Molecular studies on early tuber bulking in potato

The aim of the project was in studying the molecular mechanism of tuberisation to promote breeding early tuberising, high quality potatoes. To achieve this goal, four different sets of experiments were performed.

1. Metabolite analysis of tubers of grafted plants

Maturity is an important breeding trait because early-maturing potato cultivars complete their life cycle before stress becomes a serious constraint and, therefore, are more profitable for growers. Early tuberisation, however, may affect the quality of tubers. To test this assumption, in the first study, two Hungarian potato cultivars, 'Hópehely' (HP) and 'White Lady' (WL) with contrasting tuber metabolite compositions and differing in time of tuber initiation were homo- and hetero-grafted, and the effects of grafting investigated in comparison to non-grafted controls. A positive correlation between the growth rate of the leaves and the time of tuber initiation was detected. The time of tuber initiation was delayed in the WL rootstocks by HP scions and shortened in the HP rootstocks by WL scions supporting the previous finding that tuberisation is triggered by source-derived mobile signal. To unravel the influence of vegetative organs on the primary polar metabolite content of tubers and the effect of tuberisation on the metabolite content of leaves non-targeted metabolite analysis using gas chromatography-mass spectrometry (GC-MS) was performed. A total of 31 polar metabolites were identified in the extracts. No significant influence of grafting on the metabolite composition of tubers, including sucrose, the major polar metabolite in tubers was detected, whereas the galactinol concentrations in leaves were slightly changed in hetero-grafts. Galactinol is a precursor of galactosyl-sucrose oligosaccharides such as raffinose and stachyose. Since the direction of change in galactinol concentration was opposite with the sucrose content of tubers we speculated that there is a relation between the two phenomenon, i.e. to maintain the sucrose concentration characteristic for the the rootstock tubers less or more amounts of galactosyl-sucrose oligosaccharides are synthesised resulting in an increase or decrease of galactinol level in leaves.

In the second study, a very early- and a very late-maturing potato line, CE3130 and CE3027, respectively, were homo- and hetero-grafted. Four-week-difference in the time of tuber initiation between the two lines was detected under our greenhouse conditions, which was shortened by the CE3130 scions and delayed by the CE3027 scions in heterografts with approximately three weeks, while the phenotype and flowering time of scions were not altered indicating that there is no genetically determined signal arising from the rootstock, which could influence the scion's flowering characteristics. The tubers were harvested at the end of the scions' vegetation period and metabolite profiling was performed using GC-MS. Ninety-nine metabolites were identified and an additional 181 peaks detected in chromatograms, out of which 186 were polar and 94 non-polar compounds. The concentrations of 113 metabolites were significantly different in the tubers from CE3130 and CE3027. Hetero-grafting resulted in considerable changes in the metabolite content of tubers. Especially, the effect of CE3027 on the metabolite composition of tubers formed on CE3130 rootstocks was readily apparent. Nevertheless, 29 compounds were present at similar levels in the tubers of hetero-grafted plants as was found in the tubers of their scion counterparts suggesting that these compounds are transported from the source leaves to tubers.

Publications:

Odgerel K, Bánfalvi Z* (2021) Metabolite analysis of tubers and leaves of two potato cultivars and their grafts. PLoS One, 16:e0250858. doi.org/10.1371/journal.pone.0250858. IF: 3.240, Q1

Villányi V, Gondor OK, Bánfalvi Z* (2022) Metabolite profiling of tubers of an early- and a late-maturing potato line and their grafts. Metabolomics 18:88. doi.org/10.1007/s11306-022-01950-3. IF: 4.747, Q2

2. Studying the regulation and function of the *GIGANTEA* genes in the cultivated potato 'Désirée'

GIGANTEA (*GI*) is a plant-specific gene encoding a nuclear protein with diverse functions identified in many plant species (e.g., flowering time regulation, control of circadian rhythm, stress responses, etc.). In the wild Andean landrace *Solanum tuberosum* Group Andigena, a strict short-day plant for tuberisation, it was demonstrated that GI, as a part of a complex, is an indirect repressor of tuberisation. Nevertheless, tuberisation of the commercial potato cultivars is day-length independent. To get information on the function of GI in commercial potatoes we searched for *Arabidopsis GI* homologues in the potato genome sequence database and identified two *GI* copies located on chromosomes 4 and 12 (*StGI.04* and *StGI.12*). The *GI* promoter regions of the commercial potato cultivar 'Désirée' (DES) were cloned and sequenced. The PlantRegMap data analysis platform predicted more than ten transcription factor families binding to the *StGI* promoters including those involved in flowering, circadian clock regulation and abscisic acid (ABA) response and a binding site for the POTATO HOMEOBOX 20, which is presumably involved in tuber initiation. However, the locations of *cis*-acting regulatory elements as well as the organ specific expression and responses of the *StGI* genes to abiotic stresses and ABA were different. Thus, we hypothesised that the function of *StGI.04* and *StGI.12* are at least partially different.

To test this hypothesis we started the functional analysis on *StGI.04* using the technique of antisense repression of gene expression. Twenty *StGI.04*-repressed DES lines were obtained. The highest level of repression reached around 50%. Nevertheless, this level did not influence tuber formation and yield but did cause a reduction in tuber colour. DES is a red-skinned potato whose skin colour is determined by anthocyanins. Thus, we concluded that StGI.04 affects the anthocyanin content of tuber skin. To explore the effects of StGI.04 repression on the leaf transcription profile RNA-seq analysis was performed with a selected antisense line. It was found that, as AtGI in *Arabidopsis*, StGI.04 influences the expression of the key genes of the circadian clock, flowering, starch synthesis and stress responses in potato. Anthocyanins are synthesized through a branch of the phenylpropanoid pathway and we found that the expression of *PHENYLALANINE AMMONIA LYASE* (*PAL*), the *LEUCOANTHOCYANIDIN OXIDISING* enzyme gene *LDOX*, and the *MYB-RELATED PROTEIN Hv1* (*MYB-Hv1*), a transcription factor coding gene, which is presumably involved in the regulation of flavonoid biosynthesis, are down-regulated in the leaves of *StGI.04*-repressed plants. In sum, besides conserved functions, we detected a novel function of a *GI* gene in influencing the anthocyanin synthesis and potato tuber skin colour.

In order to get information on the function of *StGI.12*, in comparison with *StGI.04*, mutant plants were generated using the CRISPR/Cas9 gene-editing system. Two gRNAs, whose target sequences are located in an approximately 100-bp distance to each other were designed to the less similar region of the two genes, cloned into a gene-editing vector and introduced into DES. Using primer pairs surrounding the two gRNAs large deletions were detected in *StGI.04* in 29 out of 82 and in *StGI.12* in 18 out of 86 transgenic DES plants tested with PCR. Cultivated potatoes, including DES, are tetraploids. Thus, it is difficult to obtain null mutants in T_0 generation. However, we were lucky enough to obtain four null mutants in each *StGI* gene. These null mutants with one additional biallelic mutant line from each mutagenesis were tested in the greenhouse. No significant alteration in tuberisation was detected except that the anthocyanin content of the tuber skin in one of the *StGI.04* and one of the *StGI.12* null mutants was higher, while in the *StGI.04* biallelic mutant was lower than in DES. The GI proteins are in direct interaction with several other proteins. The known binding sites are mainly at the N-terminal part of GI, while our targeted region was located at the C terminal. Thus, we concluded that the generated mutations may not influence the GI functions and started a new experiment to isolate mutants with deletions closer to the C-terminal region of the GI protein.

Publications:

Karsai-Rektenwald F, Odgerel K, Jose J, Bánfalvi Z* (2022) *In silico* characterization and expression analysis of *GIGANTEA* genes in potato. Biochem Genet 60:2137–2154. doi.org/10.1007/s10528-022-10214-7
IF: 1.89, Q3

Odgerel K, Jose J, Karsai-Rektenwald F, Ficzek G, Simon G, Végvári G, 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 Biol 22:249 doi.org/10.1186/s12870-022-03636-3. IF: 4.215, D1

3. Studying the function of the *CYCLING DOF FACTOR 1* gene in the cultivated potato 'Désirée'

It was demonstrated that in the wild Andean landrace *Solanum tuberosum* Group Andigena, the CYCLING DOF FACTOR 1 (StCDF1) is an indirect inducer of the transcription of SELF-PRUNING 6A (SP6A), a mobile tuberisation signal. It was also demonstrated that the StGI complex interacts with StCDF1 and targets it for degradation by the proteasome. Earliness of the diploid potato line CE3130 is based on the lack of the interaction of StCDF1 with the StGI complex due to a deletion in the 3' end of the *StCDF1* gene compared to the *StCDF1* gene of the late tuberising line CE3027. We found that DES possesses *StCDF1* in a single copy with a sequence identical with *StCDF1* in CE3027 in its 3' end. Thus, we speculated that by getting a deletion in the 3' end of DES *StCDF1* we can probably increase the earliness of tuber initiation in a commercial potato cultivar.

The CRISPR/Cas9 system with two gRNAs was used for mutagenesis. *StCDF1* is a member of a gene family and it was impossible to find gRNAs specific for the 3' coding region. Therefore, we decided to target the 3' UTR of the gene expecting that the size of the deletions will be large enough to reach the 3' end of the coding sequence. After DES transformation

and regeneration, 75 putative transgenic plants were tested with PCR. Eight mutants were obtained with deletions ranging from 21 to 251 bp. Unfortunately, however, the deletion did not reach the coding region in any of them and none of them were null mutants. Still, based on the number of the mutated alleles and the size of the mutations, three lines were selected for a greenhouse test. We found that the development of composite leaves was delayed and the stems of all three lines were thinner than the stems of the control plants. Two mutants were shorter than the control at the beginning of the growth period. However, by the end of the vegetation period there was no significant difference between the height of the mutant and DES plants. The mutations did not change the time of tuber initiation compared to the control. At the end of the vegetation period, however, significantly more, but smaller tubers were formed in one the *StCDF1* mutant lines, whereas the tuber yield of another one was 20% higher than that of the control plants. No correlation between the size and number of the deletions and the tuberisation characteristics of the mutants was detected. Thus, the influence of off-target mutations on tuber formation cannot be excluded.

Publication:

Karsai-Rektenwald F, Odgerel K, Gyulai P, Bánfalvi Zs* (2023) A *CYCLING DOF FACTOR 1* (*CDF1*) gén szerepe a 'Désirée' burgonyafajtában. Kertgazdaság (manuscript in preparation)

4. Studying the regulation and function of the *BIG BROTHER* gene in the cultivated potato 'Désirée'

Grafting experiments between HP and WL resulted in detection of a positive correlation between the growth rate of the leaves and the time of tuber initiation (see section No.1). It is known that the ubiquitin E3 ligase *BIG BROTHER/ENHANCER OF DA1* (*BB*) gene encoding a RING finger protein is a central growth regulator in *Arabidopsis* as it restricts cell proliferation and promotes leaf senescence. Supposing that the *StBB* gene has a similar role in potato we aimed to study the regulation and function of *StBB* gene in potato.

The promoter region of *StBB* was analysed *in silico* and the level of *StBB* expression was studied in different organs of DES by RT-qPCR. A total of 48 binding sites for 15 transcription factor (TF) families were predicted. Most of them were located in the -1.5-kb promoter region. The dominating family of TFs was DOF. It was found that 20 out of the 24 TFs with known functions are involved in developmental processes such as for example, the flower-, leaf-, stem- and root development or cell cycle regulation. In line with this finding, *StBB* mRNA was detected in each organ tested with the largest amounts in petal and stamen. These results suggested a similar function of StBB in potato than that is of AtBB in *Arabidopsis*.

To test the pertinence of this assumption DES lines with antisense repression of *StBB* expression were generated (aBB lines). In three independent aBB lines, an approximately 30% reduction in *StBB* mRNA level was detected when the plants were grown in *in vitro* culture. In the first greenhouse test, the aBB plants grew faster and became 10-20% higher than DES. The yield of aBB plants was doubled compared to DES. This result, however, could not be repeated. Moreover, no significant reduction in *StBB* expression of greenhouse-grown plants was detected. The reason of the opposite results obtained in the first and the subsequent tests is unknown.

Publication:

Odgerel K*, Bánfalvi Z (2022) *In silico* promoter analysis and expression of the *BIG BROTHER* gene in different organs of potato. Columella - Journal of Agricultural and Environmental Sciences 9:31-41 doi.org/10.18380/SZIE.COLUM.2022.9.1.31