Phase Changes in Arrhenius Plots on NMR Relaxation Times for Various Organs of Crop Plants Exposed to Temperature Stresses

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(Received December 5, 2003)

Arrhenius plots of dynamic states of water in various crop plant organs exposed to cold or heat stress were studied by using ^1H -NMR. In chilling sensitive plants, phase transition in NMR spin-lattice relaxation time (T_1) occurred at 7.5°C and at 12.5°C in *Vigna radiata* and *V. mungo* hypocotyls, respectively, while that in spin-spin relaxation time (T_2) occurred at 13.5°C in sweet potato tubers. On the other hand, phase transition in T_1 occurred at 0°C in both leaves and roots of perennial ryegrass and pea epicotyls. Prolongation of T_1 was observed after the break point in these species. Furthermore, phase changes in T_2 of the ryegrass leaves and roots occurred at -20°C and -10°C, respectively. Judging from electrolyte leakage and viability test as well as T_2 , both vacuolar and cytoplasmic compartments froze between -20 and -25°C in the leaves while the freezing in the root tissues occurred between -10 and -15°C in ryegrass. Therefore, supercooling ability of the ryegrass organs were especially detected by T_2 . On the other hand, adaptation to heat stress was clearly shown by T_1 while heat-denature was shown by a marked decrease in T_2 . From these results, the phase transition temperatures were closely correlated their stress sensitivity, especially acclimation to temperatures was detectable by T_1 while critical temperature by T_2 . In conclusion, analysis of Arrhenius plots of T_1 and T_2 in plant organs provided sensitive and non-invasive way for evaluating both primary and lethal responses to the temperature stresses.

Keywords: Arrhenius plots, ^1H -NMR relaxation times (T_1 , T_2), molecular dynamics of water, temperature stresses, water compartments

INTRODUCTION

Environmental stresses dramatically affect plant survival and productivity. Different strategies are required for protection against transient stresses. Many plants acclimate to cold and/or heat stress (Sabehat et al., 1998; Thomashow, 1999 references therein). Therefore, it is important to select cold- or heat-tolerant crop plants for adaptation to cultivate in various climate regions. For temperature-stressed studies in plants, a phase transition has been shown in Arrhenius plots of membrane lipid (Lyons and Raison, 1970; Lyons, 1973; Steponkus, 1981; Martin, 1986; Wilson, 1987; Vonwiren et al., 1998) and a variety of biological reaction

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rates (Casado and Heredia, 1999 ; Queiroz et al., 2000 ; Schreiber, 2001, 2002). Non-linear temperature dependency or the presence of discontinuity has been seen as diagnostics of phase transitions of membrane lipid. Although mechanisms of thermo-tolerance in plants have been extensively studied, little is known about dynamic states of water which affect cellular metabolism. The physical state of water in plant cells is also a subject of interest relative to plant cold hardiness (Levitt, 1980). Many metabolic processes such as enzymatic reactions, transportation and accumulation of materials occur in cytosol, and thus water in living tissues is considered to play an important role in their physiological condition. Water plays an important role not only as a solvent for biochemical reactions, but also as a stabilizer of macromolecular structure. Nuclear magnetic resonance (NMR) of water protons provides a method of nondestructive research on water and water interactions in biological systems (Budinger and Lauterbur, 1984 ; Ishida et al., 2000 ; Ionenko and Anisimov, 2001 ; Van der Weerd et al., 2002). The degree of physiological activity in the tissue reflects the level of water binding. Since the mobility and characteristics of cell-associated water are closely related to the condition of the cells, NMR images represent physiological maps of the plant tissues (Kano et al., 1997 ; Ishida et al., 2000). ^1H -NMR spin-lattice (T_1) and spin-spin (T_2) relaxation times are used as indicators of dynamic states of water in biological tissues since they reflect the motion of water molecules. The magnitude of the signal following excitation recovers as a function of T_1 and the echo signal declines with that of T_2 (Farrar and Becker, 1971). Furthermore, T_1 and T_2 of water protons were applied to the studies of higher plant tissues exposed to thermal stresses (Kaku et al., 1985 ; Abass and Rajashekar, 1991 ; Iwaya-Inoue et al., 1993 ; Caldwell et al., 1997 ; Yoshida et al., 1997 ; Maheswari et al., 1999 ; Van der Weerd et al., 2002).

In this study the temperature dependencies of T_1 and T_2 of water protons in various organs such as hypocotyls, epicotyls, leaves, roots and tubers were determined in various plants such as pea plants, mung bean, black mappe, pasture plants (perennial ryegrass and tall fescue) and sweet potato when they were exposed to chilling, freezing or heat stress. Furthermore, we used ^1H -NMR to address the possibility of the early identification of physiological changes in these crop plants subjected to temperature stresses.

CHANGES IN NMR RELAXATION TIME (T_1) IN VARIOUS PLANT ORGANS EXPOSED TO CHILLING STRESS

Temperature dependencies of Arrhenius plots of NMR spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2), indicating dynamic states of water, were determined in various plant organs. A ^1H -NMR spectrometer with a magnet operating at 20 MHz or 25 MHz for ^1H was used for the measurements of T_1 and T_2 . For a cold treatment, a probe temperature was controlled by a connected thermostat to the sample chamber of the spectrometer using LN_2 . For T_1 measurements, the saturation recovery method (90° - τ - 90° pulse sequence) or inversion recovery method (180° - τ - 90° pulse sequence) was used (Kaku and Iwaya-Inoue, 1988 ; Iwaya-Inoue et al., 2004a, 2004b).

A question of "break" or "straight" in Arrhenius plots is fairly dependent on the validity of the model selection in the statistical analysis, and AIC (Akaike's Information Criterion) method is applicable as a useful statistical procedure to give the practical standard for the resolution of the problem of the "use" or "misuse" of Arrhenius plots in stress physiology of plants (Akaike, 1971 ; Iwaya-Inoue et al., 1989). Break points determined with statistical models occurred at about 7.5°C for mung bean (*Vigna radiata* [L.] Wilczek) and at about 12.5°C for black mappe (*V. mungo* [L.] Hopper) in temperatures ranging from 20 to 0°C in 2.5°C steps (Table 1). On the other hand, T_1 values in epicotyls of chilling-insensitive pea

Table 1 Changes in Arrhenius plots of NMR relaxation times (T_1 , T_2) of solutions and various plant organs exposed to chilling stress.

Plants and solutions		Response to lowering temperature				Cited references	
		Materials		Temperature dependency			
			Range (°C)	B.P. (°C) ^z			
<i>In vivo</i>	Organs					Viability	
<i>Vigna radiata</i> (L.) Wilczek	hypocotyl	fresh	20 to 0	T_1	decreased	7.5 increased	partly injured
<i>Vigna mungo</i> (L.) Hopper	hypocotyl	fresh	20 to 0	T_1	decreased	12.5 increased	partly injured
<i>Pisum sativum</i> L.	epycotyl	fresh	20 to 0	T_1	linearly decreased		alive
<i>Ipomoea babatus</i> Lam.	tuber	fresh	30 to 0	T_1	slightly increased		partly injured
	tuber	fresh	30 to 0	T_2	increased	13.5 decreased	partly injured
	tuber	stored ^y	30 to 0	T_1	linearly decreased		—
	tuber	stored	30 to 0	T_1	constant		—
	tuber	dead	30 to 0	T_1	linearly decreased		—
	tuber	dead	30 to 0	T_2	constant		—
<i>In vitro</i>							Phase
Distilled water	—	liquid	30 to 0	T_1	linearly decreased		liquid
	—	liquid	30 to 0	T_2	linearly decreased		liquid
Maltose	0.1 M	liquid	30 to 0	T_1	linearly decreased		liquid
	0.1 M	liquid	30 to 0	T_2	slightly decreased		liquid
Sucrose	0.1 M	liquid	30 to 0	T_1	linearly decreased		liquid
	0.1 M	liquid	30 to 0	T_2	slightly decreased		liquid

^z B.P., break point of Arrhenius plots.^y Sweet potato tubers stored at 15°C for one year were partly injured.

(*Pisum sativum* L. cv. Alaska) plants linearly depended on temperature ranging from 20 to 0 °C. In addition, Arrhenius plots of the T_1 in leaves of tomato (*Lycopersicon esculentum* Mill. var. *cerasiforme* cv. Minicarol) and cucumber (*Cucumis sativus* L. cv. Ochiai) indicated biphasic with temperature drop from 20 to 0 °C (Iwaya-Inoue et al., 2002). “Breaks” in Arrhenius plots of T_1 of fresh leaves in tomato and cucumber occurred at about 12.5 °C and 10 °C, respectively, while those of chilling-insensitive clone of gloxinia (*Sinningia speciosa* hybrids) indicated no break point during the same process. The phase change closely reflected their chilling sensitivity. From these results, it would be pointed out that the phase change in the chilling process suggests a much earlier symptom for primary response to chilling stress.

However, T_1 in sweet potato (*Ipomoea babatus* Lam. cv. Koganesengan) fresh tubers kept constant levels or slightly increased from 30 to 0 °C and the relaxation behavior was markedly different from those in seedlings of the other species described above. The water content of sweet potato tuber tissues was about 65% (Iwaya-Inoue et al., 2004a), whereas the water contents of the others were over 90% (Iwaya-Inoue et al., 1993, 2003b, 2004b). The different T_1 characteristics between tubers and hypocotyls were due to the water content. In distilled water, T_1 linearly decreased with decreases in temperature. Sugar solutions adjusted to the similar concentrations in fresh sweet potato tubers were prepared. T_1 of 0.1 M sucrose and 0.1 M maltose solutions linearly decreased with a decrease in temperature. The different NMR relaxation behaviors in the crop plants would be attributable to cell structure and cellular constitution as well as the water content. Thus, the structure of water would be altered by such physiological or pathological disturbance.

CHANGES IN WATER COMPARTMENTS DETERMINED BY T_1 IN ORGANS EXPOSED TO CHILLING STRESS

Perennial ryegrass (*Lolium perenne* L. cv. Friend) is widely cultivated as a pasture plant in cool temperate region (Waller and Sale, 2001 ; Ebdon et al., 2002). T_1 s in leaves and roots of 6-week-old ryegrass linearly decreased with a temperature drop from 20 to 0 °C (Fig. 1, Table 2). Furthermore, T_1 values in seedlings of chilling-insensitive pea (*Pisum sativum* L. cv. Alaska) linearly depended on temperature ranging from 20 to 0 °C (Iwaya-Inoue et al., 1989). Water in plant tissues was often characterized by different proton relaxation times (Ishida et al., 1987 ; Isobe et al., 1999 ; Iwaya-Inoue and Nonami, 2003). It is generally accepted that 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 et al., 2004b references therein). The three compartmentation of water originally identified from the vacuole, cytoplasm, and cell wall/extracellular space (apoplast), is reflected by different relaxation times in the parenchyma tissue (Snaar and Van As, 1992 ; Hills and Remigereau, 1997). In morning glory (*Pharbitis nil* Choisy.) seeds, three components of water fractions characterized by different T_1 s and chemical shifts were observed (Isobe et al., 1999).

T_1 values of two water components in the ryegrass leaves were constant or linearly decreased until 0 °C while T_1 of long fraction gradually increased below 0 °C. The relative value of signal intensity in all leaves tested was almost constant during the experiment between 20 and about -12.5 °C. On the other hand, T_1 values of two fractions in roots linearly decreased, and the relative value of signal intensity did not change between 20 and 0 °C. “Breaks” in Arrhenius plots of T_1 of perennial ryegrass leaves occurred at 0 °C followed by prolongation in T_1 . The phase change in T_1 might have responded to a physiological change as a primary event.

Table 2 Changes in Arrhenius plots of NMR relaxation times (T_1 , T_2) of solutions and various plant organs exposed to freezing stress.

Plants and solutions Materials		Response to lowering temperature					Cited references			
		Temperature dependency								
		Range (°C)		B.P. (°C) ^z	B.P. (°C) ^z	Viability				
<i>In vivo</i> <i>Lolium perenne</i> L.	Organs									
	leaf	20 to -25	T ₁	decreased	0.0 increased	-10.0	ND ^y	lethal	Iwaya-Inoue et al., 2004b <i>ibid</i> <i>ibid</i> <i>ibid</i>	
	leaf	20 to -25	T ₂	slightly increased		-20.0	decreased	lethal		
	root	20 to -15	T ₁	linearly decreased		0.0	ND	lethal		
root	20 to -15	T ₂	slightly increased		-10.0	decreased	lethal			
<i>Festuca arundinacea</i> Schreb.	leaf	25 to -30	T ₁	constant	0.0 increased	-10.0	ND	lethal	Phan et al., 2003 <i>ibid</i>	
	leaf	25 to -30	T ₂	linearly increased		-16.3	decreased	lethal		
<i>In vitro</i> Distilled water Glucose BSA								Phase		
	—	liquid	30 to -20	T ₁	linearly decreased		-10.0	ND	solid	Matsuda, 2003 <i>ibid</i>
	—	liquid	30 to -20	T ₂	linearly decreased		-10.0	decreased	solid	
	0.25 M	liquid	30 to -20	T ₁	linearly decreased		-10.0	ND	solid	<i>ibid</i> <i>ibid</i>
	0.25 M	liquid	30 to -20	T ₂	slightly increased		-10.0	decreased	solid	
	8%	solated	30 to -20	T ₁	linearly decreased		-10.0	ND	gelated	<i>ibid</i> <i>ibid</i>
	8%	solated	30 to -20	T ₂	linearly decreased		-10.0	decreased	gelated	

^z B.P., first and second break points of Arrhenius plots.^y ND, T_1 values were not detectable below the break point.

PROLONGATION IN T_1 OF ORGANS EXPOSED TO CHILLING STRESS

Long-term storage caused prolongation of T_1 in sweet potato (*Ipomoea batatas* Lam.) tubers (Iwaya-Inoue et al., 2004a). Initial T_1 values for the fresh tissues examined at 30°C were about 400 ms, whereas those for the stored tuber tissues were about 700 ms. There was not a clear thermal dependency on T_1 in the fresh tuber tissues, whereas a linear thermal dependency was observed in the case of T_1 in the stored tuber tissues of sweet potato (Table 1). The prolongation rate of T_1 values in the warming process of cool-warm cycle (20 to 0°C) correlated with the degree of chilling damage or lesion in gloxinia leaf tissues (Kaku and Iwaya-Inoue, 1987).

In many cases of disordered tissues in animals, it has been shown that T_1 relaxation times are greatly enhanced in comparison to corresponding healthy ones (Damadian, 1971; Williams et al., 1980) and the theory was widely applied as MRI diagnosis (Podo et al., 1998; Hayes et al., 2002). And T_1 of water protons in biological systems may be influenced by a variety of changes in the conformational state of macromolecules, water-membrane and water-protein interactions (Chang et al., 1981; Mathur-De Vre, 1984). In woody plants, higher ratio of ion leakage was well associated with prolongation of T_1 in galled leaves invaded by insects (Kaku and Iwaya-Inoue, 1990). Ion leakage of the stored tuber tissues indicated about 70%, thus the stored tissues were partly injured. It has been reported that T_1 values of chilling-sensitive *Vigna radiata* hypocotyls gradually increased after the tissues were exposed to 0°C for 1 h indicating reversible change while water content decreased slightly after the stress (Iwaya-Inoue et al., 1993). The distinct T_1 change in the slope after the "break" in *Vigna* two species occurred might suggest the beginning of the T_1 prolongation, assuming that the break does not occur and a linear drop of T_1 continues over the entire temperature range, and if the T_1 values between the actual data and the predicted value derived from the regression line for the first straight line segment are compared (Kaku and Iwaya-Inoue, 1988; Iwaya-Inoue et al., 1989).

It is known that proteins are reversibly denatured at low temperatures as a result of weakening of hydrophobic bonds. A reversible change in conformation of H^+ -ATPase in the tonoplast of hypocotyls of *Vigna radiata* at 0°C, may cause cytoplasmic acidification (Yoshida et al., 1989). There was a closed correlation between pH and T_1 *in vitro*; when pH of a solution of paramagnetic ions and protein, in particular, Mn^{2+} and bovine serum albumin (BSA), was reduced from 7.5 to 6.0, the T_1 value increased (Iwaya-Inoue et al., 1993). The range of pH values that affected T_1 significantly corresponded to the pH of the cytoplasm in *Vigna* cells in culture exposed to 0°C (Yoshida et al., 1989). Therefore, it seems likely that the gradual prolongation in T_1 for plant tissues exposed to chilling stress depends partly upon changes in cytoplasmic pH.

CHANGES IN NMR SPIN-SPIN RELAXATION TIMES (T_2) IN ORGANS EXPOSED TO CHILLING STRESS

T_2 was measured by the Carr-Purcell-Meiboom-Gill (CPMG) method with pulse ($90^\circ x - 180^\circ y - 2\tau - 180^\circ y - 2\tau \cdots$) sequence. T_2 values for the perennial ryegrass (*Lolium perenne* L.) leaves were almost constant or slightly increased with decreasing temperature between 20 and 0°C (Table 2, Fig. 1). Several components of T_2 for the tissues were also calculated from semi-log plots of 1H -NMR signal intensities using a curve fitting. T_2 values of two components in the leaves and roots showed no temperature dependency between 20 and 0°C.

Additionally, a strong inverse relation between temperature and T_2 was observed at

temperatures ranging from 30 and 0°C in fresh sweet potato tubers (Table 1). Initial T_2 values of the fresh sweet potato tuber tissues indicated around 100 ms, whereas those of the stored tubers for one year at 15°C ranged between 200 and 300 ms (Iwaya-Inoue et al., 2004a). The long-term storage caused prolongation in sweet potato tubers. From these results, T_2 values as well as T_1 values in the sweet potato tubers increased with long-term storage. Electrolyte leakage indicated the stored sweet potato tubers were partly injured. A change in the slope of the Arrhenius plots of the T_2 values in fresh tubers was statistically investigated. As a result, a change of the slope in the T_2 of the fresh tissues was observed at about 14°C, although there were no break points in the Arrhenius plots of T_2 in the dead tissues. It is known that the critical cold-storage temperature ranges between 10 and 15°C for sweet potato tubers (Ravi and Aked, 1996, references therein). The 15°C-stored-tubers were partly injured by this method of evaluation, and the slope change occurred at about 14°C in the T_2 of the fresh tissues, which was consistent with the storage temperature. An Arrhenius plot of metabolic heat rate revealed a break in the line at 15°C, suggesting the occurrence of a metabolic transition at this temperature (Queiroz et al., 2000).

T_2 changes in relation to chilling temperature were discussed in animal cells; when chicken eggs were stored according to the regulations between 5 and 8°C over 2 weeks storage, an increase of T_2 was observed in comparison to unchilled eggs (Schwagele et al., 2001). T_1 linearly decreased with decreases in temperature while T_2 of the 0.1 M sucrose and maltose solutions slightly depended on temperature within the range of 30 to 0°C, respectively. The difference between T_1 and T_2 observed in the sugar solutions was similar to those in the dead tuber tissues. In other words, the temperature dependency of T_2 s of the sugar solutions and the dead tissues appeared to respond to viscosity at higher temperatures. These results indicate that water mobility determined as T_2 was more suppressed at higher temperatures.

PHASE CHANGE IN T_1 OF ORGANS EXPOSED TO FREEZING STRESS

T_1 in sweet potato tuber tissues markedly decreased by treatment involving both freezing and thawing (Iwaya-Inoue et al., 2004a). Initial T_1 values for the fresh tuber tissues examined at 30°C ranged between about 300 and 500 ms, whereas those for the frozen-thawed tissues were around 200 ms. Since water contents in both the fresh and the frozen-thawed tissues of sweet potato tubers were about 65%, shortening of T_1 did not depend on decrease in the water content. An extent of leakage of electrolytes was determined with an electrolyte conductivity meter and expressed as the percentage of the total electrolytes in each sample, measured after samples were killed by a cycle of freezing and thawing. An index has been reported of injury related to ionic conductance from freeze-stressed cabbage (*Brassica oleracea* L. Capitata cv.) (Manley and Hummel, 1996) and nitric oxide-stressed wheat leaves (Mata and Lamattina, 2001). Leakage of electrolytes in the frozen-thawed tuber tissues indicated 100%, whereas that of fresh tissues was approximately 30% (Iwaya-Inoue et al., 2004a). In the dead tissues a positive temperature dependency of the Arrhenius plots was clear in T_1 determined at temperatures ranging from 30 to 0°C (Table 1). The T_1 dependency in the dead plant tissues seems to solely reflect the mobility of water in the tissues as shown *in vitro*.

T_1 determination in fresh perennial ryegrass leaves could not be carried out at about -15°C while T_1 in roots could not be determined below -5°C (Table 2, Fig. 1). Similar tendency was observed in leaves of tall fescue (*Festuca arundinacea* Schreb.) (Phan et al., 2003). Both sugar and protein solutions adjusted to the concentrations in the ryegrass and tall fescue grass leaves were prepared. T_1 s of 0.25 M sucrose and 8% BSA solutions linearly decreased with decrease in temperature and phase changes occurred at -10°C and T_1 could not be determined at less than -10°C (Matsuda, 2003). In addition, T_1 values in ryegrass leaves were markedly

shorter than those in roots. Seasonal changes in T_1 were closely correlated with the water content and supercooling ability of azalea florets (Kaku et al., 1984). The water content in both ryegrass leaves and roots was about 89%, and that of leaves and roots decreased to 82% and 83%, respectively. Therefore, changes in T_1 of ryegrass organs as well as sweet potato tubers exposed to freezing stress could not be solely ascribed to the water content.

ABRUPT DECREASE IN T_2 IN ORGANS EXPOSED TO FREEZING STRESS

T_2 in the perennial ryegrass leaves gradually prolonged in the temperature range from 0 to -20°C (Table 2, Fig. 1). Similar tendency was observed in T_2 of tall fescue leaves. A gradual increase in T_2 of long fraction and a linear decrease in T_2 of short fraction in ryegrass leaves were observed (Iwaya-Inoue et al., 2004b). Additionally, relative value of signal intensity in the long fraction gradually increased in the temperature range from 0 to -20°C . The T_2 in long fraction of the leaves is thought to be mainly associated with vacuole and the fraction with the short T_2 is thought to be associated with the cytosol and apoplastic region (Chen and Gusta, 1978). Difference in T_2 values of biological tissues is also interpreted in terms of the differences of the ratio of "free water" to "bound water" (Walter et al., 1989). The solid-echo (90° - τ - 90°) method was also applied for T_2 measurements when T_2 values were below 1 ms. Abrupt shortening of T_2 occurred in perennial ryegrass leaves at -25°C and that in roots between -10 and -15°C . In addition, the similar phase change in T_2 of tall fescue occurred at about -16°C . The T_2 values in both long and short fractions of leaves decreased to about 600 μs and 10 μs at -25°C , respectively. T_2 value below 100 μs is thought to be associated with water tightly bound to macromolecules in cells (Hills and Remigereau, 1997). The abrupt decrease in T_2 depended on decreases in T_2 values of two fraction components accompanied by a decrease in the signal intensity of long fraction component. On the other hand, T_2 in roots began to decrease at -10°C and the relative value of signal intensity of the long T_2 fraction markedly decreased. In parenchyma tissue of apple, vacuolar peak of T_2 has

Fig. 1 Schematic profile of Arrhenius plots of T_1 and T_2 in crop plant organs and solutions exposed to cold stress. Solid lines indicate those of plant organs while broken lines indicate those in solutions. Since T_1 and T_2 in DW indicate same values in the temperature range, they are shown by one straight line. Black arrows indicate changing points observed in plant tissues and a white arrow indicates that of solutions. DW, distilled water; Sugar, 0.25 M glucose, 0.1 M maltose and 0.1 M sucrose solutions; Protein, 8% bovine serum albumin (BSA) solution.

vanished showing that the vacuolar compartment has frozen at -3°C , while the two peaks corresponding to the cytoplasm and cell wall compartments were observed indicating that these compartments remain unfrozen (Hills and Remigereau, 1997). Therefore, it indicated that vacuolar water in the leaf and root tissues disappeared at the abrupt changing point in T_2 .

The leakage of electrolytes from the ryegrass leaf and root tissues after the abrupt decrease of T_2 which occurred at -25 and about -15°C , respectively, showed about 100% (Iwaya-Inoue et al., 2004b). The leakage of electrolytes indicated these organs exposed to these subzero temperatures were severely damaged. In addition, activity of 2,3,5-triphenyltetrazolium chloride (TTC) reduction in ryegrass organs which reflected the viability of the tissues vanished. The marked shortening of T_2 in perennial ryegrass suggested that intracellular freezing occurred between -20 and -25°C in leaves while it occurred between -10 and -15°C in roots.

The shortening of T_2 has been attributed to a decrease in membrane permeability under freezing injury in wheat crowns (Chen and Gusta, 1978). The T_2 of cellular water is known to be dependent on plasmalemma and tonoplast permeability (Van As, 1992). T_2 values in the ryegrass roots were also longer than those in leaves as well as T_1 . More restricted mobility of water in the leaves might play an important role against the freezing stress. These findings indicated that the abrupt T_2 decreasing was reflective of a supercooling ability in individual organs.

CHANGES IN T_1 OF ORGANS EXPOSED TO HEAT STRESS

T_1 s in leaves and roots of perennial ryegrass exposed to heat stress decreased in the temperature range between 20 and 50°C (Table 3, Fig. 2). T_1 s in the leaves were almost constant or slightly decreased from 20 to 35°C and a slope change occurred at 40°C , while those in roots linearly decreased in temperatures ranging from 20 to 50°C . Electrolyte leakage and TTC reduction indicated that leaf tissues have more heat resistance compared to roots (Iwaya-Inoue et al., 2004b). On the other hand, epicotyls of pea (*Pisum sativum* L.) plants after the subsequent thermal hysteresis of 20 – 40°C cycles exhibited tissue necrosis (Iwaya-Inoue et al., 2004c). It has been reported that treatment at 40°C to pea plants led to a serious injury of the photosynthetic apparatus while the changes in Chl fluorescence parameters at 35°C were reversible (Georgieva and Lichtenthaler, 1999). T_1 values in intact pea epicotyl tissues exposed to 120°C for 15 min treatment markedly decreased from 1.2 to 0.7 s (Iwaya-Inoue et al., 2004c). The shortening of T_1 was also observed in the heat-denatured hypocotyls of mung bean (Iwaya-Inoue et al., 2000). T_1 s for both 40°C - and 120°C -treated pea epicotyls linearly decreased with decreasing temperature while it increased with increasing temperature between 20 and 40°C . A slope change occurred at about 28°C and a prolongation in T_1 corresponding elevated temperature after the break point would reflect an occurrence of tissue injury. From these results, the temperature dependency in T_1 of the heat-denatured pea epicotyls as well as the frozen-thawed sweet potato tubers seems to solely reflect the mobility of water shown *in vitro* (Tables 1, 3).

On the contrary, it was indicated that molecular dynamics of water in pea epicotyls exposed to 30°C maintained the shortened values and was temperature-independent (Iwaya-Inoue et al., 2004c). Furthermore, the shortening of T_1 was observed at 40°C in perennial ryegrass leaves (Table 3) and mung bean hypocotyls (Iwaya-Inoue et al., 1993). They exhibited no severe injury. Therefore, such findings suggested that adaptability for heat stress was accompanied by a shortening of T_1 values.

The water content of plant tissues is a main factor affecting water status in the tissues (Burke et al., 1974). T_1 values of mung bean seedlings exposed to 40°C for 4 h decreased by

Table 3 Changes in Arrhenius plots of NMR relaxation times (T_1 , T_2) of solutions and various plant organs exposed to heat stress.

Plants and solutions		Response to elevated temperature				Cited references			
		Temperature dependency							
Materials		Range (°C)	B.P. (°C) ²						
<i>In vivo</i> <i>Lolium perenne</i> L.	Organs				Viability				
	leaf	fresh	20 to 50	<i>T</i> ₁	decreased	40.0	decreased	partly injured	Iwaya-Inoue et al., 2004b
	leaf	fresh	20 to 50	<i>T</i> ₂	linearly decreased			partly injured	<i>ibid</i>
	root	fresh	20 to 50	<i>T</i> ₁	linearly decreased			injured	<i>ibid</i>
<i>Pisum sativum</i> L.	root	fresh	20 to 50	<i>T</i> ₂	linearly decreased			injured	<i>ibid</i>
	epycotyl	fresh	20 to 40	<i>T</i> ₁	decreased	27.5	increased	lethal	Iwaya-Inoue et al., 2004c
	epycotyl	dead	20 to 40	<i>T</i> ₁	linearly increased			—	<i>ibid</i>
								Phase	
<i>In vitro</i> Distilled water	—	liquid	30 to 60	<i>T</i> ₁	linearly increased			liquid	Matsuda, 2003
	—	liquid	30 to 60	<i>T</i> ₂	linearly increased			liquid	<i>ibid</i>
Glucose	0.25 M	liquid	30 to 60	<i>T</i> ₁	linearly increased			liquid	<i>ibid</i>
	0.25 M	liquid	30 to 60	<i>T</i> ₂	linearly increased			liquid	<i>ibid</i>
BSA	8%	solated	30 to 60	<i>T</i> ₁	increased	55.0	decreased	gelated	<i>ibid</i>
	8%	solated	30 to 60	<i>T</i> ₂	increased	50.0	decreased	gelated	<i>ibid</i>

^z B.P., break point of Arrhenius plots.

10 to 15%, while the water content decreased slightly (by less than 2%) after the heat stress (Iwaya-Inoue et al., 1993), which indicated the water content did not strongly affect T_1 in hypocotyls exposed to heat stress. In addition, the heat shock treatment (40°C for 4 h) to the seedlings of *Vigna radiata* induced a 70 kDa protein and the hypocotyls showed tissue viability (Iwaya-Inoue et al., 1993). In seedlings of the same species members of heat shock protein, HSP70 family, were induced by 41°C for 4 h (Kawata and Yoshida, 1988). In yeast cells HSP70 family might stabilize denatured proteins and it might play a role as a surfactant in cells (Komatsu et al., 1990). These results suggested that gradual shortening in T_1 and no temperature dependency in intact pea epicotyls exposed to 30°C-thermal hysteresis might reflect cell adaptation to heat stress. In addition, a change in the slope of Arrhenius plots occurred at 40°C in T_1 of ryegrass leaf tissues would also indicate a primary response against heat stress. From these results, the early response in T_1 s of the epicotyl and leaf tissues was considered as a fast adaptation or mortality in cells to heat stress.

CHANGE IN T_2 OF ORGANS EXPOSED TO HEAT STRESS

T_2 values in both leaves and roots of the perennial ryegrass markedly decreased with an increase in temperature ranging from 20 to 50°C (Table 3, Fig. 2). Ion leakages in the leaves and roots after exposure to the heat stress were 27 and 56%, respectively (Iwaya-Inoue et al., 2004b). The low leakage of electrolytes in leaves exposed to heat stress indicated there was no severe damage. These results suggested that leaves had more heat tolerance compared to roots. In general, plant roots are protected from cold and heat stresses more efficiently than the other organs, because the soil temperature is more stable than the air temperature as an environmental factor. It was reported that there was a steady decrease in T_2 for the major fraction for grape leaves with an increase in temperature and sharp decreases in T_2 at 48 and 45°C, which correspond with the lethal temperature measured by both electrolyte leakage and TTC reduction (Abass and Rajashekar, 1991). In 8% BSA solution, a marked decrease in Arrhenius plots of T_2 occurred at 50°C while a slight change occurred at 55°C in those of T_1 . The BSA solution indicated gelatinization of soluble protein at the break points (Matsuda,

Fig. 2 Schematic profile of Arrhenius plots of T_1 and T_2 in crop plant organs and solutions exposed to heat stress. Lines and arrows are described in Fig. 1. Sugar, 0.25 M glucose solution; Protein, 8% BSA solution.

2003). From these results, T_1 on heat response can be used as an indicator of the reversible change while T_2 can be used as that of the irreversible change in macromolecules and plant tissues.

CONCLUSION

Arrhenius plots of both T_1 and T_2 suggested that dynamic states of water reflected the difference in thermal response in hypocotyls, epicotyls, leaves, roots and tubers in various crop plants. These results underscore an importance of selecting temperature resistant cultivars for adaptation to cool and warm climates. Based on these results, it is possible to conclude that the break points in T_1 and T_2 of the Arrhenius plots of fresh tissues might be reflective of the physiological changes. Especially, T_1 can be used to detect response to mild stress and adaptation while T_2 is useful for viability assay to more severe stress to critical temperatures. In conclusion, Arrhenius plots of NMR relaxation times (T_1 , T_2) in various organs of crop plants provided sensitive and non-invasive way for evaluating tissue response to both cold and heat stresses.

We are thankful to Mr. Soichi Nagai, JEOL, Co., Ltd., Japan, for his technical support.

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