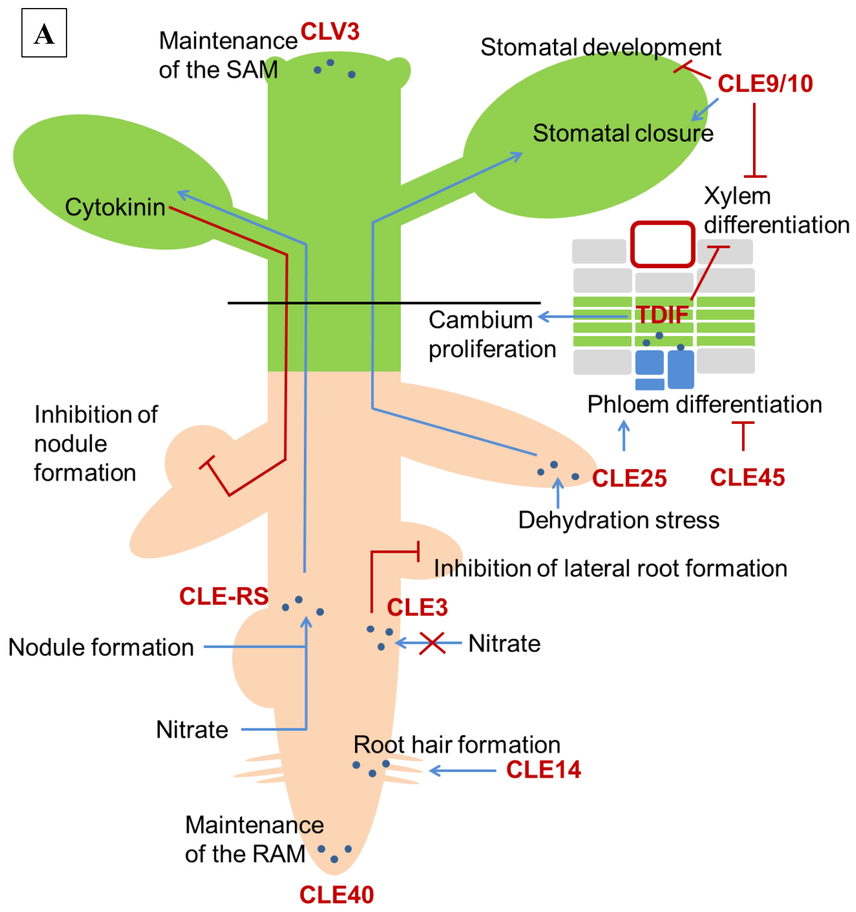
CLE and *BAM* genes in phloem development in potato (Solanum tuberosum L.)

***Losev M.,** Gancheva M., Polushkevich L., Lutova L.

Department of Genetics and Biotechnology, Saint-Petersburg State University, Universitetskaya emb.7/9, Saint-Petersburg, Russia
*e-mail: st069730@student.spbu.ru

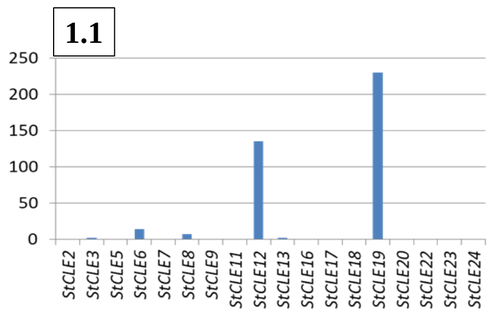
CLE peptides (CLAVATA3/ENDOSPERM SURROUNDING REGION) are small (12-14 amino acids) peptide phytohormones involved in many processes in plants. They are involved in the development of the apical meristems of the shoot and root, procambia and cambia, vascular tissues: xylem and phloem; they also mediate the response to water lack. The action of CLE hormones is based on their reception on the plasmalemma of plant cells and subsequent signal transmission to the underlying elements of the signaling pathway. CLE receptors are proteins related to leucine-rich repeat receptor-like serine/threonine-protein kinases (LRR-RLK). One class of such proteins is BAM (BARELY ANY MERISTEM). For *Arabidopsis thaliana*, the AtCLE45 peptide is shown to be involved in inhibiting phloem development, and the AtCLE25 peptide is a positive regulator (Fig. A). For potatoes normal and effective phloem development is very important, because this tissue plays a key role in the formation of the storage tuber. However, the molecular mechanism of potato phloem development is poorly understood.



1. Search for *StCLE* genes and their receptors expressed in the phloem

When using data on transcriptomic analysis of potato phloem, we found that the phloem accumulates StCLE12 and StCLE19 transcripts (Fig. 1.1). At the same time, the sequence of the StCLE19 peptide was only one amino acid different from that of AtCLE25, which involved in the development of the phloem in *A. thaliana*. The StCLE12 peptide was found to be identical to the AtCLE41 and AtCLE44 peptides, which showed accumulation in the phloem, as well as participation in the regulation of the development of cambial cells (Fig. 1.2, 1.5).

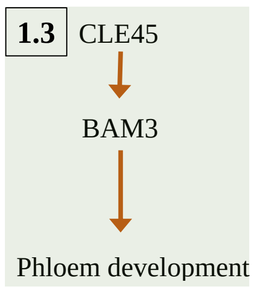
1.1

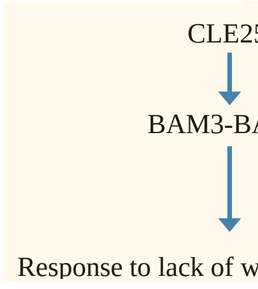


1.2

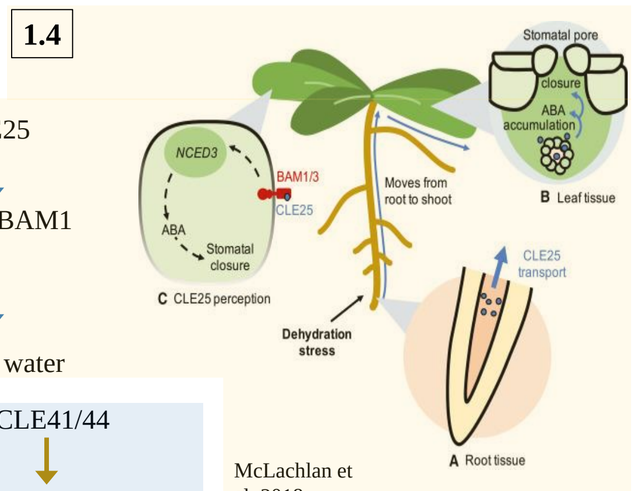
StCLE19	R	R	V	P	N	G	P	D	P	I	H	N
AtCLE25	R	K	V	P	N	G	P	D	P	I	H	N
StCLE12	H	E	V	P	S	G	P	N	P	I	S	N
AtCLE41	H	E	V	P	S	G	P	N	P	I	S	N
AtCLE44	H	E	V	P	S	G	P	N	P	I	S	N

1.3



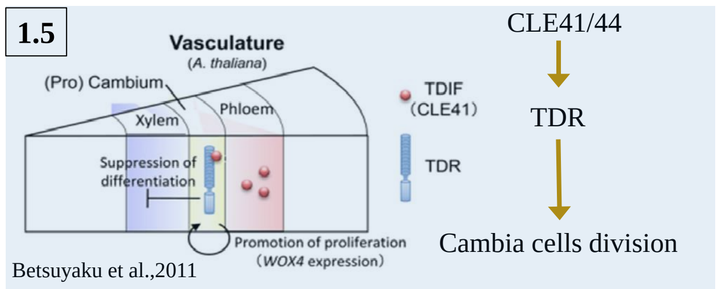


1.4



McLachlan et al, 2018

1.5



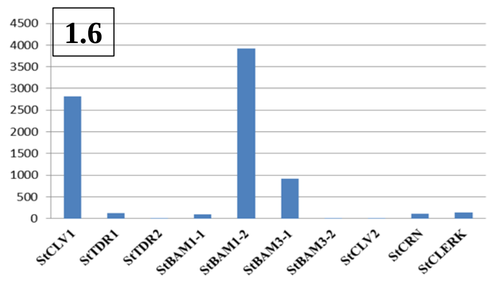
Betsuyaku et al.,2011

An accumulation of ***StCLV1***, ***StBAM2*** and ***StBAM3*** transcripts was found among the putative CLE-peptide receptors in the potato phloem (Fig. 1.6).

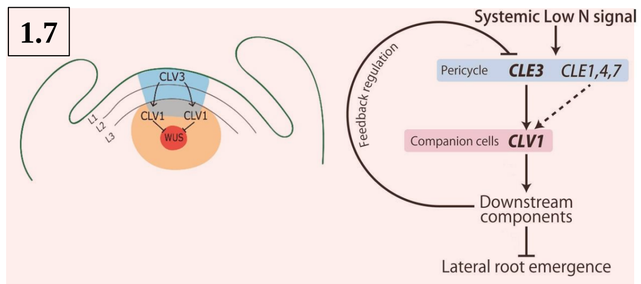
At the same time, the AtBAM3 protein is a receptor:
1) of the negative regulator of phloem development-AtCLE45 peptide;
2) of the AtCLE25 peptide in response to dehydration stress (Fig. 1.3-1.4)

CLV1 is involved in maintaining the stem cell pool in the apical shoot meristem (SAM), as well as in the formation of lateral roots (Fig. 1.7) The protein BAM1 is a coreceptor of BAM3 in the response to water shortage, and it is also able to replace the functions of CLV1 to maintain SAM (Fig. 1.4) The involvement of these proteins in the development of potato phloem has not been previously shown.

1.6

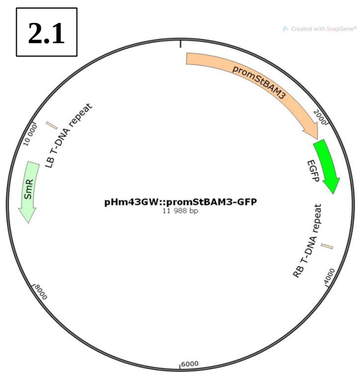


1.7



2. Analysis of the activity of promoters of the studied genes

2.1



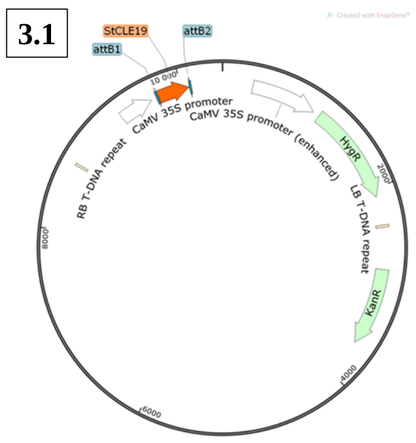
To accurately localize the expression of the *StCLE12*, *StCLV1*, *StBAM3*, and *StBAM2* genes, we developed genetically engineered constructs containing the promoter regions of the genes of interest, the green fluorescent protein reporter gene (*GFP*) with the nuclear localization signal (NLS) (Fig. 2.1). The transformation of the potato Désirée was carried out with the help of *Agrobacterium tumefaciens*. At the moment, fully regenerated plants have been obtained, ready for histological analysis of promoter activity. For the StCLE19 and StBAM2 genes, work is currently underway to clone their promoter regions into vectors.

This work was supported by the RFBR grant 20-316-80004.

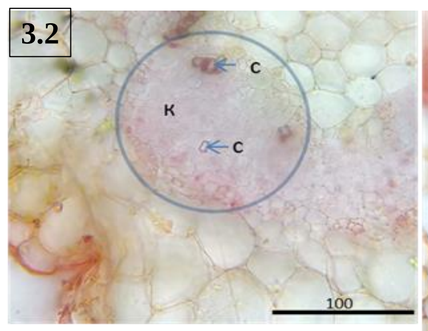
3. Analysis of the effect of overexpression of the *StCLE12*, *StCLE19* genes

To find out how the increased expression of the *StCLE12* and *StCLE19* genes affects plants, we created constructs in which these genes are placed under the control of the constitutive promoter 35S of the cauliflower mosaic virus (CaMV) (Fig. 3.1). Analysis of transformants with overexpression of *StCLE12* showed that in such plants, hypertrophy of the vascular bundles occurs due to the intensification of cell divisions in the cambial zone. In addition, there was no lignification of parenchymal cells of the xylem and phloem (Fig. 3.2). These data may suggest that the *StCLE12* gene is not actually involved in phloem formation, but affects the activity of cambium cell division. Plants with overexpression of *StCLE19* demonstrated a low ability to regenerate shoots and roots.

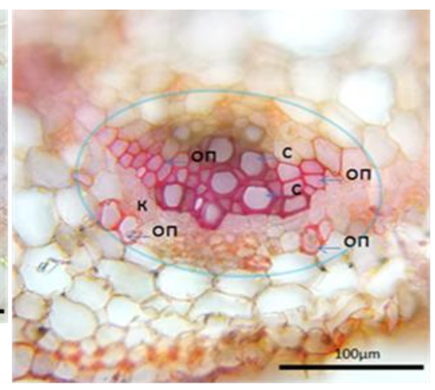
3.1



3.2



StCLE12-oe

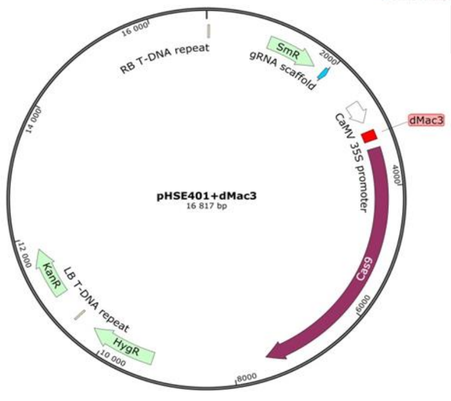


Контроль

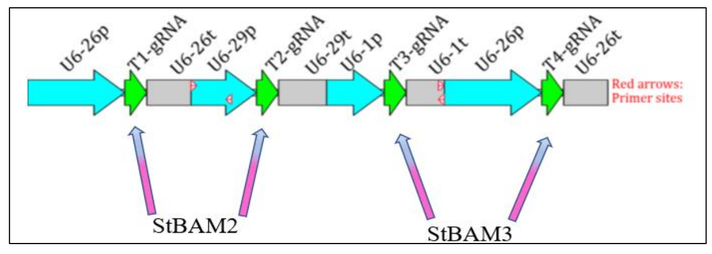
4. Genomic editing of genes of interest

To assess the effect on plants of the loss of function of genes involved in the development of potato phloem, work has begun on creating constructs for genomic editing of these genes using the CRISPR/Cas9 genome editing system. The variety Désirée is tetraploid, so for the most efficient editing, a vector with the rice translational enhancer dMac3 was used. Its presence before the *Cas9* gene increases the probability of editing four alleles of the gene. The enhancer was successfully cloned into the pHSE401 plasmid (Figure 4.1). For editing the *StBAM2* and *StBAM3* genes, 4 targets (two for each gene) and primers were selected, and work is underway to assemble the structure (Fig. 4.2).

4.1



4.2



По Xing et at., 2014

CONCLUSION

1. An accumulation of transcripts of the *StCLE12*, *StCLE19*, *StCLV1*, *StBAM2*, and *StBAM3* genes was found in the potato phloem.
2. Constructs were created to analyze the activity of promoters of the *StCLE12*, *StCLV1*, and *StBAM3* genes, plants were transformed, and regenerants were obtained. Work is underway to clone the promoters of the *StCLE19* and *StBAM2* genes into vectors.
3. Constructs for the analysis of the overexpression of the *StCLE12* and *StCLE19* genes were created. Histological analysis of transformed plants with overexpression of *StCLE12* was performed.
4. Modified the design for genomic editing of potatoes. Targets and primers for editing the *StBAM2* and *StBAM3* genes were selected.