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Influence of Seed Moisture Content and Leakage on Germination and Viability in *Pisum sativum* L. Seeds

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Abstract: The involvement of seed coat integrity, imbibition and solute leakage in effect to the moisture content ranging from 10.3 to 4.7% by treating with the temperature ranging from 35 to 55°C of *Pisum sativum* seeds during germination was evaluated. Pea seeds with below 7% moisture content show reduced viability, increased electroconductivity and damage to the testa. The appearance of hardseededness is also an important observation below the moisture content of 7%. The loss of viability of seeds after treatment at high temperature was related to the loss of membrane integrity and desiccation.

Key words: Desiccation, electroconductivity, *Pisum sativum*, seed germination, steep water

INTRODUCTION

The several aspects of seed germination include moisture content and viability (Ellis and Roberts, 1982; Ellis *et al.*, 1989, 1990), leachate conductivity (Powell and Matthews, 1981), soaking injury (Rowland and Gusta, 1977) and effect of temperature on germination (Munro *et al.*, 2004). Poor survival of seeds and seedlings and high leakage of organic and inorganic substances during imbibition are commonly observed results of imbibition and chilling stress. The conductivity of leachate was significantly related with germination and was clearly higher for samples with lower germination (Thornton *et al.*, 1990; Doijode, 1988).

The leakage of solute from seeds during imbibition is due to reorganization or repair of membrane components (Simon, 1984) and conformational changes occurring in cell membranes (Vertucci and Leopold, 1987; Senarathna *et al.*, 1988; Bewley, 1997) upon drying of seeds. The membrane phospholipid component forms a gel phase during maturation drying (Crowe and Crowe, 1992). When the seeds were subjected to higher temperature progressive removal of water occurs. Bound water, associated with macromolecules is lost first resulting in structural and functional deterioration of seeds (Bewley and Black, 1994) and changes in embryo drying rates are associated with changes in seed quality and that slow embryo drying rates to threshold levels may be crucial to acquire the ability to withstand higher drying rates without detrimental effects on seed quality (Leobigildo and Burris, 2002).

The present study was directed to evaluate the seed vigor of *Pisum sativum* seeds by accelerated aging tests, but the information on seed physiological potential seems to be incomplete. Consequently, the main objective of this research was to investigate the relationship between the viability, electrolytic leakage and germination of seeds under different temperature.

MATERIALS AND METHODS

The study was conducted at Physiology and Biochemistry division, Department of Botany, University of Calicut, Kerala, India in 2005.

Seeds of *Pisum sativum* L. cv. Bonni villa were used for the present study. The seeds were purchased from National seed corporation, Kerala, India. The seeds were conditioned to different moisture content ranging from 10.3 to 4.7% by treating with the temperature ranging from 35 to 55°C in hot air oven. The seeds of each level of moisture content were tested for mechanical damage and the damaged one was eliminated. The air dried seeds were treated as control. The seed moisture content was determined using hot air oven method (ISTA, 2005).

Germination studies, at room temperature (30±2°C) and in the refrigerator (3±1°C), of control and conditioned seeds were carried out in sterilized Petri dishes lined with Whatman No.1 filter paper. For germination studies under cold the seeds of all the samples were made into two lots. The first lot was subjected to imbibe water at room temperature for 4-5 h and after removing the excess water content was kept under cold condition and the second lot made to imbibe and germinate under cold.

For conductivity test, 50 seeds of each sample in 5 replicates were weighed, soaked in 250 mL redistilled water and incubated at 25°C. The electrical conductivity of the seed steep water was determined after 1, 2, 3, up to

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11 h of imbibition using Toshniwale TCM 15 Auto-ranging conductivity and TDS meter and expressed in μmho/cm/gram of seeds.

The integrity of the seed coat was examined through the anatomical studies of the testa. The seeds of control and conditioned at 50°C was germinated at the room temperature and after 24 h, testa were separated and collected for anatomical studies. The samples were fixed in FAA and treated with 25% hydrofluric acid for softening. The samples were dehydrated through alcohol-TBA series and embedded in paraffin wax. Using a rotary microtome the individual blocks were cut at 11 μ thickness. The sections were deparaffinised, hydrated and stained with a mixture of Azure II-Methylene blue at 60°C for 1 min (Khasim, 2002). The photographs of the stained sections were taken using Nikon Model Eclipse E-500 Image analyzer.

RESULTS

The seeds of *Pisum sativum* L. on treatment with temperature ranging from 35-55°C, gradual reduction in moisture content were observed (Table 1). Along with this reduction in moisture content the germination percentage under room temperature was also decreased. When the moisture content of the seeds was below 7% the hard seeds were observed (Table 1). In order to test the viability of the hard seeds, they were subjected to mechanical scarification by making a small incision on the testa with blade, all of them were imbibed but none of them were germinated.

The germinability tests of these seeds, after 4-5 h of imbibition under room temperature, when conducted under cold condition there was significant increase in the germination percentage. But when the imbibition and the germination were conducted under cold the seeds with the moisture content above 10% showed higher germination percentage, whereas the others were non-viable (Table 1). These results shows that at certain level the seed moisture content and the germination temperature were stressful to the viability of *P. sativum* seeds.

Leakage from over dried conditioned seeds of *P. sativum* was significantly higher than from control and seeds treated with 35 and 40°C (Fig. 1). A significant

Table 1: Effect of temperature on moisture content and germination in room temperature and cold condition

Temperature of treatment (°C)	Moisture content (%)	Germination % in room temperature	Germination % under cold	Imbibition and germination under cold	Hard seed (%)
Control	12.3±0.70	100	100	80	0
35	10.3±0.23	100	100	80	0
40	9.2±0.51	100	100	43	0
45	7.7±0.54	93	100	8	0
50	6.5±0.88	78	93	8	7
55	4.75±1.80	60	33	0	7

decrease in the viability was affected with increased leachate from the seeds. Germination was lowest in seeds having the highest electroconductivity (Fig. 1).

The testa of control seeds (Fig. 2a) was intact when compared to the seeds treated at 50°C (Fig. 2b).

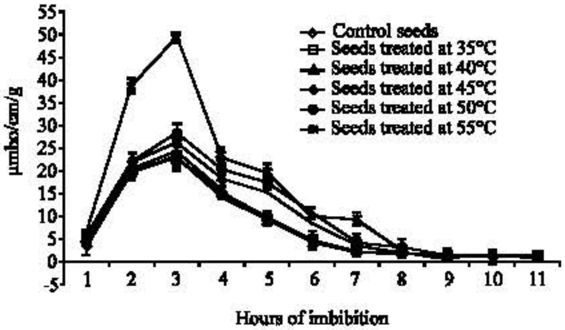

Fig. 1: Electroconductivity of pea seed exudates during 24 h of imbibition at room temperature

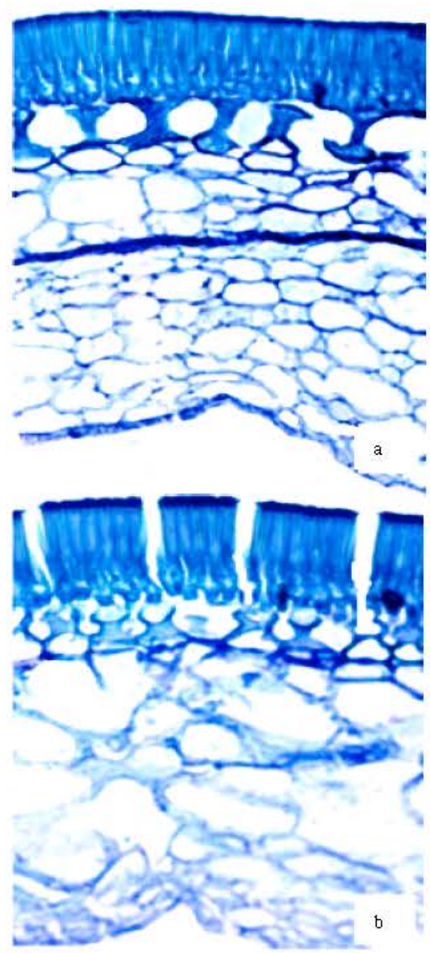

Fig. 2: Seeds coat (testa) of *Pisum sativum* L. seeds after 24 h of germination; a) Testa of control seeds and b) Testa of seeds treated at 50°C

Anatomical structures of fresh pea seed testa consist of an outer most layer of palisade cells (macroscleriеds). These cells were closely packed with a pointed outer end. This outer end had a characteristic thick wall forming terminal cap. The outer region of the macroscleried was with a thick coating of suberin. Inner to this macroscleriеds were the hourglass shaped uniformly arranged osteoscleried cells. Beneath the osteoscleried was parenchymatous layer of about 12-13 cells thick (Fig. 2a).

The testa of seeds treated at 50°C (Fig. 2b) showed numerous broken areas in the outermost palisade cell layer. The macroscleried cells were broken and were arranged in bundles, making gap in between the bundle of cells. The osteoscleriеds were unevenly distributed and some of them were broken. The parenchymatous region showed complete lysis of cells and number of cell layer also was decreased (Fig. 2b).

DISCUSSION

The moisture content distribution of *Pisum sativum* seeds treated at high temperature reveal strong influence of temperature on seed 'bound water' removal without losing viability up to 55°C when the moisture content was below 7%. The proportion of seeds germinated normally in standard germination tests declined progressively from 94 to 50% when the seeds were dried from 14.8 to 3.7% moisture content at ambient temperature (Ellis *et al.*, 1990; Ellis and Roberts, 1982). Occurrence of seed hardedness was an important observation in the present study (Table 1) and in *Pisum sativum* seeds irreversible seed hardedness was induced by desiccation at moisture content of 7.9% (Ellis *et al.*, 1990).

Chilling stress following some hours of soaking resulted in little or no distinct reduction in percentage germination or seedling vigour (Zheng *et al.*, 1980). In our observation, the pea seeds were showing reduced germination percentage when the imbibition and germination were carried out under chilling stress on comparison with the seeds germinated under cold after imbibition at room temperature (Table 1). Pea seeds show resistance to chilling stress (Munro *et al.*, 2004).

One of the important characteristic features of seeds during germination is imbibitional injury and resultant solute leakage (Simon *et al.*, 1976; Powell and Matthews, 1981). Seeds under dry storage as well as aged seeds exhibited increased leakage of solutes due to more imbibitional injury. Imbibitional injury of seeds depends on several factors such as seed maturation, age, moisture content and temperature (Tully *et al.*, 1981; Powell and Matthews, 1979; Taylor and Prusinski 1990). Rate of solute leakage is considered as a parameter of imbibitional injury and viability (Doijode, 1988; Bruggink *et al.*, 1991). Cortes and Spaeth (1993) investigated the origin and characterization of electrolytes loss as a result of ageing of seeds by compartmental analysis of potassium efflux in submerged *Pisum sativum* embryos during imbibition and suggested that aged seeds showed increased amount of potassium since the potassium ions were available for efflux from both compartments.

Greater leakage of electrolytes from the over dried and mechanically damaged seeds suggested that initial water content of the embryo (Taylor and Prusinski, 1990) and testa (Powell and Matthews, 1979) determine the result of conductivity test as well as plant establishment. During initial period the conductivity of leachate of seeds conditioned at higher temperature (45, 50 and 55°C) only showed higher electroconductivity (Fig. 1) compared to the control and others at 35 and 40°C, possibly due to the cracks on the testa (Fig. 2), which enhanced leakage of electrolytes or metabolites. Imbibitional damage was more due to more leakage through damaged testa (Simon, 1974; Powell and Matthews, 1979; Taylor *et al.*, 1993; Beresniewicz *et al.*, 1995) and these authors suggested that seed coat crack enhanced leakage of solute compared to seeds with intact testa. Several cracks are observed in the outermost layer of the testa and irregular distribution of osteoscleriеds and the parenchymatous layer in the inner region in the case of seeds treated at 50°C (Fig. 2). *Pisum sativum* seeds are readily germinable and imbibition takes place through the entire testa and the damage occurring on the testa during temperature treatment may result in more absorption of water, while intact testa imposes some limit to the leakage of solutes. Leakages of solute through testa have been used as a rapid method to assess seed quality (Simon and Mathavan, 1986). Nevertheless, leakage tests may be confounded by seed coat cracks that influence leakage rate. In these observations it has been concluded that in pea seeds shows moisture content, leachate, seed viability and integrity of testa are closely related.

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