http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 1 (3): 148-151, 1998 *Research Article*

Changes in Amino Acid and Carbohydrate Contents in Leachates of Preimbibed Wheat Seeds

M. Ashraf and M. M. Hussain

Biochemistry Laboratory, Department of Chemistry, Islamia University, Bahawalpur, Pakistan

Abstract

Amino acid and carbohydrate contents during early 30 minutes of wheat seed imbibition have not been reported earlier. In this study, wheat seeds (cv. Ingelab) preimbibed in water for 2, 6, 16 and 24 hours and dried back, were soaked in water for 30 minutes and amino acid and carbohydrate contents in leachates were measured. Untreated seeds showed 4.5 times increase in amino acid contents (2-9.1 nmoles/seed) in 30 min of imbibition. However, in preimbibed seeds, as the time to pretreatment increased (from 2 to 24 h), the amount of amino acids leached decreased and in 24 h preimbibed seeds, it was 2.8 nmoles/seed after 30 min. Changes in carbohydrate contents in leachates in untreated seeds rose from 13 to 52 μg/seed in 30 min, an overall increase of 4 times was exhibited. All the pretreated seeds exhibited lower levels of carbohydrates effluxed in 30 min. However, the significant difference was observed in the initial 10 min of start of imbibition in which preimbibed seeds effluxed carbohydrate contents at faster rates (e.g. 10 times increase in 6 h and 24 h preimbibed seeds) than the control ones (0.7 times increase). Changes in these electrolytes had no effect on seed germination of these preimbibed seeds since all seeds exhibited 100 percent germination in two days. These results demonstrate that amino acid and carbohydrate contents leakage during initial 30 min of imbibition have no effect on viability of seeds but may have implications in studying seed-soil relations.

Key words: Electrolytesi leachate, preimbibed, seed,, wheat

Introduction

Seed imbibition is pre-requisite to seed germination (Bewley and Black, 1986). The process of imbibition is always associated with the leakage of inorganic and organic electrolytes in leachates of surrounding water. Increased leakage of these electrolytes is detected by the changes in electrical conductance (E.C.) and this procedure has been used in measurements of vigour and viability of different seed species. Low vigour seeds show increased E.C. values and electrolytes leakage during imbibition because of more damage to the membrane system (Bewley and Black, 1986). Leachate conductivity measurements provide a reliable assay of vigour test and weather damage of French beans and mung beans (Pandey, 1988; Williams *et al*., 1995). When seed testa is removed, more leakage is observed in the 'naked' seeds compared with that of testa-intact seeds (Simon, 1984; Simon and Mathavan, 1986; Nisar, 1997; Ashraf and Nisar, 1998). Solute leakage has been studied in bean, pea, sunflower, carrot, dill, parsley, celery, lettuce, funnel and published work has shown that K and phosphate ions are among the dominant inorganic species observed during initial hours of seed imbibition (Simon and Harun, 1972; Simon, 1984; Simon and Mathavan, 1986; Hussain, 1997; Ashraf *et al*., 1998). Several phenolic compounds, sugar molecules, amino acids and proteins, organic acids, gibberellic acid and enzymes such as glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, cytochrome oxidase are among the organic species effluxed during seed imbibition (Simon and Harun, 1 972; McKersie and Stinson, 1980; Simon, 1984; Simon and Mathavan, 1986; Hussain, 1997; Ashraf *et al*., 1998). Inorganic and organic components of leachates of seeds imbibing in soils may help in the establishment and maintenance of seedling rhizosphere.

Changes in electrical conductance and inorganic constituents in leachates of imbibing wheat seeds have previosuly been determined (Hussain, 1997; Ashraf *et al*., 1998) wherein wheat seeds were imbibed for 2, 6, 16 and 24 hours, dried and allowed to reimbibe to determine K, Na and orthophosphate contents. These results exhibited a continual decrease in the rate of leakage of inorganic ions as the time to pretreatment of seeds increased. However, the nature of organic solutes effluxed in these pretreated seeds have not previously been determined. This study is an extension of our previous work (Ashraf *et al*., 1998) and describes the changes in amino acids and sugar contents during early minutes of imbibition of preimbibed wheat seeds.

Materials and Methods

10-20 wheat seeds of Int:pleb cultivar were placed in 10 mL double distilled water in a test tube at 16°C. With occasional shaking, after given intervals, E.C. was measured at 16°C with a pre-calibrated conductivity meter (Milwaukee-CON 1000). Leachate was poured into the other test tube and used for the determination of amino acids by ninhydrin method and carbohydrates by enthrone reagent method (Plummer, 1990). Leachates after a quick spin were read at $O.D._{254}$ nm in 0.5 cm quartz cuvettes in a uv-visible spectrophotometer (Jasco).

For preimbibition treatment, seeds were allowed to imbibe for 2, 6, 16 and 24 hours in double distilled water. After

given intervals, seeds were air-dried and dessicated for a week to their original moisture contents and used/stored. All these seeds did not show protrusion of radical.

Results

Ashraf *et al*. (1998) have measured the electrical conductance of leachates of imbibing wheat seeds during initial 6 hours of imbibition wherein conductance increased from 7,28 μS/cm/seed at zero minute to 13.57 μS/cm/seed after 30 min of imbibition for the untreated control seeds. Electrical conductance of 2, 6 and 24 hours preimbibed seeds have been found to decrease as the time to preimbibition increased. Amount of K and orthophosphate (Pi) ions effluxed clearly demonstrated changes in electrolytes as the time to imbibition increased. However, when these studies have been extended for the presence of ` organic electrolytes, i.e., uv-absorbable material, again a similar picture has been resulted as given in the present paper.

Table 1: Changes in uv-absorbable material during wheat seed imbibition. Ten wheat seeds were immersed in 10 mL double distilled water for the given time and leachate was bench centrifuged and O.D. measured in 0.5cm quartz Guyette at 254 nm

Time of imbibition (mm)	Control (0 h)	2 h	6 h	16 h	24 h
5	0.216	0.201	0.176	0.155	0.184
10	0.219	0.210	0.188	0.158	0.196
15	0.225	0.217	0.198	0.158	0.205
20	0.231	0.225	0.205	0.165	0.209
30	0.285	0.238	0.211	0.165	0.225
40	0.370	0.245	0.232	0.168	0.252

Measurement of uv-abSorbable material at $0.D._{254}$: Table 1 shows the increase in leakage of uv-absorbable material during early 40 min of start of imbibition of 10 seeds in 10 mL double distilled water. These values indicate the efflux of organic solutes in leachates. Untreated dry seeds exhibit $0.D._{254}$ of 0.161 which rose to 0.370 within 40 min. All preimbibed seeds exhibit an increase in absorbance with increase in time to imbibition. However, the absorbance values are decreased in leachates with increase in time to preimbibition treatment of 2 h, 6 h and 16 h, These absorbance values in 24 h preimbibed seeds are higher than 6 h and 16 h seeds and equivalent to 2h pretreated seeds. This indicates the accumulation of organic solutes due to mobilization of food reserves and active organic macromolecular metabolism during preimbibition treatment and their leakage when such seeds were allowed to reimbibe.

Measurement of amino acid contents: Leachates of control seeds contain soluble amino acid contents of 2 nmoles/seed at zero min and within 30 min amino acid contents reached 9.1 nmoles/seed, an overall increase of 4.5 times (Table 2). Pretreated seeds at zero time contain amino acid levels lower than the control because of leakage occurred during the preimbibition period. An increase in amino acid contents occur during the first 30 min of imbibition of these pretreated seeds and there is an increase in levels in 2 h, 6 h and 16 h pretreated seeds but levels equivalent to that of controls never reached. However, 24 h pretreated seeds showed no amino acid contents in the first 5 min of imbibition and its 30 min levels (2.8 nmoles/seed) are even less than the 5 min imbibed control seeds. It means that high $0.D._{254}$ nm values in leachates of 24 h preimbibed seeds are not indicative of soluble amino acids present but may represent only a fraction of it.

Table 2: Changes in amino acid contents during wheat seed imbibition. 30 seeds were imbibed in 10 mL double distilled water for specified time and amino acid contents measured in leachates by ninhydrin method. Results are expressed in terms of nmoles/seed. (n = 2.3) error is S.E.M

Time of imbibition (mm)	Control (0 h)	2 h	6 h	16 h	24 h
0	2.0±0.15	1.1±0.2	1.1±0.1	1.5±0.15	0.0
5	3.2±0.1	1.5±0.2	2.2±0.1	2.5±0.10	0.0
10	4.8±0.2	3.3±0.2	4.0±0.1	4.2±0.15	1.1±0.2
20	6.8±0.2	4.1±0.3	4.7±0.3	5.1±0.2	1.5±0.2
30	9.1±0.2	5.1±0.3	5.3±0.2	5.8±0.1	2.8±0.2

Measurement of carbohydrate contents: Table 3 indicates changes in carbohydrate contents have been observed during the imbibition of pretreated seeds. Zero min seeds indicate carbohydrate contents of 13 μg/seed. A 4-fold increase in carbohydrate content is exhibited by control seeds, from 13 to 52 μg/seed in 30 min of imbibition. First 10 min exhibit a slow increase in carbohydrate contents in the leachate followed by a rapid increase in the next 20 min. In pretreated seeds at zero time, minimum carbohydrate contents are seen in 24 pretreated seeds. 2 h pretreated seeds showed 3-fold reduction in carbohydrate content (4.6 μg/seed) compared with the control at zero time (13 μg/seed). However, during the first 10 min imbibition in 2 h pretreated seeds there was a 3-fold increase in carbohydrate content (4.6 to 13.3 μg/seed), in 6 h preimbibed seeds there was 10-fold increase (2 to 19.3 μg/seed) and in 24 h preimbibed seeds this jump was even more than 10 times (1.3 to 15.4 μg/seed) 16 h Preimbibed seeds did not follow these profiles as is in the case of amino acid contents. In all preimbibed seeds, increase in carbohydrate contents to 52 μg/seed (of control) was never reached by any pretreated samples. When carbohydrate contents were expressed in terms of their rates of leakage, μg/min/seed, untreated control seeds exhibited a fast leakage in the first 10 min and 2 h, 16 h and 24 h pretreated seeds also showed a similar pattern but in 6 h pretreated seeds an increased leakage 'during the first 20 min of imbibition was observed.

Discussion

All seeds leak organic and inorganic substances during imbibition occurring either in laboratory conditions or in the soil (Simon, 1984). The studies have shown that the extent

Table 3: Changes in carbohydrate contents during wheat seed imbibition. 30 seeds were imbibed in 10 mL double distilled water for specified tune and carbohydrate contents measured in leachates by anthrone method. Results are expressed in terms of μg/seed. (n = 2.3) Error is S.E.M

Time of imbibition (mm)	Control (0 h)	2 h	6 h	16 h	24 h
0	13.0±1.2	4.6±1.2	2.0±0.5	3.3±0.6	1.3±0.5
5	14.5±1.1	8.5±1.2	16.0±1.5	6.6±0.6	11.3±1.1
10	18.0±1.3	13.3±1.2	19.3±2.5	9.2±1.0	15.4±1.5
20	38.0±1.5	16.6±1.3	29.3±2.5	14.7±1.2	23.5±1.5
30	52.0±1.5	23.3±1.2	40.1±3.5	18.0±1.5	27.9±3.0

of leakage and the composition and quantity of substances released from seeds depend on the nature, type and Vigour state of the seed and is determined genetically (Vancura and Stotzky, 1976; Simon, 1984; Bewley and Black, 1986; Cieslinski *et al*., 1997; Ashraf *et al*., 1998). The types and rates of molecuels effluxed after germination are changed until the establishment of the seedling root (Cieslinski *et al*., 1997), These observations necessitate the study of the nature of substances released in understanding the mechanisms of initial hours of seed germination and seed-soil relations.

It is already documented that wheat seeds exhibit an increased leakage of electrolytes during imbibition which results in increased E.C. values and uv-absorbable material (Hussain, 1997; Ashraf *et al*., 1998). Efflux of inorganic ions like K, Na and orthophosphate species has previously been reported (Hussain, 1997; Ashraf *et al*., 1998). Changes in amounts of organic substances in leachates of imbibing seeds of different species have been studied before (Simon and Harun, 1972; Simon, 1984; Nisar, 1997) but data is lacking for wheat seeds which have been preirnbtbed for upto 24 h and then placed for germination.

In these studies, among organic species effluxed during imbibition, only amino acids (and peptides as detected by ninhydrin reagent) and total water soluble sugar contents have been detected, though there may be nucleotides, phenols, acids and a list of other compounds present in these leachates which absorb uv-light. Wheat seeds which have been preirnbibed for 24 h have less uv-absorbable material than that of control seeds and this difference is maintained when such seeds are imbibed for 40 min (Table 1). This is because the organic molecules have already been released during the preirnbibition treatment. Further, it has been seen that the amounts of substances present in these two leachates is different, that is, three times more amino acids are present in the control than 24 h pretreated seeds (9.1 and 2.8 nmoles/seed, respectively) and half of sugars (27.9 μg/seed) are present in 24 h pretreated seeds than the control (52 μg/seed). A different profile has been seen in *Salvadora oleiodes* seeds wherein testa-free seeds exhibited 8 times increase in sugar and 16 times increase in amino acids contents (Ashraf and Nisar, 1998). In wheat seeds, on the other hand, it is demonstrated that the amounts of amino acids and sugars effluxed within 30 min of imbibition of untreated control seeds is 4.5 times higher, increasing from 2 to 9.1 nmoles/seed amino acids and 4 times higher for sugars, i.e., 13 to 52 μg/seed sugars (Table 2, 3).

The biochemical significance of such changes in seed-soil interactions remains to be elucidated though theories of efflux of inorganic ions are also applicable to organic electrolytes in this case (Simon, 1984). Among the species effluxed, excess leakage of sugars may represent loss of respirable substrates from some seed species whereas, others leak more amino acids than sugars and rate of likage is not the same for all electrolytes in all the species

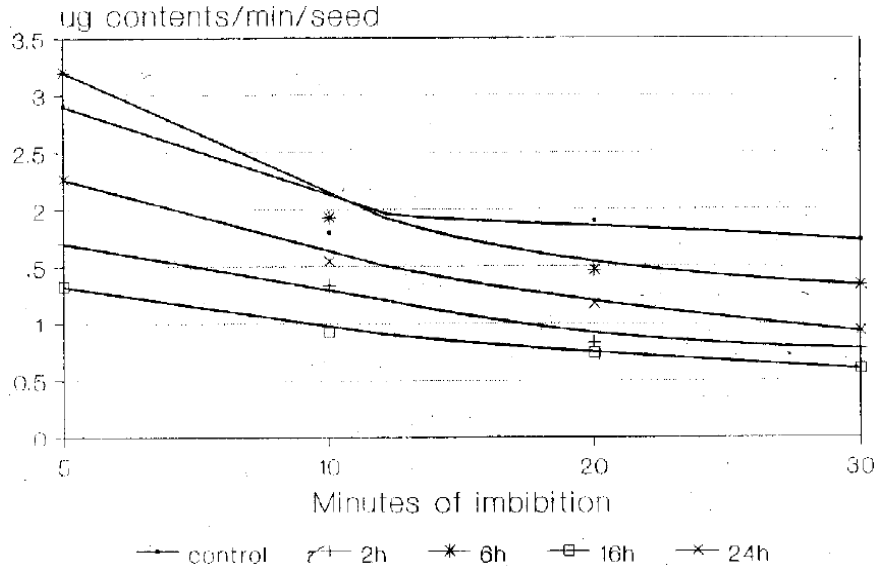

Fig. 1: Rate of change in carbohydrate contents during wheat seed imbibition. 30 seeds were imbibed in 10 mL double distilled water for specified time and carbohydrate contents measured in leachates by enthrone method. Data given in Table 3 has been expressed in terms of μg/min/seed (n = 2-3)

Ashraf and Nisar, 1998). In 24 h preimbibed seeds, no amino acid contents have been detected in the first 5 min of imbibition (Table 2) which indicates that soluble sugars or other organic molecules may be repsonsible for increase in uv-absorbance during the stated period. It is possible that the membrane properties of these seeds have been changed during the desiccation, allowing no or little molecules of amino acids and peptides to pass through the membranes (Senaratna and McKersie, 1983). These differences in membrane properties may also contribute to differential rates in the leakage of carbohydrates by 2 h, 6 h, 16 h and 24 h preimbibed seeds compared with the control (Fig. 1). In a study, soybean seeds preimbibed for 6 h and then germinated were found to be dehydration tolerant compared to 36 h preimbibed seeds which were in dehydration sensitive phase. Likewise, scarified lotus seeds leak 9 percent soluble proteins, .3.6% sugars, 3.1% phosphate, 1.4% amino acids and 13% K during the first 2 h of imbibition (McKersie and Stinson, 1980).

The data on amino acid and carbohydrate contents demonstrate the physiological, significance of these solutes in soils as carbon and nitrogen sources for the microflora around the seed and young developing seedling. It has been known that increased leakage of organic metabolites from deteriorated seeds might indirectly enhance their demise by encouraging the growth of contaminating microorganisms. However, as far as the effect of the extent of this leakage on germination percentage is concerned, the leakage of these solutes for 30 min has little importance on the germination status of these solute for 30 min has little importance on the germination status of pretreated seeds since all the seeds exhibited 100 percent germination after 2 days (data not shown). Biochemically, this is the period (30 minutes) when initiation of mRNA and protein synthesis occurs and mitochondrial biogensis with increased 'production of ATP is commenced (Bewley and Black, 1986).

In summary, in wheat seeds, amino acid and carbohydrate contents are leaked during initial minutes of imbibition along with other ions (Ashraf *et al*., 1998). Preimbibition of seeds for 24 h results in loss of electrolytes and which continue to be effluxed during re-imbibition though with different rates. Leakage of these solutes during these 30 min has no effect on percentage seed germination. However, study of prolonged imbibition of these seeds may reveal important biochemical aspects of effluxed molecules. It would be important to know the nature of other molecules effluxed, the effect on the loss of desiccation tolerance seeds and in retaining the vigour and viability of seeds. The data collectively may strengthen our ideas on understanding the mechanisms of loss of vigour and viability, preventing seed deterioration and in elucidating factors affecting aging. Work is continued on these lines.

References

Ashraf, M. and S. Nisar, 1998. Germination patterns and electrolytes leakage during imbibition of intact and 'naked' seeds of *Salvadora oleiodes*. Pak. J. Biol. Sci., 1: 287-290.

Ashraf, M., M.M. Hussain and F.H. Nasim, 1998. Changes in inorganic ions in leachates of imbibing wheat seeds. Pak. J. Bot.

Bewley, J.D. and M. Black, 1986. Seeds: Physiology of Development and Germination. Plenum Press, London, UK., Pages: 367.

Cieslinski, G., K.C.J. van Rees, A.M. Szmigielska and P.M. Huang, 1997. Low molecular weight organic acids released from roots of durum wheat and flax into sterile nutrient solutions. J. Plant Nutr., 20: 753-764.

Hussain, M.M., 1997. Imbibitional leakage of pretreated wheat seeds. M.Sc. Thesis, Department of Chemistry, Islamia University, Bahawalpur, Pakistan.

McKersie, B.D. and R.H. Stinson, 1980. Effect of dehydration on leakage and membrane structure in *Lotus corniculatus* L. seeds. Plant Physiol., 66: 316-320.

Nisar, S., 1997. Germination patterns and imbibitional leakage of intact and 'naked' seeds of Salvadora oleiodes. M.Sc. Thesis, Department of Chemistry, Islamia University, Bahawalpure, Pakistan.

Pandey, D.K., 1988. Priming induced repair in French bean seeds. Seed Sci. Technol., 16: 527-532.

Plummer, D.T., 1990. An Introduction to Pravtical Biochemistry. 3rd Edn., Tata Mcgraw Hill Publisher, Co. Ltd., New Delhi, India, Pages: 331.

Senaratna, T. and B.D. McKersie, 1983. Dehydration injury in germinating soybean (*Glycine max* L. Merr.) seeds. Plant Physiol., 72: 620-624.

Simon, E. and R.R. Harun, 1972. Leakage during seed imbibition. J. Exp. Bot., 23: 1076-1085.

Simon, E.W. and S. Mathavan, 1986. The time-course of leakage from imbibing seeds of different species. Seed Sci. Technol., 14: 9-13.

Simon, E.W., 1984. Early Event in Germination. In: The Living Seed, Murray, R. (Ed.)., Vol. 2, Acadmic Press, Australia, pp: 77-155.

Vancura, V. and G. Stotzky, 1976. Gaseous and volatile exudates from germinating seeds and seedlings. Can. J. Bot., 54: 518-532.

Williams, R.W., R.J. Lawn, B.C. Imrie and D.E. Byth, 1995. Studies on water damage in mungbean. II. Electrical conductivity of seed leachate as an assay of level of damage. Crop Pasture Sci., 46: 901-907.