Seed Science Research (2005) **15**, 99–111 DOI: 10.1079/SSR2005200

Characterization of water status in primed seeds of tomato (*Lycopersicon esculentum* Mill.) by sorption properties and NMR relaxation times

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Abstract

The enhanced laboratory and field emergence characteristics of osmo- and halo-primed tomato seeds (cv. Pusa Ruby) were related to changes in hydration–dehydration kinetics, modified sorption properties and nuclear magnetic resonance (NMR) relaxation behaviour of humidified and imbibed seeds. Water sorption isotherms were constructed for primed and unprimed seeds by equilibrating to different water activities (a_w) at 25°C. Analysis of the isotherms by the D'Arcy–Watt equation revealed that priming reduced the number of strong binding sites and the associated water content, and increased significantly the number of weak binding sites and the associated water content. This redistribution of water, which increased the availability of seed water, may be the reason for the higher speed of germination of primed seeds. The changes in transverse relaxation time (T_2) of seed water and its components, measured *in vivo* using nuclear magnetic resonance spectroscopy, showed interesting differences between primed and unprimed seeds. With an increase in humidification time, the T_2 of primed seeds could be resolved into three components with varying mobilities, while the control seeds had only two components until 10 d of humidification. During imbibition, the third component appeared after 2 and 6 h in primed and control seeds, respectively. This component disappeared after the germination process started in all treatments. The third fraction, with very low molecular mobility, which accounted for about 40% of the proton population, was assigned to hydration water of macromolecules. Hence, we propose that better performance of primed seeds may be attributed to the modifications of seed water-binding properties and reorganization of seed water during imbibition, so as to increase the macromolecular hydration water required for various metabolic activities related to the germination process.

Keywords: imbibition, *Lycopersicon*, nuclear magnetic resonance, relaxation times, seed priming, sorption isotherms, T_2, tomato

Introduction

A number of pre-sowing treatments have been used to increase the rate and uniformity of emergence in many vegetable and flower species (Parera and Cantliffe, 1994). Seed priming first allows the seed to imbibe moisture, using various protocols of osmo-, halo- and hydro-priming, followed by re-drying to permit routine handling (Heydecker *et al.*, 1973). This process controls hydration of seeds to a level that allows pre-germination activity, but does not permit radicle protrusion through the seed coat. In tomato, osmotic priming improves germination and seedling vigour of fresh and aged seeds (Pandita and Nagarajan, 2000; Nagarajan and Pandita, 2001). For example, our earlier experiment with different priming treatments on tomato seeds revealed that osmo-priming (−0.5 MPa for 3 d with PEG-6000) and halo-priming (15 mM KNO_3 for 24 h) improved the speed of germination, seedling vigour and field emergence index by 42, 47 and 15.5%, respectively (Pandita *et al.*, 2003). A hydration–dehydration treatment improved performance of medium-quality seed lots of tomato (Peñaloza and Eira, 1993). This enhanced performance of primed seeds was partially explained as due to biochemical changes, such as increased production of nucleic acids, especially rRNA (Coolbear *et al.*, 1990), or increased activities of amylase, peroxidase and dehydrogenase (Saha *et al.*, 1990; Pandita *et al.*, 2003). Morphological changes, e.g. an increase in free space between the embryo and endosperm tissue (Liu *et al.*,

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1996; Pandita *et al.*, 2003) that facilitates the uptake of water and extension of the cell wall in the radicle and weakening of the endosperm cell wall in front of the radicle tip (Karssen *et al.*, 1989), are also reported. In a recent paper, Sun *et al.* (2003) have related reduced longevity of osmo-primed *Vigna radiata* seeds to biopolymer volume contractions and enhanced water–water association in seed axes, but not in cotyledon tissues. This was confirmed by solid-state ^{1}H-NMR studies. However, no attempt has been made to explain the enhancement effect of priming on the basis of changes in seed water characteristics.

It is well acknowledged that the status of seed water influences seed quality and subsequent germination and emergence characteristics. Seed water in the present context means both the quantity of water present in the seed and its physical and chemical status. Water is an important substrate in many reactions, and its effect on phospholipid structure plays an important role in membrane stability (Leopold and Vertucci, 1989). Molecular mobility is increasingly considered a key factor influencing storage stability of biomolecular substances, as it is thought to control the rate of detrimental reactions responsible for reducing the shelf-life of germplasm. Buitink *et al.* (1998, 2000a, b) have conducted extensive studies on molecular mobility and glass transition temperatures, and have related them to seed storage stability. A simple means of studying water binding by seed tissues involves the use of moisture isotherms (Rockland, 1969; Vertucci and Leopold, 1987; Sun *et al.*, 1997). Isotherms from orthodox seeds exhibit a reverse sigmoidal shape, indicative of three regions of water binding. These regions have been classified by the strength with which water is bound and the nature of the binding site. They may be described as water that is very tightly bound to ionic groups (region I), weakly bound to polar, non-ionic groups (region II) and very weakly arrayed as bridges over hydrophobic moieties (region III). Sun *et al.* (1997) have correlated the storage stability of osmo-primed mung bean seeds with the modifications of seed water sorption properties.

Nuclear magnetic resonance (NMR) spectroscopy offers a non-destructive and non-invasive method for characterization of water status in many biological tissues, including seeds (Brosio *et al.*, 1992; Nagarajan *et al.*, 1993; Fukuoka *et al.*, 1994; Gambhir *et al.*, 1997; Krishnan *et al.*, 2003). Longitudinal and transverse relaxation behaviour of water protons can be investigated to describe the compartmentation and transport of water in tissues. The mobile and less mobile water molecules are distinguished by their different relaxation rates, and their relative amounts can be calculated (Van As, 1992; Krishnan *et al.*, 2004). In this paper, we test whether the enhanced performance of primed seeds is associated with changes in seed water status, binding and availability for various biochemical reactions, using thermodynamic principles and NMR relaxation measurements. We have used sorption isotherms and NMR relaxation times to study the changes induced by priming in seed water status and water distribution in relation to enhanced seed performance.

Materials and methods

A seed lot of tomato (*Lycopersicon esculentum* Mill., variety Pusa Ruby) was harvested in 2000–2001, stored under controlled storage [15°C and 30% relative humidity (RH)], and used as the experimental material. For NMR experiments that were conducted subsequently at New Delhi, a second lot of seeds harvested in 2001–2002 was used.

Priming treatments

Osmo-priming employed PEG-6000, and its concentration was adjusted to give − 0.5 MPa potential at 25°C (Michel and Kaufmann, 1973). The seeds (about 400) were kept on one layer of filter paper wetted with 5 ml of the osmoticum in 9-cm diameter Petri dishes, and replicated ten times. They were kept in the dark in an incubator at 25°C for 3 d, after which they were washed in running water and dried at room temperature. In the same way, seeds were imbibed in 15 mM KNO_3 for 24 h in the dark at 25°C for halo-priming, then rinsed quickly in water and dried. The control seeds were kept dry, and the hairs on the seed surface were not rubbed off in any of the treatments. The primed and control seeds were conditioned to same moisture content (*c*. 8%) by storage at 45% RH for 48 h.

Hydration and dehydration rates

Seeds were equilibrated over water in a desiccator at 25°C in wire-mesh bags and were weighed periodically for 48 h. There were three replicates for each treatment. The wire-mesh bags were removed from the desiccator, kept in the incubator at the same temperature and 45% RH for uniform dehydration to occur, and were weighed periodically for 48 h. The seeds were later dried in an oven at 110°C for 17 h. Based on the dry weight, seed moisture contents were calculated during the hydration–dehydration cycle.

Moisture dehydration–rehydration hysteresis loop

Seed lots of different treatments in wire-mesh bags in triplicate were kept in a humidified atmosphere over distilled water at 25°C for 48 h, and the initial weight was taken. Then, seeds were allowed to lose moisture

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at the same temperature and 45% RH, and weights of seeds, along with the mesh bag, were taken periodically for 24 h. Seeds were rehydrated in a humidified atmosphere at the same temperature, and the gain in weight was measured periodically. After treatment, the seeds were dried to constant weight at 110°C for 17 h. Based on this weight, the seed water contents [g water (g dry weight)$^{-1}$] were calculated during seed dehydration and rehydration cycles. Hysteresis loops were established following the procedure described by Moharir and Prakash (1995), after normalizing to equal curve heights (by dividing all values by initial moisture in the dehydration curve and by final moisture in the rehydration curve) for meaningful and effective comparison.

Moisture sorption isotherms

Seeds of the different treatments were equilibrated to constant weight at 25°C over various relative humidities maintained by saturated salt solutions, according to the method described by Vertucci and Roos (1993). In all, there were 18 salts, and concentrated sulphuric acid was used for the lowest humidity (1%). The equilibration time varied from 8 d for the highest RH to 30 d for the lowest humidity. Seed water content was measured as described above on two replicates.

Water-binding characteristics

The sorption data were fitted to the D'Arcy–Watt equation by a linear least square fit of each of the three regions of the isotherm (Vertucci and Leopold, 1984). The D'Arcy–Watt equation is:

$$\text{seed water content} = KK'(p/p^0)/[1 + K(p/p^0)] + c(p/p^0) + kk'(p/p^0)/[1 - k(p/p^0)]$$

where p/p^0 = the water activity (equilibrium relative humidity); K and K' relate to the affinity and number of strong binding sites; c is a measure of both affinity and number of weak binding sites; and k and k' relate to the affinity and number of multi–molecular binding sites.

The number of water-binding sites in seed tissues can be calculated from the derived D'Arcy–Watt coefficients. The numbers of strong, weak and multi-molecular binding sites are given as K'N/M, cN/Mp_0 and k'N/M, respectively, where N is Avogadro's number (6.023×10^{23}), M is the molecular weight of water and p_0 is the saturated vapour pressure of water at the specific temperature. The amounts of water associated with different water-binding sites in seed tissues were calculated and compared for control and primed seeds.

NMR relaxation measurements

Humidification over water

Seeds (about 220) of different treatments were equilibrated over water in wire-mesh bags in a desiccator at 25°C. The bags were weighed daily until the weight became almost constant. The wire-mesh bag with seeds was inserted into an NMR tube, corked to avoid dehydration and placed in the probe of a Bruker NMS 120 pulsed NMR spectrometer.

Imbibition in water

The primed and unprimed seeds were allowed to imbibe water in Petri dishes layered with wet filter papers at 25°C. After blotting off excess moisture, the wet weight of the seeds and NMR relaxation time (T_2) were measured periodically until all seeds had germinated. There were two replications.

The spin–spin relaxation time, T_2, was measured by the Carr–Purcell–Meiboom–Gill (CPMG) method (Snaar and Van As, 1992) at 20 MHz. Each measurement had the following settings: data points 150, pulse separation 0.5 ms, dummy echo 3 and scans 15. Gain was adjusted to maximize the signal to noise ratio. Data points were fitted using the built–in Expspel program with the single exponential decay observed in the CPMG sequence.

Components of NMR relaxation time measurement

In biological systems, including seeds, multi-exponential relaxation decay curves are generally observed, indicating the presence of three components with different relaxation times. According to Ratkovic (1987), three water components of seed systems can be identified with the spin–spin relaxation times T_{2a}, T_{2b} and T_{2c}. The component T_{2c} accounts for the hydration water of macromolecules and is tightly bound with least mobility; T_{2b} for the cytoplasmic bulk water with lower mobility; and T_{2a} for the extracellular free water with highest mobility. The three components of spin–spin relaxation times are given by the equation

$$M_t = C_a[\exp(-t/T_{2a})] + C_b[\exp(-t/T_{2b})] + C_c[\exp(-t/T_{2c})]$$

where C_a, C_b and C_c are related to the relative populations of the three components (Di Nola *et al.*, 1991; Brosio *et al.*, 1992). The components of spin–spin relaxation were analysed by least square fit analysis in the region of limits specified, based on t-values (x-axis) until the plotted curve showed a visible curvature change with a significant r^2, using a program written in C++ computer language. The general procedure of exponential peeling of curve

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decomposition was followed by locating the slowest relaxing fraction from the curve and subtracting this fraction from the observed data (Fig. 1) (Snaar and Van As, 1992; Krishnan *et al.*, 2004).

Results

Hydration and dehydration rates for primed and control seeds, kept in a humidified atmosphere over distilled water at 25°C, are shown in Fig. 2. The simple average rates of hydration and dehydration, obtained by dividing the moisture difference by the time period, were more rapid for control seeds compared to primed ones. However, the trend was different if the rate constants of water loss (β) were calculated using the expression $WC = WC_0 \exp(-\beta t)$, where WC_0 is the initial water content and t is the time of drying (Liang and Sun, 2002). The rate constants β for water loss for the first 6 h were 0.159, 0.170 and 0.212 h^{-1} for osmo-primed, halo-primed and control seeds, respectively. Similarly, when the rate constants for seed water gain were calculated using a positive exponential, the rates were 0.199, 0.188 and 0.185 h^{-1} for osmo-primed, halo-primed and control seeds, respectively, for the initial 6 h of hydration. The rate constants did not vary much among the different treatments, except for osmo-primed seeds during the initial 6-h period of hydration and dehydration. The area enclosed by the dehydration and rehydration cycle was also significantly lower for osmo-primed (348 units) and halo-primed seeds (374 units), compared to control seeds (468 units) (Fig. 3). Therefore, the difference in the dehydration and rehydration paths was much higher in control seeds compared to primed seeds.

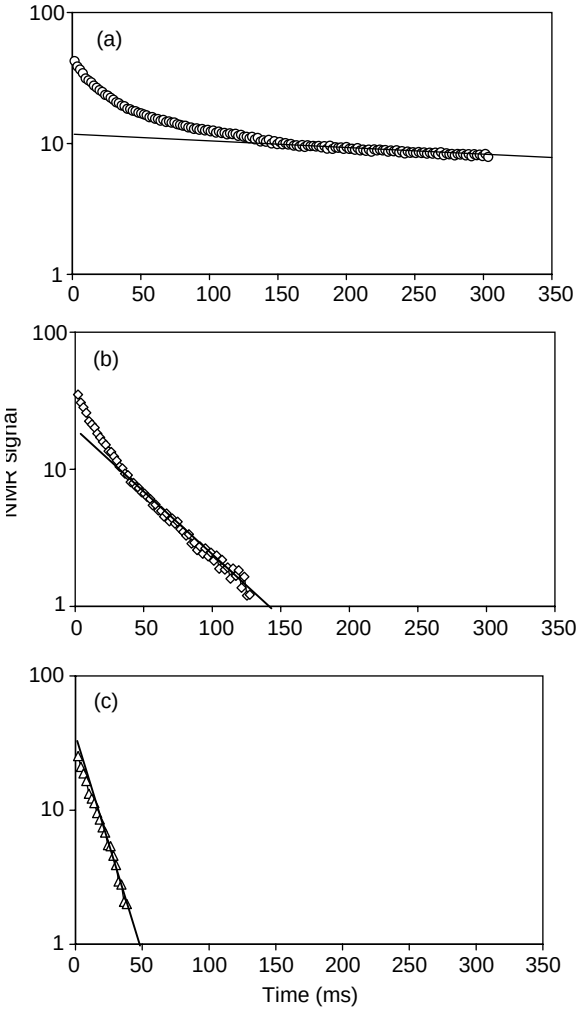

Figure 1. (a) Semi-logarithmic plot of the transverse magnetization decay curve. (b) Transverse magnetization decay of $T_{2b} + T_{2c}$ in the same sample. Curve (b) was obtained by subtracting from the curve (a) the extrapolated values of the slow-decaying component T_{2a} (line under the data points). (c) Transverse magnetization decay of T_{2c}. Curve (c) was obtained by subtracting from curve (b) the extrapolated value of the slow-decaying component of the curve (b) (line under the data points).

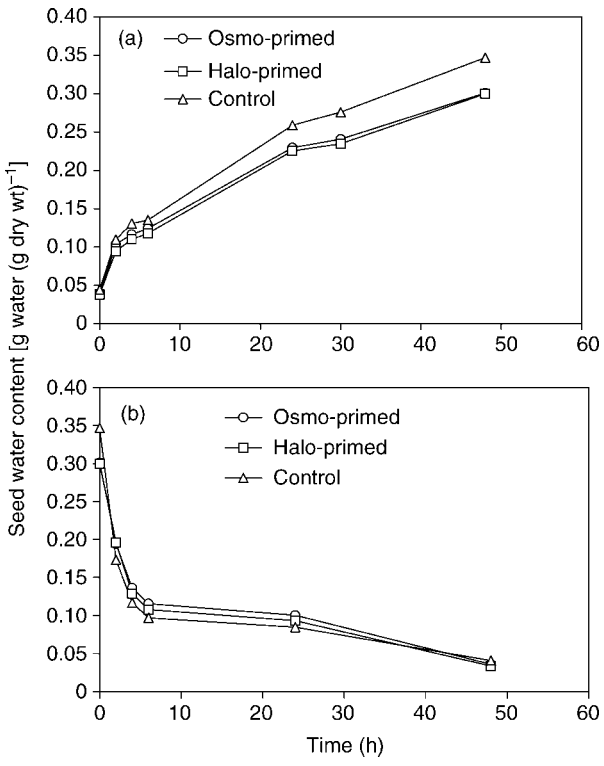

Figure 2. Changes in seed water content with time during (a) hydration at 100% relative humidity (RH) and (b) dehydration at 45% RH, 25°C, for primed and control seeds of tomato cv. Pusa Ruby. The standard errors varied between 2 and 5% of the mean measured values.

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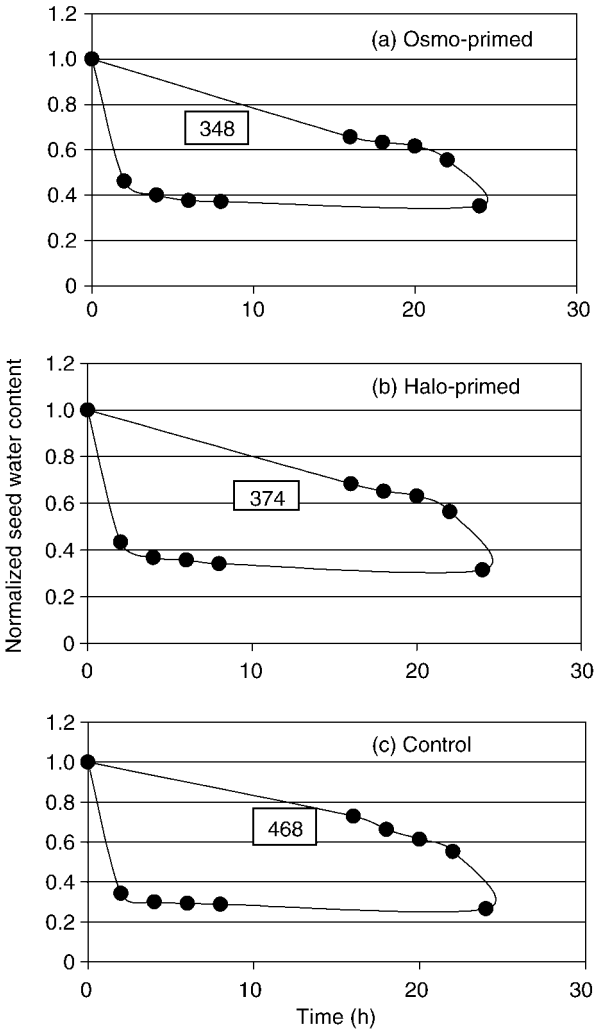

Figure 3. Normalized seed moisture dehydration–rehydration loops formed at 25°C for primed and control seeds of tomato cv. Pusa Ruby. Area enclosed, in mm^2, is indicated within the loop. Values were normalized by dividing dehydration data by the initial seed moisture content, and rehydration data by the final seed moisture content.

Water sorption isotherms of control and primed seeds developed at 25°C showed a typical reverse sigmoidal shape with three distinct regions of water binding (Fig. 4). Seeds of all treatments sorbed equally at high equilibrium water activities, but at lower water activities, the primed seeds sorbed more than control seeds. This indicated changes of water sorption properties after osmotic priming. Analysis of the isotherms using the D'Arcy–Watt equation showed that the strength of the attraction of the strong water-binding sites for water was greater for primed seeds as compared to unprimed controls (Table 1). Also, priming decreased the number of strong binding sites and increased the number of weak binding sites

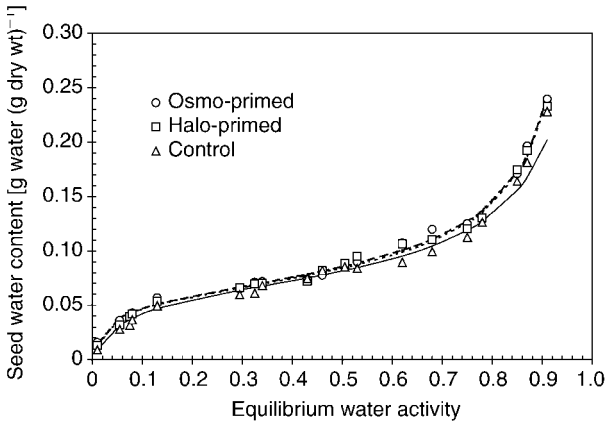

Figure 4. Water sorption isotherms of primed and control seeds of tomato cv. Pusa Ruby developed at 25°C. The minor broken line, major broken line and solid lines depict the calculated curves from D'Arcy parameters for osmo-primed, halo-primed and control seeds, respectively. The standard errors varied between 2 and 5% of the mean measured values.

(Table 2). The multi-molecular binding sites decreased only in osmo-primed seeds. The total number of water-binding sites was greater in control seeds. The amount of water associated with the three types of water-binding sites in seed tissues was also calculated using the D'Arcy–Watt model. Both osmo- and halo-priming increased the amount of water in the weak binding sites (Fig. 5). The amount of water associated

Table 1. Parameters calculated from application of D'Arcy–Watt equation to sorption isotherms constructed at 25°C for tomato seeds subjected to different priming treatments

	Sorption sites					
	Strong		Weak	Multi-molecular		
Treatments	K	K'	C	K	K'	r^2 fit
Osmo-priming	36.77	0.0581	0.171	0.889	0.0566	0.993
Halo-priming	30.30	0.0554	0.173	0.866	0.0625	0.997
Control	11.66	0.0736	0.163	0.859	0.0619	0.985

Table 2. The number of water-binding sites in primed seeds of tomato (sorption sites are calculated from derived D'Arcy–Watt coefficients)

	Sorption sites × 10^{12} [sites (g dry tissue)$^{-1}$]			
Treatment	Strong	Weak	Multi-molecular	Total
Osmo-priming	1.944	1.818	1.894	5.656
Halo-priming	1.854	1.840	2.091	5.785
Control	2.463	1.733	2.071	6.267

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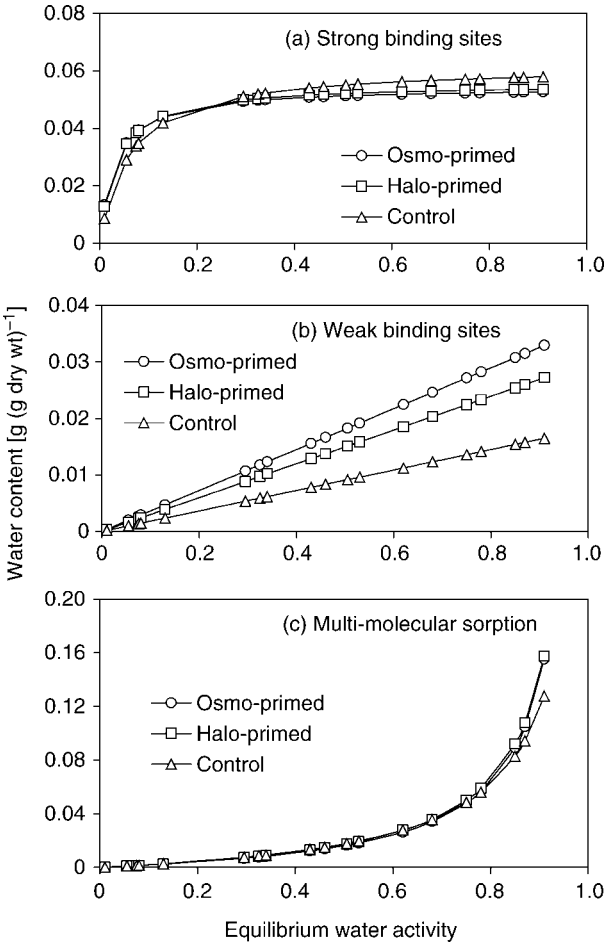

Figure 5. Water distribution among the three types of water-binding sites in control and primed seeds of tomato cv. Pusa Ruby. The amounts of water associated with different types of water-binding sites were calculated for seed tissues equilibrated with different relative humidities.

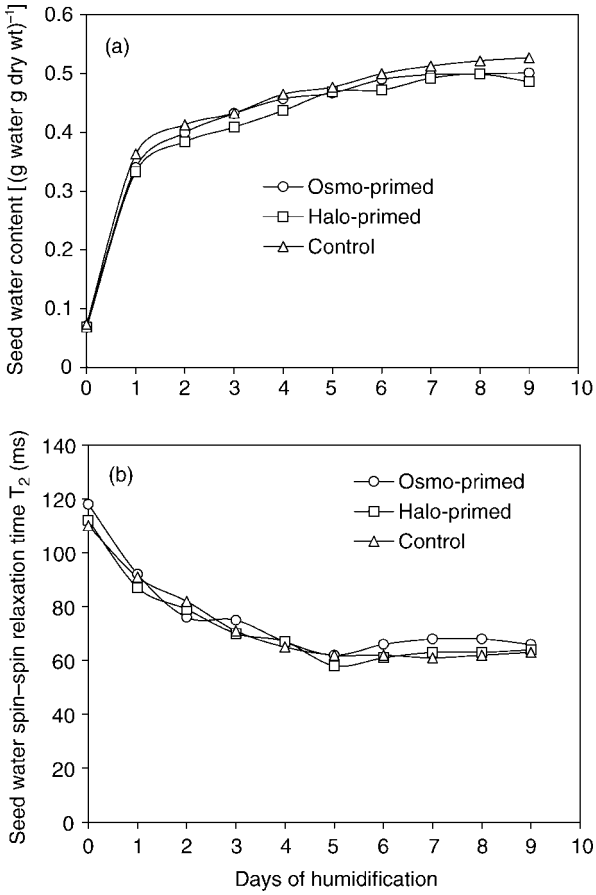

Figure 6. Changes in seed water contents and weighted average spin–spin relaxation times, T_2, of seed water with days of humidification at 100% relative humidity and 25°C in primed and control seeds of tomato cv. Pusa Ruby.

with multi-molecular sites was greater for primed seeds only at high humidities.

In the seed humidification experiment, the spin–spin relaxation time T_2 of seed water decreased initially up to 5 d, even though the seed water content increased during this period (Fig. 6). The analysis of the components of T_2 and the relative spin populations of the components revealed the redistribution of different water fractions with varying mobilities within the seed tissues (Figs 7 and 8). With an increase in humidification time, the long component of transverse relaxation time, T_{2a}, which corresponds to the relatively more mobile water fraction, decreased to a minimum value until 5 d of humidification, and then increased at different rates in the primed seeds. The control seeds continued to have low values. A similar trend was revealed by the next component, T_{2b}, of transverse relaxation time with relatively lower values (~60 ms), as it represents a less mobile water fraction. The component of transverse relaxation time (T_{2c} ~ 5 ms), which corresponds to the least mobile water in seed tissues, was detectable after 2 and 5 d of humidification in osmo- and halo-primed seeds, respectively. It was not detectable in control seeds even after 10 d of humidification. The relative spin populations of different components of relaxation times showed an interesting picture (Fig. 8). In dry seeds, the seed water was distributed between mobile and less mobile water, and the less mobile fraction was double that of the mobile fraction. The pattern of distribution was the same in primed and control seeds. With humidification of primed seeds, the relative population of protons in the least mobile fraction increased, and this happened earlier in osmo-primed seeds than halo-primed seeds. The spin population belonging to the least mobile fraction was not detected during 10 d of humidification in control

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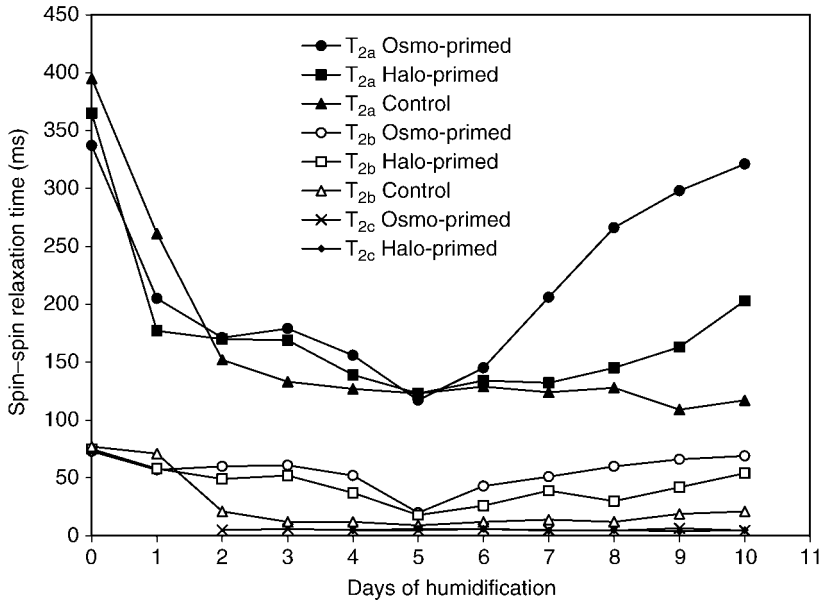

Figure 7. Changes in components of spin–spin relaxation time, T_2, of primed and control seeds of tomato cv. Pusa Ruby with days of humidification at 100% relative humidity and 25°C.

seeds, where the distribution was observed only between relatively more mobile and less mobile fractions. There was no radicle protrusion in any of the treatments until 10 d of humidification.

In the seed imbibition experiment, three distinct phases of water uptake, namely, rapid hydration (phase I), lag phase (phase II) and resumed hydration (phase III) were seen for all seeds, irrespective of their priming status. Initial rapid uptake was for 8 h, followed by a slow uptake until 50 h, when germination started at different rates in the three treatments (Fig. 9a). At 60 h of imbibition, radicle protrusion was seen in 80% of osmo-primed seeds, 40% of halo-primed seeds and only 20% of control seeds. During the initial period of imbibition, the seed water content was highest for control seeds and lowest for osmo-primed seeds. However, after 50 h, due to higher percentage germination, the seed water content of osmo-primed seeds became very high.

As during humidification, the seed water spin–spin relaxation time, T_2, decreased during the initial period of imbibition in all the seeds, remained stable until 50 h and then increased after germination (Fig. 9b). In dry seeds, it was not possible to resolve the least mobile component, and only two components, corresponding to more mobile and less mobile water fractions, were detected (Fig. 10). A third component corresponding to least mobile water appeared after 2 h of imbibition in primed seeds and was visible only after 6 h of imbibition in control seeds. Before the germination process started ($<$50 h), the relaxation times of three water fractions corresponding to three different magnetic environments decreased initially and then increased sharply after 40 h of imbibition. After the initiation of germination, again the magnetization of seed water protons could be resolved into only two components. The values of relaxation times of slow relaxing protons were between the values of T_{2a} and T_{2b} of seed water prior to germination. The values of fast relaxing second component of T_2 were between that of T_{2b} and T_{2c} of seed water prior to germination. The changes in the fractional population of water with different relaxation times revealed continuous reorganization of water in germinating seeds (Fig. 11). The relative proportion of the least mobile water fraction, which appeared after 2 h of imbibition in primed seeds, increased during initial periods of imbibition, and subsequently the fraction of intermediately mobile water increased. After germination only two fractions of water were present in all the treatments.

Discussion

The dehydration and rehydration steps of the priming procedure induce changes in seed internal morphological structure (Liu *et al.*, 1993) and the state of cell membranes (Khan *et al.*, 1978). The simple hydration and dehydration rates that did not take into account the variation in initial moisture contents were lower for primed seeds compared to unprimed controls

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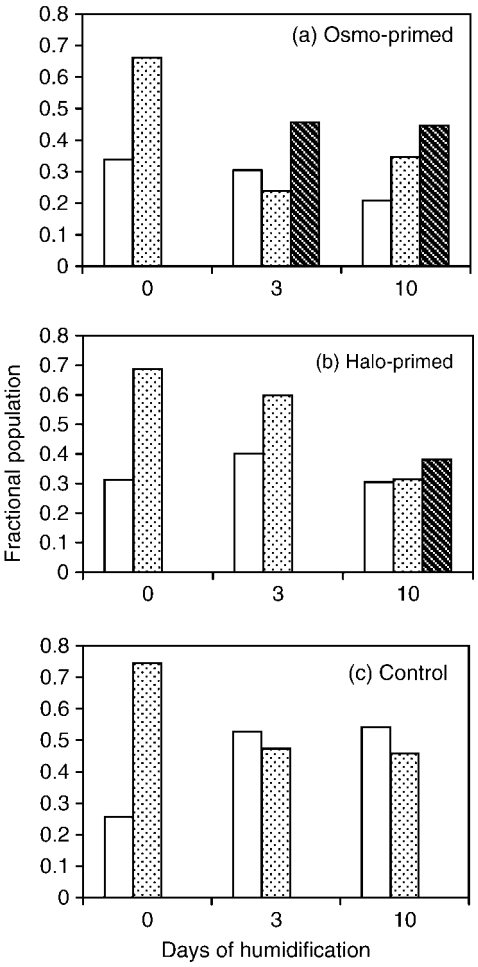

Figure 8. Fractional population of different water protons of varying mobilities with days of humidification for primed and control seeds of tomato cv. Pusa Ruby. The plain, dotted and crossed bars denote protons associated with more, less and least mobilities, respectively.

(Fig. 2). After compensating for the initial moisture contents, the calculated rate constants for water losses were lower for primed seeds, while rate constants for water gain were higher compared to control (Table 1). The dehydration–rehydration hysteresis enclosed significantly less area in primed seeds as compared to control seeds (Fig. 3). In comparison to control, seed water absorption was lower only in halo-primed seeds during humidification (Fig. 6a). But in the earlier water gain data (Fig. 2a), it was lower for both osmo- and halo-primed seeds. Perhaps the lower absolute values in the first experiment were caused by frequent opening of the desiccator in a dry environment and to use of different seed lots in the two experiments. A similar slow rate and extent of moisture absorption across the seed matrix was reported in osmo-, halo- and hydro-primed seeds of Asiatic carrot (Nagarajan *et al.*, 2003). The primed seeds have gone through one cycle of hydration and dehydration during priming treatment, and this could be the reason for the slow rate of absorption and desorption of moisture. Furthermore, in the seed imbibition experiment, the control seeds imbibed more water compared to primed seeds until phase II (Fig. 9a). This again points to the better control of the seed coat matrix for water absorption. Parera and Cantliffe (1991) demonstrated that sweetcorn seeds primed via solid matrix priming had less solute leakage and reduced water uptake rates during early imbibition than non-primed seeds.

Water sorption isotherms of primed and control seeds showed reversed sigmoid curves for water sorption isotherms (Fig. 4), but the primed seeds absorbed more moisture at both low and high water activities. This is contradictory to the results observed in primed *Ranunculus sceleratus* seeds (Probert *et al.*, 1991), but consistent with data for primed mung bean seeds in the absorption region above $0.2a_w$ (Sun *et al.*, 1997). The first study revealed a several-fold increase in mean longevity after osmotic priming, while the second reported a decrease in storage stability. The higher equilibrium moisture content of primed seeds, compared to unprimed seeds at similar RH, indicated the possibility of poor storability under conditions of high RH. It is interesting to note that while the rate of absorption was lower in primed seeds under humidified conditions, these seeds absorbed more moisture during equilibration over different relative humidities. The analysis of the isotherms using the D'Arcy–Watt equation showed that the affinity of strong binding sites (K) was greater for primed seeds compared to control seeds, while the affinities of multi-molecular binding sites were unaffected (Table 1). However, the numbers of strong binding sites (K') were greater for control seeds compared to primed seeds (Table 2). For a number of legume seeds, Vertucci and Leopold (1987) observed that the values of K were greater for axes compared to cotyledons, and those of K' were greater for cotyledons. Priming treatments modified the water-binding properties of the seed tissue and altered the number of various water-binding sites. Strong water-binding sites were exchanged for weak binding sites during priming. The total number of binding sites was greater in control seeds, which is difficult to explain from the sorption data. As compared with control seeds at the same water activity, primed seeds had more weakly bound water, less strongly bound water and nearly the same multi-molecular bound water (Fig. 5). In the case of mung bean seeds, Sun *et al.* (1997) have also shown that osmo-priming increased the number of weak binding sites. They have argued that the priming-induced water redistribution results in the depression of glass transition temperature (T_g), as water in weak

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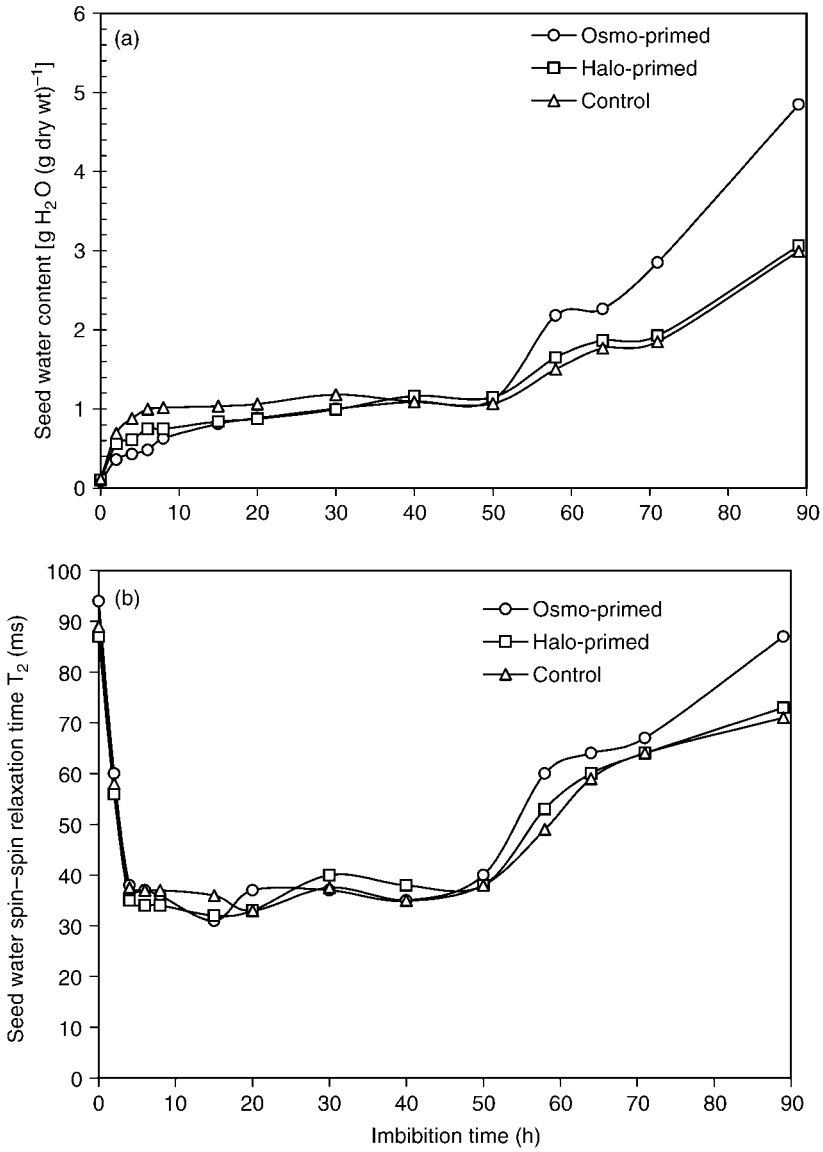

Figure 9. Changes in seed water content and weighted average spin–spin relaxation time, T_2, of seed water with hours of imbibition in water at 25°C for primed and control seeds of tomato cv. Pusa Ruby.

binding sites may serve as a plasticizer for intracellular glasses in seeds. The depression of T_g would enhance molecular mobility in seed tissue and, thereby, accelerate seed ageing (Sun and Leopold, 1994; Sun, 1997). However, they did not measure the molecular mobility of seed water directly. If we apply the same argument to our results, the increased germination percentages of primed seeds observed during imbibition might be due to increased molecular mobility of seed water.

During seed humidification, the average T_2 values were reduced for the first 5 d and then either increased slightly or remained constant (Fig. 6b). Similarly, seed water T_2 of the imbibed seeds fell sharply in phase I and remained relatively constant in phase II of absorption, and then increased after the germination process started in phase III (Fig. 9b). Such a trend has been reported in cowpea (Brosio *et al.*, 1992) and in wheat and soybean (Krishnan *et al.*, 2003) when the seeds were soaked in water. The reduction in seed water T_2 values, in spite of an increase in seed water content, can be explained on the basis of the reorganization of the different water fractions within the seed tissues. The relaxation time, T_2, is influenced by a delicate balance between total water content, macroscopic and microscopic distribution of water at different sites, macromolecular–water interactions and exchange (slow or fast) between different phases.

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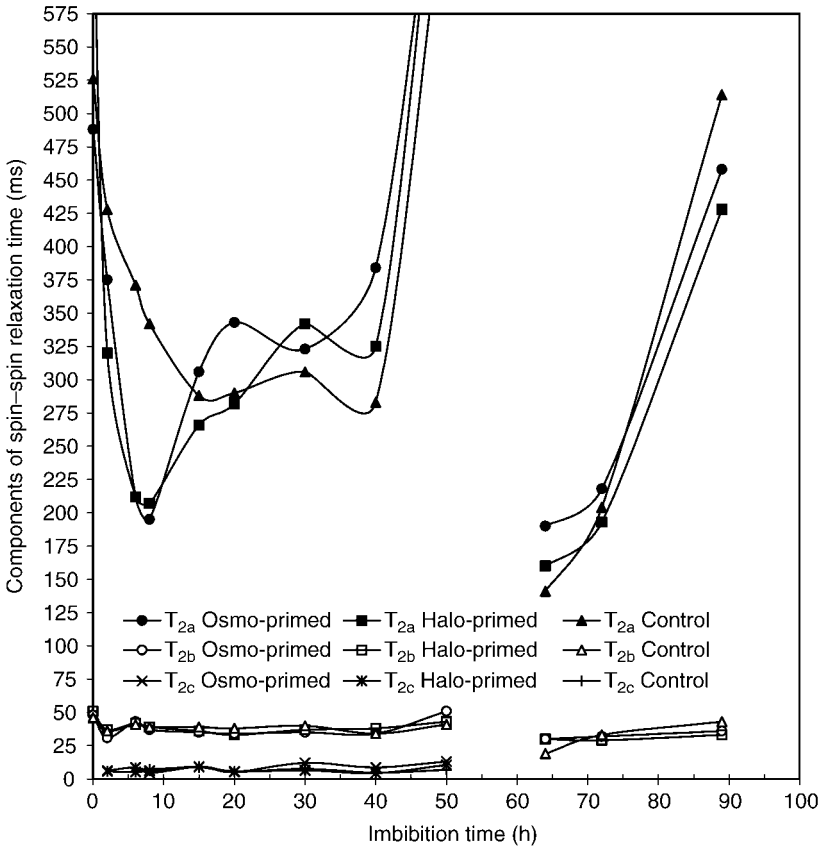

Figure 10. Changes in components of spin–spin relaxation time, T_2, of primed and control seeds of tomato cv. Pusa Ruby with hours of imbibition in water at 25°C.

With an increase in seed moisture content, either through humidification or through imbibition, there is rearrangement of cellular water. The decrease in relaxation times of the different components of water and relatively higher proportion of protons in the less mobile phase may be responsible for the initial decline in weighted average T_2.

The data on the components of transverse relaxation time of seed water during humidification and imbibition indicate the presence of three different populations of protons, each with a different magnetic environment that causes different relaxation rates in primed seeds (Figs 7, 8, 10, 11). Ishida *et al.* (1988) suggested that these three populations correspond to water molecules differing in mobility, such as extracellular free water, intracellular bulk water and solid or bound water. This suggestion has been used extensively by many researchers to explain tissue water partitioning (Di Nola *et al.*, 1988, 1991; Brosio *et al.*, 1993; Foucat *et al.*, 1993). The general conclusion is that extracellular free water is characterized by high relaxation times, intracellular bulk water by low relaxation times, and bound-structural water by very low relaxation times.

In humidified seeds, the appearance of the third population of protons with very low mobility ($T_{2c} \sim 5$ ms) in osmo- and halo-primed seeds after 3 and 5 d, respectively, is interesting. This component of water did not appear in control seeds even after 10 d of humidification. During imbibition, the third component appeared within 2 h in primed seeds and was noted only after 6 h of imbibition in control seeds. In our studies with carrot and wheat seeds, the least mobile component was present in dry seeds, as well as in humidified/imbibed seeds. As this fraction has been assigned to bound water, it is logical to expect its presence even in the dry seed. The possible explanation for not finding this fraction in tomato seeds in the present study can come from the morphology and physiology of tomato seeds. Proton relaxation is influenced by many factors, such as cell size and structure, the chemical composition and viscosity of the cellular contents and magnetic susceptibility. The tomato seed embryo possesses a much higher water-binding capacity than the

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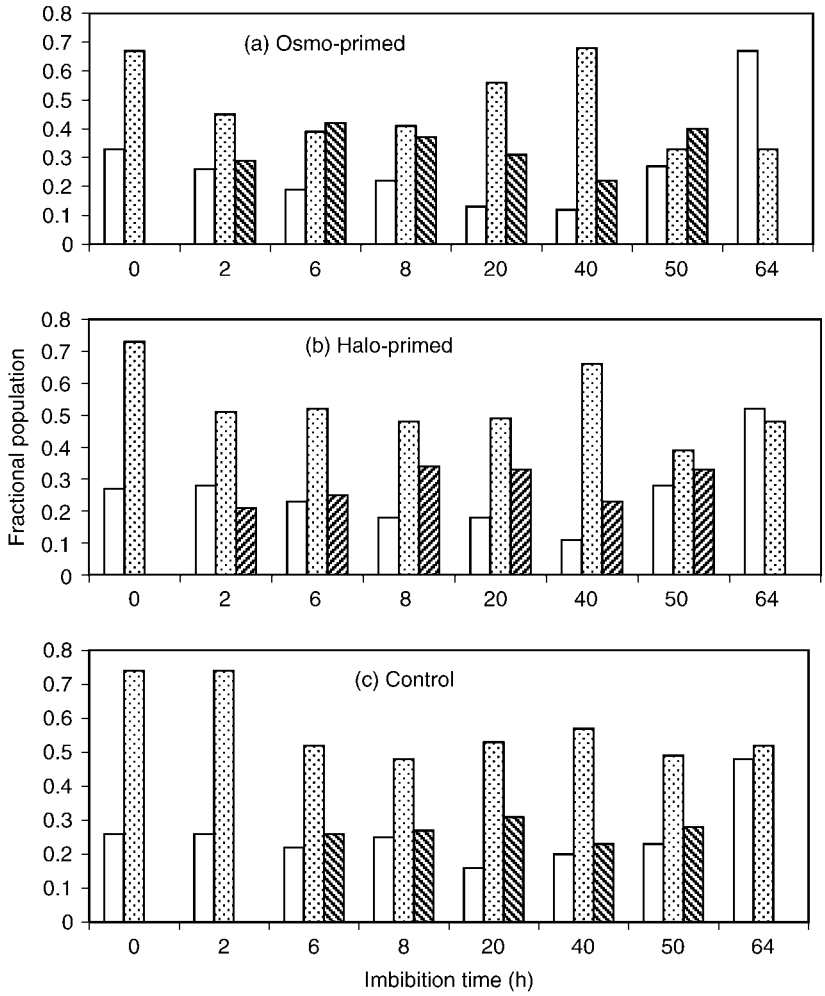

Figure 11. Fractional population of different water protons of varying mobilities with hours of imbibition in water at 25°C of primed and unprimed seeds of tomato cv. Pusa Ruby. The plain, dotted and crossed bars denote protons associated with more, less and least mobilities, respectively.

endosperm, while the embryo, which is enfolded by the endosperm, occupies about 60% of the total seed cross-sectional area (Liu *et al.*, 1993). It may be that the amount and mobility of the structurally bound water was so low that we could not detect it in our studies. Using solid-state ^{1}H-NMR, Sun *et al.* (2003) found no changes in the mobile water fraction of primed and control mung bean seeds between 0.06 and 0.20 g (g DW)$^{-1}$, and then a small increase in primed seeds beyond this seed moisture range. There was no difference in relaxation time T_2 between treatments, and a minimum value of T_2 was observed at a water content of around 0.11 g (g DW)$^{-1}$. These results cannot be compared directly with ours, as Sun *et al.* (2003) used powdered seeds conditioned to different moisture contents and have not gone beyond 0.24 g (g DW)$^{-1}$. Sun *et al.* (2003) calculated the relaxation time indirectly from the spectral width at half height, which is not a dynamic *in vivo* measurement of imbibition, as in our study.

In tomato seeds, osmo- and halo-priming significantly increased the free space surrounding the embryo (Liu *et al.*, 1993, 1996; Downie *et al.*, 1999; Pandita *et al.*, 2003). The increased air space was predicted to facilitate faster movement of water to the embryonic tissue. As the macromolecules of embryo and endosperm tissues became hydrated, a third component of hydration water with the least mobility appeared. In humidification, this water of hydration was detected only in primed seeds. Due to the much higher binding capacity, this fraction of protons showed very low mobility and accounted for nearly 40% of the proton population in primed seeds (Figs 8, 11). The synthesis and mobilization of proteins with seed hydration may be responsible for this water fraction. The proportions of hydration water and that

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of cytoplasmic bulk water, together, seem to play an important role in the germination process. Krishnan *et al.* (2004) reported complete disappearance of the bulk water fraction in non-viable wheat seeds during imbibition. In maize seeds, Bačic *et al.* (1992) have shown that the embryo water fraction had the shortest T_2 values and was essential for maintaining seed viability. During hydration after 50 h, the appearance of only two components of water with intermediate relaxation times (Fig. 10) indicates the exchange of water in different compartments by rearrangement of membrane permeability during germination and the formation of vacuoles in association with growth of the embryo. Buitink *et al.* (2000b) concluded that oligosaccharides in seeds do not affect the stability of the intracellular glassy state, and that reduced longevity after priming is not the result of increased molecular mobility in the cytoplasm. In their experiments with isolated whole embryos, rotational correlation time (which is inversely correlated with molecular mobility) of the polar spin probe 3-carboxyl-proxyl in the cytoplasm was monitored using saturation transfer electron paramagnetic resonance spectroscopy. We have used intact whole seeds and measured the transverse relaxation time of seed water protons, which is related to the translational and rotational motion of water molecules in homogeneous systems (Samuilov *et al.*, 1979). Higher relaxation times imply greater randomness of water molecules and better availability for participation in metabolic activities (Lewin, 1974). However, in heterogeneous systems such as seed tissues, the mobility of water molecules is modified by the effects of diffusion and morphology (Belton and Colquhoun, 1989).

We conclude that the better performance of primed seeds may be attributed to the modifications to seed water-binding properties and redistribution of seed water during imbibition, so as to increase macromolecular hydration water essential for germination-related metabolic activities.

Acknowledgement

We thank Dr Kent Bradford and the two anonymous referees for their comments and suggestions, which helped to improve the quality of this paper.

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Received 2 April 2004
accepted after revision 9 February 2005