Measuring bean root architecture using BeanRSAJ.ojj

Protocol below describes existing ObjectBean but it is quite easy to remove or add new measurements according to your needs (see ObjectBeanNod for an example).

1. Move images to be analyzed and ObjectBean into same folder (you can use multiple copies of ObjectBean).
2. Mark diameter of scale by marking each side. Automatically jumps to next parameter.
3. Mark stem diameter at soil surface by clicking on each side. Automatically jumps to next parameter.
4. Mark number of basal root whorls by clicking once on each file. Press tab when done.
5. Mark number of basal roots by clicking once each basal root. Press tab when done.
6. Mark number of adventitious roots by clicking once on each adventitious root. Press tab when done.
7. Measure tap root diameter approximately 2cm below the hypocotyl by clicking once on either side of the tap root. Automatically jumps to next parameter.
8. Measure length of representative lateral root on a basal root by clicking once where the lateral originates on the basal. Click again as many times as necessary to trace the lateral root to its apex. Press tab when done.
9. Make a lateral marker by clicking once on a representative section of basal root and then as many times as necessary to follow the basal root for approximately 2cm. Press tab when done.
10. Click on the number of lateral roots emerging directly from basal root in segment made in step 9. Press tab when done.
11. Measure approximate basal root growth angle by clicking on 3 points (a, b, and c with b at the vertex of the triangle). Point a should be on a horizontal line from base of hypocotyl, point b should be at vertex and point c should be where the average basal root growth angle crosses a 10 cm (estimated) circle with the base of the hypocotyl at the center. Automatically jumps to begin measuring next photo.
12. Select next photo and begin again.

When finished export data as csv and save.

Additional calculations

Convert pixels to mm

Convert all lengths (including diameters) measurements from number of pixels to mm or cm by using the number of pixels in the scale marker (diameter of scale must be measured by hand). *(see RSAJ manual for help with coding)*

Lateral root density

Lateral root density can be calculated by converting the “lateral marker line” that you drew from pixels to cm and then calculating number of lateral roots per length.

Modification 1

Measuring nodule size and number using ObjectBeanNod

Design additional measurements

1. In “Objects” window select “New Item Type”.
2. Name the new item “nodule size”, select “line” for “Item Shape”, select 3 (or more if you want more samples) for “clones”, select whichever “marker type”, “line type” and “item color” you find most beautiful.
3. Add another new item named “nodule number” and select “point” for “item shape”, 200 for “clones” and whichever “marker type”, “line type” and “item color” you like.
4. In “Columns” window make statistics friendly names; such as “ns” and “nn”. Define the appropriate operation; “Length” for ns and “Count” for nn. Then select the appropriate “item type” from the drop down menu and enter the appropriate number of clones.

Make Measurements

1. Follow all directions for ObjectBean then proceed to additional parameters.
2. Zoom in as necessary.
3. Measure the diameter of at least 3 representative nodules (you can increase the number of “clones” in the columns sheet if you wish to have more samples).
4. Click on the number of nodules and the press tab.
5. Export

Additional Calculations

Convert pixel length of nodule diameter to mm. *(see RSAJ manual for help with coding)*

ObjectRootHairs Manual

1) Move images to be analyzed (use jpg) and ObjectRootHairs into same folder (you can use multiple copies of ObjectRootHairs). It is best to obtain images from a camera integrated into a dissecting microscope. Use the same magnification for all images, including when taking a photo of the scale.
2) Measure scale, ideally a hemocytometer or something similar by clicking on 2 points representing the size of an object of known dimensions.
3) Measure parent width (length from one side of root to the other – not including root hairs) by clicking on one side and then the other. Automatically jumps to next measurement.
4) Measure length of representative root hair by measuring from edge of root to top of root hair. Automatically jumps to next measurement. You could change this to 100 clones so you can measure multiple root hairs. In that case you need to press tab to jump to next measurement.
5) Draw a density marker line along side of parent root. Automatically jumps to next measurement.
6) Click on the number of root hairs in the section of parent root you identified with the density marker in step 5. Press tab when done.
7) Proceed to next image.
8) When finished export as csv and save.

Calculations
Convert pixel lengths to mm. *(see RSAJ manual for help with coding)*