Journal of Applied Botany and Food Quality 82, 90 - 98 (2008)

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Application potential of cold neutron radiography in plant science research

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(Received July 15, 2008)

Summary

Though comprehensive knowledge of water status and water flow are important prerequisites for plant in many aspects of modern plant science truly non-destructive methods for the in-situ study of water transport are rare. Advanced imaging methods such as Magnetic Resonance Imaging (MRI) or Cold Neutron Radiography (CNR) may be applied to fill this gap. In CNR strong interaction of cold neutrons with hydrogen provides a high contrast even for small amounts of water. The combination of CNR with the low-contrast tracer D_2O allows the direct visualisation of water flow and the calculation of water flow rates in plants with a high resolution at the tissue level. Here, we give a general introduction into this method, describe their latest developments, report about studies applying neutron radiography in plant science and provide most recent results of our experiments in this field.

Introduction

Water in plants is one of the most important factors for life. As the major solvent as well as an important substrate it guaranties the well-functioning of the metabolic mechanisms of the plants, such as photosynthesis – the basic process for live on earth. Water availability, water distribution and water flow also regulate various plant physiological phenomena (VON WILLERT et al., 1995; LÖSCH, 2001). In future years water may become a limiting factor in agriculture, horticulture or silviculture production in many countries. Breeding of plants with improved drought tolerance may help to partially overcome this challenge (ARAUS et al., 2002). Conventional construction of plants with improved water uptake and transport performance such as grafting of high yield shoots on water effective roots may also be a solution (PROIETTI et al., 2008). Hence, for both breeding and grafting comprehensive knowledge about plant water relations and especially on water uptake and water flow phenomena is essential. However, non-destructive methods for the in-situ study of water transport are quite limited. Even modern heat balance method (VON WILLERT et al., 1995; LÖSCH, 2001) can't be applied without at least partially affecting the water transport pathways. Hence, non-invasive and fully non-destructive methods such as Magnetic Resonance Imaging (MRI; KUCHENBROD et al., 1996) or, less well-known, Cold Neutron Radiography (CNR, MATSUSHIMA et al., 2005a, b, 2007) seem to be appropriate methods to study water status and water transport in intact plants.

Neutron radiography is an effective imaging method, where the strong interaction of thermal or cold neutrons with hydrogen provides a high contrast even for small amounts of water. On the other hand, the neutron beam has a large penetration depth in metals (Al, Fe, Cu, Pb etc.). Therefore, neutron radiography has been mainly used for the visualizations of water and/or organic components in mechanical devices (TRABOLD et al., 2006). The full-scale entry of neutron radiography into biological field started in the early 1990th. Because soil is relatively transparent to neutron beam, neutron radiography became a good tool for non-destructive observation of plant roots (NAKANISHI et al., 1991; 1992). This imaging technique was applied to investigate the exchange of minerals between soil and plant roots (SAITO et al., 1997; OSWALD et al., 2005; MANNES et al., 2006a). The use of the CT technique allowed a 3-dimensional investigation of the water distribution around soybean roots (NAKANISHI et al., 2005; KIM et al., 2006). In postharvest technology, successful application of neutron imaging for the observation of changes in the internal structure of corn kernels during storage has been reported (CLEVELAND et al., 2006). In Japanese cedar seedlings, YAMADA et al. (2005) detected fungal induced tissue discoloration and tissue drying by neutron imaging. In this experiment, differences in the size of water deficient tissue parts resulting from damage by the impact of various fungi were identified. Neutron imaging was also applied for internal inspection and moisture mapping of tree trunks and timber (MANNES et al., 2006b). In order to study the characteristics of building materials, water absorption process of timber was investigated (LEHMANN et al., 2005).

New developments of neutron radiography using low energy neutrons and/or monochromatic neutron beams increase the potential of the method for plant science. The new developments provide better spatial resolution and a higher image contrast. In this report recent studies of the application of novel neutron radiography in plant science are introduced. Our recent attempts in this field were also described.

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. Hence, the attenuation of neutrons in a medium is random. Interestingly, certain light elements such as hydrogen and B, Be, Li, N, 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. Neutron attenuation efficiency can vary even between different isotopes of the same element.

The probability of neutron scattering and/or absorption by a matter is given by the so-called cross sections. When a material is irradiated by a neutron beam, the number of transmitted neutrons depends on the total cross section which is a sum of the scattering and the absorption cross-sections for the given material. Equation 1 explains the attenuation of neutron intensity, I, from the initial intensity I_0 to I by penetrating a material with the thickness x and the density ρ

$$\frac{I}{I_0} = e^{-\mu_m * \rho * x} \quad (1)$$

The beam detected by a two-dimensional imaging device results in an image that can be utilized to analyze the macroscopic structure of the samples interior because the mass attenuation coefficient, μ_m, of a respective material depends on its density and element composition. This means that this image of the penetrating neutrons provides a reflexion of the amount and the arrangement of certain elements in the sample.

Hydrogen is one of the elements, which have a large mass attenuation coefficient and, hence, produce clear images. The thus obtained good

contrast allows for high sensitivity to small amounts of hydrogen in complex systems. Hydrogen also forms the major constituent of living plants. It is 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 distribution of plant water are usually much more pronounced and can occur much faster than changes in other molecules.
Of course, some other elements with large total cross sections such as boron and lithium are also found in plants. However, their concentrations are normally too small to be observed by neutron radiography. Therefore, the effect of these elements is certainly negligible.
This all make neutron radiography a valuable tool to investigate the variation of water content and distribution in plant material. Nevertheless, the are some disadvantages of this method. This includes the relatively high cost and potentially radiation safety problems. Radiation safety problems are, however, rare and where they exist they are usually easily handled by shielding (BASTÜRK et al., 2005a, b).

Neutron energy and imaging

According to their energy neutrons are roughly classified as fast (high energy), thermal, cold and very cold (low energy) neutrons. This difference in their energy influences the characteristics of neutron transmission. Because the probability for interaction with the sample is reciprocal to the neutron energy the total cross section also depends on the energy of the neutrons.
By using liquid deuterium or hydrogen in the neutron source the energy of thermal neutrons can be largely reduced. This reduction in neutrons energy remarkably increases the total cross section and, hence, decreases transmission. Thus, low energy neutron beams provide high-contrast neutron imaging. This means that the dynamic range in the observation of changes of plant water is larger with the cold neutron imaging than that of the conventional method. This property should be suitable to investigate plants with thin stems, leaves and/or petals, for example small seedlings, blooming flowers, small bean pods etc. Such plant samples have spatially complex structures and the volume in the space would be relatively small.
Magnetic resonance imaging, an alternative to CNR, has a high spatial resolution (KUCHENBROD et al., 1996). It can provide various information about the status and the distribution of water (KÖCKENBERGER, 2001; GARNCZARSKA et al., 2007). MRI has also been applied to observe microscopic plant samples. For example, MANZ et al. (2005) studied the regulation of water uptake during germination of tobacco seeds by in vivo ^{1}H-nuclear magnetic resonance with a spatial resolution of 30 µm. Magnetic resonance imaging has been successfully employd to study long distance xylem flow and hydraulics in intact plants (e.g. JOHNSON et al., 1987; KUCHENBROD et al., 1996; PEUKE et al., 2001; SCHEENEN et al., 2007).
However, MRI is not suitable for small leafy plants, because both its spatial and time resolution are influenced by the ratio of the sample volume to the overall detectable space in the coil of the NMR detector. If this ratio is too small, i.e. the sample is small compared to the volume within the detector coil, the spatial and time resolution would be lower because of a low signal to noise relation (KÖCKENBERGER, 2001). This limitation is much less pronounced in CNR imaging (MATSUSHIMA et al., 2005a).
In case of CNR the resolution primarily depends on the sample thickness and not on the total sample volume to detectable space ratio. If the total thickness of the sample is smaller than the upper attenuation limit of the neutron beam, i.e. the neutrons are not fully absorbed or scattered by the sample material, the neutron radiography is able to detect water distribution with very high sensitivity.
As mentioned above, in CNR, the image contrast is affected by the energy of neutron radiation. Fig. 1 shows contrast differences in ivy leaves CNR images created by two different low energy neutron beams.

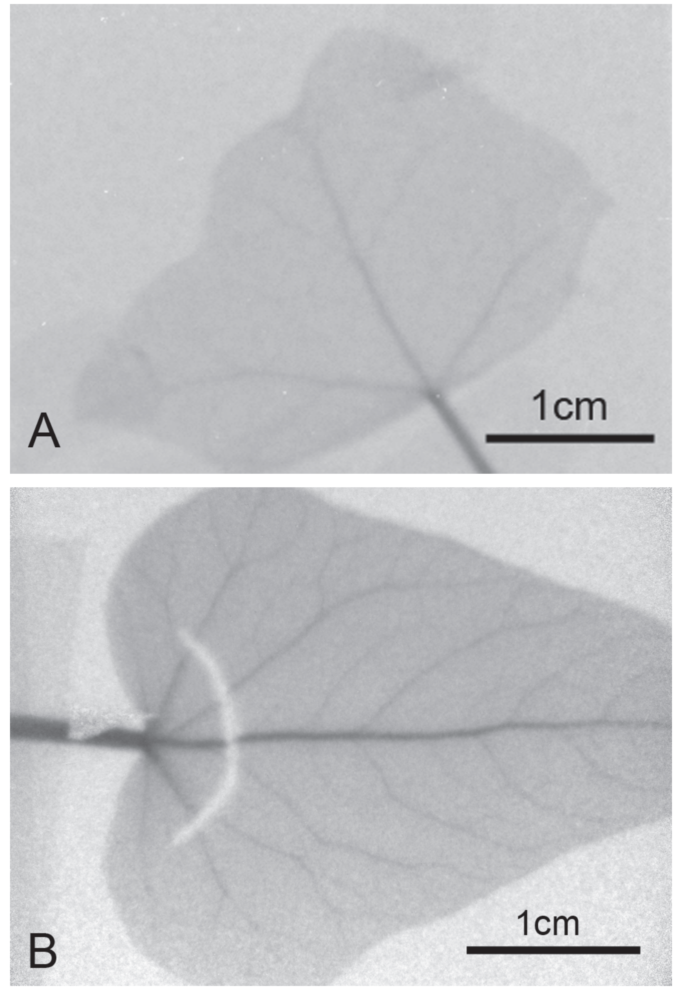

Fig. 1: Neutron images of ivy leaves taken by different radiation wavelength. A: approximately 0.3 nm (CONRAD, BER-II), B: approx. 6.0 nm (VCN port, PF2, Institut Laue-Langevin, ILL, France) (adapted from KAWABATA et al., 2005; MATSUSHIMA et al., 2005c).

The wavelength of neutron radiations used for the images in Fig. 1A and B were about 0.3 (3 Å) and 6.0 nm (60 Å), respectively. It is obvious that the contrast of the image obtained with longer wavelength radiation and hence lower energy is higher than at shorter wavelength and higher energy. In Fig. 1B the leaf veins are clearer visible than those of Fig. 1A because the low energy of the neutron beam (6.0 nm) resulted in a higher attenuation coefficient for H_2O, which, in turn, contributed to the increased contrast of the neutron image (KARDJILOV et al., 2003). In case of the image shown in Fig. 1B, water thickness could be estimated from the image at a resolution of 50 µm in 95% of confidence interval (MATSUSHIMA et al., 2005a).

Cold neutron radiography imaging system

During recent years, several experimental setups for low energy neutron imaging have been established consistently. Well known devices are CNRF („Cold Neutron beam for the Radiography Facility" at JRR-3M, Japan Atomic Energy Res. Inst., Ibaraki, Japan), ICON („Imaging with COld Neutrons" at Swiss Spallation Neutron Source, SINQ, Paul Scherrer Institut, Villigen, Switzerland), CONRAD („COld Neutron RADiography" at BER II, Hahn-Meitner Institut, Berlin, Germany), and ANTARES („Advanced Neutron Tomography And Radiography Experimental System" at FRM II, Technische Universität München,

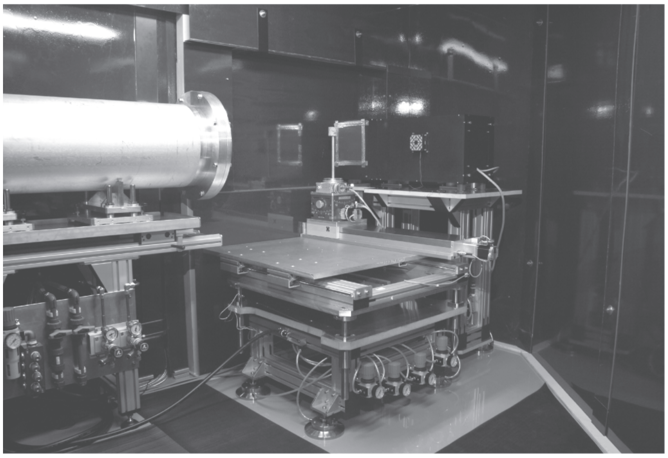

Fig. 2: Irradiation room of position II, CONRAD.

München, Germany). The wavelengths of the neutron radiation applied in these imaging facilities reflects that of a cold neutron beam of approximately 0.3-0.4 nm (3-4 Å). Hence, low energy neutron radiography performed at the facilities mentioned above will be referred to as cold neutron radiography (CNR) hereinafter.

As an example of a low energy neutron devices, CONRAD, at the Hahn-Meitner Institut (HMI) will be introduced in more detail. The experimental setup is placed at the end of a curved Ni-coated neutron guide at the experimental reactor BER II. The peak wavelength of the beam spectrum is about 0.31 nm (HILGER et al., 2006). There are two different experimental positions available. The neutron beam at Position I has a high neutron flux and a low spatial resolution. In contrast, Position II is adapted for high spatial resolution by optimization of the pinhole geometry. Users can chose the appropriate position in dependence of the specific purpose of their investigations. The beam size of Position II is 10cm×10cm and the spatial resolution is about 200 μm.

In the irradiation room there is plenty of working space for the sample setup on the right hand of beam propagation direction. More details about the experimental device are given by KARDJILOV et al. (2005) and HILGER et al. (2006). Spatial resolution has been further improved by reducing scintillator thickness and employed low energy neutron beam (KÜHNE and LEHMANN, 2006; FREI and LEHMANN, 2006; KÜHNE et al., 2006). Images appearing on the scintillator are deflected by a mirror into the 50 mm focus Nikon camera lens and is recorded by an Andor DW436N-BV CCD camera with 2048×2048 pixels, each 13.5×13.5 μm^2 large. This detector system can provide a spatial resolution of up to 25 μm.

Fig. 3 shows the root system of a tomato seedling obtained with the spatial resolution about 70 μm by the thin scintillator system. Fine roots of the seedling are clearly visible. This high resolution imaging system can be a powerful tool to investigate microscopic plant structures. However, to observe plant cells, a spatial resolution of less than 5 μm will be necessary. Images at the cellular level would clearly broaden the application of neutron imaging, thus, further technical developments are urgently required.

Applications in plant science

As mentioned above, application of neutron radiography in plant science has been primarily focused on water mapping in wood (LEHMANN et al., 2005; YAMADA et al., 2005; MANNES et al., 2006a, b), in corn (CLEVELAND et al., 2006), around and in living root systems (NAKANISHI et al., 1991; 1992; 2005; OSWALD et al., 2005; KIM et al., 2006) but also in leaves and stems of ornamentals (MATSUSHIMA et al., 2005a, b; 2007).

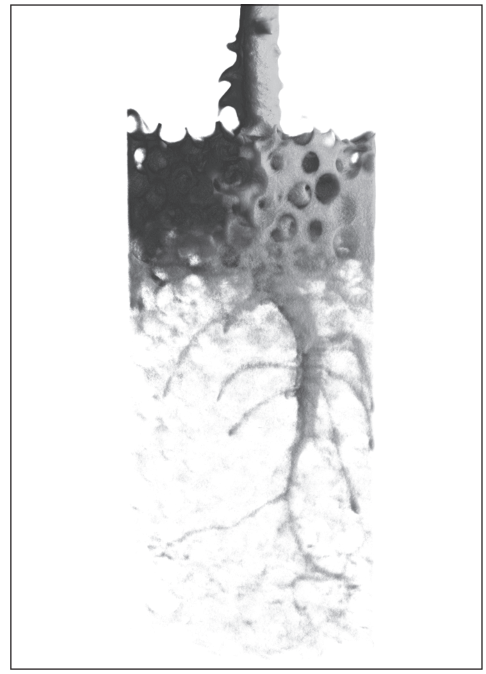

Fig. 3: Water distribution in the roots and the lower stem of a tomato seedling. The image was obtained with a spatial resolution of approx. 70 μm.

MATSUSHIMA et al. (2005b) investigated the effects of dehydration by vacuum cooling on the water content and the water distribution in chrysanthemum leaves by low energy neutron imaging at the CN-3 neutron port of the Research Reactor Institute, Kyoto University. It could be shown that during vacuum cooling overall water content declined by only approximately 5% of the initial fresh mass. However, differential water mapping before and after vacuum cooling application indicated that critical dehydration had occurred only at wound cuts of the leaf sample. Furthermore, the process dynamics of plant leaf water losses was also studied in detail using lower energy neutron beam (MATSUSHIMA et al., 2005a).

However, only further improvement of cold neutron radiographic facilities will open new perspectives for various additional usage of this method in plant science research. In the following, we present two novel applications using the advantages of CNR.

Study of water flow by D_2O tracer

In case of neutron or x-ray radiography, any growth or dehydration-induced dynamic variation in structure and/or density of plant materials causes changes of the contrast in transmission images. However, steady state flows in the biological samples such as water transport in plants that do not affect distribution or content of H_2O can not be directly detected by radiographic techniques. Therefore, contrast agents such as Iodine for x-ray radiography need to be applied as tracers to actually visualize the flow of H_2O with CNR.

In case of plant water transport deuterium oxide (D_2O) is suitable for this purpose. The physical and chemical properties of D_2O are very similar to those of H_2O (WIKIPEDIA, 2008). Hence, heavy water has been yet used in many investigations on its metabolic effects in fungi, animals and plants (ALEXANDROV et al., 1965; PITTENDRIGH et al., 1973; SIEGEL and GALUN, 1978; IGNATOV and LITVIN, 1998). In plants it has also been applied at low concentrations as a tracer to study various water exchange and water transport processes (ILVONEN et al., 2001; SEKIYA and YANO, 2004; ICHMASA et al., 2005). Concerning

CNR, the mass attenuation coefficient of D_2O is smaller than that of normal water, which allows neutron to better penetrate D_2O than normal water resulting in a large variation of image contrast.

Hence, we attempted to observe steady state water flow with CNR using D_2O as a tracer. To our best knowledge, no use of a contrast agent for plant research using neutron radiography has been documented before.

For the experiments conducted at CONRAD at HMI tomato seedlings (*Solanum lycopersicum* L. cv. Harzfeuer) were grown from seeds on sand in a climate cabinet (VB 1014, Vötsch Industrietechnik GmbH, Balingen, Germany). Climatic conditions were set to $T_{day/night}$ = 22/ 15°C and $rH_{day/night}$ = 40/70%. Plants received photosynthetic active photon fluence rates of approximately 220 µmol m^{-2} s^{-1} at a 14 h day period and were daily watered with tap water. After they had developed 2 or 3 true leaves plants were used for the experiments. Each seedling was transplanted into a quartz glass tube filled with a soda glass beads medium (Fig. 4 left). During the experiment the tomato seedlings were irradiated with a halogen lamp (Osram, Hamburg, Germany). The plants were protected by a Perspex water flow heat shield.

Fig. 4 (right) shows the experimental set up of the investigation on the D_2O-tracer application. With a custom-made PC-controlled injection system D_2O and H_2O could be automatically supplied to the samples in the quartz glass tube from bottles as requested. During the experiments, CNR images were taken every 15 seconds with an exposure time of 10 seconds and read out time of the detector of 5 seconds.

Water flow from root system to stem was clearly visualised by CNR through the positive contrast created by the D_2O tracer (Fig. 5). This clearly indicates that heavy water is a very suitable tool to comprehensively and non-destructively investigate the temporal and spatial dynamics of water movement at the plant organ level.

Furthermore, the observation of the D_2O level in the stem at different times after the exchange process enables the calculation of the velocity of water uptake and water flow in the seedling. In this measurements, flow rates of 2.6 cm h^{-1} could be estimated. These flow rates, corresponding to 0.01 mm s^{-1}, were much lower than those found (0.2 to 0.4 mm s^{-1}) for adult ricinus plants by MRI (PEUKE et al., 2001). At the respective developmental stage of the tomato seedling, xylem water flow may be slower than in mature plants due to the still developing vascular bundle system. Furthermore, the low VPD of less than 7 kPa MPa^{-1} prevailing during the entire measurement may have also reduced transpiration of leaves. Anyway, such a low flow velocity in small stems is difficult to measure with existing techniques such as heat balance systems (VON WILLERT et al., 1995).

Because the chemical structure and the physical properties of D_2O is similar to H_2O this tracer easily passes the casparian strip that selectively restricted the intake of chemical compounds. This is a big advantage of the D_2O tracer method. Boron, for example, can also be a valuable contrast agent for neutron radiography due to its high attenuation of neutron. Furthermore, boron is an essential nutrient to higher plants (BROWN et al., 2001). However, even at high external boron supply, plants do not take up enough boron to create a contrast in radiographic images. It is expected that boron, like other chemical compounds used as CNR tracers, would be partially excluded at the different transmembrane transport processes occurring during primary uptake by epidermal, cortical or endodermal cells and at the casparian strip, or during xylem loading in the root system (BASSIL et al., 2004). Therefore, tracers such as boron must be either injected by syringe, drip-infused or taken up after removal of the root system. Furthermore, at higher concentrations boron is highly toxic and it is known to concentrate in particular tissues of plant; and it is obvious that boron solution doesn't behave as normal water. Hence, this obviously indicates that D_2O is the preferential tracer for nondestructive CNR water flow studies in plants.

Furthermore, combining the D_2O tracer technique with the neutron computed tomography (NCT) imaging system it is possible to construct 3-dimensional maps of the distribution of D_2O or H_2O, respectively. Fig. 6A shows the D_2O distribution in a single vertical tomographic slice of the upper stem (peduncle) and flower bud of a rose. Using several horizontal NCT slices (Fig. 6A) taken at different but close locations it is possible to construct a D_2O replacement map. With this approach the intensity of the water movement within a vascular bundle and between the vascular bundle and the parenchyma cells could be traced (Fig. 6B).

According to the given scale, the grey scale in Fig. 6B reflect the amount of D_2O replaced in the different tissues. In this flow activity image the highly efficient vascular bundles are highlighted. The comparison of the slice of the D_2O map and a light microscope image of the peduncle cross section of a rose of the same cultivar (Fig. 6C) further indicates that the replacement of D_2O from the vascular bundles to the pith was more intensive than to the other tissues. For investi-

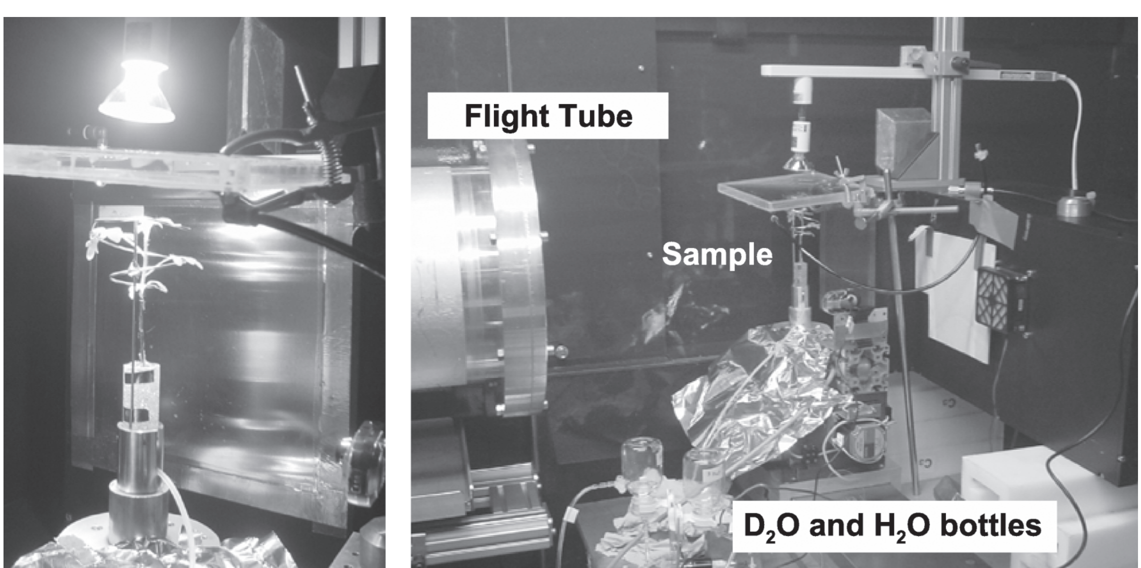

Fig. 4: Experimental setting for the application of D_2O tracer during CNR measurements. Left: Sample in a glass tube filled with soda glass beads medium. During the experiment the tomato seedling is irradiated with a halogen lamp with a Perspex water flow heat shield. Right: D_2O injection system.

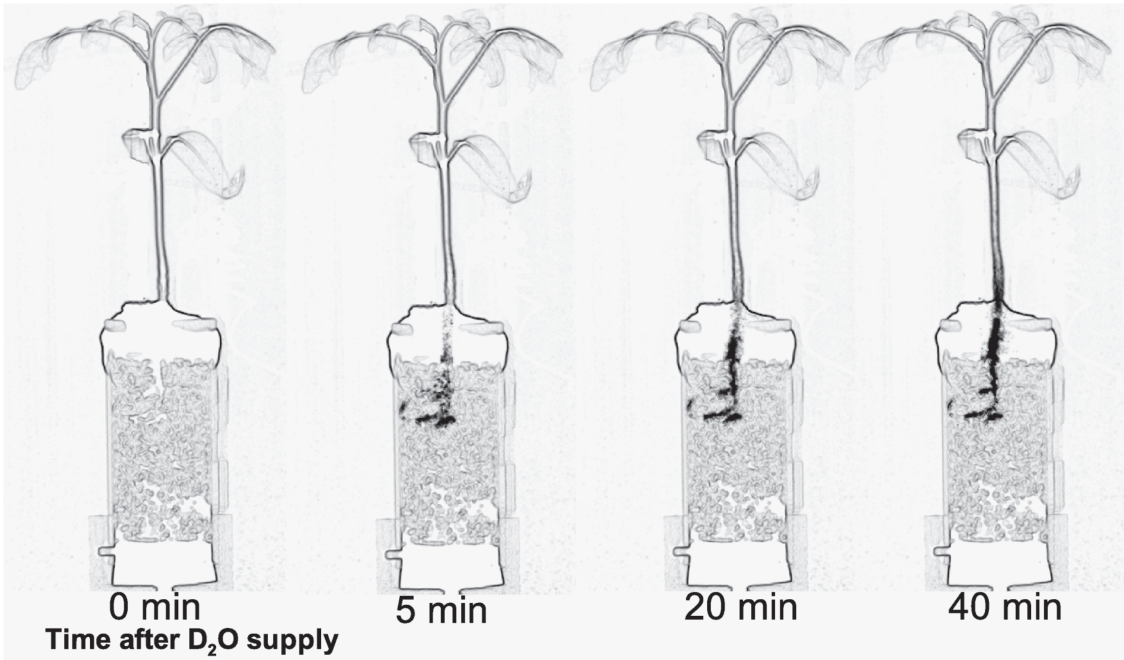

Fig. 5: An example of water flow into a tomato seedling stem indicated by the level of the D_2O tracer at different times after exchange of H_2O for heavy water. The dark area corresponds to the amount of heavy water in the stem.

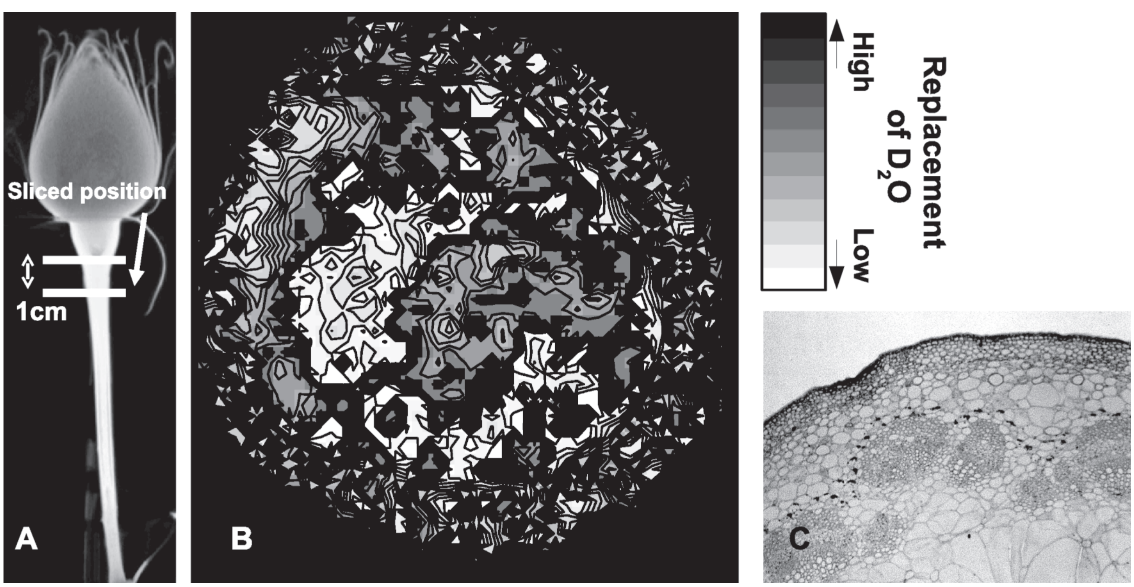

Fig. 6: D_2O map on a slice image in a neutron CT of rose peduncle. A: Vertical sliced NCT image. The lines indicates the NCT slice surface of those image used to construct the D_2O replacement map given in B. B: D_2O replacement map. C: Optical microscopic image of a rose peduncle (*Rosa hybrid* cv. Akito).

gations of mechanisms and dynamics of short and long-distance water flow in plants it is very desirable to observe 3-dimensional water flow characteristics at a high spatial resolution. For this purpose, a rapidly scanning neutron CT with improved resolution is necessary, hence further development of this technique is required.

Combination with other imaging analysis

The obvious drawbacks of MRI, compared to neutron radiography, are the lack of space and the high magnetic field around the sample which prevents the use of additional electronic devices. In case of CNR such devices can be protected from neutron and/or gamma ray by shielding, if necessary at all. This advantage of CNR was used in order to combine neutron imaging with an other method that monitors the photosynthetic activity of plants. Pulse modulation chlorophyll fluorescence analysis imaging (CF imaging) is a tool that provides deep insight into photosynthetic efficiency and integrity of plants (NEDBAL et al., 2000; HERPPICH, 2002). Especially the parameter F_v/F_m, an indicator of the potential photosynthetic efficiency, is highly related to plant stress responses (VON WILLERT et al., 1995). By the combination of neutron radiography and CF imaging, it is possible to parallely study the effects of environmental stresses on both water status and photosynthetic activity of the plant sample.

With these techniques, the effects of toxic auto-exhaust, simulate by 2 ppm SO_2 in air, on the physiological efficiency of street trees was evaluated. Hibiscus, which is a popular street tree in Okinawa, Japan, was used for experiments. Young rooted cuttings were enclosed in a temperature and humidity controlled aluminium cuvette fitted with a quartz glass window to be able to take the CF images. With their roots the plants were placed in glass tubes filled with glass beads. These tubes were connected to an automatic, PC-operated exchange system for H_2O and D_2O which could alternately supply each liquid. Before the start of the experiments, i.e. before the exposure of the plant to the simulated auto-exhaust gas an initial CF image was taken. Then, the toxic SO_2-in-air gas mixture was supplied for one hour while CF images were taken every 20 min. Afterwards, the cuvette was again flushed with normal air and plants were maintained under this condition for another hour with CF images taken regularly. During the entire experiment CNR images were recorded every 15 seconds with an irradiation time of 10 seconds. H_2O and the liquid tracer D_2O were alternately exchanged every 30 min.

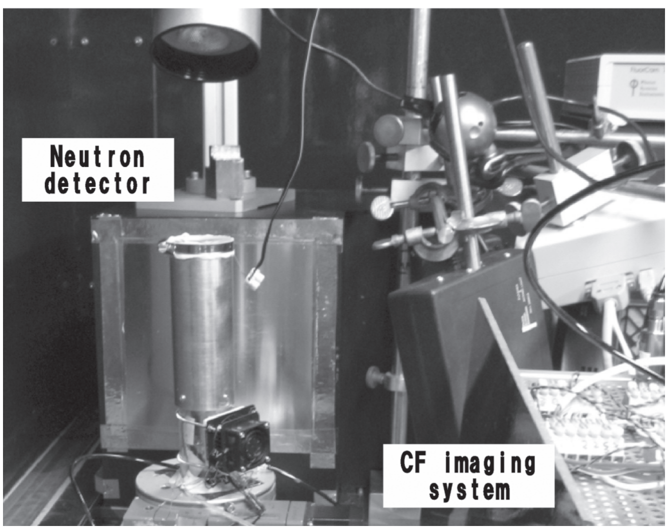

Fig. 7: CF imaging system installed in front of the CNR facility, CONRAD.

The CF imaging system was installed in front of the neutron radiography facility CONRAD (Fig. 7). It was placed vertically to the neutron beam line to avoid direct irradiation. The sample was located in the neutron beam in order to take neutron images simultaneously. Therefore, the sample cuvette was rotated by an automatic rotation table to face it to the fluorescence imaging camera.

Fig. 8 shows the variation of the maximum photochemical efficiency, F_v/F_m, of hibiscus leaves as analysed from the CF images taken during the course of the experiment. In this figure, F_v/F_m, which is a sensitive indicator of both the efficiency and integrity of plant photosynthesis, was presented in a false colour scale ranging from a minimum at 0.3 (dark) to a maximum at 0.8 (light). After the supply of the simulated auto-exhaust gas the average maximum photochemical efficiency of the exposed leaves, within few minutes, dropped by more than 30% from the initial mean of 0.64 to less than 0.40. Reduction of photochemical competence seems to be equal all over the entire leaf (Fig. 8), i.e. no clear-cut gradient developed during stress. On the other hand, these effects were fully reversible and F_v/F_m slowly recovered to its initial level after approximately 2 h in normal air (data not shown). Recovery seemed to be most rapid close to the major veins, probably also indicating a dilution effect of the cell sap, acidified by dissolved SO_2 (SCHMIDT et al., 1990). Our results clearly indicate that 2 ppm of SO_2 rapidly and seriously affect primary metabolism of hibiscus plants thus substantiating earlier findings that fumigation with SO_2 reversibly inhibited Calvin cycle activity and may even cause damage at the photosystem II level (SHIMAZAKI et al., 1984; SCHMIDT et al., 1990).

Exposure to SO_2 also rapidly affected plant water uptake and water status in the hibiscus stem as can be seen from the neutron images obtained simultaneously (Fig. 9) during the different treatments. The time interval between the four images was about 30 minutes. The D_2O tracer successfully both quantitatively and qualitatively indicated water flow and distribution in the stem of the plant sample. When the atmosphere in the cuvette was again changed from the simulated auto exhaust gas to normal air, the amount of tracer and, hence, the rate of water uptake increased. Therefore, it can be concluded that hibiscus trees may sensitively reduce stomatal conductance and transpiration in response to SO_2 stress as also found in peanut and tomato (KONDO

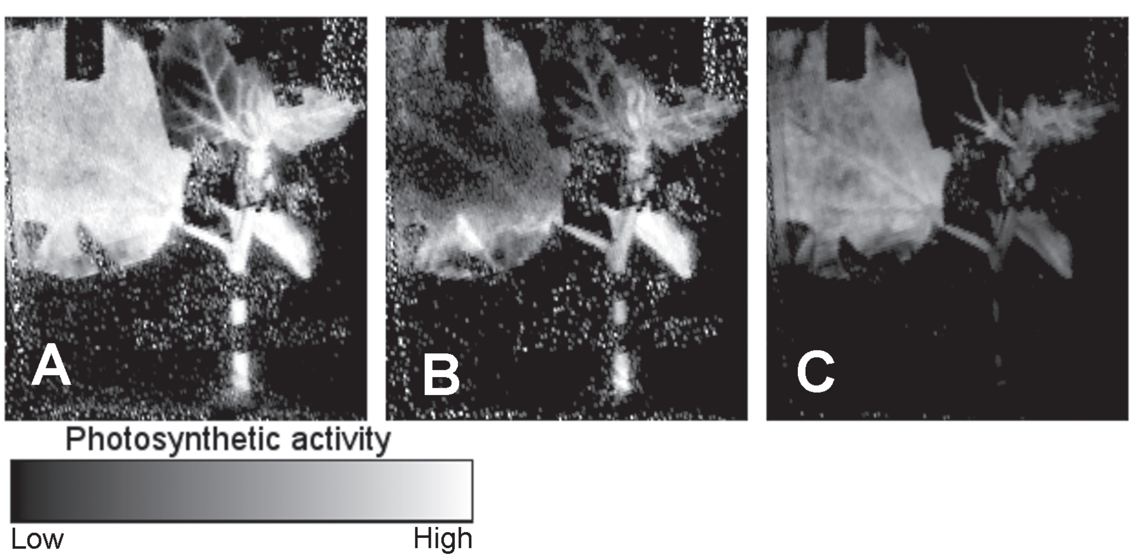

Fig. 8: CF images of hibiscus samples during the course of the experiment. A: Before supply of the SO_2 gas. B: 65 minutes after supplying 2 ppm of SO_2. C: 120 minutes after supply of normal air. Note that the low activity of the upper small leaf on the right hand side was due to a partial shading of the saturation light by the cuvette ventilator (c.f. Fig. 9).

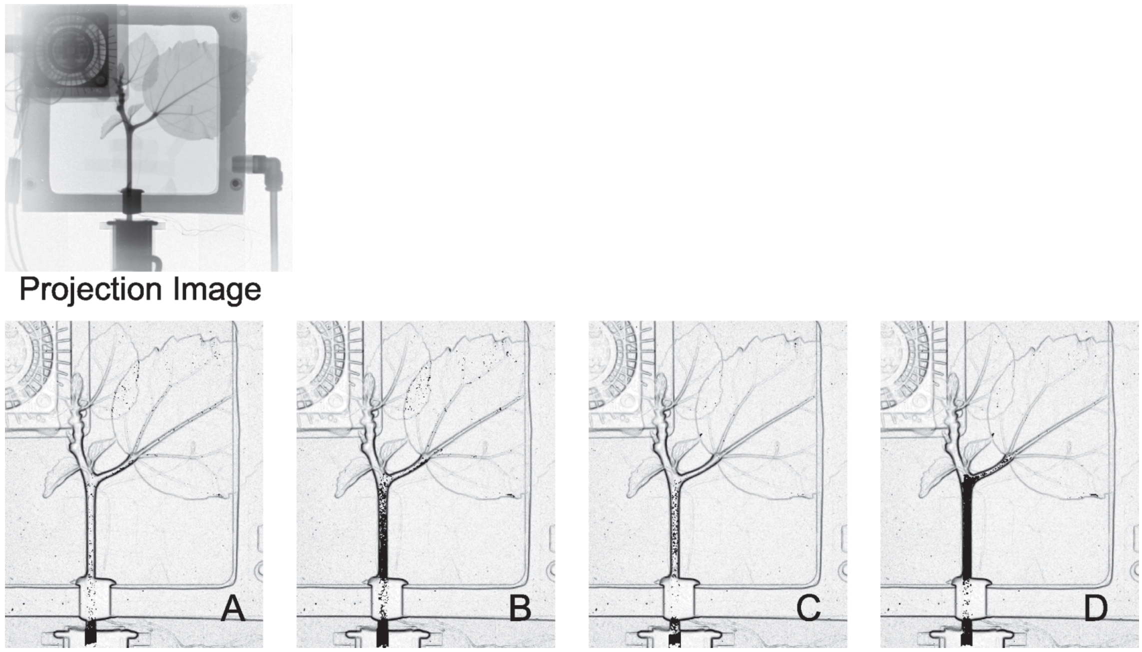

Fig. 9: Water uptake into the hibiscus stem during the different treatments as indicated by the D_2O tracer. The images were normalized to the initial state for each treatment step. A: 30 minutes after supply of SO_2. B: 60 minutes after supply SO_2 gas. C: 30 minutes after supply of air. D: 60 minutes after supplying air. The dark area corresponds to the amount of water in the stem.

and SUGAHARA, 1978). In contrast, stomata of radish, perilla, spinach (KONDO and SUGAHARA, 1978) or poplar (VAN HOVE et al., 1991) have been shown to respond much less extensive to this toxic gas. Hence, the presented results show that simultaneous CNR and CF imaging successfully visualizes the effects of air polluting gases like SO_2 on photosynthetic activity and water uptake, water movement and water distribution in plants at least in small samples. The combination of these two methods can greatly contribute to increase our understanding of the complex and interactive effects of toxic auto exhaust on the different aspects and levels of the metabolism of plants. Consequently, this is a very helpful approach to efficiently screen for street tree species and varieties with a high tolerance against auto exhaust, having the potential to increase CO_2 absorption capacity in cities (YANG et al., 2005; NORWAK et al., 2006).

Conclusions

CNR is suitable to investigate water distribution in small and/or thin plant materials, which determines a broad field of applications of this method in various field of applied plant science.

CNR combined with D_2O as a tracer directly visualizes water uptake, water flow and water distribution in seedlings and small plants. Following a further increase in spatial and temporal resolution of cold neutron radiography, water flow in microscopic pathways of plant can be monitored.

CNR can be easily and effectively combined with other advanced non-destructive and non-invasive imaging techniques such as CF, thermographic or hyperspectral imaging.

Using the combination of CNR and CF imaging, we successfully investigate the complex effects of simulated auto-exhaust gas on the physiological performance of Hibiscus cuttings. The result clearly demonstrated the integrated impact of SO_2 on both the photosynthetic activity and on transpiration.

Non-destructive imaging methods for plants are important tools to investigate plants. However, there is no imaging method suitable for all purpose. Cold neutron radiography is very effective to examine thin and spared plant materials. On the other hand, MRI or X-ray imaging can be a powerful tool for study thick compact plant materials.

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