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Monitoring the diel growth of individual *Arabidopsis* leaves using a laser scanning approach

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Highlights: A novel phenotyping approach is presented to monitor diel variation in elongation and elevation angle of individual leaves with high precision and throughput. Leaf elongation and changes in leaf elevation angle follow characteristic diel rhythms and show an overall decrease with increasing leaf age.

Keywords: Arabidopsis, phenotyping, leaf growth, laser scanning

INTRODUCTION

During their life cycle, flowering plants are frequently facing fluctuating environmental conditions, which are often sub-optimal for growth. To cope with that, plants possess a range of adaptive growth responses and protection mechanisms.

The model plant *Arabidopsis thaliana* provides a good platform to link phenotypic plasticity to the function of specific genes, proteins or protein complexes. Indeed genetic resources are well explored and available tools are extremely well developed. On the other hand, phenotyping of leaf growth have largely depended on semi-automated two-dimensional (2D) image processing (Millenaar *et al.* 2005; Wiese *et al.* 2007; De Vylder *et al.* 2012). These methods are of limited throughput and usually restricted to analyze few time-points, since imaging (in particular at night) interferes with growth processes.

We have applied a laser scanning technique using the Scanalyzer HTS (Lemnatec GmbH, Würselen, Germany) to monitor the diel growth pattern of individual leaves. Laser scanner images of growing *Arabidopsis* plants were recorded at intervals of 10 to 60 minutes. Images, containing the 3D information of individual plants were processed. As principal output, length and elevation angle of individual leaves were computed.

MATERIAL AND METHODS

Plants were grown in pots filled with a mixture of peat-rich soil and vermiculite. Before their transfer to the Scanning device ScanAlyzer HTS (Lemnatec GmbH, Würselen, Germany) they were kept 10 days in a Percival CU-36 L4 incubator (Percival Scientific Inc., Perry, IA, USA). The photoperiod was 16 h. Relative humidity inside the Percival incubator was between 80-85% and temperature was maintained at 21°C. More detailed information on growth conditions and laser scanning protocol are given by Dornbusch et al. (2012). Plants were imaged over a period of 9 days. Images were taken each 60 min. The image-processing pipeline to extract geometric features of individual leaves is illustrated in Figure 1. As a result, length l and elevation angle φ are displayed as function of time t.

RESULTS AND DISCUSSION

As an example, we present here the growth pattern of the first four leaves (two cotyledons not counted) by looking at the diel pattern of leaf elongation (l_{tip}, Fig. 2a) and leaf elevation angle (φ_{tip}, Fig. 2b) for the first four leaves (leaf 4 being the youngest). Leaf 1,2 developed at the same time and grew at similar rates. At day 4, the younger leaves 3 and 4 were shorter than leaf and 2, but they were expanding at a higher rate during this specific period and became longer starting from day 6. Leaf elongation was reduced during night periods and increased shortly after dawn (Fig. 2a) and generally decreased with increasing leaf age.

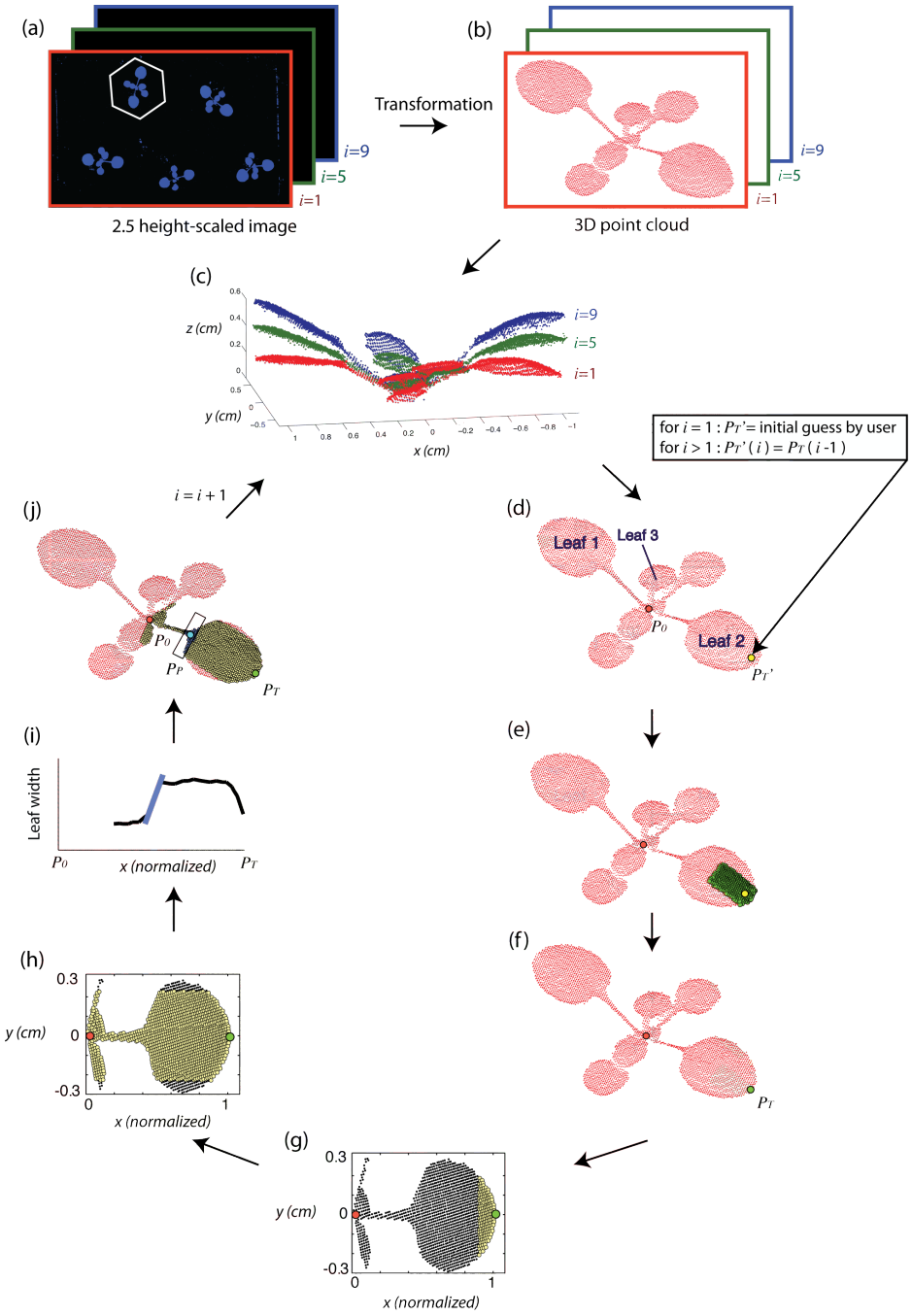

Fig. 1. Flow chart representing the different steps of the developed image processing algorithm: **(a)** 2.5D height-scaled images of plants, which are transformed into 3D point clouds, recorded at different scanning or iteration steps *i*. 3D point clouds of individual plants are obtained by segmentation, **(b)** 3D point cloud of the plant circled in Fig. 2a (example here for illustrating the following algorithm), **(c)** superimposition of point clouds of the plant in (b) at different iteration steps *i*, **(d)** As first step of the iterative loop: P_T' is determined by the user if i =1 or P_T'=P_T (i -1) if i > 1, **(e)** result of the filtering with the selection of points (in green) that are in a defined area around P_T', **(f)** computation of $P_T(i)$ from filtered points, **(g)** Point cloud in (f) rotated and normalized such that $P_T(i)$ = [1,0,0]. The considered leaf is hence in the *x*-*y* plane. In yellow, points nearby the tip of the leaf, **(h)** same projection than in (g). In yellow, points resulting from an additional filtering, which should cover most of the leaf surface (leaf 2 here), **(i)** Computing the maximum of the first derivative of the 'width' of the leaf, **(j)** Obtaining $P_P(i)$, which is the centroid of the set of points close to the computed max. derivative (highlighted by the black box); Having computed $P_T(i)$ and $P_P(i)$, the point cloud of the next time step *i+1* is processed, initiating the same loop (d) to (i) using P_T (i -1) as input for (d). Note that apart from the first iteration step i=1, the algorithm is fully automated.

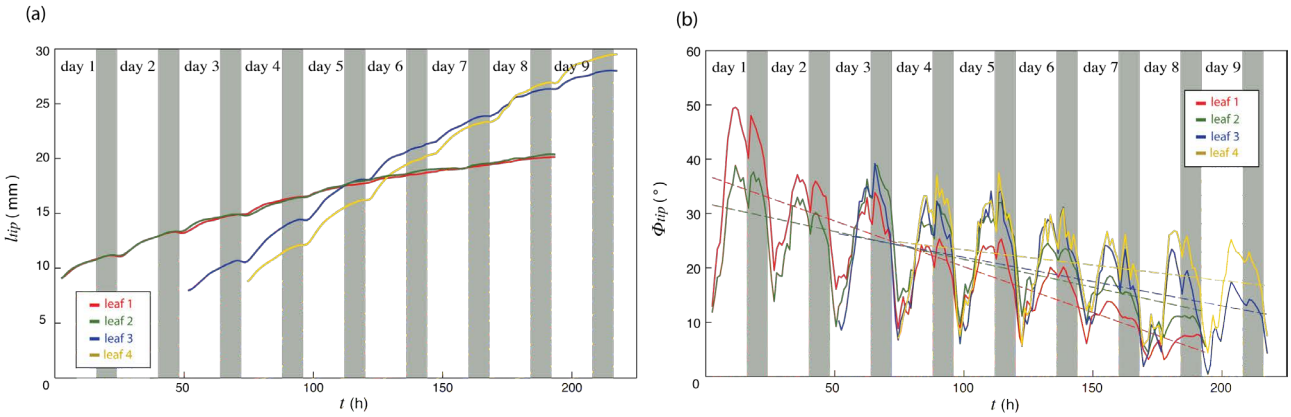

Fig. 2. **(a)** Mean leaf length l_{tip} and **(b)** leaf elevation angle Φ_{tip} for leaves 1 to 4 plotted against time (t). Grey bands represent night periods**.** One curve represents the mean value computed from 5 individual leaves, which were tracked over the whole period. The dashed lines in (b) represent the trend lines to illustrate the decreasing leaf angle.

In general, leaves followed a sinusoidal diel pattern of leaf elevation angle (φ_{tip}), being most horizontal in the early morning and most vertical in the evening (Fig. 2b). An abrupt upward movement of leaves (increase in φ_{tip}) was measured at day-night transitions for all four leaves throughout the whole period. There was a clear trend of decreasing φ_{tip} with increasing leaf age (dashed lines in Fig 2b). At the same time the maximal difference in elevation angle (amplitude during 24 h) was also reduced with leaf age (Fig. 2b) in the same fashion as leaf elongation (Fig. 2a).

This leads to the interesting question to what extent leaf elongation and concomitant changes in leaf angle of *Arabidopsis* leaves are linked and rely on the same growth mechanisms. For instance, Schuster and Engelmann (1997) have shown that circumnutations in hypocotyls (similar to changes in elevation angle in leaves) were more prominent for young hypocotyls growing at faster rates. To the best of our knowledge, no comparable study has been done for *Arabidopsis* leaves.

To conclude, we have presented a non-invasive methodology that allows phenotyping of individual *Arabidopsis* leaves at high temporal and spatial resolution. The throughput is compatible with genetic screens and, in combination, both would allow to unravel molecular mechanisms behind the dynamic aspects of leaf growth.

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