Standard Operating Procedure 21

High-throughput Phenotyping of Pea Genotypes under Drought Stress

Brief Description

The plant-to-sensor conveyor system can be used to evaluate drought tolerance differences among pea (*Pisum sativum*) accessions by assessing contrasting genotypes.

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Standard Operating Pocedure 21

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High-throughput Phenotyping of Pea Genotypes under Drought Stress

Materials and Equipment

- Pea seed (*Pisum sativum*)
- Growth chamber with controlled temperature, light, and humidity Settings
- Plant-to-sensor conveyor system
- 3D Maxi-Marvin, CropReporter and RGB SideView camera systems
- Potting soil (sterilized)
- Pots for planting
- Sticks to support the plant
- Automated Irrigation system
- Labels and Barcodes
- Data recording software
- Oven to dry samples
- Meter
- Balance
- Leaf area meter

Preparation

Growth Chamber Setup

- Set the growth chamber to maintain optimal conditions for pea growth:
  - Temperature: 22°C during the day and 18°C at night
  - Light: 16 hours of light, 8 hours of dark
  - Humidity: 60%

Pot Preparation

- Fill pots with potting soil.
- Ensure that each pot is labelled with a barcode to identify its treatment condition.

Irrigation Levels:

- Control Condition: 70% Field Capacity
- Drought Condition: 30% Field Capacity

(Each genotype should have three replications per condition).





Realising Dynamic Value Chains for Underutilised Crops (RADIANT) is a Research and Innovation Action supported by the ECs Horizon2020 programme, Grant Agreement number 101000622.

Experimental Procedure

Experimental Design and Pot Placement

Each pot should be randomly positioned on the conveyor system within the greenhouse compartments, following a completely randomized design.

Plant Monitoring and Structural Support

- During the experiment, plants should be weighed every other day to monitor water loss and determine if additional irrigation is required.

- Sticks be placed in the pots to support the pea plants and prevent them from growing out of the pots.

Imaging Systems and Measured Traits

RGB Side-View Imaging Traits (CropReporter Camera System)

- **DB_Top** – Top-view digital biomass (cm^2)
- **DB_Side** – Side-view digital biomass (cm^2)
- **CH** – Side-view convex hull area (cm^2)
- **Height** – Plant height (*cm*)
- **Solidity** – Side-view solidity

Spectral Imaging Traits (CropReporter Camera System)

- **HUE** – Hue (*degree*)
- **VAL** – Value (*unitless*)
- **SAT** – Saturation (*unitless*)
- **RRed** – Reflectance in red color (*unitless*)
- **RGreen** – Reflectance in green color (*unitless*)
- **RBlue** – Reflectance in blue color (*unitless*)

Chlorophyll Fluorescence Traits (CropReporter Camera System)

- **F0** – Minimum fluorescence of dark-adapted leaves (*unitless*)
- **Fm** – Maximum fluorescence of dark-adapted leaves (*unitless*)
- **Fv/Fm** – Maximum quantum yield of PSII photochemistry (*unitless*)

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Experimental Procedure

Water Use Efficiency Analysis

Water Use Efficiency (WUE) is analyzed using an automated greenhouse system, which monitored plant water consumption and biomass accumulation through precise irrigation control and high-throughput imaging techniques.

Final Plant Harvest and Biomass Measurement

At the conclusion of the experiment:

- Harvest plants at the soil level to measure their fresh weight (FW).
- Record dry weights (DW) separately after drying the samples in an oven at 105°C for 72 hours.
- Measure the total leaf area (TLA) using a leaf area meter.
- Record plant height (PH) in centimetres.



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