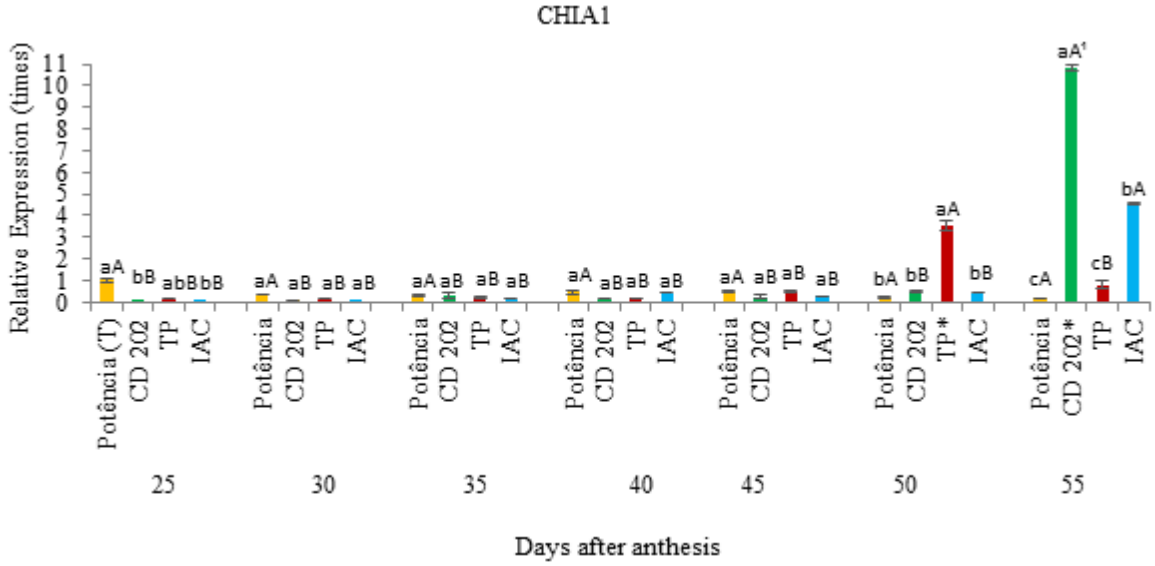

Figure 1. Relative expression (fold) of the CHIA1 gene in seed coats of four contrasting soybean genotypes collected in seven developmental stages after flowering. [1]Means followed by different lowercase letters for genotypes within each collection date, and capital letters within each genotype and between sampling dates differ by Tukey test at 5% likelihood. Genotypes marked (*) have the gene expression divided five-fold.

When comparing seed coat development phases for each genotype, it was observed that for BMX Potência RR the results of gene expression did not change, with no differences between phases. However, the CD 202 genotype showed differences, especially at 55 DAA, in which the value for gene expression was significantly higher than for other phases, in which no differences were observed. A similar situation occurred for the IAC and TP genotypes; however, in the latter the highest value of gene expression occurred at 50 DAA, differing from the other stages of seed coat development (Figure 1).

These results highlight the potential for genotype CD 202 (yellow seed coat) to breeding programs that make use of molecular tools for target gene transfer, in this case the CHIA1 gene, which may be an interesting alternative in order to increase plant resistance to fungi attack.

CHIA1 can be considered a gene of interest to the seed industry, especially in genotype CD 202, as it expresses at the final stages of seed coat formation and it may be involved in tissue protection against pathogen attacks. After reaching physiological maturity the seeds are exposed to unfavorable environmental conditions, and may suffer fungi infection, whether in the field or under storage conditions, which will cause them to deterioration rapidly. Thus, in the case of seed coats that can express this gene in the final stages of seed filling, they will likely develop enhanced resistance to fungi infection. However, research should push forward on the effect of this gene in tissues infected by fungi, much in the same way as done by Gijzen et al. (2001), who observed an enhanced expression for the gene encoding the chitinase enzyme in the soybean hypocotyl when infected by *Phytophthora sojae*, compared to the same tissue without the occurrence of infection. According to these researchers, the seed coat of soybean seeds is rich in defense proteins (PR) and peptides. This feature is particularly important because the seeds are subject to attack by pathogens during storage and in the field, after completing physiological maturity and even during the germination - emergence period.

In transgenic tobacco, genetically modified to confer resistance to *Rhizoctonia solani*, Kern et al. (2010) found that GM plants were efficient in reducing the pathogen in leaves, observing a direct relationship between the chitinase enzyme activity and a reduction on leaf area affected by fungal lesions.

As for gene SGF14c expression, it was observed that at 25 DAA (days after anthesis), genotype CD 202 yielded the highest values, followed by IAC and TP which did not differ between them and genotype BMX Potência RR showing the lowest gene expression.

At 30 DAA, the highest gene expression was also observed in genotype CD 202, followed BMX Potência RR without significant differences between them. The lowest expression was observed in genotype IAC, also without difference from the TP genotype. At 35 DAA, the highest gene expression was observed in the genotype RR BMX Potência, which did not differ from TP at 35 and 55 DAA and from genotype IAC at 40 DAA (Figure 2).

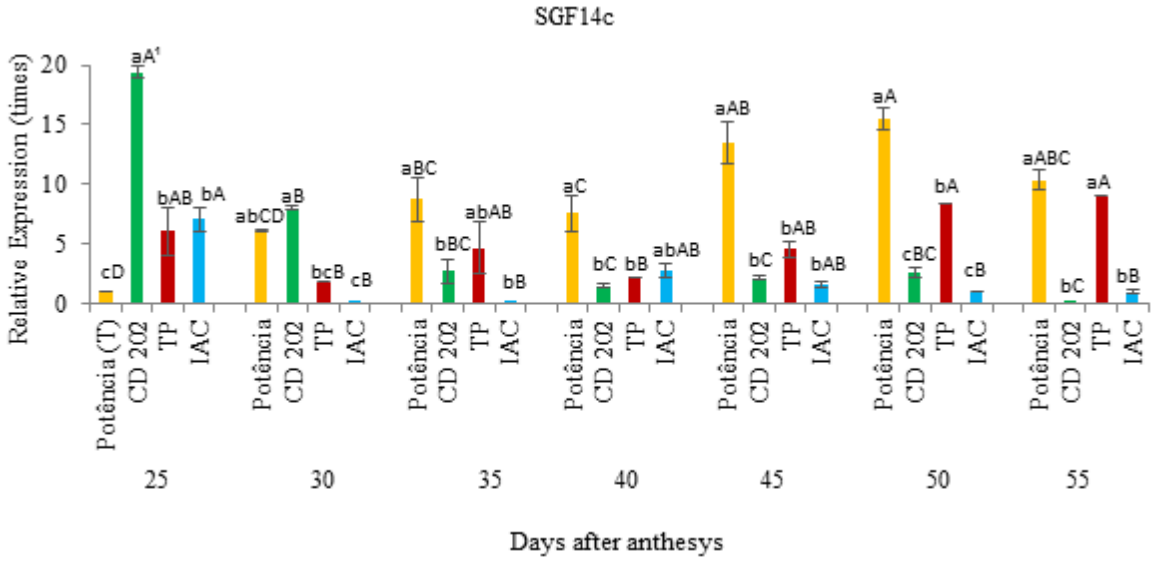

Figure 2. Relative expression (fold) of the SGF14c gene in seed coats of four contrasting soybean genotypes collected in seven developmental stages after flowering. [1]Means followed by different lowercase letters for genotypes within each collection date, and capital letters within each genotype and between sampling dates differ by Tukey test at 5% likelihood.

The lowest expression for gene SGF14c was observed at 35 DAA in genotypes IAC and CD 202. At 40 DAA the lowest expression occurred in genotypes CD 202 and TP; from DAA 45 onward low gene expression values were observed also for genotype IAC.

Eighteen genes from this multigene family (SGF14a - SGF14r) were identified in soybean by Li and Dhaubhadel (2011), sixteen of them already transcribed (SGF14a - SGF14p). All sixteen SGF14s showed higher expression in the embryo, suggesting their potential role on seed development, in agreement with the findings by Hajduch et al. (2005). Li and Dhaubhadel (2011) also observed that in particular the SGF14c gene, expressed itself intensely in the embryo between 30 and 40 days after pollination (DAP) and, to a lesser extent at 50 DAP, but still very close to the levels from other stages of development. Gene expression in seed coats was lower when compared to the embryo, but higher than what was recorded for other tissues such as root, stem, leaves and flowers.

An important function of SGF14s genes that has been recently discovered is their possible involvement in the passage of GmMYB176 between the cytoplasm and the cell nucleus. GmMYB176 is a transcription factor that interacts with the soybean protein 14-3-3 regulating the CHS8 gene expression and acting in the biosynthesis of flavonoids (DHAUBHADEL; LI, 2010).

In genotype BMX Potência RR the higher expression of gene SGF14c occurred at 50 DAA and did not differ from the levels observed at 45 and 55 DAA. The lowest level of expression for this gene in BMX Potência RR occurred at 25 DAA, not differing from that at 30 DAA.

Unlike what was observed for BMX Potência RR, genotype CD 202 showed the highest gene expression in the early stages of seed coat development i.e., at 25 DAA, followed by 30 DAA, the latter not differing from gene expression levels at 35 DAA (Figure 2).

The expression of genes SGF14s in embryos during their early stages of formation suggests that they may act in the development of soybean seeds, signaling the synthesis and transport of amino acids and metabolites that contribute to protein and reserve triglycerides synthesis as well as that of secondary metabolites (LI; DHAUBHADEL, 2011).

A different pattern for expression of the gene SGF14c was observed for the TP genotype, which showed the highest level at 50 and 55 DAA, not differing from the expression levels recorded at 25, 35 and 45 DAA. The IAC genotype had higher levels of expression in the early stages of seed coat development, decreasing thereafter, with the exception of dates 30 and 35 DAA (Figure 2).

The different pattern of expression of this gene reflects its potential for a multifunctional role in the development of plants and seeds. Because it belongs to a group of genes involved with signaling pathways and protein synthesis, it is not uncommon to observe its expression at different levels (LI; DHAUBHADEL, 2011).

Radwan et al. (2012) identified an important role of genes SGF14c and SGF14l in the first stages of the nodulation process in soybean plants. Through their silencing a small number of root nodules were recorded in soybeans, after inoculation with *Bradyrhizobium japonicum*, with many of the remaining nodules showing some kind of abnormality such as low cell decay, eliciting the critical role of these genes in this process. In *Arabidopsis thaliana*, Pignocchi and Doonan (2011) observed the key role of 14-3-3 proteins in the cell division process, which coincides with the initial stage of root nodulation in soybean.

Therefore, in addition to their alleged role on cell division that occurs along the symbiotic process, these genes can play the same role during seed formation. Thus, seeds that have higher expression of these genes may somehow experience greater cell division in the early stages of their formation, achieving a higher degree of development, originating high quality seeds due to a higher cell count for the allocation of reserve substances.

Among the many genes that comprise the family of chalcone synthase (CHS), our work evaluated the relative expression of gene CHS8*, still not characterized, in contrasting soybean genotypes for seed coat traits along seven stages of development.

At 25 DAA, the lower expression of the gene CHS8* occurred in genotype CD 202, with no differences among the other genotypes. At 30 and 35 DAA all differences in levels of gene expression among the soybean cultivars disappeared. The opposite was observed at 40 DAA, with genotype CD 202 showing higher expression for gene CHS8* together with BMX Potência RR, being the lowest expression observed in genotypes with black seed coats, i.e., IAC and TP (Figure 3).

By studying the pattern of expression of two chalcone synthase genes in developing embryos of soybean seeds, Dhaubhadel et al. (2007) observed higher transcript accumulation near seed maturity, agreeing with the findings of this work, despite in our case occurring within the seed coats.

At 45 DAA the highest expression for the gene CHS8* occurred in the BMX Potência RR genotype, whereas no differences were observed for the rest. At 50 DAA genotype CD 202, despite having shown a lower gene expression than BMX Potência RR, expressed higher levels than genotypes with black seed coats.

In soybean, the chalcone synthase is encoded by at least nine genes (CHS1 to CHS9) (TUTEJA; VODKIN, 2008), and the members are classified into two subfamilies, one corresponding to genes CHS7/CHS8 and the other encompassing the remaining members CHS (CHS1 - CHS6, CHS9) (KURAUCHI et al., 2009.). In dark seed coats CHS7/CHS8 mRNA transcripts are abundant (CHO et al., 2013) and form the vast majority of the transcripts, while the other members of the CHS subfamily occur at lower levels. In soybean seeds with yellow seed coats the mRNA levels of most CHS mem-

bers are significantly reduced, particularly for CHS7/ CHS8 (TUTEJA et al., 2004). However, according to Tuteja et al. (2004), the same expression pattern does not occur in soybean legumes, leaves, stems, roots and cotyledons, where the dominant allele is abundant and mRNA and CHS levels do not decrease.

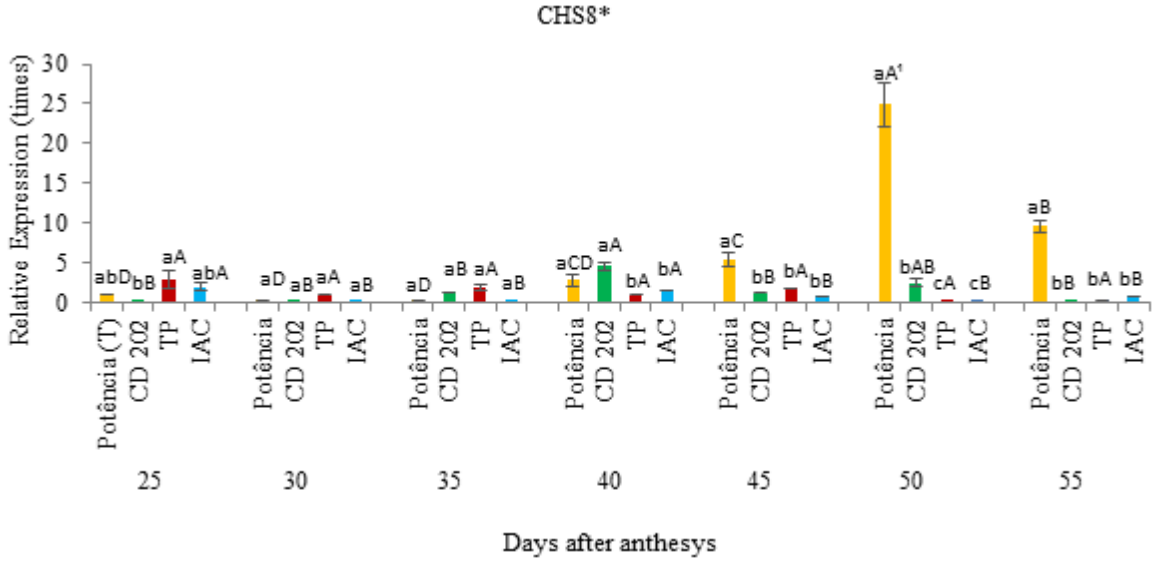

Figure 3. Relative expression (fold) of the SGF14c gene in seed coats of four contrasting soybean genotypes collected in seven developmental stages after flowering. ¹Means followed by different lowercase letters for genotypes within each collection date, and capital letters within each genotype and between sampling dates differ by Tukey test at 5% likelihood.

Based on the results of this work in relation to the level of gene expression for each genotype along the phases of seed coat development and references from the literature, it appears that, maybe, this gene is not directly related with seed coat coloring, deserving further studies to validate this possibility. It is important to note that the primer design was based on the gene CHS8, which has so far not been characterized, i.e., with a function not yet established by the scientific community. As a result, we chose the CHS8* nomenclature to differentiate it from the CHS8 gene described in the work of Tuteja et al. (2004), which is related to the dark coloration of soybean seed coats.

The greatest expression of gene CHS8* occurred at 50 DAA, followed at 55 DAA for genotype BMX Potência RR. The lowest expression for that gene was observed in the early stages of seed coat development, i.e. at 25, 30, 35 and 40 DAA; the latter did not differ from expression levels at 45 DAA (Figure 8).

For genotype CD 202, the highest level of expression was observed from 40 DAA to 50 DAA, without differences between dates. However, the latter date showed expression patterns for gene CHS8* similar to those observed in other phases of seed coat development, with no differences between them.

The soybean TP genotype did not show any differences in gene expression along the different stages of seed coat development. For genotype IAC, the highest level of expression occurred at 25 and 40 DAA, with no difference between the other phases of development.

Some explanations can be suggested for the pattern of this gene in soybean lines with black seed coats, based on work by Senda et al. (2004) and Voinnet (2005). According to the first authors, certain species exhibit a defense system based on the post-transcriptional gene silencing (PTGS), which is responsible for RNA degradation that can occur upon virus infection to the plant. When this mechanism is activated, the gene silencing of chalcone synthase can occur, which according to Senda et al. (2004) happens in soybeans with yellow seed coats. However, in the present study we observed the opposite situation, being that CHS8* expression was recorded in soybeans with yellow seed coats.

Given these results, it seems that this particular gene does not influence seed coat coloring, so we hypothesize that it may have been silenced in genotypes with black seed coats. We base our supposition on the fact that in black seed coat genotypes several other genes responsible for the plant's defense against biotic and abiotic stresses may be expressed (Webb et al., 1995), which are characteristic to this type of seed coat and can directly affect CHS8* expression.

The importance of the data obtained from this gene in this work lies on the wide variation found on the level of expression among the different genotypes. Therefore, new research can be planned in order to identify the exact protein produced by this gene, since it has not yet been characterized to this date by any research team, and its function in soybean seeds needs to be elucidated. With such infor-

mation, genotypes that show a higher expression of this gene may be considered of interest to the scientific community.

CONCLUSIONS

The CHIA1 and SGF14c genes showed higher expression in soybean cultivar CD 202. The former in the final stages of seed coat development, at 55 days after anthesis, while the latter gene during earlier stages, more precisely at 25 days after anthesis. The CHS8* gene showed higher expression in seed coats of cultivar BMX Potência RR at 50 days after anthesis.

All three genes have higher expression in genotypes of yellow seed coats, and they are important for new areas of research based on the expression of genes related to seed quality.

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