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Evaluation of Physiological Quality of Onion Seed Stored for Different Periods

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ABSTRACT

Seeds of the onion cv. Ailsa Craig stored for 1 to 4 years were evaluated for their physiological quality. Water uptake during imbibition was maximum in fresh seeds having the lowest initial moisture content and was minimum in those stored for two years and had the highest moisture content before imbibition. However, the rate of water uptake was initially high for the seeds stored for two years and low for fresh seeds. Germination percentage, rate of germination, seedling length and fresh weight were higher in seed lots stored for one or two years compared with those stored for three or four years. In cold germination test, the seeds stored for 1 year gave the best performance in respect of seed germination percentage, number of normal seedlings, seedlings dry weight and growth rate.

Key Words: *Allium cepa;* Cold germination test; Onion; Seed quality; Seed viability; Seed vigour

INTRODUCTION

The onion (*Allium cepa* L.) is a crop of major economic and dietary importance in all parts of the world. In Pakistan, the crop is grown on an area of 105.3 thousand hectares with a total production of 1496.2 thousand tonnes (Anonymous, 2001). Compared with many other crops, the onion has a fairly complex life cycle involving several distinct development phases. It is generally known that onion seed is one of the shortest-lived seeds of the common vegetable crops, rapidly losing viability after harvest unless special precautions are taken in its storage. It is therefore generally recommended that only fresh onion seed should be used for crop production (Riekels *et al*., 1976), and only seed of high germination percentage should be sold. The percentage and rate of germination of onion seeds also vary considerably among seed lots (Bedford & MacKay, 1973) and this leads to difficulties in establishing optimum plant populations in the field. It has long been known that the factors, which have the greatest influence on the longevity of seeds in storage are moisture, temperature and oxygen partial pressure. It is usually agreed that the moisture content of the atmosphere is the most critical factor, a rise in air moisture being more damaging than raising temperatures. However, it has been 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; Ellis *et al*., 1985a & b). Several other workers have also attempted to store the onion seeds under different storage conditions for different periods (Caneppele *et al.,* 1995; Pandey, 1996; Stumof *et al.,* 1997; Dong *et al.,* 1998; Yanping *et al.,* 2000). Therefore, it becomes necessary to evaluate the physiological quality of commercially available onion (*Allium cepa* L.) seed stored for different periods for proper crop stand and performance in the field.

MATERIALS AND METHODS

Seed source and storage. Seeds of onion (*Allium cepa* L.) cv. Ailsa Craig were obtained from Booker Seeds Ltd., Sleaford, England as single large aluminium foil packs, each containing 1 kg of seeds. Seeds from five different harvests (stored for 0 – 4 years) were obtained, and each seed lot was divided into smaller batches which were stored in closed glass bottles in a cold room at 5°C. Seed samples were removed from cold room and the seeds, still in their air-tight glass bottles, were kept closed overnight to equilibrate fully with laboratory temperatures before conducting tests.

Determination of seed moisture content. Two replicate samples, each of 100 seeds were taken, evenly spaced in 90 mm glass Petri dishes and placed in an oven at 103±2°C for 17 ± 1 h, cooled in a silica gel container for 15–30 min, after which they were reweighed. The moisture content was expressed as a percentage of their wet weight in accordance with the International Rules for Seed Testing (ISTA, 1985) 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 the imbibition period. 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.

Germination tests. Germination potential of the onion seeds was estimated (ISTA, 1985). Germination percentages, using four replicates of 50 seeds, were determined by placing the seed samples in 90 mm Petri dishes on filter papers (Whatman No. 1) moistened with 4 mL of distilled water. Seeds were distributed evenly within each dish. Petri dishes were covered with their lids and then placed in a Gallenkamp forced-air incubator, maintained at 20±2°C and adjusted to give 12 h photoperiod by fluorescent light illumination. Each Petri dish was watered daily with an amount of distilled water according to its requirement. 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.

Germination speed. The germination speed of the seed lots was 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 highest (G.S.) is the greatest speed.

Time to reach 50% germination (T_{50}): Time to reach 50% of final germination (T_{50}) was calculated from 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 the 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. Seedling length was measured using a ruler in mm. To record seedling fresh weight, these were counted, cut free from their cotyledons and weighed while still moist. Their weights were recorded in mg. Seedlings were then placed at 80°C in an oven for 24 h for drying. Samples were cooled in an air-tight closed glass desiccator containing silica gel for 15 – 30 min, then reweighed to record seedling dry weights.

Tetrazolium viability test. 1 g of 2,3,5-triphenyl tetrazolium chloride (TTC) was dissolved in 100 mL of distilled water to make a 1% solution of the tetrazolium salt. The test was conducted with two replicates of 100 seeds soaked in distilled water for 18 - 20 h. Each seed was cut longitudinally without completely separating the two halves. The seeds were submerged in 1% TTC solution for at least 8 h at 35°C in darkness, after which the staining patterns were recorded.

Cold germination test. Four replicate samples of 50 seeds were placed in closed plastic boxes in rows on paper tissues moistened with distilled water, at 10°C for seven days, then shifted for another four days to 20±2°C in an incubator. Over a period of 11 days, germination percentage, the numbers and percentages of abnormal seedlings, and the types of abnormalities were observed. The seedlings were classified as normal, weak normal or abnormal. 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. The abnormalities of onion seedlings were recognised as listed in Section 5.8.2 (ISTA, 1966). Average dry weights of normal seedlings were also recorded as described previously. Seedling growth rate (SGR) was calculated from the formula:

Seedling growth rate (SGR) = e/d

Where, d is total number of normal seedlings = a - (b + c), a is the seeds planted, b is the dead seeds, c is abnormal seedlings and e is seedling dry weight (mg).

RESULTS AND DISCUSSION

Initial moisture content of seeds. Replicate samples of the onion seeds of different ages were withdrawn at random from the container in which they were delivered or in the case of older seed lots, from the containers in which they had been stored. These were weighed, and their water contents determined on wet weight basis (ISTA, 1985). The lowest moisture content (8.54%) was recorded in the fresh seeds, which was probably due to their proper procurement and being vacuum-stored until they were tested. The highest moisture content (11.13%) was recorded in case of the seed stored for 2 years. Moisture content of the other seed lots varied from 10.08 – 10.39% (Table I). It was noticed that the most recent seed lot had the lowest seed moisture content, while the older ones were all in the range of 10 – 11%. This moisture content was determined with seeds on removal from their commercially sealed metal foils bags, which had been at least partially evacuated of air. All the older seed lots had earlier been transferred from foil bags to closed bottles, in which they had been stored, and in retrospect, it would obviously have been better to keep the seeds unopened in their original foil bags until they were required for use.

Moisture contents needed for full imbibition. Replicate samples of onion seeds were tested for their moisture contents and the amounts of water required for full imbibition were determined. The results presented in Table I, show that the seed moisture content after imbibition did not differ much among the seed lots tested. However, the percentages of water taken up differed depending upon the seed lot (age and/or source). Water uptake during imbibition was maximum (68.29%) in fresh seed, which contained the lowest moisture content before imbibition. The minimum water uptake (59.39%) was recorded in the seed stored for two years and had the highest moisture content before imbibition. Age, size and the initial moisture content of seeds may cause differences in the process of imbibition, and the amount of water required for the initiation of metabolism and germination. The imbibition of water into the dry seed is an essential part of germination; which has also been documented as a potentially hazardous period (Woodstock, 1988). The imbibition of water converts the seed from a quiescent body with a very low or non-detectable respiratory rate into a metabolizing organism, active in respiration and in biosynthesis, and capable of growth (Mayer & Poljakoff-Mayber, 1974). The amounts of water taken up by the seeds during imbibition prior to germination in the present study appeared within the expected range depending on their initial moisture contents.

Rate of water uptake during imbibition. Three replicates of 50 seeds from each onion seed lot were weighed and imbibed with distilled water over a period of 16 h, and the amounts of water taken up at increasing times during imbibition were determined. Table II shows that the rate of water uptake was initially high in case of seeds stored for two years and was low in fresh seeds. It is interesting to note that initial moisture contents of these lots were also in the same pattern. The final amounts of water taken up by onion seeds of the four different sources stored since purchase at 5^{o}C were generally higher when compared with the uptake by fresh seeds, determined immediately on purchase, except in case of the seeds stored for two years, which showed a smaller water uptake possibly due to their higher initial moisture content. When dry seeds are plunged into water, they imbibe water rapidly in the first few minutes, followed by a slower phase of imbibition until they become fully hydrated (Parrish & Leopold, 1977; Powell & Matthews, 1979; Murphy & Noland, 1982). Air-dried cotyledons of soybean imbibe water rapidly for 10 min followed by a slower, linear rate of uptake (Parrish & Leopold, 1977). The water uptake pattern for *Brassica napus* seeds shows rapid uptake, followed by a short plateau, then breaking up of the testa and irreversible radicle growth (Schopfer & Plachy, 1984). McCormac and Keefe (1990) found that dry seeds of cauliflower (*Brassica oleracea*) showed an immediate rapid phase of imbibition upon the addition of water. However, the rate of water uptake during imbibition may vary among the seed lots depending upon the harvesting, procurement and storage conditions.

Seed germination. Samples from the four onion seed lots stored for 1, 2, 3 and 4 years at 5^{o}C since purchase were tested for germination potential, germination speed, and time to reach 50% of the final germination percentage. The highest germination percentage was recorded in case of seed lot stored for two years, followed by that stored for 1 year. While, the lowest germination percentage was recorded for the seed lot stored for four years, followed by that stored for three years (Table III). This indicates that as storage time increased, germination percentage was affected. In the present study, the two older seed lots (stored

Table I. Initial moisture content and extra water taken up during imbibition by onion seed batches of different ages stored at 5^{o}C. Figures are the means (± SD) of two replicates, each of 100 seeds

Storage period or seed age	% m.c. before imbibition	Wt. before imbib. 100 seeds (mg)	Weight after imbibition (mg)	Weight after drying (mg)	% m.c. after imbibition	% water uptake during imbib.
Fresh seed	8.54	410.0 ± 0.5	695.0 ± 0.5	380.0 ± 0.0	45.34	68.29
1 year	10.08	437.5 ± 1.5	698.5 ± 0.5	389.0 ± 0.0	44.31	60.66
2 years	11.13	446.0 ± 1.5	708.0 ± 1.0	393.5 ± 0.5	44.42	59.39
3 years	10.39	388.5 ± 0.5	635.5 ± 0.5	342.5 ± 0.5	46.11	65.03
4 years	10.30	394.5 ± 1.5	648.5 ± 1.5	352.5 ± 0.5	45.64	64.73

Table II. Water uptake (%) after each 2-hour interval by fresh onion seeds, compared with seeds of different ages stored at 5^{o}C. Figures are the means of three replicates of 50 seeds each

Storage period	Water uptake (%) after imbibition at 20 ± 2^{o}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
Fresh seed	6.83	8.64	11.50	13.24	17.70	26.96	33.49	39.39	41.50	46.51	51.46	52.68
1 year	7.90	10.40	12.30	14.00	20.80	29.90	36.70	41.10	48.00	48.90	51.90	54.30
2 years	10.27	12.33	12.79	15.07	22.37	30.59	35.62	40.41	44.98	46.80	50.91	51.83
3 years	8.10	11.39	11.39	16.46	22.78	30.38	36.71	44.05	47.60	46.84	53.92	56.96
4 years	8.56	11.84	12.34	17.63	24.69	33.50	40.55	45.59	47.10	48.61	54.41	58.19

for 3 and 4 years) experienced delays both in emergence, and in rate of germination as shown by the germination speed and T_{50} (Table III). These results are in accordance with the findings of Bedford and MacKay (1973), who found that the percentage and rate of germination of onion seeds vary considerably among seed lots. In the present study, the germination potentials and speeds of germination reflected the ages of the seed lots, declining with age, with the exception that the seed lot stored for two years, rather than the most recent, was apparently of the best quality. This could have been due to better harvest conditions and handling of this particular seed lot.

Seedling lengths and fresh weights. The seedlings resulting from the germination tests were maintained for a period of seven days to measure their lengths and fresh weights. Table III again shows decreased performance achieved by the seeds those had been stored longer than one year. The tallest seedlings were produced from the seeds stored for one year followed by that stored for two years, while the storage period of three years resulted in minimum seedling length. However, the highest fresh weight was recorded in seedlings resulting from seeds stored for two years followed by those from the seeds stored for one year. The minimum fresh weight was recorded in seedlings produced from seeds stored for three years. This indicates that the vigour was reduced with the increase in storage period (Bedford & MacKay, 1973), depending upon the seed lot.

Tetrazolium viability test. Samples of the seeds from the different seed lots available were subjected to the tetrazolium test. The seed samples tested showed good viability, with slight decreases for the two older harvests (Table III), reflecting the age of seed. The tetrazolium test is a biochemical test, which differentiates the living and dead tissues of a seed by the presence or absence of a red stain known as formazan. A germination test can give an apparently erroneous result because some seeds may be in a state of dormancy, but the tetrazolium test includes all the seeds, which are alive either active or dormant. The test has also been used in the harvest season, when it is necessary to know whether or not frost has caused damage to seed crops (Porter *et al.*, 1947), and whether seeds have been damaged during harvesting and threshing. Moore (1972) stated that the seed production and marketing industries, educational establishments and legal control agencies need a method such as the tetrazolium test that yields results faster than those provided by germination or growth tests.

Cold germination test. Laboratory germination tests are performed in optimum conditions, but field conditions are seldom optimal. Some indications of the probable performance of a seed lot in the field may be obtained by subjecting the seeds to some degree of stress before or

Table III. Germination potential and seedling growth after seven days of germination, from onion seeds of different ages. Seeds were also tested for their viability by tetrazolium staining. Figures are the means (± SD) of four replicates of 50 seeds for germination test, 10 seedlings for seedling lengths and seedling fresh weights and two replicates of 100 seeds each for tetrazolium test

Storage period or seed age	Final germination %	Germination speed	Germination T_{50}	Seedling length (mm)	Seedling fresh weight (mg)	Seed viability % (tetrazolium test)
1 year	90.5 ± 2.6	19.92	2.69	68.5	27.8	98
2 years	96.5 ± 1.7	19.93	2.68	60.6	28.5	99
3 years	89.0 ± 1.0	18.78	3.23	52.1	23.0	92
4 years	88.5 ± 4.6	18.80	3.24	55.6	24.5	94

Table IV. The effect of applying cold germination test to onion seeds of different ages on their germination and seedling abnormalities over a period of 11 days. Figures are the means (± SD) of 4 replicates of 50 seeds each

Storage period or seed age	Germination % (all at 10 °C)	Germination % (4 days at 20 °C)	Seedling abnormalities %	Types of abnormalities (%) Ia	Ib	Ic	IVc	Va	Ve	Vg	VIa
1 year	51.0 ± 8.4	98.0 ± 1.4	13.2 ± 3.2	5.0	3.6	3.0	-	1.6	-	-	-
2 years	90.5 ± 3.0	96.0 ± 2.8	10.8 ± 1.8	4.6	2.6	1.0	2.0	-	0.6	-	-
3 years	71.5 ± 1.6	83.5 ± 3.2	15.0 ± 3.9	2.0	2.6	2.0	2.4	1.0	-	-	5.0
4 years	66.5 ± 6.6	87.0 ± 5.4	17.4 ± 7.0	2.0	0.6	1.6	1.0	0.6	1.6	2.4	7.6

Table V. Growth rates of seedlings from onion seeds of four different ages (after applying cold germination test). Figures are the means (± SD) of four replicates

Storage period or seed age	No. of seeds sown (a)	No. of dead seeds (b)	No. of abnormal seedlings (c)	Normal seedlings (d) = a – (b + c)	Seedlings dry weight (mg) (e)	Seedlings growth rate (SGR) = e/d
1 year	50	3.2 ± 1.5	6.3 ± 0.8	40.5 ± 1.1	92.8 ± 4.8	2.29 ± 0.10
2 years	50	2.2 ± 1.2	7.0 ± 3.1	37.8 ± 1.5	84.8 ± 2.8	2.25 ± 0.04
3 years	50	8.7 ± 1.3	7.0 ± 2.5	34.3 ± 2.2	68.8 ± 2.1	2.01 ± 0.09
4 years	50	5.2 ± 2.3	5.3 ± 1.7	39.5 ± 1.1	78.0 ± 4.5	1.98 ± 0.10

during the laboratory germination period. The cold germination test in which the temperature is reduced to 10°C for a period of seven days after imbibition (variable for other species), is a means of imposing a degree of stress upon the germinating seeds. A comparison of germination percentages in Tables III and IV show that all seed sources had slightly changed their relative positions, with the best performance in the cold test shown by the seed stored for two years. Seeds stored for 3 and 4 years were in the middle and seed stored for one year gave the poorest performance, when seed germination was carried out at 10°C. However, when germination was tested at 10°C with 4 days at 20°C, the situation was different. In this case, the seed stored for one year, followed by that stored for two years showed better performance. Seedling abnormalities increased slightly with the age of the seed lot (Table IV). The highest abnormalities were recorded in the seedlings resulting from the oldest seed (stored for four years) and the lowest in those from seed stored for two years. This indicates that as the seed age is increased, the quality of seed decreased. The following recognisable abnormalities of onion seedlings were found, as listed in Section 5.8.2 (ISTA, 1966) and their percentages resulting from the seed lots studied are given in Table IV.

Ia no primary root

Ib primary root short and stunted

Ic primary root short and weak or spindly

IVc poorly developed leaf-like cotyledon without a definite bend or "knee"

Va decayed cotyledon

Ve decayed primary root

Vg completely decayed seedling (an additional category to those in Section 5.8.1)

VIa short and weak, or spindly, or watery seedling

Rapid and uniform emergence is an important component of the definition of seed vigour. A test, in which dry matter production by the normal seedling fraction from a germination test gives a comparison of vigour between seed lots. The results, presented in Table V, shows that number of normal seedlings, their dry weight and growth rate was higher in the youngest seed lot (stored for one year) compared with all older seed sources, which showed declining growth rates with increase in age. The complete loss of ability to germinate is the ultimate result of seed deterioration. However, before that state is reached, various seeds in a population lose vigour at different rates. A slower and uneven rate of germination, poor seedling emergence, slower seedling growth, and an increase in the number of abnormal seedlings can be seen in a seed lot as increased sensitivity to adverse storage conditions, particularly when seeds are sown under adverse field conditions.

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