THE THESIS OF THE PH.D. DISSERTATION

Khongorzul Odgerel

Gödöllő
2022

Hungarian University of Agriculture and Life Sciences-MATE

Molecular genetics and metabolomic studies related to tuberization and metabolite composition of cultivated potatoes

Khongorzul Odgerel

Gödöllő
2022

Doctoral School: Doctoral School of Plant Science

Name: Khongorzul Odgerel

Discipline: Plant biotechnology

Head of Doctoral School: Lajos Helyes DSc
Professor, Doctor of the Hungarian Academy of Sciences
MATE, Director General of Gödöllő Campus

Supervisor: Zsófia Bánfalvi DSc
Group leader, Doctor of the Hungarian Academy of Sciences
MATE, Institute of Genetics and Biotechnology

Co-supervisor: Anikó Veres Dr
Doctor, Associate Professor
MATE, Doctoral School of Plant Science

....

Approval of School Leader Approval of Supervisor

.....

Approval of Co-supervisor

TABLE OF CONTENTS

1 BACKGROUND OF THE WORK AND ITS AIMS

Potato (*Solanum tuberosum* L.) is one of the most consumed crops in the world in accordance with its nutritional status. With increasing demand, sustainable productivity is becoming a challenging issue of potato breeding because potato yield is limited in various environmental conditions by its sensitivity to biotic and abiotic stresses such as pests, elevated temperature, drought, frost and salinity. Thus, early tuberizing potato cultivars are more profitable because early bulking potatoes can complete their life cycle before stress becomes a serious constraint.

The tuberization process of potato takes place alongside with other growth or developmental processes in the plant such as foliage development, leaf expansion, flowering and sprout development. It has been known that tuberization is triggered by mobile signals that are raised in the canopy as a source organ and transported to the tuber as a sink organ. It has not been known, however, how the canopy as a source can influence the metabolite composition of tubers and vice versa; does the developing tuber influence the metabolite composition of the source, the mature leaves? Thus, the first aim of our study was to answer these questions.

In *Arabidopsis* and other plant species, ubiquitous genes *GIGANTEA* (*GI*) and *BIG BROTHER (BB)* are known to be involved in various processes. *GI* has a conserved function in terms of flowering time determination and circadian clock regulation and has been known as an indirect repressor of tuberization in potato. The *BB* gene is referred as an ENHANCER1 OF DA1 (EOD1) which restricts cell proliferation and represses organ growth. Based on their functions repression of *GI* and *BB* expression in potato was considered to be a promising approach to influence, in a positive way, the tuberization in potato.

Based on the above described assumptions the following research objectives were defined:

1. Unravel the influence of vegetative organs on the primary polar metabolite composition of potato tubers
2. Unravel the effect of tuberization on the primary polar metabolite composition of leaves
3. Characterize the *GIGANTEA* (*StGI*) and *BIG BROTHER* (*StBB*) genes in *Solanum tuberosum* L. cv. 'Désirée'

- study the organ-specific expression of the two *StGI* genes, *StGI.04* and *StGI.12*, and *StBB*
- study the effect of stress treatments on the expression of the two *StGI* genes, *StGI.04* and *StGI.12*, and *StBB*
- repress the expression of *StGI.04* and *StBB*
- evaluate the effect of *StGI.04* and *StBB* repression on plant morphology and tuberization.

2 MATERIALS AND METHODS

2.1 Materials

Table 1. Plant materials used for the experiments

№	Potato variety
1.	*Solanum tuberosum* L. cv. 'Désirée'
2.	*Solanum tuberosum* L. cv. 'White Lady'
3.	*Solanum tuberosum* L. cv. 'Hópehely'
4.	aGI lines – *GI.04*-repressed derivatives of *S. tuberosum* L. cv. 'Désirée'
5.	aBB lines – *BB*-repressed derivatives of *S. tuberosum* L. cv. 'Désirée'

For plant culture Murashige-Skoog medium (MS), rooting medium (RM), callus induction medium (CIM) and shoot induction medium (SIM) were used (MURASHIGE and SKOOG 1962). LB, SOC and YEB media with appropriate antibiotics were used for the growth of bacteria (SAMBROOK et al. 1989). The bacterial strains *Agrobacterium tumefaciens* LBA4404 and *Escherichia coli* DH5α and the vectors pCP60, pGEM-T Easy and pRK2013 were used for cloning and mobilisation of a *StBB* fragment into *Agrobacterium* for transformation of potato leaves as described by DIETZE et al. (1995). PCR primers, enzymes and kits were used according to the manufacturers' instructions.

2.2 Methods

Methods used for studying the tuberization and metabolite composition of cultivated potatoes are shown in Figure 1.

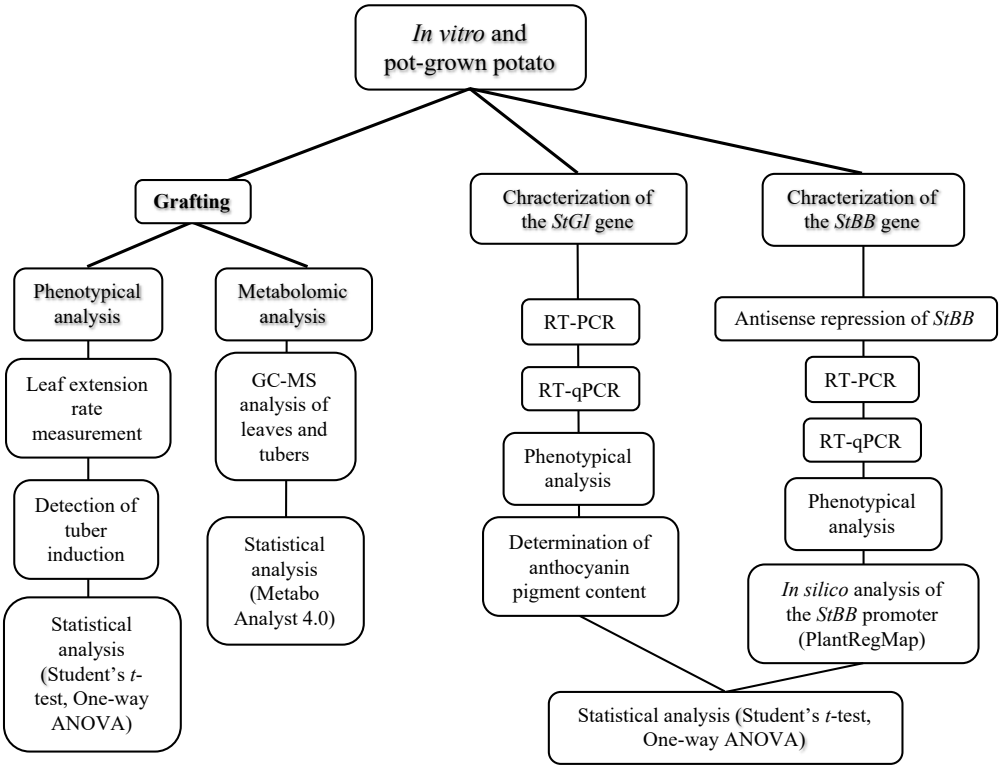

Figure 1. General scheme of the methods used in the experiments

3 RESULTS AND DISCUSSION

3.1. Metabolite analysis of tubers and leaves of two potato cultivars and their grafts

Grafting experiments were carried out with two Hungarian potato cultivars, ‘Hópehely’ (HP) and ‘White Lady’ (WL), which have different tuber morphologies. The grafting success was 64–85%. Six weeks after grafting, the number of tubers was counted and the canopy development of the plants was estimated by measuring the area of the five largest leaves of the plants. A positive correlation (R^2 = 0.6947) between the leaf growth rate and the number of tubers formed on plants was detected (Fig. 2). There was no significant difference in the average number of tubers per pot, tuber yield and tuber mass between the HP and WL plants, and the values were not changed by grafting.

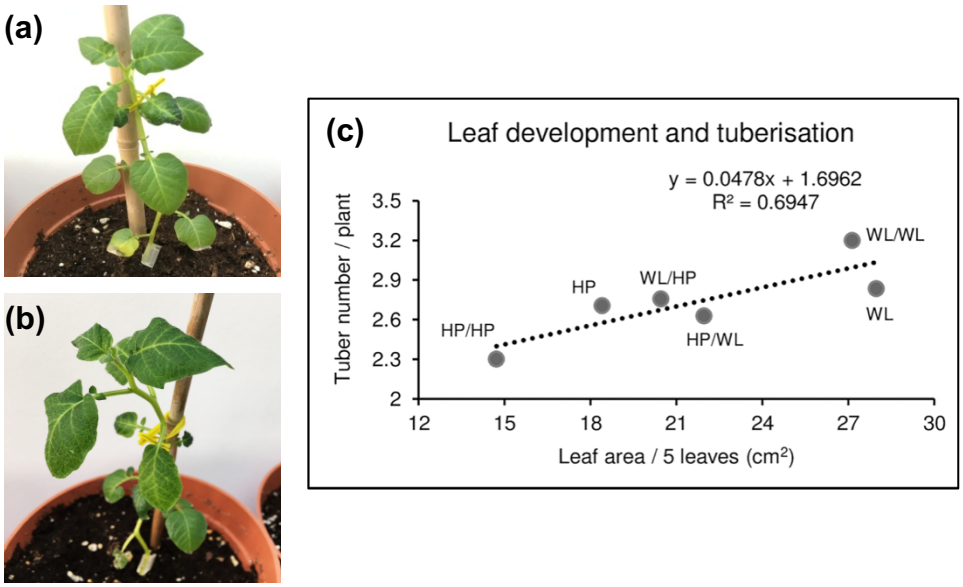

Figure 2. Grafting of two cultivars. (a) ‘White Lady’ (WL) and (b) ‘Hópehely’ (HP). (c) Correlation between leaf size and tuberization six weeks after grafting. The area of the five largest leaves per plant was measured. WL and HP, non-grafted controls; WL/WL homo-grafted WL; HP/HP, homo-grafted HP; WL/HP, hetero-graft: WL scion/HP rootstock; HP/WL, hetero-graft: HP scion/WL rootstock.

To investigate the influence of grafting on the metabolite composition of leaves and tubers, metabolite profiling was carried out using GC-MS. A total of 31 polar metabolites were identified in the leaf and tuber extracts, including amino acids, sugars, sugar alcohols, organic acids and an inorganic acid. The major compounds were sugars and malic acid in leaves and sugars and citric acid in tubers.

Principal component analysis (PCA) of the HP and WL leaf and tuber metabolite data revealed distinct profiles causing samples to cluster based on genotype. The hetero-grafting did not substantially change the metabolome of the leaves and tubers. Nevertheless, compared to the homo-grafted plants, the hetero-rootstocks and hetero-scions evoked some differences in the leaves and tubers, respectively. To determine which compounds caused the major differences between the leaf and tubers, variable importance projection (VIP) plots by partial least squares-discriminant analysis (PLS-DA) were utilized. Four compounds had a VIP score higher than 1.5 (galactinol, glutamic acid, mannitol and isoleucine) in leaves (Fig. 3a), while five compounds had a VIP score higher than 1.5 (sucrose, galactinol, pentonic acid, galacturonic acid and lactulose) in tubers (Fig. 3b).

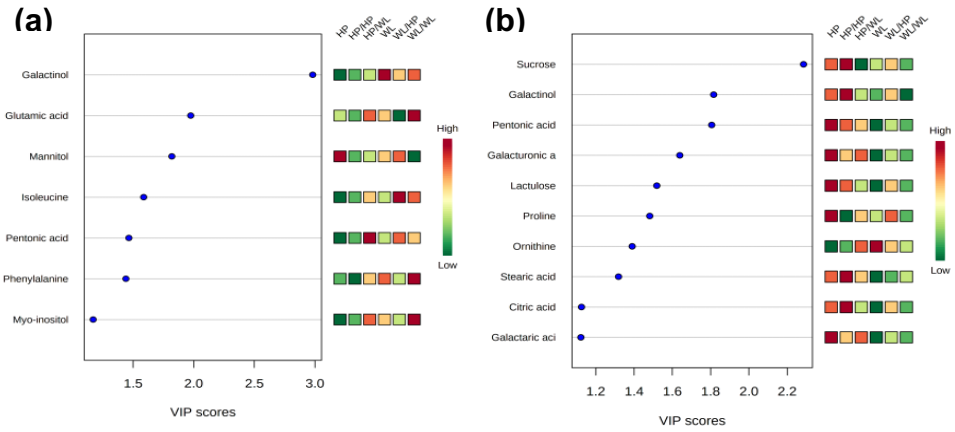

Figure 3. VIP plots calculated from PLS-DA showing the metabolite differences in the (a) leaves and (b) tubers. The plots indicate the major differences between non-, homo- and hetero-grafted plants. The labels are as in Fig. 2.

To gain insight into the effect of grafting on the concentrations of the metabolites which were highlighted as the most important features separating the different plant categories (i.e., those compounds with VIP score >1.5), bar graphs were prepared for each compound. In the leaves, galactinol showed a tendency of grafting-dependent changes (Fig. 4a). Galactinol is formed from UDP-galactose and myo-inositol. Galactosyl-sucrose oligosaccharides, as for example raffinose and stachyose, are synthesised from galactinol and sucrose and from galactinol and raffinose, respectively, producing myo-inositol as a by-product (SENGUPTA et al. 2015). The level of myo-inositol was similar in the leaves of all plants, while the amounts of raffinose and stachyose were under the detection level. Thus, we suppose that the different galactinol concentrations in different plant categories are rather related to differences in the rate of catabolism or phloem transport from source to sink organ than to galactinol or galactinol-derived oligosaccharide synthesis.

There was a 2.7-fold difference between the sucrose content of the HP and WL tubers and a 3.0-fold difference between the sucrose content of the HP/HP and WL/WL tubers, and these differences were not changed significantly by hetero-grafting (Fig. 4b).

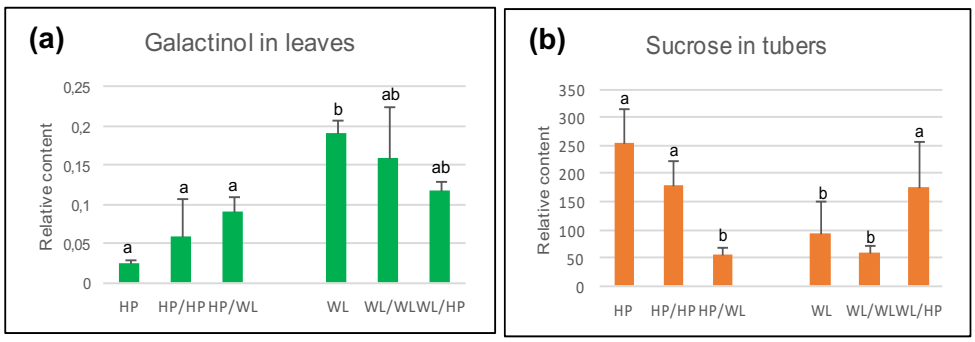

Figure 4. Differences in the galactinol and sucrose contents of (a) leaves and (b) tubers, respectively, detected by GC-MS; the relative data shown on the *Y*-axis are derived from a comparison of the peak sizes of the samples and the internal standard, ribitol.

Sugar transport between source and sink tissues has been excessively studied and found that it is mainly facilitated by the translocation of sucrose molecules (FERNIE et al. 2002). Several experiments showed that by manipulating the expression level of enzymes involved in starch synthesis the sucrose and glucose contents of tubers can be strongly influenced (MÜLLER-RÖBER et al. 1992; TAUBERGER et al. 2000; TRETHEWEY et al. 2001; HAJIREZAEI et al. 2003; JUNKER et al. 2006). In those experiments, however, expression of target genes was manipulated by expression of a suitable construct driven by the *CaMV35S*, *PATATIN* or *rolC* promoter in the whole plant or in a tuber- or vascular tissue specific manner, respectively. In contrast, in our experiments, only natural signals derived from scion could influence gene expression and/or metabolite pathway regulation in rootstock and vice versa. Since no significant alterations in sucrose concentrations of tubers developed on hetero-grafted compared to homo-grafted plants were detected it was concluded that the sucrose content of HP and WL tubers is genetically determined.

3.2. Characterisation of the GIGANTEA (GI) genes in potato

Two transcript variants of *GIGANTEA* gene were found in *S. tuberosum* Group Phureja (*StGI.04* and *StGI.12*). Expression of *StGI.04* and *StGI.12* were tested in different organs of potato using gene-specific primers. *StGI.04* mRNA was detected in each tested organ with the highest levels in root, stolon and sepal while *StGI.12* was expressed at relatively high levels in root, tuber and sink leaves, but little or no expression was detected in flower organs (Fig. 5). In general, the level of *StGI.12* expression was higher than that of *StGI.04*. In sum, these results indicated that the expression pattern of the two *StGI* genes is unique and organ-specific. Considering the results of a previous work (KARSAI-REKTENWALD et al. 2022) we speculate that the higher activity of *StGI.12* may be explained by the higher proximity of *cis*-

acting regulatory elements in the *StGI.12* promoter with the core promoter region than in the *StGI.04* promoter.

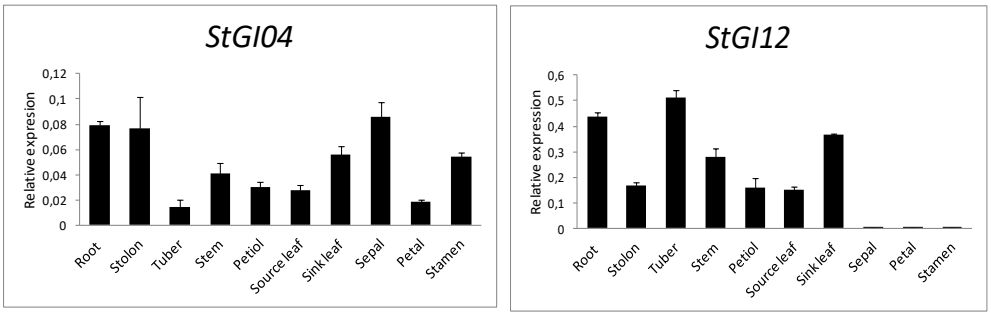

Figure 5. Organ-specific expression of *StGI.04* and *StGI.12* genes in potato. *Y*-axis shows mean relative expression values of *StGI* genes compared to the geometric mean of Ct values of *ACTIN* and *EF1α* ± standard deviation, from three technical replicates.

In other plant species, GI is known to be involved in abiotic stress regulation (BRANDOLI et al. 2020). To test the effect of abiotic stresses and the stress mediator abscisic acid (ABA) on the transcription of *StGI* genes, detached leaves of plants grown in a greenhouse were treated with various stresses and ABA. It was found that PEG, cold and heat up-regulated *StGI.04*, but down-regulated *StGI.12* and while ABA induced *StGI.12* expression it had no effect on *StGI.04*. The salt stress repressed *StGI.12*, but did not influence the *StGI.04* mRNA level. Thus, one can conclude that the two *StGI* genes respond to stresses and ABA in a different way.

The function of StGI.04 was studied by antisense repression of *StGI.04* expression. Sixty-five *StGI.04* transgenic 'Désirée' (DES) lines designated aGI lines were generated (JOSE 2019). The expression of *StGI.04* was tested with RT-qPCR in leaves of *in vitro*-grown plants and five lines with different level of *StGI-04* expression were selected for further studies (Fig. 6).

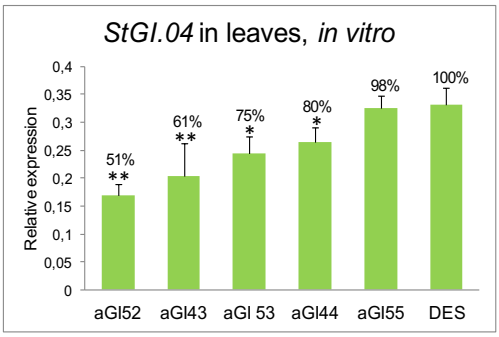

Figure 6. Level of *StGI.04* repression in aGI lines compared to the control DES. RNA was isolated from middle leaves of *in vitro* plants. *Y*-axis shows mean relative expression values of *StGI.04* gene compared to the mean expression values of *ACTIN*.

The selected aGI plants were grown further in a greenhouse and the level of *StGI.04* expression was re-tested in the leaves and tubers. The result was similar to that obtained from leaves of plants grown *in vitro*. Particularly, aGI52 showed significant ($p < 0.01$) and relatively stable, around 50% reduction in *StGI.04* expression in all three RT-qPCR analyses. Thus, this line was used later to test the specificity of antisense repression.

The height, earliness of tuberization and tuber yields of aGI plants grown in a greenhouse were measured and compared to DES. No difference in canopy phenotype or tuber yield was detected between the aGI lines and DES. Thus, it was concluded that the level of reduction in *StGI.04* transcript level that we could achieve might not be high enough for influencing the tuberization in the commercial potato cultivar 'Désirée' or *StGI.04* is not involved in tuberization in 'Désirée'.

Although the less homologous region of *StGI.04* to *StGI.12* was used for generation of aGI lines the identity of the two regions was still 71.2%. Thus, the repression of *StGI.12* expression by the *StGI.04* fragment could not be excluded. To test this possibility a *StGI.12*-specific primer pair was used in parallel with the *StGI.04*-specific primer pair in the RT-qPCR analysis of

aGI52 and DES leaves and tubers. The results showed that the repression in aGI52 was *StGI.04*-specific and did not extend to *StGI.12*.

DES is a red-skinned potato. The skin colour of aGI tubers was lighter than the skin colour of DES (Fig. 7). The reduction in colour was more or less in accordance with the level of *StGI.04* repression in tubers being the most pronounced in aGI52 and aGI53. Since anthocyanins determine the skin colour (LEWIS 1997) these compounds were extracted from tuber peels and their relative quantity measured in aGI52 and DES. In comparison with DES, 43% reduction in anthocyanin content was found in aGI52 tuber peels (Fig. 7).

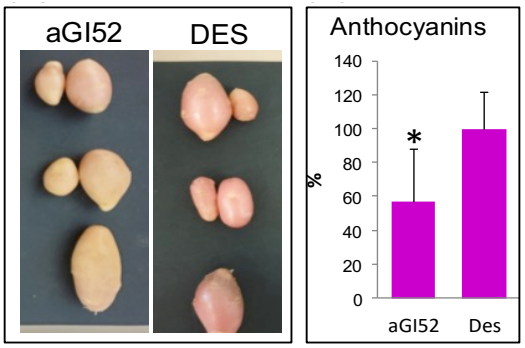

Figure 7. Morphology of mature tubers of aGI52 and the control DES plants grown under short day conditions and the relative anthocyanin pigment content of tuber peels.

It has been known for a long time that three loci, *D* (developer), *R* (red), and *P* (purple) determines tuber colour. JUNG et al. (2009) demonstrated that the *D* locus encodes an R2R3 MYB TF, a part of the MYB-bHLH-WD40 complex, regulating anthocyanin synthesis. Several alleles of R2R3 MYBs as StMYBA1 and StMYB113 were identified in cultivated tetraploid potatoes (JUNG et al. 2009) and shown that an *R2R3-MYB* is a direct target of the small RNA regulation (BONAR et al. 2018). Thus, we presume that the repression of *StGI.04*, directly or indirectly, influences the activity of these transcription factors and thereby regulates the

synthesis of anthocyanins in potato. To our knowledge, this function has not been reported for *GI* in any other plant species investigated thus far.

3.3 Characterisation of the BIG BROTHER (BB) gene in potato

The *BB* gene restricts the leaf development in *Arabidopsis* (VERCRUYSSE et al. 2020). Assuming a similar function for *BB* gene in potato we thought that down-regulation of *BB* gene expression might result in a higher leaf expansion rate and earlier tuberization in potato. In order to identify the *BB* gene in potato a search for the *A. thaliana* RING/U-box superfamily protein (*AtBB*) NM_148885.3 homologue was carried out and a *S. tuberosum* E3 ubiquitin ligase *BIG BROTHER-like* (*StBB*) gene with 70% identity to *AtBB* was identified.

To get an idea about the regulation of *StBB* The Plant Transcriptional Regulatory Map Platform was used to predict transcriptional binding sites in the 3000-bp region upstream from the *StBB* translation start site (chromosome 11, from 37009829 bp to 37012829 bp, reverse complement) in the *S. tuberosum* Group Phureja genome sequence. The search resulted in identification of 48 binding sites for 29 transcription factors (TFs) in the *StBB* promoter region at a threshold *p*-value $\leq 1e^{-5}$. These TFs belonged to 15 families. Eighteen out of the 48 binding sites served for the DOF family TFs. The other dominating families were BBR-BPC, bHLH, M-type MADS and MIKC-MADS. Location of the TF binding sites with the indication of TF families is presented in the top of Figure 8.

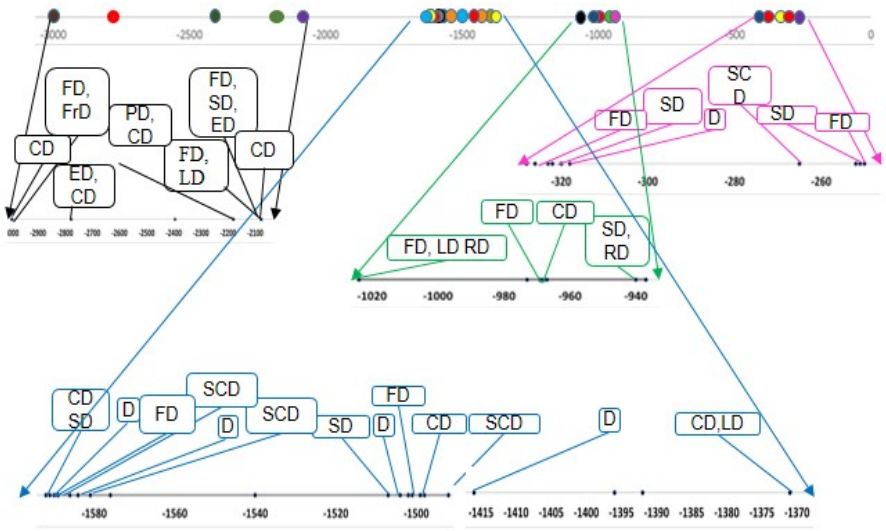

Figure 8. Predicted binding sites of TFs in the promoter region of the *S. tuberosum* Group Phureja *StBB* gene. Thin line represents the promoter region from the translation start site to -3000 bp. Round shapes in different colour represent predicted TF families: red, DOF; light blue, BBR-BPC; yellow, C2H2; green, M-type MADS; purple, MIKC-MADS; pink, B3; light green, LBD; grey, HB other; white, WRKY; orange, GRAS; dark green, ERF; black, RAV; brown, bHLH; red circle, MYB; squash, MYB-related. TFs involved in developmental processes are illustrated by boxes. The coloured arrows indicate zooming in the different regions of the promoter. Abbreviations: CD, cell development; D, development; ED, embryo development; FD, flower development; FrD, fruit development; LD, leaf development; RD, root development; SD, stem development; SCD, seed and seed coat development; PD, pollen development.

The predicted TFs are involved in a wide range of biological processes and respond to different internal and external stimuli. However, 20 out of the 24 TFs with known functions are related to developmental processes as for example, the flower-, leaf-, stem- and root development or cell cycle regulation.

Organ-specific expression of *StBB* was studied with RT-qPCR. Figure 9 demonstrates that *StBB* is expressed in each organ of potato, which is in line with the prediction of TFs involved in different developmental processes binding to the *StBB* promoter.

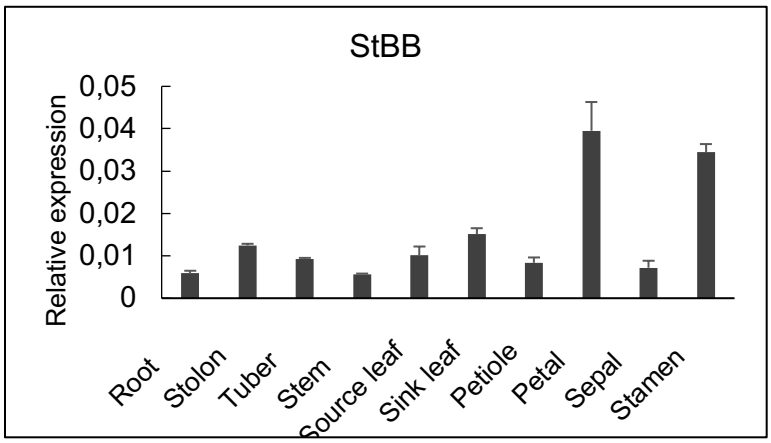

Figure 9. Expression profile of the *StBB* gene in different organs of *S. tuberosum* cv. 'Désirée' determined by RT-qPCR. Bars indicate mean relative expression values of *StBB* gene compared to the mean expression values of *ACTIN* and *EF1α* + SE (n = 3 technical replicates).

DISCH et al. (2006) also detected *BB* mRNA in all organs with highest amounts in proliferating tissues including shoot, root and floral meristems, vasculature, young organs and developing embryos of *Arabidopsis*. They examined not only the *BB* expression level in *Arabidopsis* but also tested a series of genotypes that expressed increasing amounts of *BB* mRNA from the endogenous promoter ranging from 0% to 600% of the wild-type level and concluded that *BB* is both necessary and sufficient to limit *Arabidopsis* floral organ size, floral biomass accumulation and stem thickness. We detected the highest level of *StBB* expression in petals followed by the reproductive tissue, stamen. Thus, we hypothesise that the function of *StBB* in potato may be similar to that found for *BB* in *Arabidopsis*, i.e., restricting organ overgrowth and especially, the overgrowth of petal and stamen.

To test the effect of stresses and ABA on the transcription of *StBB* gene detached source leaves of greenhouse-grown potato plants were subjected to different treatments. Under the NaCl and PEG treatments the leaves lost water and started to collapse, while the cold- and heat stress and

ABA did not influence the phenotype of leaves. Transcript level of *StBB* was slightly increased by the salt stress, however, statistically significant increase was observed only after 6 h of PEG treatment. No changes in expression were detected under cold and heat stresses or after ABA treatment (Fig. 10).

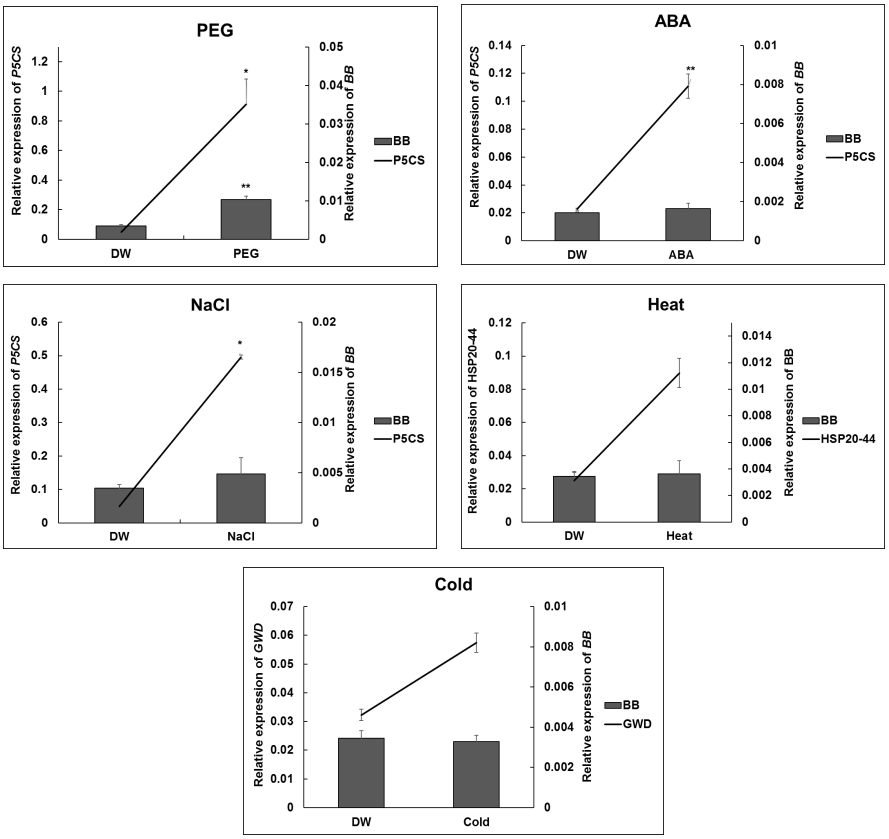

Figure 10. Relative expression level of *StBB* gene determined by RT-qPCR using total RNA from stress-treated leaves. Efficiency of treatments was tested by the up-regulation of *Δ1-PYRROLINE-5-CARBOXYLATE SYNTHETASE (StP5CS), α-GLUCAN, WATER DIKINASE (GWD)* and *HEAT SOCK PROTEIN 20-44 (HSP20-44).*

QI et al. (2020) reported that the expression of one of the RING-finger ubiquitin ligase E3 genes, *StRFP2* (potato *RING-FINGER PROTEIN 2*), is up-regulated by the PEG-elicited osmotic stress, thereby enhancing the drought tolerance of potato. Hence *StBB* and *StRFP2* both contain a RING-finger domain and *BB* is a functional E3 ubiquitin ligase (DISCH et al. 2006)

we suppose that *StBB*, like *StRFP2*, enhances the drought tolerance of potato by limiting the plant growth under drought stress condition.

On the basis of current research of *in silico* and expression analysis, *StBB* could be a promising target for potato crop improvement as repression of *StBB* may result in accelerated plant growth and early tuber bulking. Nevertheless, based on the presumed function of *StBB* under drought condition the drought tolerance of the *StBB*-repressed plants may be reduced. A future analysis of *StBB* will be required to understand how the level of *StBB* expression is determined and how *StBB* influences organ growth at the molecular level under optimal and sub-optimal growth conditions.

Functional analysis of the *StBB* gene was carried out as that of *StGI.04*; antisense repressed DES lines were generated using an *Agrobacterium*-mediated transformation protocol. After regeneration, shoots were isolated from individual leaves or distant positions of a leaf, out of which 10 rooted in antibiotic-containing medium. Three lines (aBB2, aBB3, aBB9) were found with lower level of *StBB* expression than the non-transformed control. However, only 26-33% reductions in *StBB* mRNA levels were detected, which were statistically ($p < 0.05$) not significant (Fig. 11).

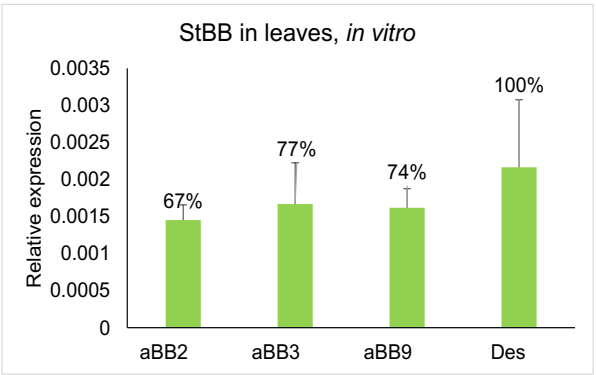

Figure 11. Level of *StBB* repression in aBB lines compared to the non-transformed control DES. *Y*-axis shows mean relative expression values of *StBB* gene compared to the mean expression values of *ACTIN*.

Despite the insignificance of reduction in *StBB* expression level we tested the plants under greenhouse conditions. We found only one line, aBB3, differing in height from DES as it was higher (61.0±15.1 cm) than the non-transformed control (47.1±6.8 cm) at significant $p < 0.05$ level. Tubers were harvested at the end of the vegetation period and measured for weight. However, no significant differences in tuber yield between aBB lines and DES were found.

It has been demonstrated that *BB* has a role in regulation of organ size in *Arabidopsis, Saltugilia*, *Nicotiana tabacum*, etc. (VANHAEREN et al. 2017; LANDIS et al. 2020). We attempted to get information on the function of *BB* gene in potato by antisense repression, however, we could not reach a significant level of reduction in any of the lines tested. A possible explanation might be related to the very low level of *StBB* expression even in control leaves (0.06-fold of the expression of *ACTIN*), which could result in a high error rate in RT-qPCR. Thus, other methods, as for example gene-editing, should be used in the future to get information on the function of *BB* gene in potato.

4 CONCLUSION AND RECOMMENDATIONS

The first part of the study provided an understanding of the effects of intercultivar grafting in potato and summarized the morphological and metabolic outcomes. We identified the major polar metabolites in leaves and tubers of two commercial potato cultivars (*Solanum tuberosum* L. cv. 'White Lady' and *Solanum tuberosum* L. cv. 'Hópehely') and found characteristic differences in the metabolite compositions in both organs of the two cultivars. In comparison to non-grafted and homo-grafted controls no major effect of hetero-scions and rootstocks on the metabolite concentrations were detected suggesting that the level of major metabolites is under genetic control. The only exception was galactinol, the concentration of which was slightly influenced by hetero-grafting in leaves. Furthermore, the grafting experiments resulted in detection of a positive correlation between the rate of leaf growth and the time of tuber initiation which support the idea that tuberization is triggered by source-derived mobile signals. To the best of our knowledge, no information at molecular level is available regarding the responses of two potato cultivars grafted on each other. Although the grafting did not have considerable influences on metabolite composition in potato leaves and tubers under greenhouse conditions similar experiments can be performed under field conditions with involvement of more potato genotypes to get more information on transported metabolites under natural environmental conditions. Moreover, hence the signal transports largely depend on vascular formation (KUROTANI and NOTAGUCHI 2021), it is important to focus on the mechanism of vascular development.

To unravel the influence of some candidate genes to tuberization we turned to the study of *GIGANTEA* (*GI*) and *BIG BROTHER (BB)* because these genes are ubiquitous in the plant kingdom and are involved in diverse processes from flowering to stress responses and from leaf sizes to

tuberization. The two genes were studied in several plant species including *Arabidopsis*, however, no details on the regulation of *GI* and *BB* gene expression in potato have been reported thus far. Thus, one of the aims of our work was the characterization of *GI* and *BB* in *Solanum tuberosum* L. cv. 'Désirée'(DES).

In this study, expression analysis of the two potato *GI* genes homologous to *Arabidopsis*, designated *StGI.04* and *StGI.12*, was performed and found that the responses of the two genes to abiotic stresses and ABA as well as their organ-specific expression is different. Thus, we presume that the function of *StGI.04* and *StGI.12* are at least partially different. This study laid foundation for further investigation of the roles of *GI* genes in potato.

Analysis of a *StGI.04*-repressed line demonstrated that *StGI.04*, as with *GI* in other plant species, influences the circadian clock, flowering, stress responses, and starch synthesis via the alteration of expression of key genes of these processes in leaves of potato plants (KARSAI-REKTENWALD et al. 2022). Expression of *StGI.04* was quantified in *StGI.04*-repressed lines and found that the highest repression achieved was around 50% compared to the non-transformed control plant DES. However, no differences either in phenotype including plant height or tuber yield were observed. Thus, we concluded that the repression of *StGI.04* may not result in alteration of tuber formation in DES or the level of reduction in *StGI.04* transcript level that we could achieve was not high enough for influencing the tuberization in this potato cultivar. Unexpectedly, however, we identified a novel function for *StGI.04* not detected earlier in other plant species, namely, promotion of the synthesis of anthocyanins in tuber skin. In order to study how *StGI.04* influences the anthocyanin level, the next step could be focused on transcriptome analysis of tuber peels of DES and the *StGI.04*-repressed lines. Moreover, considering a similar trend of phenolic levels with anthocyanin

contents, detecting the major phenolic compounds in the tuber skin of *StGI.04*-repressed lines in comparison to DES is suggested.

The function of the other potato *GI* gene, *StGI.12*, is still unknown. However, as it was concluded above, based on the different expression pattern of *StGI.04* and *StGI.12* we suppose that their function is at least partially different and *StGI.12* may have higher influence on tuberization than *StGI.04*. To investigate this hypothesis, the further study could be focused on investigating how *StGI.12* gene repression influences tuberization and gene expression at transcriptome level under stress conditions in different organs. In addition, testing the involvement of *StGI.12* gene in anthocyanin metabolism is recommended. These could be the essential studies to understand the role of GIs in commercial potato cultivars.

The *S. tuberosum* E3 ubiquitin ligase coding *BIG BROTHER-like* (*StBB*) gene is 70% identical with the *Arabidopsis BB* gene *(AtBB).* Supposing that the *BB* gene, like in *Arabidopsis*, has an important role in the development of potato, a 3.0-kb promoter sequence of the potato *BB* gene was analysed *in silico.* 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. Twenty 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. The level of *StBB* expression was studied in different organs and under different stress conditions. The *StBB* mRNA was detected in each organ tested with the largest amounts in petal and stamen. Also, statistically significant increase in *StBB* transcript level was detected after the osmotic stress. These results suggest that the function of *StBB* is similar to that of *AtBB*. We have attempted to test this by antisense repression of *StBB* expression. However, no lines with statistically different *StBB* mRNA level could be obtained. Thus, further experiments are required to understand how the level of *StBB* expression is determined and how *StBB*

influences organ growth at the molecular level under optimal and sub-optimal growth conditions.

5 NEW SCIENTIFIC RESULTS

- Grafting experiments between the commercial potato cultivars ‘Hópehely’ and ‘White Lady’ indicated that the sucrose concentration of tubers is genetically determined, whereas the galactinol concentration in the leaves can be influenced by the developing tuber.
- A positive correlation between the growth rate of the leaves and the time of tuber initiation was detected.
- A putative *BIG BROTHER (StBB)* gene homolog has been identified in *Solanum tuberosum*.
- It was found that *StGI.04, StGI.12* and *StBB* genes are differentially expressed in response to different abiotic stress treatments and in different organs.
- A new function for the *GIGANTEA* in potato was discovered by demonstrating that repression of *StGI.04* expression leads to the reduction of anthocyanin content of tuber skin.

6 REFERENCES

BONAR, N., LINEY, M., ZHANG, R., AUSTIN, C., DESSOLY, J., DAVIDSON, D., STEPHENS, J., MCDOUGALL, G., TAYLOR, M., BRYAN, G. J., & HORNYIK, C. (2018): Potato miR828 is associated with purple tuber skin and flesh color. In: *Frontiers in Plant Science*, (9) 1742. p. https://doi.org/10.3389/fpls.2018.01742

BRANDOLI, C., PETRI, C., EGEA-CORTINES, M., & WEISS, J. (2020): *GIGANTEA:* Uncovering new functions in flower development. In: *Genes*, 11(10) 1142. p. https://doi.org/10.3390/genes11101142

DIETZE, J., BLAU, A., & WILLMITZER, L. (1995): *Agrobacterium*-mediated transformation of potato (*Solanum tuberosum*). In: GENE TRANSFER TO PLANTS. Berlin, Germany. p. 24-29.

DISCH, S., ANASTASIOU, E., SHARMA, V.K., LAUX, T., FLETCHER, J.C., & LENHARD, M. (2006): The E3 ubiquitin ligase *BIG BROTHER* controls *Arabidopsis* organ size in a dosage-dependent manner. In: *Current Biology*, 16(3) 272-279. p. https://doi.org/10.1016/j.cub.2005.12.026

FERNIE, A. R., TIESSEN, A., STITT, M., WILLMITZER, L., & GEIGENBERGER P. (2002): Altered metabolic fluxes result from shifts in metabolite levels in sucrose phosphorylase-expressing potato tubers. In: *Plant Cell Environment*, (25) 1219–1232. p. https://doi.org/10.1046/j.1365-3040.2002.00918.x

HAJIREZAEI, M. R., BÖRNKE, F., PEISKER, M., TAKAHATA, Y., LERCHL, J., KIRAKOSYAN, A., & SONNEWALD, U. (2003): Decreased sucrose content triggers starch breakdown and respiration in stored potato tubers (*Solanum tuberosum*). In: *Journal of Experimental Botany*, 54(382) 477–488. p. https://doi.org/10.1093/jxb/erg040

JOSE, J. (2019): Master thesis: Molecular genetic studies on *GIGANTEA* gene in potato (*Solanum tuberosum* L.). Hungarian University of Agriculture and Life Sciences. Gödöllő, Hungary.

JUNG, C. S., GRIFFITHS, H. M., DE JONG, D. M., CHENG, S., BODIS, M., KIM, T. S., & DE JONG, W. S. (2009): The potato developer (D) locus encodes an R2R3 MYB transcription factor that regulates expression of multiple anthocyanin structural genes in tuber skin. In: *Theoretical and Applied Genetics,* 120(1) 45–57. p. https://doi.org/10.1007/s00122-009-1158-3

JUNKER, B. H., WUTTKE, R., NUNES-NESI, A., STEINHAUSER, D., SCHAUER, N., BÜSSIS, D., WILLMITZER, L., & FERNIE, A. R. (2006): Enhancing vacuolar sucrose cleavage within the developing potato tuber has only minor effects on metabolism. In: *Plant & Cell Physiology*, 47(2) 277–289. p. https://doi.org/10.1093/pcp/pci247

KARSAI-REKTENWALD, F., ODGEREL, K., JOSE, J. & BÁNFALVI, Z. (2022): *In silico* characterization and expression analysis of *GIGANTEA* genes in potato. In: *Biochemical Genetics,* https://doi.org/10.1007/s10528-022-10214-7

KUROTANI, K. I., NOTAGUCHI, M. (2021): Cell-to-cell connection in plant grafting-molecular insights into symplasmic reconstruction. In: *Plant & Cell Physiology*, 62(9) 1362–1371. p. https://doi.org/10.1093/pcp/pcab109

LANDIS, J. B., KURTI, A., LAWHORN, A. J., LITT, A., & MCCARTHY, E. W. (2020): Differential gene expression with an emphasis on floral organ size differences in natural and synthetic polyploids of *Nicotiana tabacum* (*Solanaceae*). In: *Genes*, 11(9) 1097. p. https://doi.org/10.3390/genes11091097

LEWIS, C. E., WALKER, J. R. L., LANCASTER, J. E., & SUTTON, K. H. (1997): Determination of anthocyanins, flavonoids, and phenolic acids in potatoes. II. Wild, tuberous *Solanum* species. In: *Journal of the Science of Food and Agriculture,* 77(1) 58-63. p. doi.org/10.1002/(SICI)10970010(199805)77:1<45::AID-JSFA1>3.0.CO;2-S

MURASHIGE, T., SKOOG, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. In: *Physiologia Plantarum,* (15) 473–497. p. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

MÜLLER-RÖBER, B., SONNEWALD, U., & WILLMITZER, L. (1992): Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. *The EMBO Journal*, 11(4), 1229–1238.

QI, X., TANG, X., LIU, W., FU, X., LUO, H., GHIMIRE, S., ZHANG, N., & SI, H. (2020): A potato RING-finger protein gene *StRFP2* is involved in drought tolerance. In: *Plant Physiology and Biochemistry, (*146) 438-446. p. https://doi.org/10.1016/j.plaphy.2019.11.042

SAMBROOK, J., FRITCH, E. F., & MANIATIS, T. (1989): Molecular cloning. A LABORATORY MANUAL (2nd edition). New York, USA.

SENGUPTA, S., MUKHERJEE, S., BASAK, P., & MAJUMDER, A. L. (2015): Significance of galactinol and raffinose family oligosaccharide synthesis in plants. In: *Frontiers in Plant Science*, (6) 656. p. https://doi.org/10.3389/fpls.2015.00656

TAUBERGER, E., FERNIE, A. R., EMMERMANN, M., RENZ, A., KOSSMANN, J., WILLMITZER, L., & TRETHEWEY, R. N. (2000): Antisense inhibition of plastidial phosphoglucomutase provides compelling evidence that potato tuber amyloplasts import carbon from the cytosol in the form of glucose-6-phosphate. In: *The Plant Journal: For Cell and Molecular Biology*, *23*(1) 43–53. p. https://doi.org/10.1046/j.1365-313x.2000.00783.x

TRETHEWEY, R. N., FERNIE, A.R., BACHMANN, A., FLEISCHER-NOTTER, H., GEIGENBERGER, P., & WILLMITZER, L. (2001): Expression of a bacterial sucrose phosphorylase in potato tubers results in a glucose-independent induction of glycolysis. In: *Plant Cell Environment,* (24) 357–365. p. https://doi.org/10.1046/j.1365-3040.2001.00679.x

VANHAEREN, H., NAM, Y.J., DE MILDE, L., CHAE, E., STORME, V., WEIGEL, D., GONZALEZ, N., & INZÉ, D. (2017): Forever young: the role of ubiquitin receptor *DA1* and E3 ligase *BIG BROTHER* in controlling leaf growth and development. In: *Plant Physiology,* 173(2) 1269-1282. p. https://doi.org/10.1104/pp.16.01410

VERCRUYSSE, J., BAEKELANDT, A., GONZALEZ, N., & INZÉ, D. (2020): Molecular networks regulating cell division during *Arabidopsis* leaf growth. In: *Journal of Experimental Botany,* 71(8) 2365-2378. p. https://doi.org/10.1093/jxb/erz522

7 THE PUBLICATIONS OF THE AUTHOR IN THE FIELD OF STUDIES

Articles published in peer reviewed scientific journals

Karsai-Rektenwald F., **Odgerel K.,** Jose J., Bánfalvi Z. (2022): *In silico* characterization and expression analysis of *GIGANTEA* genes in potato. *Biochemical Genetics.* (IF:1.89) https://doi.org/10.1007/s10528-022-10214-7

Odgerel K., Bánfalvi Z. (2021): Metabolite analysis of tubers and leaves of two potato cultivars and their grafts. *PLoS One.* 16:e0250858. (IF:3.24) https://doi.org/10.1371/journal.pone.0250858

Articles submitted in peer reviewed scientific journals

Odgerel K., Bánfalvi Z. (2022): Promoter and expression analysis of the *BIG BROTHER* gene in potato. *Columella – Journal of Agricultural and Environmental Sciences*. (submitted)

Odgerel K., Jose J., Karsai-Rektenwald F., Ficzek G., Gergely 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 Biology*. (resubmitted)

Articles and abstracts published in conference books

Villányi V., **Odgerel K.**, Karsai-Rektenwald F., Bánfalvi Zs. (2021): Az oltvány hatása a burgonya koraiságára és a gumók metabolit összetételére. XXVII. Növénynemesítési Tudományos Napok. Március. Összefoglaló kötet, 37.

Odgerel K. (2021): The promoter analysis and gene expression of potato *BIG BROTHER* gene. Proceeding of the "Scientific Conference of PhD. Students - 2021". Nitra, Slovakia. Nov. pp.16

Karsai-Rektenwald F., **OdgereL K.,** Jose J. (2021): Regulation of *GIGANTEA* genes in potato. Proceeding of the "Scientific Conference of PhD. Students - 2021". Nitra, Slovakia. Nov. pp.14

Odgerel K., Bánfalvi Z. (2020): The use of grafting to study the influence of canopy growth rate on earliness of tuber initiation and metabolite

composition of potato tubers. Proceeding of "The 8th Plant Genomics & Gene Editing Congress". Rotterdam, the Netherlands. Mar. pp. 19

Odgerel K., Kondrák M., Bánfalvi Z. (2019): Studying leaf-derived signalling on tuberization and tuber metabolome by grafting experiments in potato. The 2nd international conference on Plant and Molecular Biology. Amsterdam, the Netherlands. Oct. pp. 30

Bánfalvi Z., **Odgerel K.,** Csákvári E., Jose J., Kondrák M. (2019): Studying the relationship between tuber bulking and canopy growth in potato. Proceedings of the XXV. Plant Breeding Scientific Day. Budapest, Hungary. Mar. pp. 149-153

Oral and poster presentations

Odgerel K. (2021): The promoter analysis and gene expression of potato *BIG BROTHER* gene. Scientific Conference of PhD. Students – 2021. Nitra, Slovakia. Nov. 10.

Odgerel K., Bánfalvi Z. (2020): The use of grafting to study the influence of canopy growth rate on earliness of tuber initiation and metabolite composition of potato tubers. The 8th Plant Genomics & Gene Editing Congress. Rotterdam, the Netherlands. Mar. 4-5.

Odgerel K., Kondrák M., Bánfalvi Z. (2019): Effect of leaf mobile signals on potato tuber induction and metabolite composition. The MBK Days - Scientific meeting. Gödöllő, Hungary. Nov. 29.