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BRIEF COMMUNICATION

Characterisation of soybean and wheat seeds by nuclear magnetic resonance spectroscopy

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Abstract

The effects of equilibration under different air relative humidities (RH, 1 - 90 %) and temperatures (35 and 45 °C) on soybean (*Glycine max*) and wheat (*Triticum aestivum*) seeds were studied using different techniques. Seed moisture content, electrical conductivity (EC) of seed leachate and per cent seed germination were measured following standard procedures, and compared with nuclear magnetic resonance spin-spin relaxation time (T_2) measurements. Moisture contents of soybean and wheat seeds, following the reverse sigmoidal trend, were greater at 35 than at 45 °C at any particular RH. Changes in T_2 were related to the changes in germination percentage and leachate EC of both soybean and wheat seeds. Equilibrating soybean seeds at RH ≤ 11 % decreased germination percentage with corresponding decrease in T_2. On the contrary, EC of seed leachate increased. In wheat seeds equilibrated at 45 °C, T_2 was maximal at RH 5.5 %. T_2 declined in seeds equilibrated at high RH (> 80 %) together with low germination percentage.

Additional key words: *Glycine max*, NMR relaxation time (T_2), relative humidity, seed equilibration, temperature, *Triticum aestivum*.

Seeds are usually stored under variable relative humidity (RH) and temperature which affect seed water content (Fang *et al*. 1998) and germination. Nuclear magnetic resonance (NMR) spectroscopy is used to study water status and metabolic changes in many biological systems (Snarr and Van As 1992, Ratcliffe and Shachar-Hill 2001). Longitudinal and transverse relaxation behaviour of water protons have been used to describe the compartmentation and transport of water in tissues of plants and seeds (for review see Ratcliffe 1994). Mobile and less mobile water molecules can be distinguished by their different relaxation rates and their relative amounts can be calculated (Krishnan *et al*. 2003a,b). NMR techniques have been used successfully to study the pattern of seed hydration (Brosio *et al*. 1992, Fukuoka *et al*. 1994, Noda *et al*. 1998, Krishnan *et al*. 2003a,b). Compared to gravimetric methods, NMR techniques can provide more detailed information on the amount and state of water in seeds at different water content and RH during storage. In the present investigation, effects of different RH and temperatures on soybean and wheat seeds were studied using different techniques and compared them with NMR relaxation time measurements.

Freshly harvested soybean (*Glycine max* L. cv. JS 335) seeds were obtained from the National Research Centre for Soybean, Indore, India and wheat (*Triticum aestivum* L. cv. HD 2329) seeds were from the Regional Research Station, Indian Agricultural Research Institute, Karnal, India. Seeds were screened with the help of magnifying lens to remove those with visible damage. The protein, sugar, and moisture content in wheat and soybean, and oil in soybean seeds were estimated following the established procedures. Seed germination was tested according to the standard procedure (ISTA 1985).

Saturated salt solutions to obtain different RH values (1 - 90 %) at two temperature conditions (35 and 45 °C) were prepared by mixing different chemicals (H_2SO_4, NaOH, $LiCl_2$, $CaCl_2$, $MgCl_2$, K_2CO_3, $MgNO_3$, NH_4NO_3, NaCl, KCl, and KNO_3) at the required amounts with

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distilled water according to Vertucci and Roos (1993). A slurry level of 2 - 3mm above the salt crystals was maintained in all cases during the incubation period. Seeds (40 g each) in muslin bags were equilibrated over these saturated salt solutions at 35 and 45 °C. Seeds were weighed daily until they reached constant mass (about 14 - 21 d), at which point seeds were considered to be equilibrated and the following observations were made. The moisture content of seed species was determined, using triplicate seed samples (2 g each), dried in oven at 95 °C to constant mass (Walters 1998). Moisture content [%] was calculated on the dry mass basis. Germination tests were conducted by the standard method (ISTA 1985), in four replicates of 50 seeds each, at 25 °C. After 7 d, seedlings were evaluated as normal and abnormal, and the dead and hard seeds were counted. The germination percentage was recorded on the basis of normal seedlings only. Seeds (2 g) were soaked in 50 cm^3 of distilled water at 25 °C for 16 h. The electrical conductivity (EC) of seed leachates was measured using a digital conductivity meter (*Model 304*, *Systronics*, New Delhi, India).

For NMR relaxation measurements, the seed samples were collected and immediately placed into the NMR tubes of 10 mm diameter, corked to avoid dehydration and placed in the probe of a *Bruker NMS 120* pulsed NMR spectrometer (*Bruker Analytik GmbH*, Rheinstetten, Germany). The height of the sample was kept at around 2 cm. Each measurement was made with six replicates. The spin-spin relaxation or transverse relaxation time to study the decay of transverse component of magnetisation was measured by the Carr-Purcell-Meiboom-Gill method (Snarr and Van As 1992, Krishnan *et al*. 2003a,b) at 20 MHz. The settings of data points, pulse separation, dummy echo and scans were maintained for each measurement as described earlier (Krishnan *et al*. 2003a,b). The T_2 values were determined by measuring the exponential decay of the signal:

$$M_{xy}(\tau) = M_o \exp(-\tau/T_2)$$

where $M_{xy}(\tau)$ is the magnetisation at the echo time τ along xy, M_o is total magnetisation along xy just after 90 ° pulse applied, and T_2 is the spin-spin relaxation time. The gain was adjusted to maximise the ratio of signal to noise and the pulse sequence was chosen so that T_2 values obtained were not significantly different from that of the weighted average of the two components, derived by the analysis of decay curve.

Seeds were equilibrated both at 35 °C, which represents the near ambient temperature in the sub-tropical regions and at 45 °C, at which drying of seeds are generally performed. The best fit of moisture sorption isotherms for wheat and soybean seeds which give the equilibrium relationships between water content [%] and RH and, which are generated for each temperature separately, followed the reverse sigmoidal mode (Fig. 1*A*). The total amount of water absorbed by these seed species varied significantly. In spite of high protein content, soybean (contents: 35 % protein, 26 % starch and 20 % oil) attained lower equilibrium moisture content at a given humidity than wheat (contents: 64 % starch, 12 %

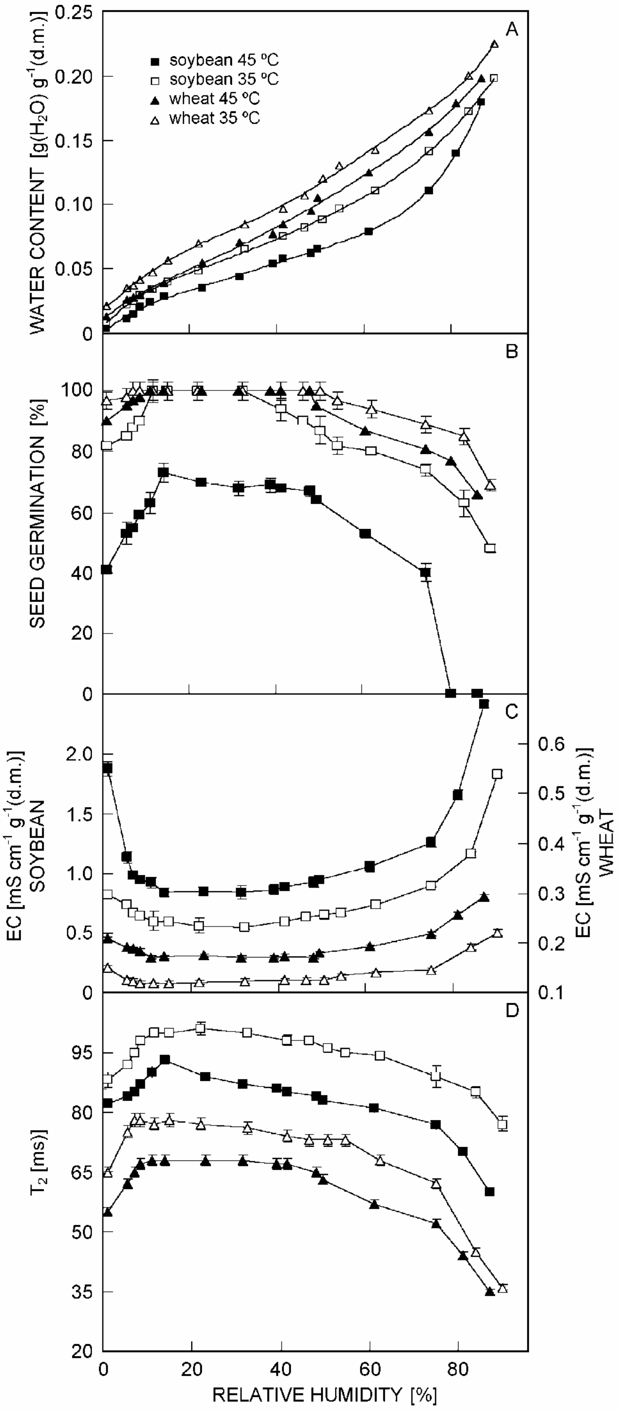

Fig. 1. *A* - Water content of seeds equilibrated at different RH and air temperature. *B* - Germination percentage of seeds aquilibrated at different RH and air temperature. *C* - Time cource of leachate conductivity of seeds aquilibrated at different RH and air temperature. *D* - Spin-spin relation time (T_2) of seeds aquilibrated at different RH and air temperature.

protein and 1.5 % oil). Equilibrium moisture contents of seeds in both species decreased with decrease in RH of air.

Under any given combination of RH and temperature, soybean had lower germination percentages than wheat (Fig. 1*B*). The germination percentage of both species decreased at very high and low RH, both at 35 and 45 °C. But, the loss in viability of both species was greater at 45 than at 35 °C. The highest germination percentage of soybean (near 100 %) was when seeds were equilibrated between 11.5 to 32.5 % RH at 35 °C. At RH ≥ 40 %, the loss of germination in soybean seeds was very rapid. A similar decline was also noted in soybean at very low humidity. At about 1 % RH, soybean seeds had germination percentage of 80 and 40 %, respectively, when equilibrated at 35 and 45 °C. Wheat seeds had 100 % germination when equilibrated at 45 °C between RH 11 - 41 %. Near 100 % germination percentage was also observed in wheat seeds at 35 °C and RH between 7.0 and 50.5 %. The decline in germination percentage was observed when these seeds were equilibrated above or below this range of RH, either at 35 or 45 °C.

The EC of soybean leachate was higher than that of wheat (Fig. 1*C*). In general, the leachates of both seed species equilibrated at 45 °C had higher EC than that of those equilibrated at 35 °C. Soybean seeds, in general, showed an increase in leachate EC when equilibrated at RH ≥ 70 % and, also when equilibrated at RH ≤ 11 %. Similar trends were observed with wheat. The T_2 measurements of seeds equilibrated at different RH and temperature conditions showed that, at a particular RH, T_2 values were lower at 45 than at 35 °C (Fig. 1*D*). In general, the T_2 values increased with a decrease in RH. Soybean seeds had higher T_2 than wheat seeds. But, the variations within T_2 values of soybean seeds were lesser, when seeds were equilibrated at RH between 12 and 32.5 % and both at 35 and at 45 °C. In wheat seeds, the variation in T_2 ranged from about 80 to 30 ms. As observed with soybean seeds, T_2 of wheat increased with decreases in RH. At 45 °C, there was a decline in T_2 when wheat seeds were equilibrated at RH ≤ 8.5 %. However, such decline was observed only at RH ≤ 3.5 %. There was a sharp decline in T_2 of wheat species when seeds were equilibrated at RH ≥ 51 % and 45 °C and those at RH ≥ 55 % and 35 °C, respectively.

In the present experiments, the moisture isotherms of soybean and wheat seeds equilibrated at two different temperatures (35 and 45 °C) over different RH (1 - 90 %) exhibited the reverse sigmoidal shape, which could be attributed to the multi-molecular adsorption (Fang *et al*. 1998). With increase in RH, the moisture contents of both seed species increase as the availability of water for seed equilibration increases. At a particular RH, the seed moisture content of both species increased under low equilibration temperature and this result corroborated the report of Buitnik *et al*. (1998). Low moisture retention capacity of soybean seeds, compared to wheat seeds at similar RH and temperature, could be attributed to higher lipid contents of soybean. The critical seed moisture content which varies among species has an inverse relationship with the lipid content of seeds. Our study showed further that, at either temperature, soybean seeds had less germination percentage at very high as well as low RH. Such loss in germination of soybean at very low RH contradicts the general notion that there is a continuous benefit to seed longevity with progressive drying. Though traditional wisdom suggests that dry seeds survive longer than moist seeds, seed life span could not be prolonged indefinitely by progressively drying seeds (Ellis *et al*. 1989).

NMR relaxation time measurements (T_2), which represent the mobility of water molecules, are modified by morphology and chemical constituents of seed tissues. Our data suggested that there were large differences in T_2 values of soybean and wheat seeds, equilibrated even at similar moisture content. The variations in T_2 measurements under the imposed equilibration conditions suggest that the T_2 values are influenced by many factors such as proton relaxation, cell size and structure, the chemical composition and viscosity of the cellular contents and magnetic susceptibility. The delicate balance between the total water content, macroscopic and microscopic distribution of water in different phases/sites, macromolecular-water interactions and exchange (slow or fast) between different water phases determine the NMR relaxation times of seed tissue water. Our results also showed that there exists a definite relationship between the T_2 measurements and the germinability of seed species under seed drying conditions. Starchy seeds like wheat exhibited gradual uptake and release of moisture at high as well as low RH while soybean with its high protein content had rapid moisture uptake but slow release, probably due to its oil rich constitution. In both seed species, T_2 showed decreasing trend in seeds equilibrated at sub- and supra-optimum RH, indicative of considerable disruption of organized cell structures. High temperature or extreme (low or high) moisture conditions are known to begin the rupture of cell membrane and change the properties of macromolecular structure in seeds, which further affect water status in their vicinity (Millard *et al*. 1996). These irreversible macromolecular structural changes can advance the seeds to nonviable state (Walters 1998). The NMR relaxation time measurements are extremely useful for the characterization of seed species during storage and in turn, the viability of seed species.

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