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Loss in Germinability of Heat-treated Wheat Seeds is Associated with Reductions in Electrical Conductance of Their Leachates

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Abstract: Wheat seeds of two high vigour cultivars, Ingelab and Pasban, were heat-treated at 55 ± 2°C and 70 ± 2°C in presence (wet) or absence (dry) of water for 1 day. Germination tests exhibited reductions in percentage seed germination with increase in the pre-treated temperature and zero percent germination was exhibited in 55 ± 2°C (wet) treated seeds. 'Pasban' seeds were more sensitive to heat-treatment than 'Inqlab' seeds. Electrolytes leakage (μS/cm/seed) and rates of electrolytes leakage (μS/cm/seed/h) measured in the first 6 hours of imbibition reduced with increase in temperature treatment compared with that of controls. The correlation between loss in germinability and electrical conductance is discussed.

Key words: Electrical conductivity, Heat-treatment, Imbibition, Seed, Wheat

Introduction

A dry seed may contain moisture content of 7-15% which can vary depending upon the storage conditions, seed age and other factors. Even in dry state, degradative cellular changes may take place which result in lesions in macromolecules such as DNA and RNA (Cheah and Osborne, 1978; Bewley and Black, 1986; Ashraf and Bray, 1993). Ribosomal and mitochondrial membranes are damaged partially because of autoxidation of membrane lipids during drying and storage and these processes continue in dry seed leading to decreased rate of germination, lower resistance to adverse conditions, abnormal seedling growth and ultimately total loss of viability (Bewley and Black, 1986). However, as a seed is allowed to imbibe, reactivation of genome takes place and repair/replacement of mitochondria and ribosomes occur and cellular integrity is restored by the synthesis of proteins (and enzymes), RNA, lipids and repair and replication of DNA (Elder and Osborne, 1993; Bewley and Black, 1994).

As a seed is placed at higher temperatures, 'over-activation' of biological molecules may result in lethal changes in macromolecules due to excessive loss of water molecules, denaturation of proteins and decreased fluidity of membranes (Ho, 1987). Rate of lipid autoxidation is increased and cellular membranes become the prime target to disorganize and rupture (Simon and Harun, 1972). Tho resulting seed desiccation state also results in disrupted cellular membranes (Senaratna and McKersie, 1983). Among several methods to measure the disruption of cellular membranes, the measurement of electrical conductance (E.C.) of the leachate of imbibing seed is considered as a good indicator of the extent of damage occurred (Bewley and Black, 1986), though other workers do not find it a consistent indicator of measurement of membrane integrity (Coolbear *et al.,* 1984) or germination performance (Dearman *et al.,* 1986).

E.C. measurements and efflux of electrolytes have previously been observed in leachates of imbibing wheat seeds (Hussain, 1997; Ashraf and Hussain, 1998). Changes in inorganic and organic constituents were measured at 16 ± 1°C in 2, 6, 16 and 24 hours imbibed seeds. Results showed no relationship with the seed germination since all seeds exhibited 100% germination. The present study was carried out to investigate the seed germination and electrolytes leakage during early hours of imbibition when seeds are introduced to supra-optimal temperatures prior to germination. The results show that the conductance of the leachate of pretreated seeds lowered associated with reductions in germination percentage.

Materials and Methods

Germination Tests: Wheat seeds of two different cultivars, Inqalab and Pasban, were allowed to germinate at 16 ± 1°C in glass petri dishes containing two layers of filter papers soaked with distilled water. Seed germination was checked until all the viable seeds had germinated.

Heat-treatments of seeds: Seeds were placed in petri plates with or without water at 55 ± 2°C and 70 ± 2°C for 1 day. Pretreated seeds were stored in sealed plastic jars until used. Seeds treated at 70 ± 2°C in presence of water were 'cooked' and therefore were discarded and not included in studies. Seeds, therefore, were designated 55 ± 2°C (dry), 55 ± 2°C (wet) and 70 ± 2°C (dry), respectively.

Measurement of Electrical Conductance: Fifteen seeds, treated or controls, were allowed to imbibe in 10 ml double distilled water. Electrical conductance (E.C.) was measured at 1 ± 1°C with a pre-calibrated conductivity meter (Milwaukee-CON 1000) within one minute of start of imbibition and named the value, zero hour. This was followed by E.C. measurements at regular intervals.

Results and Discussion

Germination performance of control and heat-treated seeds is given in Table 1. At 55 ± 2°C (dry), upto 25% reduction in percentage seed germination of Pasban occurred with a further reduction of upto 60% at 70 ± 2°C (dry) compared with that of control. Pasban cultivar was found to be more sensitive to heat-treatment than the Ingalab. However, when seeds were treated at 55 ± 2°C (wet) in presence of water, neither of the seeds of the two cultivars germinated. This expected result was attributed to loss of inorganic and organic solutes during 1 day imbibition at supra-optimal temperatures.

E.C. of the pretreated or control seeds was measured during initial six hours of imbibition (Table 1). Untreated control seeds exhibited higher E.C. values and higher rates of E.C.

Table 1: E.C. of leachates measured (μS/cm/seed) during initial six hours of imbibition. Seeds were treated at given temperatures for 1 day in absence of water (dry) or immersed in water (wet). 15 seeds were immersed in 10 ml double distilled water and E.C. measured at 16 ± 1°C. The values are expressed in terms of specific conductance (μS/cm/seed). Figures in () indicate the percent seed germination. S.E.± <5% for germination tests In = 2)

No.	Cultivar	Control	55 ± 2°C (dry)	55 ± 2°C (wet)	70 ± 2°C (dry)
1	Inqalab	17.59	12.70	9.06	4.56
		± 1.62	± 0.87	± 2.31	± 0.86
		(96.00%)	(82.00%)	(0.00%)	(64.00%)
2	Pasban	20.12	13.81	8.75	6.14
		± 2.54	± 1.88	± 1.09	± 0.91
		(97.00%)	(75.00%)	(0.00%)	(40.00%)

Table 2: Rate of E.C. of leachate measured during initial six hours of imbibition. 15 seeds were immersed in 10 ml double distilled water and E.C. measured at 16 ± 1°C. Rate is expressed as μS/cm/seed/h (n = 2)

No. Cultivar	Duration (hours)	Control	55 ± 2°C (dry)	55 ± 2°C (wet)	70 ± 2°C (dry)
1. Inqalab	0.5	30.94	21.10	8.88	5.40
	1	15.47	11.58	4.92	2.93
	2	9.05	6.35	3.06	1.70
	3	6.45	4.60	2.25	1.87
	4	5.30	3.57	1.71	1.68
	6	3.87	2.73	1.32	1.36
Mean		11.85	8.32	3.69	2.49
S.E.		± 4.17	± 2.86	± 1.16	± 0.62
2. Pasban	0.5	31.26	22.20	9.68	11.90
	1	17.69	13.24	5.56	6.74
	2	9.72	7.25	3.41	3.85
	3	7.06	5.65	2.93	2.88
	4	6.41	4.34	2.28	2.22
	6	5.16	3.10	1.70	1.59
Mean S.E.		12.88	9.30	4.26	4.88
S.E.		± 4.11	± 2.96	± 1.21	± 1.59

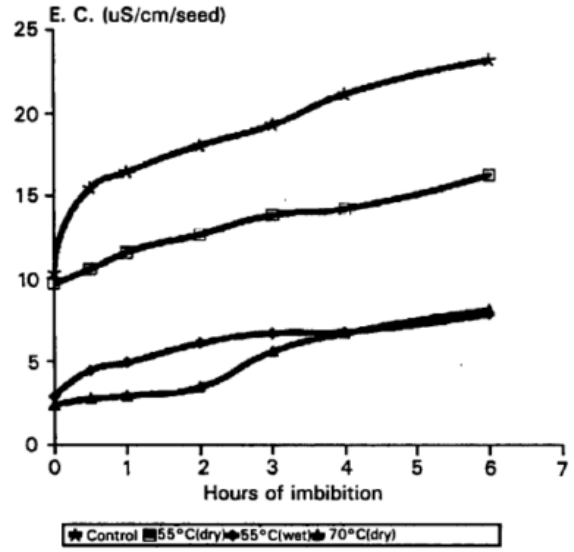

Fig. 1: Changes in E.C. during early hours of imbibition of heat-treated Inqalab cv. Fifteen seeds were immersed in 10 ml double distilled water. E.C. was measured after given intervals with a pre-calibrated conductivity meter. Results are expressed in terms of μS/cm/seed. Values indicate mean of two independent experiments carried out at 16 ± 1°C

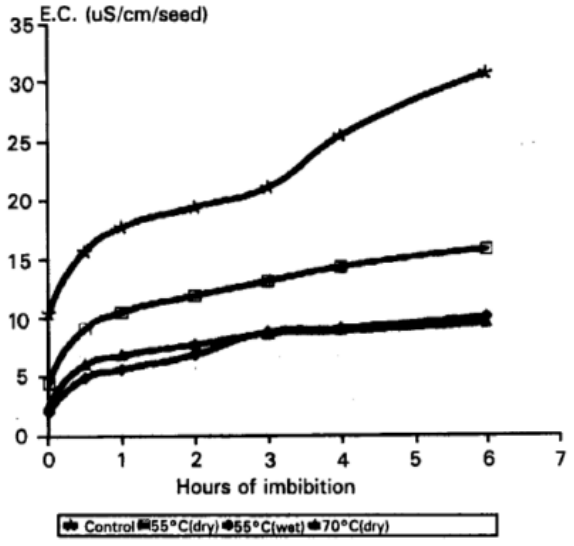

Fig. 2: Changes in E.C. during early hours of imbibition of heat-treated Pasban cv. Fifteen seeds were immersed in 10 ml double distilled water. E.C. was measured after given intervals with a pre-calibrated conductivity meter. Results are expressed in terms of μS/cm/seed. Values indicate mean of two independent experiments carried out at 16 ± 1°C

compared with that of heat-treated ones (Table 1, 2, Fig. 1, 2). The treated seeds exhibited 30-40% less E.C. values at $55 \pm 2°C$ (dry) which further decreased with increase in temperature. E.C. values of seeds heat-treated at $55 \pm 2°C$ (wet) or $70 \pm 2°C$ (dry) depicted similar patterns (Fig. 1, 2). Lower E.C. values mean that more electrolytes leakage has already been taken place during the 1 day heat-treatment, whilst in untreated controls, electrolytes are finding their first chance to efflux out of the seed.

The rates of electrolytes leakage exhibit the extent of efflux of electrolytes out of the seed (Table 2). Rate of E.C. is 30.94 μS/cm/seed/h for Inclalab control seeds during the initial 30 minutes of imbibition which decreases to 50% in the next 30 minutes. Similar profiles are seen in Pasban cv. In heat-treated seeds, similar patterns of rate of E.C. are exhibited in both the cultivars. However, a drastic reduction in initial rate is seen in the control and heat-treated seeds, i.e., 30.94 to 21.10 to 8.88 and 5.40 μS/cm/seed/h for the Incialab during first 30 minutes of imbibition. The decrease in E.C. rates with increase in 'heat stress' might be attributed to the time taken to repair the cellular membrane and other organelles during to increased membrane damage or impaired membrane ionic selectivity during the treatment (Bewley and Black, 1986). However, why $55 \pm 2°C$ (wet) treated seeds lost viability though the E.C. values are similar to that of $70 \pm 2°C$ (dry) treated seeds? The answer suggested is that at $70 \pm 2°C$ due to the absence of water during the treatment (4.30% moisture content), only volatile components and electrolytes were lost whilst in $55 \pm 2°C$ (wet) seeds, starch mobilization along with other cellular changes in protein, enzyme, and other macromolecules might have occurred and such changes proved to be irreversible. Such seeds reached the 'threshold' where initiation of germinative processes failed but such processes were still operational when pretreated seeds were back to reimbibition state.

High temperature affects the fluidity of lipid bilayers of membranes which alters the ionic transport through the membranes. Ionic balance in cell is disturbed which triggers other biochemical processes. Heat stress in aleurone layer cells of barley induces damage to membrane system of endoplasmic reticulum, which leads to arrest of protein synthesis (Baszczynski *et al.,* 1982). Moreover, proteins are thermally denatured which induce the synthesis of heat-specific proteins (hsp). Balance between different metabolic pathways may also be disturbed and one or more metabolites may be depleted or accumulated. For example, P^1, P^4-diadenosine-5'-tetraphosphate (AppppA) has been accumulated during heat stress and such rare compounds formed due to heat stress have been called 'alarmones' (Lee *et al.,* 1983). Similarly, gramine is a secondary metabolite accumulated in leaves of barley due to long-term heat stress (Hanson *et al.,* 1983).

The present data reveals that E.C. measurements during initial six hours of imbibition are good indicator of germination performance of seeds. However, Coolbear *et al.* (1984) have suggested that in tomato seeds solute leakage is not the most appropriate means of measuring membrane integrity. Similarly, Dearman *et al.* (1986) while working on priming of onion seeds found that conductivity measurements of seed leachates were not a reliable indicator of germination performance. In summary, high temperature heat-treatments of seeds in the absence of water result in loss of molecules which are important in maintaining the integrity of cellular membranes and viability of seeds. However, if water contents are higher during the treatment, seeds rapidly loose viability due to loss of inorganic and organic solutes, disturbances in membrane integrity and cellular osmotic balance and accumulation or depletion of unusual metabolites. Further studies are suggested to undertake this investigation and look into the nature of the molecules present in the leachate and the accumulation of rare compounds/metabolites which may prove good indicators of heat stress. Work is in progress on these lines.

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