HIP WP 1.2: Getting to the Roots of Stress Resilience of Potato Plants

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The goal of this project is to gain insight in the role of the root system in tolerance to stress conditions (salinity and low Nitrogen availability).
Roots are notoriously difficult to investigate, as these are underground structures. Yet, roots are the first to sense many environmental stresses like drought, salinity and low nutrient availability, and root structure and specific water, ion and nutrient uptake properties are at the basis of tolerance mechanisms that may enable crops to maintain an appreciable yield under stress conditions.
In this project we are assessing variation in and genetic control of the response of roots to soil salinity and low N availability, and the consequences for growth of the crop.

Highlights:
In the first year, we started with development of a method and protocol to assess root structure variation. Several potato lines were grown on vertical agar plates in which the roots are visible and can be imaged. We have optimized conditions (light, media) for evaluation of the salinity response using a limited set of diploid potato lines. We have established variation for salinity tolerance in a limited set of potato cultivars in a hydroponics system, displaying variation in growth as well as root formation under saline conditions. We have used potato field trials of another HIP project in which potato cultivars were grown amongst others under nitrogen limitation, and established variation in root structure under these conditions as well.
A candidate gene approach targeting genes known to be important for root architecture under stress has been initiated. We will generate expression profiles using RNAseq, and generate lines with altered expression of the candidate genes to characterize their role in root architecture plasticity under saline and N-limiting conditions.

Spring/summer 2021 we conducted a large potato greenhouse experiment with 14 genotypes (see Table 1). In this experiment we used synchronized cuttings derived from tissue culture, which were rooted at 21 ^{o}C in MS20 medium. After 3 weeks, the rooted plants were transplanted into 17 cm pots containing vermiculite No.3 as substrate in a greenhouse compartment at Unifarm, Wageningen University and Research. The greenhouse compartment was climate controlled (21 ^{o}C day and 19 ^{o}C night temperature). The experiment was set up in four parts of which each part was connected to a drip irrigation system. Each pot was individually connected to one of the systems.
The plants were transferred into the vermiculite and were acclimatized/rooted for 3 weeks. During these weeks ½ Hoagland solution was dripped twice a day to the plants. At 9.00h and at 15.00h. After the 3 weeks period both systems were drained and refreshed with new ½ Hoagland solution. In one system NaCl was added on day one to reach 50 mMol. The second day NaCl was added to reach 100 mMol and on the third day NaCl was added to reach the final concentration of 150 mMol. When the final concentration was reached, it was considered as day 0 of the salt stress experiment.

Table 1. Material used in the greenhouse experiment 2021

Genotype name		Ploidy level
Desiree		Tetraploid
Innovator		Tetraploid
HZM 11		Tetraploid
Cultivar x		Tetraploid
Colomba		Tetraploid
Festien		Tetraploid
Mozart		Tetraploid
C parent		diploid
E parent		diploid
Russet Burbank		Tetraploid
Mona Lisa		Tetraploid
Bintje		Tetraploid
Global		Tetraploid
Altus		Tetraploid

Trial setup

From each genotype, 15 plants for control and 15 plants per clone for brackish water/saline were used. The plants were in a complete random block design over the four parts.

Sampling for RNA isolations (destructive)

Early: day 3 of the experiment
3 plants per clone per treatment were sampled for RNA isolations. (84 plants) Divided into 3 types of tissue. Root, stem and leaf. Frozen in liquid nitrogen and stored at -80C
(12 plants per clone per treatment left) this time point failed due to time limitations only 2 out of 3 repeats were harvested

Mid: 7 days after reaching 150mMol salt stress.:
Sample 3 plants per clone for RNA isolations. Divide into 3 types of tissue. Root, stem and leaf. Freeze in liquid nitrogen and store at -80C
(9 plants per line per treatment left)

Late : 14 days after reaching 150mMol salt stress:
Sample 3 plants per clone for RNA isolations. Divide into 3 types of tissue Root, stems and leaf. Freeze in liquid nitrogen and store at -80C
(6 plants per line per treatment left)

During the stress
For all determinations (see below) at each time point: measurements on 3 to 4 plants per line per treatment (total 84-112 plants)

Measurements (non-destructive)

SPAD (chlorophyl content)
Total 3 time points, Day 3, day 10 and day 20.
Per plant: measurements on 3 completely opened leaves of the third leaf counted from the top.

LICOR handheld (Chlorophyl fluorescence, leaf temperature, stomata conductance and porometer)
Total 2 time points, day 10 and day 20
Per plant: measurements on 3 completely opened leaves of the third leaf counted from the top.

Final harvest for ion analysis
Day 22: Determine fresh weight of 3 plants per genotype per treatment, the plants were divided into 3 tissues: root, stems and leaves. These were weighted separately.
Day 22 to day 25: drying of the samples at 105 Celsius ovens (UNIFARM)
Day 25: Determining dry weight after oven drying
The dried samples have been grinded into 0.5 mm particles and are currently stored for ion analysis

Planning for 2022:

Ion analysis
The grinded dried material obtained from the greenhouse experiment 2021 will be processed and analyzed beginning of 2022 (February-March) after which ion concentrations will be determined. These results will assist in the selection for this year's greenhouse experiment.

Greenhouse experiment 2022
A follow-up experiment is planned in the late spring of 2022. This will be a similar experiment as 2021, but with a sub-selection of genotypes.

Gene expression 2022
A selection based on the 2021 greenhouse experiment outcome (weight & LICOR data) will be made. RNA will be isolated from these samples. A selection of candidate genes (to be determined which) will be used for gene expression on these samples.