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Comparative assessment of onion seed longevity under ambient storage and artificial ageing conditions

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ABSTRACT

A study was carried out to assess the storage potential of onion genotypes under natural and accelerated ageing conditions. Freshly harvested seeds of thirty onion genotypes were stored in ambient conditions for nine months and subjected to accelerated ageing for 3 and 6 days (42°C and 75% RH). Significant effects of seed ageing on germination per cent, seed vigour index, mean germination time and germination index were recorded. Germination per cent ranged from 56.0- 96.0 in control seeds, which declined to 31.0-82.0 and 18.0-83.0 per cent, respectively, at ambient conditions (9 months) and accelerated ageing treatments (6 days). In the current study, the seed germination and vigour indices decreased significantly, whereas the mean germination time increased for all the genotypes under both conditions. Further, cluster analysis was done to categorize the onion genotypes into good or poor storers. A significant and positive correlation was found among both natural and artificial ageing treatments. The study facilitated the categorization of onion genotypes into distinct seed longevity groups.

Key words: *Allium cepa* L., Accelerated ageing, Germination, Seed storability, Vigour

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important cultivated bulb crops of the family *Amaryllidaceae*. Due to its rich source of dietary flavonoids, onion has been valued for its medicinal properties, leading to a consistent increase in its consumption worldwide. In India, it is cultivated on 1.91 mha area with a production and productivity of 31.27 MMT and 16.33 t/ha, respectively (MoA & FW, 12). It is also one of the largest foreign exchange earners among the fresh vegetables ($ 460 million in 2021-22) in India and has around a 5% share in the global onion trade (APEDA, 2). However, poor viability, low seed vigour and non-availability of quality seeds are some of the major constraints hindering onion cultivation. Onion seeds are generally characterized as short-lived and possess poor storability, especially under subtropical conditions due to large fluctuations in temperature and relative humidity (Ellis and Roberts, 6). Considerable variability exists among onion varieties for seed longevity, which is determined by genetic factors, including maternal influence, initial seed quality, chemical composition (22-26% oil content), storage conditions (oxygen pressure, temperature, and relative humidity) and duration of storage (Hornke *et al*., 7; Thirusendura Selvi and Saraswathy, 15). Accelerated ageing test (AAT) is the most frequently used artificial ageing test for prediction of seed storability, developed by Delouche and Baskin (5), wherein seeds are kept for a given time under high temperature and RH. It is one of the best methods to estimate changes in seed vigour during storage (Tian *et al*., 16). The process mimics the natural ageing processes (Rajjou and Debeaujon, 13), but the extent of seed deterioration and reliability of prediction of storability remains debatable and needs validation.

Seed longevity is an important trait for the seed industry and for storage purposes in the genebanks. The high quality onion seed is the need of the hour, as loss of vigour during storage causes substantial economic losses to the farmers and seed producers. Therefore, assessment of seed storability is imperative for developing appropriate packaging and storage protocols, which will augment the information on understanding the storage behaviour of onion seeds. Many researchers have studied the longevity of onion seeds under different storage conditions but still there is lack of information on the comparative assessment of seed storability under natural and accelerated ageing conditions. The current investigation was carried out to evaluate the storage potential of thirty onion genotypes employing ageing protocols and classify them under different storage groups.

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MATERIALS AND METHODS

Freshly harvested seeds (*Rabi* 2021-22 produce) of thirty onion genotypes used for the study are mentioned in Table 1.

The seeds were equilibrated to 7-8% moisture content by conditioning in desiccators for three days at room temperature. They were used for further analysis under accelerated and natural ageing. Onion seeds were stored in cloth bags at room temperature (27±1 °C) in the Seed Testing Laboratory, ICAR-IARI, New Delhi. Under natural ageing, the seed germination and vigour parameters were analyzed up to nine months at three months interval. The onion seeds were subjected to AAT by maintaining seeds at 42°C temperature, 75% RH in desiccator, having a saturated solution of sodium chloride (40g/100ml) for a period of three and six days, along with an unaged control.

Table 1. Onion genotypes used for the study along with production location

S.N.	Genotypes	Production Location
1	V L Pyaz	ICAR-IARI, New Delhi
2	Sukhsagar	
3	POS 20K	
4	POS 24K	
5	Pusa Sona	
6	Arka Pragati	
7	L-355	
8	Bhima Dark Red	ICAR-DOGR, Pune
9	Bhima Red	
10	Bhima Super	
11	Bhima Kiran	
12	Bhima Shakti	
13	Bhima Shubhra	
14	RO-1	RARI, Durgapura-Jaipur
15	L-883	
16	Agrifound Dark Red	NHRDF, New Delhi
17	Agrifound Light Red	
18	Agrifound White	
19	NHRDF Red	
20	NHRDF Red-2	
21	NHRDF Red-3	
22	NHRDF Red-4	
23	Pusa Madhavi	ICAR-IARI, Karnal
24	Pusa Shobha	
25	Pusa Red	
26	Brown Spanish	ICAR-IARI, Katrain
27	Hisar-03	CCSHAU, Hisar
28	Hisar-04	
29	PRO-6	PAU, Ludhiana
30	PRO-7	

Studies related to germination were performed following ISTA Rules (Anonymous, 8). Four replicates of 50 sterilized seeds were put in petri plates in germination chamber maintained at 20°C and 100% RH for twelve days. Seed germination counts were taken each day up to twelve days. After twelve days, germination percentage (GP) was scored based on the counting of normal seedlings and calculated as

GP= No. of normal seedlings*100/Total no. of seeds.

Following the germination tests, ten normal seedlings were selected at final count and Seed Vigour Index I (SVI-I) was calculated as described by Abdul-Baki and Anderson (1).

SVI-I = Seed germination (%) x Seedling length (cm)

Germination Index (GI) was estimated as described by Kader (9).

Germination Index (GI) = $[(N_1 \times t) + [N_2 \times (t-1)] + \ldots (+N_{12} \times 1)]$.

Where N_1 to N_{12} is total number of seeds germinated on day t (12) to day 1, respectively. Mean germination time was calculated by the formula, MGT = $\sum(n.t)/\sum n$), where n = seed germinated on day t (Kader, 9). Seed quality evaluation was done under laboratory conditions. Minitab software was used for cluster analysis to categorise the onion genotypes into different classes based on seed storability. Significant differences among the various observations were examined using the ANOVA and Tukey's HSD test.

RESULTS AND DISCUSSION

Onion seeds are generally considered poor storers, as their viability and germination decrease significantly over time. Maintaining a high germination percentage of onion seeds (i.e., 70% as per IMSCS) is difficult due to rapid deterioration under the subtropical conditions in the Indian sub-continent, where the onion seeds are stored for about 6-8 months before planting. Therefore, maintaining seed longevity during storage is a prerequisite for ensuring the availability of quality seeds during sowing of onion.

The control (unaged) seeds of onion genotypes had a variable initial germination percentage which ranged from 56.0 to 96.0 % (Table 2); 'Bhima Red' and 'Bhima Super' had the highest germination (96.0%), whereas 'RO-1' exhibited the lowest germination of 56.0%. The decline in seed germination was significant after 3, 6, and 9 months of ambient storage, ranging from 37.0-94.0%, 33.0-87.0%, and 31.0-82.0%, respectively. The highest germination after nine months was observed in 'Bhima Super' (82.0%) and the lowest in 'RO-1' (31.0%). The germination

Table 2. Germination percentage of onion genotypes after natural and artificial ageing.

Genotype	Control	Natural ageing			AAT	
		3m	6m	9m	3 days	6 days
VL Pyaz	79(62.8)^{e}	75(60.0)^{def}	70(56.8)^{de}	68(55.5)^{cd}	76(60.7)^{de}	72(58.1)^{c}
Sukhsagar	84(66.4)^{cd}	79(62.7)^{de}	73(58.7)^{de}	70(56.1)^{cd}	82(65.0)^{bcd}	72(58.2)^{c}
POS20 k	60(50.8)^{hi}	48(43.8)^{i}	40(39.2)^{j}	37(37.4)^{ij}	54(47.3)^{hi}	44(41.5)^{g}
POS24 k	84(66.4)^{cd}	69(56.2)^{fg}	64(53.1)^{ef}	58(49.6)^{f}	74(59.3)^{e}	60(50.8)^{ef}
PusaSona	72 (58.0)^{fg}	70(56.9)^{fg}	53(46.7)^{gh}	47(43.3)^{h}	54(47.3)^{hi}	34(35.6)^{i}
ArkaPragati	81(64.2)^{de}	73(58.7)^{ef}	64(53.1)^{ef}	59(50.2)^{ef}	75(60.0)^{e}	61(51.3)^{ef}
L-355	90(71.6)^{bc}	75(60.0)^{def}	68(55.5)^{ef}	60(50.8)^{ef}	67(54.9)^{f}	62(51.9)^{def}
Bhima Dark Red	84(66.6)^{cd}	78(62.1)^{de}	70(56.8)^{de}	66(54.3)^{cde}	80(63.5)^{cd}	63(52.6)^{def}
Bhima Red	96(78.8)^{a}	91(72.5)^{ab}	81(64.1)^{bc}	77(61.4)^{ab}	88(69.8)^{b}	76(60.7)^{b}
Bhima Super	96(78.4)^{a}	94(76.0)^{a}	87(68.9)^{a}	82(64.9)^{a}	93(74.7)^{a}	83(65.6)^{a}
Bhima Kiran	88(69.8)^{bc}	74(59.3)^{ef}	67(54.9)^{ef}	65(53.7)^{de}	81(64.1)^{cd}	65(53.7)^{de}
Bhima Shakti	88(70.0)^{bc}	86(68.1)^{bc}	79(62.7)^{bc}	70(56.8)^{cd}	81(64.2)^{cd}	72(58.0)^{c}
Bhima Shubhra	95(77.1)^{ab}	78(62.1)^{de}	69(56.1)^{def}	64(53.1)^{de}	84(66.4)^{bc}	66(54.3)^{cde}
RO-1	56(48.5)^{ij}	37(37.4)^{j}	33(35.0)^{k}	31(33.8)^{k}	38(38.0)^{j}	18(25.1)^{k}
L-883	88(70.3)^{bc}	77(61.6)^{de}	74(59.3)^{cd}	67(54.9)^{cde}	80(63.5)^{cd}	67(55.0)^{cde}
Agrifound Dark Red	84(66.6)^{cd}	78(62.1)^{de}	72(58.1)^{de}	69(56.1)^{cd}	82(64.9)^{bcd}	68(55.5)^{cd}
Agrifound Light Red	86(68.1)^{cd}	81(64.1)^{cd}	77(61.4)^{bcd}	72(58.2)^{bc}	79(62.7)^{cde}	64(53.1)^{de}
Agrifound White	71(57.5)^{fg}	68(55.5)^{fg}	66(54.3)^{ef}	62(51.9)^{def}	67(54.9)^{f}	59(50.2)^{ef}
NHRDF Red	92(73.7)^{ab}	83(65.7)^{cd}	73(58.7)^{de}	59(50.2)^{ef}	79(62.8)^{cde}	61(51.3)^{ef}
NHRDF Red-2	86(68.0)^{cd}	83(65.6)^{cd}	80(63.4)^{bc}	72(58.0)^{bc}	81(64.2)^{cd}	69(56.2)^{cd}
NHRDF Red-3	85(67.2)^{cd}	80(63.4)^{cde}	79(62.7)^{bc}	66(54.3)^{cde}	82(65.0)^{bcd}	69(56.2)^{cd}
NHRDF Red-4	87(69.2)^{bcd}	83(65.7)^{cd}	77(61.3)^{bcd}	70(56.8)^{cd}	85(67.3)^{bc}	76(60.7)^{b}
Pusa Madhavi	94(76.7)^{ab}	90(71.6)^{ab}	83(65.6)^{b}	79(62.7)^{ab}	87(69.0)^{bc}	80(63.4)^{a}
Pusa Shobha	75(60.0)^{f}	75(60.0)^{def}	74(59.3)^{cd}	67(54.9)^{cde}	73(58.8)^{e}	65(53.7)^{de}
Pusa Red	64(53.1)^{h}	61(51.3)^{h}	58(49.6)^{g}	52(46.1)^{g}	59(50.2)^{gh}	40(39.2)^{gh}
Brown Spanish	83(65.9)^{cd}	78(62.1)^{de}	74(59.4)^{cd}	67(54.9)^{cde}	81(64.8)c^{d}	66(54.3)^{cde}
Hisar-03	63(52.5)^{hi}	59(50.2)^{h}	47(43.3)^{i}	40(39.2)^{ij}	56(48.4)^{gh}	40(39.2)^{gh}
Hisar-04	72(58.2)^{fg}	59(50.2)^{h}	55(47.9)^{gh}	41(39.8)^{i}	60(50.8)^{g}	29(32.6)^{j}
PRO-6	90(71.6)^{bc}	87(68.9)^{bc}	80(63.4)^{bc}	75(60.0)^{bc}	88(69.8)^{b}	76(60.7)^{b}
PRO-7	59(50.2)i^{j}	49(44.4)^{i}	45(42.1)^{i}	39(38.6)^{ij}	52(46.1)^{hi}	41(39.8)^{gh}
Mean	81.0(65.3)	73.9(59.9)	67.7(55.7)	61.7(51.9)	73.3(59.9)	60.6(51.3)
Factor		Genotype			Ageing	
C.D. (P≤0.05)		3.388 (2.336)			1.515 (1.045)	
$SE_{(m)}$		1.213 (0.836)			0.542 (0.374)	

Control: fresh seeds; 3m, 6m & 9m: 3, 6 & 9 months naturally stored seeds, 3 days & 6 days: 3 & 6 days artificially aged seeds; Values in parenthesis are Arc Sine transformed values

percentage range of 38.0-93.0% and 18.0-83.0% was recorded after the 3 and 6 days of accelerated ageing, respectively. The highest decline in seed germination after nine months of natural ageing was recorded in 'NHRDF-Red' (33.0 %) and lowest in 'Pusa Shobha' (8.0 %). Similar findings on the decline in germination percent after ageing have already been reported in onion (Balikai *et al.,* 3; Kumar *et al*., 10). The genotypes that maintained greater germination per cent and seedling growth after the ageing treatments were associated with higher values of seedling vigour indices. The variability in per cent germination reduction after ageing could be due to the initial variability in composition, genotypic makeup and seed quality. Natural ageing is the most direct way to study the storage behaviour of seeds, but it requires a longer period for monitoring seed deterioration assays. Also, it is affected by many factors including storage temperature, relative humidity, and the duration of storage. Accelerated ageing (controlled deterioration) mimics natural ageing and it has been reported that both lead to similar results. It is an effective strategy and is being utilized in seed quality control programs. Accelerated ageing is a viable tool to determine the changes in vigour and viability of seed (Balikai *et al*., 3; Melo *et al*., 11; Tian *et al.,* 16) and provides a reference line for the studies related to storage behaviour where the natural ageing is not practically feasible. To meet the upsurge in demand for quality seed, it is important to study the storage behaviour for supply of quality seed during the peak cropping season and also to develop the packaging and storage protocols for long-term storage of poor storer onion genotypes.

A significant variation was observed for seed vigour index-I (SVI-I) in fresh and aged seeds (both natural and accelerated) of selected genotypes (Fig. 1). The highest and lowest SVI-I was observed in 'Bhima Shubhra' (1149.7) and 'Hisar-03' (557.4) in unaged (control) seeds, respectively. Under ambient storage after nine months, the highest SVI-I was recorded in 'Agrifound Light Red' (675.3), followed by 'Pusa Madhavi' (664.2) and 'NHRDF Red-2' (649.6) whereas the least SVI-I was observed in 'POS 20 K' (213.6). Subsequent to accelerated aging treatment (6 days), the highest and lowest SVI-I were observed in 'Bhima Super' (696.9) and 'RO-1' (63.3), respectively. Similar results were reported by Melo *et al.* (11); Rastegar *et al.* (14). Highest germination index in unaged seeds was observed in 'L-883' (510.5), followed by 'Agrifound Dark Red' (506.0), and 'Bhima Red' (501.5), whereas the lowest was recorded in 'PRO-7' (312.5) (Fig. 2). Under ambient storage of nine months, the lowest and highest germination index (GI) was observed in 'PRO-7' (222.5) and 'Pusa Madhavi' (438.5). However, after three and six days of accelerated ageing treatments, the germination index ranged from 188.0-501.5 and 111.0-496.5,

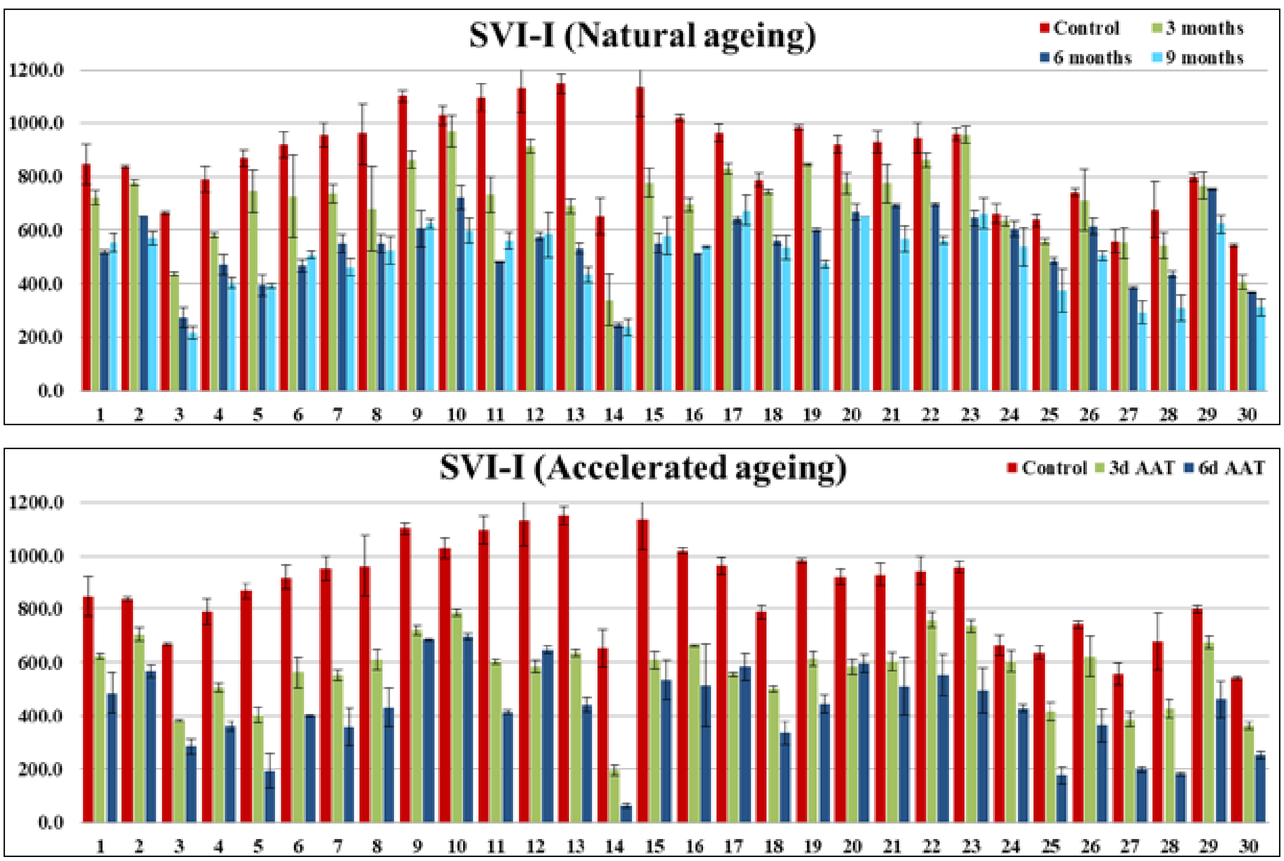

Fig. 1. Seed Vigour Index I (SVI-I) of onion genotypes after natural and artificial ageing.

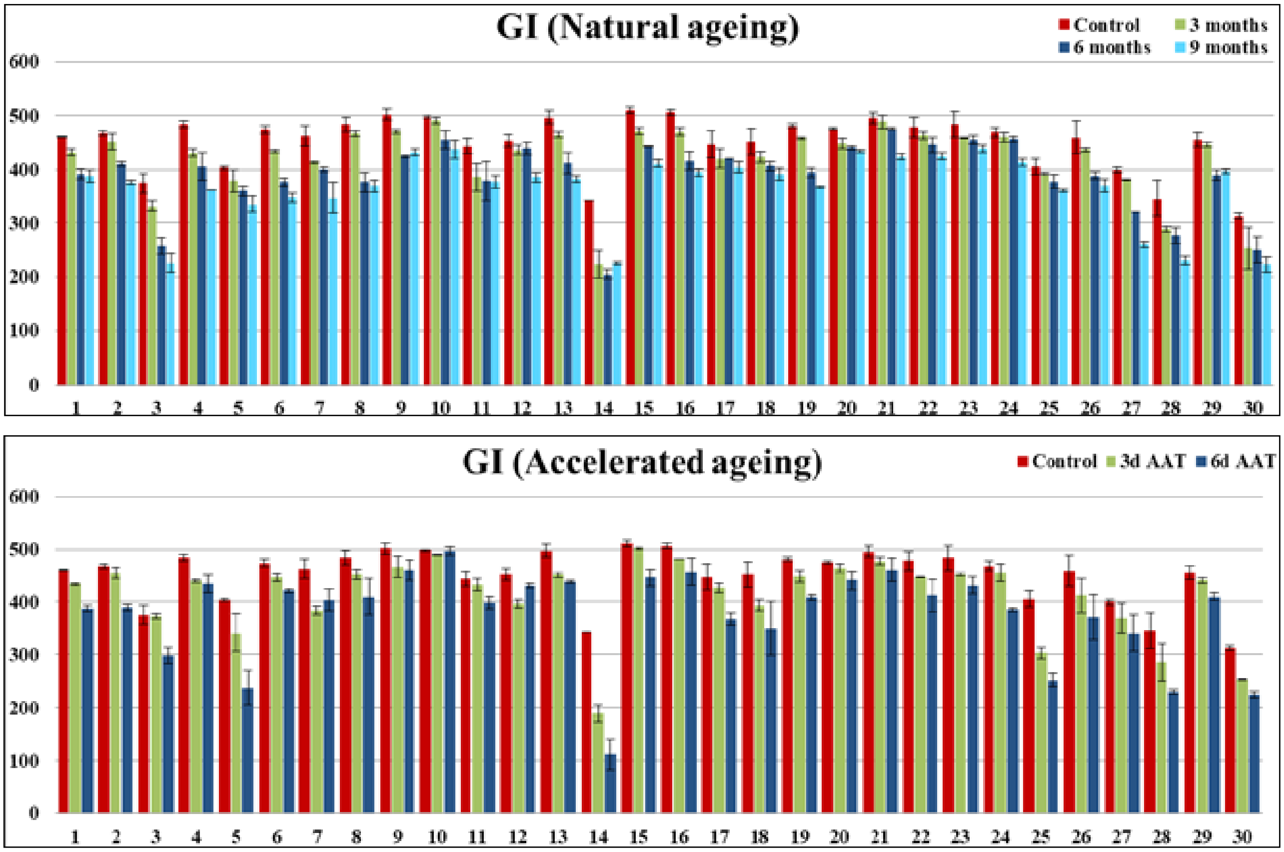

Fig. 2. Germination index (GI) of onion genotypes after natural and artificial ageing.

respectively. Germination percent, vigour indices, and germination index are the indicators of seed vigour and are essential parameters for seed aging-related studies. In the present studies, the germination and vigour indices decreased significantly after the ageing treatments (both in natural and accelerated) which were in conformation with the findings of Brar *et al.* (4); Rastegar *et al.* (14). Initially, there was a slow decline in the vigour indices but rate of decline increased with the progression from three to nine months of ambient storage. In accelerated aging, the rate of decline was related to the high temperature and relative humidity conditions. The variation for longevity among different genotypes for germination and vigour indices showed that longevity of seeds was partially determined by initial seed quality and the duration of storage (Hornke *et al.*, 7; Thirusendura Selvi and Saraswathy, 15).

The mean germination time (MGT) of genotypes in unaged seeds ranged from 2.48 to 3.93 days (Fig. 3) which indicates an average time period required to germinate by seeds. After nine months of ambient storage, the highest and lowest MGT recorded were 4.46 days for 'Hisar-04' and 3.26 days for 'NHRDF Red-3', respectively. However, after six days of accelerated ageing, the highest and lowest MGT was observed in 'PRO-7' (4.53 days) and 'L-883' (2.87 days), respectively. Therefore, the mean germination time increased in all the onion genotypes under natural as well as accelerated ageing treatments. The genotypes with lower MGT values germinated earlier than those with higher MGT values. In general, physiologically older seeds exhibited decreased rate of germination due to the ageing process (both natural and accelerated) resulting in higher MGT values than the unaged ones. In the current study, the increased values reflect adverse effect of the ageing period on seed germination and vigour of different onion genotypes which is in confirmation with Balikai *et al.* (3).

A histogram plot indicating mean values and standard deviation of germination percentage for ageing treatments is presented in Fig. 4. The histogram indicated frequency distribution curves having the highest mean germination percentage (81.07) for control as compared to ageing treatments whereas, 6 days of accelerated ageing showed the lowest mean germination (60.6). The peak of

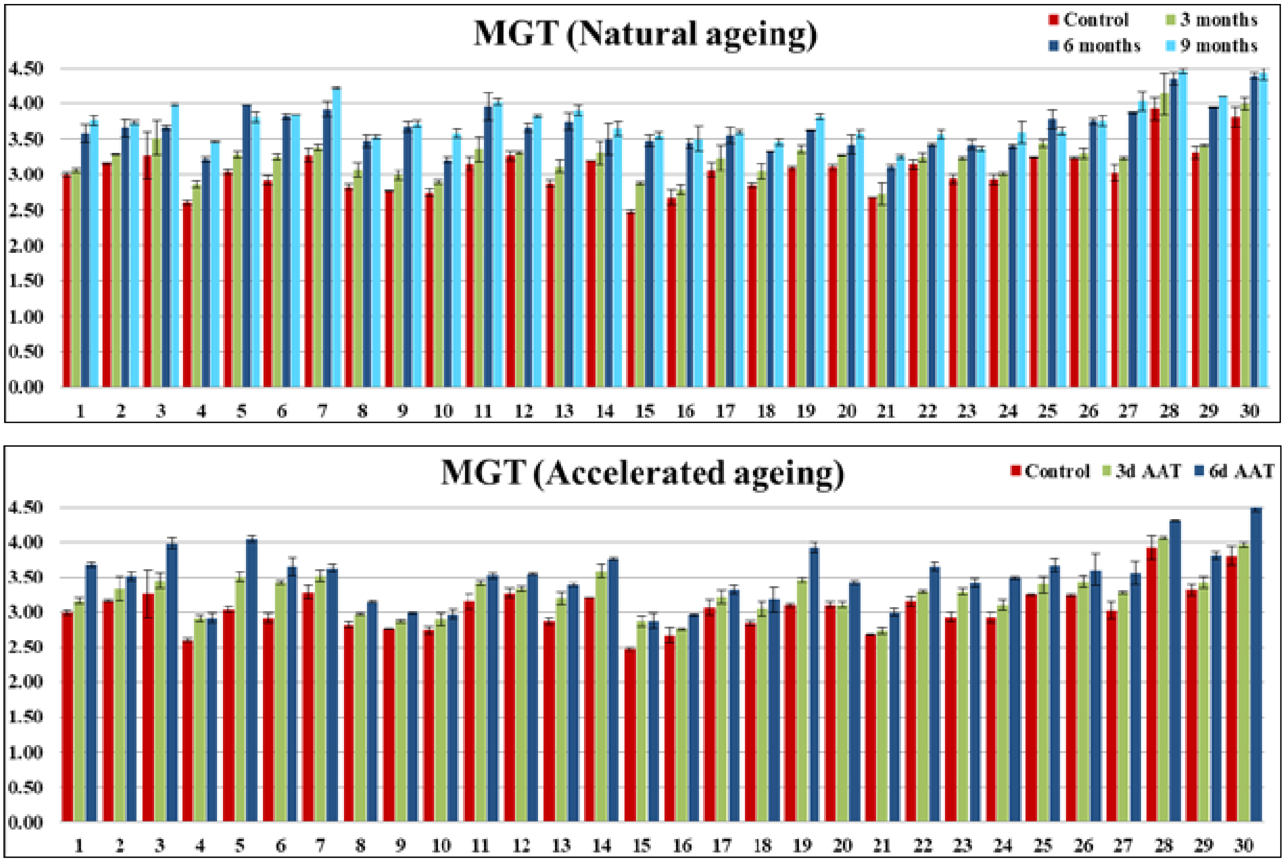

Fig. 3. Mean germination time (MGT) of onion genotypes after natural and artificial ageing.

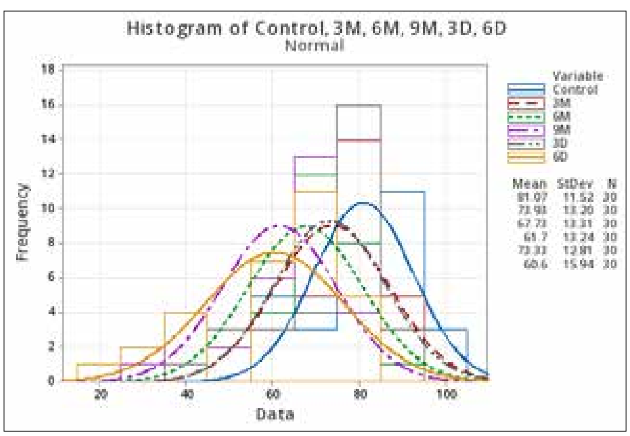

Fig. 4. Histogram of germination percentage of onion genotypes after natural and artificial ageing.

Control indicates fresh seeds; 3M, 6M and 9M indicate 3, 6 and 9 months of natural ageing; 3D, 6D indicates 3 and 6 days of accelerated ageing

a frequency distribution curve for control had the highest and most narrow peak, indicating a higher frequency of genotypes having higher germination percentage, whereas a lower and broader peak for 6 days of accelerated ageing frequency distribution curve indicated genotypes having lower germination percentage (60.6). The other ageing treatments had intermediate mean germination with intermediate peaks which is in conformity with Brar *et al.* (4). Further, the significant associations between the ageing treatments were assessed through Pearson's correlation test. The natural ageing duration of onion seeds was evaluated to determine their correlation with duration of artificial ageing. A high positive correlation ($p \leq 0.05$) was observed between artificial and natural ageing treatments. Both three and six days of artificial ageing treatments were highly correlated with natural ageing durations ranging from 0.65 to 0.83. The highest correlation coefficient of 9 months of natural ageing ($r = 0.83$) was observed with 6 days of artificial ageing. Thus, 6 days AAT can be used to simulate natural ageing equivalent to 9 months in onion seeds. Accelerated ageing has been reported to be highly correlated with field emergence, mean germination time and germination percentage (Balikai *et al.*, 3; Brar *et al.*, 4). A cluster analysis of natural and accelerated aged seeds of onion genotypes was performed using a decline in germination percentage with ageing, resulting in two clusters having good and poor storer genotypes (Fig. 5). Cluster 1 included 'VL Pyaz', 'Bhima Super', 'PRO-6', 'Sukhsagar', 'NHRDF Red-4', 'Pusa Shobha', 'Bhima Dark Red', 'Bhima Red', 'Agrifound Dark Red', 'Brown Spanish', 'NHRDF Red-3', 'Bhima Shakti', 'Pusa Madhavi', 'Agrifound Light Red', 'Agrifound White', 'NHRDF Red-2', and 'Pusa Red'. This cluster reported a significantly higher average germination percentage after 9 months of natural ageing (69.6) and 6 days of artificial ageing (68.8), with a 14.6 % and 15.4 % of fall in germination percentage as compared to control (fresh seeds). Cluster 2 included 'POS 20 K', 'POS 24 K', 'PRO-7', 'Bhima Shubhra', 'Arka Pragati', 'L-883', 'Bhima Kiran', 'NHRDF Red', 'Hisar-03', 'Pusa Sona', 'L-355', 'Hisar-04', and 'RO-1'. This cluster reported significantly lower germination percent after 9 months of natural ageing (51.3%) and 6 days of artificial ageing (49.8%) with about 25.6 % and 27.1 % fall in germination percentage as compared to a fresh seed. Therefore, the genotypes under the Cluster 1 were good storers and under the Cluster 2 were poor storers. Similar results related to cluster analysis have been reported by Kumar *et al.* (10), where onion genotypes were categorised into three clusters (good, medium and poor storers).

During the present investigation, onion genotypes demonstrated varying degrees of germination decline after aging treatments. The SVI-I, GI and MGT act as valuable indicators of seed germination and vigour, and a consistent decline was observed after aging treatments. Cluster analysis classified the onion genotypes as good or poor storers, based on germination percent. Correlation analysis demonstrates the consistency in seed deterioration patterns during storage and highlights the significant positive correlation among artificial and natural aging durations (Kumar *et al.*, 10) which can be utilized as a screening tool for assessing the storage potential of seed lots/genotypes. The results of the investigation can be used to differentiate the onion genotypes for seed longevity, developing breeding lines and mapping populations for improving storability of onion. Overall, understanding the storage behaviour of onion seeds and developing appropriate storage protocols are essential for enhancing onion productivity and ensuring a consistent supply of quality seeds.

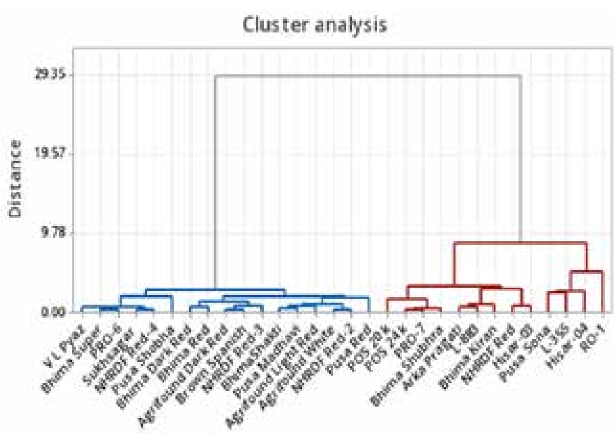

Fig. 5. Cluster dendrogram of onion genotypes for percent decline in germination over natural and artificial ageing.

AUTHORS' CONTRIBUTION

Conceptualization of research (SKL,SB); Designing of the experiments (SKL,SB,AK,SKJ,AA,VY); Contribution of experimental materials (SKL,AK,VY); Execution of field/lab experiments and data collection (NP); Analysis of data and interpretation (SKL,SB,NP); Preparation of the manuscript (NP,SB,AK).

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DECLARATION

The authors declare no conflict of interest.

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