

Katharina Wellpott<sup>1\*</sup>, Anna Maria Jozefowicz<sup>2</sup>, Hans-Peter Mock<sup>2</sup>, Philipp Meise<sup>3</sup>,  
Annegret Schum<sup>3</sup>, Traud Winkelmann<sup>1</sup>, Christin Bündig<sup>1</sup>

## **Identification of candidate proteins in drought stress tolerant and sensitive starch potato genotypes (*Solanum tuberosum* L.) for biomarker development**

---

<sup>1</sup> Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany;  
wellpott@baum.uni-hannover.de, buendig@baum.uni-hannover.de,  
traud.winkelmann@zier.uni-hannover.de

<sup>2</sup> Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), OT Gatersleben,  
Corrensstraße 3, 06466 Seeland, Germany; jozefowicz@ipk-gatersleben.de,  
mock@ipk-gatersleben.de

<sup>3</sup> [ehemals] Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen,  
OT Groß-Lüsewitz, Rudolf-Schick-Platz 3, 18190 Sanitz, Germany

\* Korrespondenz: wellpott@baum.uni-hannover.de



**DGG-Proceedings 2021, Vol. 10**

Short Communications – Peer Reviewed, Open Access

Deutsche Gartenbauwissenschaftliche Gesellschaft e. V. (DGG)

German Society of Horticultural Sciences

[www.dgg-online.org](http://www.dgg-online.org)

Annual Conference DGG and BHGL

09.03.2021, Stuttgart (online), Germany

## Identification of candidate proteins in drought stress tolerant and sensitive starch potato genotypes (*Solanum tuberosum* L.) for biomarker development

Katharina Wellpott<sup>1\*</sup>, Anna Maria Jozefowicz<sup>2</sup>, Hans-Peter Mock<sup>2</sup>, Phillip Meise<sup>3</sup>,  
Annegret Schum<sup>3</sup>, Traud Winkelmann<sup>1</sup>, Christin Bündig<sup>1</sup>

<sup>1</sup> Leibniz Universität Hannover, Germany

<sup>2</sup> Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Germany

<sup>3</sup> [ehemals] Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen,  
Germany

### Abstract

Due to the foretold climate change, droughts will become more frequent in spring and early summer. This time represents the period of highest vegetative growth in potato development, which highly correlates with later starch yield. Since potato plants are depending on a high supply of nitrogen and water during this period, breeding starch potato varieties with high nitrogen efficiency and increased drought tolerance is of great interest especially for the production of starch for industrial use. Drought tolerance is based on the activation and regulation of specific pathways that lead to physiological, morphological and biochemical stress responses of the plant. In a previous proteomic study, we found 138 differentially abundant proteins by comparing a drought stress tolerant and a sensitive starch potato genotype after application of osmotic stress in an *in vitro* test system on solidified medium.

In the present study, the drought-tolerant starch potato genotypes 'Eurostarch' and 'Tomba' as well as the more sensitive genotypes 'Kiebitz' and 'Kolibri' had been submitted to drought stress in rain-out shelter experiments in 2013 and 2015. Proteins were extracted from leaf material of both experiments to identify divergently responding pathways and potential marker proteins for drought tolerance. By means of liquid chromatography-mass spectrometry (LC-MS) and subsequent bioinformatic analysis, 1535 proteins were identified. Out of these, 233 showed a significantly different abundance between control and stressed plants in at least one genotype. Proteins with increased abundances that were exclusively detected in the tolerant genotypes in the drought stress treatment were for example involved in ROS detoxification. These proteins will be of great interest for gene expression studies and will be validated in further experiments involving drought stress *in vivo* as well as osmotic stress treatments *in vitro*.

### 1. Introduction, Knowledge, Objectives

*Solanum tuberosum* L. is the world's fourth most important crop after rice, maize and wheat. In 2020, the planted acreage for starch potatoes in Germany was about 58,000 ha (BLE 2021). More than 20 % were used for the production of starch potatoes for industrial purposes, e. g. to produce biogas, bioethanol or adhesives (Röper 2002).

Despite increasing acreage, starch yields decreased dramatically from 2017/18 to 2020. The reason was found in the increasing drought spells during the early growth phases of potato plants. Strong winds and warm or even hot days in early summer months have led to high evaporation and increased land aridity. Precipitation of previous years could not compensate for this aridity (BLE 2021). In many cases the soil-borne water was not enough to supply the shallow-rooted potato plants and irrigation became obligatory. However, irrigation is both labor- and cost-intensive (Iwama and Yamaguchi 2006).

If the vegetative growth phase of *Solanum tuberosum* coincides with the expected dry periods, this will have severely negative consequences for the potato yield and quality at harvest time. Besides inhibition of shoot growth, shift of root/shoot ratio towards the roots and reduced leaf size, drought stress also inhibits photosynthesis, reduces tuber size and –quality, and enhances ROS production (George et al. 2017; Jozefowicz et al. 2017). Thus, selecting and breeding of drought stress tolerant potato genotypes is of high economic and ecological interest.

In previous works, the proteome of potato under nitrogen deficiency and drought stress was characterized in the project PROKAR to increase the knowledge for breeding nitrogen efficient and drought stress tolerant starch potatoes. Therein, differences in the proteome were observed in differently responding genotypes. For example, proteins of proteolysis, specific stress proteins, and proteins of ROS-detoxification were found to play a role. In addition, differences in metabolite concentrations (proline, glycine, sucrose) were found and morphological differences between genotypes were detected in rain-out shelter experiments (Bündig et al. 2016a; 2016b; 2016c). The objectives of our current collaborative project VALPROKAR result from findings in the project PROKAR (FNR 2016; 2022). In VALPROKAR, we are working on the validation of candidate proteins for drought stress tolerance using leaf material of drought-stressed and non-stressed potato plants and plants from a rain-out-shelter experiment. Proteins of interest will be evaluated for their usability as biomarkers in drought stress tolerance breeding for tolerance of starch potato. In this study, we present the identification of new proteins of interest showing differences in abundance between different potato genotypes under drought stress.

## 2. Data, Methods and Approach

Based on results of Meise et al. (2019), the drought stress tolerant genotypes 'Eurostarch' and 'Tomba' and the drought stress sensitive genotypes 'Kiebitz' and 'Kolibri' were selected for the proteomic analyses. These genotypes were identified in two rain-out shelter experiments based on tuber and starch yields at the end of the growth period (Meise et al. 2019). In our study, we analyzed plant material from these two experiments. These experiments were performed in 2013 and 2015 (Meise et al. 2019). After tuber induction, plants were exposed for five days to drought stress at < 20 % water holding capacity (WHC), whereas control plants were cultivated at 60 % WHC. On the 5th day of stress treatment, the third leaflet of the youngest fully developed pinnate leaf was harvested from stressed and control plants (Fig. 1).

Per genotype, four leaf samples per experiment were analyzed. About 100 mg frozen leaf sample was grinded using 3 mm steel beads and a mixing mill (Retsch, Haan, Germany). Protein extraction was performed using the TCA method (Tsugita and Kamo 1999). TCA solutions A and B consisted of 20 mM DTT instead of 0.07 % (v/v) 2-mercaptoethanol. Next steps were the quantification of the proteins using the 2D-Quant Kit (GE Healthcare,

Munich, Germany) and the filter-based digestion with trypsin (Jozefowicz et al. 2020). Peptides were taken up in 2 % acetonitrile (ACN), 0.1 % formic acid (FA) (v/v), separated by LC (Dionex UltiMate™ 3000 RSLCnano System from Thermo Fisher Scientific, Dreieich, Germany) using analytical column Acclaim PepMap RSLC C18 (50 cm x 75 µm, Thermo Fisher Scientific) and measured by ESI-QTOF-MS/MS (Impact II from Bruker Daltonics, Bremen, Germany). LC-MS data were analyzed using Progenesis QI (Version 3.0, Nonlinear Dynamics, Newcastle upon Tyne, U.K.) for mass correction, alignment, normalization, peak picking, quantification, and statistics. All samples were normalized to one sample automatically selected as normalization reference, using 'normalise to all proteins' default method. MS/MS spectra were used for peptide identification with Mascot v2.5.1.

The database analysis was based on the potato genome Phureja DM1-3 (PGSC\_DM\_v3.4\_pep\_representative, 39,031 entries) (Potato Genome Sequencing Consortium et al. 2011) matching against NCBI entries via Blast2GO. The following search parameters were applied: 15 ppm peptide mass tolerance, 0.05 Da fragment mass tolerance, one missed cleavage allowed, carbamidomethylation as fixed modification, and methionine oxidation as variable modification. Proteins identified with at least two unique peptides and a maximum fold change > 1.5 were quantified. Only proteins that passed the significance limits were considered as differentially abundant proteins (students t-test p value < 0.05 and fold change stress/control <0.66 or >1.5).

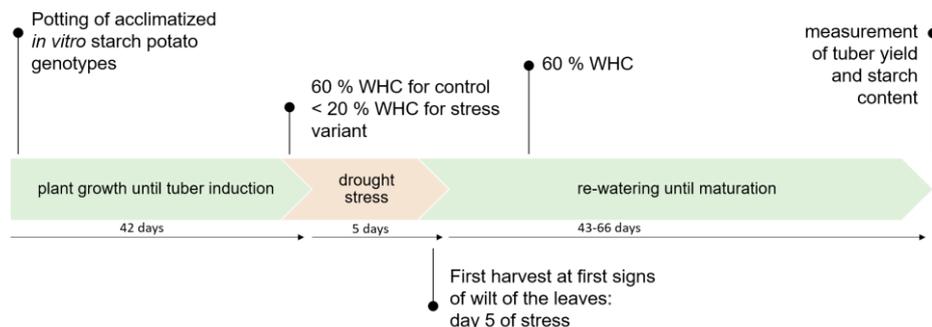


Figure 1: Experimental setup of rain-out-shelter experiments in 2013 and 2015. WHC: water holding capacity. Analyzed genotypes were 'Eurostarch', 'Tomba', 'Kiebitz' and 'Kolibri'. Plants were grown until tuber induction. Leaves were harvested on the 5th day of drought stress. Measurement of tuber yield and starch content took place after maturation.

### 3. Results

In total, 1,535 proteins were found in all samples. Out of these, 444 passed the maximum fold change > 1.5 and 233 proteins of these showed different abundance in at least one of the four genotypes when comparing stressed and control plants passing the student's t-Test (p-value < 0.05) and a fold change stress/control < 0.66 or > 1.5 (Fig. 2). Most of the differentially abundant proteins were identified in the tolerant genotypes 'Eurostarch' and 'Tomba'. For example, 93 proteins showed lower abundance in stressed plants compared to control plants of cultivar 'Eurostarch' in 2013, whereas 54 proteins showed higher abundance. In cultivar 'Tomba', there were 85 proteins less abundant in stressed plant samples, whereas in 'Kolibri' and in 'Kiebitz'

69 and 39 proteins showed lower abundance in stressed plants, respectively. Comparing proteins with different abundance in drought tolerant and sensitive genotypes, we found that 14 proteins were specifically downregulated in stressed plants of the tolerant genotypes 'Eurostarch' and 'Tomba' in 2013 (13 proteins in 2015), whereas eight proteins were of higher abundance in both experimental years. These proteins are, among others, involved in plant hormone regulation and ROS detoxification and could be a reason for higher drought stress tolerance of 'Eurostarch' and 'Tomba'.

From these proteins, a total of 15 were selected for future studies according to following criteria: All of these proteins were identified in both experimental years in all genotypes. The selected proteins were significantly differentially abundant in both tolerant genotypes in at least one experimental year. Selected proteins are listed in Table 1. Two proteins were differentially abundant in both years and both genotypes (PGSC0003DMT400011762 Protein invertase inhibitor and PGSC0003DMT40005410 Protein subtilisin-like protease-like).

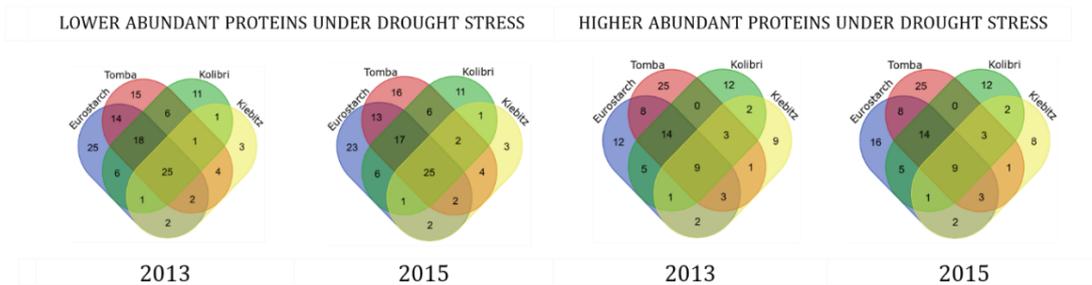


Figure 2: Venn diagrams presenting proteins with differential abundance in drought stressed and control plants of four starch potato genotypes in 2013 and 2015. n= 4 (one leaf sample from the third leaflet of the youngest fully developed leaf from four plants)

Nine proteins were higher abundant under drought stress. Besides some proteins involved in plant hormone regulation, abiotic stress response and proteins of secondary metabolism, some differentially abundant proteins were identified which support the plant in coping with oxidative stress such as 26S protease regulatory subunit 6b homolog, cbs domain-containing protein mitochondrial-like and 2-alkenal reductase (NADP(+)-dependent)-like. There is also one protein of lower abundance under drought stress which is connected to ROS detoxification (catalase isozyme 1-like protein).

Table 1: Proteins with differential abundance in drought stressed and control plants. Higher abundance in stress treatment (S) than in controls (C) in the rather tolerant genotypes 'Eurostarch' and 'Tomba': 1-9. Lower abundance: 10-15.

	<b>Protein</b>	<b>FC 'Eurostarch' [S/C]</b>	<b>FC 'Tomba' [S/C]</b>	<b>year</b>	<b>Pathway</b>
1	phosphoribosylformylglycine midine cyclo- chloroplastic mitochondrial-like	5.62	5.95	2013	secondary metabolism
2	probable carotenoid cleavage dioxygenase chloroplastic-like	3.65	2.34	2013	hormone regulation, abiotic and biotic stress response
3	26s protease regulatory subunit 6b homolog	1.62	2.53	2013	tolerance to oxidative stress, heat shock protection
4	linoleate 13s-lipoxygenase 2- chloroplastic-like	1.54	1.50	2013	oxylipin biosynthesis
5	cysteine protease inhibitor 1- like	1.52	2.74	2013	inhibition of programmed cell death
6	invertase inhibitor	2.21	1.56	2015	stress response to drought, temperature, salt, ABA
7	cbs domain-containing protein mitochondrial-like	1.87	1.89	2015	ROS detoxification
8	2-alkenal reductase (nadp(+)-dependent)-like	1.72	1.85	2015	ROS detoxification
9	serine mitochondrial-like	1.52	1.94	2015	photorespiratoric enzyme (glycine → serine)
10	catalase isozyme 1-like protein	0.62	0.60	2015	ROS detoxification
11	subtilisin-like protease-like	0.43	0.36	2015	inhibition of leaf senescence
12	peroxidase 51-like	0.64	0.63	2013	NADPH oxidase signaling pathway
13	early tobacco anther 1	0.63	0.63	2013	catalysis of structural changes of molecules
14	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex-like	0.63	0.64	2013	transferase activity
15	high mobility group family	0.48	0.60	2013	mitochondrial electron transport

#### 4. Conclusions

Drought stress in potato leads to many different stress responses. One of them, oxidative stress, can be triggered by several factors, including the imbalance of ROS synthesis and degradation/scavenging (antioxidation) mechanisms and the *de novo* synthesis of ROS as a defense and adaptation mechanism (Demidchik 2015). Hence, tolerant potato plants might reduce these radicals by synthesizing new antioxidants or ROS scavenging elements. Pieczynski et al. (2018) also found genes encoding oxidative stress-related proteins to be connected to drought stress in potato, *A. thaliana* and rice. Furthermore, Liu et al. (2018) reported a role of ROS scavenging proteins during drought stress in potato.

The tolerant genotypes 'Eurostarch' and 'Tomba' withstood short periods of drought stress better than the sensitive genotypes 'Kiebitz' and 'Kolibri'. One reason for this could be the handling of reactive oxygen species. Specific proteins involved in the formation of antioxidants might allow plants to maintain a protective mechanism against oxidative stress, resulting in less growth deprivation.

As a next step, the proteins of interest will be investigated at gene transcript level in order to draw conclusions about the level of regulation. Further metabolite analyses will be performed to characterize the observed pathways and to identify important pathways. Furthermore, the stress response of the plants will be investigated proteomically and metabolomically in further rain-out shelter experiments to study younger plant stages.

#### Literature

Bundesanstalt für Landwirtschaft und Ernährung (BLE) (2021) Bericht zur Markt- und Versorgungslage Kartoffeln 2020:1-65

Bündig C., Jozefowicz A.M., Mock H.-P., Winkelmann T. (2016a) Proteomic analysis of two divergently responding potato genotypes (*Solanum tuberosum* L.) following osmotic stress treatment in vitro. *Journal of Proteomics* 143:227-241

Bündig C., Blume C., Peterhänsel C., Winkelmann T. (2016b) Changed composition of metabolites in *Solanum tuberosum* subjected to osmotic stress in vitro. Is sorbitol taken up?. *Plant Cell Tiss Organ Cult* (2016) 127:195-206

Bündig C., Vu T.H., Meise P., Seddig S., Schum A., Winkelmann T. (2016c) Variability in Osmotic Stress Tolerance of Starch Potato Genotypes (*Solanum tuberosum* L.) as Revealed by an In Vitro Screening: Role of Proline, Osmotic Adjustment and Drought Response in Pot Trials. *Journal of Agronomy and Crop Science* 203:206-218

Demidchik V. (2015) Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. *Environmental and Experimental Botany* 109:212-228

Fachagentur Nachwachsende Rohstoffe e. V. Projektdatenbank <https://www.fnr.de/>

George T.S., Taylor M.A., Dodd I.C., White P.J. (2017) Climate Change and Consequences for Potato Production: a Review of Tolerance to Emerging Abiotic Stress. *Potato Research* 60:239-268

Iwama K. and Yamaguchi J. (2006) Abiotic stresses. In: Gopal J., Khurana S.M. (eds) *Handbook of Potato Production. Improvement and Postharvest Management*. Food Product Press, New York, 231-278

Jozefowicz A.M., Hartmann A., Matros A., Schum A., Mock H.-P. (2017) Nitrogen Deficiency Induced Alterations in the Root Proteome of a Pair of Potato (*Solanum tuberosum* L.) Varieties Contrasting for their Response to Low N. *Proteomics* 17:23-24

Jozefowicz A.M., Döll S., Mock H.P. (2020) Proteomic approaches to identify proteins responsive to cold stress. *Methods in molecular biology* (Clifton, N.J.), 2156:161–170

Liu S., Meng M., Chen Y. (2018) De novo Assembly and Discovery of Genes in Potato (*Solanum tuberosum*) under Drought Stress and Rehydration. *International Journal of Agriculture and Biology* 20:1787-1794

Meise P., Seddig S., Uptmoor R., Ordon F., Schum A. (2019) Assessment of Yield and Yield Components of Starch Potato Cultivars (*Solanum tuberosum* L.) Under Nitrogen Deficiency and Drought Stress Conditions. *Potato Research* 62:193-220

Pieczynski M., Wyrzykowska A., Milanowska K., Boguszewska-Mankowska D., Zagdanska B., Karlowski W., Jarmolowski A., Szweykowska-Kulinska Z. (2018) Genomewide identification of genes involved in the potato response to drought indicates functional evolutionary conservation with Arabidopsis plants. *Plant Biotechnology Journal* 16:603-614

Potato Genome Sequencing Consortium, Xu, X., Pan, S., Cheng, S., Zhang, B., Mu, D., Ni, P., Zhang, G., Yang, S., Li, R., Wang, J., Orjeda, G., Guzman, F., Torres, M., Lozano, R., Ponce, O., Martinez, D., De la Cruz, G., Chakrabarti, S. K., Patil, V. U., ... Visser, R. G. (2011). Genome sequence and analysis of the tuber crop potato. *Nature*, 475(7355):189–195

Röper H. (2002) Renewable Raw Materials in Europe – Industrial Utilisation of Starch and Sugar. *Starch/Stärke* 54:89-99

Tsugita A. and Kamo M. (1999) 2-D electrophoresis of plant proteins. *Methods Mol Biol.* 112:95-97