

## The Relationship Between Water Sorption Properties and Desiccation Tolerance in Contrasting Seeds

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### ABSTRACT

The desiccation tolerance in two distinctly different seeds viz., okra (resistant to desiccation) and papaya (sensitive to desiccation) was studied by constructing water sorption isotherms by equilibrating the seeds over different salt solutions at 30°C. The seeds conditioned to varying moisture levels were tested for their germination/viability. The seed viability fell sharply when the seed moisture content was below 7% in papaya and below 4% in okra. The analysis of the isotherms using D'Arcy-watt equation revealed that in okra seeds, the number and affinity of strong binding sites were more compared to papaya seeds. On other hand, the number of weak binding sites was more in papaya while the multi molecular binding sites remained same for both type of seeds. The pressure-volume isotherms were used to estimate matrix bound water in both kinds of seeds. It was estimated to be 4.4% in okra and 6.8% in papaya seeds. Hence, the loss of viability of seeds equilibrated to low relative humidities may be due to the removal of matrix water, which is essential to maintain the seed structure. We may conclude that desiccation tolerant and intolerant species differ in their seed water binding characteristics and in their proportion of matrix bound water.

**Key words:** Sorption isotherms, desiccation tolerance, seed water binding, okra and papaya seeds.

### Introduction

Seeds can be divided into at least three categories according to their ability to tolerate drying and their storage behaviour. The mechanism of desiccation sensitivity and seed longevity in different species remains unclear. Orthodox seeds like okra can tolerate high desiccation and show predictable storage behaviour. Seeds like papaya come under intermediate type and are susceptible to desiccation and store poorly. These performances are governed by the chemical constitution of the particular seed and its water binding properties. The cellular water plays an important role in protecting the macromolecules from the deleterious effects of desiccation (Vertucci and Leopold, 1987). Also, the status of water and its binding play an important role in its storage behaviour. 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 seed possess 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 nature of the binding site. They may be described as water which 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 3). Desiccation intolerance depends on the structure associated matrix bound water, the removal of which reduces seed survival (Pritchard and Manger, 1998). In the present study, an attempt has been made to explain the contrasting desiccation tolerance characteristics of okra and papaya seeds on the basis of their seed water status, their binding and the amount of matrix bound water using thermodynamic principles.

### Materials and Methods

**Seed material:** Seeds of okra (*Abelmoschus esculentus* L) cv. A-4 were obtained from fresh harvest of 1999-2000 season. Seeds of papaya (*Carica papaya*) cv. Pusa Giant were extracted from well ripe fruits in the year 2000. After removal of sarcotesta by washing, the seeds were shade dried.

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Both type of seeds were kept in controlled storage (15°C and 30% RH) for three months. Seeds were taken out of storage, conditioned to room temperature and used for experimentation.

**Moisture sorption isotherms:** The seeds of the different treatments were equilibrated to constant weight at 30°C over various relative humidities maintained by saturated salt solutions according to the method described by Vertucci and Roos (1993). In total, there were 10 salts and conc. sulphuric acid was used for very low humidity (1%). Seed water content was expressed as gram water per gram dry weight. There were three replications.

**Water binding characteristics:** The sorption data was fitted to 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 given as

$$\text{Seed water content} = \frac{KK' (p/p^0)}{\{1 + K (p/p^0)\}} + \frac{c (p/p^0)}{1 + k (p/p^0)}$$

$p/p^0$  = the water activity ( equilibrium relative humidity )

K and K' are the affinity and number of strong binding sites

c is a measure of both affinity and number of weak water-binding sites

k and k' relates 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 the Avogadro's number ( $6.023 \times 10^{23}$ ), M is the molecular weight of water and  $p^0$  is the saturated vapour pressure of water at the specific temperature. The amount of water associated with different water binding site in seed tissues were calculated and compared for the two crop species.

**Seed germination:** The germination percent of the seeds equilibrated to different humidities were also determined by following the ISTA procedure (ISTA, 1985). Seeds of okra and papaya were kept in the germinators at 25 and 30°C respectively and

final count taken after 14 and 30 days, respectively.

**Determination of matrix water:** The relation between the reciprocal of the water potential of the salt solutions and the seed relative water content were plotted. The matrix bound water fraction was determined by linear regression and extrapolation of the data for 0.20 to 0.80 RWC to the X-axis intercept (Pritchard and Manger, 1998). The water potential and Relative water content (RWC) of the seeds were calculated as given below:

Water activity of the seed  $a_w$  when equilibrated in a particular RH = RH/100,

$$\text{Chemical potential of seeds } \mu_w = \mu_w^0 + RT \ln (a_w) = \mu_w^0 + RT \ln (RH/100)$$

Where  $\mu_w^0$  is the chemical potential of standard i.e. Pure water.

Water potential  $\Psi_w$  is basic to study of water relations in tissues. It is actually a measure of pressure, which can be derived from the chemical potential, mw by dividing it by molar volume of water (v) at 20°C and 1 atm. (18.048 ml/mol) and by setting the value of  $\mu_w^0/v = 0$ . Therefore, water potential can be calculated as  $\Psi_w = RT \ln (a_w)/v = RT \ln (RH/100)/v$  (Noble, 1970). The RWC is calculated as the ratio of the water content at any equilibrium RH to the maximum water content at the highest RH.

## Results and Discussion

The equilibrium RH-moisture isotherms of okra and papaya seeds showed a reverse sigmoidal shape and indicated the three regions of water binding (Fig. 1).

Region I (0-20% RH) corresponds to mono layer adsorption, region II (20- 75% RH) represents multi-layer adsorption and region III (>75% RH) refers to free water held in capillaries (Pabis *et al.*, 1998). Okra seed tissues adsorbed less water than papaya seeds at RH less than 40% ( $a_w < 0.4$ ); however, they adsorbed more water than papaya at higher water activities. This indicated changes in water sorption properties of the two seed types. The three regions observed in the isotherm belong to strong, weak water binding sites and multimolecular sorption sites.(Vertucci and Leopold, 1987). Water in the strong water-binding sites is associated with

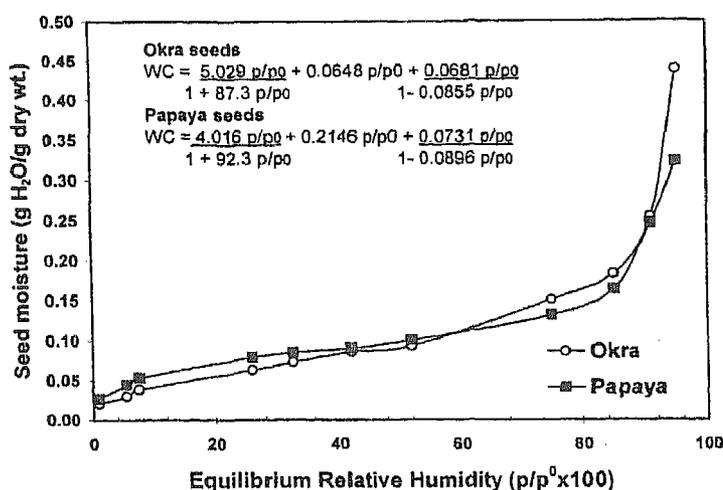


Fig. 1. Water sorption isotherms for okra and papaya seeds. Seed tissues were equilibrated over saturated salt solutions at 30°C. Data were analysed with the D'Arcy-Watt sorption model (equations and curves) to calculate the water distribution among three types of water-binding sites in seed tissues

macromolecular surfaces by ionic bonding. This type of water is tightly bound and has very negative differential enthalpy. Water in the weak water binding sites is loosely bound by hydrogen bonding. The analysis of the isotherms using D'Arcy-Watt equation revealed that the number of strong water binding sites were more in Okra seed compared to papaya (Table 1). Similarly the numbers of weak binding sites were significantly lower in okra compared to papaya. Also, it was interesting to note that the total number of binding sites per gram dry weight were significantly higher in papaya than that of okra. The amounts of water associated with three types of water binding sites in seed tissues were also calculated using D'Arcy-Watt model. In comparison to okra, the amount of water associated with strong binding sites was less and that of weak binding sites was more in okra while there was very little

difference in the multi-molecular binding sites among the species (Fig 2 a,c). It may be inferred that the reduced level of strong water binding sites and increased levels of weak binding sites were responsible for the poor storability of papaya and its desiccation sensitivity. In partially germinated soybean axes, which were desiccation intolerant, similar loss in strong water binding sites and gain in weak binding sites was observed (Vertucci and Leopold, 1987). Sun *et al.* (1997) have shown partial loss of desiccation tolerance in osmotically primed seeds of mung bean with poor storage stability. Priming was found to modify the sorption properties and significantly increased the amount of water associated with weak water binding sites.

The relationship between equilibrium moisture content and germination percent is given in Fig. 3

Table 1. The number of water-binding sites in okra and papaya seeds. (The number of sorption sites are calculated from derived D'Arcy-Watt coefficients)

Material	Sorption sites (sites/g dry tissue)			
	Strong	Weak	Multimolecular	Total
Okra seeds	$1.994 \times 10^{11}$	$2.168 \times 10^{11}$	$2.860 \times 10^{11}$	$7.022 \times 10^{11}$
Papaya seeds	$1.456 \times 10^{11}$	$7.181 \times 10^{11}$	$2.993 \times 10^{11}$	$11.630 \times 10^{11}$

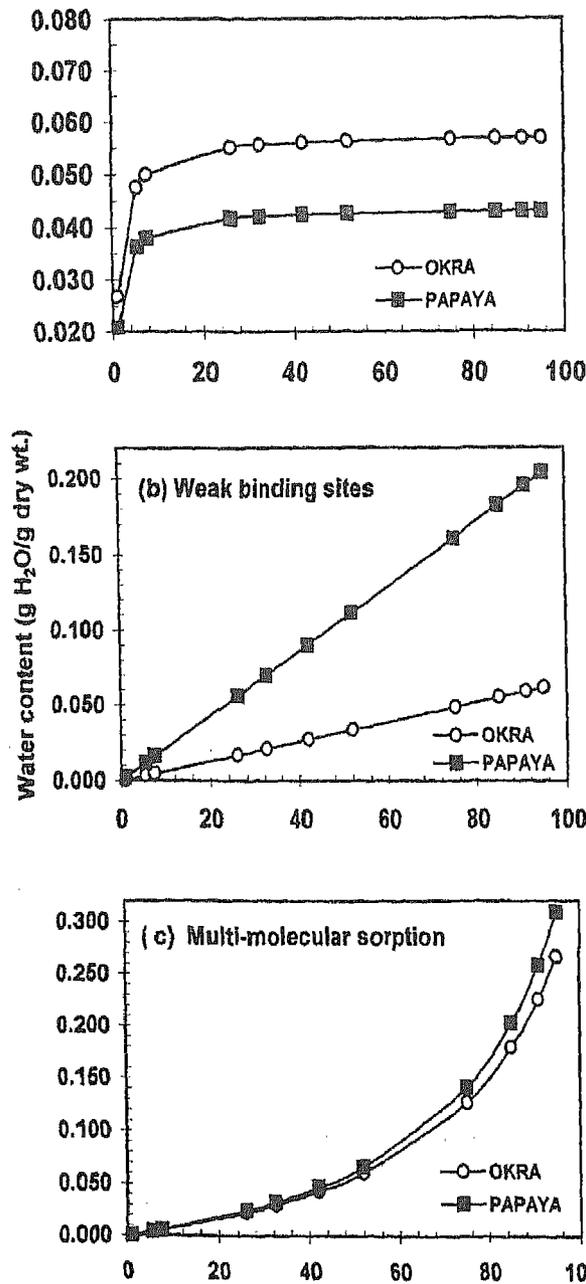


Fig. 2. Water distribution among three types of water-binding sites in okra and papaya seeds. The amounts of water associated with different types of water-binding sites were calculated for seed tissues for tissues equilibrated with different relative humidities (a, b and c)

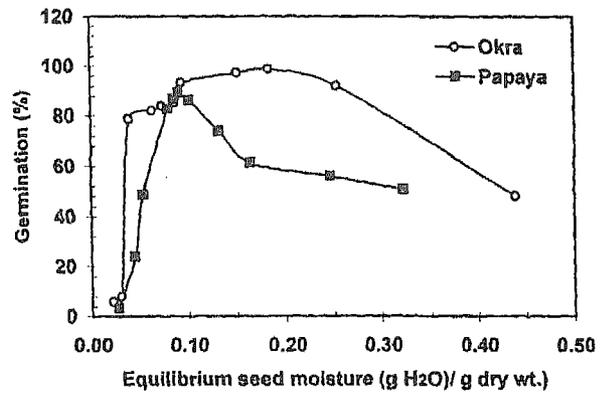


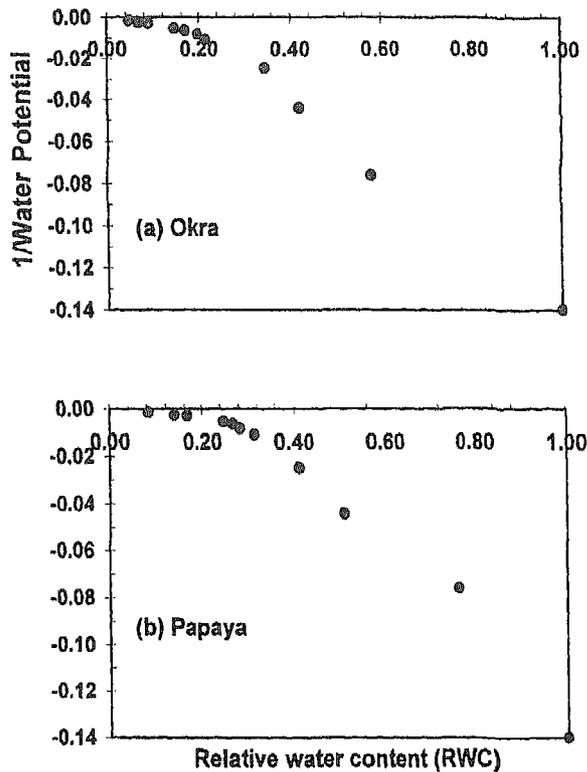
Fig. 3. The relationship between equilibrium seed moisture content and germination percent in okra and papaya seeds. The standard error varied between 1-4 and 3-5% for seed moisture and germination percent respectively

for okra and papaya seeds. The viability was lost at both ends of the curve i.e., when equilibrated over very high and very low relative humidities. Similar results were reported in *Hibiscus cannabinus* L seeds, where damage due to free radicals were dependent on hydration; seeds within first and third hydration regions were damaged by free radical producing radiation, while seeds in the second hydration region was somehow protected (Mahama and Silvy, 1982). The low germination of okra seeds below 4% seed moisture and that of papaya below 7% seed moisture could be due to imbibitional injury caused by rapid uptake of water by dry seeds (Powell and Mathews, 1978). It also clearly indicated the desiccation intolerance of papaya seeds and the critical limits of seed drying in okra and papaya seeds.

Table 2 gives the matrix water estimated by linear regression and extrapolation of RWC to X-axis intercept ( $R_0$ ) in the graph between reciprocal of seed water potential and RWC (Fig. 4). The amount of matrix water was 4.4 % okra and 6.8 % for papaya, which gave the critical limits of seed water content for both types of seeds. If dried below this limit, the viability would be lost, as this water is required to protect the seed matrix from denaturation. It can be inferred that the sudden decline in germination of okra and papaya seeds below 7 and 4% seed moisture were due to the removal of matrix water and collapse of the seed structure.

**Table 2.** Estimation of matrix bound water for okra and papaya seeds

Material	Seed moisture at saturation (%)	Extrapolated value of RWC	Amount of Matrix water (%)
Okra seeds	43.9	0.100	4.389
Papaya seeds	32.3	0.210	6.775



**Fig. 4.** The relationship between the reciprocal of water potential and relative water content (RWC) of (a) okra and (b) papaya seeds. The matrix bound water fraction ( $R_0$ ) was estimated by linear regression and extrapolation of the data for 0.20 to 0.80 RWC to the X-axis intercept

The desiccation tolerant (okra) and susceptible (papaya) differs in their seed water binding characteristics. In okra, relatively more number of strong binding sites and the large amount of water associated with it induce desiccation tolerance. Higher amount of water with weak binding makes papaya susceptible to desiccation and leads to poor storability. The removal of structure-associated matrix water is responsible for the loss of seed germination due to desiccation, which occurs at higher seed water content in papaya than in okra.

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