Schlussbericht
zum Vorhaben

Thema:
Charakterisierung des Proteoms unter Stickstoff- und Wassermangelstress als Grundlage für die züchterische Entwicklung stickstoffeffizienter und trockentoleranter Stärkekartoffeln (PROKAR)

Zuwendungempfänger:
Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK),
Corrensstraße 3,
06466 Stadt Seeland OT Gatersleben

Förderkennzeichen:
22023411

Laufzeit:
01.10.2012 bis 31.12.2015

Monat der Erstellung:
06/2016

Das diesem Bericht zugrundeliegende Vorhaben wurde aufgrund eines Beschlusses des Deutschen Bundestages mit Mitteln des Bundesministeriums für Ernährung und Landwirtschaft (BMEL) über die Fachagentur Nachwachsende Rohstoffe e.V. (FNR) als Projektträger des BMEL für das Förderprogramm Nachwachsende Rohstoffe unterstützt. Die Verantwortung für den Inhalt dieser Veröffentlichung liegt beim Autor.
Abschlussbericht zum Verbundvorhaben PROKAR

Grant recipients: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstraße 3, 06466 Stadt Seeland OT Gatersleben

Project number: 22023411

Project title: Characterization of proteome under nitrogen and water stress as the basis for development of drought tolerant and nitrogen efficient industrial potatoes (PROKAR).

Duration of the project: 01.10.2012 – 31.12.2015

Part A:

Research Group Applied Biochemistry (ABC), Gatersleben - IPK-ABC

Involved scientists: Anna Jozefowicz, Hans- Peter Mock

Work packages:

AP 5. Analysis of quick changes in the potato root proteome (main part of work)

AP 2. In vitro screening under nitrogen deficiency, investigation of proteome changes (collaboration with JKI-Groß Lüsewitz)

AP 3. In vitro screening under water stress, investigation of proteome changes (collaboration with LU Hannover)
1. **Aim of the project**

Potato is very profitable source of starch, which can be used to the production of bioethanol and biogas. The large-scale production of potato, leads however to the high environmental pollution. The main aim of the project was to provide insights on the stress tolerance mechanisms in *Solanum tuberosum*, which can be important for developing new environment friendly breeding strategies. Moreover, developed methodology should contribute to the development of breeding drought-tolerant and nitrogen efficient varieties by shortening the time required for selection and characterization of cultivars.

2. **Scientific and technological goals of the Applied Biochemistry group (IPK Gatersleben).**

The IPK Gatersleben was mostly focused on the work package 5, which was the analysis of quick changes in the potato roots under nitrogen deficiency in the selected tolerant and sensitive genotypes. To complete the knowledge about proteomic reaction to nitrogen deficiency obtained in the work package 2 two additional approaches were applied. In the first part comparison of the plasma membrane (PM) protein composition was performed in order to get an insight in the mechanism leading to the contrasting tolerance of the two genotypes to lower nitrogen levels. The information about abundance of transporters, signaling proteins and other PM proteins was expected. To complete this task the method for PM enrichment, protein digestion and LC-MS measurement needed to be adjusted to the potato root tissue. In the second approach changes in the protein phosphorylation pattern were investigated, as phosphorylation plays a crucial role in biological processes, like signal transduction and metabolic regulation. Changes in the phosphorylation state of proteins followed in form of kinetic should give information how both genotypes react to the stress signal and if there is a time related difference in the early reaction of sensitive and tolerant plants.

3. **Nitrogen deficiency- scientific background.**

The availability of nitrogen is one of the most critical and limiting factor in crop plant production. Every year about 85-90 million metric tons of nitrogenous fertilizers are added to the soil and this is predicted to achieve a level of 250 MMt by the year of 2050 (1). Among the other fertilizers nitrogen is one of the most expensive nutrients and determines therefore the high costs of crop plant production. Another concern about excessive nitrogen fertilization is its loss from the soil giving rise to the environmental pollution (2). Mineral N sources due to the microbial transformations are likely to be oxidized to nitrate and be leached to the ground water causing a great concern from environmental but also health aspects(3). Additionally about 7.5% of the calculated greenhouse effect caused by human activity is related to the carbon and nitrogen gases released from agricultural soils (4). The efficiency of nitrogen recovery from the soil is especially low in the case of potato plant and estimated at around 45%, which is explained by the shallow root system. Additionally potato cultivation is often performed on the sandy soils, which are more susceptible to nitrate leaching (5). Understanding of mechanism responsible for the higher
nitrogen use efficiency is therefore essential to obtain maximal yield and reduce nitrogen fertilization at the same time.

4. Scientific and technological achievements


Nitrogen deficiency experiment was conducted using four potato genotypes. Two of them (Lambada and Topas) were selected as a reference genotypes as it was already described by Schum et al (6) that it’s nitrogen use efficiency differ in the significant way. The second set of genotypes was selected during the rain-out shelter and in vitro screening performed by JKI Groß Lüsewitz. Due to the very low amount of root biomass produced by the starchy potato genotypes the plasma membrane enrichment experiment could only be accomplished with Lambada and Topas. In the further targeted and functional analysis all four genotypes were included were possible.

Plants were grown under constant conditions on Murashige&Skoog medium. The stress conditions were obtained by reduction of initial nitrogen concentration to 1/16. Root material was harvested after 14 days of cultivation (Figure 1).

![Figure 1. In vitro grown Solanum tuberosum after 14 days of treatment](image)
Plasma membrane enriched fraction was obtained by separation of microsomes on the aqueous two phase system according to Hynek et al (7). Plasma membrane (PM) enrichment is a critical step for the membrane proteome analysis. In order to evaluate the effectiveness of membrane isolation Western blot with the compartment antibody markers recognizing H⁺ATPase, voltage-dependent anion channel, V type ATPase, monodehydroascorbate reductase and luminal-binding protein was performed. The plasma membrane marker H⁺ ATPase was about 13 times more abundant in PM fraction than in the original crude extract and 3 times more than in the microsomal fraction (Figure 2). Cytosolic marker MDAR and mitochondrial marker VDAC, which were highly abundant respectively in soluble and lower phase, were barely detectable in the upper phase. Although BiP was still present in the PM fraction the intensity was 3 times lower than in the microsomal fraction, where the reaction of the antibodies was the strongest. The V ATPase was detected at the similar level in all membrane fractions. These results confirmed that the upper phase of two phase system contained enriched PM.

Plasma membrane proteins were further delipidated, digested with trypsin and injected to the LC-MS analysis. About 400 of proteins were identified, but only 70 of them were significantly altered in abundance as a result of nitrogen deprivation.

The differences between protein profiles were illustrated using principal component analysis. PCA clear grouping between two genotypes as well as between control and nitrogen deficient plants. The variability between stress-treated biological replicates was observed (Figure 3).
For further functional studies proteins with highest fold change, possible role in the nitrogen transport and adaptation to low nutrient level were selected. Another requirement was the number of predicted transmembrane helices (at least 1) in order to assure that chosen candidate protein is integral membrane protein.

The protein showing highest change in the expression pattern was high-affinity nitrate transporter (NRT2.1). The results of study showed opposite regulation of NRT in both genotypes. In reaction to nitrogen deficiency there is a decrease in NRT expression, whereas in Topas nitrate transporter is about nine times more abundant. The Western blot with antibodies against NRT2.1 confirmed presence of protein on much higher level in the tolerant genotype not only during low but also high level nitrogen treatment (Figure 4). The experiment included also the starch potato genotypes Tomba and Kiebitz. The difference between both genotypes in NRT expression was not as high as in case of Topas and Lambada but still we could observe higher signal coming from efficient genotype.
Nitrate transport occurs via symport mechanism driven by pH gradient across the membrane and the activity of plasma membrane H\textsuperscript{+}ATPase plays a fundamental role in this process. In our study 3 isoforms of H\textsuperscript{+}ATPase were identified and all of them showed the highest abundance in the efficient genotype during nitrogen deprivation (Figure 5).

The other proteins affected during nitrogen deficiency were the members of plasma membrane intrinsic proteins family. Two isoforms of aquaporin 1 (PIP1;1 and PIP1;3, Figure 6) were up-regulated in Topas, whereas no difference was observed for Lambada. The targeted analysis with anti-PIP1 antibodies did not confirm if the aquaporins plays a role in nitrogen deficiency response due to the high biological variability between replicates. As it has been shown that nutrient deficiency can have an impact on root hydraulic conductivity (8) but only a little is known about the role of aquaporins in the nitrogen use efficiency further functional studies were performed.
Figure 6. The expression of aquaporins during nitrogen deficiency treatment

The expression of aquaporin genes in potato was studied using quantitative real time PCR technique. Additionally three other aquaporins (PIP1;4, PIP1;5, PIP2;1) were investigated to gain deeper knowledge about their role. The water channel protein isoforms were found to be differentially regulated in both genotypes. For example, no significant difference observed in the expression of pip1;1 gene in the susceptible genotype in contrast to the tolerant, while the expression was about three times lower in the stress treated plants. The pip1;5 on the other hand was found to be about two times higher expressed in tolerant genotype under control conditions. In the applied stress treatment pip1;5 was down-regulated reaching the expression level of intolerant cultivar under both conditions. Obtained results suggest that aquaporins can be involved in the adaptations of potato plants to different nitrogen regimes.

Figure 7. The expression of aquaporins genes during nitrogen deficiency treatment
In order to elucidate the potential significance of aquaporin isoforms in the adaptation of plants to low nitrogen conditions the stress experiments with identified *Arabidopsis thaliana* T-DNA insertion mutants (pip1;1, pip1;2, pip1;3 and pip 2;1) were carried out. Plants were grown in-vitro with 10 mM and 2 mM nitrogen. After three weeks of cultivation plant material was harvested and root as well as shoot fresh weight were characterized. First signs of chlorosis were observed in the wild type plants under nitrogen deficiency. The most severe reaction to the low nitrogen treatment showed the pip1;1 mutant where not only the chlorosis was reaching much more advanced step as by the other mutants but also first symptoms of senescence were observed. In summary, knocking down particular isoforms of aquaporins resulted in phenotypic alteration in the chosen experimentall conditions, indicating the role of water channels in the adaptation of plants to the low nitrogen conditions (Figure 8).

Figure 8. Morphological characterization of *Arabidopsis thaliana* aquaporin mutants.
b. Profiling of root whole proteome under nitrogen deficiency

Complementary to the plasma membrane proteome also whole proteome of potato roots was investigated. More than 110 proteins identified in crude extract were altered due to the lack of nitrogen. Proteins were grouped into 13 functional classes according to Bevan et al (9) (Figure 9). Majority of the significantly regulated proteins were related to the metabolism (20%), energy (17%), disease/defence (16%) and protein synthesis (13%).

Many proteins associated with carbon metabolism were affected during nitrogen deficiency treatment. Malate dehydrogenases as well as citrate synthase, phosphoenolpyruvate carboxylase and succinyl-CoA ligase, key enzymes of citric cycle, were more abundant in the efficient genotype during N deprivation. Also glyceraldehyde-3-phosphate dehydrogenase was up-regulated during low N condition in the efficient genotype (Figure 10). Fructokinase and sucrose synthetase were observed to be much higher abundant in the efficient genotype, suggesting shift towards starch accumulation. In contrast there is only a small increase in formate dehydrogenase in Topas, while in Lambada the concentration is twice as high as in the control samples.
Nitrogen deprivation had a high impact on the enzymes connected to glutamine and glutamate metabolism. Two isozymes of cytosolic glutamine synthetase were identified, whereas one of them was highly up-regulated in Lambada during stress treatment and the other one was down-regulated in Lambada and strongly up-regulated in Topas. Glutamate decarboxylase and dehydrogenase concentration increase as a response to low nitrogen in both genotypes suggesting the remobilization of nitrogen (Figure 11).

The role of glutamine synthetase in the nitrogen use efficiency was deeply investigated over the decades. In order to prove the importance of GS in the potato lines functional characterisation of enzyme was performed. Western blot analysis with antisera against both GS1 (cytosolic isoform) and GS2 (plastidic isoform) was conducted (Figure 12).

The abundance of GS1 in the tolerant genotypes Topas and Tomba was similar and stayed unchanged upon nitrogen deficiency treatment. In contrast, the expression pattern of both susceptible genotypes showed opposite regulation. The expression of GS1 in Lambada was lower in the deficiency treated plants and in Kiebitz slightly higher. The abundance of GS2 isoforms revealed the same tendency in all cultivars, with a decrease during stress.
treatment. Subsequent to the analysis of protein expression during nitrogen deficiency treatment also the activity of enzyme was tested.

Specific glutamine synthetase activity was the highest in the susceptible genotype Kiebitz with slight increase in the stressed plants. In contrast, second intolerant genotype showed lower activity of GS in deficiency treatment as in control. No difference in the activity was detected for tolerant cultivar Topas, whereas for Tomba the activity was lower in the plants cultivated in low N conditions.

c. Changes in the phosphorylation pattern of proteins during nitrogen deficiency treatment

Phosphorylation is an important posttranslational modification, which plays a broad role in regulation of cellular processes ranging from signaling to metabolism. It has essential role in enzyme activating, metabolic regulation, signal transduction and biomolecular interactions.

For this purpose plants were cultivated in a liquid Murashige & Skoog medium with full nitrogen content. After 10 days of cultivation, plants were subjected to either low nitrogen medium or medium without nitrogen source. Roots were collected after 10, 30 and 60 minutes for investigation of early signaling changes, and after 3, 24, 48 and 72 hours in order to study long-term responses (Figure 14).
Proteins were precipitated according to TCA/aceton method and separated by 2D SDS-PAGE. Gels were stained with ProQ Diamond, allowing visualization of phosphoproteins and further re-stained with Coomassie. Gel images were further analyzed and compared with Progenesis SameSpot Software. Already after stress treatment for 10 min we could observe changes in the phosphorylation pattern, most likely corresponding to signaling events. Further proteins showed changes after one hour of treatment. In total eleven spots were shown to be nitrogen-deficiency responsive phosphoproteins after 72 hours of treatment. As no significant differences between 30 min, 1 and 3 hours, as well as 2 and 3 days, we decided to use only the time points 10 min, 1 hour, 24 hours and 3 days in further experiments. Short time treatment with nitrogen on lower level (1/8N) seems not be sufficient for stimulate stress reaction, as we did not observe any changes in phosphorylation pattern. In further experiments only medium without nitrogen was used.
Difference in abundance of only few spots were observed based on two-dimensional electrophoresis. This was not sufficient to get full picture of changes in the proteome profile during nitrogen deficiency. To enhance identification phosphorylated proteins identification rate enrichment method was applied. Proteins were first digested with trypsin and further incubated with titanium dioxide beads. After elution enriched phosphopeptides were measured with using technique (Figure 16).

![Workflow of phosphopeptides enrichment and measurement.](image)

Peptides were label free quantified and analyzed for their statistical significance. Comparison of 10 min and 3 days response is showed on the volcano plot (Figure 17). Analysis revealed that much more peptides show a differently expression in the short-term treatment with a nearly even distribution between up- and down-regulation. In the 3 days treatment much more phosphopeptides were up regulated than down-regulated. So far identified peptides belong to metabolic processes like nitrogen assimilation and lipid metabolism. None of the identified significantly regulated peptides was regulated at both short - and long-term treatment, pointing towards involvement of different pathways for coping with stress deficiency.
Phosphoenolpyruvate carboxylase was one of the proteins identified to differ in abundance in the selected time points of treatment. The corresponding peptide (LASIDAQLR) was monitored for changes in expression pattern using Skyline (v. 2.5). Results clearly show up-regulation in the short-term treatment (10 min, 1 hour), whereas no significant difference at the time point of 1 and 3 days was found (Figure 18). Phosphoenolpyruvate carboxylase is an important enzyme which plays a role in nitrogen assimilation and can be further investigated for its significance in response of potato to low nitrogen levels.

4. Knowledge transfer and collaborations within project partners

Selected potato genotypes were kindly provided in form of *in-vitro* cultures by Dr. Schum (JKI Groß Lüsewitz). Identification of proteins, which was planned in the work packages 2 and 3, was performed in the IPK Gatersleben using mass spectrometry techniques.
About 700 spots differentially abundant during drought stress treatment were provided by Ms. Bündig. Proteins were identified using MALDI TOF MS and in case of failure in identification further measurement were performed with LC/MS. About 30% of proteins were successfully identified. Shoot tip analysis of nitrogen deficient plants using two-dimensional electrophoresis were performed by Mr. Meise. About 150 spots were measured with MALDI TOF MS and about 90% of them were identified. The works on common publications are in progress.

5. Presentations of results

a. Posters
Anna Jozefowicz, Hans-Peter Mock (2013): Analysis of Solanum tuberosum plasma membrane proteome under nitrogen deficiency and drought stress. 9th Plant Science Student Conference, May, Halle (Saale)/Germany


b. Publications

Anna M. Jozefowicz, Hans- Peter Mock: Comparative analysis of potato root plasma membrane proteome under nitrogen deficiency (manuscript finished for submission).

Anna Maria Jozefowicz, Anja Hartmann, Andrea Matros, Annegret Schum, Hans-Peter Mock: Differential changes in root proteome of two potato (Solanum tuberosum L.) genotypes contrasting in nitrogen deficiency response (manuscript in preparation).

In cooperation with partner lab Professor T. Winkelmann, Hannover

6. References

Abschlussbericht zum Verbundvorhaben PROKAR

Zuwendungsempfänger: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstraße 3, 06466 Stadt Seeland OT Gatersleben

Projektnummer: 22023311

Projekttitel: Charakterisierung des Proteoms unter Stickstoff- und Wassermangelstress als Grundlage für die züchterische Entwicklung stickstoffeffizienter und trockentoleranter Stärkekartoffeln (PROKAR).


Teil B:
Arbeitsgruppe Teilsammlungen Nord (TEN), Groß Lüsewitzer Kartoffel-Sortimente (GLKS), Groß Lüsewitz – IPK-GLKS

Ausführende technische Mitarbeiterin: Katja Löschner
Beteiligter Wissenschaftler: Klaus J. Dehmer

Arbeitspakete:
AP 2. In-vitro-Screening unter Stickstoffmangelstress [und Untersuchung der Veränderung des Proteoms] (Kooperation mit JKI Groß Lüsewitz)
AP 3. In-vitro-Screening unter Wassermangelstress [und Untersuchung der Veränderung des Proteoms] (Kooperation mit LU Hannover)
I. **Ziele**


1. **Aufgabenstellung**

Die Groß Lüsewitzer Kartoffel-Sortimente (GLKS) der IPK-Genbank erhalten ca. 6.100 Akzessionen von etwa 140 knollenbildenden Solanum-Arten. Im Rahmen des PROKAR-Projektes wurden einige der vorhandenen Wildartakzessionen unter In-vitro-Bedingungen hinsichtlich divergenter Reaktionen auf Stickstoffmangelstress (AP 2) sowie Wassermangelstress (AP 3) analysiert. Dabei wurde bevorzugt auf Akzessionen zurückgegriffen, die a) zur Art S. chacoense gehören, welche in der sehr trockenen Chaco-Region im Norden Argentiniens heimisch sind oder b) bei früheren Untersuchungen am heutigen Institut für Resistenzforschung und Stresstoleranz des JKI hohe Stärkegehalte aufwiesen hatten.


2. **Stand der Technik**

abgewandelten Verfahren (C. Bündig, pers. Mitt.) vorgegangen, bei dem der Wassermangel mittels Sorbitolzugabe zum Medium induziert wird.

Referenzen:

3. Zusammenarbeit mit anderen Stellen
Im Rahmen des Projektes fand eine enge Zusammenarbeit mit IPK-ABC, vor allem aber mit den Projektpartnern JKI und LUH statt, weitere Kooperationen fanden nicht statt.

II. Ergebnisse
1. Erzielte Ergebnisse
-Etablierung von Genbank Akzessionen in vitro
Nach Auswahl von 28 (ursprünglich geplant: 20) Akzessionen aus zehn Kartoffel-Wildarten, die vier Serien der Gattung Solanum repräsentieren (Tab. 1), wurden Samen der zur Phänotypisierung in AP 2 und AP 3 notwendigen Genotypen unter In vitro-Bedingungen angezogen (Abb. 1).

Details zu den ausgewählten Genbank-Mustern finden sich in Tabelle 1. Hierbei wurde vor allem auf Material der Art *S. chacoense* zurückgegriffen, welches in Evaluierungen durch das heutige Institut für Resistenzforschung und Stresstoleranz des JKI einen hohen Stärkegehalt zeigte und zudem aus einem sehr trockenen Ursprungsgebiet stammt; zudem wurden Hochstärke-Akzessionen von neun weiteren *Solanum*-Wildarten ausgewählt. Die etablierten Genotypen (max. 20 pro Akzession; insgesamt 538 Genotypen; wenige davon in vitro schlecht wachsend) wurden mehrmals subkultiviert und auf Quarantäneschaderreger (Andenviren, PSTVd, *Clavibacter michiganensis* und *Ralstonia solanacearum*) durch die zuständigen Pflanzenschutzbehörden untersucht.

- Überführung der in vitro-Akzessionen ins Gewächshaus und Stärkebestimmungen

Zwischen 2013 und 2015 wurden 534 Genotypen zur Knollenerzeugung in das Gewächshaus überführt. Von fast allen Genotypen konnten Knollen geerntet werden, insgesamt mehr als 15.000 Knollen. Pro Genotyp lagen die Zahlen zwischen 1 und 88 Knolle(n), der Durchschnitt lag bei ca. 27 Knollen pro Genotyp (siehe auch Abb. 2).


Die geernteten Knollen wurden anschließend für Stärkebestimmungen verwendet. Der maximale Stärkegehalt lag bei 37,3%, das Mittel betrug 21,4%. 168 Genotypen, bei denen Stärkegehalte der Knollen unter 20% ermittelt wurden, werden nicht weiter erhalten und verworfen. Von den verbliebenen Genotypen wurden 42 Genotypen selektiert (Tabelle 2), die a) hohe Stärkegehalte aufwiesen, b) gut in vitro zu erhalten waren und c) die taxonomische Breite des Ausgangsmaterials aus Tabelle 1 möglichst komplett widerspiegelten.

Tab. 2: Testmaterial für Stickstoff- und Wassermangelstresstoleranz in vitro (4 Serien, 9 Arten, 23 Akzessionen, 42 Genotypen, vier Standards); Abkürzung der Serien siehe Tabelle 1. Fettdruck: tatsächlich verwendetes Material der Stickstoffmangelversuche; unterstrichen: tatsächlich verwendetes Material der Wassermangelversuche.

<table>
<thead>
<tr>
<th>botanische Art</th>
<th>Genotypnummer(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. chacoense</em></td>
<td>30134_01, 30135_05, 30135_19, 30154_09, 30156_01, 30156_16, 30159_05, 30160_13, 30160_15, 30177_01, 30177_02, 30177_15, 30177_17, 30177_20, 30181_06, 30181_18, 30191_16, 30197_03, 30197_14, 30916_08, 30916_13, 30995_18</td>
</tr>
<tr>
<td><em>S. commersonii</em></td>
<td>30211_07, 30211_13</td>
</tr>
<tr>
<td><em>S. handelmannii</em></td>
<td>32852_01, 32852_19</td>
</tr>
<tr>
<td><em>S. microdontum</em></td>
<td>30688_04, 30688_12</td>
</tr>
<tr>
<td><em>S. pinnatisectum</em></td>
<td>31600_05, 31600_10, 31602_06, 31605_07, 31610_13, 31610_18</td>
</tr>
<tr>
<td><em>S. sparsipilum</em></td>
<td>30944_08, 30944_10</td>
</tr>
<tr>
<td><em>S. stenotomum</em></td>
<td>31559_11, 31559_14</td>
</tr>
<tr>
<td><em>S. tarijense</em></td>
<td>31583_02, 31583_12</td>
</tr>
<tr>
<td><em>S. tuberosum ssp. andigena</em></td>
<td>34995_08, 34995_18</td>
</tr>
<tr>
<td>Standards</td>
<td>PROKAR_02, PROKAR_09, PROKAR_13, PROKAR_15</td>
</tr>
</tbody>
</table>

*Stickstoffmangeltoleranz im in vitro-System*


Im Rahmen der in vitro-Versuche zum Stickstoffmangel-Stress zeigten die einige der insgesamt 22 untersuchten Wild-Genotypen ähnliche Stressantworten wie Kulturgenotypen: ein reduzierter Stickstoffgehalt des Mediums führt zu einer verminderten Sproßbildung, während der Aufbau von Wurzelmasse leicht gesteigert wird; beim Vergleich Frisch- vs. Trockengewicht relativieren sich die Unterschiede bzgl. der Wurzelmasse, während die Unterschiede bezüglich der Sproßmasse bestehen bleiben. Dagegen zeigen andere Wildgenotypen zum Teil sehr unterschiedliche Stressantworten im Vergleich zu den Kulturgenotypen; insbesondere Genotyp 30177_01 (*S. chacoense* Bitter) zeichnet
sich durch eine deutlich höhere Wurzelmasse im Verhältnis zur Sprossmasse aus (Abb. 3 links, Abb. 4-6). Genotyp 31600_10 hingegen zeichnet sich durch eine (anatomisch bedingte) geringe Biomassebildung aus (Abb. 3 rechts), besitzt aber auch die höchsten Quotienten aus Wurzel- zu Sproßmasse bei der ⅛ N-Stressstufe (Abb. 6)

Abb. 3: gebildete Sproß- und Wurzelmasse im in vitro-Testsystem nach 21 Tagen Kultur mit ½ N (jeweils linkes Glas) bzw. ⅛ N (jeweils rechtes Glas); Einzelgenotypen: GLKS 30177_01, S. chacoense (links); GLKS 31600_10, S. pinnatisectum (rechts).

Abb. 4: Frischmasse (FM) von Sproß (S) bzw. Wurzel (W) [mg pro Einzelpflanze] bei ½ und ⅛ N (Kontrolle bzw. Stress) im in vitro-Testsystem nach 21 Tagen Kultur.

Abb. 5: Trockenmasse (DM) von Sproß (S) bzw. Wurzel (W) [mg pro Einzelpflanze] bei ½ und ⅛ N (Kontrolle bzw. Stress) im in vitro-Testsystem nach 21 Tagen Kultur.
Abb. 6: Quotient Wurzel/Sproß (R/S) von Frisch- (FM) bzw. Trockenmasse (DM) bei $\frac{1}{2}$ und $\frac{1}{8}$ N (Kontrolle bzw. Stress) im in vitro-Testsystem nach 21 Tagen Kultur.

- Wassermangeltoleranz im in vitro-System


Abb. 7: gebildete Sproß- und Wurzelmasse im in vitro-Testsystem nach 21 Tagen Kultur mit Normalmedium (Kontrolle, jeweils links) bzw. Medium mit 0,4 M Sorbitol (Stress, jeweils rechts); Einzelgenotypen: GLKS 30159_05, S. chacoense (links); GLKS 31559_11, S. stenotomum ssp. stenotomum (rechts).
Nach Stressinduktion mittels 0,4 M Sorbitol zeigten die neun untersuchten Wildgenotypen zum Teil deutliche Unterschiede zu den Kulturstandards (Abb. 8-10): Sowohl bei Kontroll- als auch bei Stressbedingungen konnten einige Wildgenotypen mehr Sproß- bzw. Wurzelbiomasse erzeugen. Genotyp 30159_05 hingegen zeichnete sich durch eine sehr geringe Wurzelbildung unter Normal- und Stressbedingungen aus (Abb. 7, 8-9), so dass hier auch die geringsten Wurzel-Sproß-Quotienten vorlagen (Abb. 10)

---

**Abbildung 8:** Frischmasse (FM) von Sproß (S) bzw. Wurzel (W) [mg pro Einzelpflanze] bei Normalmedium (Kontrolle) bzw. Medium mit 0,4 M Sorbitol (Stress) im in vitro-Testsystem nach 11 Tagen Stress.

---

**Abbildung 9:** Trockenmasse (DM) von Sproß (S) bzw. Wurzel (W) [mg pro Einzelpflanze] bei Normalmedium (Kontrolle) bzw. Medium mit 0,4 M Sorbitol (Stress) im in vitro-Testsystem nach 11 Tagen Stress.

2. **Verwertung**

- **gesammelte wesentliche Erfahrungen**


Insgesamt konnten die beiden Meilensteine von IPK-GLKS (Meilenstein 1: Etablierung Genbank Akzessionen in vitro; Meilenstein 4: Screening von Genbank Akzessionen abgeschlossen) erreicht werden, wobei Meilenstein 1 vom Umfang her deutlich übertroffen wurde (538 Genotypen von 28...

- Verwertbarkeit der Ergebnisse, wissenschaftliche und/oder technische Erfolgsaussichten nach Projektende

Mehrere der untersuchten Wildart-Genotypen könnten interessante Objekte für weitere Studien zu obigen Mangelstresen darstellen, insbesondere hinsichtlich ihrer Proteom-Zusammensetzung. Im Rahmen des jetzigen PROKAR-Projektes war dies jedoch nicht mehr möglich. Auch können beabsichtigte Arbeiten in diese Richtung im Rahmen eines Fortsetzungsprojektes wegen der Ablehnung des entsprechenden Antrags zunächst nicht umgesetzt werden.

- wirtschaftliche Erfolgsaussichten


3. Erkenntnisse von Dritten

Während der Durchführung des Vorhabens sind dem ZE keine Fortschritte auf dem Gebiet des Vorhabens bei anderen Stellen bekannt geworden.

4. Veröffentlichungen
Dehmer, K.J., P. Meise, C. Bündig, T. Winkelmann & A. Schum, 2016: In vitro screening of cultivated and wild potatoes under nitrogen and water deficiency stress. 3rd Meeting of the Section of Agronomy and Physiology of EAPR, Riga, 26.-29.-09.2016 (Vortrag, akzeptiert)
