



## GT-RootS using

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**Goal :** How to use the GT-RootS plugin

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## 1. What is GT-RootS ?

GT-RootS (*Global Traits of Root System*) is the automated Java-based open-source solution we develop for processing the root system images coming from the Rhizoscope, a Cirad's phenotyping platform configured for the study of adult cereal plants. Two modes of use are proposed. The full-automated mode applies a predefined standard processing pipeline to a preselected set of images while the semi-automated allows the user to interactively modify intermediate processing results to a specific image.

GT-RootS produces two types of results which are saved in the specified output folder. First, numerical measurements are exported in a comma-separated-value file for statistical analysis where each line corresponds to a root system of the data set, and each column to an evaluated trait. Second, output images are produced to illustrate different steps of the processing. For example, these images give the possibility to post check the root system segmentation or to display density maps.

## 2. GT-RootS launching

GT-RootS plug-in is launched from the *ImageJ*<sup>1</sup> application. So,

- Run ImageJ...
- Select the **GT-RootS** sub menu of the *plugins* menu

The GT-RootS dialog box is then opened.

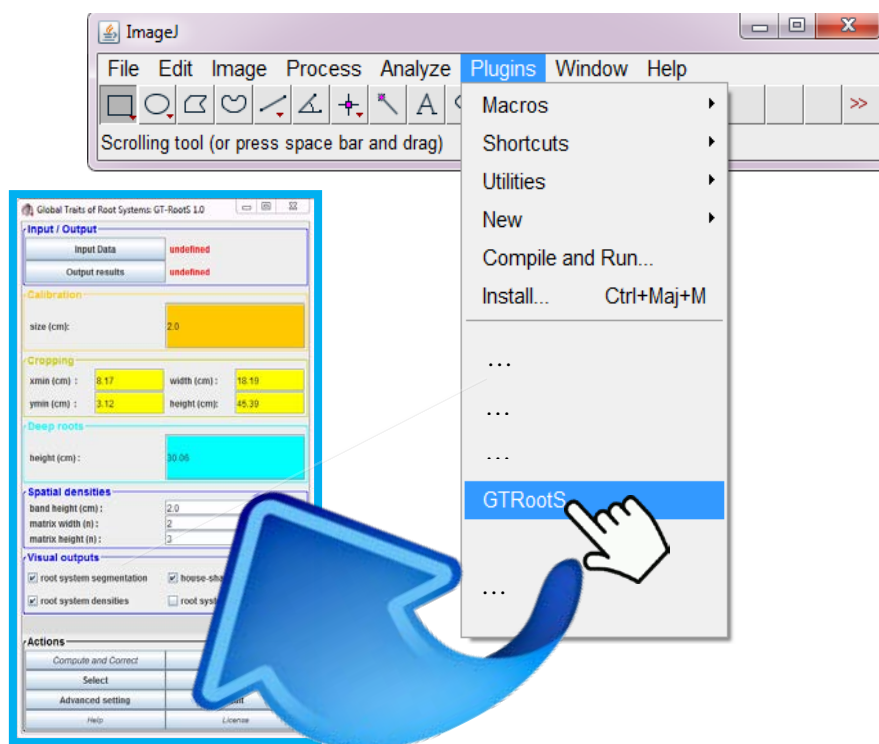


Figure 1: GT-RootS launching

<sup>1</sup> **ImageJ** is an open-source software developed by the *National Center for Biotechnology Information* (NCBI), for the images processing and analysis. It is distributed under GPL licence.

### 3. GT-RootS setting

The *GT-RootS dialog box* is composed of several blocks which allow the user to customize the processing and the global traits computation.

#### 3.1. The input and output folders

The processing pipeline works with two different folders. Each folder is selected with a browser to avoid the input errors.

**Click on** both the “input data” and the “output results” buttons to open a repertory browser and select the desired folders.

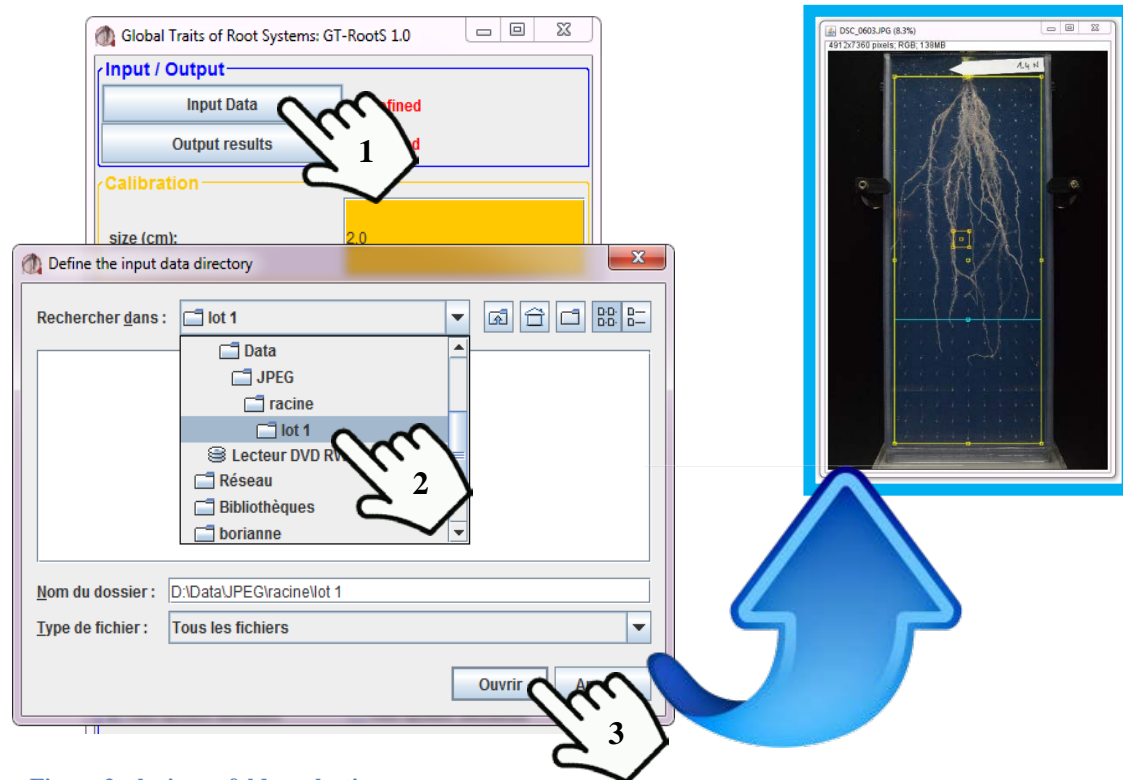


Figure 2: the input folder selection

*When the input folder is selected, the image whose file name comes first is displayed: this can take some seconds. This image allows the user to adjust the parameter setting. Changing the reference image is possible by using the selection or navigation boutons.*

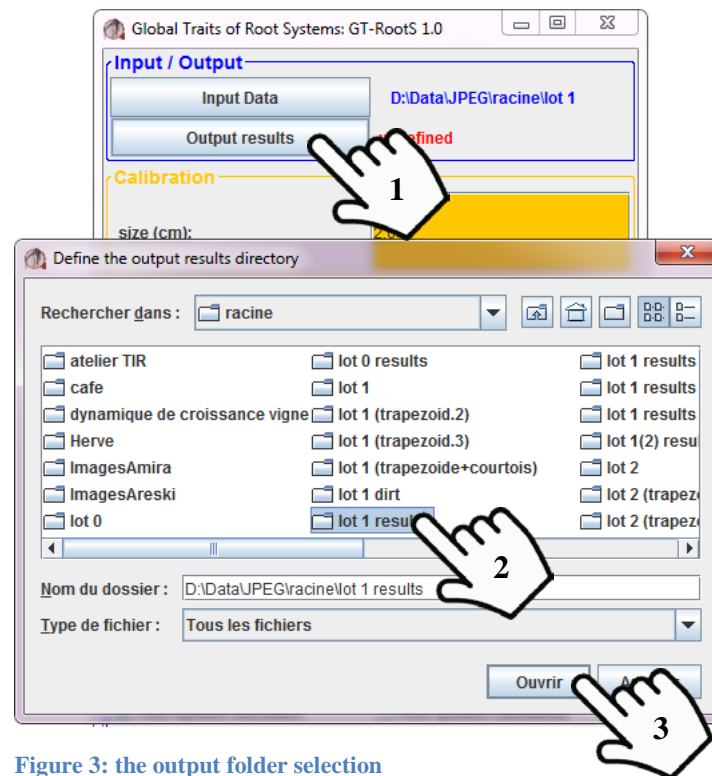


Figure 3: the output folder selection

### The fields :

input data : folder in which the plug-in takes the images to process.

output results : folder where the plug-in saves the numerical outings and the post-checking images produced during the processing.

## 3.2. Calibration

Scale calibration defines the pixel size in centimetres: the user adjusts the orange square of the pin grid on the graphical view (see Figure 4.2) or/and enters the centimetre correspondence in the numerical field (see Figure 4.1).



Figure 4: the scale calibration

**Click and drag** the corner **points** to resize the orange reference square, and the central point to move it.

*Default value and positions are pre-set from reference image of an empty rhizobox.*

### 3.3. Cropping

Cropping is used to quickly exclude sticker and rhizobox walls. The region of interest is represented by the yellow rectangle: the user adjusts it on the graphical view (see Figure 5.2) or/and enters the positions and sizes in the numerical fields (see Figure 5.1).

| Cropping    |      |              |       |
|-------------|------|--------------|-------|
| xmin (cm) : | 8.17 | width (cm) : | 18.19 |
| ymin (cm) : | 3.12 | (cm):        | 45.39 |
| Deep roots  |      |              |       |

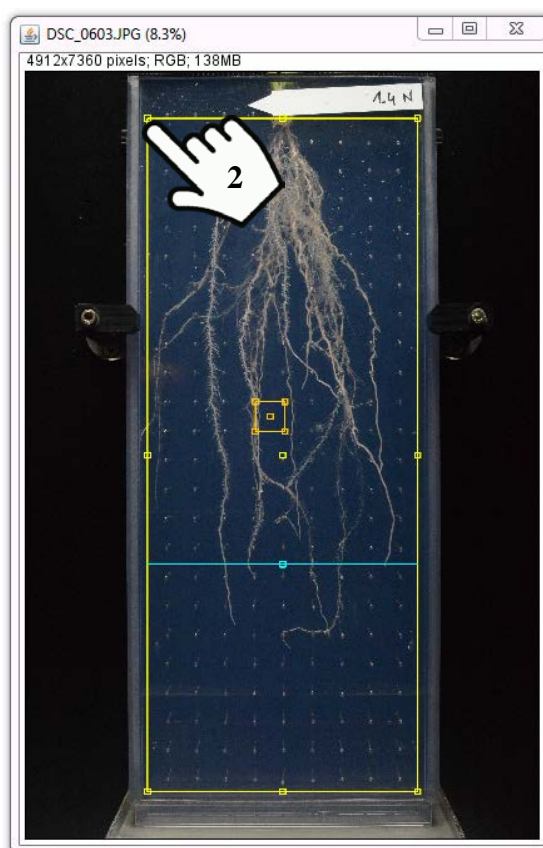


Figure 5: cropping area

**Click and drag** the corner **points** to resize the yellow rectangle, the mid-line point to stretch it and the central point to move it.

*Default value and positions are pre-set from reference image of an empty rhizobox.*

### 3.4. Deep roots

The user adjusts the depth where roots will be individualized and counted. This depth is changed by dragging the cyan line on the graphical view (see Figure 6.2) or/and by entering a numerical position in the dialog box (see Figure 6.1). **Note that the centimetre value is given from the upper edge of the cropping area.**

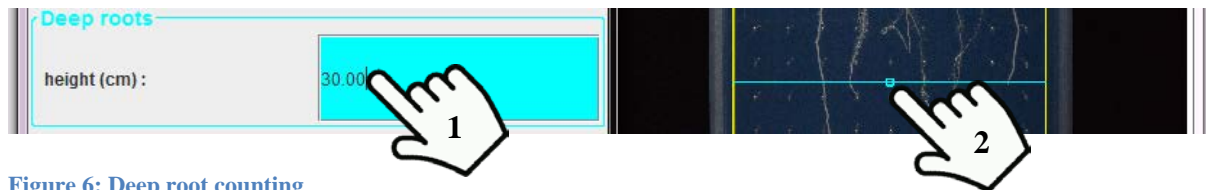


Figure 6: Deep root counting

**Click and drag** the middle **points** of the horizontal line to move it.

### 3.5. Spatial densities

The user specifies the spatial distribution of the root system densities: the vertical distribution is defined by the width of the horizontal bands, the bi-dimensional distribution by a double-entry grid.

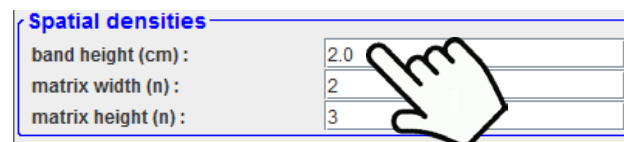


Figure 7: distributions of densities

### 3.6. Visual outputs

The user selects the images which will be saved during processing: the *root system segmentation*, *house-shaped polygon* and *root system densities* are the default visual outputs. Moreover, the *root system silhouette* is proposed for additional measurements with other software

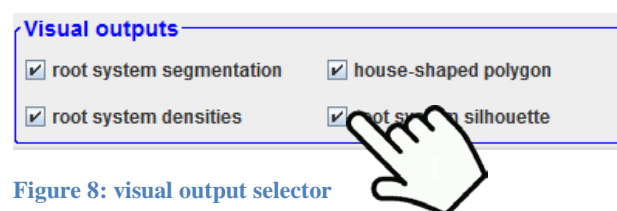


Figure 8: visual output selector



Click on the toggle buttons to select the requested outputs or unselect the unintended outputs.

**Root system segmentation:** the result of the segmentation is superposed to the original image; transparency avoids the user to appreciate the accuracy the segmentation. This image shows also the rotation applied for re-orientation of the root system (see fig. xxxx).

**House-shaped polygon:** the house-shaped polygon is superposed to the re-oriented segmented image (see fig. yyyy).

**Root system densities:** the vertical density distribution evaluated in each horizontal band and the density map of the house-shaped polygon are displayed with a density scale composed of 256 colours ranging from dark blue to bright red where the bright cyan corresponds to the mid-density (see fig. zzzz)

**Root system silhouette:** the segmented root system is saved as a binary image (see fig. wwwwww)

### 3.7. Advanced setting

The user selects the images which will be saved during processing: the *root system*

## 4. Actions

The GT-RootS GUI includes some managing functionalities to select a full-automated or semi-automated processing.

### 4.1. Compute all

The *compute-all* mode applies a predefined standard processing to the selected set of images. No action of the user is possible.

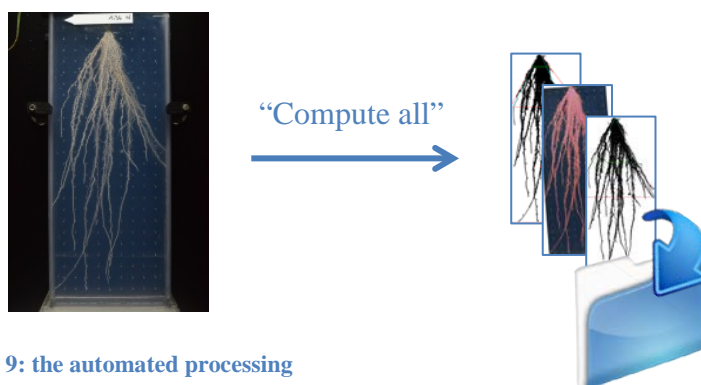


Figure 9: the automated processing

Click on this button to process all the images of the input folder. The results are saved as explained in the § XXXXXXXXXXXXXXXX.



## 4.2. Compute and correct

The *compute-and-correct* mode allows the user to indirectly modify the final results by manual corrections of the segmentation step result.

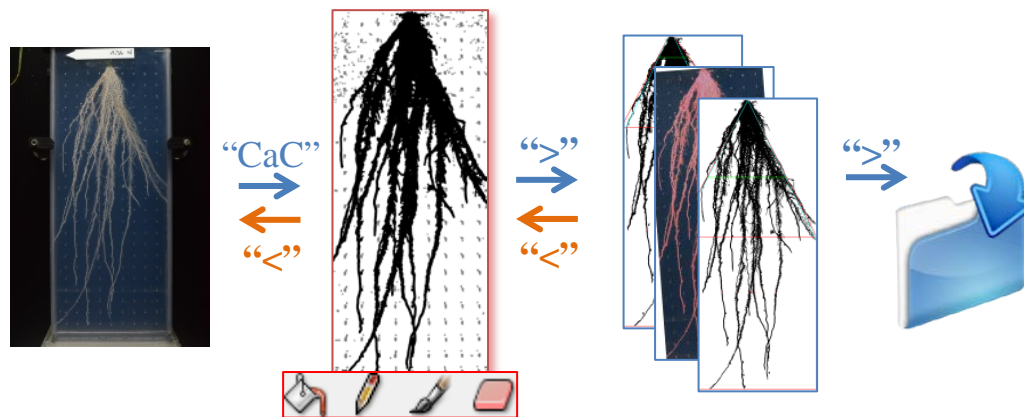


Figure 10: the semi-automated processing

The user can modified the result of the pre-segmentation step by using the different interactive tools of ImageJ. For more details, refer to the *ImageJ* user guide.

Click “<” to return to the previous step and “>” to go to the next step.

## 4.3. The file selector

The file selection allows the user to choose a specific image of the input folder for changing the image of reference, controlling the input settings or loading the image where a specific processing must be applied.

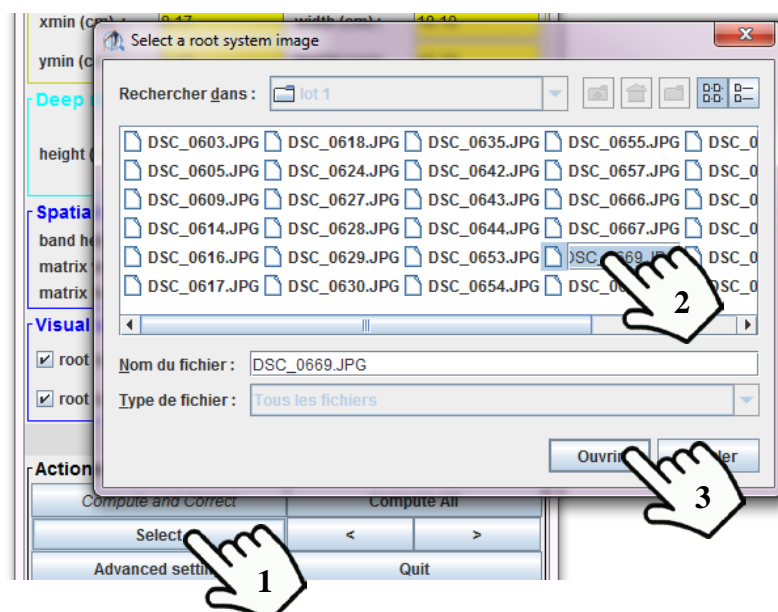


Figure 11: the file selection



**Click on** the “Select” button. A customized file browser is opened. **Click on** the desired file and on the “Open” button. *The selected image is displayed: this can take some seconds.*

Note that user may not navigate in the folder tree. If you need to access to a superior level, refer you to the “Input data” setting (cf. § 3.1).

#### 4.4. The navigation functions

- “<”: to select the previous image in the alphabetical listing of the input folder. *When the current image is the first name of the list, the function returns the last image.*
- “>”: to select the next image in the alphabetical listing of the input folder. *When the current image is the last name of the list, the function returns the first image.*

*The returned image is displayed: this can take some seconds.*

### 5. The root house (or house-shaped polygon)

The global shape of fasciculate root system is defined by a house-shaped polygon. This polygon is composed of two compartments: a trapezoid for covering the upper part of the root system and a rectangle for the downer part. The covering polygon is defined as shown in Figure 3 from both the spatial position of the four most-external points and significant vertical weighted-density profiles of the root system. The weighted density describes the root system density according to its depth; this density is weighted by the local width of the system to give less emphasis to a single very-dense root than a complex dense root tangle. The weighted density at each depth  $d$  of the root system is defined by the harmonic mean of the two between-0-and-1 ratio  $r_1$  and  $r_2$ , where  $r_1$  is the  $d$ -horizontal root quantity compared to the  $d$ -horizontal root system width and  $r_2$  the  $d$ -horizontal root system width compared to a reference width. Two profiles of weighted-densities are required to define the house-shaped polygon. The first profile is used for locating the top of the house-shaped polygon: the reference width is here pre-set to about 3 centimetres. The top edge of the root system is defined by the minimal depth where the weighted-density is superior to the limit between both the low-ratio and high-ratio classes resulting from a 2-means clustering. The second profile locates the most densely horizontal zone of the root system: it is produced setting the reference width with the greater root system width. The most densely depth of the root system is given by the minimal depth where the weighted-density is maximal. Top edge and the most densely zones are so identified by their respective depth and represented by horizontal segment corresponding to the local root system width. The extremities of these two segments define the left and right slopes of the upper trapezoidal compartment of the house-shaped polygon. The vertices of the polygon are so obtained by geometrical intersections between the oblique lines of the upper trapezoidal-compartment and the most-left, most-right and lowest edges of the downer rectangular-compartment.

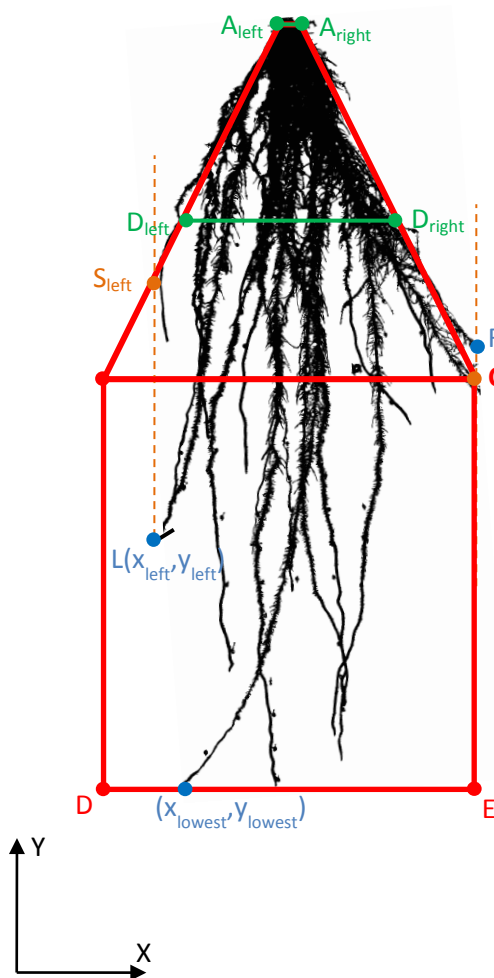


Figure 12: the house-shaped polygon

The root shape landmarks: the most-left, the most-right and the lowest points of the root system.

The root density landmarks: the left and right external points of the most densely root depths.

- $A_{left}A_{right}$  is the minimal depth where the weighted-density is superior to the limit between both the low-ratio and high-ratio classes.
- $D_{left}D_{right}$  is the minimal depth where the weighted-density is maximal

The built landmarks: the intersections between the left and right trapezoid slopes respectively defined from the density landmarks

- if  $X_{AD_{left}} \cap X=x_{left} > 0$ ,  $S_{left} = AD_{left} \cap X=x_{left}$  else  $S_{left} = L$
- if  $X_{AD_{right}} \cap X=x_{right} > 0$ ,  $S_{right} = AD_{right} \cap X=x_{right}$  else  $S_{right} = R$

The house-shaped polygon landmarks:

- $A_{left}$  &  $A_{right}$ : see above
- B: (  $X_{AD_{left}} \cap Y=y_B$ ,  $\min(Y_{S_{left}}, Y_{S_{right}})$  )
- C: (  $X_{AD_{right}} \cap Y=y_C$ ,  $\min(Y_{S_{left}}, Y_{S_{right}})$  )
- D: (  $x_B$ ,  $y_{lowest}$  )
- E: (  $x_C$ ,  $y_{lowest}$  )

## 6. Root system traits

GT-RootS is focused on simplified geometrical descriptors of adult cereal root systems for which it is hard to identify precisely each root axis. Global traits as root width or cone angulation are estimated from the root house-shaped polygon. The spatial-root distribution of the trapezoidal and rectangular compartments are estimated from elementary areas (respectively regular trapezes and rectangles) in which the root density is estimated by the ration between the black and white pixels.

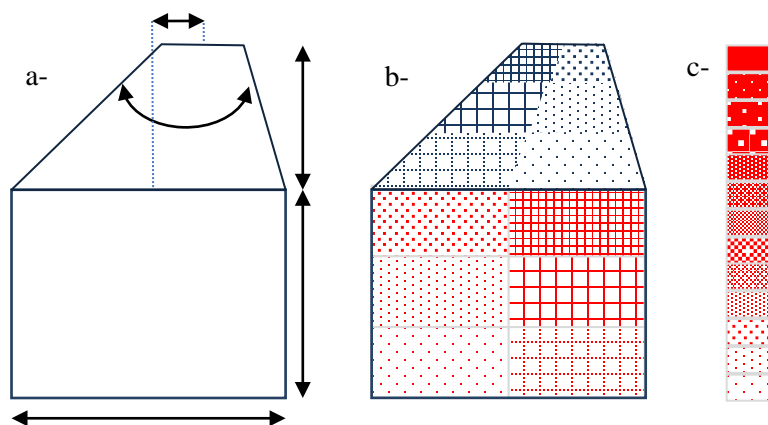


Figure 13: the house-shaped polygon measurement

Table 1 provides a summary definition of the traits evaluated for each root system.

Table 1: Traits description

| Trait                               | Units           | Description   |
|-------------------------------------|-----------------|---|
| left Edge (lE)                      | y/n             | Yes when the root system reaches the left side of the rhizobox.   |
| right Edge (rE)                     | y/n             | Yes when the root system reaches the right side of the rhizobox.  |
| bottom Edge (bE)                    | y/n             | Yes when the root system reaches the lower side of the rhizobox.  |
| root system Deviation ( $s\alpha$ ) | °               | The angle between the principal axis of inertia of the root system and the vertical axis of the image frame. <i>The value is positive for left inclination, and negative for right inclination.</i> |
| house Area (hA)                     | cm <sup>2</sup> | The area of the house-shaped polygon that covers the root system.   |
| inside root Area (irA)              | cm <sup>2</sup> | The area of the root system which is inside the house-shaped polygon.   |
| outside root Area (orA)             | cm <sup>2</sup> | The area of the root system which is outside the house-shaped polygon.  |
| house Height (hH)                   | cm              | The vertical difference between the upper and the downer edges of the house-shaped polygon.   |
| house Width (hW)                    | cm              | The horizontal difference between the most-left and the most-right edges of the house-shaped polygon.   |
| root cone Angle ( $rc\alpha$ )      | °               | The angle at the top point of the upper-house-shaped-compartment  |



|   |    |  |
|---|----|--|
| upper Height (upH)                      | cm | The vertical difference between both the upper and the downer edges of the upper-house-shaped-compartment.   |
| top Width (topW)                        | cm | The horizontal distance between the most-left and most-right top points of the upper-house-shaped-compartment.   |
| upper Offsett (upO)                     | cm | The horizontal distance between the middle point of the most-left and most-right extremities of both the upper and downer edges of the upper-house-shaped-compartment. The value is negative for the left offsets, and positive for the right offsets. |
| down Height (downH)                     | cm | The vertical difference between both the upper and the downer edges of the downer-house-shaped-compartment.  |
| 30 cm deep roots ( $N_{30\text{cm.}}$ ) | n  | The number of roots reaching the 30 cm depth. <i>This indicator can be zero for small root systems.</i>  |
| 80% deep roots ( $N_{80\%}$ )           | n  | The number of roots reaching the 80% depth of the house-shaped polygon. <i>This indicator is superior or equal to 1.</i>   |
| band Density (bandD <sub>t,h</sub> )    | %  | The percentage of the root surface in the polygonal area defined by the intersection between the house-shaped polygon and the horizontal band parameterized by its thickness t and its centre h.   |
| up Density (upD <sub>i,j</sub> )        | %  | The percentage of the root surface in the (i,j) trapezoidal area of the upper-house-shaped-compartment. <i>Trapezes result from the regular splitting of the trapezoidal upper-house-shaped-compartment.</i>   |
| down Density (downD <sub>i,j</sub> )    | %  | The percentage of the root surface in the (i,j) squared area of the downer-house-shaped-compartment. <i>Squares result of the regular splitting of the rectangular downer-house-shaped-compartment.</i>  |

## 7. Example

### 7.1. Input images

The input data are landscape images of 7,360x4,912 pixels where the rhizobox is shown on its left side: the box is viewed from the front).



Figure 14: the input images

*The rotated images are processed without problem: the re-orientation step is automatically skipped.*

### 7.2. Output images

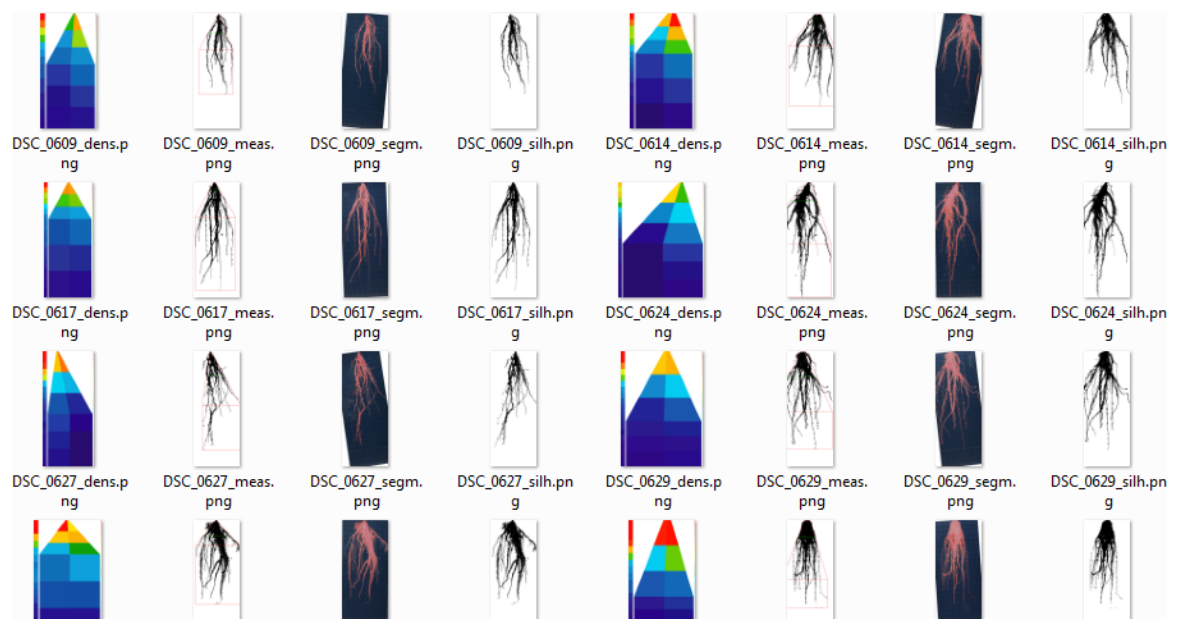


Figure 15: the output images



The different output images are saved in the output folder: their name is built by concatenating the original (root)name and particular extension :

- “dens” for the “density map”
- “meas” for the “house-shaped polygon”
- “segm” for the “root system segmentation”,
- “silh” for the “root system silhouette”

*See §3.6 for more details.*





### 7.3. Numerical results

## 8. Error and messages

*The Input and output folders must be different!*

- ➔ You must IMPERATIVELY select two different folders for managing the input data and output results. Refer to paragraph 3.1.

*The input data folder is not defined. The processing is aborted.*

- ➔ You must IMPERATIVELY select an input data folder before running the processing. Refer to paragraph 3.1.

*The output result folder is not defined. The processing is aborted.*

- ➔ You must IMPERATIVELY select an output result folder before running the processing. Refer to paragraph 3.1.

*You can either modify the pre-segmented image before ending the processing (>) or return to the parameter setting before restarting the processing (<).*

- ➔ Click on to the “>” button after modifying the pre-segmented image to go to the next step or on the “<” button to return to the previous step.

*You can either return to the previous step (<) or save the results in the output folder (>).*

- ➔ Click on to the “>” button to save results and end process or on the “<” button to return to the previous step.

## 9. How to cite

**To come**

## 10.Example

This part illustrates the inputs and outputs through some typical examples.

### 10.1. Inputs

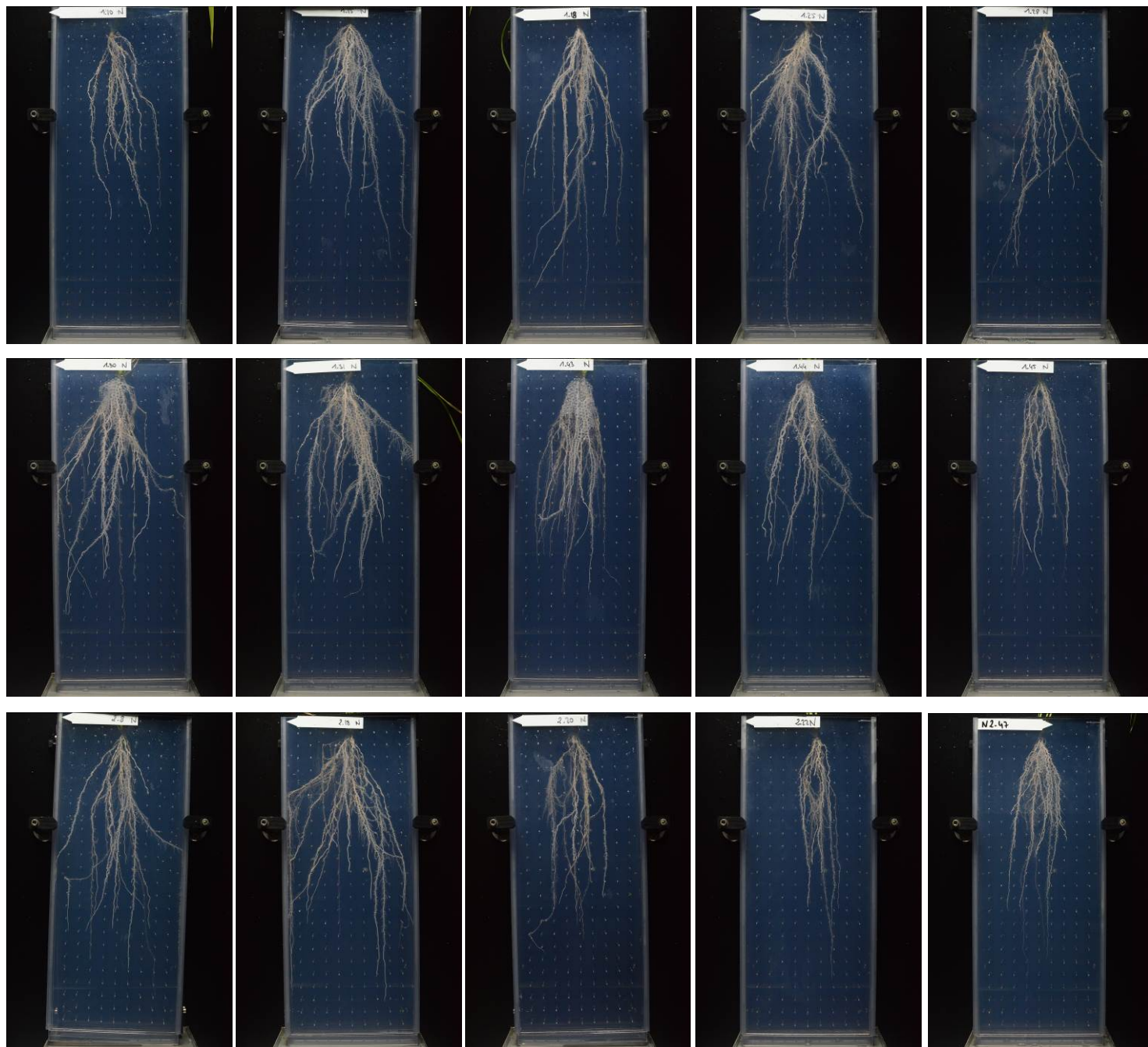


Figure 16: input images of some adult rice root systems

## 10.2. Visual outputs

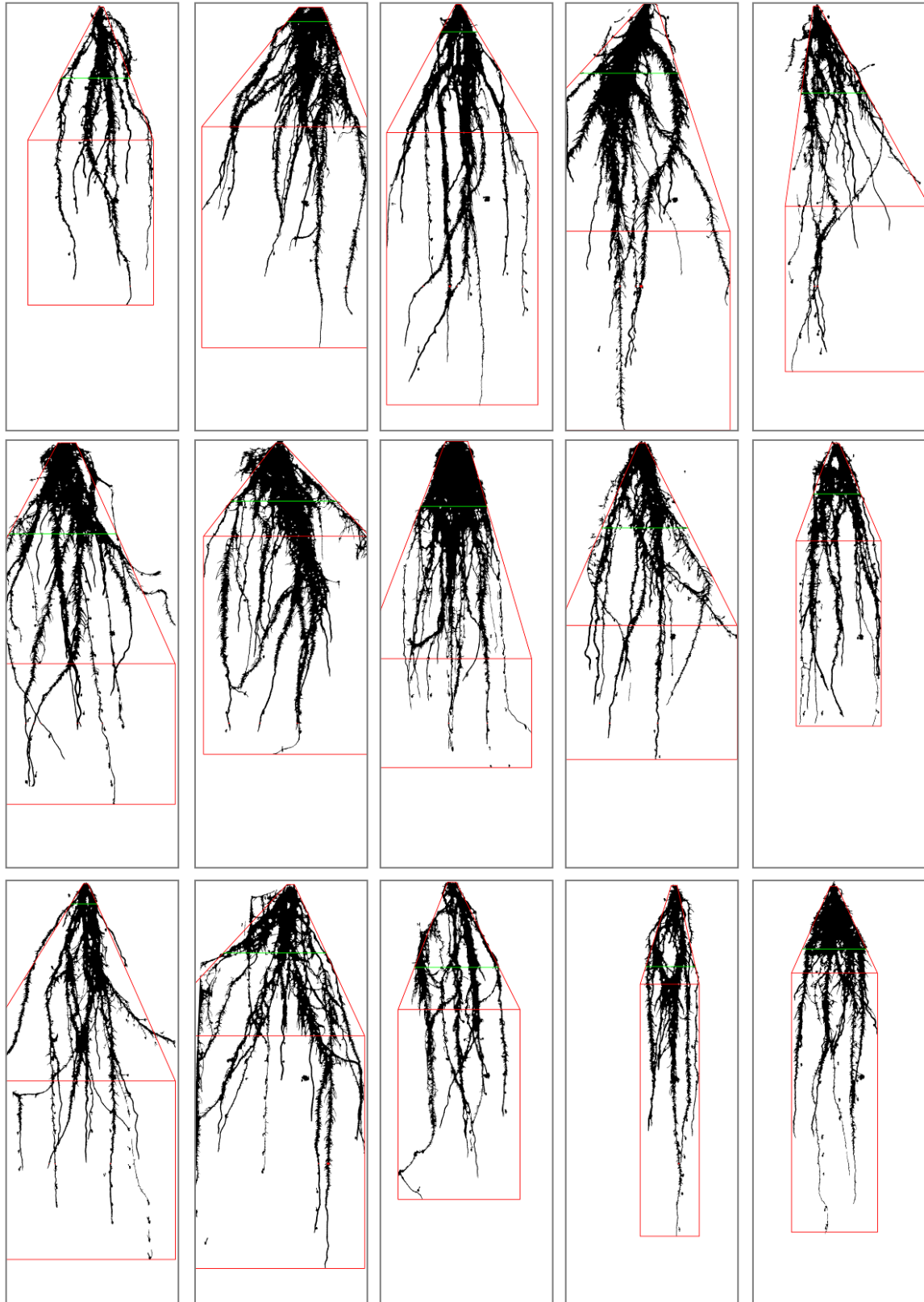


Figure 17: house-shaped polygon of the root systems shown in Figure 13

P. F. Post visual verification of the root shape descriptor: the house-shaped polygon, in red, is superposed to the root silhouette, in black; the most densely segment is drawn in green.

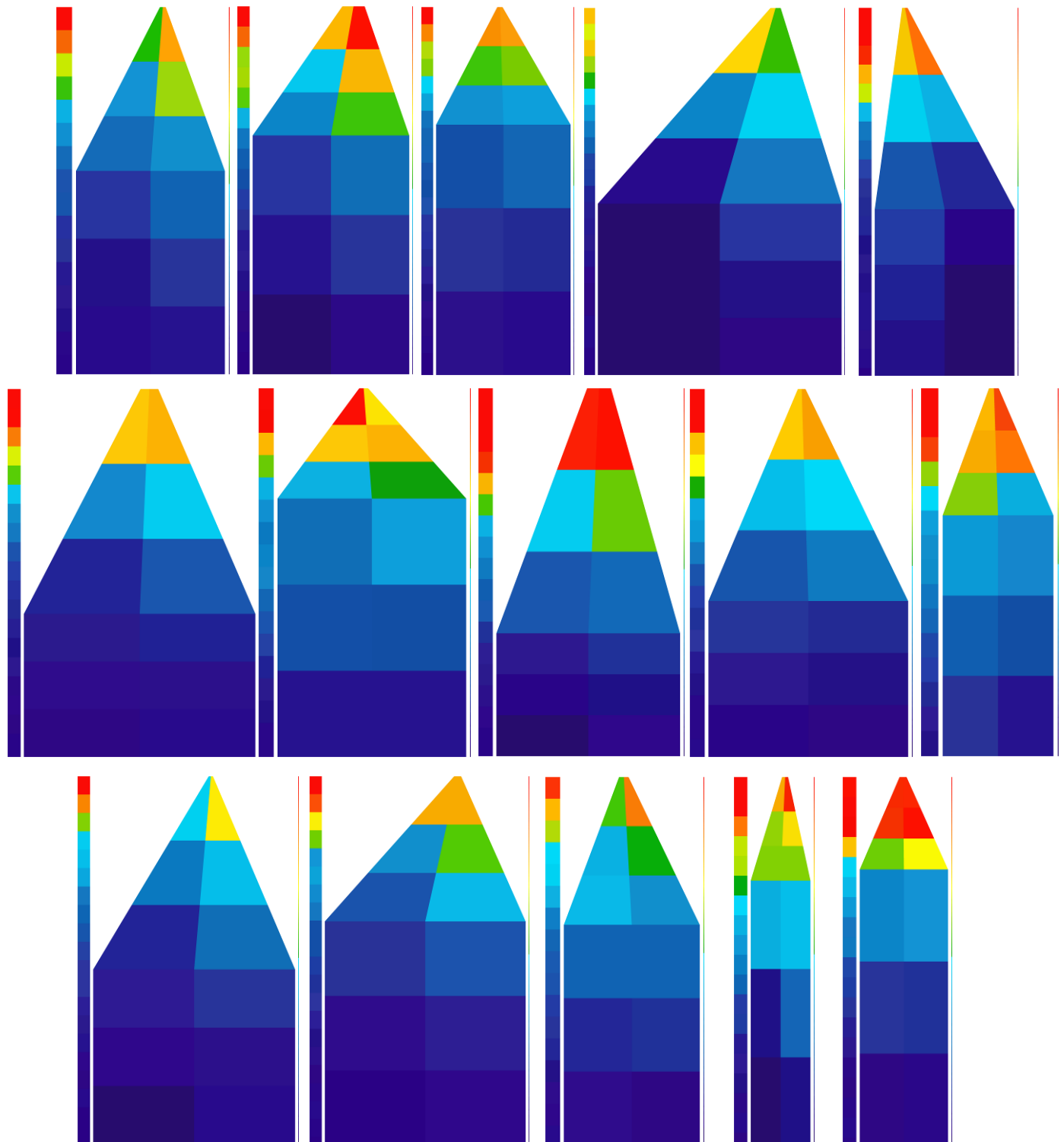


Figure 18: density maps of the root systems presented in the Figure 13

Spatial density distributions: on the left, vertical density distribution evaluated in each 2 cm width horizontal band; on centre, density map from the 2x3 decomposition of the house-shaped polygon; on right side, density scale composed of 256 colours ranging from dark blue to bright red where the bright cyan corresponds to the mid-density.



