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Water transport in leaf vein systems and the flow velocity measurement with a new method

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ABSTRACT

As an exploration to the nature, research about plants' physiological properties have never been suspended. Water transport in leaf vein systems is an essential part of plant growth and development. In this paper, a simple but efficient method combined the fluorescence labeling technology frequently used in bioresearch and the image-processing technology in the computer realm was developed to measure the flow velocity, which was used as a quantitative description to reveal the regulation of water transport in leaf vein systems. Three ordinary species of plants were selected for the experiments and the influence of the experimental conditions, such as the concentration of fluorescein and illumination intensity of LEDs, was investigated. Differences among the flow velocities of different leaf veins of the same leaf as well as the flow velocities of different species were shown in bar charts. The mean measured flow velocities of the midrib and secondary vein of *Ficus virens* Ait. var. *sublanceolata (Miq.) Corner* were 4.549 m/h and 3.174 m/h. As for *Plumeria rubra* L. cv. *Acutifolia* and *Hamelia patens*, that were 0.339 m/h and 0.463 m/h, 2.609 m/h and 2.586 m/h, respectively. With the algorithm developed in this paper, the variation of the flow velocity in leaf veins was investigated by setting a constant time interval. Then a verification of the flow velocity measured by the algorithm was performed. Finally, according to the natural conditions of a plant leaf, a simulation about the water transport in leaf vein systems was carried out, which is especially different from the previous research.

1. Introduction

As an exploration of nature, research about plants' physiological properties has never been suspended. Water transport in leaf vein systems is an essential part of plant growth and development. The leaf vein architecture can influence the transport properties a lot. As a review, Roth-Nebelsick et al. (2001) summarized knowledge of interrelationships between the form and function of leaf vein and the evolution of leaf vein patterns to investigate the functional properties of the leaf vein systems. The leaf vein networks with a large number of closed loops have been proven to be the optimal transport networks which have resilience to damage and fluctuations in load (Bohn and Magnasco, 2007; Corson, 2010; Katifori et al., 2010). Yet, there still lacks quantitative description of the water transport in real leaf vein systems. As a quantitative parameter, flow velocity can be a description to reveal the regulation of water transport in leaf vein systems. However, the measurement of flow velocity in leaf veins is seldom found in the previous research. Particle image velocimetry (PIV) is a new flow measurement technology developed from the 1980s, which combines optical technology, computer technology, photographic technology and image-processing technology (He, 2009). Hao et al. (2011) investigated the dynamic wetting characteristics of water droplets on silicon wafers with microscale regular pillars structures and fresh lotus leaves experimentally. The internal velocity distribution of water droplets on both of these super hydrophobic surfaces were studied with a PIV system. However, the situation will be of great difference when the fluid region to be measured is too small to be beyond the optical resolution or particle size, then the traditional PIV technology is no longer applicable (He, 2009). In order to solve this problem, a Micro-PIV technology was developed, whereas, no application on plant leaves could be found. Soares et al. (2013) presented an alternative protocol for use with the PIV technique in fluid and particle flow monitoring, without the use of external particles seeded as targets in the cross-correlation of the flow images. The method was based on the dynamic laser speckle patterns, or

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bio-speckle laser (BSL) patterns, with grains varying over time. A test used a simulated speckle pattern in a micro-flow in a torn leaf reacting to the broken internal pressure was taken. The results confirmed this hypothesis regarding the use of BSL associated to a PIV technique and illustrated a protocol to deal with the boiling effect that undermined the translational information in the speckle patterns. It was a significant breakthrough of flow measurement in plant leaves, but the method and experimental conditions were too difficult and complex to be followed. Furthermore, there was not any quantitative data reported about the flow velocity in the article. Garbe et al. (2002) used a technique of active thermography for estimating the water velocity in plant leaves. In his experiment, a part of the leaf was heated by a laser or heating wire and the movement of the heated water parcel was then visualized by an infrared camera. The water velocity, which must be calculated from a complex set of equations, was not given, too. Furthermore, the difference of temperature might have a significant impact on the water velocity and the heated water flowing from the petiole was opposite to the nature flow. Hüve et al. (2002) used the dyes neutral red and acid fuchsin for staining of the water transport pathways in leaf veins. The acid fuchsin was observed under a binocular microscope with a magnification of 2.5–5 times, to measure the time interval up to the first visibility of the dye reaching tiny dots of ink placed along the two or three selected veins every 5 mm. Then the transport velocity of the dye acid fuchsin in veins of different size, within intact and damaged leaflets, was calculated. The experimental results indicated that the transport velocity in small veins of damaged leaves was increased several fold compared to the measurement of transport velocity in veins of the same size in intact leaves, which ensured water transport to leaf areas distal from the cut. So far, this is the only flow velocity data in leaf veins reported by the literature. But it should be noticed that the measurement under the binocular microscope could only obtain the data from two or three selected veins once, which meant that the whole data of the same sample could not be obtained at the same time, and that might bring in serious errors caused by the diversity of different plant leaves, even of the same species.

In order to use the flow velocity as a quantitative description to reveal the regulation of water transport in leaf vein systems, a simple but efficient method must be developed for the flow velocity measurement. The method of using a fluorescence probe as a tracer to discover the mysteries inside plants is well developed. As being mostly used by researchers, fluorescent proteins play an indispensable role in unraveling the mechanism that governs the plant growth and development. For example, Chapman et al. (2005) reviewed the engineering of fluorescent proteins which continued to produce new tools for in vivo studies. The motivation of these improvements was to make the fluorescent proteins brighter and easier to be imaged. Zwiewka and Friml (2012) introduced the fluorescence imaging-based forward genetic screens as a developed approach which had progressed at a remarkable pace in the field of biological imaging in the last decades to identify trafficking regulators in plants. Compared with the conventional organic fluorescent dye, quantum dots (QDs) is a new fluorescence material and has the advantages of abundant color, stable photochemical properties, little fluorescence scattering and photo-bleaching, and low biological toxicity. Consequently, it is widely applied in the fields of biological tag, human pathology, material science, plant cell detachment and mark, genomics, proteomics, microorganism, biological imaging, biochip and so on (Chen et al., 2010; Pang et al., 2009). However, the application of QDs on plant research just began a few years ago and it is mainly used in roots and stems, not in leaves (Al-Salim et al., 2011; Hu et al., 2010). Besides fluorescent proteins, fluorescent dyes are also effective tracers for plant physiology studies, especially for the visualization of water transport through the plant leaves. Salleo et al. (2003) perfused *Prunus laurocerasus* L. and *Juglans regia* L. with a 2% Phloxine B solution under pressure. Infiltration of leaves with Phoxine B revealed that *P. laurocerasus* major veins were largely leaky in the radial direction whereas those of *J. regia* leaflets showed prevailing axial water transport. Katifori et al. (2010) injected fluorescein post injury at the stem of a lemon leaf, of which a circular cut was made on the main vein. It was observed that the fluorescein flowed through the vein network around the injury, closing a number of loops and eventually reaching the tip of the leaf. The experimental results indicated that the existence of a high density of loops in transport networks, such as leaf veins, has resilience to damage and fluctuations in load. Both Salleo and Katifori have studied the characteristics of water transport through the plant leaves using fluorescent dyes as tracers. But they haven't quantitatively analyzed the flow velocity of water transport in leaf vein systems.

With the development of computer technology, various efficient algorithms were applied for leaf veins recognition and extraction (Chen et al., 2011; Li and Zhao, 2012; Li et al., 2006; Radha and Jeyalakshmi, 2014; Zheng and Wang, 2010). Whereas, they were only fit for static leaf images. Inspired by the achievements in plants image-processing, the authors of this paper have tried an algorithm based on image-processing to recognize the position of the fluorescent dye flowing through the leaf veins and then automatically calculate the flow velocity in a selected leaf vein in a definite time interval.

In this paper, a simple but efficient method is developed to measure the flow velocity, which is used as a quantitative description to reveal the regulation of water transport in leaf vein systems.

2. Materials and methods

2.1. Plant material and tracer

For plant material preparation, three ordinary species of plants with heat-resistance in the campus, *Ficus virens* Ait. var. *sublanceolata (Miq.) Corner* (Fig. 1a), *Plumeria rubra* L. cv. *Acutifolia* (Fig. 1b) and *Hamelia patens* (Fig. 1c) were selected. During the experiments, the leaves of the three selected plants were cut and fed with a tracer fluorescein ($C_{20}H_{12}O_5$). The diameter of the fluorescein molecule is about 0.870 nm, which is much smaller than the pits pores (about 5 nm). Thus the movement of fluorescein could be representative of the velocity of the water. Legible and smooth flowing processes were observed. Fig. 1 shows the flowing images of the leaves of the three selected plants during the experiment, on which the tracer fluorescein can be easily detected.

In order to achieve good results, we selected the intact leaf samples from the three selected plants above, which were picked up with the stem remaining and placed in a plastic bag with wet towels to prevent damage to the veins. In the laboratory the leaves were stored in water and examined on the day of sample preparation.

2.2. Experimental setup

What we are interested in is the fluid flow in leaf veins. Therefore, we need to make the fluid visible. Our preferred approach is the one that could make the flow information digitized. Then, we tracked the flow by supplying the leaf with water dissolved fluorescein and recorded the flow from the petiole to leaf tip.

After a lot of trial-and-error experiments, we arrived at the setup with good effect shown in Fig. 2, which is described in the following.

The leaf's upside was fixed to a black piece of cardboard, which was just of the same size and shape of the leaf, with double-faced adhesive tape, due to the veins running near the backside of the leaf in the investigated species and therefore being more easily perceivable from that side. Then the cardboard with the leaf on