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Effect of Drying Treatments on the Germination and Imbibitional Leakage of *Salvadora oleiodes* Seeds

Sarvat Nisar and Mohammad Ashraf
Biochemistry Laboratory, Department of Chemistry, Islamia University, Bahawalpur, Pakistan.

bstract

mature and developing seeds of *Salvadora oleiodes* (locally called *Pelun*) were collected in May/June and graded into small and large (L) and either dried under the sun (sun-dried) or in the laboratory (shade-dried) upto 8 ± 2 percent moisture ntent. When electrical conductance (E.C.) of leachates measured was taken as an index of membrane damage, leachates seeds with testa ('intact') exhibited lower E.C. values after 6 hours of imbibition than that of without testa ('naked'). ade-dried (S) 'naked' seeds had lower E.C. values (30.9 μS/cm/seed) than their 'intact' counterparts (61.84 μS/cm/seed). ien nature of molecules effluxed during the first 30 minutes of imbibition was determined, K ions were dominant inorganic ecies than Na ions, and with higher rates of leakage in sun-dried 'intact' seeds (12-77 ppm/seed) than the shade-dried 2-54 ppm/seed) ones. Na ions levels remained constant (14-15 ppm/seed) in shade-dried 'intact' seeds than the sun-dried es (5-18.5 ppm/seed). When amino acids and total sugar contents were determined in 'naked' seeds, sun-dried seeds iibited higher amino acids leached (5-15.8 μmoles/seed) than the shade-dried ones; 7-9.5 μmoles/seed in 'L' and 10-16.8 oles/seed in 'S' seeds. Soluble sugar contents in seeds of both the treatments varied in the range 18.75-33.7 μg/seed d were statistically insignificant to draw any conclusions between the two drying methods. It was also seen that the ure of the molecules effluxed was not correlated with the germination behaviour of seeds since only shade-dried ('S' and seeds showed 100 per cent germination whilst sun-dried ('L') had 40 per cent germination and immature as well as sun-ed ('S') seeds did not germinate at all.

troduction

e process of imbibition involves repair and restoration of naged cell membrane during early minutes of contact ween the seed and the surrounding water (Bewley and ck, 1986). It is this time during which solutes leak out the cells. The increase in electrical conductivity (E.C.) in chates of imbibing seeds is due to the increased leakage these electrolytes (Simon, 1984; Ashraf and Hussain, 98). Intact seeds leak little in the surrounding medium. en seed testa is removed, more leakage is observed in 'naked' seeds (Simon and Mathavan, 1986; Ashraf and ar, 1998). Pea seeds after removal of seed testa show immediate and rapid leakage of potassium and other ctrolytes. Increased electrolytes leakage has also been erved in dehydrated isolated axes and an inverse tionship has been found between germination and ctrolytes leakage (Senaratna and McKersie, 1983).
adora oleiodes (locally called *jhal* or *pelun*) is an green medicinal tree of desert ecosystems (Kirtiker and u, 1991). Its blackish seeds possess tough seed coat. d development starts in the last week of April and uration lasts upto July (Arshad and Rao, 1993). Little is wn about the imbibitional leakage and nature of solutes uxed during early hours of imbibition in *S. oleiodes* ar, 1997; Ashraf and Nisar, 1998). The optimal nination temperatures have been studied earlier (Ashraf Nisar, 1998) but the studies on the vigour and viability developing seeds are lacking. The effect of drying tments on the germination and imbibitional leakage of eloping seeds have also not been studied earlier even igh it is a desert tree and bears several environmental stresses in the desert during seed development. The reported work is an extension of our previous work (Ashraf and Nisar, 1998) on understanding the early hours of seed imbibition and nature and amounts of solutes effluxed after drying of developing seeds with two different methods.

Materials and Methods

Seeds of *S. oleiodes* were collected in summer 1997 during their early development phase, mid of development and late developmental phase (mature seeds). Mature large ('L') and small ('S') seeds were dried under the sun (sun-dried) or in the laboratory (shade-dried) whilst immature seeds collected during early developmental stage were dried in the laboratory (shade-dried) only. Seeds, prior to start of imbibition, with un-damaged testa were designated 'intact' and with manually removed seed coat, testa-free, were designated 'naked' seeds. Moisture contents of seeds were maintained at 8 ± 2 per cent. Germination tests were performed by placing the sets of 10 seeds (in triplicate) on presoaked double layered filter papers in glass petri dishes at 25°C. Number of seeds germinated were noted after 24 hours for two weeks. Protrusion of radical marked the seed 'germinated'. 10-20 seeds ('intact' or 'naked') were immersed in 10ml double distilled water for the specified time and E.C. was measured by immersing the precalibrated electrode of conductivity meter (Milwaukee-CON1000) in the leachate. These leachates were then used for the measurement of K and Na ions by flame photometer (Corning) after suitable dilutions. The amino acids and carbohydrate contents were measured by ninhydrin and anthrone methods as described earlier (Ashraf and Nisar, 1998; Ashraf and Hussain, 1998). All chemicals used were of analytical grade.

Results

Effect of drying on the germination of seeds: Shade-dried seeds ('S', 'L') exhibited 95-100 per cent germination. However, sun-dried drying resulted in loss of viability and only 40% germination was achieved in 'L' seeds whilst 'S' seeds did not germinate at all. Seed drying of immature seeds in shade also proved lethal which most probably could be attributed to immature and under-developed seed embryo (Fig. 1).

Table 1: Changes in E.C. in 'intact' seeds of *S. oleiodes* (with seed coat). Results are expressed in terms of specific conductance, μS/cm/seed.

Time (Min)	Sun-dried (L)	Sun-dried (S)	Shade-dried (L)	Shade-dried (S)	Immature seeds
0	4.40	6.31	6.54	4.64	7.73
5	10.35	10.95	16.90	38.08	28.08
10	12.14	17.14	21.18	51.88	44.51
20	12.97	18.21	21.54	64.02	48.55
30	13.92	20.11	23.44	69.97	53.31
60	15.11	20.47	25.59	71.16	63.31
120	16.66	21.54	29.90	76.75	80.68
240	18.68	24.51	33.32	87.23	107.46
360	20.65	26.77	33.68	92.11	120.90
Mean	13.87	18.45	23.57	61.84	61.61
± SE	± 1.6	± 2.13	± 2.85	± 9.0	± 12.10

Table 2: Changes in E.C. in 'naked' seeds of *S. oleiodes* (without seed coat). Results are expressed in terms of specific conductance, μS/cm/seed.

Imbibition time	Sun-dried (L)	Sun-dried (S)	Shade-dried (L)	Shade-dried (S)
0	2.62	2.26	2.97	3.21
5	9.64	11.07	7.50	10.47
10	12.26	15.95	9.52	14.99
20	14.28	18.21	11.30	17.97
30	17.61	22.68	13.09	21.30
60	24.63	34.99	17.25	29.39
120	39.00	47.96	24.87	46.41
240	54.29	69.02	33.56	64.14
360	65.21	76.87	39.27	70.21
Mean	26.61	33.22	17.7	30.90
± SE	± 7.2	± 8.73	± 4.11	± 7.98

Measurement of E.C. as an index of membrane injury: E.C. measurements of leachates of imbibing seeds (upto 6 hours) were taken as an index of membrane damage occurring during the course of drying treatments. Table 1 shows that E.C. of shade-dried ('S') seeds is the highest (61.84 ± 9 μS/cm/seed) and equivalent to immature ones. Sun-dried seeds have lower E.C. values (13.87 and 18 μS/cm/seed for 'L' and 'S', respectively) than the sha dried ones (23.57 and 61.84 μS/cm/seed for 'L' and respectively).

Seed coat of 'intact' seeds was removed with hands w care not to damage the 'naked' seeds. It was not poss to remove seed testa of immature seeds because of under-development, therefore, the E.C. measurements h not been taken for the 'naked' seeds. Seeds were immer in water and E.C. was measured. Results (Table 2) sh that sun-dried 'naked' seeds have E.C. values (26.61 for and 33.22 μS/cm/seed for 'S' seeds) and the shade-d 'naked' seeds have (17.7 for 'L' and 30.9 μS/cm/seed 'S' seeds); small seeds have higher E.C. values than large seeds. E.C. of sun-dried 'naked' seeds were hig (almost double) than the 'intact' seeds (compare Tabl and 2).

Changes in inorganic electrolytes: Leachates were analyz for K and Na ions (Table 3). Results show that leakage K ions is higher in 'S' seeds compared with that of seeds. Sun-dried seeds have higher leakage of K io compared with shade-dried seeds. 12 ppm/seed K effluxed from sun-dried 'L' seeds with 5.5-fold increa within 30 minutes of imbibition (Table 3). Comparativ slightly higher levels are seen in 'S' seeds. However, sha dried seeds exhibit higher levels at zero time but there is fold increase in K ions levels within 30 minutes imbibition. It means shade-dried seeds have so mechanism of repair/ replacement of damaged membra which needs completion within 10-20 min so the furth leakage is reduced, especially for processes relating K io in this case.

Na ions in leachates remain 5 to 18.5 ppm/seed ran during 30 minutes of imbibition (Table 3). Sun-dried see ('L' and 'S') exhibit 5 ppm/seed levels with 3.5-fo increase in the next 30 minutes of imbibition when the le is 18.5 ppm/seed. On the other hand, Na levels in shad dried seeds remain constant both in 'S' and 'L' seeds at 1 15 ppm/seed throughout the studied period of 30 minut

Changes in organic electrolytes: The effect of drying amino acids leakage during imbibition reveals that 'L' see exhibit insignificantly lesser leakage of amino acids than seeds; the amino acid levels remain between 5 to 15 μmoles/seed for sun-dried (both 'L' and 'S') and 7 to 16 μmoles/seed for shade-dried (both 'L' and 'S') seeds (Ta 4). A 3-fold increase in amino acids (5 to 15.8 μmoles/see levels is seen in sun-dried 'L' seeds during 30 minutes imbibition in 'naked' seeds which is maximum of all th the other drying method for 'S' seeds. Changes in solu sugar contents in leachates of imbibing seeds have al been demonstrated (Table 4). It is seen that 'S' see exhibit higher sugar contents in leachates compared wi the 'L' seeds and sun-dried seeds exhibit greater sug leakage than the shade-dried seeds though these chang

Table 3: Determination of K and Na ions (ppm/seed) in leachates of *S. oleiodes* intact seeds during the initial minutes of imbibition. Standard error is <5% (n = 3).

Time	Sun-dried (L)		Sun-dried (S)		Shade-dried (L)		Shade-dried (S)	
Min.	K	Na	K	Na	K	Na	K	Na
0	12.0	5.0	20.0	5.0	22.0	15.0	24.0	14.0
10	26.0	9.0	36.0	9.0	28.0	15.0	36.0	14.0
20	41.0	13.5	56.0	15.5	36.0	15.0	41.0	14.0
30	69.0	18.5	77.0	18.0	40.0	15.0	54.0	14.0

Table 4: Determination of amino acids (aa) contents (μmoles/seed) and sugar contents (μg/seed) in leachates of *S. oleiodes* 'naked' seeds during the initial minutes of imbibition. Standard error is <8% (n = 3).

Time	Sun-dried (L)		Sun-dried (S)		Shade-dried (L)		Shade-dried (S)	
Min.	aa	sugar	aa	sugar	aa	sugar	aa	sugar
0	5.0	20.00	6.0	23.5	7.0	18.75	10.0	21.75
10	7.0	22.25	8.8	26.1	8.0	20.75	11.7	25.5
20	10.0	25.25	10.6	30.5	9.0	22.50	14.0	28.6
30	15.8	28.0	14.0	33.7	9.5	25.5	16.8	31.4

were statistically insignificant. These results demonstrate that sun-dried seeds are comparatively at higher risks to damage than the shade-dried seeds.

Discussion

Drying of seeds is a final process occurring during later stages of seed development which brings the seed to minimum moisture contents to the level that developed seeds could survive for longer hours and hence can retain vigour and viability, and when the conditions are favourable, can germinate (Bewley and Black, 1986). The results in this study show that loss of germinability is probably due to damage occurred to seeds exposed under direct sun-light rather than slow shade-drying method. Since, drying in shade retains maximum seed germinability (100 and 95 per cent) than the sun-drying method (40 and 0 per cent germination) and it reveals that 'L' seeds can bear severity of drying treatment better than the small 'S' seeds. In the previous studies, germination upto 85 per cent was obtained for *S. oleiodes* seeds which were collected when mature with moisture content of 6.05 per cent. Those seeds were also collected from locations different to that of the present studies and from several different trees in 1996 (Ashraf and Nisar, 1998). In these studies, seeds were collected from a single tree during development and allowed to dry to moisture contents upto 8 per cent. Therefore, it is most probable that the differences in the amounts of electrolytes may be different though the patterns of leakage remain the same. These findings are in consistent with other studies that germination behaviour of seeds varies with the tree, the size of the seed, the moisture contents and desiccation treatments effect the viability and germinability of seeds (Bewley and Black, 1986; Nisar, 1997).

It has already been documented that the lower the vigour or viability of seeds, the greater the proportions of electrolytes leached into the imbibing medium (Adebona and Odu, 1972). Williams *et al.* (1995) have shown that mung bean seeds put to artificial weathering conditions (loss of vigour) exhibited high electrical conductivities of their leachates. Immature seeds (with seed coat) on imbibition release electrolytes rapidly due to active metabolism ongoing at the developing stage and the proportion of seed constituents which are able to be lost into an imbibing medium. Shade-dried seeds also show higher E.C. values than the sun-dried seeds. This may probably be due to the extended loss of solutes or metabolites or volatile electrolytes during the sun-drying method (Williams *et al.*, 1995). Significantly higher rates of electrolytes leakage have been seen during imbibition of radish seeds and sugar pine embryos (Murphy and Noland, 1982). McKersie and Stinson (1980) have also indicated that dehydration induces a reorganization of the membranes in dehydration sensitive seeds.

When the seed testa is removed and seed are allowed to imbibe, leachates contain enhanced levels of electrolytes and hence increased E.C. values are resulted. Sun-dried seeds showed this pattern and E.C. levels almost doubled in 'naked' seeds than the 'intact' ones both for 'L' and 'S'. In contrast, shade-dried 'naked' seeds exhibited comparatively lower E.C. values than the intact seeds, i.e., 17.7 and 23.57 μS/cm/seed for 'L' seeds. These results suggest that the greater E.C. values (almost doubled) in sun-dried 'naked' seeds (compared with 'intact' seeds) were due to lack of seed testa and most of the electrolytes leached directly without a hard barrier to restrict efflux. However, the higher E.C. values in shade-dried 'intact' seeds (compared with 'naked' seeds) indicate that some electrolytes, which were restricted to efflux through the seed coat in the drying process in 'intact' seeds, are leaked through the seed testa. The efflux of inorganic ions especially Na ions (preliminary results) has been seen mainly from the seed testa though K is mainly leached through the embryo and endosperm of seed (McKersie and Stinson, 1980). It is possible that the chemical nature of electrolytes is different in leachates of seeds with and without seed-coats and findings may reflect the severity of these two different drying methods.

The levels of inorganic electrolytes were investigated in 'intact' seeds with sun-dried or shade-dried treatmetns. Sun-dried seeds exhibit higher K and Na levels (upto 77 or 18.5 ppm/seed, respectively) than shade-dried seeds which show upto 54 and 15 ppm/seed, respectively (Table 3). The levels of these electrolytes in leachates emphasize the involvement of seed coat in regulation of efflux of these ions (McKersie and Stinson, 1980). Similar findings have been reported (Simon and Raja-Harun, 1972; Simon, 1984; Ashraf and Hussain, 1998; Ashraf and Nisar, 1998).

Among organic electrolytes, only amino acids and sugars were measured in the leachates. Table 4 shows that the two drying methods have not affected the amino acids leakage during seed imbibition, though the values for shade-dried 'L' seeds were smaller than the rest of the treated seeds. The insignificant differences in the amino acids contents between sun-dried and shade-dried seeds suggest that sun-dried damaged membranes are not highly permeable to soluble amino acids and proteins (McKersie and Stinson, 1980). Similarly, the effects of these two seed drying methods on the soluble sugar contents of leachates of imbibing seeds have been minimal (Table 4). Large seeds possess similar sugar contents profiles (25 to 28 μg/seed) and small seeds possess slightly higher sugar contents (31.4 to 33.7 μg/seed). Greater leakage of sugar contents is probably due to the higher contents of low molecular weight oligosaccharides or monosaccharides than the larger seeds which may contain high molecular weight polysaccharides. McKersie and Stinson (1980) have reported that ungerminated *Lotus corniculatus* seeds leaked 3.6 per cent sugar and 1.4 per cent amino acids during 2 hours of imbibition. These results suggested that efflux of all solutes was not uniform and an increase in membrane permeability was considered.

Changes in phospholipids, fatty acids and sterols in the membrane fractions may contribute to control the fluidity of membrane and micro-environments of the surrounding proteins, so affecting the permeability properties of membranes. These changes may also affect the transport mechanism across the membranes and activities of membrane-bound enzymes (Bewley and Black, 1986). It would be interesting if the amounts of saturated fatty acids, which account for almost 49 per cent of total fatty acids in the plasma membrane, are determined by sophisticated methods and relationship is established with the leakage properties of the membranes (Surjus and Durand, 1996).

In summary, shade-dried method seems preferred method to collect seeds for 100 per cent germination than t seeds dried by sun-dried method since the later meth involves a lot of damage to membrane systems.

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