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Ontogenetic changes of the water status and accumulated soluble compounds in developing and ripening mume (*Prunus mume*) fruit measured by ^{1}H-NMR analysis

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Abbreviations: CPMG= Carr-Percell- Meiboom-Gill; FAA= formalin acetic acid alcohol; NMR= nuclear magnetic resonance; T1= spin-lattice relaxation times; T2= spin-spin relaxation times.

Key words: Histological observation, membrane integrity, mobility of water, NMR relaxation times (T1, T2), water content.

Abstract: The physiological changes of intact mume (*Prunus mume* Sieb. et Zucc. cv. Rinshu) fruit tissues were examined by measuring the physical states of cell-associated water in the fruit tissues with developing and ripening using ^{1}H-NMR spectroscopy. We found that the water molecules in mume fruit tissues existed in several different compartments with different mobilities. Additionally, spectral recovery in the water proton indicated reverse relationships between the pericarps and seeds at the immature and mature stages. In the pericarp tissues, the longest *T*1 and longer *T*2 markedly increased, while those in the seeds decreased. From these results, the change in the water status with growth stage had reverse trajectories in the pericarp and seed of the fruit. In the pericarp tissues, both water uptake and dry weight prominently increased with ripening. The epidermis and inner parenchymal cells of the pericarp tissues remarkably enlarged as a sigmoidal growth curve. Membrane permeability, indicating a loss of membrane integrity, increased in the pericarp tissues. The elongation in the fully vacuolated cells and changes in the membrane permeability in the pericarp tissues with ripening correlated to the longest *T*1. In contrast, the high mobility of water in the seeds began to decrease with maturation, while oil began to accumulate. Thus, the mobility of water, as analyzed in this study, is considered to reflect the results of physiological changes such as cellular heterogeneity and spatial arrangements both in the pericarp and in seed tissues for mume fruit with development and ripening.

1. Introduction

The Japanese apricot, mume (*Prunus mume* Sieb. et Zucc.) fruit, is widely distributed among the different climate regions of East Asia, and is harvested at the yellow-peel stage for pickles called "umeboshi" or at green-peel stage for its juice. Mume is known to be a climacteric fruit that produces large amounts of ethylene as it ripens (Inaba and Nakamura, 1981; Koyakumaru, 1997; Mita *et al*,. 1999), making it difficult to preserve for long periods of time as the characteristic taste and firmness is quickly lost. The reason for the degradation of the fruits and measures to prevent this degradation have never been thoroughly investigated. Investigating harvest timing and improving conditions for fruit quality preservation during commercial processes may make the fruit more common.

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NMR spectroscopy is a promising tool for exploring new aspects of plant science, especially given that there are various types of plant materials in which the physical states of cell-associated water change naturally occur, e.g. in the maturation process of red raspberry (Williamson *et al.,* 1992), gooseberry (Williamson *et al.,* 1993), olives (Gussoni *et al.,* 1993), kiwifruit (Callaghan *et al.,* 1994), coconuts (Jagannathan *et al.,* 1995), cherry tomatoes (Ishida *et al.,* 1994), cherry fruits (Ishida *et al.,* 1997), in floral malformation in mangos (Usha *et al.,* 1994) and maturation in apples (Wang *et al.,* 1988). In addition, the detection of water in different subcellular organelles is often used to understand the changing water distribution among plant components. It has been suggested that multi-exponential ^{1}H-NMR relaxation times (*T*1 and *T*2) in plant tissues reflect water in different plant cell compartments (Burke *et al.,* 1974; Stout *et al.,* 1978; Gusta *et al.,* 1979; Bacic and Ratkovic, 1984; Hills and Duce, 1990; Isobe *et al.,* 1999), and can be ascribed to several different causes: cellular heterogeneity and subcellular compartmentation (Belton

and Ratcliffe, 1985; Snaar and Van, 1992; Kumamoto *et al.,* 1998; Iwaya-Inoue *et al.,* 2004 a, b). The movement of water is controlled by cellular organization, such as compartmentalization by membrane structures (Tanner, 1978, 1983), and water is ordered by macromolecules, such as proteins and polymers of organic compounds (Hazlewood, 1995). Thus, the dynamic states of water in cells closely correlate with the organic properties of macromolecular structures.

The objectives of the present research were (1) to examine the ontogenetic changes in the state of water by ^{1}H-NMR spectroscopy, water content, membrane permeability and histochemical observation during development and ripening of Japanese apricot fruit, and (2) to study the interrelationships among them.

2. Materials and Methods

Plant materials

Japanese apricot (Prunus mume Sieb. et Zucc, cv. Rinshu) fruits cultivated in the orchard at the University Farm of Kyushu University were harvested at each stage (a total of four times) from April to June. The fruits were defined as belonging to four ripening stages as follows (Fig. 1 and Table 1): Stage 1, fruits at approximately 2.0 g in fresh weight and 1.5 cm in transverse diameter, peels green, and seeds immature and 0.1 g in fresh weight; Stage 2, fruits at 15.0 g in fresh weight and 3.0 cm in transverse diameter, and seeds 0.5 g in fresh weight; Stage 3, fruit at their maximum size, seeds 0.7 g in fresh weight; Stage 4, fruit at maximum size and 45.0 g in fresh weight, 4.0 cm in transverse diameter, the firmness in the pericarp softening, the peels yellow, and seeds hard with a fresh weight of 0.8 g. Fruits were separated into pericarp (including epicarps) and seed, and both tissues were used for experiments. Tissue water contents were determined by obtaining the weight loss after drying in an oven at 90°C for 20 h. Eight to ten fruits were used in each stage in the following experiments.

^{1}H-NMR analysis

An NMR spectroscope with a magnet operating at 89.5 MHz for ^{1}H (JEOL EX 90A) was used for the measurement of spin-lattice relaxation times ($T1$), ^{1}H-NMR spin-spin relaxation times ($T2$) and ^{1}H-NMR spectra. A piece of pericarp or seed of an intact Japanese apricot fruit was placed in an NMR tube (8 mm in diameter) which was then placed in an outer glass tube (10 cm in diameter) containing 99.8% D_2O as an internal lock signal; spectroscopic measurement at 25±1°C was then undertaken. The repetition time was 15 s with four accumulation transients for each tissue. The decay between scans was always greater than five times $T1$.

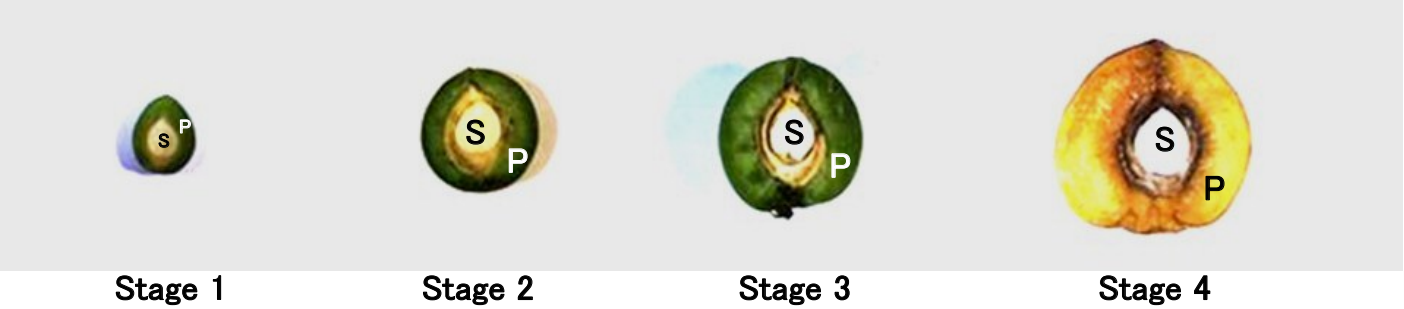

Fig. 1 - Mume (*Prunus mume* Sieb.) fruit during four ripening stages. P= pericarp; S= seed.

Table 1 - Characteristics of mume fruit tested

Characteristics		Stage			
		1	2	3	4
		Harvested day			
		April 9 ~ 24	May 6 ~ 12	May 29 ~ June 4	June 13 ~ 30
Skin color		green	green	green	yellow
Endocarp		hard	hard	soft	soft
Size (cm)	Longitudinal diameter	1.6±0.1	3.2±0.4	3.9±0.1	4.2±0.1
	Transverse diameter	1.3±0.1	2.9±0.1	3.5±0.9	4.2±0.1
Weight (g)	Whole [z]	1.7±0.2	14.8±1.1	32.8±3.0	45.8±2.4
	Pericarp	1.1±0.3	11.2±1.1	27.7±2.4	35.6±2.6
	Seed	0.1±0.0	0.5±0.1	0.8±0.1	0.7±0.1

Values represent the mean of eight to ten fruits±SE.
[z] The weight of the stone is not included.

*T*1 measurements were determined by the inversion recovery (180°- τ -90°pulse sequence) method. τ is the time between the radio-frequency pulses in the sequence, and the indicated angles are between the average direction of the original proton spins and that induced by the radio-frequency pulse (Farrar and Becker, 1971). Twenty-two values of pulse interval (τ) ranging from 0.001 s to 15 s were used to acquire a *T*1 data set. *T*1 was determined from the slope of ln (M0 - M) /2M0 versus τ, where M is the amplitude of the FID of the water proton signal following the 90° pulse at τ, and M0 is the limiting value of M. The existence of water components with different *T*1 values was revealed from spectral recovery and semi-log plots of signal intensity of ^{1}H-NMR according to Ishida *et al.* (1994). A graphical method was used to determine the relaxation time of water and the percentage of the fraction (Hazlewood and Nichlos, 1969; Belton and Packer, 1974). Short *T*1 values including those below 0.1 s were considered from spectral recovery ranging in the pulse intervals of 0.03 s and 0.05 s.

*T*2s measurements were determined by the CPMG (Carr-Percell-Meiboom-Gill) method from the slope of ln M/M0 versus t, where M0 is the magnetization amplitude of the water proton signal occurring at time τ after the initial 90° pulse in the CPMG (90°-τ-180°-2τ-180°-2τ---) pulse sequence. t = 2nτ, where n is the number of refocusing pulses (25 points from 2 to 4000 loops) and τ was 0.001s. *T*2 was also determined from a semi-log plot of signal intensity as in the case of *T*1.

Histological observations

Materials were fixed in FAA (formalin acetic acid alcohol; 80% ethanol: 100% acetic acid: formalin = 90:5:5), and 20-μm sections were cut using a microtome (Cryostat HM500-OM, Microm Co. Ltd) at -20°C. The sections were stained with 0.01% Ruthenium red for pectic substances in the middle lamella (Fig. 2A) and by Sudan III for lipid substances (Fig. 2B), respectively. They were then subjected to microscopic observations (Axiphot, Carl Zeiss Co. Ltd).

Leakage of electrolytes

Pericarp tissues of Japanese apricot fruit (about 3.0 g) were cut into pieces of about 2-mm square. These small pieces were immersed in distilled water (50 ml) and stirred at 180 cycles/min. The extent of leakage of electrolytes was determined with an electrolytes conductivity meter (Toa conductivity meter, Model CM-20E, Toa Electronics Ltd.) and expressed as the percentage of the total electrolytes in each sample measured after samples were killed by a cycle of freezing and thawing (Iwaya-Inoue *et al.,* 2004 a, b).

3. Results

Histochemical characteristics of fruit tissues with ripening

Mume fruit are characterized by four stages (Fig. 1 and Table 1). The histochemical changes of the mume fruit tissues with ripening are shown in figure 2. The individual pericarps and seeds are mainly comprised of the parenchymal tissue, beneath the epidermal tissue and around the vascular tissue. Pectic substances stained by Ruthenium red are abundant in the middle lamella of cell walls in the pericarp parenchymal tissues from the small green fruit to the large green fruit (Fig. 2A, Stages 3 and 4). The components in the cell wall of pericarp tissues began to change with matura-

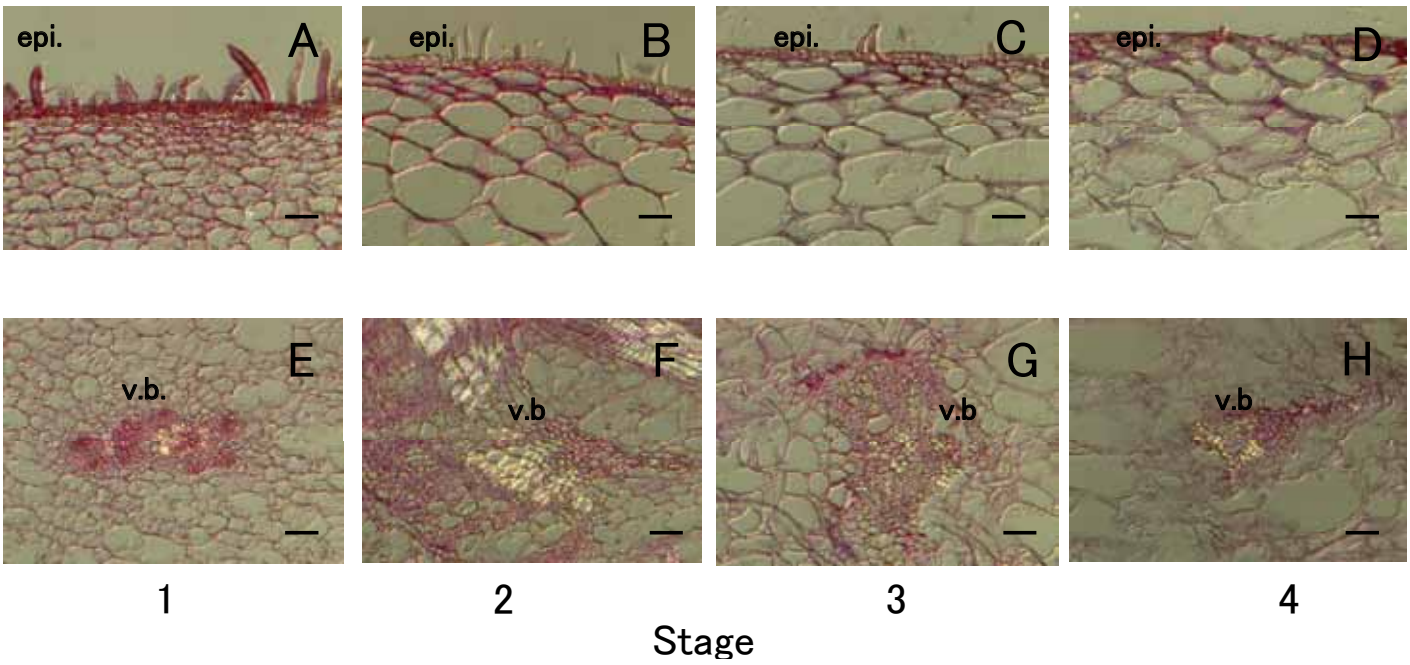

Fig. 2A) Photomicrographs of cross-sections of the pericarp tissues in mume fruit with ripening (A ~ D). Parenchymal tissues beneath epidermis. A= Stage 1; B= Stage 2; C= Stage 3; D= Stage 4. (E ~ H) Vascular bundles. E= Stage 1; F= Stage 2; G= Stage 3; H= Stage 4. epi= epidermis; v.b.= vascular bundle. Tissues were stained with Ruthenium. Photomicrographs of cross-sections of the pericarp tissues in mume. Bars indicate 50 μm.

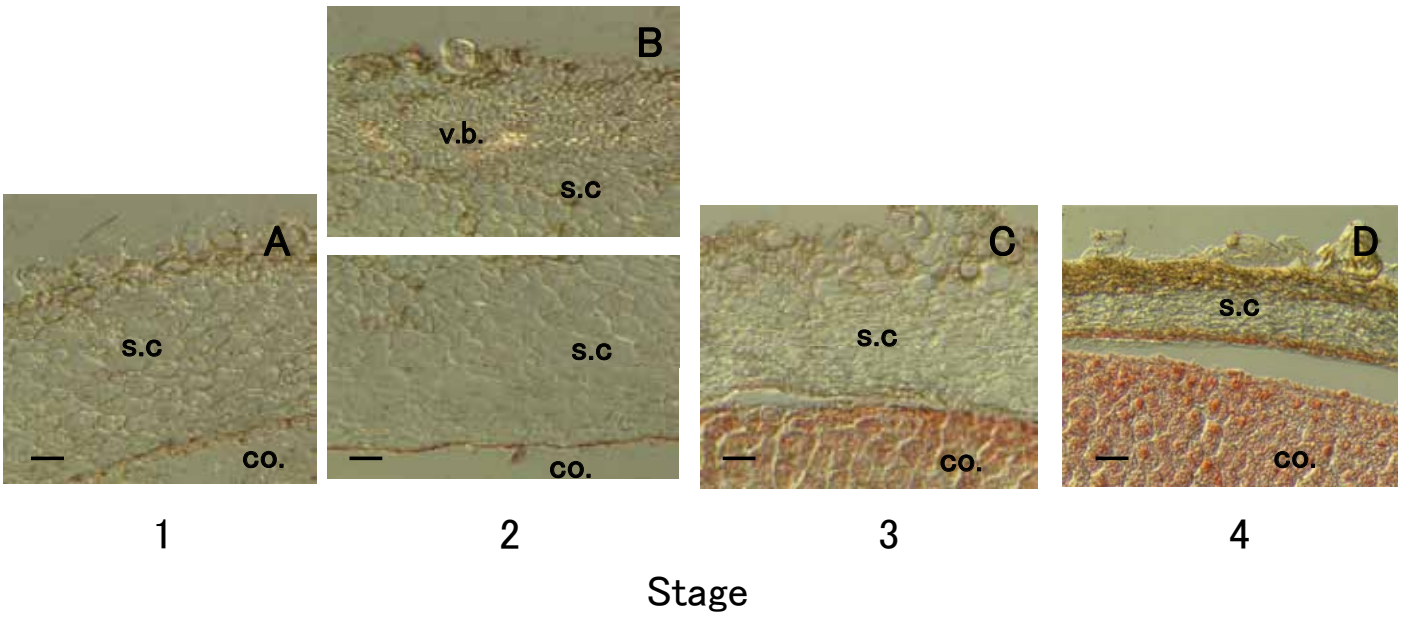

Fig. 2B - Photomicrographs of cross-sections of the seed tissues in mume fruit with ripening (A ~ D). Parenchymal tissues beneath epidermis. A= Stage 1; B= Stage 2; C= Stage 3; D= Stage 4. s.c.= seed coat; co.= cotyledon. v.b= vascular bundle. Tissues were stained with Sudan III. Bars indicate 50 µm.

tion of the fruit (Stage 3). As the peel of the fruit assumes a yellow color, pectic substances in the cell wall structures in the parenchymal tissue collapse, except for the epidermis and vascular bundles (Fig. 2A, Stage 4). On the other hand, lipids in the seeds stained with Sudan III were observed in the epidermis of seed tissues, indicating that the cells of the epidermis are abundant in suberin (Fig. 2B). Moreover, oil bodies were also observed in the parenchyma of the cotyledons in the matured and ripened fruit seeds (Stages 3 and 4).

Water uptake in relation to cell enlargement

In the pericarp tissues, both water uptake and dry weight prominently increased during ripening, especially from the large green-fruit stage to the matured-fruit stage (Fig. 3). Vacuoles occupy a large part of individual cells in the tissues at these stages (data not shown). The epidermis and inner parenchymal cells of the pericarp tissues remarkably enlarge during ripening in a sigmoidal growth curve (Table 2). The enlargement of cells in the pericarp region closely correlated with that for fruit diameter, accompanied by a marked increase in water uptake and dry matter accumulation (Tables 1 and 2). In seed tissues, water uptake did not change, while dry weight remarkably increased in both the matured and ripened fruit (Fig. 2). Therefore, the seed water content remarkably decreased in the mature stages (Fig. 4, Stage 3).

Membrane integrity in the pericarp tissues

Changes in the membrane permeability as well as the cell wall integrity of mume fruit with ripening were considered. Leakage of electrolytes from the pericarp tissues increased during ripening, and 90% of the total electrolytes were found to have leaked at fruit maturation (Fig. 4, Stage 3). Thus, membrane permeability, indicating a loss of membrane integrity, arises in the pericarp tissues.

Tissue specificity of 1H-NMR spectra with various pulse intervals between 180 and 90°

The physiological changes of intact tissues were examined by measuring the physical states of cell-associated

Table 2 - Changes in cell size of pericarp during ripening of mume fruit

		Cell size (µm)			
		Stage			
		1	2	3	4
Epidermis	Long side×short side	25.1× 9.9	39.0×12.3	38.9×22.9	49.5×49.5
Parenchyma	Small cells				
	Long side×short side	44.7×18.6	97.9×54.4	119.7×68.9	142.9×102.9
	Larage cells				
	Diameter	61.4	145.5	181.2	243.9

Values represent the mean of ten cells of the pericarp tissues in three fruits.

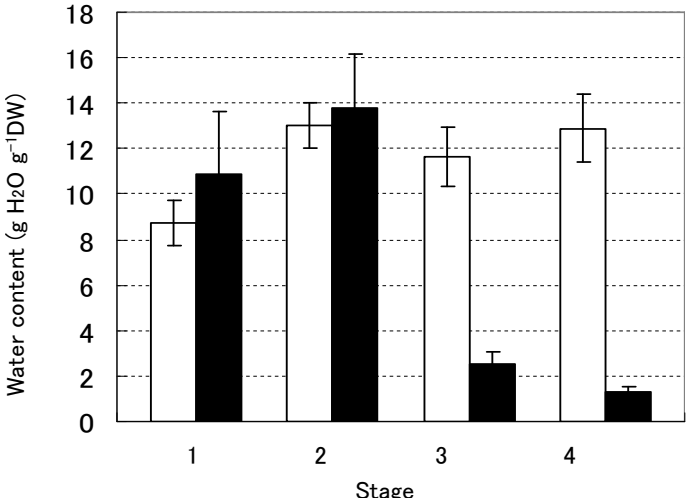

Fig. 3A - Changes in the water content of pericarp (□)and seed (■) for mume fruit with ripening. Values represent the mean of eight to ten fruits ± SE.

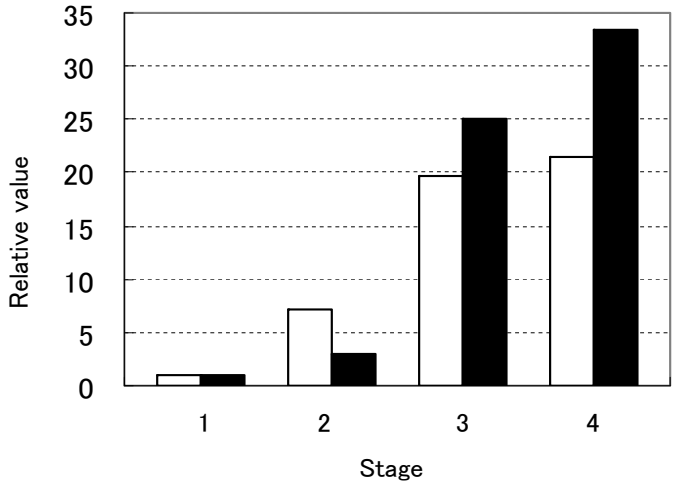

Fig. 3B - Changes in the dry weight of pericarp (□)and seed (■) for mume fruit with ripening. Values represent the mean of eight to ten fruits ± SE.

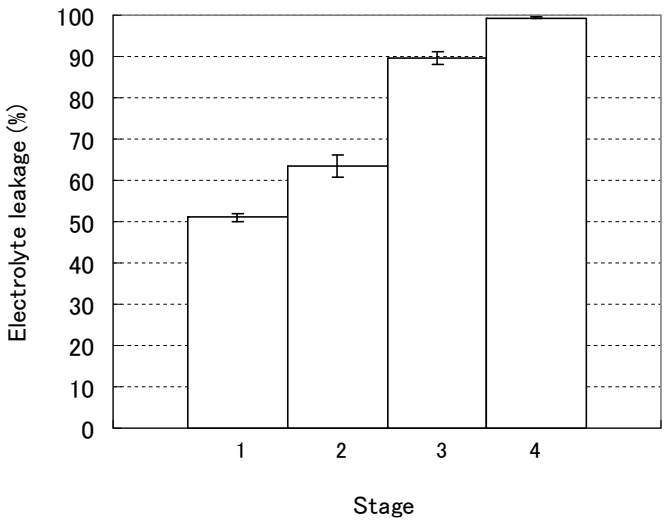

Fig. 4 - Changes in electrolyte leakage of pericarp during ripening of mume fruit. Values represent the means of three fruits ± SE.

water in fruit tissues with ripening using ^{1}H-NMR spectroscopy. ^{1}H-NMR spectra of both intact pericarp and seed in the fruit tissues were examined by spectral recovery of ^{1}H-NMR with the inversion recovery method. The pulse interval was varied between 180° and τ- 90° pulses. An ^{1}H-NMR spectral peak was observed at 4.8 ppm of chemical shift, which corresponds to the ^{1}H nuclei of water. ^{1}H-NMR spectra of both the pericarp and seed tissues were not symmetrical, indicating that the peak consists of components with different chemical shifts and various recovery times (Fig. 5).

In pericarp tissues of the small green fruit, major spectral recoveries were observed in the pulse intervals of 0.3 s and 0.5 s, while in the ripened fruit, these were 0.9 s and 1.5 s (Fig. 5A and B, Stage 1). On the other hand, in seeds of the small green fruit, several peaks of spectral recovery were mainly observed at pulse intervals of 0.8 s and 1.0 s,

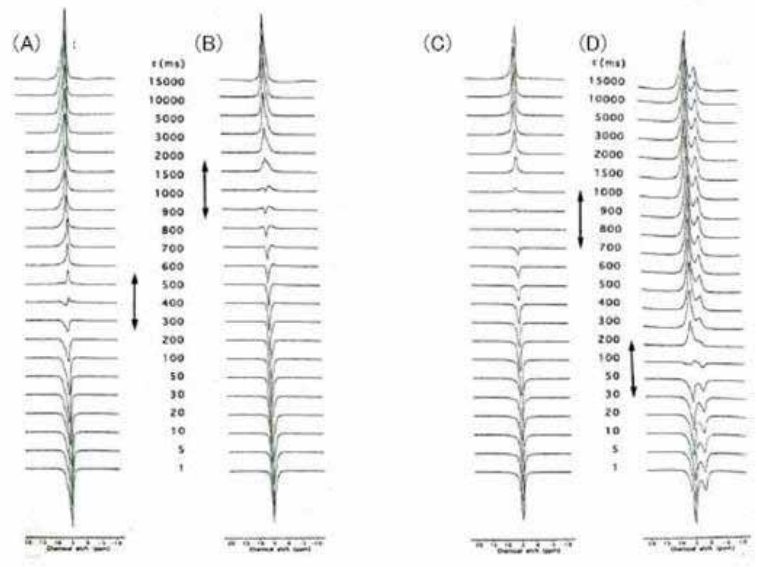

Fig. 5 - Spectral recovery of 1H-NMR of mume fruit with inversion recovery method. T: pulse interval. A= Spectra of pericarp tissues at Stage 1; B= Spectra of pericarp tissues at Stage 4; C= Spectra of seed tissues at Stage 1; D= Spectra of seed tissues at Stage 4. 1; D= Spectra of seed tissues at Stage 4.

while they occurred between 0.05 s and 0.2 s in ripened fruit (Fig. 5C and D, Stage 4). The individual values of the *T*1 components can be obtained by pulse interval (τ) at null point, *T*1 = null / ln2. Thus *T*1 values that are due to major spectral recovery times in pericarp tissues became longer in the ripened fruit, while the tendency was reversed in the seed tissues.

Changes of T1 components in the fruit tissues

The relaxation times and estimated amounts of the individual water fractions calculated from semi-logarithmic plots of ^{1}H-NMR signal intensities in the fruit tissues with ripening processes are listed in Table 3. The spin-lattice NMR relaxation time (*T*1) describes the process of realigning the magnetic moment with the external magnetic field.

Cellular water exists in two to three components, which are shown by NMR relaxation times; in plant tissues, these water components consist of three states of water: i.e. free water, loosely bound water, and tightly bound water (Iwaya-Inoue and Nonami, 2003). The three compartmentalizations of water originally identified as the vacuole, cytoplasm, and cell wall/extracellular space (apoplast) are reflected by the different relaxation times in the parenchymal tissues of apples (Snaar and Van As, 1992; Hills and Remigereau, 1997). Thus, differences in the relaxation times (*T*1, *T*2) of biological tissues can be interpreted as differences between the ratio of free water to bound water. Semi-logarithmic plots of ^{1}H-NMR signal intensity with the inversion recovery method of mume fruit were multi-exponential. The long *T*1 fraction could be understood as highly mobile water (free water) derived from the vacuoles, while the short *T*1 fraction with restricted mobility represents loosely bound and bound water from the cytoplasm and the apoplastic region, respectively.

In the pericarp tissues, *T*1 values of the longest water component markedly increased from 0.8 to 2.0 s from the small green fruit to the large green fruit (Stages 1 and 2). The major fraction of the longest *T*1 was about 80% and the ratio was constant during the ripening stage. The fraction of the shortest component of *T*1, at 0.4 s was about 10% and was also constant through all stages. In addition, vacuoles stained by neutral red were observed in a large proportion of individual cells in the pericarp tissues with ripening (data not shown). Thus, water in the pericarps was considered to increase the amount of free water with ripening. On the other hand, in the seeds of the small green and large green fruits, *T*1 values and the fractions of the longest component were about 2.0 s and 75%, respectively (Table 3, Stages 1 and 2). *T*1 values of the longest water component in the seeds markedly decreased from 2.0 to 0.4 s from the large green-fruit to matured-fruit stages (Stage 2 and 3). The *T*1 values did not change further for the ripened fruit. However, the fraction ratio of the longest water component in the seed decreased from 45 to 30 % during that stage (Stage 4).

Table 3 - Changes in components of ^{1}H-NMR spin-lattice relaxation times (*T*1)s during ripening of mume fruit

	Stage							
	1		2		3		4	
	*T*1 values (ms)	Fraction (%)	*T*1 values (ms)	Fraction (%)	*T*1 values (ms)	Fraction (%)	*T*1 values (ms)	Fraction (%)
Pericarp	412±13	12.1±3.2	416± 1	12.1±0.5	413± 5	12.3±0.8	424±13	11.3±1.7
	603±55	7.4±3.4	1171±44	9.2±2.4	1332±63	11.9±1.1	1723±28	10.2±2.1
	758±54	80.5±1.6	1367±34	78.7±1.7	1619±91	75.8±2.1	2057±94	78.5±1.1
Seed	349±11	13.5±4.5	785± 6	12.2±1.1	181± 3	20.2±1.1	138± 2	18.5±1.2
	1414±28	13.4±4.3	1443±25	15.9±1.3	321±49	33.7±3.6	193± 3	50.6±1.2
	2216±15	73.0±0.4	2040±19	75.6±0.6	443±66	46.1±2.6	370± 2	30.9±0.8

Values represent the means of eight to ten samples±SE..

Table 4 - Changes in components of ^{1}H-NMR spin-spin relaxation times (*T*2)s during ripening of Japanese apricot fruit

	Stage							
	1		2		3		4	
	*T*2 values (ms)	Fraction (%)	*T*2 values (ms)	Fraction (%)	*T*2 values (ms)	Fraction (%)	*T*2 values (ms)	Fraction (%)
Pericarp	151±12	28.1±6.0	289±17	26.4±1.2	394±34	25.9±0.9	404±38	26.9±6.7
	211±10	71.9±6.0	442±10	73.6±1.2	731±26	74.1±0.9	713±14	73.1±6.7
Seed	143±11	12.8±2.8	205±19	16.7±6.3	36± 2	16.7±2.8	27± 2	44.8±2.8
	323±24	87.2±2.8	317±14	83.3±6.3	56± 2	83.3±2.8	47± 6	55.2±2.8

Values represent the means of eight to ten fruit±SE.

Changes of the T2 components in the fruit tissues

The spin-spin NMR relaxation time (*T*2) describes the time-dependent decay of NMR signal due to the dephasing process of the individual spins with respect to each other. The water component estimated by *T*2 was divided into two fractions (Table 4). The regions with long *T*2 values in *Glycine max* seed tissues had high concentrations of free water, while the regions with short *T*2 values had high concentrations of loosely bound and bound water (Ishida *et al.,* 1987). In the pericarps of the mume fruit, the fractions in *T*2 comprised about 70% through the four stages. *T*2 values of both long and short components increased until fruit maturation (Stage 3); however, they did not prolong further at the ripened-fruit stage (Stage 4). By contrast, *T*2 values of the longer water component in seeds markedly decreased from about 300 ms to 60 ms from the large green fruit to the matured fruit (Stage 2 to Stage 3).

4. Discussion and Conclusions

Relationship between NMR relaxation time T1 and the characteristics in mume fruits during development and ripening

Nuclear magnetic resonance (NMR) spectroscopy is a useful technique to follow physiological changes with respect to the state of water in developing Japanese apricot fruits.

Water in living tissues is known to consist of several components with regard to the relaxation of the magnetized protons (Hazlewood *et al.*, 1969; Hazlewood, 1995; Isobe *et al.,* 1999; Iwaya-Inoue, 2004 a, b). In developing Japanese apricot fruits, three components with different *T*1 values were distinguished on the semi-logarithmic plots of the recovery of the ^{1}H-NMR signal (Fig. 5 and Table 3). Multicomponent water fractions are similar to those reported for other plant tissues (Stout *et al.,* 1978; Gusta *et al.,* 1979; Isobe *et al.,* 1999). Referring to the line width of ^{31}P-NMR signals (Kano *et al.,* 1990; Takagishi *et al.,* 1991), the longest component is ascribed to that of vacuoles containing small molecules, such as metabolic intermediates, secondary products and inorganic ions, and the middle component to that of cytoplasm in plant tissues. The water with the shortest *T*1 is considered to be exchangeable water around macromolecules, such as starches, proteins and strings of macromolecules in vesicles or between cell walls (Rorschach and Hazlewood, 1986).

Large amounts of highly mobile water were detected in the seeds of small green and large green fruits. The mobility of exchangeable water in the seed tissues became higher as fruit developed. However, the mobility became low at fruit maturation; thereafter, it continued to be low. On the other hand, the mobile water in the pericarp of small green fruit was low. This suggests that concentrations of cell components that bind water were high in these tissues. Thereafter, the mobile water in the pericarp increased with enlargement and even more so upon coloring of the fruit (Figs. 1 and 5, Tables 1 and 3). These trends agree with previous reports (Ishida *et al.,* 1989; 1994; 1997). Changes in the mobility of water are considered to be the result of physiological changes in fruit growth including seed development and maturation, which is the most important natural function of the fruits (Crane, 1964).

Relaxation times are strongly influenced by the availability of the water and the presence of macromolecules to which water molecules can be "bound". It was shown that *T*1 closely correlated with water content in developing and maturing rice grains (Funaba *et al.,* 2006), in azalea buds subjected to low-temperature stress (Kaku *et al.,* 1984), and in the heat-tolerant and heat-sensitive cultivars rice grains subjected to high-temperature stress (Tanaka *et al*., 2009).

In the pericarp tissues, the changes in water contents were not correlated with the values of the longest *T*1 component with ripening (Fig. 3A, Tables 3). However, our results suggest that other factors may contribute to the motional restriction of water. A similar tendency was observed in sweet potato tubers exposed to cold stress (Iwaya-Inoue *et al.,* 2004 b).

We found that membrane permeability, indicating a loss of membrane integrity, increased in mume pericarp tissues during ripening. An increase in membrane permeability also has been reported in ripening wild-type tomato fruit, but not in the ripening-inhibited (rin) mutant (Poovaiah *et al.,* 1975). Moreover, the permeability of the plasma membrane increased and the membrane-lipid composition changed in ripening *Malus domestica* fruit (Lurie *et al.,* 1987) and during the development and senescence of *Cucumis melo* fruit (Lester and Stein, 1993). This coincided with our results from the electrolyte leakage study for mume fruit. In wood plants (i.e. galled leaves invaded by insects), a higher ratio of ion leakage was strongly associated with prolongation of the NMR relaxation times (Kaku and Iwaya-Inoue, 1990). In addition, it was demonstrated that the relaxation times strongly related to the size and geometry of the vacuolated cells in mushroom tissues during postharvest senescence (Donker *et al.,* 1997). In our experiment, elongation in fully vacuolated cells and changes in membrane permeability in the pericarp tissues may have contributed to the correlation of the longest *T*1 components with fruit ripening.

However, in seed tissues, a considerable decrease in the longest *T*1 components in seeds of mume fruit was accompanied with markedly decreased water content, from 14 to 1 g H_2O/g dry weight from Stage 2 to Stage 4 (Fig. 3A and Table 3). Phenomena such as cold acclimation in plant tissues correlated with the decrease both in *T*1 and in water content (Burke *et al.,* 1974; Kaku *et al.,* 1984; Fennell *et al.,* 1996; Yoshida *et al.,* 1997). The decrease in water content of the seed tissues during maturation is considered to be associated with a reduction in free water, perhaps due to an increase of dry matter, that is, an increase in the accumulation of cellular substances (Fig. 2A and B and Table 3).

Relationship between NMR relaxation time T2 and characteristics in mume fruits during development and ripening

The larger fraction of the two, with the long *T*2, can still be assigned to vacuolar water, whereas the small fraction with short *T*2 represents water from the cytoplasm and perhaps the contribution of water inside the cell wall and extracellular water (Scheenen *et al.,* 2002).

The amount of water or *T*2 in the pericarp and seed changed inversely according to the progression of growth stages. Mobile water in the pericarp of small green fruit was low (Table 4). Thereafter, the mobile water in the pericarp increased with enlargement, but did not change further at the coloring fruit stage. Thus, the changes in water contents were not correlated with the values of a longer *T*2 component with ripening (Fig. 3A and Table 4).

During the stage at which the fruit peel became yellow, however, pectic substances in the cell wall structures in the parenchymal tissue collapsed except for in the epidermis and vascular bundles (Figs. 1 and 2A, Stage 4).

Kaneko *et al.* (1989) reported changes in the pectic substance components during mume fruit ripening. The cellular changes, such as the changes of polysaccharide components or pectic substances, specific to the pericarp tissue ripening characteristics, may contribute to a more ordered state of water, thereby resulting in unchanged relaxation time. In previous reports, McCarthy *et al.,* (1995) measured a decrease in *T*2 in bruised regions of apples. In addition, marked shortening of *T*2 values compared with *T*1 values in the fruit tissues may reflect a correlation between the relaxation times and compartment size of cross-linked polymer gels (Murase and Watanabe, 1989).

On the other hand, in seed tissues, a considerable decrease in the longer *T*2 components in seeds of mume fruit is accompanied with markedly decreased water content from Stage 2 to Stage 4 (Fig. 3A and Table 4).

Water status can provide useful information about the characteristics of mume fruit development and ripening

When the seed of a small green mume fruit was gellified, the mobility of water was high. Thereafter, when the color inside the seed of a small green mume fruit turned milky white, the mobility of water, water derived from the vacuole and exchangeable water around macromolecules, became low (Fig. 1 and Tables 3 and 4). The decrease in water content of the seed tissues during maturation is associated with a reduction in free water, perhaps due to an increase of dry matter, that is, an increase in the accumulation of cellular substances such as oil bodies (Fig. 2B and Fig. 3B).

Japanese apricot fruit pericarp tissues may act as a sort of storehouse where photosynthates are temporarily accumulated for further transport into the seed during seed formation. Therefore, seed formation, which is the primary object of fruit growth, may require larger amounts of energy than are available in the pericarp. The parenchymal tissues of the pericarps in matured mume fruit began to collapse in the yellow-peel stage (Figs. 1 and 2A), probably because the pericarp tissues are no longer necessary to physiological functions. These are considered to be the result of a loss of membrane integrity and efflux of pectic substances in the cell wall structures (Figs. 2A and 4). Regardless of the constant high water content, the level of high-mobility water, i.e. water derived from vacuoles and exchangeable water, was high (Fig. 3A, Tables 3 and 4). This increase is considered to result from the fact that the increase of the mobile water by cell wall breakdown is greater than the decrease of the free water by cellular accumulation. In addition, the enlargement of cells in the pericarp region closely correlates with increases in fruit diameter, water uptake, and dry matter accumulation. Changes in the fully-vacuolated-cell volume were closely correlated with changes in the values of the longest *T*1 component during ripening (Tables 2, 3 and 4). Thus, the water compartments and the mobility of water analyzed in this study are considered to reflect the result of physiological changes such as cellular heterogeneity and spatial arrangements both in the pericarp and in seed tissues for mume fruit with development and ripening.

In conclusion, the analysis of water components derived from ^{1}H-NMR spectroscopy provides useful information about the characteristics of fruit ripening.

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