

Chapter 17

Neutron Imaging Applied to Plant Physiology

T.M. Nakanishi

Abstract Until recently, the in situ study of roots and dynamic measurements of water uptake in plants or trees has relied on physical, and therefore invasive and often destructive, sampling or sensor installation in the surrounding soil. These methods can, at the very least, alter the equilibrium of the rhizosphere of the plant or require the removal of the tree or plant. The sensitivity of neutrons to hydrogen atoms provides a noninvasive, nondestructive experimental approach to understand real-time processes in plants (e.g., water uptake). This chapter attempts to illustrate the effectiveness of neutron imaging techniques in studying plant physiology, soils, and wood.

Keywords Botany · Neutrons · Cold Neutrons · Thermal Neutrons · 2D imaging · 3D imaging · Charge-coupled device · in situ · Soil · Water uptake · Wood · Flower · Carnation · Plant physiology · Roots · Growth · Sand

17.1 Introduction

As discussed in Chapter 1, neutrons are particularly sensitive to hydrogen atoms and can therefore provide information on their location with high spatial resolution ($\sim 50 \mu\text{m}$). When a neutron beam passes through a sample, the attenuation coefficient of some light elements — including hydrogen as well as rare earth elements such as gadolinium, dysprosium, and samarium — is extremely high, that is, 100–1000 times higher than those of the other common elements [1, 2]. In the case of X-rays, the attenuation coefficient changes by factors rather than orders of magnitude, from a light element to a heavier one, making it difficult to distinguish the change in the contrast due to a

T.M. Nakanishi (✉)

Graduate School of Agricultural and Life Sciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
e-mail: atomoko@mail.ecc.u-tokyo.ac.jp

specific element from other neighboring ones. Neutron imaging techniques are naturally suited for materials containing hydrogen atoms and other low atomic weight attenuating materials, permitting nondestructive in situ measurements of the spatial and temporal distribution of water (and other fluids rich in hydrogen) in plants and trees as well as in soils.

17.2 Nondestructive Water Observation in Plants and Soils Using Neutron Transmission Imaging Techniques

Nondestructive techniques for analyzing processes within tissues of plants have the potential to provide extremely important information for studying plant activity. Neutrons are efficient at probing plant roots, which have greater water content (between 70 and 95%) than their surrounding media (between 5 and 30%), as explained in [3]. Clearly the size of the root is limited to ensure a “reasonable” transmission thickness for neutrons, which may be up to several millimeters depending on the neutron wavelength (for an example of neutron transmission calculations, see Chapter 1). Water movement in particular, which plays an important role both in biochemical processes and in physical structure, has not been studied in detail. To acquire nondestructive in situ images of plant or soil water distribution, the use of X-ray computed tomography (CT) measurements has been reported [4]; however, the resolution obtained with this method was insufficiently high to acquire microscopic-level images. X-ray imaging has been able to resolve spatial patterns of root distribution in soil, although this requires destructive collection of soil slices [5]. The most promising approach to obtaining images of water, as well as images of other biological chemicals, is nuclear magnetic resonance spectroscopy. Although the resolution of these images can theoretically reach 10 μm [6], the sample size is limited and measurements are time-consuming.

Plant morphological development is reflected by water distribution within tissues, which can be monitored using neutron imaging techniques [7]. Neutron imaging of intact plant tissues can indeed yield exquisite microscopic images from a unique and novel perspective, and without causing much radiation damage.

Although neutron imaging is the most suitable method for the study of living plant behavior based on water uptake, historically, a few researchers in plant physiology have employed it [8–10]. Recently, however, with the development of neutron beam techniques such as cold neutron radiography [11–13], new technological advances such as thin neutron sensitive imaging scintillators [14], and advances in charge-coupled device cameras (Chapter 4), the use of neutrons for plant research has increased significantly. Dynamic imaging (i.e., the study of water movement) has especially increased in application but still remains limited compared to other techniques.

In thin plant tissues such as flower petals or leaves, changes in the water content are too small to image with thermal neutrons; therefore, cold neutrons can be applied to a thin tissue to enhance the contrast, as, for instance, in the

case of a chrysanthemum leaf [13]. Although cold neutrons are limited in their use because of a low transmission, small veins in the leaf can be observed, and the contrast is approximately 70% higher than that of a thermal neutron image. In addition, the range of changes in water thickness observed in the leaf is smaller than 30 μm [15].

Neutron beam is perfectly suitable for analysis of the tissue level of the sample, such as water distribution within a cut flower [3, 16–20] or a wood sample [21–24]. However the resolution afforded by the technique, typically tens of microns, is insufficient to study the process taking place within a single cell because much higher resolution is needed for observation within a cell.

To obtain 2D neutron transmission images of living plants and their root system in soil, samples are often grown in thin aluminum containers (2–3 mm thick). The total cross section (absorption and scattering) of aluminum is approximately 1/50 that of hydrogen; therefore, aluminum is almost transparent to thermal neutrons, and images of samples are not much affected by it.

17.2.1 Plant Roots Imbedded in Soil

Understanding of root growth is very limited because of the difficulty in observing root systems without obvious damage of the surrounding media and/or the root system itself. Often, destructive physical sampling or invasive sensor or minirhizotron installation is necessary and ultimately perturbs the rhizosphere (i.e., immediate area around the root system). However, observation of growth rates and root systems can be successfully imaged with neutron [25] or X-ray [26] imaging.

Figure 17.1 shows a neutron radiograph of a soybean plant root, after (A) 8 and (B) 15 days of growth in an aluminum container (of size $10 \times 150 \times 2 \text{ mm}^3$) packed with standard sand (silt type, 197–203 μm grain size, in pore size), containing 18% (by weight) of water. Since the water content in the root (in white in Fig. 17.1) is much higher than that in the surrounding sand, the root image was clearly shown even in the presence of soil [27].

The water-deficient part of the plant (dark in the image) is clearly visible near the upper part of the main root. To distinguish the water content of the root more clearly, a part of the main root initiating the growth of lateral roots is magnified in Fig. 17.1C. The apparent 3D image in Fig. 17.1D is produced by converting the degree of whiteness, which is directly related to the water content and to the height. The lower area near the root indicates a large amount of water uptake by the root. The activity of the root is well seen in a 1-mm area from the root surface. In this region, not only nutrients or water uptake, but also a corporative interaction between root and microorganisms occurs. From the neutron radiograph, it can be seen that the amount of water was very low around some parts of the root, suggesting the possibility of water vapor absorption rather than water solution. Possible future studies include the measurement of heterogeneity in water distribution and uptake at the rhizosphere. The latter phenomenon is expected to play an important role in

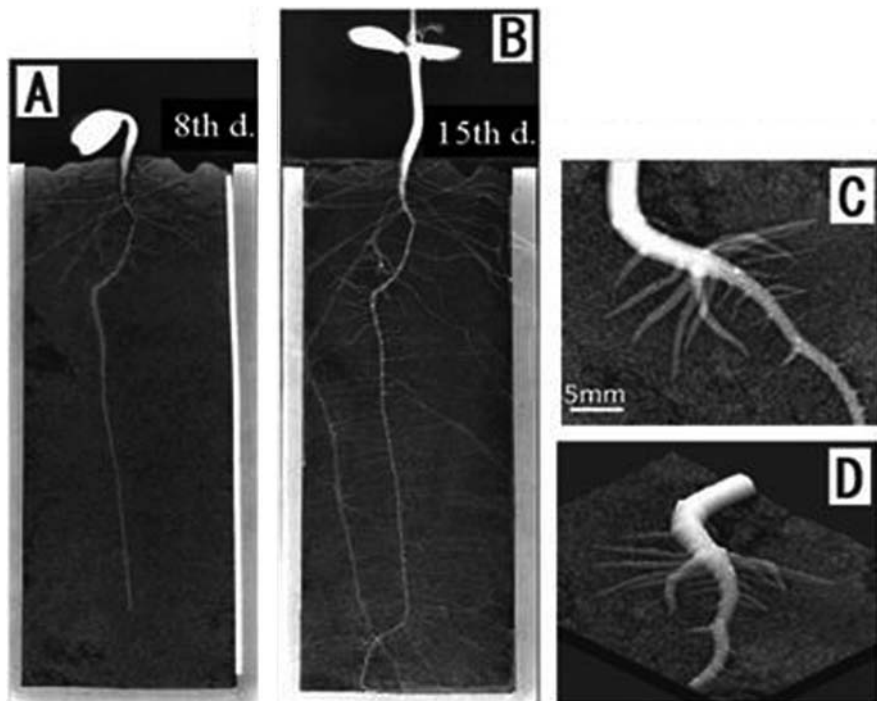


Fig. 17.1 A neutron radiograph of a soybean plant root embedded in soil after (A) 8 and (B) 15 days of growth; (C) magnification of the root; and (D) conversion of (B) to the 3D image

understanding the fundamental activities of the plant root system. Furthermore, neutron imaging may help explain the discrepancy between the amount of nutrients the plant actually absorbs from its environment and the amount “invasively” measured by soil sample extraction.

17.2.2 Observation of Root Development Under Different Soil Conditions

Noninvasive neutron imaging methods play an important role in the observation of growth rates and root development under different soil conditions, as discussed in [25]. In general, roots seek the most favorable place to grow, and adapt their growth orientation or pattern to the soil condition. For example, when water-rich soil is provided at one lateral side of the main root, the secondary roots preferably develop on that side. Similarly, Fig. 17.2A displays changes in root development due to the presence of vanadium in the soil. A soybean seedling is grown in an aluminum container where water-absorbing polymer material [28] containing 50 mM of vanadium is embedded in the soil. When vanadium is placed below the main root, the growth of the root ceases at

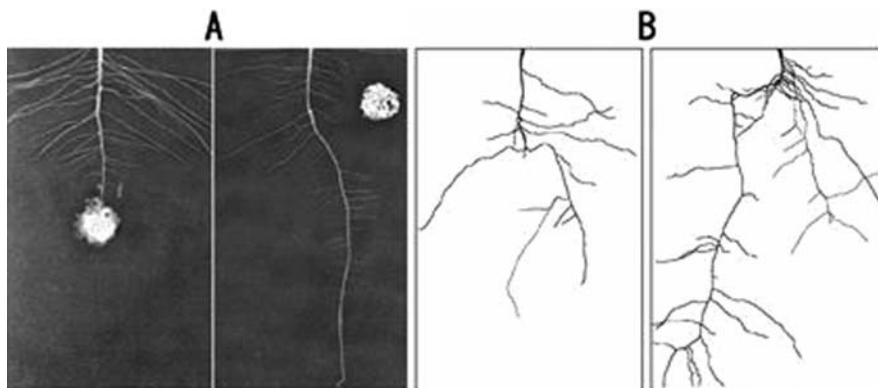


Fig. 17.2 Root development pattern. **A:** Soybean root development in the presence of 50 mM of vanadium (*white aggregate*). **B:** Radish root development. (*Left*) Low-pH soil containing 23 mM of aluminum; (*right*) Modified lignin mixed with the soil shown in the image on the left to reduce inhibition of growth by the aluminum

the polymer, but the side roots grows larger to compensate. On the other hand, when the polymer “doped” with vanadium is placed on one side of the root, the lateral root facing the polymer side does not grow, but the root system on the other side is longer. The position at which growth stops and the direction of root growth are dependent on the vanadium concentration inside the polymer [29]. It is also shown that the amount of water uptake at a certain height of the main root increases before side root growth begins.

Since root development is an indicator of the soil condition, neutron imaging can be applied to evaluate soil conditioning agents as well as the effect of a chemically modified fertilizer. To study lignin derivatives used as a soil conditioning agent, a radish plant is employed to observe the root profile in an acidic soil [30]. The root growth pattern captured in the neutron image in Fig. 17.2B clearly shows that the modified lignin is effective as a soil conditioning agent. Subsequently, the total root length is measured through image analysis, and the soil conditioning effect of different chemicals is analyzed. The root pattern shown in Fig. 17.2B is similar to patterns found in lightning or in the production of cracks in a pane of glass. There is no reproducibility in the pattern—a fundamental feature, especially for roots, for maintaining biological diversity. Because of this, experimental errors cannot be quantified properly when analysis of root growth is measured using invasive techniques such as the line intersection method, in which a lattice board is placed on the root and then removed from the soil, allowing the number of intersections between the root and the lattice to be counted. Neutron imaging, because it is nondestructive, can use the same sample to trace the root activity over time, which reduces the fundamental errors that arise from using different samples.

Recent laboratory experiments reproducing water infiltration in soil, plant, and the atmosphere have reported water redistribution processes as a function

of different conditions such as, for example, root structure, soil structure, and moisture [31].

17.2.3 Neutron Tomography of Root Systems

Since water movement in a plant is slow (approximately several minutes to hours), computed tomography can be used to evaluate “static” spatial water distribution inside and around root systems. For example, Fig. 17.3 illustrates a soybean root grown in a 3-cm diameter aluminum container packed with standard sand [27].

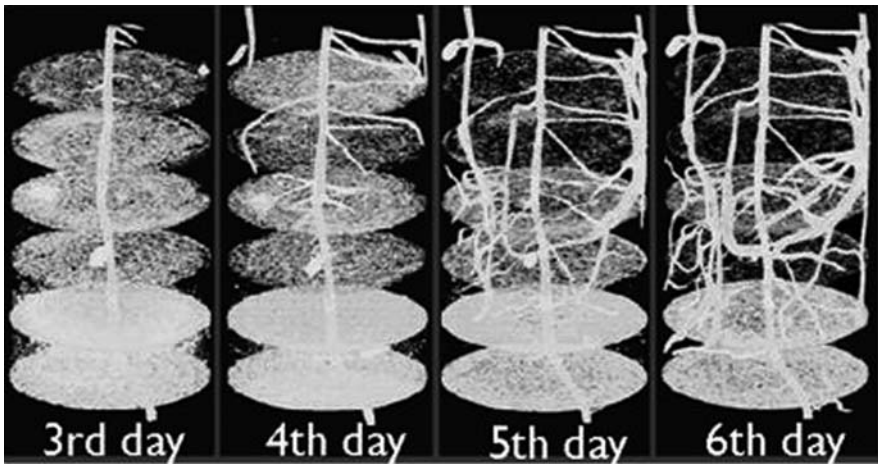


Fig. 17.3 In situ neutron tomography of the root system of a soybean during different days

Six CT reconstructions corresponding to different heights of the root system permit comparison of water content changes. Within a few days, the amount of water in the upper part of the container decreases, corresponding to an increased formation of roots. Using tomographic root images, positions and lengths of the side roots, as well as water movement around them, can be quantified (Fig. 17.4) [31].

There is an obvious increase in side root growth around the upper part of the container (up to 20 mm down from the air/soil interface) compared to side roots located farther down the container (Fig. 17.4, left). This correlates to a decrease in the water amount found in the soil after several days (6 days, for example) in the same region (Fig. 17.4, right).

Root surfaces and volumes can also be calculated using neutron imaging techniques. In the case of a soybean root, when 10 mM of AlCl_3 solution is applied to the soil, a decrease in both root surface area and root volume is observed [32]. The presence of aluminum ions is one of the main factors inhibiting plant growth

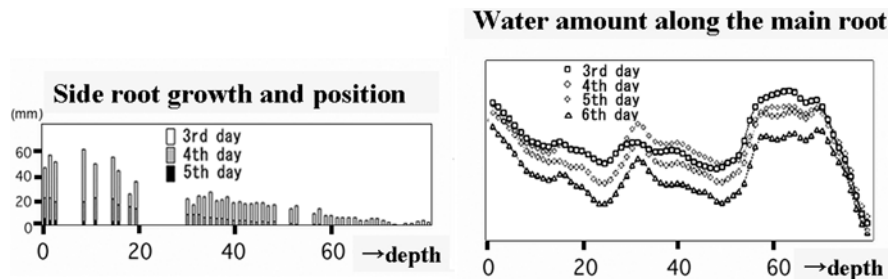


Fig. 17.4 Quantitative measurements of (left) side root growth according to their position along the main root and (right) water distribution around the main root days after watering the specimen. Depth is in mm

in an acidic soil. Moreover, root development in soil contaminated with heavy metals has also been analyzed via neutron imaging, as illustrated in [33].

17.2.4 The Aboveground Portion of the Plant

Neutron imaging is commonly used to analyze the aboveground portion of a plant. For example, neutron imaging of a cowpea plant can provide information on stem internode tissue, whose function is to store water [34]. Under water-deficient conditions, water is primarily moved from the internode to other tissues. A complementary study of water labeled with a positron emitter, ^{15}O , has measured real-time movement of water in a plant, as referenced in [35].

Extending the life of cut flowers is a key issue in the floral industry, and the amount of water in a flower plays a key role in maintaining its flowering stage. Neutron imaging of cut flowers shows detailed images of water content in flowers such as lilies, morning glories, and chrysanthemums. Figure 17.5 shows an example of carnation flowers imaged with thermal neutrons.

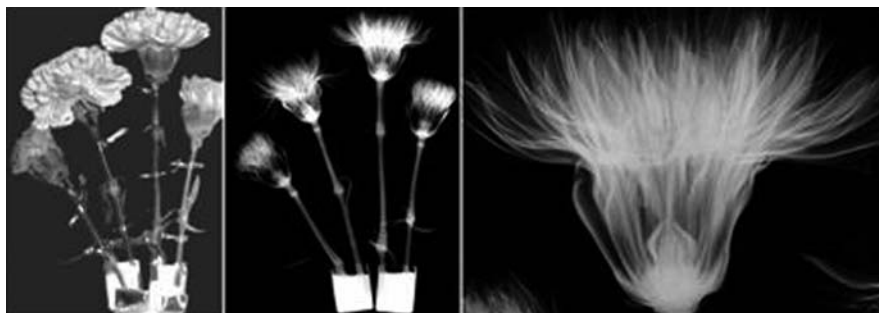


Fig. 17.5 Images of a carnation flower. A photograph is on the left, a neutron image in the center, and a magnification of the neutron image on the right. In the left and center images, the two flowers on the inside are controls and the two on the outside were dried before the neutron radiograph was taken [36]

Increasing the viscosity of water (i.e., xenon is dissolved under high pressure into water) can help prolong the life of a carnation flower after it has been harvested, as illustrated in Fig. 17.6A [36]. This helps control the metabolism of the flower, which slows down the deterioration process mediated by an enzymatic reaction. An example of a 3D neutron imaging study is shown in Fig. 17.6B. The flower respiration rate while conducting neutron imaging during the senescent (i.e., aging) stage has been evaluated [3, 18, 19]. Furthermore, the analysis of 3D images of a carnation flower demonstrates the importance of water inside the ovule to maintain the flowering stage longer. It is

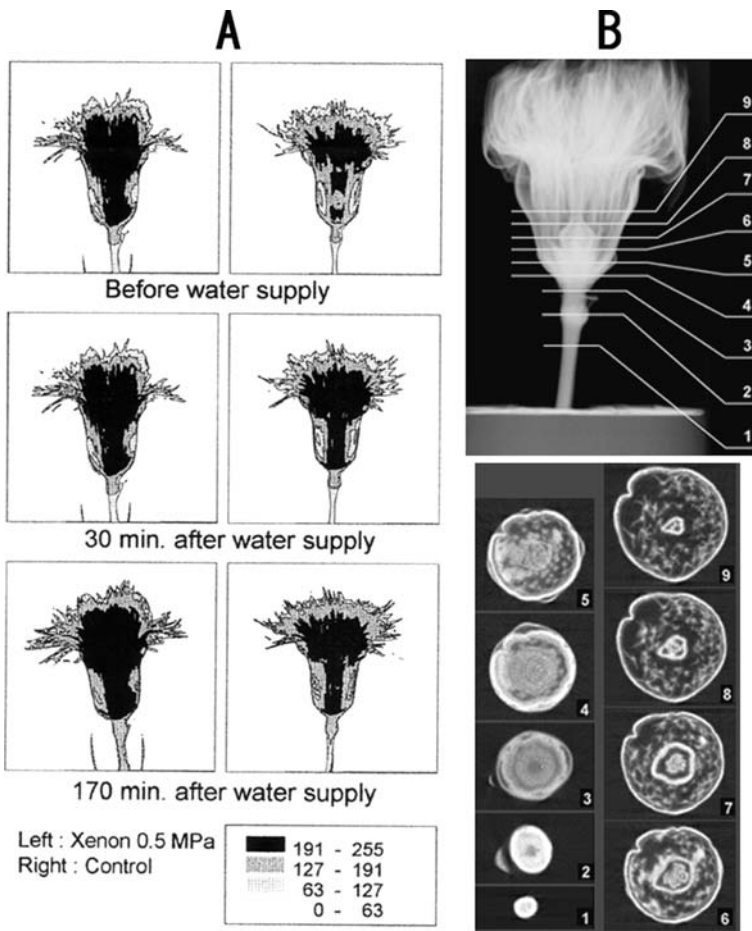


Fig. 17.6 Neutron image of a carnation flower. (A) Neutron radiographs of a carnation flower after being supplied with water containing xenon. (B) CT images at different heights along the flower head [36]

shown that the ovary and pistil areas continue to increase in size after the respiration rate stops increasing [36].

17.3 Investigation of Wood Samples

The study of physiological activity of a tree mainly relies on knowing the water distribution inside the wood. The example below illustrates the use of neutron imaging on wood disks extracted from a tree cut only a few hours before experiments, which therefore permits the observation of the green moisture image [23, 24]. A stem section, covered as a precaution to prevent water loss, is further cut to obtain 1 cm thick wood disks just prior to being exposed to neutrons. Figure 17.7 shows the neutron images of disks from a Japanese cypress (*Chamaecyparis obtusa*; Fig. 17.7A) and a Sugi (*Cryptomeria japonica*; Fig. 17.7B), whose ages are estimated to be about 19 and 24 years, respectively. The upper images in the figure are photographs of wood disks, and the

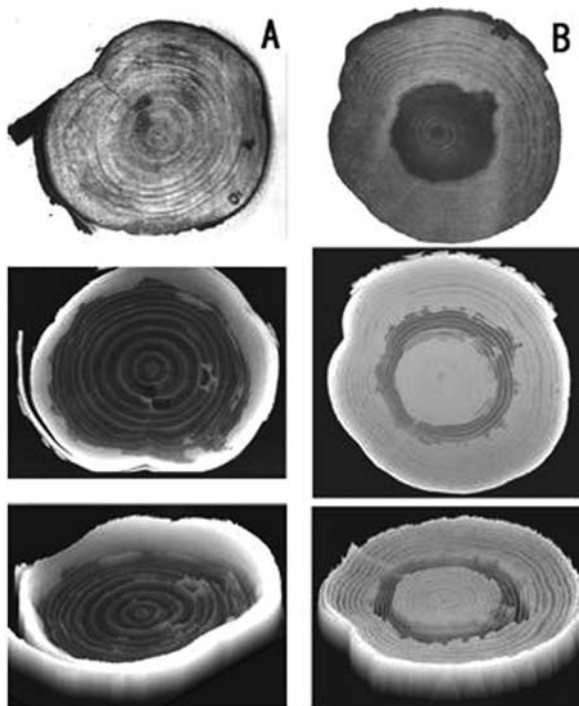


Fig. 17.7 Images of wood disks, each 1 cm thick. Photographs of disks are shown in the *top row*; neutron images in the *center row*; and reconstructions of the middle images, with high points corresponding to water-rich areas, in the *bottom row*

center images are the corresponding neutron images. The lower images are reconstructed ones based on the images in the center row with the degree of whiteness indicated as height.

In the Japanese cypress, the neutron image shows higher water content in the outer rings, adjacent to the bark. This zone includes the living phloem tissue and recently developed xylem tissue that transports water from the roots to the leaves. Both tissues have high water content. Water content declines radially from the bark into the older, less functional, air-filled xylem tissue. Water distribution in some areas shows a wavy pattern that might indicate tissue damage or buildup of specific chemicals within the rings. In the heartwood region, rings appear to have much lower water content, likely indicating buildup of decay-resistant extractive chemicals. Besides the large hydrogen-rich outer part of the disk, many rings corresponding to the annual rings of the wood are shown to contain a higher level of water than their immediate surroundings.

Sugi is a popular wood in Japan for building houses and furniture. Although there is only one species of Sugi, neighboring trees of the same cultivar show different water content at the heartwood. It is not known what causes the differences in moisture content at the heartwood. When lumber is processed, residual moisture causes serious problems. When the moisture content at the heartwood is high in green lumber, it is difficult to remove water completely during the drying process. However, moisture is gradually lost after the lumber is used in houses or furniture, causing the shape to warp over the years.

When neutron radiographs of wood discs are taken during the drying process, the mechanism by which moisture is lost can be directly analyzed [24]. In the case of Sugi, another feature in the disk is observed in the heartwood. As shown in Fig. 17.7B, there is always a water-deficient area between heartwood and sapwood. Because of the lighter color of this zone, it is called the white zone. As the tree grows, the area of heartwood increases. However, the water content throughout the heartwood is maintained at a high level as the tree grows. It is not known how the water in sapwood moves into heartwood across the white zone.

A rapid decrease in water content can be observed when the plant tissue is injured. For example, neutron imaging shows that when Sugi wood is inoculated with a canker fungus, a water deficiency occurs and spreads from the inoculated site [30]. The dynamic process of water absorption in a wood sample can be evaluated with neutron imaging techniques [22]. Imaging of solvent absorption and water-loss processes in small wood samples ($4 \times 4 \times 2.7$ cm) has been reported [21] when acryl resin solvents are applied to protect the surface of the wood, which is of importance for the development of wood-protective materials for preserving wood art with historical and cultural value.

Several other neutron imaging studies of wood can be found in [37–41].

17.4 Other Agricultural Applications

Besides the examples presented above, neutron imaging has been used in many other agricultural applications. The water uptake of seeds during germination provides useful information, for example, in investigating the method of storing seeds because in some seeds, physical damage or the storage condition may change the germination rate (Fig. 17.8) [42]. Even at an early stage of germination, the germination rate of seeds can be estimated using neutron images of water absorption.

Recently, a tomographic study of corn kernels has aimed at understanding the effect of *Aspergillus flavus* (*A-flavus* fungus) to resistant species. Anatomic changes are observed when resistant inoculated and uninoculated species are compared to their nonresistant inoculated counterparts [43].

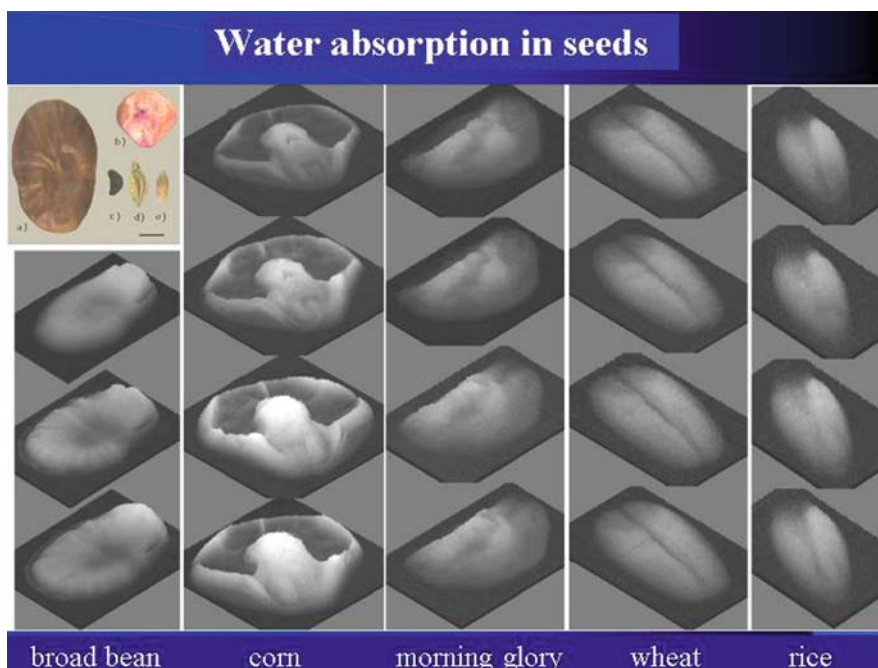


Fig. 17.8 Water absorption process in five kinds of seeds. Photographs of the seeds are shown in the upper left corner (the black bar is 5 mm long). Seeds are soaked in water and neutron images are taken after 0, 2, 4, and 6 h (top to bottom). For the broad bean, images taken after 0, 2, and 4 h are shown

17.5 Concluding Remarks

Nondestructive imaging methods for imaging water or H-rich fluids in living plants, soils, and wood using neutrons provide the highest resolution yet obtained for in situ water content analysis. Due to the high sensitivity to H atoms, this technique is capable of providing high-resolution images of water movement in seeds, in roots embedded in soil, in wood, and in meristems during development. The images presented in this chapter provide a taste of the intriguing new areas of investigation in the field of plant physiology that are being opened by new developments in neutron imaging methods.

Recently, the powerful methods derived from molecular genetics have resulted in a tendency to focus research on the molecular aspects of biology and to ignore important aspects of the intact plant. However, the intact plant itself has a high potential to integrate many functions and to respond to many diverse environmental conditions. Nondestructive techniques are essential to studying and understanding the activity of a living plant, its development, and its adaptation to environmental changes. The method reported here seems to be the most promising new tool for doing so.

References

1. K.A. Garrett and H. Berger, *Atom. Rev.* **15**, 125 (1977).
2. J.F.W. Markgraf and R. Matfield, Neutron beam, in J.C. Domanus, ed., *Practical Neutron Radiography*, Kluwar Academic Publishers, Dordrecht, Boston, London, pp. 26–50 (1992).
3. U. Matsushima, Y. Kawabata, C-M. Sim, K-Y. Nam, and T. Nishizawa, Proc. VIIIth IS Postharvest Phys. Ornamentals, Eds. N. Marissen et al., *Acta Hort.* **669**, 111 ISHS (2005).
4. J.M. Hainsworth and L.A.G. Alymore, *Soil Sci. Soc. Am. J.* **50**, 841 (1986).
5. C.J. Moran, A. Pierret, and A.W. Stevenson, *Plant Soil* **223**, 101 (2000).
6. P.T. Callaghan, Diffusion-limited resolution, in P.T. Callaghan ed., *Principles of Nuclear Magnetic Resonance Microscopy*, Oxford Science, Oxford, pp. 203–206 (1991).
7. T.M. Nakanishi and M. Matsubayashi, *J. Plant Phys.* **151**, 442 (1997).
8. S.T. Willatt et al., *Agron. J.* **70**(4) 581–586 (1978).
9. S.T. Willatt et al., *Ann. Bot.* **43**, 415–422 (1979).
10. P. Couchat et al., *Agron. J.* **72**, 321–324 (1980).
11. A. Hilger, N. Kardjilov, M. Strobl, W. Treimer, and J. Banhart, *Physica B* **385**(86), 1213 (2006).
12. Y. Kawabata, M. Hino, T. Nakano, H. Sunohara, U. Matsushima, and P. Geltenbort, *Nucl. Instrum. Meth. Phys. Res. A* **542**, 61 (2005).
13. Y. Kawabata, U. Matsushima, T. Horie, T. Nakano, and R. Maruyama, *J. Radioanal. Nucl. Chem.* **264**, 319 (2005).
14. G. Frei and E. Lehmann, *Proceedings of 8th World Conference of Neutron Radiography*, Gaithersburg, USA, p. 21, October 16–19 (2006).
15. U. Matsushima, K. Kawabata, M. Hino, P. Geltenbort, and B. Nicolai, *Nucl. Instrum. Meth. Phys. Res. A* **542**, 76 (2005).
16. U. Matsushima, T. Ooshita, T.M. Nakanishi, M. Matsubayashi, Y. Seo, and Y. Kawagoe, *J. Jpn. Soc. Agr. Machin.* **62**, 70 (2000).

17. U. Matsushima, K. Kawabata, T. Nakano, M. Hino, P. Geltenbort, and B. Nicolai, *Proc. 5th Int. Postharvest Symp.* **682**, pp. 1411 ISHS (2005).
18. U. Matsushima, Y. Kawabata, and T. Horie, *J. Radioanal. Nucl. Chem.* **264**, 325 (2005).
19. U. Matsushima, E. Lehmann, P. Vontobel, G. Fre, B.M. Nicolai, T. Nishizawa, and Y. Kawamitsu, *Proceedings of the Asia-Pacific Symposium on Quality Management for Agri-Foods in Supply Chains*. Bangkok, Thailand, Aug. 7–10, p. 119 (2006).
20. T.M. Nakanishi, J. Furukawa, and M. Matsubayashi, *Nucl. Instrum. Meth. Phys. Res. A* **424**, 136 (1999).
21. E. Lehmann, S. Hartmann, and P. Wyer, *Nucl. Instrum. Meth. Phys. Res. A* **542**, 87 (2005).
22. D. Mannes, E. Lehmann, S. Oswald, and P. Niemz, *Proceedings of 5th Plant Biomechanics Conference*, Stockholm, August 28–September 1, pp. 393 (2006).
23. T.M. Nakanishi, I. Karakama, T. Sakura, and M. Matsubayashi, *Radioisotopes* **47**, 387 (1998).
24. T.M. Nakanishi, T. Okano, I. Karakama, T. Ishihara, and M. Matsubayashi, *Holzforschung* **52**, 673 (1998).
25. M. Menon, B. Robinson, S.E. Oswald, A. Kaestner, K.C. Abbaspour, E. Lehmann, and R. Schulin, *Euro. J. Soil Sci.* **58**, 802 (2007).
26. A. Pierret, M. Kirby, and C. Moran, *Plant Soil* **255**, 361–373 (2003).
27. T.M. Nakanishi, Y. Okuni, J. Furukawa, K. Tanoi, H. Yokota, N. Ikeue, M. Masubayashi, H. Uchida, and A. Tsuji, *J. Radioanal. Nucl. Chem.* **255**, 149 (2003).
28. T.M. Nakanishi, S. Matsumoto, and H. Kobayashi, *Radioisotopes* **42**, 26 (1993).
29. J. Furukawa, T.M. Nakanishi, and M. Matsubayashi, *Nucl. Instrum. Meth. Phys. Res. A* **424**, 116 (1999).
30. T. Yamada, Y. Aoki, M. Yamamoto, M. Komatsu, D. Kusumoto, K. Suzuki, and T.M. Nakanishi, *J. Radioanal. Nucl. Chem.* **264**, 329 (2005).
31. J. Furukawa, T.M. Nakanishi, and M. Matsubayashi, *Nondestr. Test Eval.* **16**, 335 (1999).
32. K. Saito and T.M. Nakanishi, *Mokuzai Gakkaishi* **43**, 669 (1997).
33. T.M. Nakanishi, S. Matsumoto, and H. Kobayashi, *Radioisotopes* **41**, 638 (1992).
34. T.M. Nakanishi, K. Don-Jin, T. Kitamura, R. Ishii, and M. Matsubayashi, 242, 353 (1999).
35. Y. Okuni, J. Furukawa, M. Matsubayashi, and T.M. Nakanishi, *Anal. Sci.* **17**(Supplement), i1499 (2001).
36. T.M. Nakanishi, J. Furukawa, and M. Matsubayashi, *Nucl. Instrum. Meth. Phys. Res. A* **424**, 136 (1999).
37. E.H. Lehmann, P. Vontobel, and P. Niemz, *Investigation of Moisture Distribution in Wooden Structures by Neutron Radiography*. PSI Annual Report Annex VI, p. 53 (1999).
38. E.H. Lehmann, P. Vontobel, P. Niemz, and P. Haller, The method of neutron radiography and its use for wood properties analysis. In *Proceedings of the International Conference on Wood and Wood Fibre Composites*, Stuttgart, Germany (2000).
39. E.H. Lehmann, P. Vontobel, P. Scherrer, and P. Niemz, Application of neutron radiography as method in the analysis of wood. *Holz als Rohund Werkstoff* **59**(6), 463–471 (2001).
40. P. Niemz, E.H. Lehmann, P. Vontobel, P. Haller, and S. Hanschke, Investigations using neutron radiography for evaluations of moisture ingress into corner connections of wood. *Holz als Roh-und Werkstoff* **60**(2), 118–126 (2002).
41. V. Bucur, *Nondestructive Characterization and Imaging of Wood*, Springer Series in Wood Science, (Hardcover), ISBN 3-540-43840-8 (2003).
42. T.M. Nakanishi and M. Matsubayashi, *Bioimages* **5**, 45 (1997).
43. T.E. Cleveland et al., *J. Cereal Sci.* **48**, 517 (2008).