Research Article

Simulated Storage Germination and Growth Responses in Carrot Seedlings Grown from Artificially-Aged Seeds

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Abstract | Seed deterioration behavior during storage of carrot seeds was assessed by subjecting them to controlled-ageing for different temperatures and incubation time during 2016 at Seed Preservation Laboratory, Bio-resources Conservation Institute, NARC, Islamabad. Forced seed ageing was carried out at 25, 30, 35 and 40°C for Day-one (D1) through day-six (D6) of incubation period. Observations were recorded for percent germination, shoot length (cm), root length (cm), fresh seedling biomass (g) and dry seedling biomass (g); revealing significant differences at all temperature regimes, controlled incubation period for ageing as well as their interaction. Rising temperatures was much steeper and negatively correlated as compared to the incubation period. The germination rate declined gradually at all temperatures, whereas, at higher temperature of 45°C accompanied with longer incubation periods (D5 and D6) the germination process ceased to progress. Similarly, the growth performances of the seedlings were slower at higher temperatures coupled with longer storage periods. The aging led to decline in seedling fresh and dry biomass as well as root and shoot development capacity; while the deleterious effect of higher temperature (45°C) were more pronounced and severely affected the seedling growth and development from start. The information generated on seed behavior under storage conditions in this study; would be helpful to devise better strategy for short- and long-term storage of carrot seeds as well as seed of other crops of similar category.

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Introduction

All plant seeds have a maximum potential for germination at point of natural dispersal which decline gradually with the passage of time (Al-Maskri et al., 2003). The physiological potential at maximum is normally achieved near to seed maturity and then seeds start deteriorating depending on time of harvesting and method, environmental conditions, seed moisture content, post-harvest handling procedures adopted for drying, processing and seed storage (Marcos-Filho, 2015). Aged and old seed is among prominent factors that is responsible for the poor crop stand of various food crops particularly vegetables. The loss of vigor due to ageing is normally observed as delayed emergence, quite slow growth, over responsive to stress susceptibility (McDonald, 1999). The deterioration rate varies substantially among species and the ageing in stored seed is quite natural; where tendency to lose viability has been recorded in seeds even stored under ideal conditions (Jatoi et al., 2001). Diverse pathway and processes

have already been highlighted that may possibly have involved in the seed deterioration like disruption of internal membrane integrity, reduction in ATP production (McDonald, 1999). In storage, different external and internal factors affect the seed longevity among which temperature, relative humidity and seed moisture content (SMC) are sufficiently investigated for various crops (Khan et al., 2017).

The deterioration behavior of many seeds in long term storage is quite different. To keep viable for long period of time, large number of seed samples is stored in gene bank under optimal storage conditions. However, it is recommended to monitor its viability as well as vigour regularly at specific interval. Forced aging is a valuable tool to determine viability and seed vigour. Artificially-ageing as a tool to study the biochemical changes and physiological attributes in seeds has been employed for various investigations (Shibata et al., 2012), predict storability of seed lots (Baalbaki et al., 2009). The forced aging in some cases also favored seed germination (Shibata et al., 2012). The information on seed deterioration for each crop under different temperature and storage conditions is useful for forecasting seed longevity in long term seed storage. Proteomics is also another approach to uncover the controlling molecular-mechanisms playing vital role in seed viability and vigour under ageing conditions (Nguyen et al., 2015). Documented information on forced aging in carrot and its response to varying conditions is sparsely available in literature. The current study deals with the seed deterioration behavior of carrot during storage using artificially created aging conditions that may help to understand carrot-seed response particularly under storage conditions in the genebanks. Therefore, to plan the regeneration calendar and categorize the germplasm into different categories this assessment of storage behavior is necessary.

Materials and Methods

The experimental work under this study was conducted in Seed Preservation Laboratory at Bio-Resource Conservation Institute (BCI), National Agricultural Research Centre (NARC), Islamabad in 2016. The seed for this study was obtained from National Genebank of Pakistan at BCI. Quality of carrot seeds was examined by employing standard germination and seedling growth rate test outline by the International Seed Testing Association (ISTA, 2016) and the Association of Official Seed Analysts (AOSA, 1983).

Accelerating aging (AA)

For creating accelerated aging conditions, seed was exposed to high relative humidity (approximately 100%) created in a leakage proof-chamber at different temperatures (35°C, 40°C and 45°C) for one (D1) to six days (D6). Each day from D1 to D6, samples from incubators were removed after required incubation and used for germination testing. Control consisted of original conserved seed lot subjected to germination directly at 25°C.

Germination testing

Germination testing was performed using between papers (BP) method. For each treatment 100 seeds per replication were used following ISTA Rules (ISTA, 2016). For germination testing in Lab, victory brand paper towel (22cm x 23cm) prepared by Shinbashi Paper Co., Japan was used. For this purpose, double sheets of paper towel were moistened with distilled water and seeds were placed on paper towel in different rows keeping appropriate distance among seeds as well as rows. Another sheet of paper towel was placed over it and all the sheets were then gently rolled to make kind of sandwiches. The rolled paper towels were placed in plastic beakers vertically. After covering beakers with polythene bags, was placed in incubator with temperature at 25±2 for 15 days. Seeds with up to 0.5cm radical and plumule were considered as germinated. Final germination count was done on 15th day. Percent germination was recorded on the basis of number of normally grown seedlings.

Seedling growth rate

For each treatment, control as well as artificially aged seeds, 30 seeds were used to determine the rate of seedling growth and each treatment was replicated three times. Normal seedling with complete shoots and roots were used for seedling growth rate. The data was recorded at 15th day of sowing. For each normally grown seedling, shoot and root length was recorded and then averaged. The weight of fresh and dry seedling biomass of each replication was recorded. The complete seedlings along with seed remains were dried at 100°C in an oven for 24 hours and expressed as g/seedling for dry seedling biomass.

Data was subjected to analysis for variance using RCBD with storage periods and different temperature

regimes as two factors following Steel and Torrie (1997). Mean separation was conducted through DMR Test.

Results and Discussion

Germination (%)

Seed transformation into a plant is the ultimate goal and a proper crop stand is ensured with high seed germination. The carrot seeds in response to forced ageing revealed significant difference for germination at different temperatures (Table 1) as well as seed incubation periods (Table 2). Germination in carrot ranged from 96.9% (25˚C; control) to 6.44% (45˚C) with a sharp decline in germination. For the different incubation periods, germination in carrot ranged from 72.33% (D1) to 34.5 (D6) with a gradual decline from D1 to D6.

The interactive impact of temperature (25˚C to 45˚C) and periods (D1 to D6) on germination rate was highly significant (Figure 1A). For the control temperature at 25˚C non-significant differences were observed from D1-D6 where germination ranged between 99.0% to 95.67%. At 35˚C the germination at D1 was 86.67%, followed by 64.33 (D2), 49.00 (D3), 41.00 (D4), 31.00 (D5) and 24.33 at 6th day while at 40˚C the germination recorded at D1 was 74.66%, followed by 52.00 (D2), 35.67 (D3), 27 (D4), 22.00 (D5) and 18.00 at 6th day. Similarly, at 45˚C the germination recorded at D1 was 29.00%, followed by 9.67 (D2), and it was zero at remaining days. On the other hand, germination observed at any of the given day of incubation displayed significant differences at all temperature regimes tested. At D1 it was 99.00 at 25˚C followed by 86.67% (35˚C), 74.67 (40˚C) and 29.00 (45˚C). At D2 it was 98.00 (25˚C), 64.33 (35˚C), 52.00 (40˚C) and 9.67 (45˚C). For rest of the four days germination was recorded only at 25, 35 and 40˚C; and no germination was observed at 45˚C that appeared devastating for carrot seeds.

The germination was drastically affected even at D1 when artificially aged at 45˚C displaying this temperature as deleterious for carrots. Lipid peroxidation might be responsible for this behavior as due to high temperatures the membranes are agitated (Chang and Sung, 1998) and the membranes of aged seed subjected to such alterations may lead to electrolyte leakage in the process of imbibitions thus causing reduced germination (Mohamed et al., 2010) reflecting the sensitivity of carrot seeds to high temperature.

The differences in germinability revealed that the rate of germination was highly affected by the temperature and ageing period as noticed in carrot-seed by recording high germination (> 85%) in non-aged seeds (Al-Maskri et al., 2003). The reduction in seed viability with the ageing interval revealed 77, 36 and 12% in carrot seeds at 3, 5, and 7 days of ageing, respectively. A similar type of experiment performed by Mohamed et al. (2010) revealed increase in temperature had a negative significant effect on germination percentage of oilseed crops like sesame, peanut and canola.

Speed of germination

Seed vigour recorded through speed of germination was influenced both by temperature and time period (Figure 1B). At control temperature it was 8.694; and reduced to 6.383 (35˚C), 5.654 (40˚C) and 1.787 (45˚C). The decline in speed of germination as well as reduced seedling size and rise in number of abnormal seedlings are the strong indicators of initial seed aging (Marcos-Filho, 2015). Speed of germination was quite low and gradually decreased at 25˚ from D1-D6 whereas it was fast on rest of temperatures (35, 40 and 45˚C); however, speed of germination was highly affected at 45˚C revealing 0.035 at D2, zero at D3 to D6. Compared with the control, the percent decline in speed of germination was 79.5 even at one day of incubation at 45˚C that reached to 95.4% at D2 and finally 100% at D3 and onward (Figure 2B).

Shoot length (cm)

The shoot length at seedling stage for ageing at varying temperatures (Table 1) and ageing periods (Table 2) depicted significant differences. At various temperatures the shoot length was 5.67cm, 2.5cm, 1.47cm and 0.26cm at 25, 35, 40 and 45˚C, respectively (Table 1). Similarly, for various incubation periods shoot length recorded was 3.88cm, 3.03cm, 2.53cm, 1.89cm, 1.8cm and 1.73cm at D1, D2, D3, D4, D5 and D6, respectively (Table 2).

The inter-action of the temperatures and periods had significantly different impact on shoot length (Figure 1C). At control (25˚C), there was a non-significant trend in shoot length across the number of ageing days showing a least impact of this temperature on different ageing periods (Table 2). However, at 35˚C

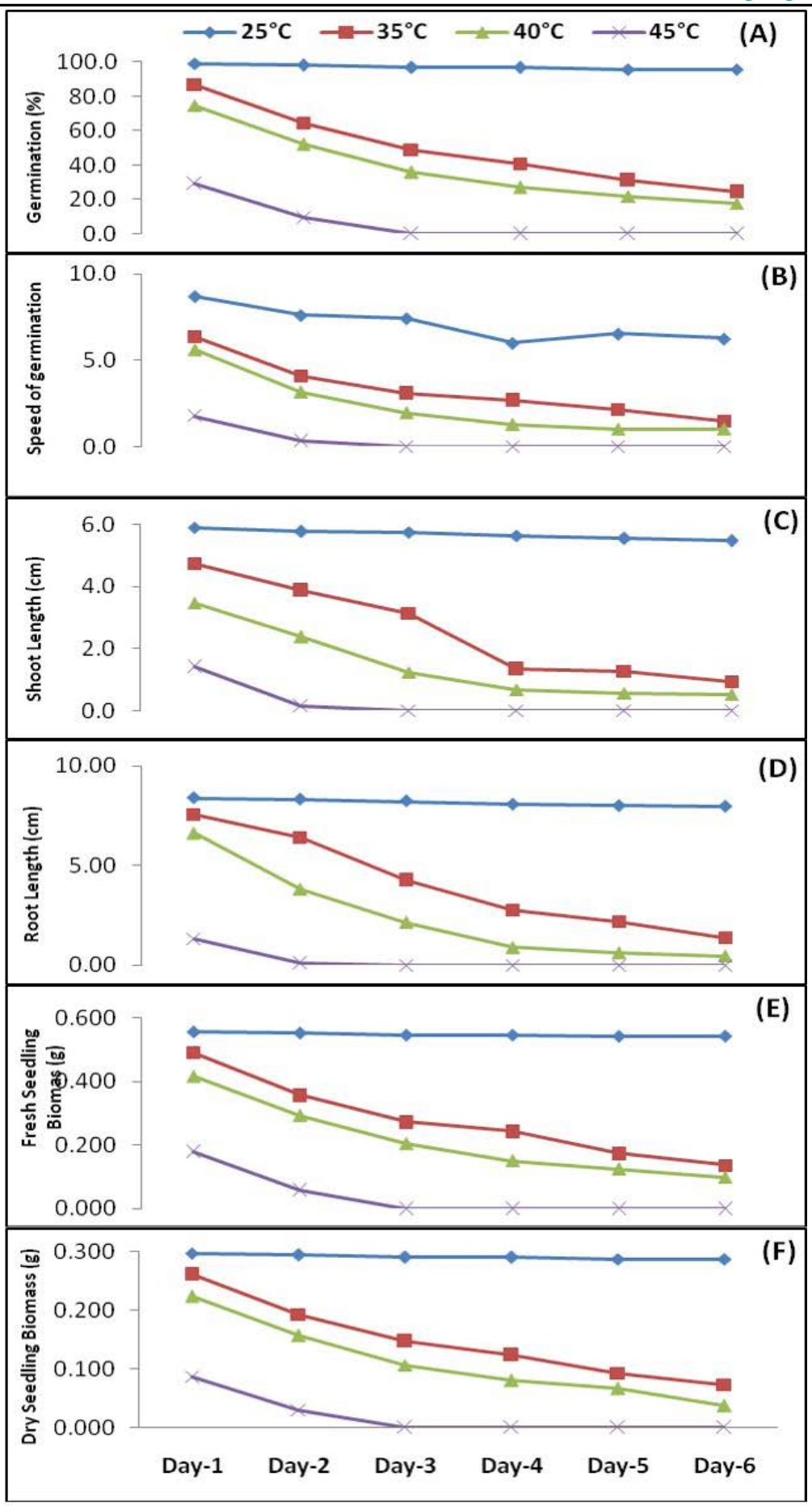

Figure 1: *Interactive effect of different temperatures and storage periods on different parameters observed in carrot seeds subjected to germination after forced ageing; (A) germination (%); (B) Speed of germination; (C) Shoot length (cm); (D) Root length (cm); (E) Fresh seeding biomass (g) and (F) Dry seeding biomass (g).*

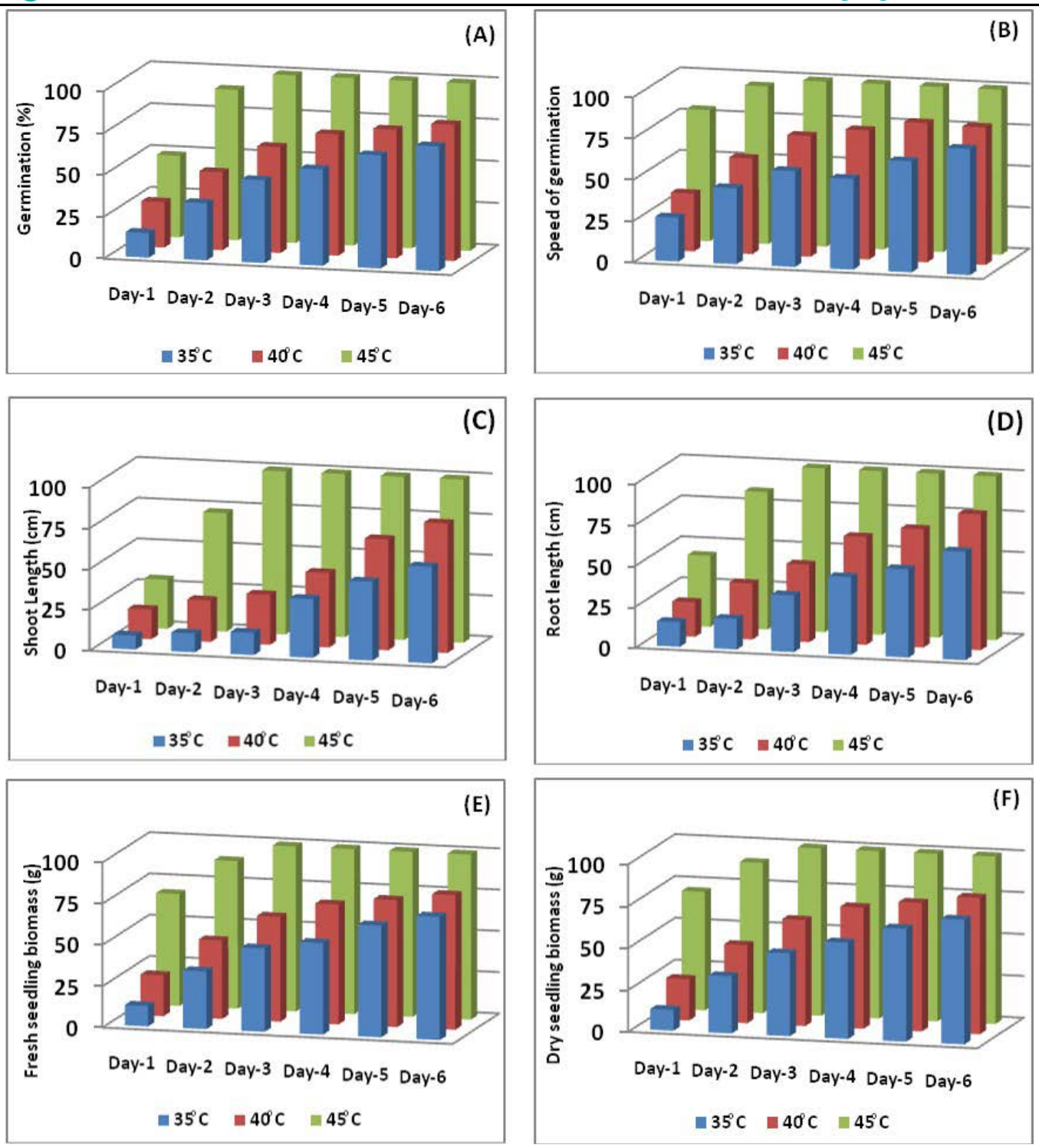

Figure 2: *Percent decrease observed in various attributes in carrot aged seed against varying temperature and incubation period; (A) Germination (%); (B) Speed of germination; (C) Shoot length (cm); (D) Root length (cm); (E) Fresh seeding biomass (g) and (F) Dry seeding biomass (g).*

Table 1: *Seed germination and various seedling attributes in carrot as affected by different temperature regimes under artificial ageing.*

	Germination (%)	Shoot length(cm)	Root length(cm)	Fresh seedling biomass (g)	Dry seedling biomass (g)
T1 (25°C)	96.94a	5.67a	8.16a	0.55a	0.29a
T2 (35°C)	49.39b	2.55b	4.1b	0.28b	0.15b
T3 (40°C)	38.22c	1.47c	2.44c	0.21c	0.11c
T4 (45°C)	6.44d	0.26d	0.24d	0.04d	0.02d

Means followed by the same letters in column are statistically non-significant at 5%level of probability.

Table 2: *Seed germination and various seedling attributes in carrot as affected by forced ageing under different storage periods.*

	Germination (%)	Shoot length(cm)	Root length(cm)	Fresh seedling biomass (g)	Dry seedling biomass (g)
D1	72.33a	3.88a	5.97a	0.411a	0.217a
D2	56.00b	3.03b	4.68b	0.315b	0.168b
D3	45.33c	2.53c	3.68c	0.256c	0.136c
D4	41.17d	1.89d	2.93d	0.235d	0.123d
D5	37.17e	1.85d	2.71de	0.211e	0.112e
D6	34.50e	1.73d	2.46e	0.194e	0.103e

Means followed by the same letters in column are statistically non-significant at 5% level of probability.

shoot length ranged between 4.73cm (D1) to 0.93cm (D6). At 40°C it was the longest (3.47cm) at D1 that gradually declined to 0.53 cm at (D6). On the highest temperature assayed (45°C) shoot length was recorded only on D1 and D2 that was 1.40 cm and 0.13cm, respectively. On the other hand, the impact of a given ageing period across the temperatures was also statistically significant for shoot length (Figure 1C) where the gradual decrease in shooting capacity was confined up to 40°C and no shoot emergence was recorded on 45°C from D1 to D6. The pattern of shoot length observed at different ageing temperatures and periods corresponded to the pattern observed for germination. Another study on radish revealed similar results of decreased shoot length (from 7.36 at D1 to 1.41 Day-6) during accelerated aging (Jain et al., 2006).

Root length (cm)

Like in all plants, root is an important water absorbing organ and for carrot it is also the storage organ of economic value. Rooting capacity in carrot seedlings differed significantly both for temperatures as well as time (Tables 1 and 2). The root length observed at different temperatures remained in between 8.16cm (25°C) and 0.24cm (45°C). A linear and sharp decrease in root length was recorded from low temperature to high temperature. For the ageing period root length ranged from 5.97 (D1) to 2.46cm (D6) and displayed the decrease in root length in a gradual mode from D1 to D6.

The interaction of all temperature and ageing days remained significant except for control (25°C) where it showed non-significant differences for root length (Figure 1D). At 25°C (control) the longest root (8.39cm) was recorded at D1 whereas the shortest root (7.97cm) at D6 showing negligible differences. At 35°C root length ranged between 7.57cm and 1.40cm at D1 and D6, respectively whereas it ranged between 6.63cm (D1) to 0.47cm (D6) at 40°C. Poor root growth was recorded at 45°C, where the longest root length was only 1.3cm that was recorded in seedlings with one-day ageing. The smallest root (0.13cm) at the same temperature was recorded at D2. For rest of the days no root development was recorded because no seedling could survive at this temperature from D3 to D6. On the other hand, differences in root length from D1 to D6 across the temperatures were significant (Figure 1D). The seed ageing at D1 and D2 induced roots at all temperatures; however, from D3 to D6 root induction was recorded only at 25°C, 35°C and 40°C (Farhadi et al., 2012).

Fresh seedling biomass (g)

The production of biomass is an indicator of plant growth and development. In this study fresh seedling biomass (FSB) was significantly affected by varying temperature and number of days (Table 2). The highest FSB was noted at control (0.5983); it was followed by 0.2794g (35°C), 0.2139g (40°C) and 0.040g (45°C). The similar fashion was observed for seed aging periods. The highest FSB was recorded on D1 (0.411g) that was followed by 0.315g (D2), 0.256g (D3), 0.235g (D4), 0.211g (D5) and 0.194g (D6). In both cases FSB decreased from T1 (25°C) to T4 (45°C) and D1 to D6.

This interactive response of temperature and storage period for FSB was also found significant (Figure 1E). At control (25°C) FSB was displayed non-significant difference at D1to D6 while the FSB at 35°C was significant reveling highest biomass at D1 (0.4900g) and the lowest (0.1367g) at D6. Similarly, at 40°C FSB was more at D1 (0.411g) and lowest at 0.0967g (D6), whereas the highest FSB at 45°C was recorded at D1 (0.1800g) and it was followed 0.060g at D2. However, for rest of the days and the temperature no FSB was recorded.

Dry seedling biomass (DSB) g

Seedling biomass is the net effect of photosynthesis and alignment of physiological mechanism to support growth. In this study dry seedling biomass (DSB) displayed significant differences at different temperatures (Table 1) and varying ageing periods (Table 2). DSB ranged between 0.2906g (25˚C) to 0.0222g (45˚C) whereas for ageing periods it ranged from 0.2176g (D1) to 0.1033g (D6).

The cumulative impact of different temperatures and ageing periods also revealed significant difference for DSB (Figure 1F). At control treatment (25˚C) DSB displayed non-significant difference whereas at 35˚C it ranged between 0.2600 (D1) to 0.0733g (D6) that was a 3.5-fold decrease in DSB. Similar pattern was observed at 40˚C, however at 45˚C, DSB was recorded only at D1 (0.0867g) and D2 (0.0300g) and for rest of ageing periods no DSB was recorded. On the other hand, DSB at D1 was ranged between 0.2967g (25˚C) and 0.0867g (45˚C). Similarly, at D2 it ranged from 0.2933 (25˚C) to 0.0300g (45˚C). For rest of the storage duration DSB was only at 25˚C, 35˚C, 40˚C and no DSB at 45˚C was recorded on D3 to D6 (Figure 1F).

Percent decline rate as compared to control observed for each parameter displayed a gradual increment at each increasing temperature as well as days (Figure 2). The cumulative response of carrot revealed through various seedlings attributes provided good insight into seed storage behavior. From perusal of the data set, the performance of the seedlings was very low at high temperatures coupled with long duration e.g. D5 and D6. The decline in the characteristics of various seed related parameters may be attributed to poor enzymatic activity in seeds caused by forced ageing. Seed exposure to unfavorable conditions usually causes critical degeneration in metabolic processes like reduction in storage reserves, decreased enzyme activity, protein denaturation, reducing sugars levels and increases in free fatty acids that in-fact are produced by disintegration and degeneration of the membrane system in seed (Marcos-Filho, 2015). At initial stage of ageing in maize, germination characteristics and enzyme activity were higher as compared to the final stages of the aging (Kapilan, 2015). Moreover, reduction of enzyme activity particularly of α-amylase and other hydrolytic enzymes and the quantity of carbohydrate storage (Bailly et al., 1996) or denaturing tendency of the proteins due to accelerated ageing (McDonald, 1999) could have been responsible for it. Similar results were obtained by Farhadi et al. (2012) while working on similar aspects in Basil (*Ociumum basilicm* L.) seeds observing decrease in various attributes of seedling like germination, speed of germination, root length and shoot length.

The result of this study showed that seed deterioration rate flared-up with an increase both in aging period and temperature. As a common practice, seed vigour and viability of stored material in gene banks is monitored regularly at specific intervals through germination and other tests. The seed material displaying low percent germination needs to be rejuvenated that would ensure the germplasm housed in gene bank has substantial longevity. It also emphasizes the need to further investigate the causes of rapid seed deterioration at intra-specific level in particularly focusing different varieties belonging to same species. Detailed studies would improve our understanding on deterioration process that might offer suitable ways to prolong the seed longevity particularly in the gene bank.

It has been reported that artificially ageing may have led to increase in germination. The accelerated aging, in some cases, favored seed germination (Shibata et al., 2012). Similarly, enhanced germination was also recorded in *Cedrela fissilis* (noble wood tree) that was subjected to artificial aging for 72 h of (Borges et al., 1990). The mechanism of cell repair is activated due to high moisture content in the process of artificial ageing and similar effects were observed in peanut seeds (Schmitt, 2000). However, in all the ageing conditions in present study, no such observation has been noted in carrot.

Although, there is no single test that would be accepted universally for determining physiological potential of a given seed belong to one or group of species; however, the level of knowledge has tremendously increased on seed vigor testing and its relationship with seed performance (Marcos-Filho, 2015). For the reliable results to depend on, it is imperative to have multiple tests conducted simultaneously on seed vigour and germination as one test can never portray the true picture of seed performance. This study will help in better planning of regeneration calendar putting the carrot in less frequent category.

Conclusions and Recommendations

Seed storage of various crop groups for short and long-term periods is a regular practice in the gene-banks. Ageing of seeds under artificially created different storage conditions help to understand deterioration behavior of seed. The impact of forced ageing investigated against varying temperature and storage periods on carrot seed revealed varying seed deterioration responses for germination and various seedling attributes. It improved our understanding on the relationship of temperature and storage periods on germination and other seedling parameters in carrot. Rising temperatures was much steeper and negatively correlated as compared to the incubation period. Similarly, the growth performances of the seedlings were slower at higher temperatures coupled with longer storage periods. The current study would be helpful to make better decision for keeping carrot seeds alive in the genebank for short- and long-term storage.

Author's Contribution

Uzma Arif: Conducted experimental work.
Sadar Uddin Siddiqui: Statistical analysis.
Muhammad Fareed Khan: Conceived the idea and Review.
Muhammad Arshad: Review and Technical Input.
Shakeel Ahmad Jatoi: Manuscript write-up.

Conflict of interest

The authors have declared no conflict of interest.

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