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IMAGE ANALYSIS OF 2-DIMENSIONAL ROOT SYSTEM ARCHITECTURE

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Abstract. Root system architecture is essential for plant nutrient and water uptake and is therefore crucial for plant development. Root system responses to heterogeneous soil conditions are of highest interest in plant nutrition, plant hydrology as well as plant breeding. An entire quantification of these root responses i.e. phenotypical expressions calls for (a) elaborate experimental work (b) appropriate imaging techniques (c) advanced image analysis. In the last years there has been enormous progress in imaging techniques in terms of cost and image resolution, while there has been less progress in automated data evaluation. In this work we want to discuss existing as well as new ideas how to analyse 2-dimensional images of root system architecture, and how to extract relevant parameters describing it.

Key words. root system architecture, phenotyping, image analysis, root labelling

AMS subject classifications. 00A72 , 46N60, 97M10

1. Introduction.

Crucial factors for plant development are radiation interception as well as water and nutrient availability in soils. Regarding water and nutrient uptake, root architecture is the main aspect of plant productivity [12] and needs to be accurately considered when describing root processes. Currently, understanding the impact of roots and rhizosphere traits on plant resource efficiency is of highest relevance [5]. Development in this area will increase food security, by enabling a more sustainable production with reduced fertilizer input by improving cropping systems and cultivars for resource limited environment [3].

Root architectural traits comprehend architectural, morphological, anatomical as well as physiological traits. For the systematic investigation of such complex biological systems mathematical modelling is inevitable [17]. Ideally, experiments and theoretical models are developed mutually supporting each other. In this way models are created which include state of the art knowledge and have significant parameters. There are various root architectural models incorporating a multitude of processes (e.g. [9, 16]) which are originally based on [15] and [4]. In this work we will present techniques how image analysis like automatic root labelling can be used to retrieve some of the model parameters. In this way we want to simplify the systematic investigation of root architectural traits, especially phenotypical expressions in heterogeneous environments.

There are already many software tools for root morphology and root architecture measurements in 2-dimensions. Most important are WinRhizo a semi-automatic commercial root analysis tool, EzRhizo [1] a semi-automatic and free software, DART [8] a manual root labelling tool, and SmartRoot [11] an automatic tool for small root systems. An entire overview of available software is given in [8], Table 1. These analysis tools are easy to use, have a graphical user interface, and are often coupled with a database. The disadvantage of such solutions is that they are mostly not expandable and cannot be adjusted to a specific experimental set-up or mathematical model.

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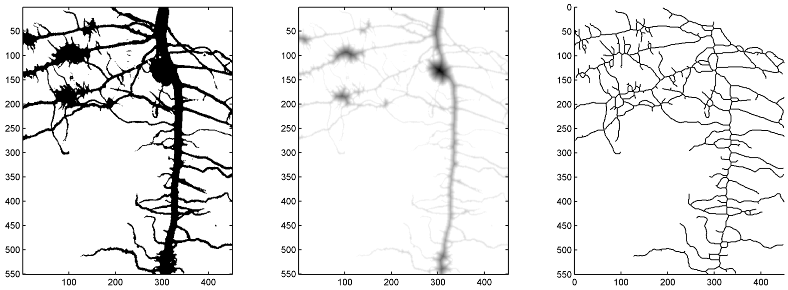

FIG. 2.1. *(left) binary image (mid) distance function of the binary image (right) skeleton of the root system.*

In this work we present a new algorithm to analyse young root systems regarding root architectural topology and root tropism, and we want to discuss the underlying techniques that are used in semi-automatic image analysis tools. Furthermore, we show how to extract parameters frequently used in root architecture modelling. The methods and algorithms are implemented in Matlab, a modelling environment which is highly suitable for prototyping and testing of the proposed techniques.

2. Methods. In the following we present basic morphological and graph theoretic methods that can be used for automatic and semi-automatic root labelling.

2.1. Thresholding. In a first step we turn 2-dimensional grey scale image u into a black and white image u_b, by comparing the intensity to some threshold value v:

$$u_b = \begin{cases} 1 & \text{if } u > v \\ 0 & \text{if } u \leq v \end{cases} \tag{2.1}$$

If there are holes within the roots due to noise or other artefacts, these gaps can be filled by morphological dilation. The resulting image is shown in figure 2.1(left). In the following morphological operations will be applied to the binary image.

2.2. Distance function. Next we perform an Euclidean distance transform of the binary image u_b. In the resulting distance map D each pixel value holds the distance to the closest boundary of the root. We use the Matlab function `bwdist` which uses the algorithm described in [13].

The resulting image is shown in figure 2.1(mid). In the following D is used to quickly look up the root radius along a given centreline.

2.3. Skeletonization. We derive a morphological skeleton u_s of a binary image u_b by iteratively applying morphological operations: (a) closing (i.e. dilation followed by erosion) and (b) thinning [7]. The resulting skeleton is shown in figure 2.1 (right).

The morphological skeleton is only a coarse approximation of a realistic centreline and can produce artefacts. Typical artefacts are: (1) an extra edge between two roots that are close (2) close roots appear as one edge (3) the points where roots branch are displaced.

The advantage of this approach is that it works on complex geometries and that the basic topology is always captured. If the skeleton is used as a centreline an artefact reduction can be applied (see section 2.5).

2.4. Graph representation. The skeleton u_s can be used to create a graph of the root system. Every leaf and branching point represents a node. Thus, each pixel which has either one neighbour (i.e. a leaf) or more than two neighbours (i.e. a branch) in an eight pixel neighbourhood is a node in the graph. Nodes are connected by an edge if they are connected by pixels in the skeleton u_s. For each edge the corresponding pixel positions are stored in a list.

In this way we can create a symmetric connectivity matrix S of the undirected graph, where the matrix entries represent the edge indices. Each edge has a corresponding list of pixel positions representing the edge in the skeleton u_s.

A big advantage of this approach is that it enables a systematic search of possible paths within the graph. Furthermore, graph theoretical algorithms like minimal spanning tree, Dijkstra algorithm, or transportation in graphs can be applied to analyse the root system. Graph theoretical methods often rely on edge weights, which are calculated from the pixel positions of the corresponding edges and from the distance map D. Typical edge weights are length or average radius.

2.5. Smoothing the edges. In order to smooth the lines of the skeleton and thereby the edge positions of the graph we regard the lines as active contours [6]. The image forces are calculated from the distance function D. In this way we achieve smoother edges due to internal forces of the active contour, and movement towards centre of the root due to the image forces. This procedure enables a more accurate measurement of length and radius.

2.6. Possible paths in a graph. The main idea of the following root labelling algorithm in section 2.8 is to evaluate all possible paths according to some objective function, and to follow the winning path.

To calculate all possible paths which are smaller than a predefined length r we can use the following recursive function:

```
function paths = getPaths(path,r)
n = path(end); % current node is the last node in path
N = neighbours(n); % neighbouring nodes of n that are not in path
c=1;  % path counter
for i = 1 : length(N)
    l=lengthOfEdge(n,N(i));
    if l<r
        npaths = getPaths([path,N(i)],r-l); % recursively find new paths
        paths(c:c+length(npaths)-1) = npaths; % copy into final list
        c=c+length(npaths); % increase counter
    else
        paths{c} = path; % don't add node
        c=c+1;  % increase counter
    end
end
```

2.7. Evaluation of a path. Which path is optimal is decided based on the properties of the edges it contains. Different features are of special importance:

- Mean volume of the path (maximize), approximated with $a^2\pi l$, where a is the radius derived from the distance map D evaluated at the edge positions and l which is the path length.
- Variance in growth direction (minimize)
- Variance in radius along the edges (minimize)

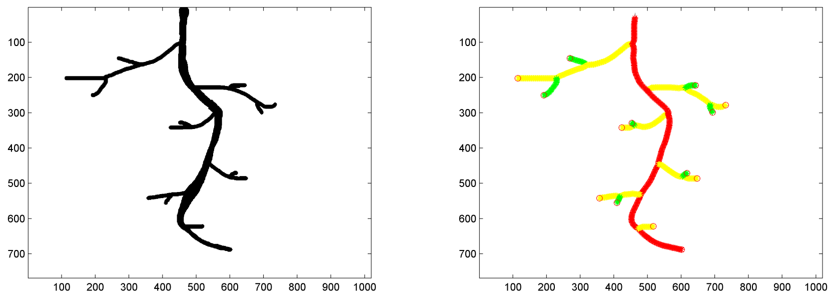

FIG. 3.1. *A small artificial root system (left) with the resulting labelled roots (right): tap root (red), first order laterals (yellow), second order laterals (green).*

Furthermore, if an edge has already been visited it is penalized. In this way edges can be visited multiple times, but other ways are favoured.

2.8. Root labelling in a graph. We use morphological knowledge about root system development to label the roots. The algorithm uses two basic assumptions: First, the root system in the image has developed from a seed: Thus, the root system has an initial node n_0, where it started to grow. This is the initial node for the algorithm. Second, the roots follow a herringbone topology. Therefore, the root is described by a branching zone, where lateral roots can emerge, which is followed by an apical zone towards the root tip, where no lateral roots emerge.

The root tips grow at a constant rate. For each time step we calculate the length each growing root should have, and add edges accordingly. For each root this is done in the following way:

1. Find all possible grow paths (see section 2.6)
2. Choose optimal paths (see section 2.7)
3. If this path is good enough, add the first edge of the optimal path to this root and denote this edge visited (to penalize multiple visits in the path evaluation).
   Else, the root is finished and stops growing.
4. Create new root tips in all other possible path directions. These tips start to grow after a time delay, in order to let the apical zone of the base root emerge.

The algorithm dynamically assigns each edge in the graph to one or more roots. Furthermore, the topology of the assigned roots is determined.

3. Results. We demonstrate the proposed methods with artificial data, which makes it easier to systematically test the algorithm in different situations.

Figure 3.1 shows a small root system. The roots were automatically labelled with the algorithm proposed in section 2.8. In this simple example all roots labelled correctly, red denotes the tap root, yellow the laterals, green second order laterals. The parameters that can be extracted for root architecture models are mean length and standard deviation of first and second order laterals, as well as the inter-branching distance along the tap root. Furthermore, the variation along growth direction can be analysed, and parameters for underlying root tropism can be determined (e.g. according to [2]).

Figure 3.2 shows a slightly larger root system containing an overlap of a first order

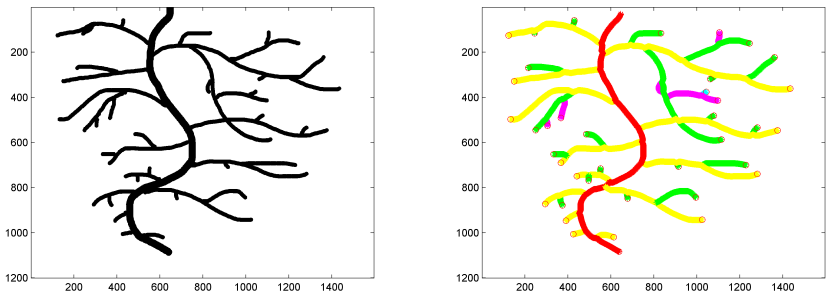

FIG. 3.2. *An artificial root system with an overlap: tap root (red), first order laterals (yellow), second order (green), third order (magenta), fourth order (cyan).*

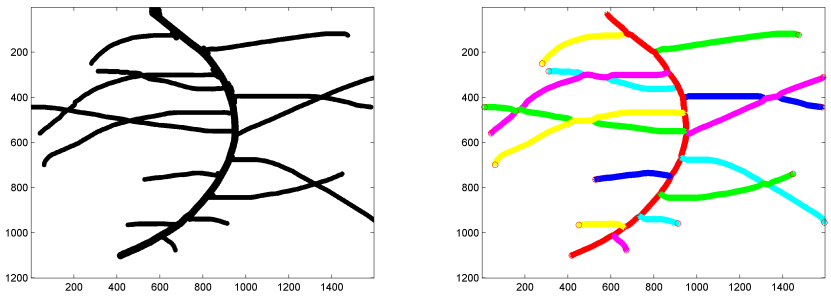

FIG. 3.3. *A simple root system with overlaps. The colours illustrate continuous roots.*

lateral with a second order lateral. The algorithm is capable of handling such overlaps and labels the roots correctly. However, situations can arise where it is hard to find the right coherence between the roots. Another challenge is that in real root systems the topological order of the roots do not always coincide with the morphological type of the root [16]. Therefore, for real root system architectures the extracted root parameters have to be assigned to certain root type by a cluster analysis.

In figure 3.3 we show that the proposed algorithm handles multiple overlaps correctly. In real images there are situations where the choice is not unique. The proposed algorithm always finds a good solution by minimizing the variation i.e. angular movements along the edges (see section 2.7).

4. Discussion and outlook. The proposed algorithm is similar to EzRhizo [1], since both algorithms use a skeleton approach, and are based on a greedy algorithm. However, we extend the approach for the graph presentation of the skeleton, which proofs very useful. Analysing all possible paths with a certain length does increase the scale on which decisions are made, and therefore, makes it more likely to find a good solution.

A different approach is to work directly on the image data [11, 14]. Generally, there is more information in the image, then in methods based on its binary segmentation. For this reason such approaches can better seize small scale image features to find good paths. However, some of the approaches lack the ability to use global information about connectivity, which is the strength of graph based approaches. A combination of these methods could increase the quality of root labelling algorithms.

While the literature for automatic root labelling is limited, there is vast literature for biomedical applications, like vessel tracking (e.g.[10]). While this problem is different in many areas, the algorithms sometimes share similar features. Ideas from this area could benefit image analysis for root architecture.

In this work we used artificial data to demonstrate specific features of the algorithm. The algorithm strongly relies on the evaluation of the possible paths (see section 2.7). There is ongoing work to find good objective functions, and to adjust them to different experimental set-ups and image acquisition techniques.

Plant root experiments are extreme in terms of labour and time. Therefore, we hope that the proposed methods can help to extract the maximal information from real image data.

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