Summary and Perspective

First, let us look again at the water imaging and movement presented. It was shown how an exquisite water-specific image could be shown by neutron beam imaging. It provided a beautiful profile of water in living plants. Since plant activity is rather slow, successive images of the plant acquired by the neutron beam could show slow movement of water that was not able to be visualized before. In the case of fast movement of water, outside our devices, it was noted that water circulation occurred in the internode. When the role of water is discussed, focus is often on water absorption or the movement of newly absorbed water, rather than the water already present in the plant. One of the points suggested by our research was that both newly absorbed water and the water already present in the plant seemed to have their own role. When water was labeled with ^{15}O, whose half-life is extremely short, 2 min, the trace of water movement could be measured by our measuring system. What the ^{15}O-water movement showed was that a tremendous amount of newly absorbed water was constantly flowing out from the xylem tissue horizontally and pushing the water already present into the xylem, where it was transferred upwards. However, the water flow in the xylem tissue was kept constant. Is there any difference between the newly absorbed water and the water already present in the tissue? How could the newly absorbed water be distinguished from the water already present in the internode? It was the first measurement of water circulation within the internode.

Although we could measure the water circulation movement only in the internode, there might be other circulation movements in other tissues. If this is the case, then there is a further question of why there is a circulation movement of water within the internode. Since the water movement was different from that of the dissolved ions in it, there must be an important role for the water movement itself. This movement might be changed during the developmental stage of the plant, which could be estimated from the development of bellows-like lignin around the xylem tissue. The pitch of the lignin bellows is large during the young stage and allows water to flow out of the xylem easily; however, the pitch gradually becomes narrower towards the senescent phase and hardly allows water to leak out at the end. It was suggested that the amount of water leaking out from the xylem was adjusted

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by this lignin, that is, the young tissue contains a high amount of water, and the amount of water reaches a minimum at the senescent phase. It was also estimated that the development from the juvenile to the adult phase might be created from the degree of water circulation activity, not only the amount of water but also its movement and speed.

The water image of the rape plant pod was acquired to study whether the water flow changed the phase of the developmental stage of the seed. When the water amount was measured by harvesting the seeds in the pod, it was found to increase before oil formation started, and then, the water content decreased with increasing oil formation. Although the change in the water amount in the seed stem could not be distinguished because of the fine structure, it was suggested that the change in the water flow might trigger oil formation in seeds.

When water is present, biochemical reactions proceed, and the plant can grow to the next stage. However, if it is possible to regulate the water flow, especially when it is possible to stop a specific water flow, this could be a survival strategy for plants. Considering the environmental conditions of the plant, biochemical reactions could cease if the water supply was stopped. As a result, without further futile use of energy, the plant can sterilize a specific tissue for further development. The water movement in a rose flower causing the bent neck phenomenon shown by neutron beam imaging suggested this possibility for the water regulation strategy by the plant.

Whether there is enough water in the environment is an important factor for plant survival; however, only water absorption activity, high or low, tends to attract attention to study the drought tolerance of plants. As shown in our study, naturally developed drought-tolerant cowpea usually absorbed less water than sensitive cowpea. However, under drought conditions, tolerant plants began to absorb much higher amounts of water, whereas sensitive plants could not absorb water. This result was unexpected. Generally, we expect drought-tolerant plants to have higher water absorption activity than sensitive plants, which is why tolerant plants can survive under semi-arid conditions. Therefore, it was natural for us to study the mechanism of water absorption and try to introduce strong water absorption activity to the sensitive plant. However, our results in naturally created drought-tolerant and drought-sensitive plants suggested that the drought tolerance activity might be related to water movement within the plant. It might be that the drought-tolerant plants utilized a small amount of water more effectively than the sensitive plants under normal conditions, which might be derived from the different water movement activities. However, it is not known how tolerant plants can absorb more water than before under drought conditions.

Regarding the velocity of water absorption, there are many questions that cannot be solved. One of them is what the normal speed of water absorption is. As shown in the comparison of water culture and soil culture, the plant grows much faster in water culture. In the case of plant factories, water culture is generally employed to grow vegetables, since plants grow much faster in water culture than in soil culture. However, in cereal plants, the amount of grain produced, the yield, is much lower in water culture than in soil culture. Therefore, we have to depend on soils to grow cereal plants, such as wheat, corn, or rice, even though the growth is slow. Considering the drastic difference in yield between water and soil culture, one of our expectations was that it might also be related to water circulation in the plant. In water culture, the plant grows too fast, especially during the juvenile phase, and might be unable to regulate water movement.

The root tip moves during growth, known as circumnutation. Because of this movement, the soil is pushed aside to facilitate root growth and guide the orientation of the growth direction. As a result, this movement makes a space adjacent to the root surface in soil. As is known, soil contains roughly similar amounts of soil matrix, water, and air. Therefore, combined with circumnutation, it is conceivable that there could be an air space close to the surface of the root.

The neutron imaging clearly showed how the roots were growing in soil and the water amount in the vicinity of the root and revealed that there was hardly any water solution touching with the root surface. The neutron image of the root imbedded in soil suggested that the root was absorbing water vapor, not water solution. Then, what about the metals is the next question. Is the root absorbing metal vapor? We have no knowledge of what chemical form of water or elements the roots are absorbing.

In the case of root movement, a very interesting phenomenon was found using the Super-HARP camera, which enabled the visualization of root movement in the dark. When there was a chemical change in the environment, although the circumnutation of the root tip ceased, the root was able to elongate, and it was interesting that after a while the root movement resumed. Although the first data on plants show that the harmful effect is growth inhibition, the first effect of the toxicity was to stop the rotation movement of the roots before growth inhibition occurred. In the case of a rice root, one round of movement of the rice root tip showed a constant time of approximately 50 minutes. However, this movement ceased when Al ion was supplied. Al ion is known to be toxic for plant growth. When the Al concentration was low, the root could grow even if the circumnutation ceased and resumed after a while. However, the roots could not grow or resume movement when the concentration of Al was increased. The time needed for resuming the movement of the root tip was dependent on the Al ion concentration. It is not known what triggers the resumption of the movement of the root tip. This visualization of the root movement indicated that it is very important to consider plants as moving, not immobile organisms. Immobility and mobility are often judged only out of our sense of time scale. It seemed that there was a regulatory system of root movement that might be related to animal movement.

The concentration profile of each element in plant tissue was found to spread systematically throughout the developmental stage of the plant by neutron activation analysis (NAA), which allows nondestructive multielement analysis and is the only method to measure the absolute amount of the elements. The features of the concentration profile in a plant differed from element to element, and each element concentration showed differences between tissues. This concentration gap was also element specific and occurred throughout the plant. These profiles of the concentrations of the elements and their concentration gaps seemed to regulate the activity of each tissue. For example, there was a diurnal change in Mg and Ca concentrations, especially in the apical meristem. However, Mg-specific features have not been well studied because of the lack of tools to separate Mg behavior from Ca behavior. There is no suitable RI to trace Mg movement, and Mg cannot be visualized by staining because Mg is always superposed by an overwhelming amount of Ca. We tried to produce ^{28}Mg, whose half-life is 21 h, by the $^{27}Al(\alpha, 3p)^{28}Mg$ reaction and applied ^{28}Mg as a tracer for the first time in a plant study. The properties of Mg were gradually revealed, and the study is currently under development, but the role of Mg in maintaining the homeostasis of the plant has not yet been successfully clarified.

Another element with an interesting diurnal change in concentration is Al. Since the sensitivity of NAA to Al is extremely high, the trace amount of Al contained in a morning glory seedling was measured. A ng level of Al was regularly secreted from the root tip, and the amount of Al outflow decreased with time. The movement of this Al was not due to artificially added Al but to Al already contained in the seed. This secretion movement at the root tip was another interesting phenomenon suggesting that there might be a rhythm in root tip activity.

From the features of the elemental profile, not only the plant itself but also the elemental conditions of the environment can be analyzed. Plants acquired methods to adapt to environmental conditions through their long history of evolution. Some of them evolved to survive under high concentrations of toxic elements, such as Se. On the other hand, when the same plants were grown in different districts, the elemental profiles could be different, reflecting the features of the soil. The agricultural production district could be identified by analyzing the trace elements absorbed in the products. Not only the plants but also animals fed with the plants grown in different districts showed elemental profiles corresponding to the soil where the plants grew.

The real-time RI imaging system (RRIS) was developed to visualize and analyze how each element is absorbed and moves within the plant. As shown, each element had its own specific movement and accumulation pattern when absorbed by the plant. Comparing these patterns with the movement of water raised the question of why the movement of each element could be so different from that of water, showing its own movement pattern, and how the movement of the elements dissolved in water could be regulated differently. There must be many kinds of different transporters for the elements at many different sites in the plant. Even if we could identify these transporters, it still seems impossible to know why the elements are moving or what triggers each transporter to maintain different activity to maintain homeostasis of the plant. These results seem to suggest that to understand these features, the activity of the whole plant should be studied at the same time.

Another interesting result shown by the RRIS was that the element movement in the water or soil around the root could be visualized. In the case of water culture, when ^{14}C-glutamine was supplied to the culture solution, the ^{14}C signal accumulated at a certain distance from the rice root tips, and then, when the concentration of glutamine was high, all of the ^{14}C signal suddenly moved together to the roots to be absorbed. The absorption of ^{14}C-glutamine did not occur constantly but with a rhythm. Until this visualization, it had never been thought that there was a method to visualize real-time chemical reactions in water, which many chemists are interested to see.

In the case of soil culture, for example, when ^{32}P-phosphate was supplied, the site in the soil from which the root absorbed the phosphate could be visualized. Since phosphate is adsorbed from soil, the root could absorb only the phosphate close to the root, which resulted in a depleted image of ^{32}P-phosphate in soil as an enlarged shape corresponding to the roots.

The physiology of plants growing in soil has not been well studied. One of the reasons is that soil itself has a very complicated structure and function; therefore, it is difficult to discuss the plants growing in this complicated soil. The water absorbing activity of roots in soil is also not well understood. The difference in the movement of water from the movement patterns of nutrients suggested a large number of possible hypotheses. For example, only the amount of water absorption could be regulated to a low level while enough nutrients were supplied, it might cause a change from the juvenile phase to the adult phase, and it could be one of the solutions to growing cereal plants in a plant factory.

Since the speed of the movement was different among the elements, as a result, the accumulation profile of each element was shown to be different. Most of the heavy elements accumulated in roots, except for Cr and Mn, and were not transferred to the aboveground part of the plant. Why heavy elements are absorbed is the next question. When they are absorbed and stay in the roots, there must be some role of the heavy elements only in the roots.

In the case of the main essential elements, when transferred to the aboveground part, they were first transferred to the youngest tissue. After enough of an element accumulates in the meristems, it moves to the other tissue by phloem flow.

When the microautography (MAR) method was modified and the distribution of heavy elements in the grain was visualized, they did not accumulate in the tissue to be grown as meristems, suggesting protection of the next generation from contamination.

The visualization of the carbon fixation process using $^{14}CO_2$ gas was very interesting. First, invisible CO_2 gas could be visualized as $^{14}CO_2$ gas in the air, and then, the gas was fixed by the plant tissue. This fixed carbon metabolite, photosynthate, was visualized and observed to transfer to the meristems and create new tissue, which means that the whole process of photosynthesis and the proliferation to produce the new tissue could be visualized. Since the main carbon source in plants is from CO_2 gas in the air, the visualization of the carbon assimilation process meant that we could trace the whole creation process of the plant structure.

Moreover, it was noted that the orientation of the photosynthate movement was different according to the tissue where CO_2 gas was fixed. In the case of Arabidopsis, as presented, the carbon fixed at the rosette leaves was transferred to the root and the main stem, whereas the carbon fixed in tissues other than the rosette leaves was transferred to the branch internode and hardly moved to the root. However, the route changed according to the developmental stage. As the developmental stage progressed, the rosette leaves supported the young stem, not the old main stem. It is not known how the route of photosynthate movement is regulated or how the orientation of phloem flow is controlled. The image analysis of the $^{14}CO_2$ gas fixation process enabled us to analyze the phloem flow in detail. For example, even carbon fixed in the small leaves in the branch stem, close to the meristems, was transferred to other tissues after enough carbon was transferred to the meristem. In the case of pod formation in a soybean plant, most of the photosynthate was supplied by the closest trifoliate leaves. Photosynthate movement is crucial to the development of new tissue, and photosynthate movement must reveal the most effective way to create the tissue.

Radioisotope imaging provides specific and direct imaging possibilities for many ions where no alternative solution with fluorescent probes exists. The RRIS offers quantitative and nondestructive access to mineral nutrition, from the short term to the long term, including the developmental stage of the plants. As shown, the RRIS offers a broad range of applications, especially in combination with fluorescent imaging.

Last, I want to reiterate that radiation and radioisotopes are indispensable and promising tools to trace water and element movement in plants and provide many fundamental but new questions to be studied. I also hope that these newly introduced methods of in vivo nondestructive imaging or measurement might open a new field of plant research, not only to reveal new functions or to evaluate intact systems but also to find ways to bridge the microscopic world of living plants with the macroscopic world.