www.ThePharmaJournal.com

The Pharma Innovation

Rifat M Bhat
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Kashmir,
Shalimar, Srinagar, Jammu
Kashmir, India

Farooq A Khan
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Kashmir,
Shalimar, Srinagar, Jammu
Kashmir, India

Imtiyaz Murtaza
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Kashmir,
Shalimar, Srinagar, Jammu
Kashmir, India

Gulzaffar
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Kashmir,
Shalimar, Srinagar, Jammu
Kashmir, India

Moinuddin
Shri Guru Ram Rai University,
Dehradun, Uttarakhand, India,
Lovely Professional University,
Phagwara, Punjab, India

Mohammad Amir
Lovely Professional University,
Phagwara, Punjab, India

Effects of accelerated aging and subsequent priming on seed quality and biochemical change of onion (*Allium cepa* L.)

Rifat M Bhat, Farooq A Khan, Imtiyaz Murtaza, Gulzaffar, Moinuddin and Mohammad Amir

Abstract
A study was performed at SKUAST-Kashmir to understand the mechanisms of seed deterioration under AA and its reversal through subsequent OP. The seeds were exposed to AA treatment for 0, 48, 72 and 96 hours at 45±2 °C and 100% R.H. Each lot of treated seeds was further divided into four sub-lots and osmo-primed with PEG 6000 for 0, 24, 48 and 72 hours at 25±2 °C. Fifty seeds from each sub-lot were placed in petri dishes and placed in an incubator at 25 °C for recording various germination parameters. AA significantly reduced the GP and CVG from 85.56 and 43.78 (A0) to 12.66 and 9.00 (A3). There were significant reduction in shoot and root length (5.94 → 2.93cm; 4.83 → 2.99cm), seedling biomass (21.18 → 15.84md), SVIs (18.12 → 2.01; 921.5 → 75.0) in A3 compared to A0. However, these attributes were found to increase to maximum of 59.84%, 29.38, 5.38cm, 4.45cm, 19.69mg, 11.71 and 5.80 due to different OP treatments. In line, biochemical attributes like shoot chlorophyll, sugar, protein, MSI and anti-oxidant potential were also found to deprive due to AA effects and build up after OP treatments.

Keywords: Accelerated ageing, onion, osmopriming, chlorophyll, antioxidant and seed germination

Introduction
Onion (*Allium cepa* L.) is an important bulbous vegetable that fetches highest foreign exchange among the fruits and vegetables. It is a herbaceous monocot plant cultivated as annual for vegetable but as biennial for seed production. The diverse agro-climatic conditions enable India to produce onion in one or the other part of the country round the year. Its popularity is due to its aromatic volatile oil, the allyl propyle disulfide ($C_3H_5S_2C_3H_7$) which imports a cherished flavour to food. Onion also has an important role as a medicinal herb and is claimed to minimize high blood pressure and other heart diseases due to its favourable action on the elasticity of blood vessels.

A good quality of seeds with high physiological potential is a basic requirement for higher crop productivity and quality. However, seed quality losses gradually after harvest due to progressive deterioration of the structure and function of seed (ageing) that lead to the loss of viability (Mohammed, 1991) [33]. Loss of seed viability following ageing has been attributed to a series of metabolic defects that accumulate in embryonic and non-embryonic structures (Sisman, 2005) [50]. At the cellular level, seed ageing is associated with loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis, and DNA degradation (Kibinza *et al.*, 2006) [26]. Increased respiration of seeds due to high temperature, moisture content and O_2/CO_2 ratio is considered as the main cause of seed deterioration. However, seed ageing and deterioration is inexorable and the best that can be done is to lower its rate (Coolbear, 1995) [9]. The detrimental effects of ageing may also be nullified (repaired) by exposure of aged seeds to osmopriming (Mouradi *et al*., 2016) [36] which may be described as a pre-sowing seed treatment in osmotic solution of low water potential followed by re-drying. It allows seeds pre-germinative metabolic activities to proceed (first stage of germination) but prevents radicle protrusion through the seed coat. Polyethylene glycol (PEG), mannitol, or salts are generally used to lower water potentials of the solutions which improve the speed and uniformity of germination, especially under adverse conditions. Seed deterioration under natural conditions takes longer time to affect the seed qualities. Artificially induced accelerated ageing (AA) is a useful technique to lower the seed viability quickly for experimental purposes (Rodo and Marcos-Filho, 2003) [43] which involves exposing seeds to

Corresponding Author:
Farooq A Khan
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Kashmir,
Shalimar, Srinagar, Jammu
Kashmir, India

highly adverse storage conditions *i.e.*, high humidity and temperature for specific periods of time. This test is able to provide information with a high degree of consistency, and 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.

Onion seeds exhibit very poor longevity after harvest and lose its viability within 1-2 years (Khan *et al.,* 2004) [24] resulting in poor germinability with less vigour and posing serious economic loss to the onion industry. Besides, onion seeds take more time (10 to 14 days) to germinate under normal conditions and it may further enhanced up to 30 days when under suboptimal conditions of temperature. Crusty soils can reduce plant numbers especially where seed vigour is not strong. Therefore, invigouration of poor quality onion seeds is of prime importance. The present investigation was performed to understand the effect of accelerated ageing and subsequent osmopriming on physio-biochemical attributes of germinating onion seeds.

$$\text{Water potential (bar)} = -(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,CT + (8.39 \times 10^{-7})\,C^2T \;\dots\dots\; (I)$$

Where C is Poly Ethylene Glycol concentration, T is temperature (°C).

Each lot of deteriorated onion seeds achieved through AA was again divided into four sub-lots having 250 seeds in each and submerged in aerated solutions of PE G6000 (-1.5 MPa) using 100ml beakers by maintaining seed to solution ratio of 1:5 (w/v). Seeds submerged in osmotic PEG solution were incubated in a B.O.D at 25±2°C temperature for 0 (P0), 24 (P0), 48 (P0), and 72 (P0) hours. After the specified periods of time, seed samples were removed from the B.O.D., rinsed in tap water and shade dried to original moisture level (8.5 percent).

The seeds were then surfaced sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. Four replicates of 50 seeds from of 16 sub-lots were placed in petri dishes (14.0 cm dia) lined with ten layers of Whatman No.1 filter paper moistened with 40 ml of distilled water and kept in seed germinator at 25±2°C temperature for germination. The experiment was arranged in factorial manner following completely randomized design (CRD).

Observations recorded
Germination was observed daily according to the Association

Materials and Methods
Accelerated Ageing (AA) treatments
Healthy and uniform seeds of Yellow globe onion were obtained from the division of Vegetable Science, SKUAST-Kashmir and divided into four lots of 1000 seeds. Each seed lot was kept in plastic boxes and placed in a seed germinator (**S**CIENO**C**RAFT) at 45±2°C temperature and 100% relative humidity for 0 (A_0) 48 (A_1), 72 (A_2) and 96 (A_3) hours to get the seeds with different degree of deterioration. After specified periods of exposure, seeds were removed from the seed germinator and air dried under shade until their original moisture content (8.5%) was achieved.

Osmopriming (OP) treatments
Each lot of artificially-aged (AA) seeds was again divided into four sub-lots having 250 seeds in each. Osmopriming (OP) of artificially aged seed lots were done using -1.5MPa solution of PEG 6000. Amount of PEG required to prepare the osmotic solution of -1.5MPa was calculated with the help of equation I (given by Michel, 1973) [31].

of Official Seed Analysts method (AOSA, 1990) [2] until a constant count was achieved. A seed was considered as germinated when the radicle emerged emerged out of seed coat (Sedghi *et al.*, 2011) [46]. Germination percentage (GP) was calculated using equation-II (Ashraf and Abu-Shakra, 1978) [5]

$$GP = \frac{\text{Total germinated seed after 12 days}}{\text{Total number of seeds}} \;\dots\; (II)$$

The shoot and root length were measured from the base of the hypocotyle to the tip of the primary leaf and root, respectively. The dry weight of the seedlings was also recorded and expressed in grams. Coefficient of velocity of germination (CVG) which gives an indication of rapidity of germination was calculated as per the equation III (Kader and Jutzi, 2004) [20].

$$CVG\ (\%d^{-1}) = 100 \times \sum Ni / \sum (Ni \times Ti) \;\dots\dots\; (III)$$

Where, Ni is the percentage of germinated seed in day I; Ti is the sequence of day from sowing seed.

First and second seedling vigour indices (SVI-I and SVI-II) were calculated using equation IV and V, respectively (Abdul-Baki and Anderson, 1973) [1]:

$$\text{SVI-I} = \text{dry weight of 10 seedling (g)} \times \text{germination (percent)} \;\dots\dots\; (IV)$$

$$\text{SVI-I} = \text{length of 10 seedlings (root + shoot) (cm)} \times \text{germination (percent)} \;\dots\dots\; (V)$$

Chlorophyll extraction was done following the method of Hiscox and Israelstam (1979) [17], using Dimethyl sulfoxide (DMSO) as extractant and the absorbance of the supernatant was recorded at 663, 645nm. Total chlorophyll content was determined by using the formula given by Arnon (Arnon, 1949) [3] and expressed as mg g^{-1} of fresh leaf.

$$\text{Total Chlorophyll} = \frac{[20.2\,(D_{645}) + 8.02\,(D_{663})]}{1000 \times W} \times V \;\dots\dots\; (VI)$$

$$\text{Total Chlorophyll} = \frac{[20.2\,(D_{645}) + 8.02\,(D_{663})]}{1000 \times W} \times V \;\dots\dots\; (VI)$$

Where, D = Absorbance specific wave length, V = Final volume of DMSO (ml), W = Weight of fresh leaf (g).

The amount of total soluble sugars was estimated using phenol sulphuric acid method (Dubois *et al.*, 1956) [11]. Five hundred mg of fresh leaf materials was kept in 10 ml of alcohol for 1 hour at 600C in incubator. Extraction of protein

from the shoot was done following the method of Bradford (1976) [7] using Bovine Serum albumin (BSA) as standard. Sairam (1994) [44] was followed for determining the shoot membrane stability index (MSI) in the sample (equation VII)

Membrane stability index (MSI) = [1 - (C_1/C_2)] × 100 …(VII)

Whereas, C1 is the EC of leachates obtained by heating the sample in double distilled water at 40 °C for 30 min and C2 is the EC of leachates obtained by boiling the same sample in at 100 °C for 10 min.

Malondialdehyde (MDA) content of shoots generated as product of lipid peroxidation was estimated according to Cakmak and Horst (1991) [8] following equation VIII.

$$[MDA] = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} …….. (VIII)$$

Where, A_{532}, A_{600} and A_{450} represent the absorbance of the mixture at 532, 600, and 450 nm, respectively.

The total antioxidant capacity of the plant extracts was measured by the method described by Prieto *et al.* (1999) [40]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. Data were subjected to the statistical analysis following analytical procedures as described by Gomez and Gomez (1984) [15]. Level of significance used for F; and t; tests were $p < 0.05$ from the Table given by Fisher (1970) [13].

Results

Accelerated ageing treatments significantly decreased the germination from 85.56 percent in control (A_0) to 75.02, 53.60 and 12.66 percent in 48 (A_1), 72 (A_2) and 96 (A_3) hours of ageing treatments, respectively (Table 1). In contrast, seed osmopriming with -1.5MPa of PEG 6000 improved germination percent from 51.91 in control (P_0) to 55.62, 59.46 and 59.84 in 24 (P_1), 48 (P_2) and 72 (P_3) hours of priming duration. However, percentage of seed germination due to 48 hours of osmopriming was in fact statistically at par with 72 hours of osmopriming. Two-way interaction of the treatments (A×P) clarified that two factors in combinations also exert significant influence on seed germination percent which varied from 11.30 ($A_3 \times P_1$) to 89.27 ($A_0 \times P_3$). Different durations of artificial ageing treatments with no or zero hours priming showed a germination percent value of 82.24 ($A_0 \times P_0$), 70.63 ($A_1 \times P_0$), 45.30 ($A_2 \times P_0$) and 9.50 ($A_3 \times P_0$) but they were found to increase up to 89.27 ($A_0 \times P_3$), 77.37 ($A_1 \times P_3$), 58.30 ($A_2 \times P_3$) and 15.40 ($A_3 \times P_3$).

Accelerated ageing of different durations also caused a decrease in shoot and root length of onion seedling measured at 20 days after sowing (Table 1). A maximum shoot and root length were recorded as 5.94 and 4.83cm which were found to decrease as 4.63 and 4.59cm, 3.94 and 4.70cm, and 2.93 and 2.99cm, respectively due to 48, 72 and 96 hours of ageing treatments. However, reduction in shoot length due to ageing was statistically non-significant. Seed osmopriming with -1.5MPs solution of PEG6000 resulted in an increase in both shoot as well as root length but the increase in root length was non-significant. Highest increase in shoot length (4.41cm) was recorded with 72 hours of priming duration which was statistically at par with 48 hours of priming. Interaction effects of ageing and priming (A×P) revealed that $A_0 \times P_0$ resulted in maximum shoot length of 4.2cm compared to the recorded shoot length of 3.57, 2.63 and 2.30cm in $A_1 \times P_0$,

$A_2 \times P_0$ and $A_3 \times P_0$, respectively. Likewise, interaction of different ageing treatments with 48 hours of priming ($A_0 \times P_2$, $A_1 \times P_2$, $A_2 \times P_2$ and $A_3 \times P_2$) showed highest shoot length of 7.50, 5.30, 5.30 and 3.40cm compared to interaction of different ageing treatments with other priming durations. Interaction effect of different ageing and priming durations, however, was found as non-significant.

Findings on seedling dry biomass (Table 2) clarified that accelerated ageing treatments significantly reduced the seedling dry biomass from 21.18mg/seedling in un-aged seeds to 20.25, 17.98 and 15.84 mg/seedling by 48, 72 and 96 hours of ageing treatments. In contrast, osmopriming treatments with -1.5MPa solution of PEG 6000 were found to increase the seedling dry biomass from 17.65 mg/seedling in un-primed seeds to 18.53, 19.9 and 19.38 mg/seedling in 24, 48 and 72 hours of priming duration, respectively. Interaction accelerated ageing and priming (A×P) additionally stipulate that ageing treatments markedly reduced the seedling dry biomass and gone from 20.30mg/seedling ($A_0 \times P_0$) to 14.20mg/seedling in 96 hours of aged seeds ($A_3 \times P_0$). However, osmopriming of aged seeds caused a significant increase in seedling dry biomass and the maximum (22.40mg/seedling) was recorded with ($A_0 \times P_3$) followed by $A_0 \times P_2$ (21.30mg/seedling), $A_0 \times P_1$ (20.70 mg/seedling) and $A_0 \times P_0$ (20.30mg/seedling).

Accelerated ageing treatment of different durations adversely affected the seedling vigour indices (SVI) (Table 2) measured in terms of SVI-I and SVI-II. A SVI of 18.12 (SVI-I) and 921.5 (SVI-II) recorded with A_0 (control) were gone down to 2.01 (SVI-I) and 75.0 (SVI-II) in 96 hours of accelerated ageing treatments (A_3). SVIs were found to increase due to different priming durations with -1.5MPa of PEG 6000. The maximum improvement in seedling vigour indices (SVI-I and SVI-II) was observed with 48 hours of priming treatments with absolute values of 11.71 and 580.3, respectively as compared to seedling vigour indices values of 9.16 and 389.8, respectively. Interaction of ageing and priming treatments also explained that ageing treatments declined both SVI-I and SVI-II from 16.69 and 756.6 to 13.84 and 610.2, 7.47 and 300.3, and 1.35 and 52.9, respectively due to 48, 72 and 96 hours of ageing treatments. Conversely, osmopriming was found to enhance the seedling vigour indices significantly but osmopriming for 24 hours of duration was established as the most prominent among all other priming durations.

Coefficient of velocity of germination (CVG) of onion seeds measured under varying degree of accelerated ageing showed (Figure 1) a decreasing trend with increased ageing intensity as A_0 showed a CVG value of 43.78% day^{-1} compared to CVG values of 29.03, 17.45 and 9.00% day^{-1}, respectively with 48 (A_1), 72 (A_2) and 96 (A_3) hours of ageing treatments. However, the average value of CVG (20.38% day^{-1}) with P_0 got increased due to 22.90, 26.60 and 29.38% day^{-1} due to 24 (P_1), 48 (P_2) and 72 (P_3) hours of seed priming with PEG 6000 of -1.5 bar strength. Two way interaction of the data also exhibited identical trends that showed CVG values of 39.20% day^{-1} ($A_0 \times P_0$), 22.80 ($A_1 \times P_0$), 14.60% day^{-1} ($A_2 \times P_0$) and 04.90% day^{-1} ($A_3 \times P_0$) under different ageing treatments while as 72 hours of priming treatment (A_3) showed better CVG under different degrees of ageing treatments with absolute values of 49.20% day^{-1} ($A_0 \times P_3$), 35.40% day^{-1} ($A_1 \times P_3$), 20.20% day^{-1} ($A_2 \times P_3$) and 12.70% day^{-1} ($A_3 \times P_3$).

Shoot chlorophyll content (Figure 2) of was found to decrease through increasing durations of ageing treatments. The

highest chlorophyll content (0.74mg.g.$^{-1}$FW) was recorded with control (A_0) which was decreased to 0.65, 0.47 and 0.19 mg.g.$^{-1}$ FW under 48 (A_1), 72 (A_2) and 96 (A_3) hours of ageing treatments. As anticipated, the shoot chlorophyll content (0.44 mg.g.$^{-1}$FW) of un-primed seeds (P_0) was got intensified to 0.46, 0.53 and 0.62 mg.g.$^{-1}$FW under 24 (P_1), 48 (P_2) and 72 (P_3) hours of priming durations. Decreased chlorophyll content due to accelerated ageing treatment had also been evident through interaction of ageing and priming treatments which showed the absolute chlorophyll values of 0.62, 0.36 and 0.14 mg.g.$^{-1}$FW under 48 ($A_1{\times}P_0$), 72 ($A_2{\times}P_0$) and 96 ($A_3{\times}P_0$) hours of ageing treatments against a chlorophyll value o 0.64 mg.g.$^{-1}$FW of chlorophyll in control ($A_0{\times}P_0$). Interaction treatments also verified the encouraging effect of osmopriming on shoot chlorophyll content which caused significant improvement under different ageing treatments and highest values of 0.87, 0.71, 0.63 and 0.26 mg.g.$^{-1}$ FW were observed with $A_0{\times}P_3$, $A_1{\times}P_3$, $A_2{\times}P_3$ and $A_3{\times}P_3$ interactions, respectively.

The values of soluble sugar and protein of onion seedling (Figure 3 & 4) got deprived in A_1 (0.65 & 0.75%), A_2 (0.51 & 0.42%), A_3 (0.32 & 0.27%) of ageing treatments compared to A_0 (0.77 & 0.74%), while as different osmopriming treatments *viz* P_1 (0.57 & 0.51%), P_2 (0.60 & 0.57%), P_3 (0.59 & 0.61%) showed a recuperating trends over least values (0.49 & 0.50%) of both sugar and protein of non-primed seeds (P_0). Interaction of accelerated ageing and osmopriming (A×P) showed the highest values of sugar and protein contents with $A_0{\times}P_3$ (0.87 & 0.78%) followed by $A_1{\times}P_3$ (0.68 & 0.78%), $A_2{\times}P_3$ (0.54 & 0.43%) and $A_3{\times}P_3$ (0.36 & 0.34%). However, the least values of both sugar ans protein were recorded with $A_3{\times}P_0$ (0.25 & 0.23%) followed by $A_2{\times}P_0$ (0.44

& 0.32%), $A_1{\times}P_0$ (0.64 & 0.69%) and $A_0{\times}P_0$ (0.64 & 0.79%). Individual effect of accelerated ageing treatments indicate (Table 3) that accelerated ageing treatments decreased MSI values from 44.09% (A_0) to 41.64 (A_1), 34.93 (A_2) and 33.43% (A_3) while increased the lipid peroxidation values from 08.90 μmol MDA.g^{-1} FW (A_0) to 09.78 (A_1), 11.12 (A_2) and 12.39 (A_3) μmol MDA.g^{-1} FW. However, different osmopriming treatments did not increase the MSI values but conversely, they were able to decrease the lipid peroxidation of seeds from 11.81 (P_0) to 10.86 (P_1), 10.08 (P_2) and 9.43 (P_3) μmol MDA.g^{-1} FW. It is also evident from the interaction data that two factors in combination (A×P) influenced the MSI and lipid peroxidation wherein respectively, highest and lowest values (46.81% & 07.53 μmol MDA.g^{-1} FW) were recorded with $A_0{\times}P_3$. However, the lowest value of MSI (31.67%) and highest value of lipid peroxidation (13.16 μmol MDA.g^{-1} FW) were recorded with $A_3{\times}P_2$ and $A_3{\times}P_0$, respectively.

Findings presented in table 3 also revealed that seedling anti-oxidant capacity increased from 40.50 mg GAE.g^{-1} DW (A_0) to 51.00 mg GAE.g^{-1} DW due to relatively lower degree of ageing (A_1). However, further intensification of ageing treatments (A_2 and A_3) decreased the antioxidant capacity to 42.50 and 33.75 mg GAE.g^{-1} DW. Anyway, osmopriming treatments with PEG 6000 (-1.5MPa) was found to build up the antioxidant capacity from 38.00 mg GAE.g^{-1} DW (A_0) to 41.25, 43.00 and 45.50 mg GAE.g^{-1} DW in A_1, A_2, A_3, respectively. Interaction of A×P, however, resulted in highest antioxidant capacity of 52.0 mg GAE.g^{-1} DW with $A_1{\times}P_2$ and $A_1{\times}P_3$ followed by $A_1{\times}P_0$ (47.0 mg GAE.g^{-1} DW) and $A_0{\times}P_3$ (46.0 mg GAE.g^{-1} DW) against the minimum antioxidant capacity (29.00 mg GAE.g^{-1} DW) observed with $A_3{\times}P_0$.
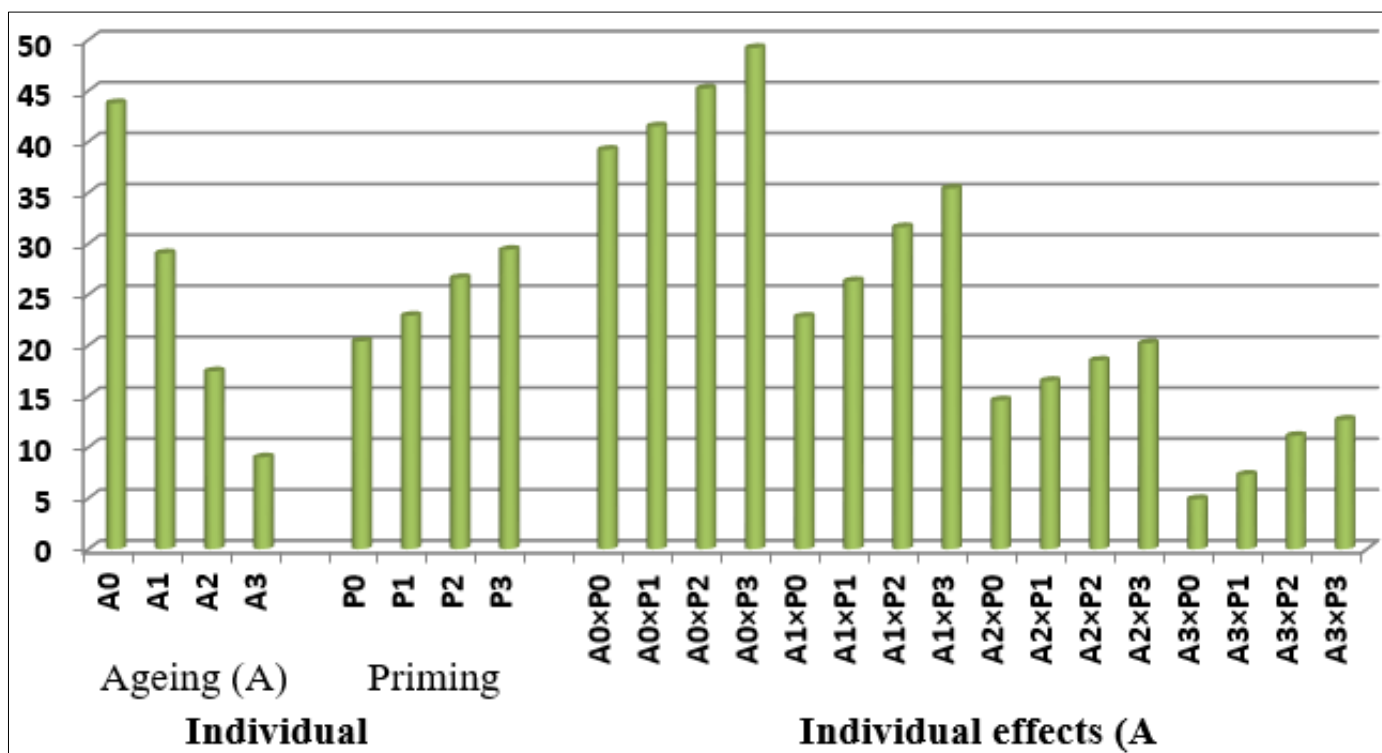

Fig 1: Effect of accelerated ageing of seed and subsequent priming on coefficient of velocity of seed germination (% day^{-1}) in onion
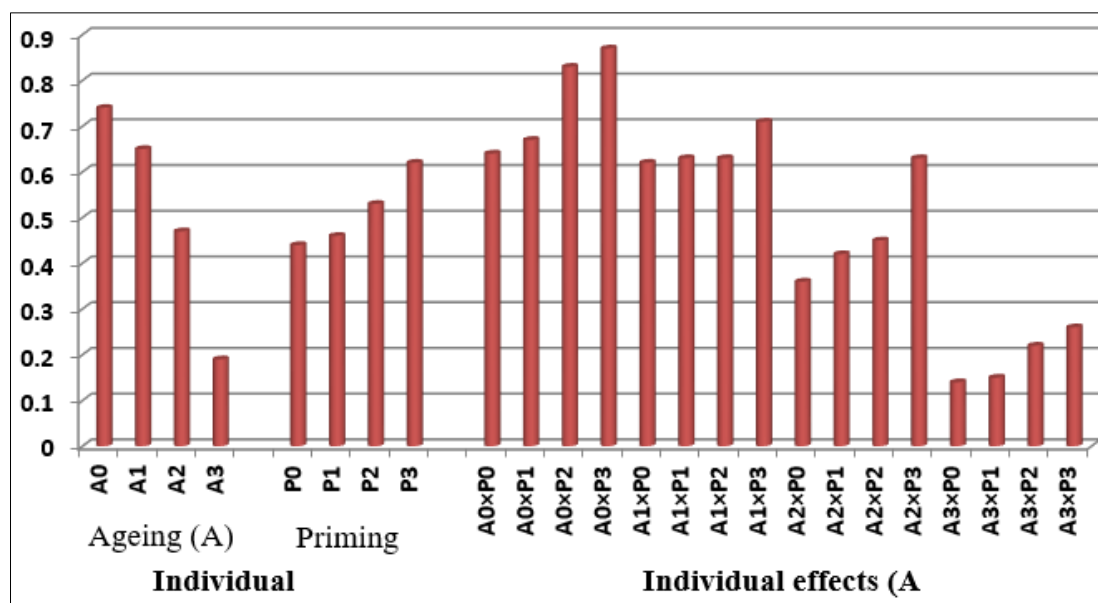
Fig 2: Effect of accelerated ageing of seed and subsequent priming on total chlorophyll content (mg^{-1}.FW) of onion seedlings
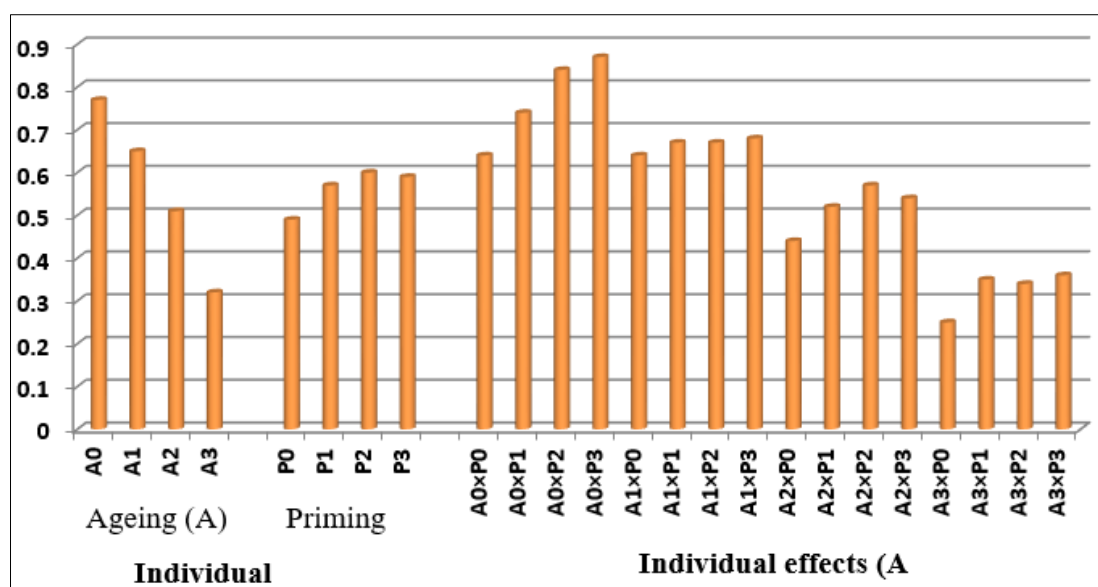

Fig 3: Effect of accelerated ageing of seed and subsequent priming on leaf soluble sugar content (%) of onion seedlings
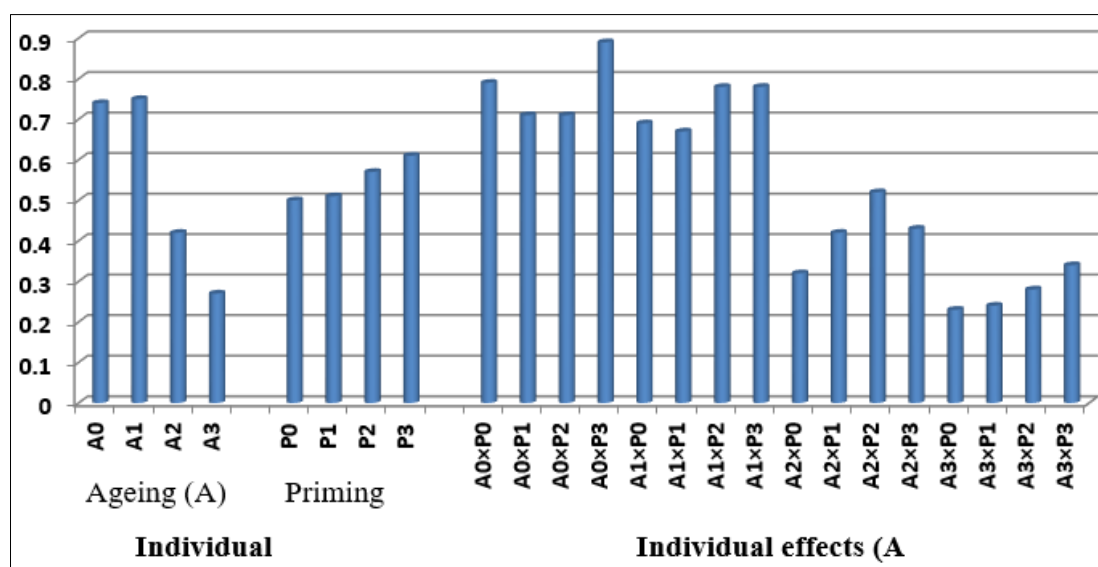

Fig 4: Effect of accelerated ageing of seed and subsequent priming on leaf protein content (%) of onion seedlings

Table 1: Effect of accelerated ageing of seed and subsequent priming on seed germination and seedling growth in onion

Treatments	Seed germination attributes		
	Final germination (%)	Shoot length (cm)	Root length (cm)
Individual effects of accelerated Ageing (A)			
00 hours – A_0	85.56 (9.3)	5.94 (2.61)	4.83 (2.40)
48 hours – A_1	75.01 (8.71)	4.63 (2.36)	4.59 (2.35)
72 hours – A_2	53.60 (7.38)	3.94 (2.20)	4.70 (2.38)
96 hours – A_3	12.66 (3.68)	2.93 (1.72)	2.99 (2.05)
CD (0.05P)	0.049	NS	0.125
Individual effects of osmopriming (P)			
00 hours – P_0	85.56 (9.3)	5.94 (2.61)	4.83 (2.40)
24 hours – P_1	75.01 (8.71)	4.63 (2.36)	4.59 (2.35)
48 hours – P_2	53.60 (7.38)	3.94 (2.20)	4.70 (2.38)
72 hours – P_3	12.66 (3.68)	2.93 (1.72)	2.99 (2.05)
CD (0.05P)	0.049	0.29	NS
Interaction effects of accelerated ageing and osmopriming (A × P)			
$A_0 \times P_0$	82.24 (9.12)	4.27 (2.29)	4.93 (2.43)
$A_0 \times P_1$	83.33 (9.18)	5.50 (2.54)	4.33 (2.30)
$A_0 \times P_2$	87.40 (9.40)	7.50 (2.91)	5.20 (2.48)
$A_0 \times P_3$	89.27 (9.50)	6.50 (2.73)	4.87 (2.42)
$A_1 \times P_0$	70.63 (8.46)	3.57 (2.13)	5.07 (2.45)
$A_1 \times P_1$	74.37 (8.68)	5.23 (2.49)	4.90 (2.42)
$A_1 \times P_2$	77.67 (8.86)	5.30 (2.50)	4.40 (2.32)
$A_1 \times P_3$	77.37 (8.85)	4.40 (2.32)	4.00 (2.23)
$A_2 \times P_0$	45.30 (6.80)	2.63 (1.90)	4.00 (2.23)
$A_2 \times P_1$	53.50 (7.38)	3.50 (2.12)	5.37 (2.52)
$A_2 \times P_2$	58.30 (7.70)	5.30 (2.50)	5.03 (2.45)
$A_2 \times P_3$	57.33 (7.63)	4.33 (2.30)	4.40 (2.32)
$A_3 \times P_0$	09.50 (3.24)	2.30(1.81)	3.27 (2.32)
$A_3 \times P_1$	11.30 (3.50)	3.60 (2.14)	3.23 (2.05)
$A_3 \times P_2$	14.47 (3.93)	3.40 (2.09)	2.90 (1.96)
$A_3 \times P_3$	15.40 (4.04)	2.40 (1.84)	2.57 (1.88)
CD (0.05P)	0.49	0.083	NS

Table 2: Effect of accelerated ageing of seed and subsequent priming on seedling dry biomass and vigour indices in onion

Treatments	Seed germination attributes		
	Seedling dry biomass (mg)	SVI-I	SVI-II
Individual effects of accelerated Ageing (A)			
00 hours – A_0	21.18 (4.70)	18.12 (4.26)	921.5 (30.36)
48 hours – A_1	20.25 (4.60)	15.19 (3.90)	691.6 (26.30)
72 hours – A_2	17.98 (4.35)	9.64 (3.10)	463.1 (21.52)
96 hours – A_3	15.84 (4.09)	2.01 (1.42)	75.0 (8.66)
CD (0.05P)	0.018	0.38	1.33
Individual effects of osmopriming (P)			
00 hours – P_0	17.65 (4.30)	9.16 (3.03)	389.8 (19.74)
24 hours – P_1	18.53 (4.40)	10.31 (3.21)	495.6 (22.26)
48 hours – P_2	19.69 (4.54)	11.71 (3.42)	580.3 (24.09)
72 hours – P_3	19.38 (4.50)	11.60 (3.41)	500.9 (22.38)
CD (0.05P)	0.018	0.38	NS
Interaction effects of accelerated ageing and osmopriming (A × P)			
$A_0 \times P_0$	20.30 (4.61)	16.69 (4.09)	756.6 (27.51)
$A_0 \times P_1$	20.70 (4.65)	17.25 (4.15)	819.1 (28.62)
$A_0 \times P_2$	21.30 (4.72)	18.62 (4.32)	1110.0 (33.32)
$A_0 \times P_3$	22.40 (4.83)	20.00 (4.47)	1015.0 (31.86)
$A_1 \times P_0$	19.60 (4.53)	13.84 (3.72)	610.2 (24.70)
$A_1 \times P_1$	20.60 (4.64)	15.32 (3.91)	753.4 (27.45)
$A_1 \times P_2$	20.60 (4.64)	16.00 (4.00)	753.4 (27.45)
$A_1 \times P_3$	20.20 (4.60)	15.63 (3.95)	649.9 (25.49)
$A_2 \times P_0$	16.50 (4.18)	7.47 (2.73)	300.3 (17.33)
$A_2 \times P_1$	17.43 (4.29)	9.33 (3.05)	474.5 (21.78)
$A_2 \times P_2$	19.57 (4.53)	11.41 (3.38)	602.2 (24.54)
$A_2 \times P_3$	18.40 (4.40)	10.55 (3.25)	500.5 (22.37)
$A_3 \times P_0$	14.20 (3.89)	1.35 (1.16)	52.9 (7.27)
$A_3 \times P_1$	15.37 (4.04)	1.74 (1.32)	77.2 (8.79)
$A_3 \times P_2$	17.30 (4.27)	2.50 (1.58)	91.2 (9.55)
$A_3 \times P_3$	16.50 (4.18)	2.54 (1.59)	76.5 (8.75)
CD (0.05P)	0.026	0.55	1.88

Table 3: Effect of accelerated ageing of seed and subsequent priming on membrane characteristics during seed germination in onion

Treatments	Seed germination attributes		
	MSI (%)	MDA (µmol.g⁻¹ FW)	Anti-oxidant (mg GAE.g⁻¹ DW)
Individual effects of accelerated Ageing (A)			
00 hours – A_0	44.09 (6.71)	08.90 (3.11)	40.50 (6.50)
48 hours – A_1	41.64 (6.52)	09.78 (3.25)	51.00 (7.19)
72 hours – A_2	34.93 (6.01)	11.12 (3.48)	42.50 (6.66)
96 hours – A_3	33.43 (5.84)	12.39 (3.65)	33.75 (5.96)
CD (0.05P)	0.071	0.042	0.031
Individual effects of osmopriming (P)			
00 hours – P_0	38.40 (6.26)	11.81 (3.57)	38.00 (6.29)
24 hours – P_1	38.21 (6.27)	10.86 (3.41)	41.25 (6.48)
48 hours – P_2	38.06 (6.23)	10.08 (3.29)	43.00 (6.66)
72 hours – P_3	39.42 (6.33)	9.43 (3.21)	45.50 (6.87)
CD (0.05P)	0.037	0.040	0.028
Interaction effects of accelerated ageing and osmopriming (A × P)			
$A_0 × P_0$	42.08 (6.56)	10.54 (3.39)	37.00 (6.24)
$A_0 × P_1$	43.00 (6.63)	08.82 (3.07)	37.00 (6.24)
$A_0 × P_2$	44.45 (6.74)	08.69 (3.06)	42.00 (6.63)
$A_0 × P_3$	46.81 (6.91)	07.53 (2.91)	46.00 (6.92)
$A_1 × P_0$	42.03 (6.58)	11.21 (3.49)	47.00 (6.99)
$A_1 × P_1$	41.50 (6.51)	10.54 (3.39)	43.00 (7.16)
$A_1 × P_2$	40.70 (6.45)	08.82 (3.07)	52.00 (7.27)
$A_1 × P_3$	42.33 (6.55)	08.55 (3.06)	52.00 (7.34)
$A_2 × P_0$	34.17 (5.92)	12.32 (3.64)	39.00 (6.40)
$A_2 × P_1$	33.60 (5.88)	11.21 (3.49)	42.00 (6.63)
$A_2 × P_2$	35.40 (6.03)	10.52 (3.39)	44.00 (6.78)
$A_2 × P_3$	36.53 (6.12)	10.44 (3.38)	45.00 (6.85)
$A_3 × P_0$	35.03 (5.98)	13.16 (3.76)	29.00 (5.56)
$A_3 × P_1$	34.73 (5.96)	12.88 (3.70)	33.00 (5.91)
$A_3 × P_2$	31.67 (5.70)	12.30 (3.63)	34.00 (5.99)
$A_3 × P_3$	32.30 (5.75)	11.21 (3.49)	39.00 (6.40)
CD (0.05P)	0.11	0.081	0.104

Discussion

Seed germination percentage (GP) determines the crop stand establishment. Seed ageing and deterioration decreased GP and enhance the seed requirement that can be dropped off by improving the GP using various techniques of seed priming. A progressive decrease in seed germination due to increased duration of accelerated aging and healing effect of osmopriming have been reported through the present study. The possible explanation for declined seed germination due to ageing is reduced quality of seed due to lipid peroxidation and cell membrane damage (Barreto and Garcia, 2017) [6] that have also been observed in the present study. The increase in germination may be due to the activity of α-amylase due to osmopriming (Farooq *et al*. 2011; Lemmens *et al*., 2019) [12, 28]. Amylases are key enzymes that play a vital role in hydrolyzing the seed starch reserve, thereby supplying sugars to the developing embryo. Ghassemi-Golezani *et al*. (2008) [14] stated that during seed priming, the embryo expands and compresses the endosperm. The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration, producing free space and facilitating root protrusion after rehydration. The improved seed performance after priming has also been explained by completion of DNA repair during priming (Osborne, 1983) [39].
Accelerated ageing of onion seeds tend to decrease root length, shoot length and seedling dry biomass where reductions were more with increased durations of ageing. On the other hand seed osmopriming with PEG 6000 (-1.5MPa) resulted in an increase in these attributes especially with 24 and 48 hours of priming durations.). Decrease in seedling

growth of various crops due of accelerated ageing has also been reported by earlier researchers; Govindraj *et al*., 2017; Mirmazloum *et al*., 2020) [16, 32]. Reduced seedling growth due to seed ageing is the consequence of declined seed reserve depletion percentage (Mohammadi *et al*., 2011) [34]. A better seedling growth in terms of root and shoot length and seedling dry biomass due seed priming treatments has also been supported by previous workers (Lemmens *et al*., 2019; Kanatas *et al*., 2020) [28, 22]. Improved seedling root and shoot length and seedling biomass might be due to earlier germination and emergence that stimulated seedling growth and vigour causing enhanced plant growth while as early emergence of primed seeds might be due to enhanced expression of germination metabolites such as amylase activity (Farooq *et al*. 2011) [12].
The coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination and reciprocal to the mean germination time. Thus CVG increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically the highest CVG possible is 100 and this would occur if all seeds germinated on the first day (Jones and Sanders, 1987) [19]. Accelerated ageing of seeds greatly declined the CVG value that was found to deepen with increased ageing durations. In opposition, osmopriming of onion seeds with PEG6000 (1.5KPa) brought about a constructive change in CVG which was further improved by increasing priming durations. The altered CVG values due to accelerated ageing as well as osmopriming could be attributed to the discrepancy in the synthesis of DNA, RNA and proteins (Waterworth *et al*., 2019) [54]. Rapid ageing treatments have also been reported to

have negative effect on CVG of seed germination (Rastegar *et al.*, 2011; Kumar *et al.*, 2021) [41, 27]. Results of the present investigation with respect to osmopriming of seeds corroborate the earlier researches (Shahriari *et al.*, 2014; Aryal *et al.*, 2020) [47, 4].

Seedling vigour indices are taken as a measure of seed quality and can be worked out in two ways (i) seedling vigour index-I (SVI-I), also known as seedling length vigour index (SLVI) and (ii) seedling vigour index-II (SVI-II) which is also known as seedling weight vigour index (SWVI) (Abdul-Baki and Anderson, 1973) [1]. Results of the present experiment revealed that both SVI-I and SVI-II were declined with increasing duration of accelerated ageing whereas osmopriming treatments caused optimistic changes in these attributes. The pattern of seed weakening due to accelerated ageing or invigoration due to osmopriming was also validated in various interaction treatments of ageing and priming. Corroborating to the present study, previous studies have shown a significant reduction in SVIs owing to accelerated ageing treatments (Govindraj *et al.*, 2017 [16]; Matera *et al.*, 2019) [16, 29] and enhancement due to osmopriming (Singh *et al.*, 2017; Mirmazloum, *et al.*, 2020) [49, 32]. Improved seedling growth and vigour by osmopriming with PEG may be attributed to increased cell division within the apical meristem and regulated plant growth through enhanced cell enlargement and cell division (Vanacker *et al.* 2001) [52].

The developing seedling of onions obtains nutrients from the endosperm by way of the cotyledon. In addition, the green cotyledon of onion functions as a photosynthetic leaf, contributing significantly to the food supply of the developing seedling. The photosynthetic potential of a plant is directly proportional to the quantity of chlorophyll present in the leaf tissue. Accelerated ageing of seeds greatly lowered the shoot chlorophyll content while as seed priming treatments comprehensively re-established the lowered chlorophyll content. The alterations in chlorophyll content were controlled through the duration of ageing as well as priming treatments.

Decrease in chlorophyll content due to accelerated ageing of seeds might be because of the loss of nutrients from the seeds because of the membrane disintegration during ageing process (Kaewnaree *et al.*, 2011) [21]. The declined chlorophyll content of aged seeds may also be attributed to the reduced biosynthetic potential for chlorophyll (ide) accumulation in comparison to those from un-aged seeds (Singh and Amritphale, 1993) [48]. Declined or improved chlorophyll contents due to seed ageing or priming, respectively have also been reported through earlier studies (Kanto *et al.*, 2014; Khan *et al.*, 2016) [23, 25].

Moori and Eisvand (2017) [35] reported that seed degradation after accelerated ageing is an indicator of the decrease in enzymatic activity and in total soluble protein content in wheat. High protein content in the seeds helps to support vigor and viability (Rejeendran *et al.*, 2018) [42]. The oxidation in proteins contribute to the decrease in the seeds' germination rate (Sano *et al.*, 2016) [45]. Decrease in sugar and protein contents is considered as a common indicator and index of age-induced seed deterioration (Wang *et al.*, 2018; Jiang *et al.*, 2018) [53, 18]. In the present study, accelerated ageing treatments resulted in a significant decline of seedling sugar as well as protein content while as different osmopriming treatments showed recuperating trends of these constituents. Onder *et al.* (2020) [38] also reported a conforming decrease in seedling sugar and protein contents

due to accelerated ageing of safflower seeds (*Carthamus tinctorius* L.). Decreased protein contents due to accelerated ageing of seeds may be associated with various cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity (Kibinza *et al.*, 2006; Waterworth *et al.*, 2019) [26, 54]. Earlier reports also clarified that accelerated ageing caused as significant reduction in total soluble sugar while the amounts of monosaccharides and disaccharides increase (Murthy *et al.*, 2003) [37] and finally converted to reducing sugar. Reducing sugars can cause damage to DNA, RNA and changes the structure of proteins, reducing the viability (McDonald, 1999; Sun and Leopold, 1995) [30, 51].

Deteriorative changes of living tissues are known to mediate chiefly through membrane disintegration which leads to metabolic dysfunction of the cells. Measurement of solute leakage from the seed tissue are used to estimate the damage to the cell membrane of seeds caused by ageing (Barreto and Garcia, 2017; Wiebach *et al.*, 2019) [6, 55], which suggest that ageing has damaging effect on seed membrane resulting through lipid peroxidation and electrolyte leakage. Outcome of the experiment showed that different degree of seed ageing described a severe decrease in MSI coupled with a marked increase in malondialdehyde contents due to lipid peroxidation. Osmopriming of onion seeds showed significant reduction in malondialdehyde contents and thus lipid peroxidation. However, osmopriming did not show any constructive effect on MSI in the present study. Khan *et al.* (2004) [24] showed that onion (*Allium cepa* L.) seeds exhibit increased electrolyte leakage significantly with the ageing duration while the leaching from viable seeds (control) was negligible. Unlike the results of obtained in the present study with respect to osmopriming, most of the earlier studies showed an increase in membrane stability (lower electrolyte leakage) because of the osmopriming Kaewnaree *et al.* (2011) [21] described that malondialdehyde (MDA) was the major product of lipid peroxidation which was associated with an increase in total antioxidant activity when ageing was carried out.

There was a significant increase in antioxidant activity during the initial periods of accelerated ageing. However it was decreased with increase in the periods of accelerated ageing. Conversely, seed priming indicated enhanced levels of these compounds and the activity further increased with increasing duration of priming. Barreto and Garcia (2017) [6] also reported an increased antioxidant activity (CAT and SOD) during initial periods of ageing treatment and then decreased when duration of ageing was enhanced. However, antioxidant activities (CAT and SOD) were found to decrease in three onion cultivars due to seed ageing (Demirkaya *et al.*, 2010) [10].

References
1. Abdul-Baki AA, Anderson JD. Vigour determination in soybean by multiple criteria. Crop Science. 1973;13:630-633. Doi:http://dx.doi.org/10.2135/cropsci1973.0011183X001300060013x.
2. AOSA. Association of Official Seed Analysis. 1990. Rules for testing seeds. Journal of Seed Technology. 1990;12:1-112. Doi:https://analyzeseeds.com/wp-

content/uploads/2018/03/2017-AOSA-Rules-Volume-3.pdf

3. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. Plant Physiology. 1949;24:1-15. Doi: 10.1104/pp.24.1.1.

4. Aryal K, Shrestha A, Subedi R. Effect of various seed priming methods on germination characteristics of black gram. HSOA Journal of Protein Research and Bioinformatics. 2020;2:9.
doi: 10.24966/PRB-1545/100009

5. Ashraf CM, Abu-Shakra S. Wheat seed germination under low temperature and moisture stress. Agronomy Journal. 1978;70:135-139.
doi:https://doi.org/10.2134/agronj1978.00021962007000010032x

6. Barreto LC, Garcia QS. Accelerated ageing and subsequent imbibition affect seed viability and the efficiency of antioxidant system in macaw palm seeds. Acta Physiologiae Plantarum. 2017;39:72.
doi: 10.1007/s11738-017-2367-z.

7. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72:248-254.
doi: https://doi.org/10.1016/0003-2697(76)90527-3

8. Cakmak I, Horst WJ. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiology Plantarum. 1991;83:463-468.

9. Coolbear P. Mechanisms of seed deterioration. In: Seed Quality (Basra, A.S. Eds.). Basic Mechanisms and Agricultural Implications, Food Products Press, New York. 1995, 223-277pp.

10. Demirkaya M, Dietz KJ, Sivritepe HO. Changes in antioxidant enzymes during ageing of onion seeds. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2010;38:49-52.
doi: https://doi.org/10.15835/nbha3814575.

11. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;26:350-356.
doi: https://doi.org/10.1021/ac60111a017

12. Farooq M, Aziz T, Rehman HU, Cheema SA, *et al*. Evaluating surface drying and re-drying for wheat seed priming with polyamines: effects on emergence, early seedling growth and starch metabolism. Acta Physiologiae Plantarum. 2011;33:1707-1713.
doi: 10.1007/s11738-010-0707-3.

13. Fisher RA. Statistical Methods for Researchg Workers (14th Ed.), Oliver an Boyd, Ltd. London. 1970.

14. Ghassemi-Golezani K, Khomari S, Valizadeh M, Alyari H. Changes in chlorophyll content and fluorescence of leaves of winter rapeseed affected by seedling vigor and cold acclimation duration. Journal of Food, Agriculture and Environment. 2008;6:196-199.
doi: 10.15258/sst.2008.36.3.26

15. Gomez KA, Gomez AA. Statistical procedure for Agricultural Research (2nd Edition), John Wiley and Sons, Inc., New York. 1984 [15].

16. Govindraj M, Masilamani P, Selvaraju P, Alex Albert V. Effect of accelerated ageing on germination and seedling vigour of manually and mechanically harvested and

threshed rice seeds. International Journal of Agricultural Science and Research. 2017;7:39-48.
doi: https://www.researchgate.net/publication/334082399

17. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany. 1979;57:1332-1334.
doi: https://doi.org/10.1139/b79-163

18. Jiang FL, Bo LP, Xu JJ, Wu Z. Changes in respiration and structure of non-heading Chinese cabbage seeds during gradual artificial aging. Scientia Horticulturae. 2018;238:14-22.
doi: 10.1016/j.scienta.2018.04.011

19. Jones KW, Sanders DC. The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. Journal of Seed Technology (USA). 1987;11:97-102.

20. Kader MA, Jutzi SC. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19°C. Journal of Agronomy Crop Science. 2004;190:35-38.
doi: https://doi.org/10.1046/j.0931-2250.2003.00071.x.

21. Kaewnaree P, Vichitphan S, Klarnit P, Siri B, Vichitphan K. Effect of accelerated ageing process on seed quality and biochemical changes in sweet pepper (Capsicum annum Linn.) seeds. Biotechnology. 2011;10:175-182.
doi: 10.3923/biotech.2011.175.182.

22. Kanatas P, Dellaportas V, Kakabouki I, Papastylianou P. Seed priming effects on germination and first growth of the medicinal plant Achillea millefolium L. Journal of Phytology. 2020;12: 20-23.
doi: 10.25081/jp.2020.v12.6324.

23. Kanto U, Jutamanee K, Osotsapar Y, Chai-arree W, Jattupornpong S. Rice seed priming with swine manure extract to ameliorate vigor and nutrient status of seedlings. Kasetsart Journal-Natural Science. 2014;48: 333-347.
doi:https://www.researchgate.net/publication/282182900.

24. Khan M, Iqbal MJ, Abbas M, Raza H, Waseem R, *et al*. Loss of vigour and viability in aged onion (*Allium cepa* L.) seeds. International Journal of Agriculture and Biology. 2004;6:708-711.
doi: http://www.ijab.org.

25. Khan FA, Maqbool R, Narayan S, Bhat SA, Narayan R, *et al*. Reversal of Age-induced Seed Deterioration Through Priming in Vegetable Crops-A Review. Advances in Plant & Agriculture Research. 2016;4:00159.
doi: 10.15406/apar.2016.04.00159

26. Kibinza S, Vinel D, Come D, Bailly C, Corbineau F. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. Physiologia Plantarum. 2006;128:496-506.
doi: https://doi.org/10.1111/j.1399-3054.2006.00771.x

27. Kumar S, Basu S, Anand A, Lal SK, Tomar BS. Identification of the best germination indices represents seed quality status in unaged and aged onion seeds. International Journal of Current Microbiology and Applied Science. 2021;10:76-85.
doi: https://doi.org/10.20546/ijcmas.2021.1002.009.

28. Lemmens E, Deleu LJ, Brier ND, De Man WL, De Proft M, *et al*. The impact of hydro-priming and osmo-priming

on seedling characteristics, plant hormone concentrations, activity of selected hydrolytic enzymes, and cell wall and phytate hydrolysis in sprouted wheat (*Triticum aestivum* L.). ACS Omega. 2019;4:22089-22100. doi: 10.1021/acsomega.9b03210.

29. Matera TC, Pereira LC, Braccini AL, Krzyzanowski FC, Scapim CA, *et al*. Accelerated aging test and its relationship to physiological potential of soybean seeds. Journal of Seed Science. 2019;41:301-308. doi: http://dx.doi.org/10.1590/2317-1545v41n3212746.

30. Mc-Donald MB. Seed deterioration: physiology, repair and assessment. Journal of Seed Science Technology. 1999;27:177-273.

31. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant Physiology. 1973;51:914-916. doi: 10.1104/pp.51.5.914

32. Mirmazloum I, Kiss A, Erdelyi E, Ladanyi M, Nemeth EZ, *et al*. The effect of osmopriming on seed germination and early seedling characteristics of *Carum carvi* L. Agriculture. 2020;10:94. DOI:10.3390/agriculture10040094.

33. Mohamed YY. Onion seed ageing and plant regeneration *in vitro*. Ph.D. Dessertation, University of Illinois at Urbana-Champaign. 1991.

34. Mohammadi H, Soltani A, Sadeghipour HR, Zeinali E. Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. International Journal of Plant Production. 2011;5:65-70. doi:https://www.researchgate.net/publication/279526414.

35. Moori S, Eisvand HR. Plant growth regulators and ascorbic acid effects on physiological quality of wheat seedlings obtained from deteriorated seeds. Pakistan Journal of Botany. 2017;49:1811-1819. doi:https://www.researchgate.net/publication/320457670.

36. Mouradi M, Bouizgaren A, Farissi M, Makoudi B, Kabbadj A, *et al*. Osmopriming improves seeds germination, growth, antioxidant responses and membrane stability during early stage of Moroccan alfalfa populations under water deficit. Chilean Journal of Agriculture Research. 2016, 76. doi: 10.4067/S0718-58392016000300002.

37. Murthy UMN, Kumar PP, Sun WQ. Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. Journal of Experimental Botany. 2003;54:1057-1067. doi: https://doi.org/10.1093/jxb/erg092.

38. Onder S, Tonguc M, Guvercin D, Karakurt Y. Biochemical changes stimulated by accelerated aging in safflower seeds (*Carthamus tinctorius* L.) Journal of Seed Science. 2020;20:1-12. doi: http://dx.doi.org/10.1590/2317- 1545v42227873.

39. Osborne DJ. Biochemical control of system operating in the early hours of germination. Canadian Journal of Botany. 1983;61:3568-3577. doi: https://doi.org/10.1139/b83-406

40. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Annals of Biochemistry. 1999;269:337-341.

doi: 10.1006/abio.1999.4019.

41. Rastegar Z, Sedghi M, Khomar S. Effects of accelerated aging on soybean seed germination indexes at laboratory conditions. Notulae Scientia Biologicae. 2011;3:126-129. doi: https://www.researchgate.net/publication/324651996

42. Rejeendran HV, Lakshmi S, Ambika S. Changes of enzymes activities in botanical treated aged seed of soybean (*Glycine max* (L.) Merrill) cv. CO_3 seeds. Legume Research. 2017;41:73-78. doi: 10.18805/LR-3589

43. Rodo AB, Marcos-Filho J. Onion seed vigor in relation to plant growth and yield. The Journal Horticulture Brasileira. 2003;21:220-226. doi: 10.1590/S0102-05362003000200020

44. Sairam RK. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. Indian Journal of Experimental Biology. 1994;32:594-597. Doi:https://www.researchgate.net/publication/316189772

45. Sano N, Rajjou L, North HM, Debeaujon I, Marion-Poll A, *et al*. Staying alive: molecular aspects of seed longevity. Plant and Cell Physiology. 2016;57:660-674. doi: https://doi.org/10.1093/pcp/pcv186

46. Sedghi M, Khomari S, Amanpour-Balaneji B. Effect of seed vigour and hormone priming on glyoxylate cycle enzymes activity in Persian silk tree (Albizia julibrissin Durazz.). World Applied Sciences Journal. 2011;13:541-544. doi:https://www.researchgate.net/publication/267766191.

47. Shahriari, Puteh ADB, Saleh GB, Rahim ABA. Germination at low osmotic potential as a selection criteria for drought stress tolerance in sweet corn. African Journal of Biotechnology. 2014;13:294-300. doi: 10.5897/AJB11.3435.

48. Singh B, Amritphale D. Effect of seed ageing on chlorophyll (ide) accumulation and Hill activity in greening soybean seedling cotyledons. Retrieved on 02-5-2021. 1993. doi:https://agris.fao.org/agris-search/search.do?recordID=CZ9300742

49. Singh S, Lal GM, Bara BM, Mishra SN. Effect of hydropriming and osmopriming on seed vigour and germination of Pea (*Pisum sativum* L.) seeds. Journal of Pharmacognosy and Phytochemistry. 2017;6:820-824.

50. Sisman C. Quality losses in temporary sunflower stores and influences of storage conditions on quality losses during storage. Journal of Central European Agriculture. 2005;6:143-150. doi: https://doi.org/10.5513/jcea.v6i2.260.

51. Sun WQ, Leopold AC. The Maillard reaction and oxidative stress during ageing of soybean seeds. Physiologia Plantarum. 1995;94:94-105. doi:https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-3054.1995.tb00789.x

52. Vanacker H, Lu H, Rate DN, Greenberg JT. A role for salicylic acid and NPR1 in regulating cell growth in Arabidopsis. The Plant Journal. 2001;28:209-216. doi: https://doi.org/10.1046/j.1365-313X.2001.01158.x.

53. Wang T, Hou L, Jian H, Di F, Li J, *et al*. Combined QTL mapping, physiological and transcriptomic analyses to identify candidate genes involved in Brassica napus seed aging. Molecular Genetics and Genomics. 2018;293:1421-1435. doi: https://link. springer.com/article/10.1007/s00438-

018-1468-8.
54. Water worth WM, Bray CM, West CE. Seeds and the art of genome maintenance. Frontiers in Plant Science. 2019;31:1-11. https://doi.org/10.3389/fpls.2019.00706.
55. Wiebach J, Nagel M, Borner A, Altmann T, Riewe D. Age-dependent loss of seed viabilityis associated with increased lipid oxidation and hydrolysis. Plant, Cell and Environment. 2019;43:303-314.
doi: https://doi.org/10.1111/pce.13651.