PLANT SIGNALING & BEHAVIOR
https://doi.org/10.1080/15592324.2018.1486144

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SHORT COMMUNICATION

Screenplay of flax phloem fiber behavior during gravitropic reaction

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ABSTRACT

Flax phloem fibers act as constitutively formed "muscles" that support the vertical position of the high but narrow stem. The specific mechanical properties of flax fibers and of similar fibers in other plant species are provided by the development of tertiary cell wall with tensed cellulose microfibrils. The work of phloem fibers becomes especially pronounced during the restoration of stem vertical position if it was disturbed. Gravistimulation of flax plants induces considerable modification of phloem fibers at the pulling stem side – the lumen diameter increases, while the cell wall thickness goes down. Here we show that the action of phloem fibers as motors of stem vertical position restoration is coupled to the cell wall remodelling as well as the increase of osmolytes (mainly potassium and malate) content, and accumulation of the γ-amino-butyric acid that may be involved in signalling events. The molecular players that take part in these processes are suggested.

ARTICLE HISTORY
Received 11 April 2018
Accepted 28 May 2018

KEYWORDS
Gravitropic reaction; phloem fibers; osmolytes; potassium channels; malate; GABA; cell wall remodelling; flax

Text

The capability for movement is one of the basic features of living bodies, both animal and plant. Plant organism as a whole is usually fixed in the soil, making the possibility to change the position of its parts or organs in space especially important. There are several kinds of plant movements; the most known ones are various tropisms that are usually considered to occur only in the growing part of the plant and to be based on the unequal elongation rate of cells located at the opposite sides of the organ.[1] We have recently described the formation of stem curvature far away from the elongating stem part in the gravistimulated flax plants.[2,3] Interestingly, the restoration of stem vertical position occurs even in the absence of stem top portion that included both apex and elongating zone. The motors of the gravitropic behaviour of flax plants are phloem fibers with the constitutively deposited tertiary cell wall. Fibers with such cell wall type serve as a kind of "plant muscles" and are developed in many plant species and in many ecophysiological situations.[4]

The restoration of stem vertical position is accompanied by changes in cell morphology and cell wall structure of phloem fibers located in the pulling stem side: lumen diameter increases, while cell wall thickness decreases, cell portions are widened with the formation of "bottlenecks" between them, leading to the "sausage-like" shape of a cell.[2] The transcriptomic analysis of fibers from gravistimulated plants has revealed the overall changes in the pool of mRNA that differed fibers located in the pulling stem side from those of the opposite side and control plants.[3] In the current publication, we put together the results on differential expression of genes (both in phloem fibers[3] and in xylem tissues) with the data on the uneven distribution of several low-molecular weight substances in different stem parts to suggest the processes that lead to the changes in fiber morphology in the region of stem curvature formed in gravistimulated plants.

Osmolyte accumulation in phloem fibers from pulling side of the stem

Transcriptome analysis of samples collected several hours after plant gravistimulation revealed the considerable enrichment of transcripts associated with two genes of highly selective inward-rectifying potassium channel AKT1 (Lus10017766 and Lus10033052) among the most obvious differences that distinguish fibers isolated from the pulling side of flax stem curvature region from those of control plants.[3] To substantiate the changes in the ion distribution in the course of the plant gravitropic response, we analyzed the potassium content in the pulling and opposite sides of stem curvature as well as in control plants, sampling both the phloem-fiber-enriched peels and the xylem stem portion 8 h after stem inclination (Figure 1A). The 2.4-fold increase of potassium content was detected in the fiber-enriched peels from the pulling stem side (Figure 1B).

The increased import of K^+ into the fibers was coupled to the increased accumulation of malate: gene encoding malate transporter (ALMT12) was specifically up-regulated in phloem fibers of the pulling side (Figure 1C) and was highly co-expressed with genes of potassium transporters (AKT1_1 and AKT1_2) that were used as the bite-genes (Figure 1F). Analysis of low-molecular metabolites by means of the metabolome analysis revealed malate as the substance with the most different content between fiber-enriched peels of the pulling stem side and other samples (Figure 1B).

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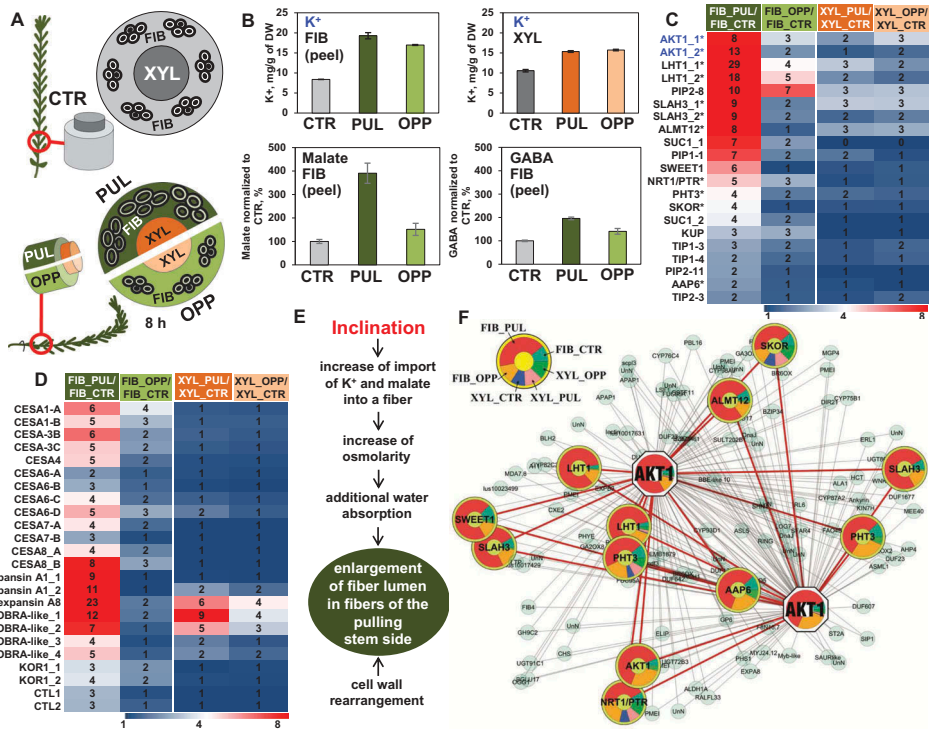

Figure 1. A – The scheme of sample collection from flax plants and stem cross sections of control plants (CTR) and plants after 8 h of gravistimulation with the designation of the pulling (PUL) and opposite (OPP) stem sides. B – Concentration of potassium ions as determined by inductively coupled plasma optical emission spectrometry (mg/g of dry weight (DW)) and content of malate and 4-amino-butyrate as determined by the approaches of metabolome analysis (233 m/z for malic acid 3TMS-derivative and 100 m/z for GABA 3TMS-derivative) in analysed samples (percentage of CTR). C, D – Gene expression, based on RNA-Seq data[3] and presented as fold change between the analysed samples (PUL and OPP) and control plants for genes of various transporters (C) and for genes of proteins involved in cell wall formation and modification. E – Summary of the considered events. F – The truncated co-expression network of flax genes of potassium channels (AKT1) as bait-genes and their highly co-expressed genes in analyzed samples. The edge (grey and red lines) connecting two genes indicates that the two genes were co-expressed (r-value is above 0,99). Red edges indicated co-expression relationship between bait-genes and genes of other transporters. The bait-genes and genes of transporters are represented by large ellipses and octagons, within which the expression profile is presented as a chart that displays the expression level of this gene (in percent) across all samples within a dataset. The network was obtained by CoExpNetViz[11] and visualized in Cytoscape v3.6[12]. FIB – isolated phloem fibers (gene expression) or phloem fiber-enriched peels (determination of potassium and metabolome analysis); XYL – xylem part of the stem, PUL – pulling part of the stem, taken from gravistimulated plants, OPP – opposite part of the stem, taken from gravistimulated plants. * – transcripts, that are part of the co-expression network (Figure 1F). Identification information on the presented genes is given in the supplementary file (Table S1).

The accumulation of K^+ and its counter ion malate^{2-} that serve as osmolytes may initiate additional water absorption into a fiber. Besides, several other transporters could add osmotically-active low-molecular substances. The activation of sucrose transporters (SUC1) and additional potassium transporter (KUP, close homolog to K^+ uptake permease) was detected in fibers from pulling side of the stem (Figure 1C). The co-expression network with the two *AKT1* genes revealed genes for slow type anion channel SLAH3, amino acid transporters (lysine histidine transporter LHT1, amino acid permease AAP6), sucrose transporter (SWEET), mitochondrial phosphate carrier protein (PHT3), members of the family NRT1/PTR identified as involved in nitrate assimilation and oligopeptide transport, and stelar K^+ outward rectifying channel (SKOR) among the genes with the highest correlation coefficient (over 0.99) (Figure 1F). The listed genes had a higher level expression in phloem fibers taken from pulling side of the stem in comparison with fibers from opposite side, control plants as well as with xylem tissues.

The water uptake induced by osmolyte accumulation can be provided by aquaporins: the activation of expression of genes for aquaporins located in tonoplast (TIPs) as well as in plasma membrane (PIPs) was revealed in fibers of the pulling stem side (Figure 1C, Table S1). The uptake of water during graviresponse could induce the expanding of flax phloem fiber lumen that has been described in our previous papers.[2,3] We didn't detect the up-regulation of the listed genes encoding transporters in xylem tissues taken from the flax stem curvature region at the same stage of graviresponse (Figure 1C), same as we didn't observe the changes in the xylem fiber lumen size.

Notably, 4-amino-butyrate, also called gamma-aminobutyric acid (GABA) was the other metabolite, next to malate, with the pronounced increase of content in the fibers of the pulling stems side (Figure 1B). In animals, GABA is a known neurotransmitter that has its own receptors; in plants, GABA interacts with ALMT proteins, which are suggested as prime candidates to mediate GABA-based signalling in plants.[5] The simultaneous increase of *ALMT12* expression and of GABA content in fibers from the pulling stem side may be considered from such point of view.

In addition, the LHT1 transporters, which expression was highly up-regulated (Figure1C, Table S1), were recently shown to be involved not only in the transport of a wide

range of amino acids but also of 1-aminocyclopropane-1-carboxylic acid – a biosynthetic precursor of ethylene.[6] Besides, the mentioned above NRT1/PTR proteins were demonstrated to transport several plant hormones, including auxin, abscisic acid, jasmonates, and gibberellins.[7] Thus, the activity of transporters in phloem fibers of the pulling stem side may also be involved in signalling events.

Cellulose biosynthesis and cell wall remodelling in phloem fibers are activated during gravitropic response

The tertiary cell wall is known to have high water content.[8] The redistribution of osmotically active substances during graviresponse may have a consequence in thinning of fiber cell wall due to the escape of some water into the fiber lumen. This may affect the mechanical function of fibers with tertiary cell wall and also cause the rearrangements in cell wall structure. Indeed, according to the transcriptome analysis, the largest number of up-regulated genes in fibers from the pulling stem side belongs to the cell wall category.[3]

Notably, the molecular machinery of cellulose biosynthesis was activated in fibers from pulling part of the stem: genes for isoforms of CESAs, COBRAs, KORRIGANs, CTL2 – the known components of the cellulose-synthesising complexes[9] – were up-regulated (Figure 1C, Table S1). The deposition of the tertiary cell wall in flax phloem fibers recruits isoforms of CESAs that are characteristic both for primary and secondary cell wall.[10] In accordance, the transcription of *CESA1, 3* and *6*, same as *CESA4, 7* and *8* was activated in fibers of the pulling side.

The modification of fiber cell wall upon gravistimulation of flax plants was indicated by the specific up-regulation of several genes for *FLAs, PMEIs* and expansins in fibers from the pulling side of the stem.[3] In all analysed xylem tissues (control plants, pulling and opposite sides of the stem), the listed genes had a similar level of expression (Figure 1C). This fact can suggest that at least at the early stage of gravitropic response, the "main blow" of gravistimulation falls on the phloem fibers bearing the thickened tertiary cell wall that accomplish the main work on the restoration of the stem. Later on, xylem fibers start to form the gelatinous layers (tertiary cell wall) and serve as additional an enforcement for the stem[2].

The obtained data permit to reveal the processes that occur in fibers with tertiary cell wall and involve the increase of turgor pressure and the rearrangements of cell wall structure (Figure 1E) in order to improve mechanical properties of fiber cells under the increased load caused by gravistimulation.

Acknowledgments

The study was supported by the Russian Science Foundation (project #1614-10256; transcriptome analysis). The authors acknowledge the Research Park of Saint Petersburg State University Center for Molecular and Cell Technologies. G.P. (analysis of metabolome) acknowledges Saint Petersburg State University for the research grant1.40.492.2017.

Funding

This work was supported by the Russian Science Foundation [16-14-10256] and by Saint Petersburg State University [1.40.492.2017].

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References

1. Toyota M, Gilroy S. Gravitropism and mechanical signaling in plants. Am J Bot. 2013;100:111–125. DOI:10.3732/ajb.1200408.
2. Ibragimova NN, Ageeva MV, Gorshkova TA. Development of gravitropic response: unusual behavior of flax phloem G-fibers. Protoplasma. 2017;254:749–762. DOI:10.1007/s00709-016-0985-8.
3. Gorshkov O, Mokshina N, Ibragimova N, Ageeva M, Gogoleva N, Gorshkova T. Phloem fibres as motors of gravitropic behaviour of flax plants: level of transcriptome. Funct Plant Biol. 2017;45:203–214. DOI:10.1071/FP16348.
4. Gorshkova T, Chernova T, Mokshina N, Ageeva M, Mikshina P. Plant "muscles": fibers with a tertiary cell wall. New Phytol. 2018;218:66–72. DOI:10.1111/nph.14997.
5. Ramesh SA, Tyerman SD, Gilliham M, Xu B. γ-Aminobutyric acid (GABA) signalling in plants. Cell Mol Life Sci. 2017;74:1577–1603. DOI:10.1007/s00018-016-2415-7.
6. Shin K, Lee S, Song WY, Lee RA, Lee I, Ha K, Koo JC, Park SK, Nam HG, Lee Y, et al. Genetic identification of ACC-*RESISTANT2* reveals involvement of LYSINE HISTIDINE TRANSPORTER1 in the uptake of 1-aminocyclopropane-1-carboxylic acid in *Arabidopsis thaliana*. Plant Cell Physiol. 2015;56:572–582. DOI:10.1093/pcp/pcu201.
7. Corratgé-Faillie C, Lacombe B. Substrate (un)specificity of Arabidopsis NRT1/PTR FAMILY (NPF) proteins. J Exp Bot. 2017;68:3107–3113. DOI:10.1093/jxb/erw499.
8. Schreiber N, Gierlinger N, Putz N, Fratzl P, Neinhuis C, Burgert I. G-fibres in storage roots of *Trifolium pratense* (Fabaceae): tensile stress generators for contraction. Plant J. 2010;61:854–861. DOI:10.1111/j.1365-313X.2009.04115.x.
9. McFarlane HE, Döring A, Persson S. The cell biology of cellulose synthesis. Annu Rev Plant Biol. 2014;65:69–94. DOI:10.1146/annurev-arplant-050213-040240.
10. Mokshina N, Gorshkov O, Ibragimova N, Chernova T, Gorshkova T. Cellulosic fibres of flax recruit both primary and secondary cell wall cellulose synthases during deposition of thick tertiary cell walls and in the course of graviresponse. Funct Plant Biol. 2017;44:820–831. DOI:10.1071/FP17105.
11. Tzfadia O, Diels T, De Meyer S, Vandepoele K, Aharoni A, van de Peer Y. CoExpNetViz: comparative co-expression networks construction and visualization tool. Front Plant Sci. 2016;1194. DOI:10.3389/fpls.2015.01194.
12. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Gen Res. 2003;13:2498–2504. DOI:10.1101/gr.1239303.