

## PROTOCOL SEMI-FIELD PHENOTYPING OF FABA BEANS GROWN IN RHIZOTUBES

### Materials:

The experimental rhizotubes were made of the transparent sealed bottom, polyethylene terephthalate glycol tube (Mocap LTD, Telford, UK.) filled with soil. The tubes were 1.8 m long, 78 mm in inner diameter 7.8 mm in outer diameter. The tubes' bottoms were pierced (with four holes of approximately 10mm), and a strip of cotton fabric was run through each hole to allow for better drainage. Tubes were fixed on a wooden frame holding 21 tubes on each side (Fig. 1). Each tube rhizotube was seeded with two seeds. Once emerged, plants were thinned out, and only one plant was left in each rhizotube. The experiments were carried out under a rainout shelter in a semi-field in Flakkebjerg, Zealand, Denmark.

### Soil

The soil filled into the tubes was taken from a field at the experimental farm. The air-dried soil was sieved to get rid of rocks and big clumps of soil. Each tube received approximately 20 kg topsoil which soil was filled stepwise in five portions. An internal steel piston was used to compact each portion by dropping it 30 cm 10 times per portion. Each rhizotube was irrigated for two weeks to allow for the soil to settle.

### Insulation and exclusion of light

Each rhizotube was covered with a white plastic tube (Albe Emballage A/S). Frames with secured tubes were further wrapped with a dark mat (Henofa BV., Hendevelde, NL) The 20 tube rhizotron on each frame were then further wrapped within a curtain of the same plastic product.

### Irrigation

Rhizotubes were continuously irrigated individually from the top using a drip water dispenser, adjusted throughout the experiment according to air temperature and to keep soil humidity at approximately 30% as measured with Flower Power sensor (Parrot, Paris, FR).

### Additional management procedures

Plants received one treatment with pesticides when aphids were observed.

### Phenotyping

#### Maximal penetration depth.

Maximal root length was recorder once a week over eight weeks. Maximal penetration depth was measured using a graduated ruler, perpendicularly from the soil surface to tip of the longest visible root on the tube's front side.

#### Root phenotyping

Root intensity was measured in the individual rhizotubes as follows. Two 8 cm x 50 cm rectangles were marked at two heights, 30 cm and 80 cm below the soil's surface. Images of the roots growing within marked rectangles were captured weekly, and during intense growth period biweekly using a custom made PhotoBox (Fig. 2) with a Sony DSC-RX0 camera, controlled by iOS/android app ImagingEdge Mobile (Sony Corporation, Tokyo, JP).



Fig.1 Frames with rhizotubes covered with plastic tubes and installed drip irrigation (the picture does not show the mat covering the frames)



Fig2. Phenotyping with the PhotoBox