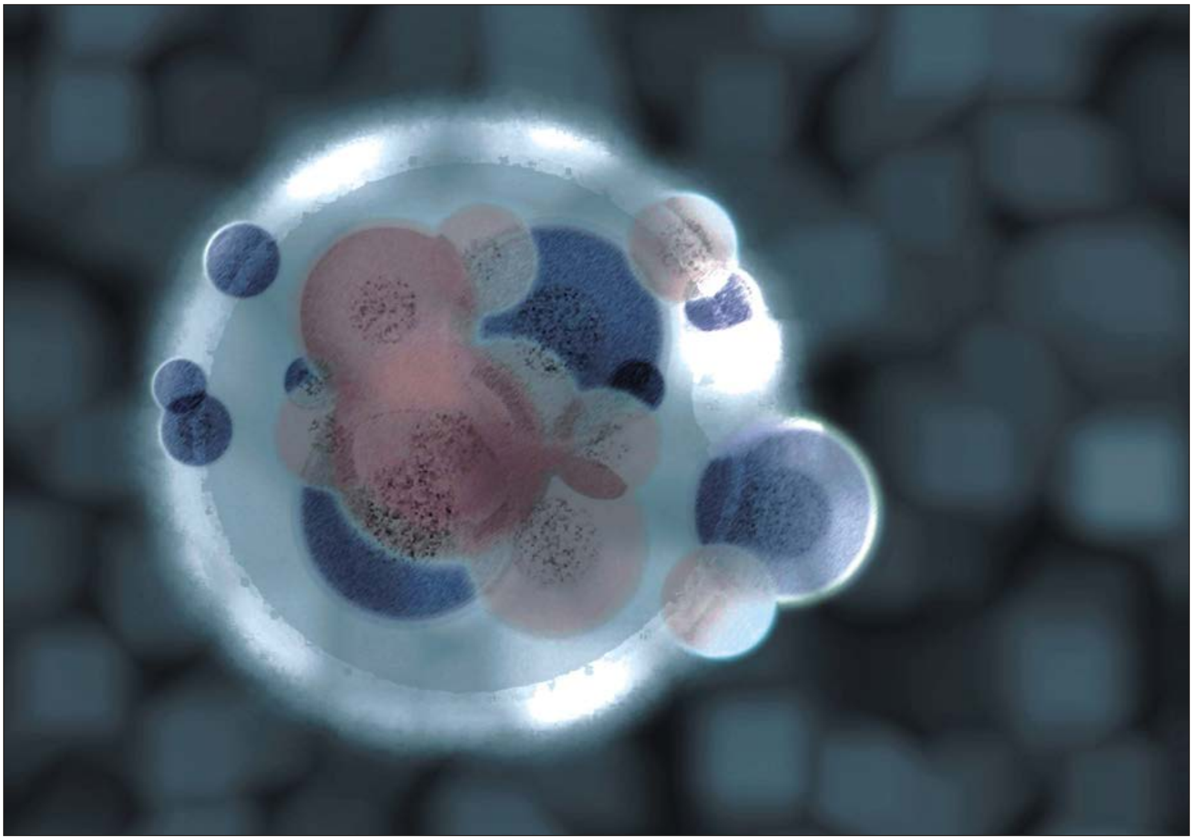

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Efflux of Inorganic Ions in Leachates of Wheat Seeds

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Abstract: Changes in Na^+, K^+ and inorganic phosphate (Pi) have been quantified in the leachates during early hours of imbibition of untreated and 2, 6, 16 and 24 hours of water pre-treated wheat seeds at 16±1°C. During the course of 24 hours imbibition of untreated seeds, Na^+ effluxed ≤1.2 ppm/seed. K^+ concentration at zero hour observed was 2.27 ppm/seed which increased to 7.2±0.9 ppm/seed in leachates of 24 hours of imbibition of control seeds. Rate of K^+ leakage from the seed was faster (4.54 ppm/seed/hour) during the initial 30 minutes which decreased to 0.3 ppm/seed/hour at 24 hours of imbibition. Pi leakage remained upto 8.1±1.2 ppm/seed during 24 hours of imbibition in control seeds. When seeds were pretreated in water for 2, 6, 16 and 24 hours, air-dried for a week to their original weights and allowed to re-imbibe for 1-3 days, decrease in ionic concentrations were noticed in their leachates with increase in duration to pre-treatment and least ionic concentrations were noticed in leachates of 24 hour pre-treated seeds. The significance of these changes during early hours of imbibition is discussed.

Key words: Electrolytes, Imbibition, Seed, Wheat.

Introduction

Seed desiccation, the final step in development, results in a partial loss of membrane integrity. When such a dry seed is allowed to imbibe, membrane integrity lost during drying is restored during the initial hours of imbibition. It is this time during which solutes leak out of the cells. Water imbibition is always accompanied with the leakage of inorganic ions, mainly K^+ and orthophosphate ions (Pi), amino acids, glucose and other sugars and a lot of other compounds (Simon and Harun, 1972; Bewley and Black, 1986; Ashraf and Hussain, 1998). The increase in electrical conductivity (E.C.) of leachates of imbibing seeds is thus due to the increased leakage of these electrolytes (Ashraf and Hussain, 1998). High vigour seeds show decreased E.C. values and electrolytes leakage during initial hours of imbibition than the low vigour seeds because the membrane system of latter is more damaged than the former (Bewley and Black, 1986).

Imbibitional leakage has been studied in pea, *Ricinus*, bean, soybean, celery, sunflower, rape, mustard, carrot, dill, caraway, fennel, parsley, lettuce, okra and *Lotus corniculatus* seeds (Simon and Harun, 1972; Powell and Matthews, 1979; McKersie and Stinson, 1980; Dadlani and Agrawal, 1983; Simon, 1984; Simon and Mathavan, 1986). Among inorganic ions, greatest leakage through membrane has been seen for K^+; imbibing celery seeds loose more than 60% K^+ originally present in the seeds (Simon and Mathavan, 1986). Potassium has been involved in the turgor maintenance, charge balance and protein synthesis and one or more K^+ transport systems reside on the plasma membrane (Kochian and Lucas, 1989). Na^+ is not an essential factor for growth and even salt-tolerant plants require it as a micronutrient and little role has been suggested in germination and growth (Marschner, 1990). Inorganic phosphate (Pi) is the other ion of great importance involved in nucleotide metabolism and has been monitored in leachates of *Lotus corniculatus* seeds (McKersie and Stinson, 1980). Due to its significance in metabolism and energetics, greater turnover of Pi is expected during the early hours of imbibition (Bewley and Black, 1986).

The efflux of organic molecules like water soluble carbohydrates and amino acids has been reported earlier by the authors during early hours of wheat seed imbibition (Ashraf and Hussain, 1998). Seeds were pre-treated in water for 2, 6, 16 and 24 hours, dried back and imbibed to determine efflux of organic molecules in their leachates. The present work was carried out to demonstrate the levels of inorganic solutes effluxed during the early imbibitional period in untreated and pretreated wheat seeds. The results show that K^+ and Pi are the major inorganic ions leaked during early hours(days) of imbibition and suggest disturbances in membrane permeability and selectivity.

Materials and Methods

Seed germination: Fifteen wheat seeds of Inqalab cultivar were immersed in 10ml double distilled water in a test tube at 16±1°C. With occasional shaking, after given intervals, leachate was poured into other test tubes and used for the determination of Na^+, K^+ by flame photometry (Corning), or orthophosphate determination spectrophotometrically by molybdate method.

Seed Pre-treatments: Seeds were immersed in double distilled water for 2, 6, 16 and 24 hours. After given time, seeds were air-dried for a week to their original weights and used or stored until used.

Results and Discussion

Measurement of inorganic ions in leachates: The concentration of Na^+, K^+ and Pi in leachates during 24 hours of imbibition of control seeds is given in Table 1. Na^+ concentration remains constant throughout the imbibing period (i.e., ≤1.2 ppm/seed). In leachates, K^+ leakage is 2.27 ppm/seed after 30 minutes of start of imbibition which is almost double after 6 hours (4.07 ppm/seed) and on 24 hours the value is reached 7.2±0.9 ppm/seed. However, the rates of K^+ leakage are higher in the initial 30 minutes of imbibition, i.e., 4.54 ppm/seed/h and these rates are decreased tremendously as time to imbibition increases. There is slow and steady increase in Pi contents which are 1.5 ppm/seed in leachates of 30 minutes imbibed

Table 1: Concentration of inorganic ions in leachates during early hours of imbibition of wheat seeds. 30 seeds were immersed in 10ml double distilled water for given time and after suitable dilutions, K^+ and Na^+ ions concentration was measured with flame photometer. Inorganic phosphate (Pi) was measured by molybdate method (n = 2, s.d.).

Hours of imbibition control seeds	K^+ (ppm/seed)	Rate of K^+ leakage	Na^+ (ppm/seed)	Pi (ppm/seed)
0.5 h	2.27±1.1	4.54	≤1.20	1.5±0.9
2 h	3.20±1.1	1.60	≤1.20	2.1±1.1
6 h	4.07±1.1	0.68	≤1.20	3.5±1.2
24 h	7.20±0.9	0.30	≤1.20	8.1±1.2

Table 2: Na^+ concentration in leachates during early days of imbibition of wheat seeds. (n=2,s.d.).

Imbibition	Control	2 h	6 h	16 h	24 h
1 day	1.10±0.2	1.05±0.2	0.95±0.2	0.75±0.2	0.45±0.1
2 day	0.95±0.2	0.65±0.1	0.50±0.2	0.50±0.1	0.40±0.1
3 day	0.40±0.1	0.35±0.1	0.35±0.1	0.25±0.1	0.30±0.1

Table 3: K^+ concentration in leachates during early days of imbibition of wheat seeds. (n=2,s.d.).

Imbibition	Control	2 h	6 h	16 h	24 h
1 day	7.20±0.9	5.25±1.1	3.06±1.2	3.94±1.1	4.90±1.1
2 day	9.18±1.1	7.59±0.9	7.11±1.1	6.42±1.0	6.81±0.8
3 day	8.73±1.1	6.33±1.1	5.49±1.1	5.04±1.2	7.95±1.2

Table 4: Pi concentration in leachates during early days of imbibition of wheat seeds. (n=2,s.d.).

Imbibition	Control	2 h	6 h	16 h	24 h
1 day	8.10±1.2	7.5±1.8	6.5±0.9	4.5±0.8	1.5±0.7
2 day	9.50±1.1	8.1±1.6	7.1±1.4	7.2±1.6	3.5±1.2
3 day	14.5±2.1	6.2±1.3	5.2±1.5	4.1±0.9	2.2±0.9

seeds and reach 8.1±1.2 ppm/seed in leachates after 24 hours of imbibition.

When the measurements of changes in ions in leachates were extended to 3 days imbibition period, the results obtained are given in Table 2-4. A decrease of 2.5-fold in Na^+ has been seen in leachates of untreated seeds by 3 day of imbibition (Table 2). In 2, 6, 16 and 24 hours pretreated seeds, further decrease in Na^+ has been detected in leachates as the time to imbibition increased. Little difference in Na^+ in the control and pretreated seeds was observed. K^+ in leachates of control seeds show slight increase with increase in time of imbibition (Table 3). In pretreated seeds, K^+ are decreased in leachates due to the reason that some amount has already been leached out during the pretreatment duration.

Increasing patterns of Pi in leachates of untreated seeds have been monitored which rose from 8.1 to 14.5 ppm/seed (Table 4). In leachates of pretreated seeds, Pi levels remained below 8.1 ppm/seed which decreased with increase in pre-treatment period and hence lowest value was detected in 24 hours preimbibed seeds. Similar changes have also been seen in seeds of *Lotus corniculatus* L. (McKersie and Stinson, 1980).

Leakage is the most rapid during the early stages of imbibition when air dry seeds or embryos are placed in water. As imbibition progresses, leakage and rates of leakage decline. Results presented here are consistent with the findings in other seeds (Simon and Harun, 1972; Simon and Mathavan, 1986). There is a suggestion in the literature that cell membranes may be damaged by the in-rush of water during imbibition. Components of plasma membranes may be displaced and disorganized due to turbulent flow of water and hardly any of the solutes remaining within the cells (Simon, 1984). It has also been suggested that due to in-rush of water, membranes are re-organized and repaired depending upon the physiological state (vigour) of the seed. Dry seeds do not leak due to lack of solvent and once seed is hydrated, a short period of leakiness is experienced before the membrane system is fully operational to semi-permeability (Simon, 1984).

Seed pretreatments such as hydration or osmo-priming prevents imbibitional injury and membrane damage is repaired during early hours of leakiness as shown in Table 2 and 3 (Bewley and Black, 1986). Six times greater electrolytes leakage in aged soybean seeds have been observed compared with the high vigour seeds (Woodstock and Tao, 1981). Removal of seed test in pea seeds enhances the rate of electrolytes leakage such as K ions and other electrolytes which results in higher E.C. values. Similar results have been seen in seeds of *Salvadora oleiodes* wherein higher E.C. values and K^+ were present in leachates of 'naked' seeds than seeds with seed test (Ashraf and Nisar, 1998).

Some seeds may leak more K ions than others. More than 60% K ions have been released in imbibing celery seeds (Simon and Mathavan, 1986). In our findings, 33% of total K leakage (7.2 ppm/seed in 24 hours) has been seen within 30 minutes of imbibition (Table 1) and the initial rate of K leakage was very high (4.54 ppm/seed/h). In the field conditions, these inorganic ions with organic solutes may stimulate the growth of fungi, mycorrhiza and bacteria in the soil; if beneficial, will stimulate the growth of seedlings and, if harmful, may invade the seedling leading to its deterioration (Bewley and Black, 1986; Chanway, 1997). In the present studies, all pre-treated seeds showed 96-97% germination, which suggest that solute leakage may not affect the viability of seeds, though vigour of seeds may be altered (McKersie and Stinson, 1980). Similar results have been reported earlier in wheat seeds (Ashraf and Hussain, 1998).

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