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Cell wall gene expression in two sub-species of flax

Dmitry Galinousky and Tsimafei Padvitski

Abstract

In this work, we attempted to elucidate mechanisms of flax fiber biogenesis by measuring, using qPCR, expression of the cell-wall related genes (cellulose synthase, cellulose synthase-like and chitinase-like genes) in stems of two contrasting fiber quality subspecies of flax (*Linum usitatissimum* L.). We studied *elongatum* Vav. et Ell. (cultivar Blakit, Belarus) and *crepitans* Boenn. (dehiscent flax) subspecies, which are differed in the height of plants, width of stems etc. Amongst all measured genes chitinase-like *Ctl19 and Ctl21* genes showed drastic difference in expression between stems of the two flax varieties, showing higher expression level in the fiber flax versus the dehiscent flax. In contrast, cellulose synthase-like *CslG4* gene had lower expression levels in the stem of fiber flax than in dehiscent flax. We suggest that hemicellulose composition and cellulose - non-cellulose glycan organisation can vary between stalk cells of different flax subspecies.

Laboratory of Ecological Genetics and Biotechnology, Institute of Genetics & Cytology, Belarus

Corresponding author: Dmitry Galinousky

E-mail: D.Galinousky@igc.by

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Introduction

The flax fiber is an important raw material for innovative biotechnology and traditional industry (1, 2). The flax fiber is classified as a bast fiber formed by cell wall deposition (3). One of the promising approaches to investigate flax fiber biogenesis is quantitative elucidation of expression of genes involved in the cell wall biosynthesis. The study of the expression pattern of genes associated with the cell wall biogenesis in various forms of flax may help identify genes that determine the flax fiber quality. In the present research, we identified cell wall biogenesis genes that were differentially expressed in two flax subspecies.

Materials and Methods

Subject of our study was two varieties of flax: Blakit (Belarus), belonging to *elongatum* Vav. et Ell. subspecies, is used to produce high-quality fiber, and dehiscent flax (subsp. *crepitans* Boenn.), which is essentially different in morphological and biological characteristics and is not used in the textile industry.

Flax plants were grown in experimental plots according to conventional techniques. RNA was isolated from stem parts that were taken from below the snap point (4). Plants were harvested during rapid growth stage (36 days after planting).

RNA was extracted using TRI REAGENT (Sigma). Quality control of the extracted RNA was performed by electrophoresis of RNA samples in a 1.5% agarose gel and by spectrophotometric measurements. cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) with an oligo dT_{18} primers.

qPCR was carried out in a 96-well plate (Thermo Scientific). The reaction volume per sample was 20 ul and contained 10 ul 2X buffer-T (S) for DNA polymerase (Primetech,

Table 1. Primers used in the study

Gene	Target ID	Primer sequences, 5'→3'	Reference
CesA1	Lus10018902/ Lus10028597	ggagcaaaaagtactctgacaaaag	(7, 11)
		cgcttctcgagacttctctg	
CesA4	Lus10008226	tacggttatgatccaccagtgtc	(7, 11)
		cgcttccctcccattttcttctt	
CesA6	Lus10002939/ Lus10002940	acgagcactttatgagctat	(7, 11)
		ggttttgattcttctctcc	
CesA7	scaffold464	cggccaaagatggtaa	(7, 11)
		gcagggctagaggatgga	
CslE	Lus10016625	atcgtcccttccctctgtct	-
		tgtggtggtagtttggtcgc	
CslD1	Lus10000755/ Lus10011736	aaaaacaacccactcctcgc	-
		tccctccaatcacccaaaac	
CslD2D3	Lus10038008/Lus10009248/ Lus10026610/ Lus10030455/ Lus10026609/ Lus10030453/	catcgtctactgcttcctccc	-
		tcagcaaactcgtcgtccac	
CslD4.3	Lus10013851/ Lus10026586	acaaaggcgaatccagcaac	-
		atgagaaggcaaaccagagc	
CslD4.4	Lus10001619/ Lus10022982	ctcagcaagacaacgcagg	-
		gacggcatttcaaacttctcc	
CslD5	Lus10010024/ Lus10025046	gctatggtgctaatgggtttg	-
		attgcttgcctgtttgggtg	
CslD6.1	Lus10012119	tggtgaggtggatttggtcg	-
		gggcggagtttagggagttg	
CslD6.2	Lus10002134	ccgctaacaccatcctctcc	-
		cttgacccgccttctatccc	
CslG3	Lus10003196	tccgccgtgactctgttttc	-
		gcttccttctcttggggtcc	
CslG4	Lus10023056/ Lus10023057/ Lus10032415/ Lus10032416	gctcgctctcctcaataatgtc	-
		tgctctgttcctcgtccac	
Ctl1	Lus10010860	aataaggtcccctcagcaca	(7)
		ccttttcgccagagtgtcat	
Ctl11	Lus10041831	cgtccatccatagcgtgatt	(7)
		tacccgggaactctgttgg	
Ctl19	Lus10010864	aaggttgacatactgagtgatcca	(7)
		caaacccacaaagcagtcttc	
Ctl21	Lus10024366	aaggtttcggagagaccatactt	(7)
		cgactttggacggtctcg	
Ctl23	Lus10035620	gccggcaagtcattctaca	(7)
		tgccgaagctagggtagga	
Ctl24	Lus10003231	tccgtaagattaacagcatgga	(7)
		agtagctgacccgagcgtta	
GAPDH	CV478202	aggttcttcccgctctcaat	(5)
		cctccttgatagcagccttg	
ETIF1	GR508906	ccttgtagggctgagggatt	(5)
		ctcatcaagaccaccagcaa	

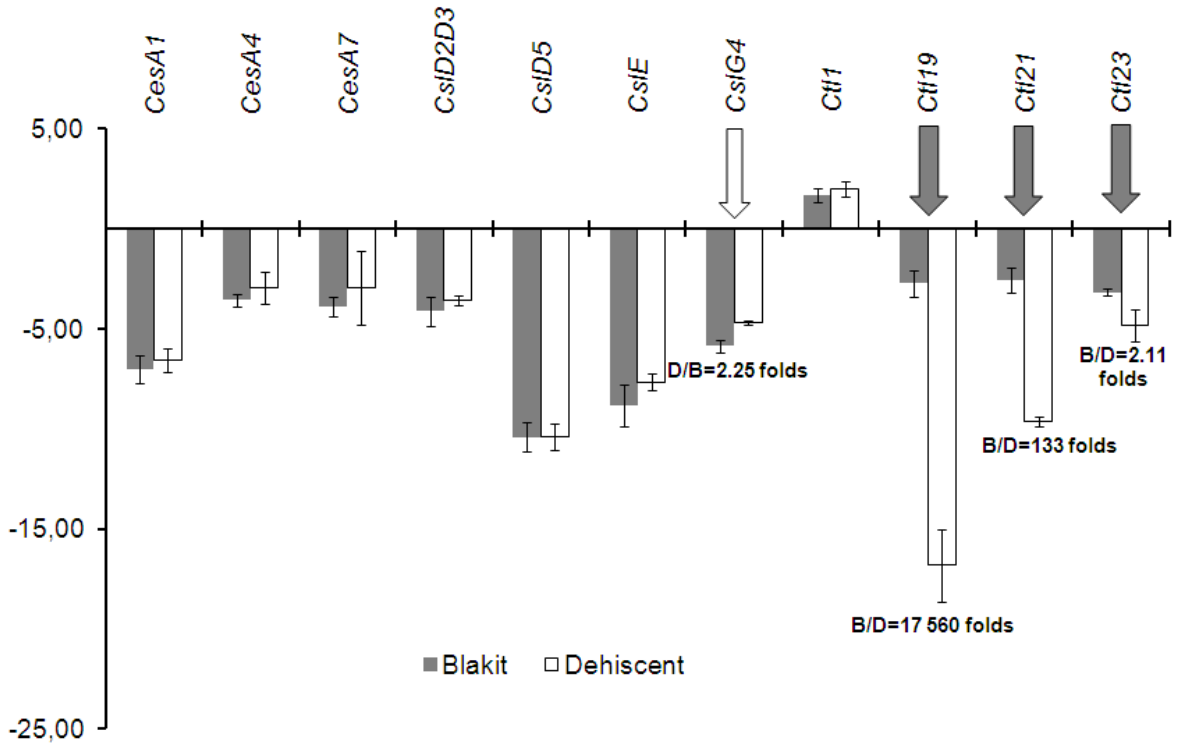

Figure 1: Expression of *CesA, Csl* and *Ctl* genes in two flax subspecies (*elongatum* – Blakit and *crepitans* – Dehiscent) relative to the expression of reference genes in stems at the rapid growth stage. The genes that had ΔCt value lower than -10 are not shown in the chart. The white arrow shows the gene, that had higher expression level in dehiscent flax (Dehiscent) than in fiber flax (Blakit). On the contrary, the gray arrows indicate genes, that had higher tanscripts levels in fiber flax (Blakit) than in dehiscent flax (Dehiscent).

Belarus), 0.2 mM of each nucleotide, 0.2 uM of forward and reverse primers, 50-100 ng of cDNA, 0.1 unit of DNA polymerase Tornado (Primetech, Belarus) and 0.16 ul of the fluorescent dye Zubr Green 100X (Primetech, Belarus). Each experimental reaction was accompanied by NRT and NTC control reactions. The primers for the genes used in this work are shown in **Table 1**. GAPDH and ETIF1 genes were used as reference genes (5). Primers to *Csl* and *CesA*-genes were designed by Primer3plus and Primer Blast.

The amplification reactions were performed using CFX96 (BioRad) thermocycler. The program of the qPCR is as follows: (1) 95,0° 15:00 min, (2) 99,0° - 1 sec, (3) 58,0° 10 sec, (4) 72,0° 10 sec, (5) plate read, repeat steps (2) – (5) 44 times, (6) 95,0° 10 sec, (7) melt curve from 65°C to 95°C with an increment 0,1°C. For each reaction, a high specificity of amplification was corroborated by the presence of a single peak on the melting curve. Expression analysis was performed using the ΔΔCt method (6). Pairwise comparison of ΔCt values was performed using t-test with non-pooled standard deviation and Benjamini & Hochberg multiple testing correction.

Results

The mean expression level of *Ctl* genes was significantly higher than the mean expression level of CesA and *Csl* genes in both subspecies studied. The transcript level of *Ctl1* was the highest among all studied genes and it was 3.2–3.9 folds higher (figure) than the transcript level of the reference genes. Substantial differences in the expression of *Ctl19*, *Ctl21* and *Ctl23* genes (17560, 133 and 2,11 folds respectively) were found between the two subspecies of flax (**Fig. 1**).

The mean transcript levels of *CesA* and *Csl* genes were approximately similar and both lower than the transcript level of reference genes. Among *CesA*s, secondary cell wall genes (*CesA4*, *CesA7*) exhibit higher levels of expression, as compared to the genes attributed to the primary cell wall synthesis (*CesA1*, *CesA6*). Among *Csl*-genes, the highest expression is demonstrated by the group of *CslD2D3* genes (figure).

The expression level of *CslG4* genes was 2.2-4.9 and *CslE* gene showed 16.9-37.5 times lower expression level than *CesA*-genes. Although, significant differences in expression patterns of *CslG4* were found for various subspecies (figure).

The transcript abundance of the cellulose synthase genes (*CesA1, CesA4, CesA6 and CesA7*), *Csl*-genes (*CslD2D3, CslD5, CslE*) and the *Ctl1* gene was similar in stems of fiber flax and dehiscent flax. The *CslD1, CslD4, CslD6, CslG3, Ctl11* and *Ctl24* genes were not expressed or expressed weakly in both subspecies of flax studied.

Discussion

The flax fiber cells formation takes place in two successive stages – (1) intrusive cell growth and (2) cell wall thickening (3). We collected plant samples at a rapid growth stage and evalu-

ated the expression of genes, when a cell wall is deposited. The studied genes affected cell wall biosynthesis: *CesA* genes encode catalytic subunit of cellulose synthase (8), Cellulose synthase-like genes (*Csl*) are related to *CesA*-genes, CSL proteins are believed to encode synthesis of cell wall hemicelluloses (9), and chitinase-like genes (*Ctl*) are likely to play a key role in establishing of interactions between cellulose and hemicelluloses (10). Substantial differences in the transcript levels of *Ctl19, Ctl21, Ctl23* and *CslG4* genes were found between the *elongatum* and *crepitans* subspecies of flax. The *Ctl*-genes were highly enriched in developing fiber cells (7). On the basis of this, we speculate that hemicellulose composition and interactions between cellulosic and non-cellulosic glycans of the cell wall of stem cells can vary in different subspecies of flax plant.

The transcript abundance of the cellulose synthase genes (*CesA1, CesA4, CesA6 and CesA7*), any *Csl*-genes (*CslD2D3, CslD5, CslE*) and *Ctl1* gene was similar in stems of fiber flax and dehiscent flax. *CslD1, CslD4.3, CslD4.4, CslD6.1, CslD6.2, CslG3, Ctl11* and *Ctl24* genes were not expressed or expressed weakly in both subspecies of the studied flax.

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Conflict of Interest Statement

The authors declare the absence of any conflict of interest.

References

1. Kvavadze E, Bar-Yosef O, Belfer-Cohen A, Boaretto E, Jakeli N, Matskevich Z, Meshveliani T. 30,000-Year-Old Wild Flax Fibers. Science 2009; 325(5946): 1359.
2. Dissanayake NPJ, Summerscales J, Grove SM, Singh MM. Life cycle assessment of flax fiber for the reinforcement of composites. J Bio-Based Materials and BioEnergy 2009; 3: 245-248.
3. Gorshkova T, Brutch N, Chabbert B,I Deyholos M, Hayashi T, Lev-Yadun S, Mellerowicz EJ,Morvan C,Neutelings G, Pilate G. Plant Fiber Formation: State of the Art, Recent and Expected Progress, and Open Questions. Critical Reviews In Plant Sciences 2012; 31 (3): 201-228.
4. Gorshkova TA, Sal'nikov VV, Chemikosova SB, Ageeva MV, Pavlencheva NV, van Dam JEG. The snap point: a transition point in Linum usitatissimum bast fiber development. Industrial Crops and Products 2003; 18: 213–221.
5. Huis R, Hawkins S, Neutelings G. Selection of reference genes for quantitative gene expression normalization in flax (*Linum usitatissimum* L.). BMC Plant Biol 2010; 10: 1–14.
6. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C_T$ method. Methods 2001; 25: 402-408.
7. Mokshina N, Gorshkova T, Deyholos MK. Chitinase-Like (*CTL*) and Cellulose Synthase (*CESA*) Gene Expression in Gelatinous-Type Cellulosic Walls of Flax (*Linum usitatissimum* L.) Bast Fibers. PLoS ONE 2014; 9 (6): e97949.
8. Chantreau M, Chabbert B, Billiard S, Hawkins S, Neutelings G. Functional analyses of cellulose synthase genes in flax (*Linum usitatissimum*) by virus-induced gene silencing. *Plant Biotechnol J* 2015; 13: 1312–1324.
9. Doblin MS, Pettolino F, Bacic A. Plant cell walls: the skeleton of the plant world. Funct Plant Biol 2010; 37: 357–381.
10. Sanchez-Rodriguez C, Bauer S, Hematy K, Saxe F, Ibáñez AB, Vodermaier v, Konlechner C, Sampathkumar A, Rüggeberg M, Aichinger E, Neumetzler L, Burgert I, Somerville C, Hauser MT, Persson S. CHITINASE-LIKE1/POM-POM1 and Its Homolog CTL2 Are Glucan-Interacting Proteins Important for Cellulose Biosynthesis in Arabidopsis. Plant Cell 2012; 24: 589–607.
11. Pydiura NA, Bayer GYa, Galinouski DV, Yemets AI, Pirko YaV, Padvitski TA, Anisimova NV, Khotyleva LV, Kilchevski AV, Blume YaB Bioinformatic search for cellulose synthase genes in flax (*Linum usitatissimum*) and their phylogenetic analysis. Cytol. Genet. 2015; 49(5): 279-287.