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CLAVATA3/EMBRYO SURROUNDING REGION (*CLE*) Gene Family in Potato (*Solanum tuberosum* L.): Identification and Expression Analysis

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Abstract: *CLE* genes encode a group of small secretory peptides, which regulate cell proliferation and differentiation in plants. *CLE* genes have been studied in many plants; however, little is known about this gene family in potato. In this study, we characterized members of the *CLE* gene family in potato *Solanum tuberosum* (*StCLE*) and comprehensively analyzed their phylogenetic relationships, structure, and expression patterns. Using available transcriptomic data, we found a relative high expression level of *StCLE8*, *StCLE12*, and *StCLE13* in stolons and tubers. Real-time PCR analysis showed that the *StCLE23* gene was upregulated by water deficiency, whereas the expression of *StCLE4* and *StCLE10* was induced by nitrogen supply. Besides that, using data from transcriptomic studies obtained previously for plants with the induction the *StBEL5* gene, a positive regulator of tuber development, we found that *StCLE4* was among genes upregulated in response to *StBEL5* induction, suggesting that *StCLE4* could be a target of StBEL5 transcription factor. However, we did not reveal a direct binding of StBEL5 to the regulatory sequences of *StCLE4* using yeast one-hybrid assay. Taken together, our data provide basic information for future functional studies of CLE peptides in potato growth and tuberization and in response to various environmental stimuli.

Keywords: potato *Solanum tuberosum* L.; *CLE* genes; nitrogen; dehydration; BEL5

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1. Introduction

CLAVATA3/EMBRYO SURROUNDING REGION (CLE) peptides are a group of post-translationally modified peptide hormones that act as mediators of cell-to-cell communication. CLE peptides are produced from larger polypeptide precursors, which contain an N-terminal signal peptide, a variable domain, and conserved CLE domain near the C-terminus [1,2]. Then, CLE domain undergoes proteolytic processing and some post-translational modifications to yield the functional CLE peptide. In *Arabidopsis*, CLE peptides control cell divisions of stem cell in different types of plant meristems including the shoot apical meristem (AtCLV3) [3], the root apical meristem (AtCLE40 and AtCLE19) [4,5], and the vascular meristem (tracheary element differentiation inhibitory factor (TDIF) peptides, which are encoded by *AtCLE41* and *AtCLE44*) [6]. CLE peptides also play roles in symbiosis, parasitism, and responses to abiotic cues (reviewed in [7]). Although the *CLE* gene family has been studied in many plants, there is still a lack of research on the phylogenetic relationship and expression of the *CLE* gene family in potato *Solanum tuberosum* L. (*StCLE*). Potato, a perennial herbaceous plant that forms edible tubers, is one of the world's most important food crops. In the present study, we annotated 41 *StCLE* genes and analyzed expression levels of the *StCLE* genes in different tissues and conditions. Tuber development is associated with active cell divisions that led to thickening of the stolon. TDIF peptides in

other plants were shown to participate in regulation of secondary thickening in the root and shoot [8,9]. Using transcriptomic data, we found relative high expression level of potato *TDIF-like* genes in stolons and tubers, suggesting these CLE peptides could be involved in tuber growth regulation. Tuberization is regulated by environmental factors such as nitrogen supply, day length, water availability, and temperature. Here, we identified candidate CLE peptides that could mediate signaling from these environmental factors. We found that the *StCLE10* and *StCLE4* were induced in potato roots under N-rich growth conditions. Furthermore, we found that *StCLE23* expression was induced in roots under dehydration stress condition. In addition to this, based on available transcriptomic data we found that *StCLE4* could be regulated by StBEL5 transcription factor, which was previously characterized as a short-day activated inductor of tuberization. Therefore, in this study we identified CLE peptides that could be potentially involved in response to water stress, nitrate-induced reactions, and regulation of tuberization.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The study was performed with the widely used *Solanum tuberosum* L. cv. Désirée. Plants were propagated by two-node stem cuttings and grown in vitro on Murashige-Skoog (MS) medium with 0.8% (*w*/*v*) agar and 1% sucrose under long-day conditions (16 h light: 8 h dark) at 22 °C. Induction of dehydration stress was performed as described in [10] using 14 days-old plantlets. MS medium for different N-availability conditions was modified as described in [11]. Two-node stem cuttings were kept on N-depleted or N-rich medium for 14 days (shoots were transferred to the fresh medium of the same composition every 7 d) until roots were formed on the shoots.

2.2. Identification and Phylogenetic Analysis of CLE Genes

To identify *CLE* genes in potato, BLASTN and TBLASTN analyses with all the *Arabidopsis thaliana* and known *Solanum lycopersicum CLEs* as queries were used against genomic sequences of *S. tuberosum* group Phureja DM1-3 516 R44 available at https://www.ncbi.nlm.nih.gov/genome/?term=txid4113[orgn] (accessed on 13 May 2021). Alignment of CLE amino acid (AA) sequences (Table S1) was generated by MEGA7 software using the muscle algorithm [12]. A phylogenetic tree with 1,000 bootstrap replicates was constructed using MEGA7 neighbor-joining method with default parameters [12].

2.3. Gene Structure and Conserved Domain Identification

The exon–intron structures and chromosomal locations for the *StCLE* genes were retrieved from the Phytozome v12 (http://www.phytozome.net/ (accessed on 13 May 2021)). The chromosomal locations were visualized by MapInspect software (http://www.softsea.com/download/MapInspect.html (accessed on 13 May 2021)). The conserved residues of CLE peptides were visualized by the MEME program (version 5.1.0, http://alternate.meme-suite.org/tools/meme (accessed on 13 May 2021)) [13] with default parameters. Signal peptides were predicted by Signal P 5.0 server [14,15].

2.4. RNA-seq Data Analysis

RNA-seq data were obtained from NCBI project PRJEB2430. Reads quality control was performed with FASTQC (v. 0.11.5) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 13 May 2021)). The bbduk program from bbtools suite (v. 37.23) (https://jgi.doe.gov/data-and-tools/bbtools/ (accessed on 13 May 2021)) was used to filter reads from mitochondrial, plastid, ribosomal potato DNA, and technical artifacts. TPM (transcripts per kilobase million) values of *StCLE*s for various tissues were counted using kallisto [16]. As target sequences coding sequences of DM_1-3_516_R44 v6.1 from SpudDB (http://solanaceae.plantbiology.msu.edu (accessed on 13 May 2021)) were taken. For heatmap construction the heatmap.2 function of the R package was used.

2.5. Quantitative RT–PCR Analysis

Total RNA was isolated using a Trizol reagent (Invitrogen, Waltham, MA, USA). First-strand cDNA was synthesized from 500 µg of total RNA using oligodT primers and RevertAid reverse transcriptase (Thermo Scientific, Waltham, MA, USA). Quantitative RT–PCR was performed with EvaGreen intercalating dye (Syntol, Novosibirsk, Russia) using CFX96 (BioRad, Hercules, CA, USA). Relative expression was normalized against the ubiquitin gene (PGSC0003DMT400011939). Primers used are listed in Table S2. Each sample was tested in three technical repeats. At least three biological replicates were used in each experiment. The statistical significance was evaluated by Student's *t*-test.

2.6. Yeast One-Hybrid Assay

Transformation of *Saccharomyces cerevisiae* (strain Y2H Gold (Clontech)) was performed as described in [17]. The promoter fragments of *StCLE4* and *StSP6A* genes (Table S3) were cloned into the pHISLEU2GW (kindly provided by Dr Rogers from Cardiff University) destination vector. Coding sequences of *StBEL5* was introduced into pDEST22 (Invitrogen, Waltham, MA, USA). A yeast one-hybrid assay was performed as described in [18]. The primers used for cloning are listed in Table S2.

3. Results

3.1. Identification and Phylogenetic Analysis of the CLE Family Genes in Solanum tuberosum

Previously, Goad et al. [19] analyzed genomic data from 57 plant species, and as a result, 21 *CLE*-like sequences were identified in potato genome database. However, no comprehensive analysis of these sequences was carried out, and other *CLE* genes may remain unidentified in potato. In this study, we identified previously unknown *CLE* genes in potato database and analyzed their sequences. To achieve this, the sequences of all previously reported *CLE* genes from *Arabidopsis thaliana* and *Solanum lycopersicum* (*AtCLE*s and *SlCLE*s, respectively) were used as queries to perform BLASTN and TBLASTN searches against potato genome at SpudDB (http://solanaceae.plantbiology.msu.edu (accessed on 13 May 2021)) and NCBI (*S. tuberosum* group Phureja DM1-3 516 R44). As a result, we identified 41 *CLE* genes in potato (Table S4), including 20 new genes that have not been identified by Goad et al. [19]. Eighteen genes (*StCLE24-41*) have not been annotated previously as coding sequences possibly due to fact that *StCLE* genes are often missed by many standard annotation pipelines [19].

Analysis of chromosomal location showed that the *StCLE* genes were distributed throughout all 12 potato chromosomes except for chromosome 6, which does not contain *CLE* genes (Figure 1). The *CLE* genes *StCLE26* and *StCLE27* are located very close to each other (approximately 4 kb apart) and have similar sequences (88% identity), suggesting that they possibly emerged from tandem duplications.

Most of the *StCLE* genes lacked introns, with the exception of five genes (*StCLE14*, *StCLE15*, *StCLE19*, *StCLE29,* and *StCLE30*). For the *StCLE14* gene, two protein isoforms with different length (164 AA for isoform 1 and 144 AA for isoform 2) were found in NCBI database due to the presence of second intron in transcript variant X2. The StCLE proteins contain 66 (StCLE31) to 163 (StCLE14) AA. Like the CLE proteins in other plants [1], potato CLEs also have a signal peptide at the N-terminus, a central variable domain, and a 12 AA CLE domain at the C-terminus. Similar to SlCLEs and AtCLEs, all identified StCLE proteins contain only one CLE domain. The online MEME software was used to visualize the conserved residues within 12 AA StCLE domains (Figure 2a). Moreover, we also searched for *CLE* homologs in the *Solanum lycopersicum* genome and found 16 previously unidentified *CLE* genes in addition to previously characterized 15 *CLE* genes (*SlCLE1-15*) in the tomato genome [20] (Table S5). To do this, BLASTN search against *Solanum lycopersicum* cv. "Heinz-1706" genome using potato and *Arabidopsis CLE* genes as queries were performed. The sequence consensus over the whole StCLE family was slightly different from one generated for AtCLEs and SlCLEs (Figure 2a); however, six residues

(R_1, P_4, G_6, P_7, P_9, and H_{11}) within CLE domain were found to be highly conserved in *Arabidopsis*, tomato, and potato CLEs (Figure 2a).

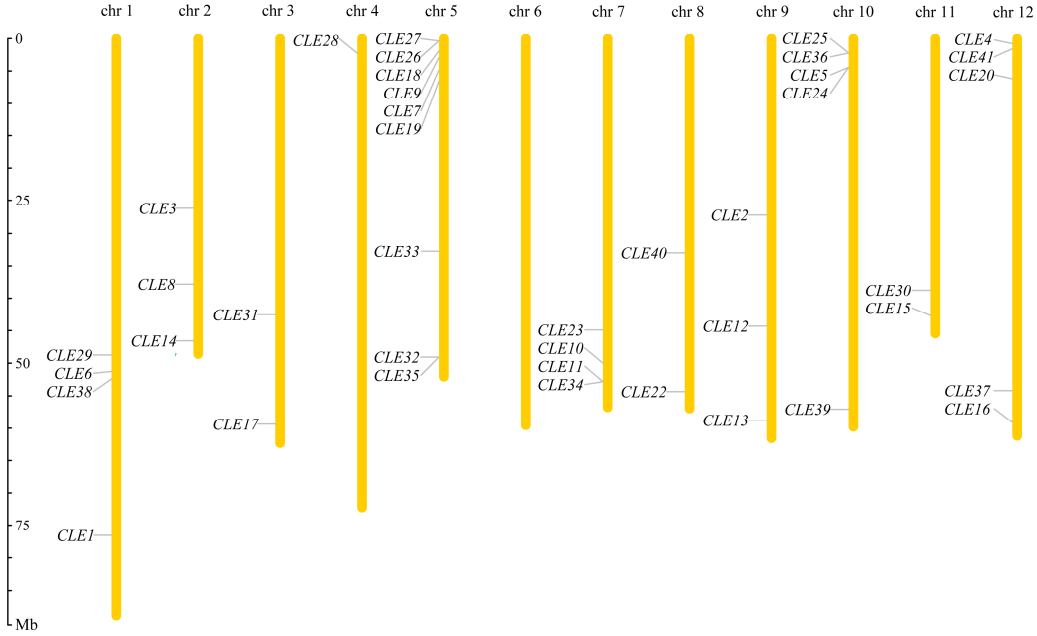

Figure 1. The distribution of *StCLE* genes on *Solanum tuberosum* chromosomes.

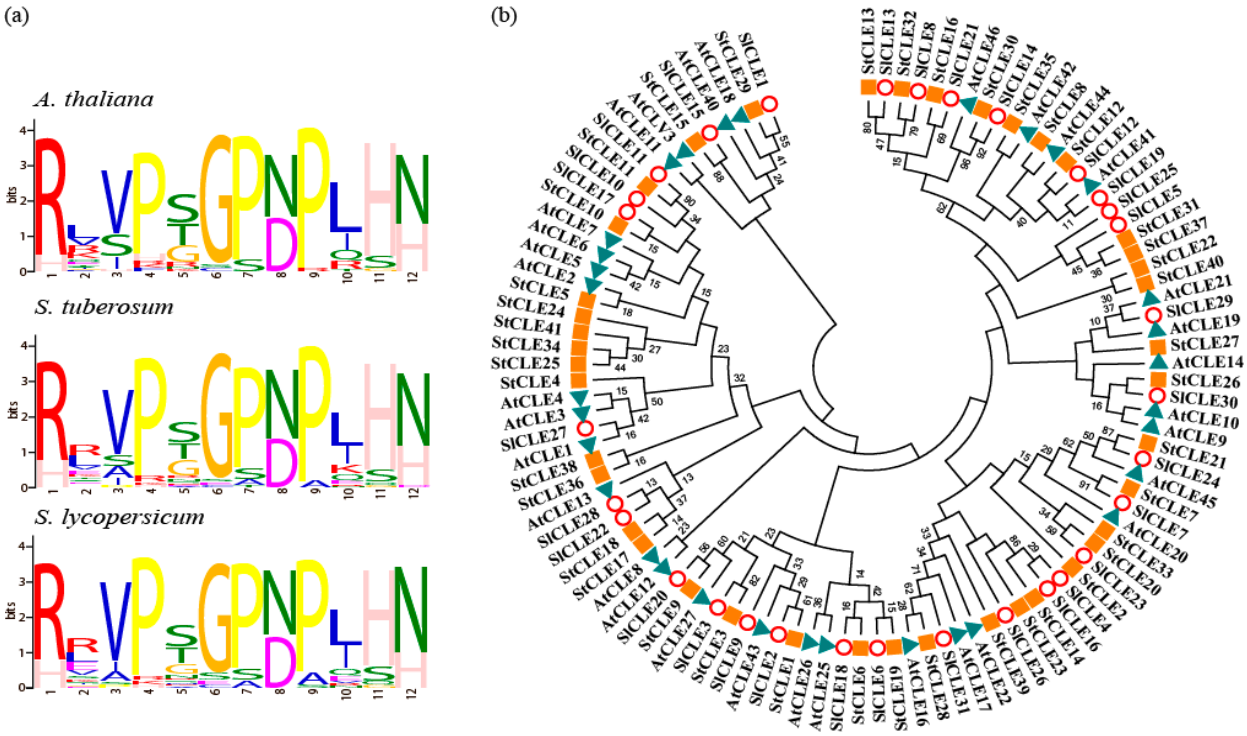

Figure 2. Phylogenetic tree and sequence analysis of CLE peptides. (**a**) Sequence logos for conserved CLE domain of AtCLEs, StCLEs, and SlCLEs visualized by MEME; (**b**) phylogenetic tree of *A. thaliana* (green triangles), *S. lycopersicum* (red circles), and *S. tuberosum* (orange squares) CLE protein families. Alignment was done by MEGA7 software with the muscle algorithm using the AA sequences of CLE proteins.

To study the phylogenetic relationship of the StCLE family members to *Arabidopsis* and tomato CLEs, an unrooted neighbor-joining phylogenetic tree, based on multiple alignments of the AA sequences of the CLEs, was created (Figure 2b, Figure S1). As expected, a perfect match was observed for potato CLEs with tomato CLEs (for example, StCLE7 and SlCLE7). Phylogenetic analysis also showed that there were some closely related homologous CLEs between potato and *Arabidopsis*. For example, StCLE7 and StCLE21 demonstrated high similarity with AtCLE45 that is involved in regulation of pollen tube growth and protophloem differentiation [21,22]. StCLE12 and StCLE8 had identical CLE domain to AtCLE41 and AtCLE44, which play crucial roles in vascular meristem maintenance [23]. AtCLE46 and StCLE30 grouped together, but little is known about functions of AtCLE46. The StCLE4, StCLE5, StCLE10, StCLE11, StCLE24, StCLE25, StCLE34, and StCLE36 group is close to nitrate-regulated CLE peptides of *A. thaliana* (*AtCLE1,-3,-4,-7*) [24,25]. For other proteins, no clear homology with AtCLEs was found.

3.2. Expression Profiles of StCLE Genes in Different Tissues According to Transcriptomic Data

To provide some clues on the roles of the *StCLE* genes in potato growth, the expression profiles of the *StCLE* genes in shoot apex, flower, leaf, petiole, stem, stolon (modified shoot, which gives rise to potato tuber), tuber (young and mature), and root were analyzed using transcriptomic data from NCBI database (Figure 3). Reads were filtered and counted with kallisto [16]. Fourteen genes (*StCLE2, -5, -10, -15, -18, -25, -27, -29, -32, -34, -35, -38, -40*, and *-41*) were excluded from the heatmap analysis because they had zero expression levels in all examined tissues. Twelve *StCLE* genes (*StCLE3, -4, -6, -11, -14, -20, -21, -22, -24, -26, -31*, and *-36*) demonstrated low expression levels or lack of expression in most of the analyzed tissues. Five other *StCLE*s (*StCLE9, -12, -13, -23*, and *-30*), on the contrary, demonstrated high expression levels in almost all analyzed tissues. Ten *StCLE* genes (*StCLE1, -7, -8, -16, -17, -19, -28, -33, -37*, and *-39*) are highly expressed in some tissues. Several *StCLE* genes showed tissue-specific expression patterns. For example, *StCLE8* was found to show abundant expression in shoot apex, stolon, tubers, and root, but had lower expression levels in flower, petiole, and leaf. Three *StCLE* genes, namely *StCLE12*, *StCLE8*, and *StCLE13*, were highly expressed in stolon and tubers, suggesting that corresponding CLE peptides could be involved in tuber growth regulation.

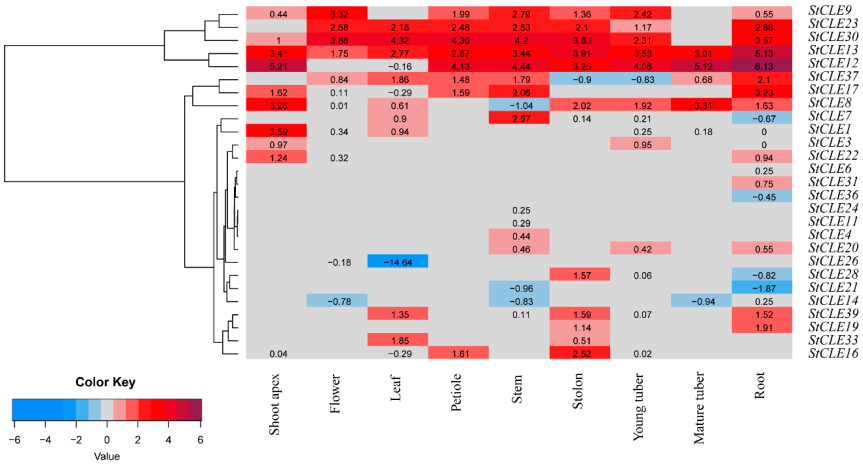

Figure 3. Expression profiles of *StCLE* genes with hierarchical clustering in different parts of potato and at two stages of tuber development (young and mature tubers). Log2 based TPM value was used to create the heatmap. The color scale represents the relative signal intensity of TPM values; red indicates high transcript abundance, and blue indicates low abundance. Zero expression (TPM = 0) or low expression are represented by the grey color.

3.3. Expression Profiles of StCLE in Response to Environmental Factors

3.3.1. Expression of StCLEs in Response to Dehydration Stress

In *Arabidopsis* and other plants, several CLE peptides were found to be involved in responses to abiotic cues. Specifically, the *AtCLE25* gene expression is induced in the root in response to dehydration stress, and AtCLE25 systemically activates signaling pathway that leads to stomatal closure thereby enhancing the resistance to dehydration stress [10]. Interestingly, AtCLE25 is also involved in protophloem cell differentiation [26]. In potato, StCLE6 and StCLE19 are grouped together with AtCLE25 according to phylogenetic analysis (Figure 2b). According to expression data collected previously by [27], *StCLE19* was expressed in phloem, as well as *StCLE12*, which showed a closer relationship to *Arabidopsis* CLE41/44 peptides involved in regulation of cambium cell proliferation and vascular development [21,28]. In our experiment after dehydration for four hours, we found no activation of *StCLE6* and *StCLE19* in roots in response to dehydration stress. However, the expression of *StCLE23* was increased in response to four hours of dehydration (Figure 4). StCLE23 does not demonstrate any clear homology with known *Arabidopsis* CLE peptides, and its possible role in dehydration stress remains to be investigated in more details.

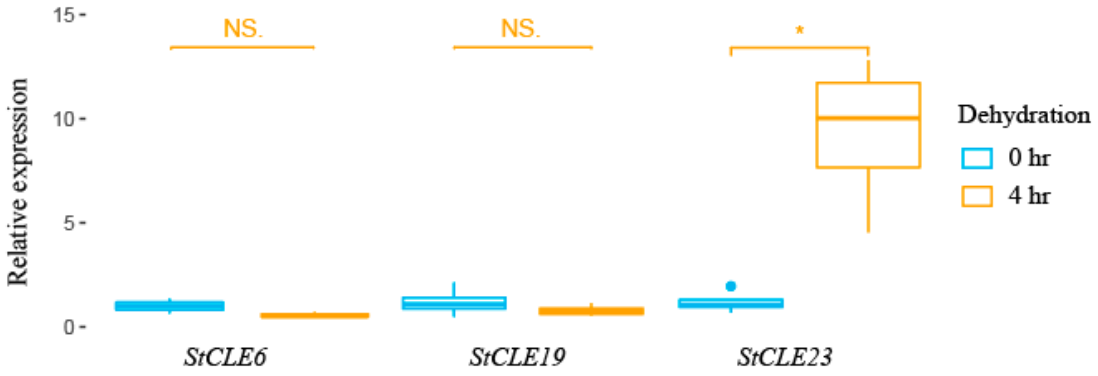

Figure 4. Expression of *StCLEs* in response to dehydration stress. NS., Not Significant; *, *p*-value < 0.05.

3.3.2. Expression of StCLEs under Low and High Nitrogen Conditions

In *Arabidopsis*, the expression levels of *AtCLE1*, *AtCLE3*, *AtCLE4*, and *AtCLE7* genes were found to be induced in roots under nitrogen (N) deficient conditions [24,25]. To identify genes specifically induced by N deficiency in potato, we evaluated the expression of *StCLE* genes (which were grouped with nitrate-regulated CLE peptides of *A. thaliana* according to phylogenetic analysis) in the roots of plants exposed to different N-availability conditions. To do this, we cultivated plants on N-rich medium for two weeks, after which control plants were kept on the same media (N-rich, 10 mM nitrogen), whereas others plants were transferred to N-depleted medium without a nitrogen source (0 mM nitrogen). After 48 h, we estimated the expression levels of *StCLE* genes in the roots of control and N-depleted plants. No upregulation of *StCLE* genes was detected in the roots of plants cultivated on N-depleted medium. However, we observed downregulation of *StCLE4* and *StCLE10* expression levels under N-depleted conditions (Figure 5).

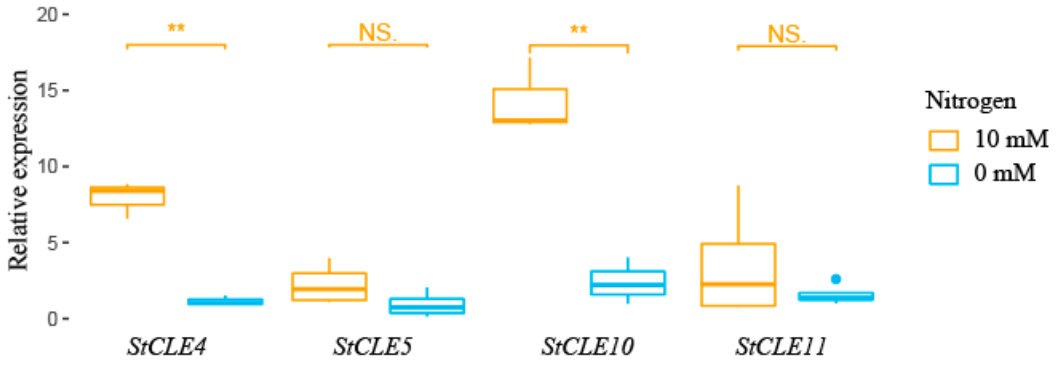

Figure 5. Expression of *StCLE4* and *StCLE10* are downregulated in roots exposed to the N-depleted medium (no N). NS., Not Significant; **, *p*-value < 0.01.

To check the hypothesis that *StCLE4* and *StCLE10* gene expression is activated by high nitrogen content, we first cultivated plants on N-depleted media and then exposed them to the N-rich or N-depleted media for 48 h. We detected upregulation of *StCLE4*, *StCLE5*, and *StCLE10* expression levels (Figure 6) on N-rich media, suggesting that these genes are activated under high nitrogen conditions. In legume plants, the *CLE* genes have been described that are activated in response to high concentration of nitrate (10 mM) and mediate nitrate-dependent inhibition of symbiotic nodulation [29–33]. These nodulation-specific *CLE* genes belong to the same clade as nitrate-regulated CLE peptides of *A. thaliana* (*AtCLE1*, *-3*, *-4*, and *-7*) [24,25]. Therefore, we suggest that *StCLE4*, *StCLE5*, and *StCLE10* genes, which also belong to the same clade of nitrate-regulated genes, upregulated by high N condition could mediate nitrate-dependent processes in potato.

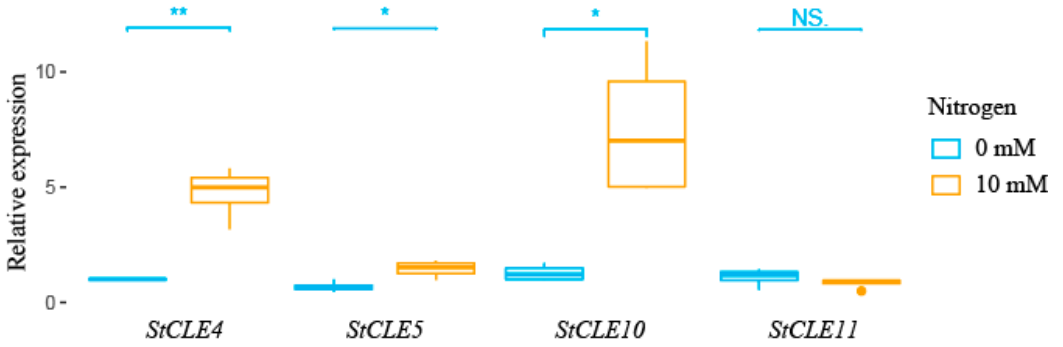

Figure 6. *StCLE4*, *StCLE5*, and *StCLE10* are expressed in roots exposed to the N-rich medium (10 mM NO_3^- and 10 mM NH_4^+). NS., Not Significant; *, *p*-value < 0.05, **, *p*-value < 0.01.

3.3.3. StBEL5 Transcription Factor as a Possible Regulator of the StCLE4 Gene

StBEL5 acts as a signal mRNA in potato, which moves from leaves to stolons and mediates the activation of tuberization [34]. The SP6A protein, also known as tuberigen, is another mobile regulator of tuber initiation that moves from shoots to stolons and induces tuberization. Expression of both *StBEL5* and *SP6A* genes is activated by short-day conditions, which are inductive for tuberization in potato (reviewed in [35]). Sharma et al. [36] performed transcriptomic studies of plants with elevated *StBEL5* expression levels obtained in ethanol-inducible system and identified approximately 10,000 potential target genes, including genes involved in the biosynthesis or bioactivity of phytohormones that regulate tuber development [36]. Interestingly, according to the list of differentially expressed genes from this study, transcription factor StBEL5 upregulates the expression of *StCLE4* (Figure S2). StBEL5 is known to interact with the tandem TGAC motif in the promoters of target genes [36]. We found that there are three TGAC motifs in a genomic

DNA sequence corresponding to 500 bp upstream of the start codon of the *StCLE4* gene (Table S3). To check if these motifs could be bound by StBEL5 transcription factor, we performed yeast-one-hybrid screening. According to our data, StBEL5 did not bind to regulatory sequences of the *StCLE4* gene (Figure 7). At the same time, in our yeast-one-hybrid screening StBEL5 demonstrated binding with regulatory sequences from the promoter of the *SP6A* gene (Figure 7), which was previously shown to be the direct target of StBEL5 according to the results of electrophoretic mobility shift assay [36].

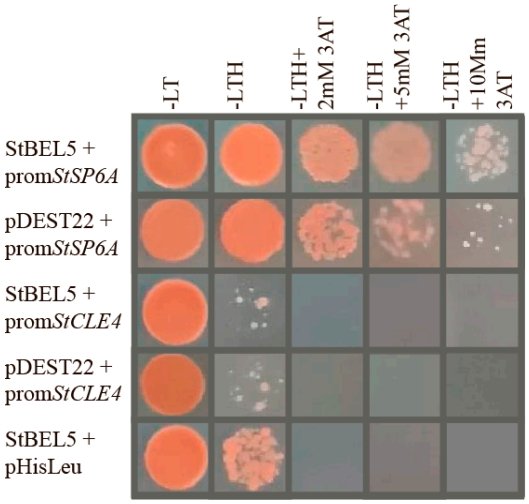

Figure 7. Yeast-one hybrid assay for transcription factor StBEL5 and *StSP6A, StCLE4* promoters. Cells were spotted on selective media lacking Leu and Trp (-LT) and selective media lacking Leu, Trp, and His (-LTH), contained the indicated concentrations of 3-Amino-1,2,4-triazole (3AT).

4. Discussions and Conclusions

In this study, we annotated 41 *CLE* genes in *S. tuberosum* and analyzed their structures, phylogenetic relationships, and expression profiles in different tissues of potato and in responses to abiotic cues.

Expression profiles for *StCLEs* in different organs suggest that corresponding CLE peptides could play various roles in potato growth. Specifically, *StCLE8* and *StCLE12* are expressed at high levels in stolon and tubers. According to phylogenetic analysis, StCLE8 and StCLE12 peptides showed a closer relationship to *Arabidopsis* AtCLE41 and AtCLE44 peptides, which promote cambium cell proliferation and inhibit xylem cell differentiation (TDIF) [21,28] (Figure 2b). It is known that TDIF homologs stimulate radial growth of shoots and roots in various plants [8,9]. However, during tuberization, active cell divisions resulting in thickening of stolons occur in the perimedullary region of the stolon, but not in the cambium [37,38]. The molecular mechanisms that control the changes in cell growth and proliferation during tuberization have not yet been studied in details, and the increased expression of TDIF-related *StCLE8* and *StCLE12* genes in potato stolons and tubers may suggest that these genes could be possible regulators of tuberization in the potato.

CLE peptides are known as regulators of plant responses to different environmental factors, such as nutrient availability and dehydration stress (reviewed in [39]). The potato is very sensitive to water stress: drought could decrease the photosynthetic rate, leaf area, tuber number, and weight [40]. We found that expression of the *StCLE23* gene was activated under water stress. In *Arabidopsis*, the AtCLE25 peptide acts as a water-deficiency signal and mediates stomatal control [10]. However, StCLE23 does not show high similarity to AtCLE25. StCLE23 clustered together with AtCLE10 that was reported to regulate protoxylem vessel formation in roots [41]. However, in comparison with AtCLE10, the StCLE23 peptide has two substitutions in the CLE domain sequence (R_2-to-L_2 and T_5-to-S_5), that might confer novel functions to this peptide in the potato. Single AA substitutions in the CLE domain are known to essentially affect function of the corresponding CLE

peptide. For example, *Arabidopsis* AtCLE25 is involved in dehydration response, but its close homologue, AtCLE26, which differs from CLE25 only by one AA substitution, is not involved in the dehydration stress response [10]. Future studies should elucidate whether the StCLE23 peptide is involved in the dehydration response pathway similar to AtCLE25-activated one, or it acts through other signaling pathways.

In addition to dehydration stress, CLE peptides were also shown to be involved in the N-nutritional response. In *Arabidopsis*, AtCLE1, -3, -4, and -7 peptides locally regulate lateral root development under N-limited growth condition [25]. In legume plants, CLE peptides were found that mediate systemic response to nitrate and transmit signal about N availability from the root to the shoot [42]. In our study we found that expression of *StCLE4, StCLE5,* and *StCLE10* was activated in the roots, which were exposed to the N-rich medium. These genes are closely related to nitrate-regulated CLE peptides of *A. thaliana,* suggesting that they could be involved in nitrogen-depended regulation of potato development and a possible negative regulation of tuberization, the latter is known to be inhibited by a high nitrogen amount. The low amount of nitrogen is one of the signals indicating the end of the growing season, and it induces tuberization in the potato. We can speculate that downregulation of *StCLE4, StCLE5,* and *StCLE10* under N-depleted conditions could have stimulating effect on tuberization; however, further experiments are required to check this hypothesis.

Other environmental factors that control tuberization include day length and temperature. Tuberization is inhibited by long days and high temperatures and is stimulated by short days and low positive temperatures [43,44]. Therefore, in a changing environmental condition, different kinds of mobile signals can mediate organ-to-organ communication regulating tuber formation. Two mobile signals, which are produced in the leaves under short-days condition and transported to the stolons to induce tuber formation, are known. The first one represents the StSP6A protein, a member of flowering locus T (FT)-like protein family, involved in photoperiodic regulation of flowering and some other developmental processes (reviewed in [39]). It is known that heat-mediated inhibition of tuber growth correlated with downregulation of *StSP6A* expression [45]. Another mobile signal transported from leaves to stolons and stimulating tuberization represents mRNA of homeodomain transcription factor StBEL5 [36]. Using transcriptomic data, we found that the *StCLE4* expression level was increased upon *StBEL5* activation, suggesting that *StCLE4* could be a potential target of StBEL5 transcription factors. Importantly, *StCLE4* is also upregulated at high-nitrogen conditions according to our data; therefore, this gene can combine regulatory signals derived from different environmental regulators, including photoperiod-dependent *StBEL5*-mediated pathway and nitrogen-induced one. To check the possibility that the *StCLE4* gene could be directly regulated by StBEL5 transcription factor, we performed yeast-one-hybrid assay. However, the direct interaction of StBEL5 with the regulatory sequences of the *StCLE4* gene was not found in our assay. Therefore, either StBEL5 transcription factor activates *StCLE4* by binding other regulatory sequences within its promoter that were not included in the 500 bp-upstream region used in this assay, or StBEL5 indirectly regulates *StCLE4* expression. Functional analysis of the *StCLE4* gene and its possible regulation by different environmental cues is of great interest and should be addressed in future studies.

Taken together, the present study provides comprehensive analysis of the *CLE* genes in the potato that allowed selecting candidate genes for further functional analysis in potato tuber development and response to environmental cues.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11050984/s1, Figure S1: Phylogenetic tree of the CLE proteins of *Solanum tuberosum (St)*, *Arabidopsis thaliana (At)*, *Solanum lycopersicum (Sl)*, *Marchantia polymorpha (Mp)*, *Selaginella moellendorffii (Sm)*, *Oryza sativa (Os)*, and *Picea abies (Pa)*, Figure S2: Effect of ethanol induction of *pAlc:StBEL5* in stolons on the activity of *StCLE4,* Table S1: AA sequences of CLE proteins used for phylogenetic tree construction, Table S2: List of primers, Table S3: Promoter regions of *StSP6A* and *StCLE4* used for Yeast One-Hybrid Assay, Table S4: Potato *CLE* genes, Table S5: Tomato *CLE* genes.

Author Contributions: Conceptualization, all authors; methodology, all authors; validation, M.G.; investigation, M.G.; writing—original draft preparation, M.G.; writing—review and editing, I.D., M.L. and L.L.; visualization, M.G.; supervision, I.D. and L.L.; funding acquisition, L.L. All authors have read and agreed to the published version of the manuscript.

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