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Effect of Relative Humidity and Ageing Period on the Quality of Onion Seed

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ABSTRACT

Various moisture contents of onion cv. Ailsa Craig seeds were obtained by equilibrating over mixtures of sulphuric acid and water for five days at 25°C. As relative humidity of the atmosphere increased, the seed moisture content also increased. Weight of seed samples before imbibition varied depending upon their moisture contents. During imbibition, the rate of water uptake was initially higher in the seeds with lower moisture contents and *vice versa*. However, weights of all the seed samples after imbibition and later after drying were in a normal range. Water uptake during imbibition was the maximum in the seeds with lower moisture contents and decreased as moisture content of seeds increased. The final moisture contents of the seeds after imbibition were almost same (45%) in all the seed samples. During accelerated ageing at 42°C and 100% RH, seed moisture contents increased with passage of time. As ageing period was increased, germination percentage, germination speed and seedling growth were decreased, while time to reach 50% germination (T_{50}), percentage of abnormal seedlings and electrical conductivity of the seed leachate increased.

Key Words: *Allium cepa;* Accelerated ageing; Onion; Relative humidity; Seed

INTRODUCTION

Onion (*Allium cepa* L.) seed is one of the shortest-lived seeds among the vegetable crops seeds, rapidly losing viability after harvest unless special precautions are taken in its storage. The loss of viability is more rapid as temperature and seed moisture content is increased (Ellis & Roberts, 1977). Therefore, for extended periods of storage, seed moisture content and storage conditions are the more important factors to control. It is usually agreed that the relative humidity of storage atmosphere is the most critical, as it determines the seed moisture content. Most deteriorative reactions take place in the seed more readily if the moisture content is higher, and consequently it imposes a threat to survival and longevity. Murata *et al.* (1979) observed that the rate of decline in germinability of barley increased as seed moisture and storage temperature increased. They further found that as the seed germinability decreased, the speed of germination also decreased, and the number of abnormalities of roots and shoots increased, usually resulting in seedling death. Halder *et al.* (1983) found a considerable decline in germinability when sunflower seeds were stored for 20 days under high relative humidity (95%), and preimbibition caused more rapid deterioration. Stumof *et al.* (1997) tested 154 commercial seed lots of onion, stored for 1 – 10 years according to the storage period and seed moisture content intervals. They concluded that as moisture content and storage period increases the viability equation becomes less accurate, being particularly useful up to three years. The nature of the relationship between metabolic activity in seeds and their moisture contents is not known. Many studies of the rapid resumption of physiological activity during imbibition are based on timed measurements, but the actual moisture contents at each point are unknown. On the other hand, excessive dryness in stored seeds has also been found to reduce longevity. It has been demonstrated that very dry seeds can be damaged when later imbibed in standard germination test regimes (Ellis *et al*., 1982). It is therefore recommended that dry and very dry seeds should be humidified prior to germination in order to raise their moisture contents slowly in the initial stages (Powell & Matthews, 1979). Zhang *et al.* (1999) found that the ageing process of short-lived onion seeds was delayed by ultra-dry storage. Imbibitional injury of ultra-dried seeds (breakage of embryonic roots) was alleviated by rehumidification before soaking.

Accelerated ageing treatment involves exposing seeds to highly adverse storage conditions of high humidity and high temperature for specific periods of time. The biochemical changes during these artificial ageing conditions are assumed to be similar, if not identical, to those during natural ageing, and the difference being only the speed at which these changes occur (Liklatchev *et al*., 1984). Accelerated ageing is used as a vigour test to determine the resistance of a seed lot to adverse conditions, and hence its suitability for long-term storage. Loss of vigour precedes a decrease in germination potential (Coolbear *et al*., 1984) and induces certain changes at the cellular level, e.g., the leaching of metabolites from seeds (Doijode, 1985). Changes in soybean (*Glycine max*) seeds, which occur during accelerated ageing (41°C, 100% RH)

showed subsequent loss of vigour, a decline in early respiratory activity and increased leakage of electrolytes. These changes with ageing were interpreted as resulting from deteriorative changes in membranes (Parrish & Leopold, 1978). Caneppele *et al.* (1995) stored seeds of onion cv. Pica Quro at 6% moisture content in six types of packaging at 20°C and 50% RH, 30°C and 80% RH or under laboratory conditions for 0 – 12 months. Seed quality, assessed by moisture content, standard germination test, seedling count, accelerated ageing and radical length varied among the treatments. The difference in performance was due to changes in seed moisture content in impermeable packing and to the degree of hygroscopic equilibrium between seeds and their surroundings. Dong *et al.* (1998) observed high seed viability after cold temperature storage in seeds of Welsh onion (*Allium fistulosum* L.) with low or medium moisture contents compared with storage at room temperature of seeds with high moisture content. There was a significant negative correlation between electrical conductivity value and percentage germination and percentage field emergence. Yanping *et al.* (2000) investigated the effect of storage temperature and moisture content on the vigour of Welsh onion seeds. After two years storage, the seed quality declined as storage temperature and seed moisture content increased. It is evident from the literature that the moisture content is the most important factor involved in the deterioration of seeds. The majority of seeds come into an equilibrium between their internal moisture content and the relative humidity of the atmosphere in which they are stored, and as a general rule, within the usual limits of moisture for storage, the life of the seed is halved for each 1% increase in moisture content of the seed (Bass, 1981). It is therefore imperative to determine the moisture content of a seed lot at the time of purchase and before and after storage, as this may determine the quality of the seed lot.

MATERIALS AND METHODS

Seeds of onion (*Allium cepa* L.) cv. Ailsa Craig were obtained from Booker Seeds Ltd., Sleaford, England as a single large aluminium foil pack, containing one kilo of seeds. Seed was divided into small batches, which were stored in closed glass bottles in a cold room at 5°C. Samples of seeds were removed from cold store (5°C) and the seeds, still in their air-tight glass bottles, were kept closed overnight to equilibrate fully with laboratory temperatures before conducting tests.

Atmospheric humidity control. To prepare a series of containers with known atmospheric humidities (Table I), the required amounts of water were weighed in heat-resistant glassware, and then the required amounts of sulphuric acid were added into the respective containers slowly, stirring with a glass rod (Solomon, 1951). Prepared sulphuric acid solutions were added immediately into the desiccators, covered with lids carefully and sealed with lanoline. The following day, batches of onion seeds (2 g each) were spread evenly in 90 mm Petri dish lids and placed on glass stands in these desiccators separately. Freshly-prepared acid dilutions were introduced from time to time to replace acids which might have absorbed water from the atmosphere on opening the desiccators, and also from the contained seeds. Seeds were brought to equilibration during 5 days in the required relative humidities at 25 ± 2°C to obtain a series of seed moisture contents for experimental purposes (Table I). Seed moisture contents were determined as described below.

Table I. The amounts of concentrated sulphuric acid required to be added to distilled water to produce 100 g of final solution, giving headspace atmospheres of known relative humidities (RH) (Solomon, 1951) and moisture contents of onion seed samples equilibrated for five days at 25±2°C in atmospheres of these RH

Sulphuric acid (g)	Relative humidity (%) at 25°C	Seed moisture content (%)
100.00	0	2.92
69.44	5	4.09
64.45	10	4.89
57.76	20	6.03
52.45	30	6.87
47.71	40	7.89
43.10	50	8.88
38.35	60	9.91
33.09	70	11.63
26.79	80	13.29
17.91	90	17.98
0.00	100	26.42

Determination of seed moisture content. Two to four replicates of 50 seeds were weighed and evenly spaced in 90 mm Petri dishes placed in an oven at 103 ± 2°C for 17 ± 1 h (ISTA, 1985), cooled in a silica gel container for 15-30 min, after which they were reweighed. The moisture content was expressed as a percentage of the wet weight basis, and calculated as:

$$Percentage\ moisture\ content\ (\%m.c.) = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where, M_1 is the weight of dish, M_2 is the weight of dish and its contents before drying and M_3 is the weight of dish and its contents after drying.

Water uptake during imbibition. Seed samples were counted, weighed, and then imbibed in Petri dishes on double sheets of filter paper moistened with 10 mL of distilled water in an incubator at 20 ± 2°C. Dishes were removed from the series at intervals, and the seeds were drained and surface water removed by blotting between sheets of paper towel. The seeds were then immediately weighed, and the percentages of water taken up were recorded and calculated as:

$$Percentage\ \ water\ \ uptake\ = \frac{W_1 - W_2}{W_2} x\ 100$$

Where, W_1 is the weight of the seeds after imbibition and W_2 is the weight of seeds before imbibition.

Accelerated ageing test. Small amounts of onion seeds were placed in open Petri dishes on a stand above water in a glass desiccator with an air-tight lid. The desiccators were then incubated for the required period e.g., 12, 24, 36, 48 and 60 h at 42°C (ISTA, 1987). The seeds were then removed, and used in standard germination test. Germination percentage, germination speed, time to reach 50% of final germination, shoot length, root length, overall seedling lengths and number of abnormal seedlings were recorded. Electrical conductivity (EC) of the seed leachate was also measured and recorded.

Seed germination. Germination percentages, using four replicates of 50 seeds, were determined by placing the seed samples in 90 mm Petri dishes on 90 mm filter papers (Whatman # 1), moistened with 4 mL of distilled water in accordance with the International Seed Testing Association (ISTA, 1985) rules. Seeds were distributed evenly within each dish. Each Petri dish was covered with its lid then placed in an incubator maintained at 20 ± 2°C and adjusted to give 12 h of fluorescent light illumination alternating with 12 h of darkness. Each Petri dish was watered daily according to its requirement with distilled water. Germination in terms of radicle emergence (at least 2 mm) was assessed each day from the second day until no further radicle emergence was noted on two successive days. Germination capacity was expressed as a percentage of all seeds with fully emerged radicle in a given batch. The average germination speeds were derived from the formula of Kotowski (1926).

$$Germination\ speed\ (G.S.) = \frac{\sum n}{\sum (n \times D_n)} x\ 100$$

Where, n is the number of seedlings germinated on day D_n. D_n is the number of days from sowing, corresponding to n, and the highest (G.S.) is the greatest speed.

Time to reach 50% of final germination (T_{50}) was calculated by using the formula of Coolbear *et al*. (1984):

$$T_{50} = t_i + \left[\frac{(N+1)/2 - n_i}{n_j - n_i} \right] x\ (t_j - t_i)$$

Where, $n_i < (N+1)/2 < n_j$. N is a number of seeds germinated, and n_i and n_j are total number of seeds germinated by adjacent counts at times t_i and t_j, respectively.

Seedling growth and abnormalities. After 10 days from the seed germination, average shoot lengths, root lengths, and overall seedling lengths were measured using a ruler, and recorded in mm. The seedlings were also examined for their morphology. The abnormal seedlings in each replicate were counted and their percentages calculated. Seedlings are classed as abnormal when one or more of the essential structures fails to develop normally because of previous damage to the embryo, or when development as a whole is weak or out of proportion compared with that of a normal seedling germinated at the same time and in the same conditions.

Electrical conductivity test. Four replicates of onion seeds, 100 seeds in each replicate, were placed in 100 mL beakers separately, each containing 75 mL distilled water. The seeds were gently stirred to ensure that all seeds were completely immersed and evenly distributed. The beakers were placed in an incubator at 20 ± 2°C. After 24 h, the seeds were gently stirred and the conductivity of the soaking water was measured without filtration using a digital conductivity meter (JENWAY, Model 4070).

RESULTS AND DISCUSSION

The effect of relative humidity of the storage atmosphere on seed moisture content. After determining the moisture content of onion seeds, separate samples were placed for five days in closed glass vessels containing acid/water mixtures giving a wide range of relative humidities (0 – 100%) at 25°C. During this period, the seed samples attained different moisture contents. Seeds placed at 0% RH had 2.92% moisture content. As relative humidity of the atmosphere increased, the moisture content of the seeds also increased, finally reaching to 26.42% at 100% RH (Table I). Ellis *et al.* (1989) have studied in detail the moisture contents of various seeds and their atmospheric storage humidities. When orthodox seed tissues become dried, physiological activity is reduced, and resumes as the tissue is rehydrated. Low moisture content is beneficial for the storage of the seeds of most agricultural crops. Generally, the rate of deterioration will be slow if the seeds are stored at lower relative humidity and temperature, but even so, it is almost impossible to predict with any accuracy the probable longevity of any particular batch of seeds when placed in storage. More moisture allows them to respire, subsequently shortening their storage life, causes spoilage through extraneous water and also by the metabolic water produced in respiration. This applies also to the great majority of seeds from dry-climate plants. In the present study as the relative humidity of storage atmosphere increased, the moisture content of the seeds also increased due to the equilibrium between seed and environmental moisture content.

Effect of seed moisture content on water uptake during imbibition. Replicate samples of the seeds held at 25°C in different relative humidities were then set to imbibe and their water uptakes were recorded at frequent intervals up to 16 hours (Table II). As expected, the rate of water uptake was most rapid into the very dry seeds, and became slower

Table II. Water uptake (%) at 2-hour intervals by untreated onion seeds, and by seeds with different initial moisture contents, having been held five days at 25°C at a range of relative humidities. Figures are the means of three replicates of 50 seeds each

Initial seed m.c. (%)	Water uptake (%) after imbibition at 20 ± 2°C											
	15 min	30 min	45 min	60 min	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h
Untreated												
8.54	**6.83**	**8.64**	**11.50**	**13.24**	**17.70**	**26.96**	**33.49**	**39.39**	**41.50**	**46.51**	**51.46**	**52.68**
	Seeds held 5 days at 25°C in a range of relative humidities before imbibition											
2.92	8.16	10.82	11.62	20.10	24.38	32.99	42.41	46.11	48.74	52.76	57.22	61.05
4.09	7.33	8.61	11.68	16.67	21.67	31.00	37.69	45.23	48.28	55.73	56.85	58.25
4.89	8.42	9.14	13.07	13.57	21.61	29.56	36.04	42.36	47.69	53.14	55.56	56.00
6.03	5.91	9.62	12.06	13.73	19.23	28.92	35.29	45.23	50.24	54.19	54.33	58.21
6.87	5.53	7.39	11.37	13.43	19.70	27.96	34.41	40.67	50.50	52.88	53.69	56.37
7.89	6.50	8.74	10.89	13.11	18.72	27.83	38.31	43.35	48.08	53.85	55.45	55.56
8.88	5.74	8.87	10.33	12.50	18.91	28.08	34.83	41.35	48.31	48.33	51.67	51.69
9.91	4.29	8.65	8.91	13.24	24.26	28.23	35.85	41.06	45.54	49.76	52.17	52.86
11.63	5.91	9.76	11.44	13.46	17.41	28.57	32.39	42.86	43.66	50.47	52.86	53.62
13.29	4.27	7.32	7.32	10.37	18.93	30.81	32.08	45.77	45.85	53.14	51.05	52.94
17.98	2.79	8.21	8.33	12.50	18.78	27.36	27.36	40.65	42.65	48.80	46.98	50.23
26.42	2.47	2.86	4.26	4.41	7.06	15.05	16.87	18.58	24.21	27.55	27.39	29.58

Table III. Initial moisture contents, and extra water taken up during imbibition by untreated onion seeds, and also seeds held five days at 25°C at a range of relative humidities. Figures are the means (± SD) of two replicates, each of 100 seeds

Relative humidity (RH)	% m.c. before imbibition	Weight before imbibition (mg)	Weight after imbibition (mg)	Weight after drying (mg)	Water uptake (%) during imbibition	% m.c. after imbibition
Untreated	**8.54**	**410.0 ± 0.5**	**695.0 ± 0.5**	**380.0 ± 0.0**	**68.29**	**45.34**
	Seeds held 5 days at 25°C in a range of relative humidities					
0 %	2.92	385.0 ± 1.0	679.0 ± 0.5	375.0 ± 0.5	76.04	44.77
5 %	4.09	398.0 ± 0.5	697.0 ± 0.0	372.0 ± 0.5	77.57	46.63
10 %	4.89	398.5 ± 0.0	717.5 ± 0.5	390.0 ± 0.0	77.27	45.61
20 %	6.03	409.0 ± 1.0	696.0 ± 0.	377.0 ± 0.0	71.97	45.83
30 %	6.87	403.0 ± 1.5	654.0 ± 0.0	366.0 ± 0.5	64.59	44.04
40 %	7.89	430.0 ± 0.5	718.0 ± 0.5	394.0 ± 0.0	67.46	45.13
50 %	8.88	411.0 ± 1.0	679.0 ± 0.5	377.0 ± 0.0	64.60	44.48
60 %	9.91	409.0 ± 0.0	675.0 ± 1.0	372.0 ± 0.5	64.17	44.89
70 %	11.63	420.0 ± 0.5	679.0 ± 0.5	374.0 ± 0.5	60.99	44.92
80 %	13.29	421.0 ± 0.5	683.0 ± 0.5	379.0 ± 0.0	58.92	44.51
90 %	17.98	453.0 ± 0.5	684.0 ± 0.0	377.0 ± 0.5	49.79	44.88
100 %	26.42	517.0 ± 1.5	674.0 ± 0.5	375.0 ± 0.5	31.41	44.36
Mean	-	-	-	-	-	45.03

as the seed moisture content increased. It is also seen that the total amount of extra water taken up during 16 h imbibition period was inversely related to the initial water content of each seed sample. Weight of the seed samples before imbibition varied depending upon their moisture contents or the relative humidity of the atmosphere in which they were kept. The minimum weight of the seed samples was recorded in those kept on 0% RH and having only 2.92% moisture content. The maximum seed weight was recorded in those kept on 100% RH and had moisture content of 26.42%. The weight of other seed samples was in the middle of these limits, depending upon the moisture contents. This difference in seed weights was due to the amount of water present in these seeds. Weights of all the seed samples increased after imbibition, due to the absorption of water. Weights of these seed samples, recorded after drying, were decreased due to elimination of water and were in a normal expected range. However, amounts of water uptake during imbibition varied depending upon the seed moisture contents. The maximum water was absorbed by the dry seeds and minimum by those with the highest moisture content (26.42%). In spite of the differences in initial seed moisture contents, and in the rates of water uptake during imbibition, the final seed moisture contents were very close to a common amount within the limits of experimental error. This figure was around 45% moisture at full imbibition (Table III).

The imbibition of water into the dry seed is an essential part of germination. The imbibition of water converts the seed from a quiescent body with a very low or non-detectable respiratory rate into a metabolising organism, active in respiration and in biosynthesis, and capable of growth (Mayer & Poljakoff-Mayber, 1974). When the rates of water uptake were examined in the present study, it was found that low (2.9%), medium, and high (26.4%) moisture content seeds took up water at

Table IV. Effect of accelerated ageing treatment for increasing times at 100% RH and 42°C on onion seeds. Seed moisture content of each sample was determined immediately after removal from the ageing treatment and before testing. Figures are the means (± SD) of four replicates of 50 seeds for all germination tests, 100 seeds for electrical conductivity, and 10 seedlings for seedling lengths

Aging period (hours)	Seed moisture (%)	Germination			Seedling length (mm)			Reduced growth (%)	Abnormal seedlings (%)	EC / 100 seeds (µS/cm)
		final %	speed	T_{50}	shoot	root	overall			
0	10.39	91±1.7	12.63	3.28	43.0	20.8	63.8	-	10.87	44 ± 0.0
12	16.24	54±4.1	11.31	5.06	41.0	17.8	58.8	7.84	38.71	46 ± 2.0
24	18.60	42±1.9	11.34	5.19	37.6	13.0	50.6	20.69	43.48	50 ± 2.0
36	23.14	32±3.5	10.95	6.38	30.2	8.0	38.2	40.13	58.82	58 ± 2.0
48	24.79	11±1.9	10.69	6.63	17.3	5.3	22.6	64.58	66.67	61 ± 1.7
60	25.50	04±0.9	10.62	6.00	13.3	4.3	17.6	72.41	100.00	67 ± 3.3

different rates, especially in the early stages, but all arrived at closely approximating final moisture contents (Tables III). Water uptake during germination is generally triphasic (Bewley & Black, 1985). This pattern of water uptake during germination was confirmed with tomato seeds by Haigh and Barlow (1987). Phase I is rapid due to the very steep gradient of water potential between the seed and its environment. Phase II is a period of varying duration, when little change occurs in seed water content or when the seed water potential is in equilibrium with the water. In phase III, water uptake again occurs because of cell enlargement occurring during the onset of radicle extension, without change in water potential or the lowering of embryo water potential (Haigh & Barlow, 1987). Air-dry cotyledons of soybean imbibe water rapidly for 10 min followed by a slower, linear rate of uptake (Parrish & Leopold, 1977). The water uptake pattern of *Brassica napus* seeds shows rapid uptake, followed by a short plateau, then breaking of the testa and irreversible radicle growth (Schopfer & Plachy, 1984). McCormac and Keefe (1990) found that dry cauliflower seed *(Brassica oleracea)* showed an immediate rapid phase of imbibition upon the addition of water.

Accelerated ageing test. The accelerated ageing test was originally devised as a means of obtaining information about the suitability of a seed lot for long-term storage (Buchvarov & Gantcheff, 1984) on the assumption that if the seeds are able successfully to withstand the effects of raised moisture content at warm temperatures, the seed lot is likely to retain its viability for a long period in good conditions of storage. Low quality seeds deteriorate more rapidly than high quality seeds under these conditions. The test is considered standardized and correlates with field emergence under a variety of seedbed conditions (Egli & TeKrony, 1996). In the present study, samples of onion seeds (initial moisture content 10.39%) were withdrawn from storage, and subjected for increasing periods of time to 42°C in a high humidity (100% RH) container. Table IV shows that the seed moisture content gradually increased during the ageing treatment. It increased from the normal storage moisture content (10.39%) to a probable maximum (25.50%) for seeds in equilibrium with a highly saturated atmosphere during a period of two and a half days (Table IV). An important consideration for the present investigation was that when the seeds were placed at various atmospheres of relative humidities (0 – 100% RH) at 25°C, the moisture contents of the seed samples became equilibrated to various levels, including drying down to low levels and raising to the maximum of 26.42%, during a period of five days (Table I). Even though the seeds were held at 25°C rather than at the 42°C level required by an accelerated ageing treatment, this is in itself a form of stress.

There was a severe decline in final germination percentage as ageing period increased, even after only 12 h of the ageing treatment, and viability was reduced to 4% after 60 h. Compared with seeds not subjected to accelerated ageing, not only the final germination percentages but germination speed was greatly reduced as well with increasing periods of accelerated ageing. Table IV further shows that there was increase in the time taken to reach 50% of final germination indicating the effects of high temperature and high humidity during accelerated ageing. The decrease in germination capacity is a function of the age of seeds. When seeds are maintained experimentally under conditions of high humidity and temperature, the rate of ageing becomes greatly accelerated, and their viability falls rapidly with time. It is generally accepted that the decline in viability of "naturally" or artificially aged seeds results mainly from damage to nucleic acid and the deterioration of cellular membranes (Dawidowicz-Grzegorzewska & Podstolski, 1992). The damage accumulates progressively both during normal seed storage and during accelerated ageing, and is affected by seed moisture content and temperature. The extent of damage and its reversibility depend on the efficiency of metabolism and on the ability to restore or replace deteriorated membranes (Elder *et al.*, 1987). These findings are in close conformity with the results of Murata *et al.* (1979), Halder *et al.* (1983) and Caneppele *et al.* (1995). In the present study, growth of the seedling was also severely affected and there was 72.41% growth reduction in the seedlings produced from the seeds subjected to accelerated ageing for 60 h, exhibiting the harmful effects of seed ageing. The percentage of abnormal seedlings also increased with the increase in ageing period and all the seedlings resulting from 60 h aged seeds were abnormal (Table IV). Similar results have been reported by Murata *et al.* (1979) in case of

barley seeds. When the conductivity of the seed leachates was measured, it increased progressively with extended times of accelerated ageing of seeds (Table IV), indicating the seed deterioration. The conductivity test is used for the measurement of electrolytes leaking from seed tissues during imbibition. Since low quality seeds often leak more exudates than high quality seeds during the first hours of imbibition, the conductivity test has become an important approach to monitoring seed quality (Hampton, 1995; McDonald, 1998). In the present study, as the ageing period increased the electrical conductivity of the seed leachate also increased. These results are in accordance with the findings of Parrish and Leopold (1978) and Ram and Wiesner (1988) who showed that artificial ageing increased membrane permeability and enhanced loss of seed electrolytes in soybean and wheat, respectively.

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