SZENT ISTVÁN UNIVERSITY

Effects of drought stress on the metabolite contents and drought tolerance of transgenic potato lines

Theses of PhD dissertation

Bózváriné Juhász Zsófia Mária

Gödöllő

2014

PhD School

Name: Plant Sciences

Head of School: Lajos Helyes
Professor, Szent István University
Faculty of Agricultural and Environmental Studies
Institute of Horticultural Sciences

Scientific field: Agricultural sciences

Research field: Plant production and horticulture

Leader of Research: Zsófia Bánfalvi
Doctor of the Hungarian Academy of Sciences
National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute

…… ……

Approved by Head of School Approved by Leader of Research

Introduction and aims

The increasing temperature leads to more and more arid agricultural areas worldwide. Hence, to have drought tolerant crops for the growing demand of food supply is reasonable.

Potato (*Solanum tuberosum*) is the fourth most important crop in the world, and very sensitive to drought. Application of molecular and transformation techniques in the last decades enable the breeders to develop new varieties with improved drought tolerance.

Trehalose, a non-reducing disaccharide composed of two glucose molecules is a very abundant sugar in nature. In bacteria, yeast and desiccation tolerant plants it accumulates under osmotic/dehydration stress, and helps cells to survive by protecting membranes and proteins (IORDACHESCU & IMAI 2008; JAIN & ROY, 2009). Expression of trehalose biosynthetic genes in plants can improve the drought tolerance (YEO et al., 2000**;** CORTINA et al., 2005; KARIM et al., 2007; MIRANDA et al., 2007). STILLER et al. (2008) have previously introduced the *TPS1* gene of yeast into the potato cultivar White Lady under the control of a drought-inducible potato promoter, *StDS2*. Although the transgenic plants became drought tolerant, it was determined that the transgene was expressed at a very low level even under optimal growth conditions and the transgenic plants displayed certain morphological and physiological changes when compared with the wild-type. For example, they grew slower, had a lower CO_2 fixation rate and reduced stomatal density. Changes in expression of the genes implicated in photosynthesis and carbohydrate metabolism were also detected (KONDRÁK et al., 2011, 2012). Transcriptional changes can lead to metabolic changes, which can modify the metabolic composition of the plants. With this object, the first issue of my dissertation is:

1. **To compare the metabolic content of the leaves and tubers of the wild-type and the T1, T2 transgenic lines expressing the yeast *trehalose-6-***

phosphate synthase 1 **gene under optimal-, and drought stress conditions.**

Infection of plants by necrotizing pathogens or colonization of plant roots with certain beneficial microbes causes the induction of a unique physiological state called priming. The primed state can also be induced by treatment of plants with various natural and synthetic compounds. Primed plants display either faster, stronger, or both, activation of the various cellular defense responses that are induced following attack by either pathogens or insects or in response to abiotic stress (PRIME-A-PLANT GROUP, 2006). The phenomenon of priming is strongly related to the pathogen-related (PR) proteins and their coding genes. The expression of *PRLIP* (pathogenesis related lipase) genes is very similar to the expression of *PR* genes (JAKAB et al., 2003). It has been proven that ß-aminobutyric acid (BABA) can activate priming in different plant species, including *Arabidopsis* (JAKAB et al., 2001, 2005). Treatment with BABA, salycilic acid and pathogens induces the expression of *PRLIP1* and *PRLIP2* genes in *Arabidopsis* (JAKAB et al., 2003). In potato, no homologues proteins to PRLIP1 and PRLIP2 have been found to this end. Hence we decided to introduce the recombinant plasmid pPZP111 harbouring the *PRLIP2* gene (JAKAB et al., unpublished) into potato to improve the drought tolerance of transgenic lines. For this reason, the second issue of my dissertation is:

1. **To aquire knowledge on *Agrobacterium tumefaciens*-mediated transformation of the cultivar Desireé and to produce new, stable transgenic potato lines.**
2. **To prove the integration and expression of the transgene with molecular methods.**
3. **To examine the drought tolerance of the transgenic lines expressing the *PRLIP2* gene in greenhouse.**

Materials and methods

Plant material

The metabolic profiling was carried out in T1 and T2 transgenic lines of the Hungarian *S. tuberosum* cv. White Lady expressing the *TPS1* gene (STILLER et al., 2008). The *PRLIP2* gene was introduced into the Dutch *S. tuberosum* cv. Desirée.

Potato transformation

The potato transformation was performed according to DIETZE et al. (1995) by *Agrobacterium*-mediated leaf transformation using the *A. tumefaciens* strains ALG0 (HOOD et al., 1984) and C58C1(pGV2260) (DEBLAERE et al., 1985).

Growth conditions

Six-week-old *in vitro* plants were transferred to pots containing A260 sterile soil (Stender, Germany) and were grown in a greenhouse under natural light, at 20-28°C, and at a soil water content of 70%. Four weeks after planting into soil, we started the drought stress by withholding irrigation. This process was continued until the leaves of the wild-type plants started wilting. At this point, we started again the irrigation, and after one week, a new drought stress period was begun. During a growing season 4-7 drought stress cycles were applied.

Plant phenotyping

Biomass, shape, size and structure of the plants and their leaves, wilting habit, tuber yield-, shape and colour, duration of dormancy and the sprouting behaviour of tubers were assessed visually.

Analytical methods

Extraction, derivatisation and analysis of potato leaf carbohydrates were carried out as described by SCHAUER et al. (2004) using a quadrupole-type GC-MS system (Finnigan Trace/DSQ, Thermo Electron Corp.). The chromatograms and mass spectra were evaluated using the XCALIBUR software (Thermo Electron Corp.) and the NIST 2.0 library, and the Golm Metabolome Database.

Quantification of trehalose-6-phosphate (T6P) was carried out by the method of LUNN et al. (2006) with some modification. The starch was measured

using the method of YU et al. (2001). The protein assay was made by the method of BRADFORD et al. (1976). The dry matter content was measured from cleaned, grinded tubers dried at 80°C for 24 hours.

Significant differences were established using Student's *t*-test. Principal component analysis (PCA) was carried out using the Multibase Excel Add-Ins program, which can directly process Excel data. For the MANOVA (Multivariate ANOVA) statistical analysis the program of IBM SPSS Statistics 19 was applied.

Molecular biological methods

Bacterial plasmid DNA was isolated by the alkaline lysis method (SAMBROOK et al., 1989). The plasmid DNA was digested with the restriction enzyme *Bam*HI using the method of the producer (Fermentas) in 30 µl reaction volume. The fragments were separated on agarose gel, cut and cleaned by QIAEX II Gel Extraction Kit or MinElute Gel Extraction Kit (QIAGEN) and introduced into the pBluescript II KS cloning vector. Transformation of *E. coli* was carried out according to INOUE et al. (1990).

Isolation of genomic DNA of the leaves was performed by the method of SHURE et al. (1983). Total RNA was extracted according to STIEKEMA et al. (1988).

The PCR reaction volume was 50 µl, including ca. 100 ng of DNA template, 1.5 mM $MgCl_2$, 0.3-0.5 µM primer, 0.2 mM dNTP mix (dATP, dCTP, dGTP, dTTP), 1xPCR Taq puffer and 1 U Taq polimerase enzyme. Denaturation was at 95^{o}C for 5 min, and the reaction volume was incubated for 40 cycles at 95^{o}C for 30 sec, at 60^{o}C for 30 sec and at 72^{o}C for 45 sec. The product was elongated at 72^{o}C for 10 min. 400 ng of total RNA was used for cDNA synthesis by the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), and 40 ng cDNA was used for PCR.

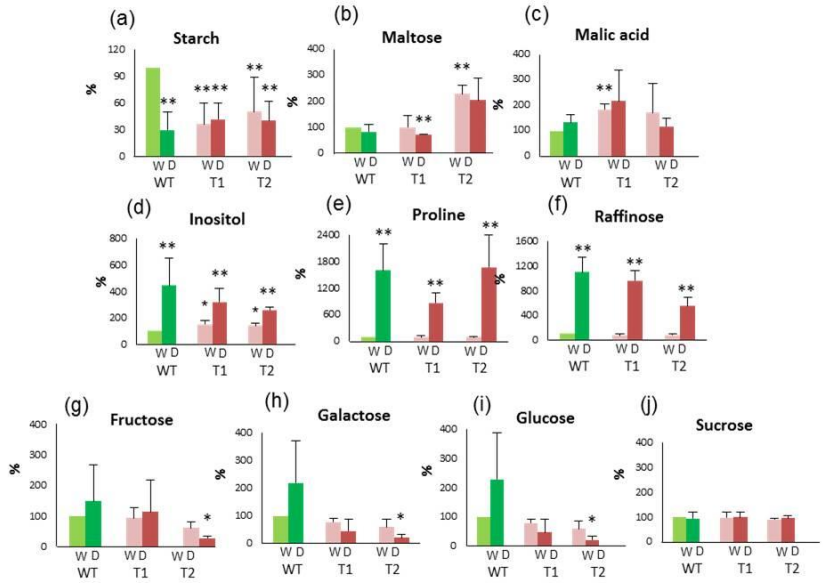
Results

Total protein, starch, and metabolite content of the *TPS1* transgenic potato lines

Analysis of the leaves

The vegetatively propagated *in vitro S. tuberosum* cv. White Lady and the T1, T2 lines expressing the *TPS1* gene (STILLER et al., 2008) were transferred into pots in greenhouse, and were further grown under optimal conditions. After four weeks the plants were devided into two groups. Three plants of each line were grown under optimal conditions, while the other three plants of each line were subjected to drought stress. Two weeks after starting the drought stress, all of the mature source leaves from three plants of each line-treatment combination were collected four hours after sunrise. The entire process was independently repeated three times to obtain three biological replicates for each line.

The starch content of the leaves of irrigated wild-type (WT) plants was higher than that of the T1 and T2 lines. Drought stress reduced the starch content of the WT leaves by about 65%. In contrast, no significant change in the otherwise lower starch content of *TPS1* transgenic plants was measured under stress condition (Fig. 1/a).



Exp. No.	Starch h. eq. g^{-1}	Maltose µmol g-1	Malic acid µmol g-1	Inositol µmol g^{-1}	Proline µmol g^{-1}	Raffinose nmol g^{-1}	Fructose µmol g^{-1}	Galactose µmol g^{-1}	Glucose µmol g^{-1}	Sucrose µmol g^{-1}
1	49	0,04	90,18	7,34	3,1	3,2	240	84	14,4	106
2	18,5	0,02	108,44	9,95	3	3	156	95	17	101
3	5,4	0,02	62,91	9,45	2,6	4,7	139	104	18,3	82

Figure 1: Relative amounts of sugars, starch and proline in wild-type (WT) and TPS1 transgenic (T1, T2) leaves compared to the well-watered WT control. Bars and error bars represent the mean ± SE derived from three independent experiments. Asterisks and dots denote significant differences at the *P* = 0.01 and *P* = 0.05 (*t* test) levels, respectively, as compared with the well-watered WT control. The absolute concentration of the different compounds in well-watered WT leaves in three independent experiments is shown in a table. Concentrations are calculated relative to dry weights (DW). The concentration of starch is given in hexose equivalents (h. eq.) g-1 DW. The high variation in leaf metabolite and starch content might be explained by the small differences in day length, light intensity and temperature in the greenhouse during the three consecutive plant tests, as environmental conditions can strongly influence metabolite content and starch accumulation in leaves. Therefore, concentrations of compounds in the well-watered WT leaves were regarded as 100% for comparison with other samples originated from the same plant test. Mean ± SE of the percentage values obtained from the three consecutive plant tests were calculated and are presented by bars and error bars.

22 compounds were detected in the leaves by GC-MS. A 4.4-fold increase in the inositol level was detected in the drought-stressed WT leaves relative to the unstressed WT controls, while this increase was only 3.2-fold in T1 and 2.6-fold in T2 leaves. The high inositol level correlated with the low starch level (Figure 1/a,d).

Drought stress induced the accumulation of proline in both WT and *TPS1* transgenic leaves (Figure 1/e), however, the leaves of the WT plants started to wilt,

while the transgenic ones did not. This result suggests that the proline content is not linked to the drought tolerance of potato plants.

The concentration of raffinose was quite low under optimal conditions in the leaves compared to other sugars, but a very strong 11-, 9.5-, and 5.5-fold increase in raffinose content in response to drought treatment in WT, T1 and T2 leaves, respectively, was observed (Figure 1/f).

Unlike inositol, the raffinose level was not elevated under well-watered conditions in the *TPS1* transgenic lines compared to WT plants. The regulatory mechanisms that underlie these increases in inositol and raffinose contents are likely quite different. While inositol synthesis is influenced by the transcriptional and/or biochemical changes triggered not only by drought but also by the expression of yeast *TPS1* in potato, raffinose synthesis is induced by water loss and is negatively correlated with the relative water content (RWC) of leaves (KONDRÁK et al., 2011, 2012). LEGAY et al. (2011) have experienced the same changes under drought stress in leaves of drought tolerant potato clones.

The sucrose level was the same in each line and did not change after the treatment (Figure 1/j). We therefore speculate that a constant sucrose level may be very important for potato plants. Since stress reduces the rate of photosynthesis maintenance of a constant sucrose level under drought stress conditions may require the plants to reduce starch synthesis and channel the carbohydrates to sucrose synthesis.

Analysis of the tubers

For testing tubers a new experiment was set up. *In vitro* White Lady plantlets were potted in greenhouse. Four weeks after planting, the plants were divided into two groups. Seven plants per line were continuously irrigated to maintain 70% soil moisture content, and eight plants per line were exposed to drought stress by withholding irrigation for 7-10 days while the water content of the soil decreased to

30%. Seven dry cycles were created within a growing season. Between the dry cycles, the plants were irrigated with an optimal amount of water for one week.

The tubers were harvested at full maturity four months after planting. The tubers were devided into two groups. Tubers of the first group were cleaned, milled, frozen in liquid nitrogen, and stored at -70°C until use. The second group was stored in darkness at room temperature for 12 weeks. The entire experiment was repeated twice.

The T1 and T2 tubers were longitudinal in shape, while the WT tubers were oval (Figure 2/a). Under well-watered conditions the number of tubers per plant and the total tuber biomass were reduced in both transgenic lines compared to the WT control (Figure 2/b,c). The average tuber number per plant was similar in both experiments, however, both parameters were lower in T1 and T2 than in WT. Under optimal conditions the average loss of the yield was 60% and 50% in T1 and T2, respectively, compared to WT. Periodic drought caused an increase in the number but a reduction in the size of the WT tubers, and in general, resulted a 50% yield loss (Figure 1/a-c). Drought had a milder effect on the *TPS1* transgenic plants and tubers. The increase in the number of tubers was less pronounced, and the yield loss did not exceed the 20-40% (Figure 2/b,c).

WT tubers started sprouting in a quite synchronized manner after ca. 12 weeks of storage. In contrast, sprouting in T1 and T2 tubers was significantly delayed (Figure 2/d). After six months of storage, the sprouts on the T1 and T2 tubers were 1-5 cm in length, while those on the WT tubers reached 15-20 cm (Figure 2/d).

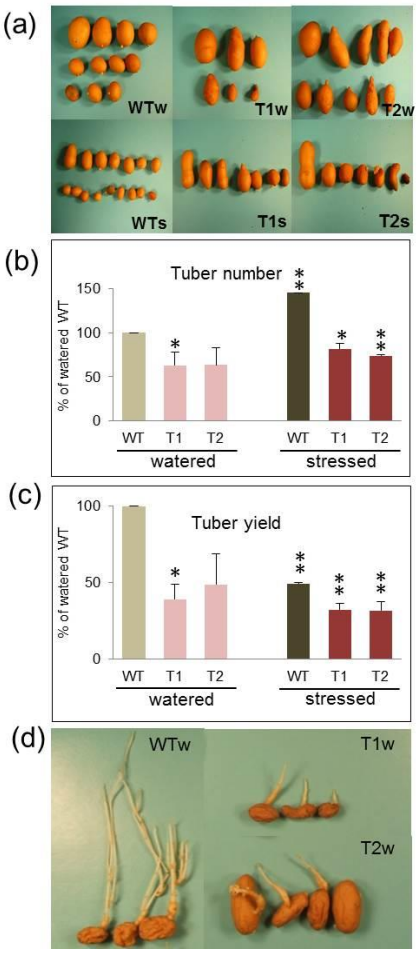


Figure 2: Tuber number and tuber yields of wild type (WT) and *TPS1* plants under well-watered (w) and drought stress (s) conditions. (a) tubers at 12 weeks after harvest stored at room temperature in darkness (b) relative tuber number per plant (c) relative tuber yield per plant (d) tubers at 6 months after harvest stored at room temperature in darkness. Bars and error bars represent the mean ± SE derived from two independent experiments. In each experiment, the tubers were harvested from seven well-watered and eight stressed plants per line. The average numbers of tubers per well-watered WT plants in the two independent experiments were 3.9 and 4.0, while the tuber yields were 11.8 g and 17.6 g. These values are considered 100% for comparisons with the other samples in the same experiments. *$P \leq 0.005$, **$P \leq 0.001$ (Student's *t*-test)

The dry matter content increased by 20% during storage due to water loss. The total protein and starch content was the same in the tubers developed under optimal conditions in all lines. However, the stress decreased the starch level and increased the total protein level in tubers. Concentration of both compounds elevated during storage indicating that the tubers remained metabolically active after harvest. The metabolite composition of tubers was analysed by GC-MS. A total of 33 compounds, including mainly amino acids, organic acids, sugars, and sugar alcohols, were identified. The *TPS1* transgene significantly influenced the amounts of 13 metabolites including asparagine, the concentration of which was significantly higher in the *TPS1* tubers under optimal and stressed conditions than in WT tubers. Not only the asparagine, but the phenylalanine level also increased during storage in tubers developed under well-watered conditions. However, the amount of phenylalanine was not changed, or even was reduced during storage in the tubers of stressed plants. We experienced a great increase in proline level in the tubers of stressed plants. An increase in proline content triggered by stress in the leaves of various plant species is a well-documented response (OBATA & FERNIE,

2012), and proline accumulation was found to be induced by high salinity, drought, and selenium in tubers (TEIXEIRA & PEREIRA, 2007; MAGGIO et al., 2008; JEZEK et al., 2011). We also observed 2-fold increases in glutamine and glutamate content in stressed tubers. Glutamine synthetase plays a key role in nitrogen metabolism and has been implicated in the regulation of proline levels in plants (BRUGIERE et al., 1999). This enzyme is activated in growing tubers in response to drought (TEIXEIRA & PEREIRA, 2007). In a field experiment, glutamine and glutamate showed the greatest changes in response to various treatments (MAGGIO et al., 2008).

To assess whether transgene expression affects the lentgh of the dormancy and T6P content in tubers, the T6P level was measured in samples from *TPS1* transgenic tubers and WT control tubers, but we did not find any correlation between the transgene expression and the T6P concentration.

Isolation and characterization of transgenic potato lines expressing the *PRLIP2* gene

Isolation of transgenic potato lines expressing the PRLIP2 gene

The binary vector pPZP111 carrying the *PRLIP2* gene was constructed by Prof. Gábor Jakab at the University of Pécs. To check the insert of the plasmid, it was cloned into the pBluescript vector and sequenced. The pPZP111::PRLIP2 plazmid was transferred by triparental mating into the *A. tumefacierns* strains ALG0 and C58C1(pGV2260). Potato leaves were infected with *Agrobacterium* for two days then regenerated on callus-, and shoot inducing medium selective for transgenic events. The regenerated shoots were cut, and put on selective rooting medium (Figure 3).

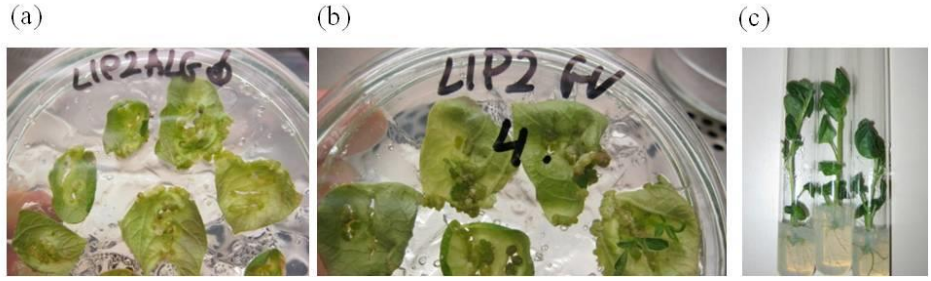


Figure 3: Leaf transformation with *Agrobacterium* (a) on callus inducing (CIM) and (b) shoot inducing (SIM) medium. (c) presumable transgenic lines on rooting medium

For control transformation an „empty" pPZP111 vector was used. To test whether the transformation was successful DNA was isolated from the *in vitro* plants, and tested by the primer pairs designed for *PRLIP2* and *nptII* genes. The PCR positive *in vitro* lines were selected, and the expression of the transgene tested by RT-PCR from the isolated RNA samples.

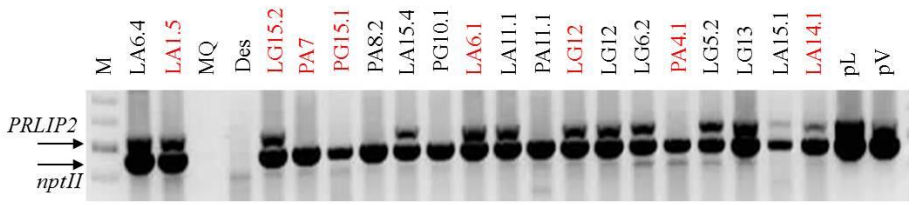


Figure 4: Expression level of *PRLIP2* and *nptII* from *in vitro* plants by RT-PCR. M: 100 bp DNA marker, LA, LG: lines with *nptII* and *PRLIP2* transgenes, PA, PG: lines with *nptII* transgene, pL: pPZP111::PRLIP2, pV: pPZP111, Des: control, non-transformed Desirée, MQ: destilled water. Red letters refer to those lines, which were selected for further experiments.

Five transgenic lines expressing *PRLIP2* with low-, medium-, and high level, and three „empty" control transgenic lines were selected for further experiments (Figure 4).

Characterization of transgenic potato lines expressing the PRLIP2 gene

Phenotypization and the level of drought tolerance of the *PRLIP2* transgenic lines were tested in greenhouse. Under well-watered conditions the LA6.1 and LA14.1 lines grew slower than the control, however, possessed higher tolerance

and biomass production under drought conditions compared to the non-transformed Desirée (Figure 5.).

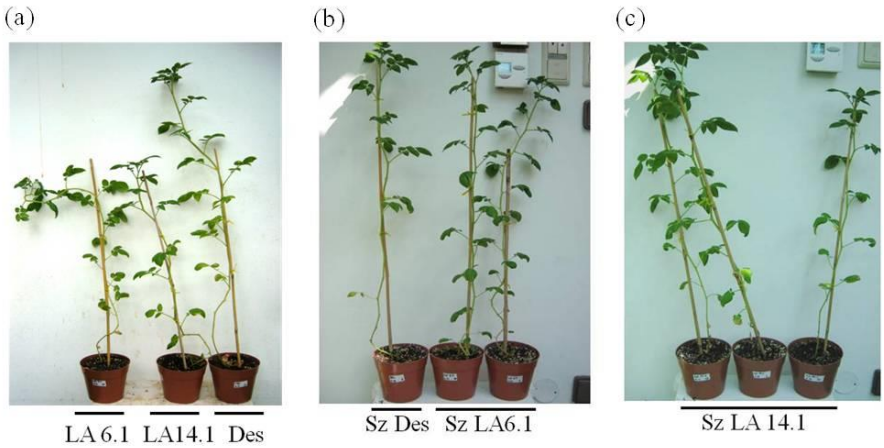


Figure 5. : Plants grown under well-watered (a) and drought stress conditions in greenhouse (b,c).

The tuber yield of the stressed plants was lower in each line than that of the well-watered plants.

After observing the phenotypical changes shown in Figure 5, we started a new trial with more biological replicates. We found that the RWC of all the stressed plants significantly decreased compared to the well-watered Desirée. Thus the expression of *PRLIP2* gene did not defend the leaves from water loss. The RWC content of LA6.1 leaves was significantly lower under optimal conditions than that of the non-transformed leaves. In this experiment, the tuber yield of LA6.1 trangenic line was significantly decreased compared to WT (Figure 6). Like in the first experiment, there were more, but smaller tubers under the stressed plants than under the well-watered plants (Figure 6/b).

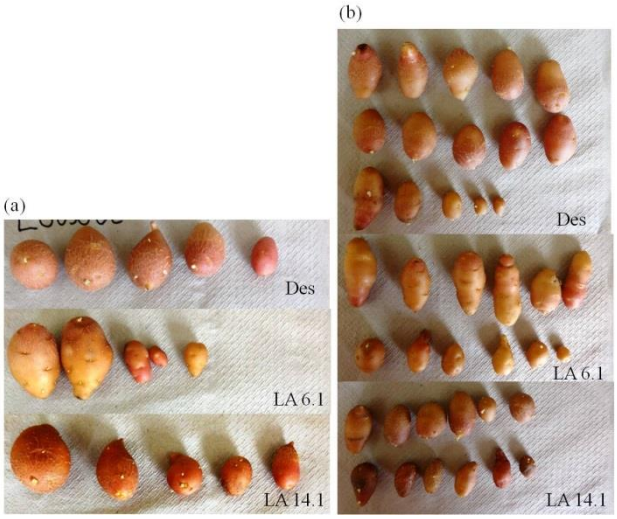


Figure 6.: Tubers developed under (a) well-watered and (b) drought stress conditions in greenhouse.

The sprouting tubers of LA6.1 and LA14.1 lines together with WT were potted in greenhouse. The development of LA14.1, but mostly of LA6.1 plants was slower under optimal conditions than that of the WT plants (Figure 7). As the LA6.1 showed the highest *PRLIP2* expression, we could have concluded that the *PRLIP2* gene is responsible for the slow development. However, there were other lines, like LA1.5, LG12 and LG15.2, which had higher *PRLIP2* expression than LA14.1 (Figure 4), however, had no phenotypical alterations. Thus further eperiments are necessary to make sure whether the phenotypical changes observed were due to *PRLIP2* gene expression or not.

Figure 7.: Well-watered (a) LA6.1, (b) LA14.1 and (c) Desirée plants

New scientific achievements

1. We provided evidence that the expression of *TPS1* gene enhances the drought tolerance of potato. Nevertheless, it alters the phenotype and tuber yield of the plants, and changes the metabolite composition and duration of dormancy in tubers.
2. The metabolite analysis and the applied statistical methods revealed that even small genetical, environmental or physiological changes can highly influence the metabolism.
3. We were succeded in expressing the *PRLIP2* gene of *Arabidopsis* related to priming in potato.
4. We demonstrated that potato lines expressing the *PRLIP2* gene senescence later under drought conditions than wild-type plants, however, this is not reflected by the tuber yield.
5. We hypothesise that there are metabolic and/or hormonal changes in tubers highly expressing the *PRLIP2* gene and these changes are responsible for the developmental delay of the plants grown from tubers.

Conclusions and suggestions

Effects of *TPS1* gene expression on potato

1. The effect of drought stress on metabolite composition of wild-type and TPS1 transgenic leaves is different:

- The starch content of the WT leaves under optimal conditions was much higher than that of the T1 and T2 lines.
- The starch level in the leaves of WT plants decreased drastically during stress, while no significant change in the amount of starch in *TPS1* leaves was detected.
- The level of fructose, glucose and galactose increased only in the WT leaves during stress.

2. The effect of drought stress on tuber development and metabolite composition of wild-type and TPS1 transgenic plants is different:

- In our study, periodic drought resulted in an average yield loss of 50% in WT, while only 20-40% in *TPS1* transgenic plants.
- The *TPS1* tubers differed morphologically from the WT tubers.
- 33 metabolites were detected in tubers. Based on MANOVA statistics, amounts of 13 metabolites were significantly affected by the *TPS1* transgene in tubers.

3. The effect of storage on metabolite composition of wild-type and TPS1 transgenic tubers is very similar:

- The level of asparagine increased in both WT and *TPS1* tubers during storage.

- In tubers of WT and *TPS1* transgenic lines developed under optimal conditions the mannose and phenylalanine content increased during storage. In contrast, concentrations of these compounds were not altered or even were decreased during storage in tubers developed under stress conditions. The physiological importance of these differences remains obscure.

4. Expression of the TPS1 gene affects the duration of tuber dormancy

- *TPS1* tubers were delayed in sprouting compared with WT tubers.
- The general changes in metabolite concentrations in stored WT and *TPS1* tubers were very similar and depended mainly on the environmental conditions in which the plants were grown rather than on the genotype of the plants. This result indicates that the differences detected between the freshly harvested and stored tubers are more likely to reflect the tuber aging process rather than the developmental stage of sprouting.
- The T6P content of tubers did not correlate with the level of *TPS1* expression.

The expression of the *E. coli* TPS-encoding gene *OtsA* driven by the strong tuber-specific patatin promoter *B33* significantly delayed tuber sprouting compared with WT. The delay in the sprouting of the *B33-OtsA* lines correlated with the T6P content of the tubers. Thus, it was concluded that the T6P levels either directly or indirectly affect tuber dormancy (DEBAST et al., 2011). In the T1 and T2 transgenic lines, the yeast *TPS1* gene was expressed from the drought-inducible *StDS2* promoter. However, due to "leaky" transcriptional regulation, the *TPS1* gene was also expressed in the tubers of well-watered plants. Although this expression did not result in a significant increase in the T6P level, the dormancy period of the transgenic tubers was prolonged. Thus, although our result supports the previous finding that TPS has a role in the maintenance of dormancy, it indicates that T6P is unlikely to have an effect on the duration of dormancy. Results of ANTAL et al. (2013) reflects on this finding by providing indirect evidence on interaction of TPS1 with the StubGAL83 subunit of the protein kinase complex StubSNF1. SNF1

is a key transcriptional regulator that responds to carbon and energy supply (COELLO et al., 2011). Disruption of its balance results in changes in metabolism and development. Considering all these, in the fututre, one should select those genes to improve drought tolerance of potato by biotechnological methods, which do not influence the plant metabolism.

Effects of *PRLIP2* expression on potato

- Expression of the *PRLIP2* gene is not able to prevent the water loss of leaves upon drought stress.
- Expression of the *PRLIP2* gene is not able to prevent the tuber yield loss under drought stress conditions.
- Expression of the *PRLIP2* gene has no effect on the duration of tuber dormancy.
- Two out of five *PRLIP2* lines grew slower than WT, however, no correlation between the expression levels of the transgene and the growth rate of the plants could be established.

The RT-PCR results showed no obvious correlation between the transgene expression and the phenotypical changes. Still, we presume that there is a kind of relationship between the *PRLIP2* expression and the pleiotrophic alterations, since the probability that two out of five independent transgenic lines have highly similar phenotype is very low. It is worth considering quantifying the expression levels of the transgene by qRT-PCR. Measuring and correlating the presumed lipase activity of PRLIP2 to the phenotypical changes would also facilitate understanding the function of the gene. In *Arabidopsis*, it was found that the level of lipase expression is related to morphogenesis (MATSUI et al., 2004; HONG et al., 2005), and that lipase activity is influenced by biotic-, and abiotic stress, pathogen infection, and by salycilic acid and etilene treatment (JAKAB et al., 2003; NARUSAKA et al., 2003; LO et al., 2004).

The novelty of our work is in introducing and expressing the *PRLIP2* gene in potato, and testing the new transgenic lines under optimal and drought stress conditions in greenhouse. We found two *PRLIP2* lines with increased drought tolerance, a similar phenomenon found by HONG et al. (2008) testing the *CaGLIP1* transgenic *Arabidopsis* lines. Expression of *PRLIP2* avoided the two lines from early senescence under drought conditions. Since the vegetative development of these *PRLIP2* lines was also delayed, we believe that further investigation of them would help to discover the relationship between plant development and senescence.

References

ANTAL F, KONDRÁK M, KOVÁCS G, BÁNFALVI Z (2013): Influence of the StubSNF1 kinase complex and the expression of the yeast TPS1 gene on growth and tuber yield in potato. *Plant Growth Regul.,* 69, 51-61.p.

BRADFORD, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72, 248-254. p.

BRUGIERE, N., DUBOIS, F., LIMAMI, A. M., LELANDAIS, M., ROUX, Y., SANGWAN, R. S., HIREL, B. (1999): Glutamine synthetase in the phloem plays a major role in controlling proline production. *The Plant Cell*, 11, 1995–2012. p.

COELLO, P., HEY, S.J., HALFORD, N.G. (2011): The sucrose non-fermenting-1-related (SnRK) family of protein kinases: potential for manipulation to improve stress tolerance and increase yield. J. Exp. Bot., 62, 883-893. p.

CORTINA, C., CULIÁÑEZ-MACIÀ, F.A. (2005): Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Sci.,* 169, 75-82. p.

DEBAST, S., NUNES-NESI, A., HAJIREZAEI, M.R., HOFMANN, J., SONNEWALD, U., FERNIE, A.R., BÖRNKE, F. (2011): Altering Trehalose-6-Phosphate Content in Transgenic Potato Tubers Affects Tuber Growth and Alters Responsiveness to Hormones during Sprouting. *Plant Physiology,* Vol. 156, 1754–1771. p.

DEBLAERE, R., BYTEBIER, B., DE GREVE, H., DEBROEK, F., SCHELL, J., VAN MONTAGU, M., LEEMANS, J. (1985): Efficient octopine Ti plasmid

derived vectors of Agrobacterium mediated gene transfer to plants. Nucl. Acids Res., 13, 4777-4788. p.

DIETZE, J., BLAU, A. AND WILLMITZER, L. (1995) Agrobacterium-mediated transformation of potato (Solanum tuberosum). In Gene Transfer to Plants (Potrykus, I. és Spangenberg, G. szerk.). Berlin: Springer-Verlag, 24–29. p.

HONG, J.K., CHOI, H.W., HWANG, I.S., KIM, D.S., KIM, N.H., CHOI, D.U. S., KIM, Y.J., HWANG, B.K. (2008): Function of a novel GDSL–type pepper lipase gene, CaGLIP1, in disease susceptibility and abiotic stress tolerance. *Planta*, 227(3):539-558. p.

HONG, J.K., HWANG, B.K. (2005): Induction of enhanced disease resistance and oxidative stress tolerance by overexpression of pepper basic PR-1 gene in Arabidopsis. *Physiol Plant.*, 124:267–277. p.

HOOD, E.E., CHILTON, W.S., CHILTON, M.D., FRALEY, R.T. (1984): T-DNA and opine synthetic loci in tumors incited by Agrobacterium tumefaciens A281 on soybean and alfalfa plants. *J Bacteriol.*, 168(3):1283–1290. p.

INOUE, H., NOJIMA, H., OKAYAMA, H. (1990): High efficiency transformation of Escherichia coli with plasmids. Gene, 96, 23-28. p.

IORDACHESCU, M., IMAI, R. (2008): Trehalose biosynthesis in response to abiotic stresses. *Integr Plant Biol.*, 50: 1223–1229. p.

JAIN, N.K., ROY, I. (2009): Effect of trehalose on protein structure. *Protein Sci.*, 18: 24–36. p.

JAKAB, G., COTTIER, V., TOQUIN, V., RIGOLI, G., ZIMMERLI, L., MÉTRAUX, J.P., MAUCH-MANI, B. (2001): Beta-aminobutyric acid-induced resistance in plants. *Eur. J. Plant Pathol.*, 107:29-37. p.

JAKAB, G., MANRIQUE, A., ZIMMERLI, L., MÉTRAUX, J.P., MAUCHI-MANI, B. (2003): Molecular characterization of a novel lipase-like pathogen-inducible gene family of *Arabidopsis*. *Plant Physiol* 132:2230–2239. p.

JAKAB, G., TON, J., FLORS, V., ZIMMERLI, L., MÉTRAUX, J.P., MAUCH-MANI, B. (2005): Enhancing Arabidopsis salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiology*, 139, 267–274. p.

JEZEK, P., HLUSEK, J., LOSAK, T., JUZL, M., ELZNER, P., KRACMAR, S., MARTENSSON, A. (2011): Effect of foliar application of selenium on the

content of selected amino acids in potato tubers (Solanum tuberosum L.). *Plant, Soil & Environment*, 57, 315-320. p.

KARIM, S., ARONSSON, H., ERICSON, H., PIRHONEN, M., LEYMAN, B., WELIN, B., MÄNTYLÄ, E., PALVA, E.T., VAN DIJCK, P., HOLMSTRÖM, K.O. (2007): Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. *Plant Mol Biol*;64(4):371-86. p.

KONDRÁK, M., MARINCS, F., ANTAL, F., JUHÁSZ, Z., BÁNFALVI, Z. (2012): Effects of yeast trehalose-6-phosphate synthase 1 on gene expression and carbohydrate contents of potato leaves under drought stress conditions. *BMC Plant Biol.*, 30;12:74. p.

KONDRÁK, M., MARINCS, F., KALAPOS, B., JUHÁSZ, Z., BÁNFALVI, Z. (2011): Transcriptome analysis of potato leaves expressing the trehalose-6-phosphate synthase 1 gene of yeast. *PLoS One*. 6(8):e23466

LEGAY, S., LEFÈVRE, I., LAMOUREUX, D., BARREDA, C., LUZ, R.T., GUTIERREZ, R., QUIROZ, R., HOFFMANN, L., HAUSMAN, J.F., BONIERBALE, M., EVERS, D., SCHAFLEITNER, R. (2011): Carbohydrate metabolism and cell protection mechanisms differentiate drought tolerance and sensitivity in advanced potato clones (Solanum tuberosum L.). *Funct Integr Genomics*, 11:275–291. p.

LUNN, J.E., FEIL, R., HENDRIKS, J.H., GIBON, Y., MORCUENDE, R., OSUNA, D., SCHEIBLE, W.R., CARILLO, P., HAJIREZAEI, M.R., STITT, M. (2006): Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. *Biochemical Journal, 397*, 139-148. p.

MAGGIO, A., CARILLO, P., BULMETTI, G.S., FUGGI, A., BARBIERI, G., DE PASCALE, S. (2008): Potato yield and metabolic profiling under conventional and organic farming. *Europ. J. Agronomy*, 28, 343–350. p.

MATSUI, K., FUKUTOMI, S., ISHII, M., KAJIWARA, T. (2004): A tomato *lipase homologous to DAD1* (*LeLID1*) is induced in post-germinative growing stage and encodes a triacylglyceral lipase. *FEBS Lett*., 569:195–200. p.

MIRANDA, J.A., AVONCE, N., SUÁREZ, R., THEVELEIN, J.M., VAN DIJCK, P., ITURRIAGA, G. (2007): A bifunctional TPS-TPP enzyme from yeast confers tolerance to multiple and extreme abiotic-stress conditions in transgenic *Arabidopsis*. *Planta, 226*, 1411-1421. p.

NARUSAKA, Y., NARUSAKA, M., SEKI, M., FUJITA, M., ISHIDA, J., NAKASHIMA, M., ENJU, A., SAKURAI, T., SATOU, M., KAMIYA, A., PARK, P., KOBAYASHI, M., SHINOZAKI, K. (2003): Expression Profiles of *Arabidopsis Phospholipase A IIA* Gene in Response to Biotic and Abiotic Stresses. *Plant Cell Physiol*. (11):1246-52. p.

PRIME-A-PLANT GROUP: CONRATH, U., BECKERS, G.J.M., FLORS, V., GARCÍA-AGUSTÍN, P., JAKAB, G., MAUCH, F., NEWMAN, M.A., PIETERSE, C.M.J., POINSSOT, B., POZO, M.J., PUGIN, A., SCHAFFRATH, U., TON, J., WENDEHENNE, D., ZIMMERLI, L., MAUCH-MANI, B. (2006): Priming: Getting Ready for Battle, *The American Phytopathological Society,* Vol. 19, No. 10, 1062–1071. p.

SAMBROOK, J., FRITCH, E.F., MANIATIS, T. (1989): Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor, NewYork: Cold Spring Harbor Laboratory Press.

SCHAUER, N., ZAMIR, D., FERNIE, A.R. (2004): Metabolic profiling of leaves and fruit of wild species tomato: a survey of the Solanum lycopersicum complex. *Journal of Experimental Botany,* Vol. 56, No. 410.

SHURE, M ., WESSLER, S ., AND FEDOROFF, N . (1983): Molecular identification and
isolation of the Waxy locus in maize, *Cell*, 35, 225-233. p.

STIEKEMA, W.J., HEIDKAMP, F., DIRKSE, W.G., VAN BECKUM, J., DE HAAN, P., BOSH, T., LAUWERSE, J.D. (1988): Molecular cloning and analysis of four tuber specific mRNAs. Plant Mol. Biol., 11, 255-269. p.

STILLER, I., DULAI, S., KONDRÁK, M., TARNAI, R., SZABÓ, L., TOLDI, O., BÁNFALVI, Z. (2008): Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of Saccharomyces cerevisiae. *Planta*, 227 299–308. p.

TEIXEIRA, J., PEREIRA, S. (2007): High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. *Environmental & Experimental Botany*, 60, 121-126. p.

YEO, E.T., KWON, H.B., HAN, S.E., LEE, J.T., RYU, J.C., BYUN, M.O. (2000): Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (TPS1) gene from Saccharomyces cerevisiae. *Mol. Cells*, 10, 263-268. p.

YU, T.S, KOFLER, H., HAUSLER, R.E, HILLE, D., FLŸGGE, U.I., ZEEMAN, S.C., SMITH, A.M., KOSSMANN, J., LLOYD, J., RITTE, G., STEUPE, M., LUEA, W-L., CHEN, J., WEBER, A. (2001) The Arabidopsis sex1 mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. *Plant Cell*,13:1907-1918. p.

Publications

Scientific articles

1. Kondrák M, Marincs F, Kalapos B, **Juhász Z**, Bánfalvi Z (2011) Transcriptome analysis of potato leaves expressing the *trehalose-6-phosphate synthase 1* gene of yeast. PLoS ONE 6(8): e23466 doi: 10.1371/journal.pone.0023466

IF: 4.411, Cit: 9

2. Kondrák M, Marincs F, Antal F, **Juhász Z**, Bánfalvi Z (2012) Effects of yeast trehalose-6-phosphate synthase 1 on gene expression and carbohydrate contents of potato leaves under drought stress conditions. BMC Plant Biology 12:74 doi: 10.1186/1471-2229-12-74

IF: 4.09, Cit: 7

3. **Juhász Z**, Dancs G, Marincs F, Vossen M, Allefs S, Bánfalvi Z (2014) Vitamin C, B5, and B6 contents of segregating potato populations detected by GC-MS: a method facilitating breeding potatoes with improved vitamin content. Plant Breeding 133: 515-520 doi: 10.1111/pbr.12169)

IF: 1.338, Cit: 0

4. Uri C, **Juhász Z**, Polgár Z, Bánfalvi Z (2014) A GC-MS-based metabolomics study on the tubers of commercial potato cultivars upon storage. Food Chemistry 159: 287-292 doi: 10.1016/j.foodchem.2014.03.010

IF: 3.259, Cit: 1

5. **Juhász Z,** Balmer D, Sós-Hegedűs A, Vallat A, Mauch-Mani B, Bánfalvi Z (2014) Effects of drought stress and storage on the metabolite and hormone

contents of potato tubers expressing the yeast *trehalose-6-phosphate synthase 1* gene. Journal of Agricultural Science 6: 142-166 doi: 10.5539/jas.v6n5pxx

IF: 2.891, Cit: 0

6. Sós-Hegedűs A, **Juhász Z**, Poór P, Kondrák M, Antal F, Tari I, Mauch-Mani B, Bánfalvi Z (2014) Soil drench treatment with ß-aminobutyric acid increases drought tolerance of potato. PLoS ONE 9(12): e114297. doi: 10.1371/journal.pone.0114297

IF: 3.534, Cit: 0

Proceedings

1. **Juhász Z**, Kocsy G, Galiba G, Bánfalvi Z (2012) Metabolic changes in a *VRN-1* mutant wheat line. Proceedings of the 11th Alps-Adria Scientific Workshop, Smolenice, Slovakia, 26th-31st March, Harcsa M (ed), Növénytermelés Suppl. 5 pp. 443-446. doi: 10.1556/Novenyterm.61.2012.Suppl.5
2. **Juhász Zs**, Boldizsár Á, Kocsy G, Marincs F, Galiba G, Bánfalvi Zs (2014) A búza 5A kromoszómája által befolyásolt metabolit változások a hideghez való alkalmazkodás és a vegetatív/generatív átmenet idején. XX. Növénynemesítési Tudományos Nap, Budapest, 2014. márc. 18., Növénynemesítés a megújuló mezőgazdaságban c. könyv, szerk.: Veisz O., kiadó: MTA Agrártudományok Osztályának Növénynemesítési Tudományos Bizottsága, 210-214. old.

Abstracts

1. **Juhász Z**, Kondrák M, Marincs F, Bánfalvi Z (2010) Transcriptional and metabolic changes in drought tolerant potato expressing the *TPS1* gene of yeast. Advances in Metabolic Profiling, Firenze, Italy, November 9-10, Abstract p. 119

2. **Juhász Zs**, Bánfalvi Zs (2011) A *TPS1* gént expresszáló, szárazságtűrő burgonya vonalak gumóinak metabolit vizsgálata. XVII. Növénynemesítési Tudományos Napok, Budapest, április 27, Összefoglaló 106. old.
3. Kondrák M, Marincs F, **Juhász Zs**, Antal F, Bánfalvi Zs (2011) A *TPS1* gént expresszáló, szárazságtűrő burgonya vonalak leveleinek transzkripciós és metabolikus jellemzése. XVII. Növénynemesítési Tudományos Napok, Budapest, április 27, Összefoglaló 107. old.
4. Kondrák M, Marincs F, Antal F, **Juhász Z**, Bánfalvi Z (2012) Molecular changes elicited by drought stress in *TPS1*-expressing potato plants. Plant Abiotic Stress Tolerance, International Conference, Vienna, Austria, February 22-25, Abstract p. 63
5. **Juhász Zs**, Kocsy G, Galiba G, Bánfalvi Zs (2012) A búza *Vrn-1* génjének szerepe a vegetatív/reproduktív átmenet metabolikus folyamataiban. XVIII. Növénynemesítési Tudományos Napok, Budapest, március 6, Összefoglaló 92. old.
6. **Juhász Z**, Balmer D, Mauch-Mani B, Bánfalvi Z (2012) Metabolic and hormonal changes in tubers of drought tolerant potato plants expressing the *TPS1* gene of yeast. Advances in Plant Breeding and Plant Biotechnology in Central Europe, Pannonian Plant Biotechnology Workshops, Debrecen, June 4-6, Abstract p. 45-46
7. Kondrák M, Marincs F, Antal F, **Juhász Z**, Bánfalvi Z (2012) Transcriptional and metabolic changes elicited by drought stress in *TPS1*-expressing potato leaves. Advances in Plant Breeding and Plant Biotechnology in Central Europe, Pannonian Plant Biotechnology Workshops, Debrecen, June 4-6, Abstract p. 47-48
8. Sós-Hegedűs A, **Juhász Z**, Antal F, Kondrák M, Mauch-Mani B, Bánfalvi Z (2012) Chemical priming for drought tolerance in potato. 9th Solanaceae Conference, Neuchatel, Switzerland, August 26-30, Abstract p. 185

9. Bánfalvi Z, **Juhász Z**, Dancs G, Marincs F, Vossen M, Allefs S (2012) Vitamin C, B5, and B6 contents of segregating diploid potato populations. 9th Solanaceae Conference, Neuchatel, Switzerland, August 26-30, Abstract p. 210

10. Bánfalvi Z, Kondrák M, **Juhász Z**, Sós-Hegedűs A, Marincs F, Stiller I, Balmer D, Mauch-Mani B (2013) Improving drought tolerance of potato. The 17th Joint Meeting of EAPR Breeding and Varietal Assessment Section EUCARPIA Section Potatoes, Hévíz, Hungary, June 30-July 4, Abstract p. 12

11. Juhász Z, Uri C, Polgár Z, Bánfalvi Z (2013) Metabolomics study on stored potato tubers of Hungarian potato cultivars. The 17th Joint Meeting of EAPR Breeding and Varietal Assessment Section EUCARPIA Section Potatoes, Hévíz, Hungary, June 30-July 4, Abstract p. 21.