Phenotypic screening of drought stress tolerance of starch related mutants in Arabidopsis thaliana by using Chlorophyll Fluorescence Imaging in Plantscreen system

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Aim:
The aim of the study is to screen the starch related mutants for progressive drought stress tolerance by means of high throughput kinetic Chlorophyll Fluorescence Imaging and RGB imaging.

Background:
Starch is a major carbohydrate storage molecule and a major product of photosynthesis in the leaves of plants. Starch synthesis occurs during the day in chloroplast and collapse in the night. The α-amylase (BAM) is a major enzyme involved in starch degradation producing maltose from Glucans. Besides the normal pathway of starch degradation, an alternative stress-induced pathway has been proposed. Such pathway includes enzymes BAM1 (β-amylase 1),AMY3 (α-amylase 3) and PG M1 (phosphoglucomutase 1) and might be activated in response to drought stress resulting in starch degradation. Such a pathway would enable the degradation of starch in the leaf, providing metabolites and osmo-protectants leading to an increased drought stress tolerance. In this study, we have used the high-throughput phenotyping platform, the PlantScreen System developed in Photon System Instruments, s.r.o. for drought stress tolerance screening in various starch related mutants.

Methods:

- Quenching Kinetics measures and calculates set of photosynthetic parameters which is used to study the photosynthetic apparatus
- Analyzed parameters are: Fo, Fm, Fv, Fo’, Fm’, Ft, F1, Fp1, Fp2
- Calculated parameters are:
  1. Fv/Fm(maximum Quantum yield) = (Fm-Fo)/Fm
  2. Dryf_4 (Drying factor_4) = Fp1/F0

Results:

<table>
<thead>
<tr>
<th>Starch Mutant</th>
<th>Fo</th>
<th>Fm</th>
<th>F0</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>col-0</td>
<td>0.75</td>
<td>0.85</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>pgm-1</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

- From Fv/Fm, no difference was observed between wild type and starch mutants and also between watered and non-watered plants

- An observed significant difference between col-0 and pgm-1, which suggests pgm-1 might be resistant to drought stress, but not bam-1 and amy-3

- Also, the early stress response was observed on day 5 after drought stress initiation

- Dryf_4 detect early stress response and also allows to discriminate between drought stress resistant and drought stress sensitive

- Visual images of watered and non-watered col-0, bam-1 and amy-3 plants during progressive drought stress treatment:

- Images of drought stress col-0 and pgm-1 plants on day 13:
  - pgm-1 showed higher survival rate compared with col-0 and other mutants

Conclusion:

- Under progressive drought stress condition, pgm-1 mutant showed higher survival rate than bam-1 and amy-3 and this seems to correlate with fluorescence kinetics data.
- This results suggests pgm-1 mutant has enhanced capacity to withstand water deprivation than other starch mutants.

Reference:

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Acknowledgement: I would like to acknowledge HARVEST(research programme of European union) for the financial support for our research