Calculation of flow vector in plants by non-destructive imaging using neutron radiography

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Abstract: *Water transport in plants is one of the most important factors for life, because it guaranties plants photosynthesis, the basic process for live on earth. Cold neutron radiography (CNR) with D_2O tracer provides visualization images of water flow in plants non-destructively. In order to calculate flow vector in plants, three optical flow algorithms, Block Matching, Horn-Schunck and Lucas-Kanade, were evaluated. The results showed that Block Matching was the optimum algorithm to calculate plant water flow from D_2O tracers. This algorithm was successfully applied to in situ investigate the flow vectors in the peduncles of three rose cultivars ('Akito', 'Milva' and 'Red Giant', respectively) having high, middle and low bent-neck susceptibility. The highest flow velocities obtained were approx. 45, 80, 19 mm hour^{-1} for 'Akito', 'Milva' and 'Red Giant', respectively. Also, for further grafting studies, calculations of flow vector in tomato seedlings were examined. Even if the seedling stem was thin, the high resolution of the CNR images allowed the exact calculation of flow vectors. Thus, this D_2O tracer vector method could most probably be applied in a great variety of plant water relations studies.*

1 Introduction

Water transport in plants is one of the most important factors for life, because it guaranties plants photosynthesis, the basic process for live on earth. Cold neutron radiography (CNR) with D_2O tracer is a useful method to non-destructively visualize water flow in small plants from root via stem to leaf or flower (NAKANISHI *et al.* 2005, MATSUSHIMA *et al.* 2005, MATSUSHIMA *et al.* 2007). Velocity of water flow can be an indicator of water sink and source strength in plant tissues and/or organs. We developed a method to observe steady water flow by cold neutron radiography using D_2O as a tracer (MATSUSHIMA *et al.* 2007, 2008). Compared to H_2O the mass attenuation coefficient of D_2O is smaller which allows for a better penetration of neutrons. As a result, the flow of D_2O

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was clearly observed in small plants such as tomato seedlings and rose peduncles. The second but essential step is to estimate the vectors of water flow in plant samples. The vectors would indicate plant parts that require water. This information would e.g. be useful for proper irrigation of plants under water deficit. This paper describes observations of water flow using CNR technique with D_2O tracer and introduces the applications for agricultural samples.

2 Experiments and calculation approaches

Cold neutron radiography

Neutron radiography visualizes the attenuation of neutrons through a medium. The probability of the neutron's interaction with a nucleus depends on the structure and the stability of the core. Some light elements such as hydrogen and beryllium, boron, lithium, nitrogen, etc. absorb and/or scatter neutrons rather well. On the other hand, neutrons penetrate very heavy elements such as lead, titanium and others rather easily. Elements having adjacent atomic numbers can have a widely differing absorption of neutrons. Hydrogen is one of the elements, which have a large mass attenuation coefficient and, hence, produce clear images. Hydrogen also forms the major constituent of living plants being incorporated in water, sugars, fibers and lipid molecules. By far the most ubiquitous molecule in living plant material is water. Hence, changes in the amount and the distributions of plant water are usually much more pronounced and can occur much faster than changes in other molecules. Experiments were conducted at Position II, CONRAD that is the CNR facility in BER-II, Helmholtz Center Berlin for Materials and Energy (HZB). The neutron flux and *L*/*D* at Position II was 10^7 neutrons/cm^2/s and 250, respectively. TAn advantage of this facility is the low noise in the obtained images. The bended cold neutron guide reduces high energy neutron that creates white spots on neutron images. Kardjilov et al. (2005) and HILGER *et al.* (2006) described CONRAD in more details.

Usage of D_2O tracer and application of algorithms

Steady state water flows or water diffusion can not be detected by radiographic techniques. Therefore, contrast agents, such as Iodine for x-ray radiography are needed to indicate water movement. In our most recent tested approach we use D_2O as a tracer to observe steady water flow. **Figure 1** shows a schematic diagram of the function of D_2O as a tracer in a plant. D_2O, showing much less interactions with cold neutrons than normal water, proved to be a suitable low-contrast tracer. Hence, the combination of CNR with the low-contrast tracer allows the direct visualization of water flow and the calculation of water flow with a high resolution at the tissue level.

To apply D_2O tracer, samples were placed in a crystal glass tube. H_2O and D_2O were alternately injected into the glass tube by a PC-controlled supply system. Due to their different attenuation coefficients for cold neutrons D_2O and H_2O creates positive and negative contrasts. In order to obtain flow vectors of the contrast images, three different optical flow algorithms, Block Matching, Horn-Schunck (HORN & SCHUNCK 1981) and Lucas-Kanade (LUCAS & KANADE 1981) were applied. For the calculations the IntelR Open source Computer Vision library, OpenCV® (opencv.jp; 2009) with C programming language was employed.

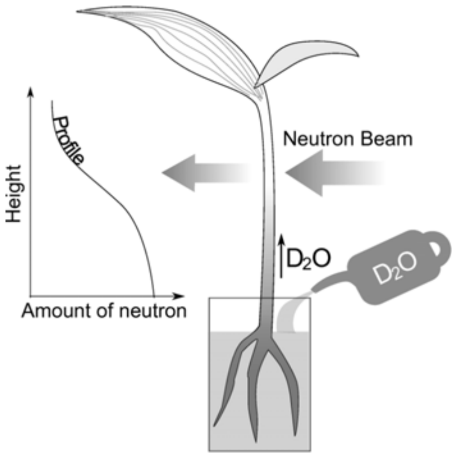

Figure 1: Schematic diagram of the function of D_2O in a plant as a tracer.

3 Water flow vector in plants

Evaluation of three algorithms

First, three optical flow algorithms were evaluated. Roses (*Rosa hybrida* L. 'Milva') were used as plant samples. **Figure 2-A, B** shows D_2O tracer images of adjacent frames. Approximately, tracer flowed vertically, because the sample was nearly uplight. The vertical movement distance ,the double-end arrow, was obtained from the adjacent binarised frames (**Figure 2-E**). **Figure 3-A, B, C** shows calculated vector using the tested algorithms respectively. The mean length of vector obtained using the Block Matching approach was similar to that of the double-end arrow. On the other hand, calculations by Horn-Schunck and Lucas-Kanade resulted in much shorter length of vector. More over, most vectors calculated by Block Matching indicated flow directions that were vertically upwards. However, vectors calculated by Horn-Schunck and Lucas-Kanade randomly indicated flow directions. Therefore Block Matching was the most suitable of the three algorithms tested. Details of this evaluation are given by MATSUSHIMA *et al.* (2009).

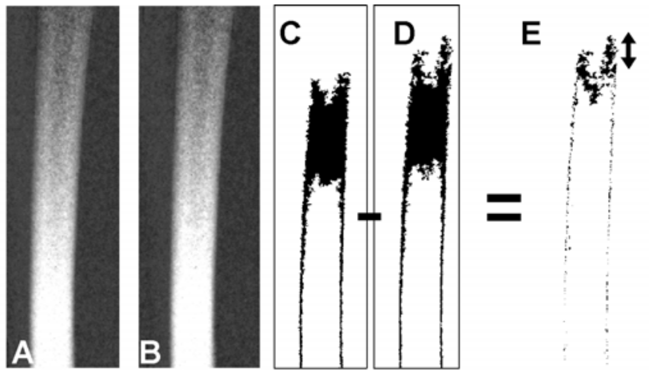

Figure 2: Adjacent frames of images indicating D_2O tracer flow in 5 min (A and B), and those of binarised images (C and D). Difference of C and D is vertical movement distance.

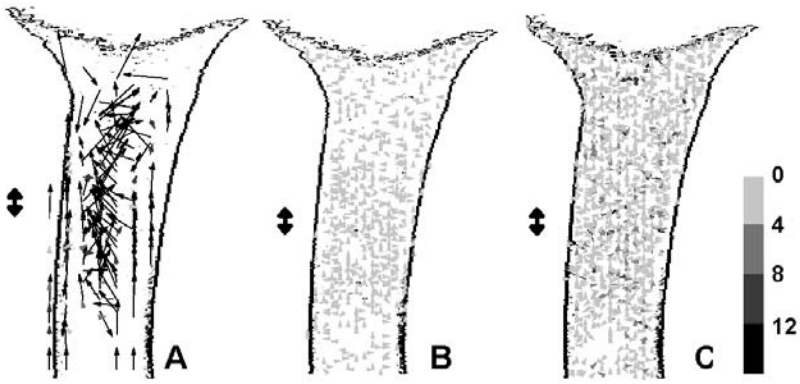

Figure 3: Flow vectors calculated using three different algorithms; Block Matching (C), Lucas-Kanade (D) and Horn-Schunck (E). Aspect ratio of the vector image was changed to show the detail of vector distribution. The unit of grey-scale bar is pixels/frame.

Application for a postharvest study of roses

This method was applied to visualize water flow in rose peduncles in order to compare “Bent-neck” resistance among three rose cultivars. Bent-neck syndrome, an important postharvest problem of cut roses, is probably due to water supply limitations and/or the structural weakness of vascular bundles of the peduncle tissue. The varieties ’Akito’, ’Milva’ and ’Red Giant’, respectively, having low, middle and high bent-neck resistances were employed as samples. Vectors were calculated by Block Matching algorithm. The highest flow velocities of 'Akito', 'Milva' and 'Red Giant' were approximately 45, 80, 19 mm hour^{-1}, respectively. The results indicated that water uptake of ‘Milva’ was the fastest. In contrast, roses of 'Red Giant', which generally show the highest resistance against bent-beck among the 3 cultivars obviously exhibited the lowest water uptake rates. Hence, water uptake velocity alone did obviously not reflect bent-neck resistance.

Application for a study of grafting

Grafting is a method to transplant e.g. weakly growing but effectively fruiting shoots (scion) onto strong root stocks. The grafting process is difficult to investigate by yet existing destructive methods. However, it is very important to better understand the grafting process to be able to rise the success rate. Hence, the final aim of the presented investigation is to evaluate the onset of water transport from root stocks to scions after transplantation. In a first step, it was attempted to obtain water flow vectors in small tomato seedlings (**Figure 3**) of 'Kagemusya' tomatoes. Even in the thin seedlings, D_2O tracer flow vectors were successfully obtained because of the high spatial and temporal resolution of cold neutron radiography images. Thus, this method is applicable to determine quantities of water flow in grafted tomato seedlings.

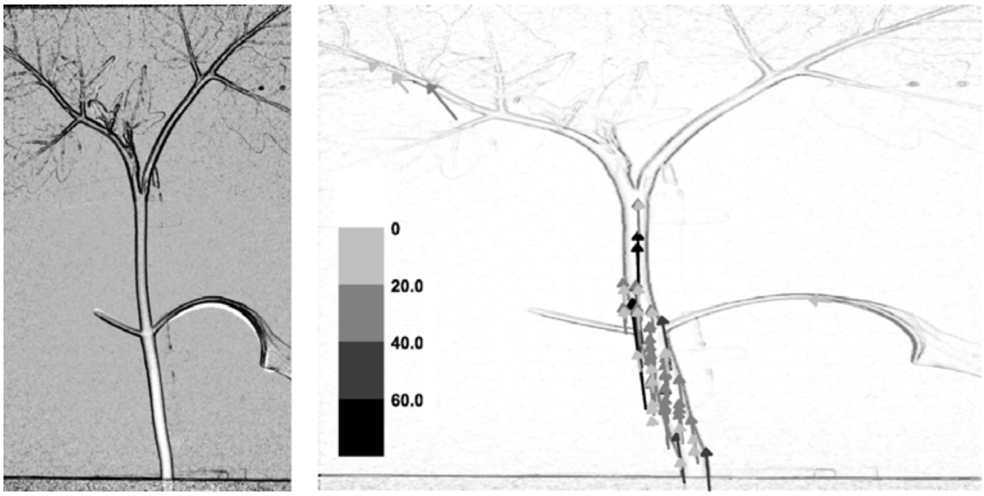

Figure 4: Flow vectors of D_2O tracer in tomato seedlings. Left: Image of D_2O tracer. Right: Calculated vectors using Block Matching. Aspect ratio of the vector image was changed to show the detail of vector distribution. The unit of grey-scale bar is pixels/frame.

4 Conclusions

In case of calculating flow vector of D_2O tracer in plants, Block Matching was the most suitable among three optical flow algorithms. CNR with D_2O tracer and vector calculation by Block Matching were successfully applied to study the bent-neck syndrome of roses and the success of grafting of tomato seedlings. Thus, evaluation of D_2O tracer vectors could most probably be applied in a great variety of plant water relations studies.

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