HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

INVESTIGATION OF THE MOLECULAR PROCESSES AFFECTING THE EARLINESS OF POTATO

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1. BACKGROUND OF THE WORK AND ITS AIMS

Today's basic tasks are climate change, land depletion and the fight against pathogens and pests. Food shortages are a major problem due to the growing size of population. However, healthy eating has also come to the fore. Thus, vegetable crops rich in vitamins and minerals play an increasingly important role in everyday's food consumption.

One of the most important cultivated food crops in the world is potato (*Solanum tuberosum* L.). It has favorable nutritional and physiological properties and contains valuable minerals and vitamins. According to the FAO, there were more than 17 million hectares of potatoes planted in the world in 2022. Potato is an annual plant, which is grown for its thickened stem, the tuber. Use of potato is diverse. It also plays an important role in industrial processing and animal feeding. Therefore, the research on tuber development, both from an economic and a biological point of view, is an important task. Two types of potatoes are distinguished: there are early and late maturing potato varieties. Early maturing varieties are less exposed to extreme weather and disease, and because they can be harvested within a short time, early varieties are economically advantageous. Therefore, one of the main goals of potato breeding is increasing earliness.

Timing of the plant reproductive cycles is strongly influenced by environmental factors. Tuber development of the ancestors of cultivated potato varieties depends on the day length; short day conditions stimulate, while long day conditions inhibit tuber formation. Tuberisation of modern potato varieties, however, does not depend on day length, but their temperature dependence has remained. Under favorable environmental conditions, phloem-mobile signals from the leaf initiate tuber development, which then reach the modified underground stem, the stolon. Three main regulatory elements are involved in this process: SP6A, BEL5 and CDF1. CDF1 (CYCLING DOF FACTOR 1) belongs to the DOF transcription factor family and determines the start time of tuber development in the obligate short-day tuberising *S. tuberosum* Andigena landscape variety. One of the aims of our experiments was to study the role of CDF1 in a light length independent tuberisating commercial potato cultivar, namely 'Désirée'.

Regulation of tuber formation in potato is similar to the regulation of flowering in *Arabidopsis*, in which GIGANTEA (GI) also plays a role. In *S. tuberosum* Andigena, GI, as part of the complex formed with FKF1, participates in the binding and delivery of CDF1 to proteasomes, where it is degraded. Thus, GI, in an indirect manner, participates in determining the time of tuberisation. Another goal of our experiments was to explore the role of GI in tuber formation in the potato cultivar 'Désirée'.

Research goals:

In order to reveal the role of CDF1 and GI in the potato cultivar 'Désirée' the following sub-tasks were defined:

- Generation of targeted mutations in the *CDF1* gene of 'Désirée' using the CRISPR/Cas9 system
- Identification of CDF1 mutants by PCR and DNA sequence analysis
- Studying the morphological and tuber formation characteristics of three selected CDF1 mutant lines under greenhouse conditions compared to the non-transformed 'Désirée'

- Identification of *GI* gene(s) in 'Désirée'
- *In silico* prediction for the regulation and role of *GI* gene(s)
- Transcriptome analysis of the leaf of a 'Désirée' line silenced in *GI* expression
- Targeted mutagenesis of the *GI* gene(s) of 'Désirée' using the CRISPR/Cas9 system
- Identification of GI mutants by PCR and DNA sequence analysis
- Studying the morphological and tuber formation characteristics of the GI mutant lines under greenhouse conditions compared to the non-transformed 'Désirée'

2. MATERIAL AND METHODS

2.1. Bacterial strains and plant lines

Escherichia coli DH5α and *Agrobacterium tumefaciens* LBA4404 bacterial strains were used for the experiments. *Solanum tuberosum* L. cv. 'Désirée' was used as a wild type control. *CDF1* and *GI* mutant lines were generated from 'Désirée'. At the transcriptome level, aGI.52 antisense plants generated by Odgerel et al. (2022) were analyzed.

2.2. Media

E. coli was cultured on LB, SOB or SOC media, *A. tumerfaciens* strains were grown on YEB as described by Sambrook et al. (1989). Media were supplemented with antibiotics (rifampicin/kanamycin/ampicillin) when required. Murashige-Skoog (MS; Murashige and Skoog 1962) and RM (MS medium without vitamins) media were used for potato propagation, *in vitro*. Callus and shoot induction was carried out on CIM and SIM medium, respectively (Dietze et al. 1995).

2.3. *In vitro* plant growth and transformation

Potato plants were grown *in vitro* in a 24°C culture room, with a 16/8 hour day/night cycle, in test tubes or jars. Plants with apical shoots were placed in fresh RM medium every 4 weeks and propagated vegetatively from stem segments. The leaves of 'Désirée' potato plants grown in MS medium in jars for three weeks were transformed according to Dietze et al. (1995).

2.4. Molecular biological methods

From the leaves of potato plants grown *in vitro* the genomic DNA was isolated as described by Shure et al. (1983).

Amplification of the DNA fragments was carried out by PCR. The reaction conditions were adjusted depending on the melting temperature of primers and expected length of the PCR fragments. The PCR fragments were separated on agarose gel stained with ethidium bromide and photographed in a Gel Doc™ EZ Imager.

Competent cells from *E. coli* were prepared according to Inoue et al. (1990). For DNA sequencing, DNA fragments were cloned into pGEM®-T Easy vector (Promega, Madison, WI, USA) and transformed into *E. coli* competent cells according to Sambrook et al. (1989). The pGEM®-T Easy recombinant plasmids were selected by blue-white selection on LB medium supplemented with ampicillin, X-Gal and IPTG. Plasmid DNA was isolated from white colonies presumably carrying a recombinant plasmid (Sambrook et al. 1989).

The pKSE401 plasmid constructs were introduced into *A. tumefaciens* according to Höfgen and Willmitzer (1988). Plasmid DNA was isolated from the grown-up colonies and checked using PCR.

2.5. DNA sequence analysis

The DNA sequences were determined by Biomi Kft. (Gödöllő), and the BioEdit program was used to read the sequences. The NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) programs were used to analyze the sequences. The obtained sequences were compared to the *S. tuberosum* group Phureja DM1-3 v6.1 genome sequence available at the Potato Genomic Resource Spud DB (http://spuddb.uga.edu/).

2.6. *In silico* DNA sequence analysis of *GI* promoters

The 3000-bp sequence from the translation start site of the promoter regions of the two *GI* genes located on chromosomes 4 (SoltuDM.04G027760) and 12 (SoltuDM.12G007510) was obtained from the Potato Genomic Resource Spud DB, while *Arabidopsis thaliana* (AT1G22770.1) promoter sequence of the *GI* gene was downloaded from the TAIR website (https://www.arabidopsis.org/). The Plant Regulation Data and Analysis Platform (PlantRegMap) (http://plantregmap.gao-lab.org/) was used to predict transcription factor binding sites.

2.7. Transcriptome analysis

Libraries of RNAs isolated according to the protocol of Stiekema et al. (1988) were prepared by Novogene Company Ltd (Cambridge, UK, https://www.novogene.com/eu-en/). After sequencing on the Illumina NovaSeq 6000 platform and the subsequent quality control, the resulting data were aligned to the *S. tuberosum* group Phureja DM1-3 v.6.1 reference genome. Bioinformatics analysis such as gene ontology (GO) and 'Kyoto Encyclopedia of Genes and Genomes' (KEGG) analysis was also performed by Novogene.

2.8. Construction of gene editing vectors

For targeted mutagenesis of the *CDF1* gene based on the CRISPR/Cas9 system, gRNAs were incorporated into the pAGM4723 vector (Weber et al. 2011). For a detailed description of the construct generation, see Karsai-Rektenwald et al. (2023).

For the CRISPR/Cas9-based mutagenesis of the *GI.04* and *GI.12* near the 3' end of the genes (eGI.04 and eGI.12 lines), gRNAs were inserted into the pKSE401 vector (Xing et al. 2014) using the same method as for the generation of mutations towards the middle of the genes (mGI.04 and

mGI.12 lines). The gRNAs were designed using the CRISPOR tool (http://crispor.tefor.net). The synthesized gRNA oligos, which also contained some bases of the pCBC-DT1T2 plasmid at their ends, were PCR amplified by adding pCBC-DT1T2 template DNA according to Xing et al. (2014), cleaved with *Bsa*I enzyme, ligated into the *Bsa*I digested pKSE401 vector and transformed into *E. coli*. Plasmid DNA was purified from some of the transformant colonies and the presence of the inserts in the plasmid DNA was verified by PCR.

2.9. Greenhouse growth conditions and morphological studies on plants

Potato plants grown under *in vitro* conditions for 5-7 weeks were planted in pots containing sterile Tobaccosubstrate soil and grown under greenhouse conditions at 20-28°C. In winter, the ambient light was supplemented with artificial lightening. Optimal growth conditions for the plants were ensured by watering twice a week. The plants were treated weekly with Mospilan pesticide. Under greenhouse conditions, plant height, leaf morphology (shape, development, wilting) and stem thickness were measured. The tubers were counted on young plants at the beginning of the growing season. At the end of the growing season, the tubers were collected, counted and weighed. The tubers were stored in the dark at room temperature and the time and extent of germination were checked during storage.

2.10. Determination of the anthocyanin content of tuber skins

The anthocyanin measurement was performed according to the simplified method of Toguri et al. (1993). Spectrophotometric absorbance measurements (540 nm) were used to determine the relative amount of chloride forms of anthocyanin pigments compared to the control.

2.11. Statistical analysis

Tukey's analysis of variance (ANOVA) (https://astatsa.com/) was used to detect significant differences between data. Metaboanalyst 5.0 (https://www.metaboanalyst.ca) was used to generate heat maps.

3. RESULTS AND DISCUSSION

3.1. Characterization of the *CDF1* gene in potato

To understand the role of the *CDF1* gene in potato, *CDF1* mutants were generated from the potato cultivar 'Désirée' using the CRISPR/Cas9 genome editing system. The gRNAs were designed to the 3'-UTR of *CDF1* in the hope of obtaining large deletions that reach the 3' coding region of *CDF1*.

To search for mutations, genomic DNA was isolated from the rooted shoots of *in vitro* plants. The genomic DNA of 75 putative mutant plants was tested by PCR, from which 11 mutant plants with deletions large enough to be clearly visible on agarose gels were identified. *Figure 1A* shows the deletion carrying fragments of the three mutant lines (gCDF1/1, gCDF1/2 and gCDF1/3) selected for further experiments.

'Désirée' is a tetraploid potato variety. No mutant plants were identified that were null mutants and no mutants were found in which the deletion reached the coding region (*Figure 1B*).

To map the deletions, the PCR fragments were cloned into the pGEM-T® Easy vector and the PCR fragments of the plasmid DNAs were sequenced. The resulting sequences were compared with the *CDF1* genome sequence in the *S. tuberosum* Phureja Spud DB (*Figure 1C*). The size of the deletions in gCDF1 mutants ranged from 49 to 117 basepairs.

It was hypothesized that the *CDF1* mRNA end structure was altered by deletions in the gCDF1 mutants. To confirm this, computational prediction was performed using the CentroidFold program (http://rtools.cbrc.jp/centroidfold/). The computational analysis confirmed our hypothesis that the end sequence structure of *CDF1* mRNA is very different from that of the wild-type *CDF1* mRNA (*Figure 1D*).

To investigate the morphological and tuberisation characteristics of the plants, 4-week-old individuals vegetatively propagated from the three gCDF1 mutant lines were further grown under greenhouse conditions. Untransformed 'Désirée' plants were used as controls in all cases. The development of compound leaves was delayed in all plants of the three gCDF1 lines compared to the 'Désirée' control (*Figure 2A*). Nevertheless, at a later growth stage, gCDF1 plants developed compound leaves similar to the control plants. All three gCDF1 mutant plants had thinner stems and elongated stem segments compared to the 'Désirée' control plants (*Figure 2B*). However, by the middle of the growing season there was no significant difference in height between gCDF1 and control plants.

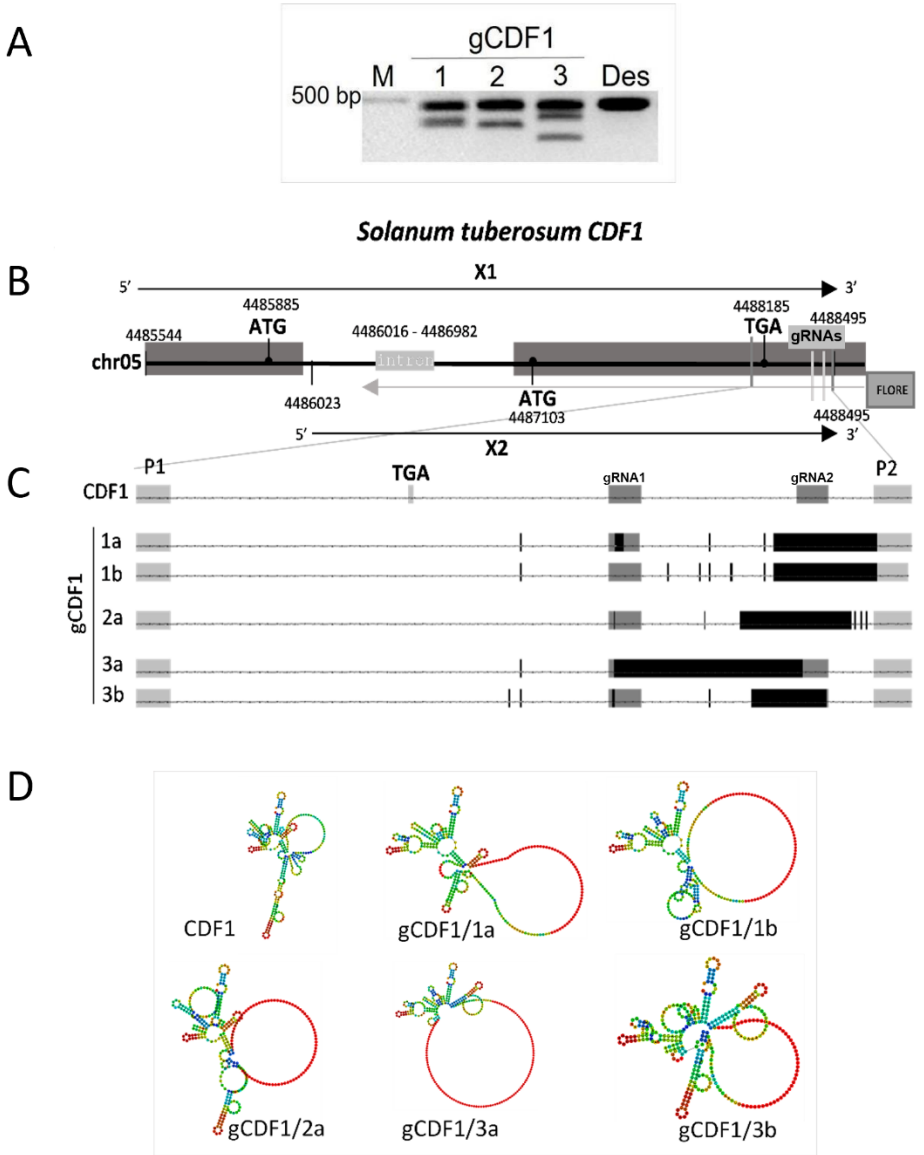

Figure 1: (**A**) Identification of gCDF1 mutants by PCR with CDF1.1 3' end FW and CDF1.1 3' end R primers. M, marker; 1, 2, 3, selected mutant lines; Des, 'Désirée' control. **(B**) Schematic drawing of the *CDF1* gene and its transcripts. The X1 (XM_006355049.2), *X2* (XM_015312183.1) and *FLORE* (Gonzales et al. 2021) transcripts are indicated by arrows. Numbers indicate chromosomal position; chr05, chromosome V; ATG, translational start codon; TGA, translational stop codon. (**C**) Location and size of deletions compared to the *CDF1* sequence of 'Désirée'. Black rectangles indicate deletions, lines indicate basepair differences. P1, CDF1.1 3' end FW primer; P2, CDF1.1 3' end R primer; gRNA1, CDF1.1 gRNA1, "guide" RNA; gRNA2, CDF1.1 gRNA2, "guide" RNA. (**D**) Putative secondary structure of *CDF1* mRNA from *S. tuberosum* Phureja and gCDF1 mutants from the translational stop codon to the polyadenylation signal.

The earliness of tuber development was estimated by counting the number of tubers on 1-month-old plants. The tuber number of mutants did not differ significantly from the control. After counting, the plants were replanted and grown further under greenhouse conditions. The tubers were counted again at the end of the growing season. The gCDF1/2 plants had significantly more tubers than the control. To determine tuber yield, the weight of tubers developed on mutant plants and control plants was measured. The tuber yields of gCDF1/1 were significantly higher, while the tuber yields of gCDF1/2 were significantly lower than those of control plants.

'Désirée' is a red skin potato. The tubers of gCDF1/1 and gCDF1/3, although in different degrees, but were lighter in colour than the control 'Désirée' tubers (*Figure 2C*). Since anthocyanins determine the colour of tubers, we measured the anthocyanin content of the tuber skins. The measurement confirmed that the gCDF1/1 and gCDF1/3 lines indeed had lighter skin colour than the control, although this difference was not significant.

The measured tubers were stored at room temperature in the dark. The first sprouts appeared on day 59 after harvesting. At day 84 after harvest, germination was found on all tubers of gCDF1/1, gCDF1/2 and 'Désirée', while germination of tubers of gCDF1/3 was below that of the other lines, with germination on only 50% of tubers. However, on day 129 after harvest, all tubers of gCDF1/3 were also germinated (*Figure 2C*).

One month after planting, shoots emerged from all tubers of both gCDF1/2 and control 'Désirée', while this was delayed for gCDF1/1 and gCDF1/3 tubers. The greatest delay in shooting was observed from gCDF1/3 tubers (*Figure 2C*).

Six weeks after transplanting, gCDF1 mutants showed differences in height (*Figure 2D*) and leaf morphology compared to the control. The gCDF1/1 and gCDF1/3 plants were shorter and developmentally lagged behind the control plants. The development of compound leaves at the beginning of the growing season was delayed, similar to the plants outplanted from *in vitro*.

At the end of the growing season, tubers were harvested and weighed to determine if there was a difference in tuber yield of plants transferred to greehouse from *in vitro* or grown from tubers. The tuber yield of gCDF1/3 plants was significantly lower than that of 'Désirée'. Compared to plants planted from *in vitro*, the tuber yields of gCDF1/1 and gCDF1/3 were lower, while the tuber yield of gCDF1/2 increased slightly.

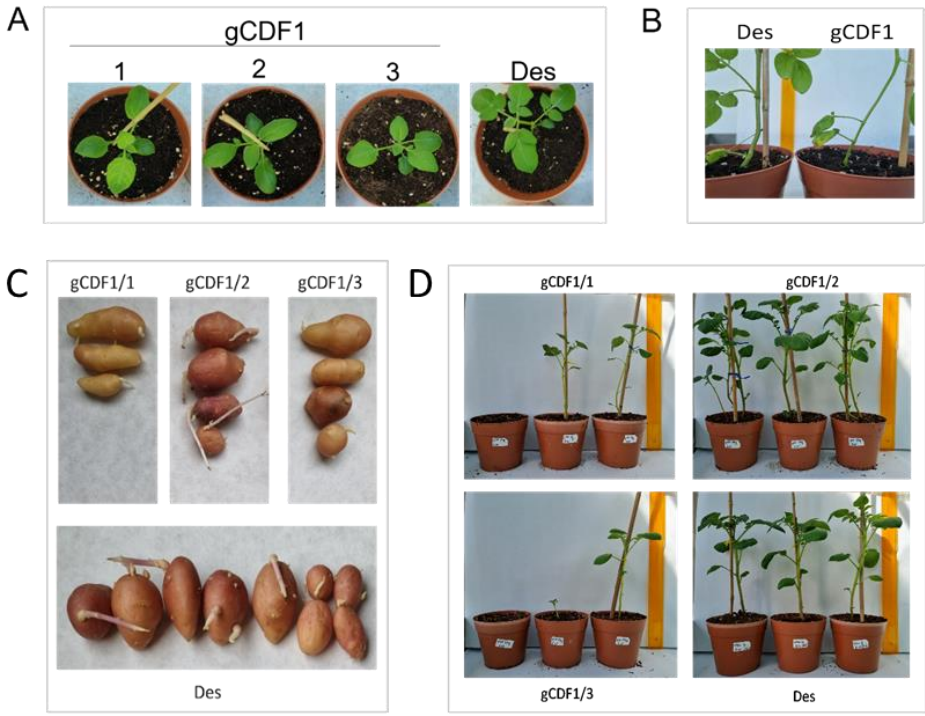

Figure 2: (**A**) Leaf morphology of gCDF1 mutant lines at the beginning of the growing season compared to control 'Désirée'. (**B**) Stem thickness of gCDF1 mutants at mid-growing season compared to 'Désirée'. (**C**) Sprouted tubers of gCDF1 mutant and 'Désirée' control lines before planting. (**D**) Height of gCDF1 mutant plants planted from tubers.

3.2. Discussion of results obtained with gCDF1 mutant plants

The CRISPR/Cas9 system proved to be efficient for the generation of CDF1 mutants, as mutant lines carrying deletions of 49-117 bp in length were obtained with a frequency of 15%. Three mutants with different sizes and locations of delations (gCDF1/1, gCDF1/2, gCDF1/3) were selected for detailed analysis. Both gCDF1/1 and gCDF1/3 mutant plants developed either from *in vitro* or tubers had delayed compound leaf development, thinner stems and elongated stems segments compared to control 'Désirée' plants. Previous publications demonstrated that CDF1 is involved in initiating tuber development and attenuation of drought tolerance in potato (Kloosterman et al. 2013; Gonzales et al. 2021), while in other plant species, it is involved in several other processes such as for example, light response or determination of flowering time. Our experimental results have shown that, in addition to tuber development, CDF1 may also have an other function in potato, namely influencing shoot development.

Different tuber developmental characteristics were obtained in all three *CDF1* mutant lines. While one of the mutant lines showed delayed tuber development, the other line had a higher total tuber weight than the control.

The germination of tubers also differed in the three mutants. Thus, we cannot be sure that *CDF1* plays a role in tuber development in the potato cultivar 'Désirée'. The different tuber development and germination of the three mutant lines can be explained by *CDF1* mutations that have not yet been identified, but we cannot exclude off target mutations. In addition, since none of the three mutants is a null mutant, they have the ability to continuously generate new mutations by Cas9 at cellular level, which, although not identified by PCR, may have an impact on tuber development and germination.

None of the deletions detected reached the coding region of *CDF1*, but still caused a phenotypic change. The 3' non-translated regions of mRNAs may play a role in mRNA stability and transport. They may bind to microRNAs or proteins that can affect the efficiency of translation (Nyitra and Pál 2013). In gCDF1 mutants, deletions are outside the polyadenylation domain, but the mutations were predicted to cause a significant change in the structure of the 3' end of the *CDF1* mRNA. It is possible that this change prevents binding of regulatory proteins to the *CDF1* mRNA and reduces the efficiency of translation. The FLORE regulatory lncRNA was also shortened by deletions, which may also play a role in the phenotypic changes, but the mechanism of this is currently unknown.

3.3. Identification and *in silico* promoter analysis of *GI* genes in potato

To identify the *GI* genes in potato, the homologue of *A. thaliana GI* (*AtGI*) NM_102124 was searched using NCBI Blast. Two transcript variants were found that were 71.8 and 72.9% identical to *AtGI*. Surprisingly, we found two *GI* genes in potato, whereas *Arabidopsis* and many other plant species have only one *GI* gene. One was located on chromosome 4 (*GI.04*) and the other on chromosome 12 (*GI.12*). The coding region of the two variants showed 83.7% identity.

In *S. tuberosum* Phureja Spud DB, the promoter sequences of the two *GI* variants were searched. The two promoter sequences were compared with each other and with the *AtGI* promoter using NCBI BLAST. No significant similarity was found between the two potato *GI* promoters or between the *AtGI* and potato *GI* promoters.

To understand the putative promoter region of *GI.04* and *GI.12*, DNA was isolated from 'Désirée' potato and primers were designed based on the *GI* promoter sequence on chromosomes 4 and 12 of *S. tuberosum* Phureja. They were cloned and sequenced. The sequencing results showed that the *GI.04* promoter of 'Désirée' was identical to the promoter of the *GI.04* gene of Phureja, with a few basepair differences. The promoter of 'Désirée' *GI.12* was very similar to that of Phureja, but compared to Phureja, three clones had a 14-basepair insertion at approximately -1.7 kb and four clones had an

8-9-basepair insertion at around -0.3 kb. Since not all four clones had an insertion at -1.7 kb, we concluded that 'Désirée' has at least two *GI.12* alleles.

Using the PlantRegMap program, we analyzed the promoter sequence of the potato *GI* genes to find the binding sites of transcription factors (TFs). PlantRegMap predicted 73 binding sites in the *GI.04* promoter, which could bind 45 TFs belonging to 14 families. While 32 binding sites were found in the *GI.12* promoter, 27 different TFs belonging to 13 families can bind to the binding sites. In the *GI.04* and *GI.12* promoters, TF binding sites related to ABA, circadian clock and flower development were identified, however, TF binding sites related to e.g. stress were also found.

To determine how similar the regulation of the potato *GI* genes is to that of the *Arabidopsis GI* gene, TF binding sites in the -3.0 kb sequence of the *Arabidopsis GI* gene were also identified using PlantRegMap and compared to the promoter binding sites of the *S. tuberosum* Phureja *GI* genes (*Figure 3*). The program predicted 160 binding sites for 106 TFs in the promoter region of *AtGI*. Many TFs belonged to the same family. Furthermore, overlapping binding sites were also detected.

All TF families associated with *AtGI* and *GI.04* promoters were identical. The most similarities between *AtGI* and *GI.12* were concentrated in the two locations of the *AtGI* promoter. Three different TF binding sites involved in circadian rhythm regulation (LHY1, REV1 and/or REV8) were also found in the *AtGI* promoter. TF binding sites associated with circadian rhythm in the *AtGI* promoter were also found in the *GI.04* promoter, but only the REV1/8 binding sites were present in *GI.12*. *AtGI* is involved in the regulation of flowering time (Rédei 1962). TF binding sites affecting flower development were found in the *AtGI* promoter. We identified SOC1-like (Lee et al. 2000), MYB17, REV1/8 (Singh and Mas 2018) and CMB1-like binding sites associated with flowering in the potato *GI.04* promoter. In the *GI.12* promoter, we found bHLH30, ATHB51, SOC1, FBP1 and REV1/8 binding sites associated with flowering. The tuberisation-associated binding site POTH20 (KNOX1) was present in both *GI* promoters. From the -1.8 kb segment of the *AtGI* promoter in the 5' direction, we found few similar motifs between *Arabidopsis* and *GI.04*. In *GI.12*, no homology was detected with the *AtGI* promoter from -1.3 kb in the 5' direction.

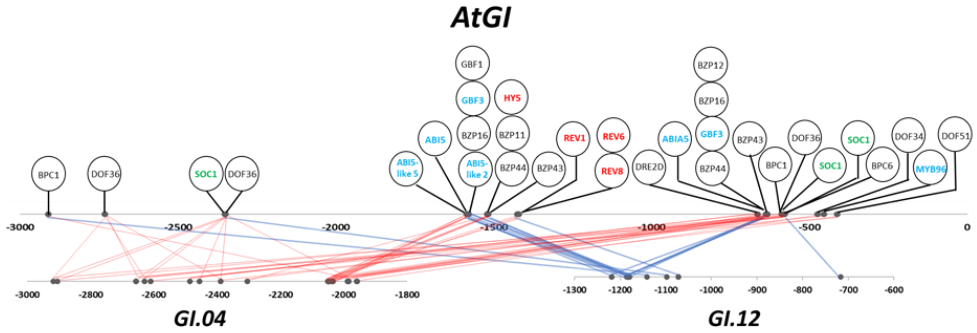

Figure 3: Schematic drawing of the location of transcription factors binding to both the *GI.04* and *GI.12* promoters of *Arabidopsis* and *S. tuberosum* Phureja. The names of TFs related to circadian rhythm, flower development and response to ABA are highlighted in red, green and blue, respectively. BPC1, BASIC PENTACYSTEINE 1; DOF36, DNA BINDING WITH ONE FINGER 36; SOC1, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1; ABI5-like5, ABA-INSENSITIVE 5-LIKE 5; ABI5, ABA-INSENSITIVE 5; GBF1, G-BOX BINDING FACTOR 1; GBF3, G-BOX BINDING FACTOR 3; BZP16, BASIC LEUCINE-ZIPPER 16; ABI5-like2, ABA-INSENSITIVE 5-LIKE2; HY5, LONG HYPOCOTYL 5; BZP11, BASIC LEUCINE-ZIPPER 11; BZP44, BASIC LEUCINE-ZIPPER 44; BZP43, BASIC LEUCINE-ZIPPER 43; REV1, REVERSIONLESS 1; REV6, REVERSIONLESS 6; REV8, REVERSIONLESS 8; DRE2D, DROUGHT-RESPONSIVE ELEMENT 2D; BZP12, BASIC LEUCINE-ZIPPER 12; BPC6, BASIC PENTACYSTEINE 6; DOF34, DNA BINDING WITH ONE FINGER 34; MYB96, MYELOBLASTOSIS 96; DOF51, DNA BINDING WITH ONE FINGER 51

3.4. Discussion of results from promoter analysis of *GI* genes

It was found that the potato cultivar 'Désirée' has two *GI* genes that differ in both promoter and coding regions. The expression of *GI* genes in different plant species shows a diurnal regulatory cycle, which is also the case in *S. tuberosum* ssp. Andigena and Neo-Tuberosum potato (Morris et al. 2014). We found the binding site of REV1/8 in the promoter of both *GI* genes of 'Désirée', whose function may be related to circadian regulation. We therefore hypothesise that the regulation of *GI.04* and *GI.12* expression may be linked to the circadian cycle.

The *GI* genes play a key role in the regulation of the floral signalling pathway (Brandoli et al. 2020). PlantRegMap predicted binding sites for TFs involved in floral development (e.g. SOC1 or ABI5) for both potato *GI* promoters, although previously Odgerel (2022) found that *GI.04* is expressed in all floral organs, whereas *GI.12* is not expressed in any floral organ. *GI.12* is likely to be active during the early stages of flower development, whereas it is no longer required for its function in the mature flower.

The *GI* gene plays a key role in tuber development in *S. tuberosum* ssp. Andigena (Kloosterman et al. 2013). We found a POTH20 binding site in the promoters of both *GI* genes of 'Désirée'. Rosin et al. (2003) showed that POTH1 is 73% identical to POTH20. Overexpression of POTH1 enhances *in*

vitro tuberization under both short-day and long-day conditions in *S. tuberosum* ssp. Andigena. If POTH20 can substitute for POTH1 in 'Désirée', it could be an alternative positive regulator of *GI* expression and thus tuber development.

GI is involved in the regulation of abiotic stress responses and in some plant species *GI* expression in leaves has been shown to be affected by stress (Jose and Bánfalvi 2019). Expression of *GI.04* is induced by cold, heat and osmotic stress, whereas *GI.12* is upregulated by ABA and downregulated by salt, cold, heat and osmotic stress, i.e. the two *GI* genes respond differently to stress (Odgerel, 2022). One reason for the differences may be that the *GI.04* promoter has MYB TF binding sites, whereas the *GI.12* promoter does not.

GI.12 expression levels were about five times higher in root and shoot organs and thirty times higher in tubers than *GI.04* expression level (Odgerel, 2022). The regulation of transcription is a complex process that depends on the availability and activity of TFs and the type, number, position and combination of regulatory elements present in and around the promoter (Hernandez-Garcia and Finer 2014). Higher activity can be explained by the greater proximity of *cis*-regulatory elements to the translational start site in the *GI.12* promoter region than in the *GI.04* promoter.

3.5. Transcriptome analysis of aGI52 leaves

To investigate the function of GI.04 Jose (2019) generated *GI.04* expression silenced 'Désirée' lines, of which the greatest silencing compared to the 'Désirée' control was in aGI52 plants, where a relatively stable 49% reduction in *GI.04* expression was detected (Odgerel 2022). Therefore, this line was selected for further studies.

To investigate the effect of *GI.04* inhibition at the transcriptome level, RNA sequence analysis was performed by the company Novogene on RNA isolated from aGI52 leaves grown in the greenhouse. 454 genes expressed only in 'Désirée' and 247 genes expressed only in aGI52 were identified. 488 differentially expressed genes (DEGs) were detected. GO analysis showed that photosynthesis-related genes were expressed at higher levels in aGI52 than in 'Désirée', while the expression of peptidases and peptidase inhibitors was inhibited in aGI52. KEGG analysis showed that certain pathways, such as carbohydrate metabolism, were activated, while the metabolism of certain amino acids, such as nitrogen metabolism, was inhibited. In aGI52, the expression of 14 TFs, including *IBH1*, *REV1* and some TFs involved in plant defense (*ERF1B, ZAT10, WRKY11, MYB1R1, TGA2.1*), was upregulated, while the expression of 11 TFs (e.g. ethylene-responsive *RAP2-7*) was downregulated (*Figure 4A*). The transcription of genes encoding key

enzymes in starch synthesis, *AGS* (*ADP-GLUCOSE SYNTHASE*) and *SS* (*STRACH SYNTHASE*), *SP* (*STARCH PHOSPHORYLASE*), was inhibited, but the expression of *TPS (TREHALOSE PHOSPHATE SYNTHASE*) increased. Among the genes involved in defence, *SOD* (*SUPEROXIDE DISMUTASE*) was activated, but *CAT* (*CATALASE*) was repressed (*Figure 4B*).

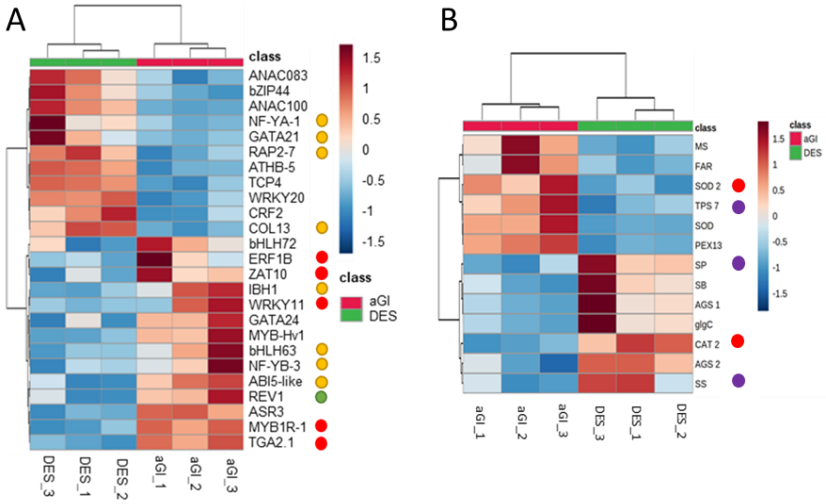

Figure 4: (**A**) TFs differentially expressed in aGI52 leaves compared to untransformed control 'Désirée' leaves and plotted on a heatmap. TFs related to flower development are indicated by yellow dots, those related to the circadian clock by green, and those related to stress by red. (**B**) Genes differentially expressed in aGI52 leaves compared to untransformed 'Désirée' control leaves, involved in glyoxylate and dicarboxylate metabolism, carbohydrate metabolism and peroxisomal pathways. Red dots indicate TFs related to stress and purple to carbohydrate metabolism.

3.6. Discussion of the results of the transcriptome analysis

The role of *GI* has been investigated in several plant species and it appears that the regulation of flowering time and circadian clock is a general function of GI (Liu et al. 2024). Transcriptome analysis has shown that inhibition of *GI.04* affects the expression of several TFs involved in flowering regulation. For example, inhibition of *GI.04* induced an increase in *REV1* expression. REV1 links the circadian clock and auxin networks to the positive regulation of the expression of the auxin biosynthetic gene *YUCCA8* and thereby promotes plant growth (Rawat et al. 2009). Thus, our results show that, like in other plant species, GI is involved in the regulation of the circadian clock in potato.

Besides flowering, GI is also involved in plant responses to environmental stress. We identified activated TFs involved in defense mechanisms in aGI52, including *ERF1B, WRKY11* and *TGA2.1*. It has been

shown that activation of *ERF1* and *WRKY11* leads to an increase in tolerance to *Bacillus* species in *Arabidopsis* and tomato (Lorenzo et al. 2003, Jiang et al. 2016). In contrast, *TGA2.1* activity delays rice plants in defense against *Xanthomomas oryzae* (Fitzgerald et al. 2005).

The antioxidant enzymes SOD and CAT are an integral part of the plant's defence mechanisms to avoid damage caused by reactive oxygen species. Interestingly, however, while *SOD* was upregulated, *CAT* was downregulated in aGI52 leaves. *GI* is also a repressor of *SOD* in *Arabidopsis*, as indicated by the increased tolerance to oxidative stress in the *gi-3* mutant (Cao et al. 2006). However, in contrast to potato, *CAT* genes expression levels in *Arabidopsis* were 1.5-2-fold higher in *gi* mutants than in the wild type (Cha et al. 2019).

The key genes for starch synthesis, *AGS1*, *AGS2*, *SS* and *SP*, were inhibited, while *TPS*, a sugar messenger, was activated in aGI52. It is known that the *Arabidopsis* circadian system is sensitive to sucrose, GI protein is stabilised by sucrose overnight, and starch content is increased in GI mutants (Dalchau et al. 2011, Eimert et al. 1995, Haydon et al. 2017). In rice plants with null mutations for *GI*, leaves had significantly increased sucrose and starch content (Izawa et al. 2011). Our transcriptome results suggest that potato may be the third known plant species in which *GI.04* expression reduces leaf starch content. A recent study has identified TPS8 as a direct interactor of GI in *Arabidopsis* (Krahmer et al. 2018) and we identified *TPS7* as an upregulated gene. Thus, our results support the previous conclusion that TPS is involved in mediating GI effects.

3.7. Isolation and characterisation of GI mutants

To understand the role of *GI* genes in potato, we generated *GI.04* and *GI.12* mutants from 'Désirée' using CRISPR/Cas9 genome editing. Most of the sequence of *GI.04* is identical to that of *GI.12*, so we first designed the gRNAs near the 3' end, where the two *GI* genes have the most divergent regions.

Genomic DNA from 82 putative mutant plants was tested for *GI.04*- and 86 for *GI.12* mutations. 29 and 18 mutant lines carrying large deletions in GI.04 and GI.12, respectively, were identified and designated "end" mutants (eGI.04 and eGI.12).

Three selected eGI.04 mutant lines were assumed to be null mutants (eGI.04/1, eGI.04/2, eGI.04/3) based on the gel image (*Figure 5A*). To determine the size and location of the deletions, PCR fragments were cloned into pGEM-T® Easy vector and sequenced. The resulting sequences were compared to the *GI.04* genome sequence in the Spud DB (*Figure 5B* and *C*). Deletions of different sizes (3-711 bp) and locations were found in the eGI.04 mutant lines (*Figure 5D*).

GI.04 protein consists of 1173 amino acids. A schematic drawing of the protein is shown in *Figure 5E*. By translating the nucleotide sequence of the mutants into amino acids, we showed that all but one allele of the deletions led to an early stop and truncation of the GI.04 protein (*Figure 5F*).

Three of the eGI.12 mutant lines carrying large deletions were also tested by PCR with gRNA primers. We found that two of the three mutant lines (eGI.12/1, eGI.12/2, eGI.12/3) were most likely null mutants, while the third line was assumed to be without mutations in all alleles (*Figure 6A*). Sizes and locations of deletions were determined in a similar way as for eGI.04. The resulting sequences were compared to the *GI.12* genomic sequence in the Spud DB (*Figure 6B* and *C*). As in the eGI.04 mutants, deletions of different sizes (between 2-197 bp) and locations were obtained in the eGI.12 mutants compared to the wild-type sequence (*Figure 6D*).

The original GI.12 protein consists of 1171 amino acids and is schematically shown in *Figure 6E*. Also, in eGI.12 mutants, deletions usually led to an early stop and truncation of the GI.12 protein, but there were also cases where after the deletion the original amino acid sequence was restored or where the deletion led to only a single amino acid difference (*Figure 6F*).

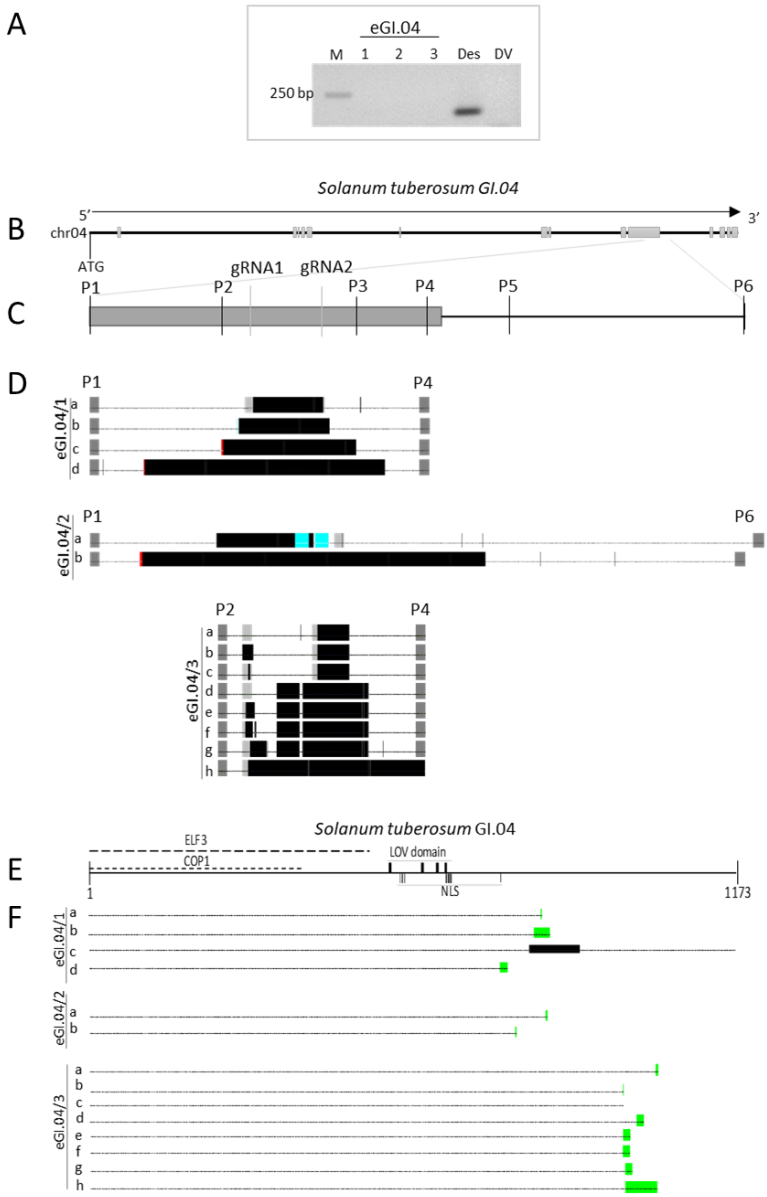

Figure 5: (**A**) Identification of eGI.04 mutants by PCR with primer pairs GI04 gRNA1 Fw and GI04 gRNA2 R. M, marker; 1, 2, 3, the selected mutant lines; Des, 'Désirée' control; DV, distilled water. (**B**) Wild type *GI.04*. chr04, chromosome 4; ATG, translation start codon; rectangles, exons (**C**) Enlarged drawing of a part of exon 10 with gRNAs and primers (P1-P6). (**D**) Location and size of deletions in the eGI.04 mutant lines. The deletions are indicated by the black rectangles, the vertical lines indicate a single bp difference. Primers are dark grey rectangles, gRNAs are light grey rectangles. In red, deletions acan be placed on either side, while in blue, duplicated sequence regions are highlighted; letters a-h indicate different alleles. At least one mutant allele in eGI.04/2 is not yet mapped. (**E**) Wild-type GI.04 with functional domains. The ELF3 (EARLY FLOWERING 3) and COP1 (CONSTITUTIVE PHOTOMORHOGENIC 1) binding domains are indicated by dashed

lines, the LOV domain (light-oxygen-voltage domain) and NLS (nucleus localisation signal) are indicated by black rectangles. The numbers indicate the first and last amino acids of the protein. (**F**) Proteins of the eGI.04 mutant lines. Black highlighting indicates the location and size of the deletion, green indicates the altered protein sequence, letters a-h indicate the different alleles.

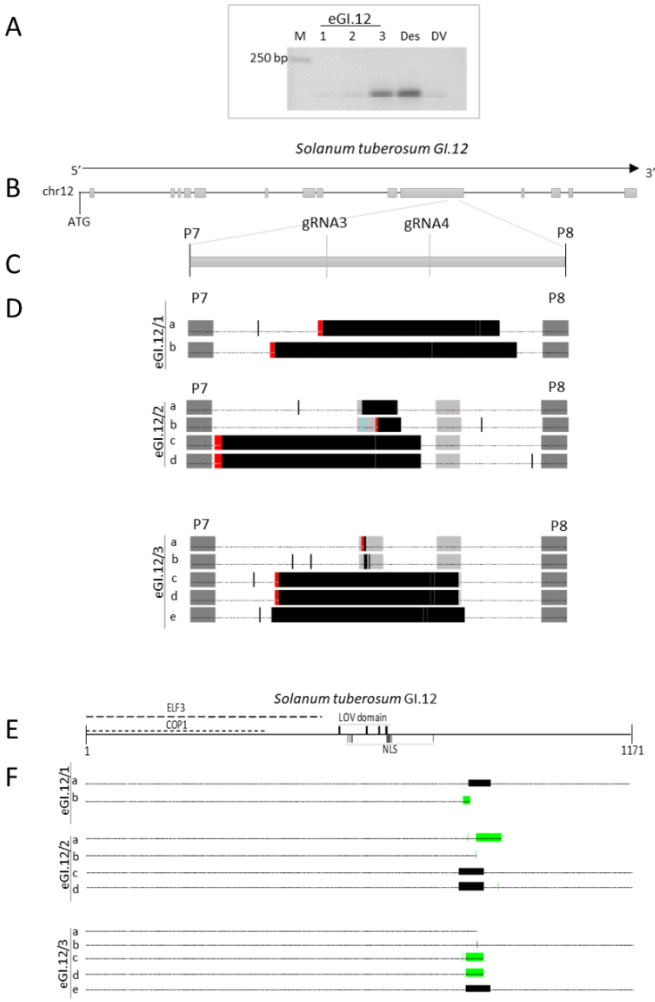

Figure 6: (**A**) Identification of eGI.12 mutants by PCR with GI12 gRNA1 Fw and GI12 gRNA2 R primers. Labels are identical to *Figure 5*. (**B**) Schematic drawing of the potato *GI.12* gene. Wild-type *GI.12*. chr12, chromosome 12 (**C**) Enlarged drawing of part of exon 10 with gRNAs and primers (P7 and P8). (**D**) Location and size of deletions in eGI.12 mutant lines. There is likely to be a wild-type allele in eG12/3 not shown in the figure. (**E**) Schematic drawing of wild-type and eGI.12 mutant proteins. (**F**) Proteins in eGI.12 mutant lines.

The phenotypic and tuber formation traits of eGI.04 and eGI.12 mutant plants were investigated in two experiments under greenhouse conditions. In both cases, untransformed 'Désirée' plants were used as controls.

None of the eGI.04 mutants differed significantly from the control in the earliness of tuber development. The number of tubers on eGI.04/1 and eGI.04/2 mutants was significantly less than on control plants in the first experiment, but in the second experiment the number of tubers on each mutant was not significantly different from the control. In the first experiment, no significant difference in tuber yield was obtained for either line, but in the second experiment, the tuber yield of the eGI.04/1 line was significantly lower than that of the control.

No significant difference in early and late tuber number was obtained from eGI.12 mutants in either line compared to the control. However, the eGI.12/2 mutant had significantly lower tuber yield than 'Désirée' in the first experiment. In the second experiment, not only eGI.12/2 but also eGI.12/3 had significantly lower tuber yields than the control.

As already mentioned, 'Désirée' is a red-skinned potato variety, the colour of which is due to the anthocyanins. Obviously, eGI.04/1 and eGI.04/2 tubers appeared darker than the tubers of 'Désirée', although to a different extent (*Figure 7A*). The eGI.04/3 tubers did not differ in colour from the control. The anthocyanin content measurement confirmed that the skin colour of eGI.04/1 tubers was indeed more intense than the control. However, the anthocyanin content of eGI.04/2 and eGI.04/3 tubers did not differ significantly from the control (*Figure 7B*). By eye, eGI.12/1 tubers appeared darker and eGI.12/2 tubers appeared lighter than 'Désirée' tubers (*Figure 7C*) and this was confirmed by the anthocyanin measurement (*Figure 7D*).

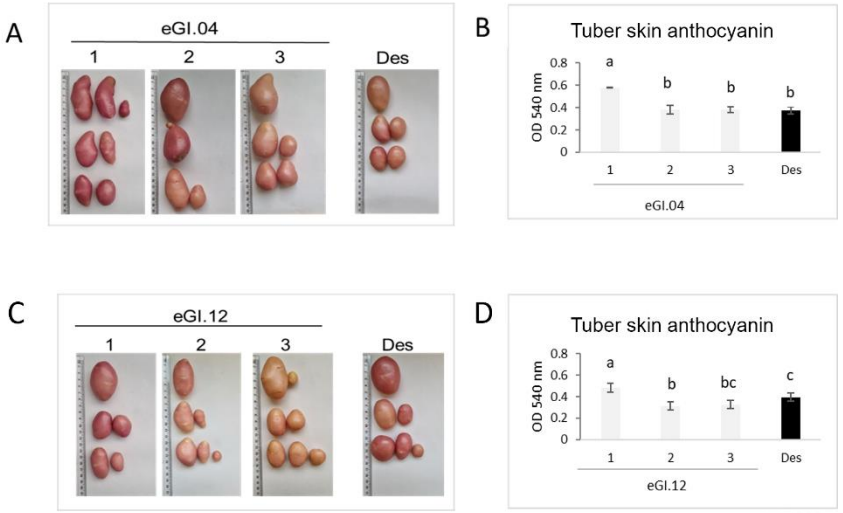

Figure 7: Tubers and anthocyanin content of eGI tuber skins compared to the control 'Désirée'. **(A**) Tubers of eGI.04 and 'Désirée'. (**B**) Anthocyanin content of eGI.04 tuber skins. (**C**) Tubers of eGI.12 and 'Désirée'. (**D**) Anthocyanin content of eGI.12 tuber skins.

Since the mutants obtained with gRNAs designed to the end of the *GI* genes of ´Désirée' showed no significant difference from the wild type, new mutants were generated. The so-called LOV domain is located in the middle of the GI protein, to which three photoreceptors, ZTL, LKP2 and FKF1, can bind (Kwon et al. 2022). We attempted to target this middle region and generate 'middle' (mGI) mutant lines. To do this, *GI.04* and *GI.12* specific gRNAs were designed, but also tried to hit both genes simultaneously by introducing the gRNAs into the ´Désirée' simultaneously using co-transformation. The resulting *GI.04* mutant lines were designated mGI.04, the *GI.12* mutant lines mGI.12 and the lines mutant for both *GIs* as mGI.4/12. Genomic DNA of 148 putative mutant plants was tested for mGI.04, 22 for mGI.12 and 108 for mGI.4/12. For mGI.04 and mGI.12, we focused only on mutants with mutations in all four alleles.

PCR testing of mGI.12 mutants with gRNA primers (*Figure 8A*) yielded 14 null mutants, of which three were selected for further analysis (mGI.12/1, mGI.12/2 and mGI.12/3) (*Figure 8B*).

Greenhouse experiments were initiated to study the phenotype and tuber formation of mGI.12 mutants. Untransformed 'Désirée' plants were planted as controls. Plant height was measured at the beginning of the growing season, 23 days after planting, and 10 days later. At both 23 days and 33 days after planting, mGI.12/2 and mGI12/3 mutant plants were significantly taller than the control 'Désirée' (*Figure 8C* and *D*). There was no significant difference in early or late tuber number or tuber yield between mGI.12 and control plants. The shape and colour of tubers did not differ from the control either.

At the end of the growing season, before harvesting the tubers, we saw that the mGI.12 mutants started to turn yellow and their leaves dried, while this was not yet observed in the 'Désirée' plants. The mGI.12 mutant lines thus showed signs of ageing earlier than the control plants (*Figure 8E*).

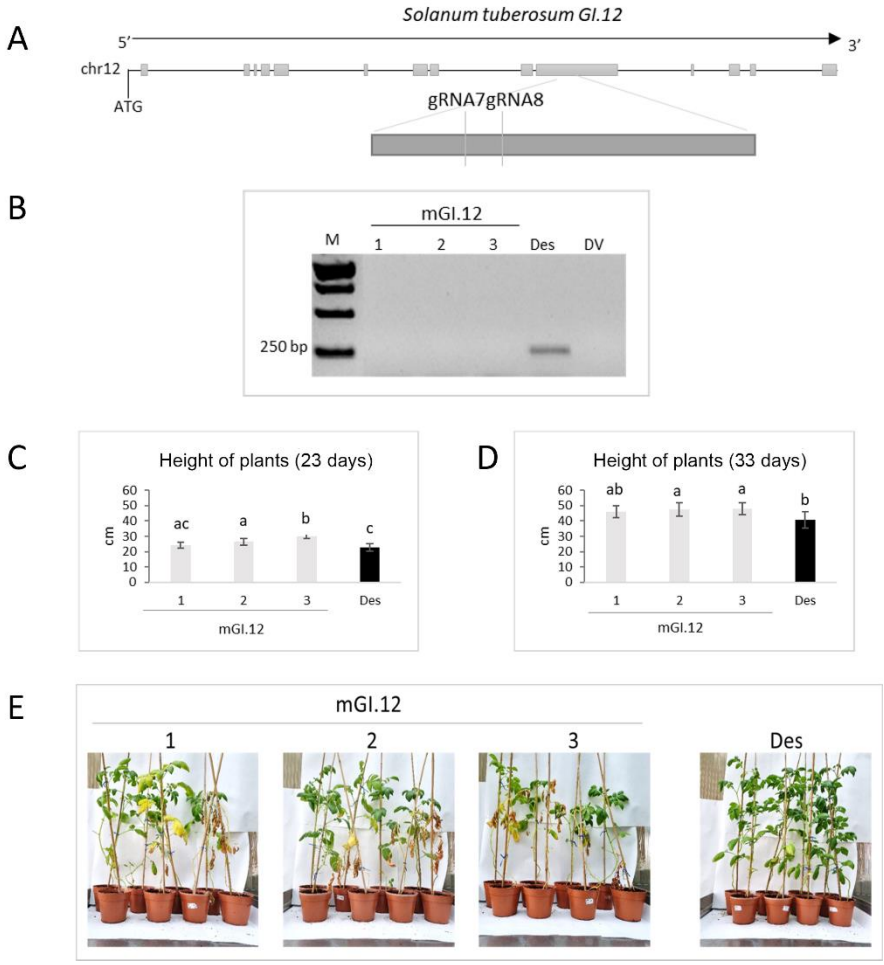

Figure 8: (**A**) Schematic drawing of the potato *GI.12* gene and enlarged image of part of exon 10 with gRNAs also used as primers. chr12, chromosome 12; ATG, translational start codon; rectangles, exons. (**B**) Identification of mGI.12 mutants by PCR with GI12 gRNAs and separation of PCR fragments on agarose gel. M, marker; numbers, selected mutant lines; Des, 'Désirée' control; DV, distilled water. (**C**) Height of mGI.12 plants compared with 'Désirée' control at 23 days after transplanting (**D**) and 33 days after transplanting. (**E**) Morphology of mGI.12 mutants at the end of the growing season compared with the untransformed 'Désirée' control.

3.8. Discussion of results obtained with GI mutant plants

To understand the role of *GI* genes in potato, we created several CRISPR/Cas9 constructs and introduced them into a commercial potato

cultivar, 'Désirée', and examined the effect of the mutations on plant development and tuber formation.

The CRISPR/Cas9 system was effective in producing *GI* mutants. For eGI.04, the frequency of occurrence of visible large deletions was 35%, while for eGI.12 mutants it was 21%. In the case of mGI lines, only null mutants were searched. Such mutants were obtained with a frequency of 2% in mGI.04, 63% in mGI.12, 5%, in mGI.4/12 for GI.04 and 15% for GI.12.

Tuber weight was different in some GI mutants compared to untransformed controls. Lines eGI.04/1, eGI.12/2 and eGI.12/3 had significantly lower tuber yields, while others showed no difference compared to the control. Notable among these is eGI.12/2, which gave the same result in both experiments performed, while for the others the lower tuber yield was not reproducible. The lower tuber yield of eGI.12/2 may be related to the size of the deletion, as it harbours two truncated proteins and two proteins in which several amino acids are deleted, but then the original amino acid sequence is restored. The eGI.12/1 also has at least one mutant allele similar to eGI.12/3, but the amino acid sequence of the other protein copies is not yet known precisely. The eGI.12/3 has a protein copy with only one amino acid missing. So it is possible that eGI.12/1 and eGI.12/3 are weaker mutants than eGI.12/2 and this may explain why they do not show a decrease in tuber yield under different greenhouse conditions. But it is also possible that eGI.12/2 has an "off target" mutation and that this causes the difference in tuber yield.

As mentioned several times, 'Désirée' is a red-skinned potato variety. The tuber skin of the antisense aGI lines has a lower anthocyanin content than the control 'Désirée'. Although to a different extent and not in all, we also observed changes in anthocyanin content in eGI mutants. The anthocyanin content of eGI.04/1 and eGI.12/1 tubers was significantly higher than that of the control, while that of eGI.12/2 was significantly lower than that of the control, for which we cannot yet provide an explanation.

The protein modifications in eGI mutants did not reach the LOV domain located in the middle of the GI protein, to which photoreceptors bind, nor the NLS sequences that allow the entry of the GI into the nucleus. Therefore, we designed new constructs targeting the middle region of GI (mGI mutants). Based on the first experiment, we found that the mGI.12 mutants grow slightly taller than the 'Désirée' and have a shorter life cycle than the control. However, they do not differ from the 'Désirée' control in terms of tuber setting time, tuber yield and tuber colour and shape. Mapping of deletions is ongoing.

4. CONCLUSSION AND RECOMMENDATIONS

The molecular regulation of tuber development initiation is known from experiments with the short-day tuberising *S. tuberosum* Andigena. The most prominent genes affecting tuber development are *SP6A*, *BEL5* and *CDF1*. The CDF1 plays a mediating role between circadian clock signals and the SP6A mobilization signal in the leaf. Indirectly, GI also plays a role in tuber formation by forming a complex with FKF1 to transport CDF1 to the proteasomes for degradation (Dutta et al. 2024). The aim of our experiments was to determine the role of CDF1 and GI in a day length independent tuberising cultivated potato cultivar, 'Désirée'.

To achieve this goal, we generated targeted mutations in *CDF1* and *GI*, of which two alleles were found in 'Désirée', one on chromosome 4 (*GI.04*) and one on chromosome 12 (*GI.12*). The mutants were generated using the CRISPR/Cas9 system. The method proved to be efficient, as we obtained mutant lines carrying large deletions with a frequency of 15% for *CDF1*, 35% for eGI.04 and 21% for eGI.12 with gRNAs designed to the 3' end of *GI.04* and *GI.12*, respectively. Of these, three eGI.04 mutants and two eGI.12 mutants were null mutants. Since we were able to isolate null mutants with gRNAs designed to the end of the *GI* genes, we only looked for null mutants among plants transformed with gRNAs designed to the middle of the *GI* genes (mGI.04 and mGI.12). Only a low frequency (2%) of such mutants was obtained from mGI.04. In contrast, the frequency of null mutants in mGI.12 was 64%. The large difference could be explained by the efficiency of the gRNAs, but it could be that *GI.04* plays a more important role in 'Désirée' than *GI.12*, so that, if the deletion reaches a certain point in *GI.04*, the plant is nonviable. Mutation mapping, which is ongoing, may bring us closer to understanding this frequency difference.

In our *CDF1* mutants, deletions were restricted to the 3' untranslated region. Although this did not significantly affect tuber development initiation time, tuber number and yield, we found differences in the stem thickness of the mutants, which were thinner than in controls, and also in delayed formation of compound leaves. Thus, we concluded that CDF1 plays a role in shoot development in 'Désirée'. In two of the three mutants tested, the dormancy period of the tubers was longer and the tubers had smaller sprouts than the control when planted. Thus, consistent with this, there was a delay in the development of shoots from the tubers. The breakdown of starch, the availability of sucrose, is a prerequisite for germination. Another important factor is the change in meristem activity, which plays a key role in germination. The reactivation of meristematic function coincides with the end of dormancy (Ferreira et al. 2017). Therefore, it would be worthwhile to

analyse CDF1 mutant tubers at the metabolite level and to investigate the transcriptional level of genes associated with meristematic activity.

The two *GI* genes, *GI.04* and *GI.12*, showed differences in both promoter and coding regions, but also many similarities. For example, we found the REV1/8 binding site, which can be linked to circadian regulation, in the promoter of both *GI* genes. This suggests that the expression of the two *GI* genes of 'Désirée' may be linked to the daily cycle, as is the transcription of the *GI* genes of *S. tuberosum* Andigena and Neo-Tuberosum (Morris et al. 2014). This would be worthy of experimental demonstration.

A transcriptome analysis of the leaves of aGI52, a line with 50% inhibition of *GI.04* expression, showed that inhibition of *GI.04* induces an increase in *REV1* expression. Our results thus show that, similar to other plant species (Liu et al. 2024), GI is involved in the regulation of the circadian clock in potato and has a negative effect on *REV1* expression, which in turn reverts the transcription of both *GIs*. The binding of REV1 to *GI* promoters could be investigated in a yeast 1-hybrid system, and the putative interaction could be demonstrated by generating *REV1*-inhibited antisense lines and examining the expression pattern of *GI* genes in these lines.

PlantRegMap predicted flowering-related TF binding sites in both *GI* promoters (e.g. SOC1, ABI5) and inhibition of *GI.04* expression also altered the levels of 7 flowering-related TF mRNAs in leaves. This suggests that, like in other plant species (Liu et al. 2024), GI is involved in the regulation of flower development in potato. Flowering-related experiments performed in the future that could prove or disprove this hypothesis would be worthwhile.

Odgerel (2022) found that the two *GI* genes respond differently to stress. While *GI.04* expression is activated by heat, cold and osmotic stresses, *GI.12* expression is elevated only by ABA, and is inhibited by abiotic stresses. One explanation for this may be that the promoter of *GI.04* has MYB TF binding sites, whereas *GI.12* does not. Although binding sites for ABA-responsive TFs were found in both promoters, *GI.04* is not ABA-responsive. Stress-related experiments designed with GI mutants may help to understand the stress responses of both *GI* genes.

We also identified POTH20 TF binding sites in the promoter of *GI* genes. POTH20 is 73% identical to POTH1 (Rosin et al. 2003). POTH1 also regulates the transcription of *BEL5* itself and *GA2-OXIDASE 1* by forming a heterodimer with BEL5, thereby affecting tuber formation (Lin et al. 2013). The question is whether POTH20 can substitute for POTH1 and indeed bind to the promoter of *GI* genes. Yeast hybrid systems could be used to answer this question.

So far, neither *CDF1* nor the *GI* mutants tested so far have shown any change in the timing of tuber initiation. However, this does not exclude the possibility that they may play a role in the initiation of tuber development, because *CDF1* deletions are outside the coding region, and in eGI mutants, they may not reach the region important for protein function. However, considering that mGI.12 mutants are not altered in the timing of tuber development initiation, we hypothesize that CDF1, and in association with GI, is not as important for tuber development in the daylength-independent tuberising 'Désirée' as in the *S. tuberosum* Andigena landscape cultivar that develops tubers only under short-day conditions. However, to confirm this, we need to continue the mapping of deletions in mGI mutants and test mGI.04 mutants under greenhouse conditions.

5. NEW SCIENTIFIC RESULTS

- We successfully applied the CRISPR/Cas9 genome editing system to the targeted mutagenesis of the *CDF1*, *GI.04* and *GI.12* genes in the cultivated potato variety 'Désirée'.

- In the gCDF1 mutants, the deletions caused a significant change in the structure of the 3' end of the *CDF1* mRNA, and we found that *CDF1* plays a role in shoot development in 'Désirée'.

- In 'Désirée', we found two *GI* genes (*GI.04*, *GI.12*), which show many similarities, but also differences in both the promoter and coding regions.

- From the elements present in the promoters of both *GI* genes, we deduced that the expression of the two *GI* genes of 'Désirée' can be linked to the daily cycle and that the *GI* genes have a role in the initiation of flowering and in stress responses, which was confirmed by the differential expression of TFs related to flowering and stress in a line silenced in *GI.04* expression compared to 'Désirée'.

- We created null mutant lines carrying deletions near the 3' end of *GI.04* and *GI.12*. The deletions were delimited at the DNA sequence level and it was established that the deletions at the 3' end of the *GI* genes have no effect on the initiation of tuber development.

- We created null mutants that carry deletions in the middle of *GI.04* and *GI.12*, and also those that are null mutants for one gene and carry large deletion(s) in the other gene.

- We showed that mutants carrying a deletion in the central part of *GI.12* are taller than 'Désirée', have shorter life cycles, but do not differ from it in terms of the time of tuber development.

6. AUTHOR'S PUBLICATIONS IN THE FIELD OF STUDY

Articles:

KARSAI-REKTENWALD, F., ODGEREL, K., JOSE, J., BÁNFALVI, Z. (2022): *In silico* characterization and expression analysis of *GIGANTEA* genes in potato. Biochemical Genetics, 60(6):2137-2154.

ODGEREL, K., JOSE, J., **KARSAI-REKTENWALD, F**., FICZEK, G., SIMON, G., VÉGVÁRI, GY., BÁNFALVI, Z. (2022): Effects of the repression of *GIGANTEA* gene *StGI.04* on the potato leaf transcriptome and the anthocyanin content of tuber skin. BMC Plant Biology, 22:249.

KARSAI-REKTENWALd, F., ODGEREL, K., GYULA, P., BÁNFALVI, Zs. (2023): A *CYCLING DOF FACTOR 1* (*CDF1*) gén szerepe a 'Désirée' burgonyafajtában. Kertgazdaság, 55(2023)2.

Conference publications:

KARSAI-REKTENWALD, F., ODGEREL, K., JOSE, J. (2021): Regulation of *GIGANTEA* genes in potato. In: Scientific Conference of PhD Students of FAFR, FBFS and FHLE SUA in Nitra with international participation. Proceedings of abstracts on occasion of the Science and Technology Week in the Slovak Republic. November 10, Nitra, Slovak Republic, Abstract pg. 14.

VILLÁNYI, V., ODGEREL, K., **KARSAI-REKTENWALD, F**., BÁNFALVI, Zs. (2021): Az oltvány hatása a burgonya koraiságára és a gumók metabolit összetételére. XVII. Növénynemesítési Tudományos Napok, Martonvásár, március 24-25. (ISBN 978-963-8351-48-7)

KARSAI-REKTENWALD, F., ODGEREL, K., JOSE, J., BÁNFALVI, Z. (2022): Regulation and function of the *GIGANTEA* gene *StGI.04* in potato. Fiatal Biotechnológusok Országos Konferenciája, Gödöllő, április 11-12. (ISBN 978-963-269-999-8)

KARSAI-REKTENWALD, F., ODGEREL, K., JOSE, J., BÁNFALVI, Zs. (2022): A *GIGANTEA* gének szerepe és szabályozása burgonyában. XVIII. Növénynemesítési Tudományos Napok, Keszthely, május 11-12. (ISBN 978-963-269-987-5)

BÁNFALVI, Z., **KARSAI-REKTENWALD, F**., ODGEREL, K., JOSE, J. (2022): Regulation and function of *GIGANTEA* genes in *Solanum tuberosum* cultivar 'Désirée'. Book of Abstracts, 4th International Conference on Plant

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