¹H-NMR Imaging of Tomato Fruits

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The distribution and the relaxation times of water in tomato fruits were measured by ¹H-NMR imaging with a resolution of 0.2 × 0.2 mm² area and 1 mm thickness. Water with a long relaxation time was preferentially accumulated in seeds and seed envelopes in immature green fruit. The total amount of water increased in mature red fruit, where water with a long relaxation time was localized in outer walls of the pericarp and water with a shorter relaxation time was distributed throughout all tissues except for seeds and seed envelopes. ¹H-NMR imaging apparently distinguished the physiological variations among different types of tissues and the physiological changes during maturation of tomato fruit.

NMR (nuclear magnetic resonance) is widely used for the measurement of compounds contained in plant materials and for the tracing of metabolism and reactions proceeding in plant cells.¹⁾ ¹H-NMR spectroscopy is expected to give information about the water phases^{2~4)} where many reactions in living cells are done. The drying of soybean seeds during maturation is accompanied by the shortening of water relaxation times.⁵⁾ The cold hardness of buds also has a close relationship with the amount and the relaxation times of internal water.^{6,7)} These facts showed that water content and its physical state are important for the regulation of cell activities. Therefore, in this investigation, the changes of distribution and the mobility of water in tomato fruits at different stages were investigated using ¹H-NMR imaging, called magnetic resonance imaging (MRI). MRI is a new technique used in medical diagnosis in which maps of the mobile water distribution in living tissues are acquired by using a static gradient and radio-frequency magnetic field. There are few reports of MRI applied to plant materials.^{8~11)} Lauterbur (1974)⁸⁾ applied the MRI technique to the living tissues

of a pine branch and intact pecan nuts as well as animals. Mansfield and Pykett (1978)⁹⁾ observed the cross sectional MRI of a whole okra seed pod, comparing cross sectional photograph of the same region of it. Recently, Bottomley *et al.* (1986)¹⁰⁾ demonstrated the water transport of the *Vicia faba* root system in soil medium with the help of a paramagnetic NMR contrast reagent. Moreover, differences in water content and degree of binding in the various stem tissues of a geranium plant were investigated by Johnson *et al.* (1987)¹¹⁾ using a high resolution MRI apparatus. The technique is expected to offer a means of access to structural and physiological aspects of plant systems. Based on the results of MRI, the changes of physiological activities along with the maturation in the various tissues in tomato fruits were discussed.

Materials and Methods

Plants. Common tomato (*Lycopersicon esculentum*) plants were cultivated in a growth chamber under natural light at 28°C during the day and 23°C at night. An immature green fruit and a mature red fruit were used as materials.

Measurements of water distribution and its relaxation times. A NMR-spectrometer with a superconducting magnet operating at 270 MHz (JEOL GSX-270) was used for obtaining ^1H -NMR images. An intact tomato fruit was fixed on a sample holder (Fig. 1) and set at the center of the 5.0 cm diameter detection coil of an imaging probe and inserted into a magnet which was equipped with another coil for producing a gradient (15 mTesla/m) for the magnetic field of the apparatus. A spin-echo imaging technique was used and the delay between the excitation and the acquisition of the spin-echo was 36 msec. A 256×256 array was applied twice to create a proton image with a resulting spatial resolution of $0.2 \text{ mm} \times 0.2 \text{ mm}$ and a 1.0 mm slice thickness (0.04 mm^3). Three imaging pulse sequences with different repetition times (0.5, 1.5, and 5.0 sec) were used to measure the relaxation times (T_1) of

the water by the saturation recovery method.¹²⁾ The images were displayed on a color TV display (256×240 point). After the measurement of ^1H -NMR images, samples were cut according to the slice section and pictures of the section were taken.

Results and Discussion

Cross sections of immature and mature tomato fruits and their imaging maps of water distribution obtained by ^1H -NMR are shown in Fig. 2 (top and middle). The fluctuation of the amounts of water measured, along with the lines drawn on the fruits horizontally, are also shown in Fig. 2 (bottom). The water detected is a sort of free water which is highly mobile as deduced from the nature of ^1H -NMR. Free water is the major field where biological reactions occur, therefore, the mobility of water in the cells is considered to regulate the physiological activities in the tissues.^{5,13,14)} The distribution of water with high mobility involves the places where active metabolism occurs and that with low mobility resulted in slow metabolism.

Water was differently distributed among tissues in the fruits. In the immature green fruit, large amounts of water were located in seeds and seed envelopes. There was considerable water in the outer wall of the pericarp and less water in dissepiments and the columella. No detectable water was observed in the placentas. The total amount of water increased^{15,16)} and a large amount of water was observed in the outer wall of pericarp, dissepiments, and columella in the mature red fruit. A

Fig. 1. Imaging Probe with 5.0 cm Detection Coil Used for the Measurement of ^1H -NMR Images.

Detection coil (A) and sample (B).

Table I. SIGNAL INTENSITY AND AVERAGE VALUE OF PROTON RELAXATION TIMES (T_1) IN DIFFERENT TYPES OF TOMATO FRUIT TISSUES

	Green		Red	
	Intensity	T_1 (sec)	Intensity	T_1 (sec)
Outer wall of pericarp	+++	1.39 ± 0.13	+++++	1.56 ± 0.14
Dissepiment	++	1.48 ± 0.08	++++	0.98 ± 0.17
Seed and seed envelope	+++++	1.95 ± 0.08	N.D.	
Placenta	N.D.		+	0.57 ± 0.09
Columella	+++	0.93 ± 0.08	+++	0.64 ± 0.17

The signals in 2×2 to $5 \times 5 \text{ mm}^2$ segments in image of ^1H -NMR were sampled and averaged to calculate one relaxation time (T_1). Symbol N.D. in the table indicates no water was detectable under the experimental conditions used.

Fig. 2. ^1H -NMR Images of Immature Green and Mature Red Tomato Fruits.

Top, the cross sections of fruits corresponding to the images; middle, ^1H -NMR images, where the increase of white color indicates the increase of the amount of water; bottom, intensities of signals measured at the lines drawn horizontally on the images of fruits.

little water was observed in mature placentas. Water in seeds and seed envelopes was not detected.

The distribution maps of water with different relaxation times (T_1) which were calculated from the images obtained using different repetition times, are shown in Fig. 3. The water in seeds and seed envelopes had a longer T_1 in the immature green fruit. On the other hand, the water with a shorter T_1 increased and covered all areas of the tissues except for seeds and seed envelopes in the mature red fruit. The amount of water with a shorter T_1 was less than that with longer T_1 in the immature green fruit.

The average values of T_1 in various tissues measured by the saturation recovery method¹²⁾ and the relative amount of water are listed in Table I. There was a considerable amount of

water with a long T_1 , which indicated a high mobility of water in seeds, seed envelopes, and the outer wall of pericarp in the immature green fruit. Water in seeds and seed envelopes showed the longest T_1 . This indicates that seeds and outer wall of the pericarp were active sites for development and enlargement. The intensity of the signal was low in dissepiments and was weak in the placentas and the columella. Though the content of water was low, water in dissepiments maintained a long T_1 . Most of the water in the columella was considered to be bound water because the T_1 was short. The water in placentas was also considered to be bound because free water with longer T_1 was not detected. Unlike the immature green fruit, a large amount of water accumulated in the outer wall of the pericarp, dissepiments, and columella of the

Fig. 3. ^1H -NMR Images with Different Relaxation Times.

The images were created from those acquired with different repetition times used for the measurement of relaxation times by the saturation recovery method. long, [the image with 5 sec repetition time]—[that with 1.5 sec]; medium, [the image with 1.5 sec repetition time]—[that with 0.5 sec]; short, the image with 0.5 sec repetition time.

mature red fruit. The water in the outer wall of the pericarp has a long T_1 but that in the dissepiment and columella shortened. The signal of water were detectable in placentas, the relaxation times of which was shortened significantly.

^1H -NMR images clearly detected differences in the distribution and the characteristics of water among various tissues in tomato fruits. This might account for the physiological changes occurring in tomato fruits during development from green immature to red mature conditions,^{15,17,18)} because the observed water was free water, the amount and the mobility of which have a close correlation to the biological reactions in enzymatic and metabolic processes in tissue cells.

Photosynthates flowing into a fruit are used for development and enlargement of seeds and fruit flesh, in an immature fruit.¹⁷⁾ The pla-

centa may play a role in storage of the photosynthates before transport to the active site, the seeds. Therefore, water in the placenta is bound with a large amount of stored materials¹⁹⁾ such as starch and proteins, resulting in the decrease of the amount and the mobility of water. Dissepiments and the outer wall of the pericarp are developing tissues and water in them showed a long T_1 , however, the amount of water in dissepiments was less than in the outer wall of the pericarp. Photosynthates in dissepiments are considered to be accumulated in temporary storage compartments where most of the water was in bound conditions⁵⁾ other than active cytoplasm (Table I).

In a mature fruit, the situation is different from that of an immature fruit. Seeds have already ceased their growth, lost water, and filled with stored materials which are to be

used at germination. Stored materials in seed envelope parenchyma cells do not flow out but remain and are converted to polysaccharides and pectic substances which form gels^{18,20)} where water was in a bond state and signals were not observed by broadening because of the short relaxation times (Figs. 2, 3). The outer wall of the pericarp is still active and enlarging at a slow rate. The influx of photosynthates decreases its rate, while water increases and the content of stored materials in the dissepiments decreases. These situations in fruits, together with the hydration of stored materials which have accumulated in vacuoles,¹⁵⁾ result in a little restoration of the mobility of water in placentas and columella (Figs. 2, 3 and Table I). Remaining materials and newly incorporated photosynthates are stored in dissepiments which are growing fat, consequently the mobility of water in dissepiments is restricted (Table I).

Magnetic resonance imaging with a resolution of 0.04 mm³ is able to distinguish several different tissue types in plant materials. The signal intensity and the relaxation times reflect the differences in the water content and the degree of binding, indicating the variations of physiological conditions and roles among tissues. Further improvement of resolution will provide us with a means for more detailed examination of water dynamics at the cellular level.

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