

# Automatic root hair measurement to quantify abiotic stresses in microscopy images

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## Abstract

Improving nutrient and water uptake in crops is one of the major challenges to sustain a fast-growing population that faces increasingly nutrient limited soils. Root hairs, which are specialized epidermal cells, are important drivers of nutrient and water uptake from the soil. Microscopy provides a mean to record root hairs as digital images. However, due to their geometry and complex spatial arrangements quantifying root hairs in microscopy images manually remains a bottleneck. Manual selection of representative root hairs can result in inaccurate estimations of root hair traits and misrepresentation of root hair functions. We present a method to quantify phenotypes automatically by measuring all individual root hairs in digital microscopy images. Our method uses random forests classification to separate root hair from the parent root and the image background. We define metrics to evaluate segments of root hairs that intersect or form blobs of two or more root hairs. Using simulated annealing for combinatorial optimization, we reconstruct individual root hairs by resolving intersections in a globally optimal way. As a result, we measure root hair length, its distribution, and root hair density in each image. We validate our method on examples of three maize cultivars under phosphorus, nitrogen, and potassium stress. Results show that our measurements of root hair traits strongly correlate with manually measured validation data in mean root hair length (Pearson-correlation: 0.74 to 0.88,  $p < .001$ ), as well as in root hair density (Pearson-correlation: 0.65 to 0.84,  $p < .001$ ). We show that our method distinguishes subtle differences between genotypes and treatments based on the extracted traits and believe that our study paves a way towards identifying the genetic control of root hair traits and increased agricultural production.

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