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RESEARCH ARTICLE

Esterase isozyme degradation and ROS-mediated protein oxidation as indicators of ageing induced seed deterioration in onion (*Allium cepa* L.)

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Abstract

Saturated NaCl salt-induced accelerated ageing is a critical marker test for assessing the viability and vigour of small-seeded, high–value crops with poor storability, such as onions. This study aimed to fill existing knowledge gaps in the biochemical changes associated with onion seed ageing and their impact on germination. The current investigation was conducted in Punjab Agricultural University, Ludhiana, Punjab, India. Seeds were aged in plastic chambers under controlled conditions (42 ± 2°C and 75 ± 1% RH) for 0, 12, 24, 48, 72, 144 and 288 hours. A 72-hour ageing treatment reduced germination to 50%, effectively producing low-vigour seeds, while 288-hours ageing resulted in complete loss of viability. Seed deterioration was primarily driven by increased malondialdehyde accumulation and moisture content along with loss of membrane integrity. Decline in germination and an increase in abnormal seedlings were linked to reduced activities of enzymes-dehydrogenases and α-amylase involved in respiration and reserve mobilization, respectively. Simultaneously, spectrophotometric analysis revealed reduced activities of chief enzymatic antioxidants such as catalase and peroxidase in conjunction with elevated levels of malondialdehyde and advanced oxidation protein products following prolonged ageing, indicating a diminished capacity of aged seeds to neutralize reactive oxygen species due to inadequate antioxidant capacity. Electrophoretic analysis demonstrated substantial degradation of esterase bands (Rf 0.39 and 0.45) while 288-hours-aged seeds exhibited a 6.39-fold increase in malondialdehyde content and a 26.91-fold increase in accumulation of advanced oxidation protein products compared with fresh seeds. The findings of the study suggest that esterase isozyme profiling, particularly the marked decline of bands at Rf 0.39 and 0.45 coinciding with a 99 to 100% loss of viability, may serve as a potential biochemical marker for assessing seed viability and vigour.

Keywords: Accelerated ageing, Advanced oxidation protein products, Isozymic patterning, Malondialdehyde content, TTC.

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Introduction

Onion (*Allium cepa* L.) is the most widely consumed and cultivated Indian spice-cum-bulb vegetable globally due to its significant culinary, nutritive and therapeutic value. Onion seeds have high market value but possess a short shelf life of only about 9 to 12 months. This is the cause for huge economic loss for onion farmers and seed producers, with factors like temperature and moisture chiefly responsible for seed deterioration. Additionally, limited access to quality seeds due to inadequate storage, poor handling, substandard quality and elevated seed moisture further constrain onion productivity in the Indian agri-sphere (Peter, 2018). Saturated salt-based accelerated ageing technique, a modification of the conventional test, helps to assess the physiological quality of high-value small-seeded crops prone to rapid seed deterioration due to temperature, humidity and moisture. The usage of saturated salt slows the rate of water uptake and microflora growth, leading to more precise and consistent seed vigour measurements (Singh et al., 2015). Seed deterioration is an irreversible process linked

with various physio-biochemical and metabolic alterations. These include lipid peroxidation and auto-oxidation, facilitated loss of membrane integrity, increased permeability, changes in unsaturated fatty acids and antioxidant systems – both enzymatic and non-enzymatic. Key biochemical markers associated with seed ageing *viz*., elevated MDA content, altered enzymatic antioxidants (CAT, POD and SOD), α-amylase and esterase activities, have been reported as reliable ageing indicators in vegetable crops (Bhanuprakash et al., 2010).

Esterases are important hydrolytic enzymes involved in reserve mobilization during seed germination and their activity is frequently associated with seed vigour and viability. Progressive deterioration of esterase isozymes has been reported during seed ageing and may reflect impairment of membrane integrity and metabolic activity (Varier & Dadlani, 1992; Bhanuprakash et al., 2010). Similarly, excessive accumulation of reactive oxygen species (ROS) during ageing promotes oxidative modification of proteins, resulting in the formation of advanced oxidation protein products (AOPPs), loss of enzymatic functionality and disruption of cellular metabolism. Therefore, esterase isozyme degradation and ROS-mediated protein oxidation may serve as sensitive biochemical indicators of seed deterioration. To address these challenges, we conducted a series of laboratory experiments under controlled conditions using the saturated NaCl salt induced accelerated ageing test. The objectives were to standardize the methodology for accelerated ageing of onion seeds and gain deeper insights into their deterioration patterns under simulated ageing conditions. These tests may also provide a theoretical basis for translating findings into breeding strategies for onion varieties with improved storage characteristics.

Materials and Methods

Plant material

Seeds of onion cv. Punjab Naroya were obtained from the Office of Director (Seeds), Punjab Agricultural University, Ludhiana, Punjab, India (30° 54′ N, 75° 48′ E, 247 m above mean sea level). Saturated NaCl accelerated ageing was conducted according to ISTA (2021) and Singh et al. (2015) using three biological replicates maintained at 42 ± 2°C and 75 ± 1% RH for 0, 12, 24, 48, 72, 144 and 288 hours. Untreated seeds served as control.

Seed moisture content

Seed moisture content was determined using 2 g seed samples in a moisture analyzer (AND MX-50) set at 103°C as per the ISTA (2021) guidelines for seeds with high oil content and the average value was presented as percentage.

In-vitro germination studies

In-vitro germination studies were carried out using the "between the paper" method with 100 seeds in an incubator (Model CI-10S) at 25 ± 2°C as suggested by ISTA (2021). On the 12th day, normally germinated seedlings (considered as germination percentage), abnormal seedlings and dead seeds were recorded. Ten representative seedlings were selected at random. The shoot and root lengths of seedlings were measured using a centimeter scale and added to calculate the total seedling length. Fresh weight of the same ten seedlings was recorded using an electronic balance (Model Precisa 310M). Vigour indices were calculated by multiplying the germination (%) to the respective average seedling length for SVI-I (Abdul-Baki & Anderson, 1973) and to the respective fresh weight of the ten seedlings for SVI-II (Huang et al., 2018).

Germination speed index (GSI) and mean germination time (MGT) were calculated using Eq. 1 and 2 as described by AOSA (1983) and Ellis & Roberts (1981), respectively, with 50 seeds per biological replicate placed on moistened filter paper in petriplates in an incubator (Model CI-10S) set at 25 ± 2°C with GSI expressed as seeds day^{-1} and MGT expressed as days. The data on the number of seeds germinated to at least 2 mm radicle length was recorded daily and the germinated seeds were removed from the petriplates.

$$GSI = \left\{\left(\frac{\text{No. of seed germinated}}{\text{First day count}}\right) + \left(\frac{\text{No. of seed germinated}}{\text{Second day count}}\right) + \ldots + \left(\frac{\text{No. of seed germinated}}{\text{Final day count}}\right)\right\} \quad (1)$$

$$MGT = \frac{\sum(\text{No. of seeds germinated on that respective day} \times \text{No. of days after germination})}{\text{Total number of seeds germinated}} \quad (2)$$

Electrolyte leakage

Electrical conductivity (EC) and membrane stability index (MSI) were determined in imbibed control and aged onion seeds using a conductivity meter (Systronics Conductivity Meter 304) following standard procedures and equations 3 and 4 by ISTA (2021) and Sairam et al. (1997); expressed as mS cm^{-1} g^{-1} and %, respectively.

$$\text{Electrical conductivity} = \frac{\text{Electrical conductivity of seeds-Electrical conductivity of DW}}{\text{Weight of seeds}} \quad (3)$$

$$\text{Membrane stability index} = \left(1 - \frac{\text{Electrical conductivity before boiling}}{\text{Electrical conductivity after boiling}}\right) \times 100 \quad (4)$$

Seed viability enzymes

Embryos of imbibed onion seeds were exposed by cutting the seeds longitudinally along the hilum. The exposed embryos were incubated in 0.5% TTC solution prepared in 50 mM sodium phosphate buffer (pH 7.0) for 6 hours to facilitate the maximum possible reagent penetration. Staining patterns obtained were analysed as per AOSA

(2000) and the percentage of stained, unstained and partially stained embryos was recorded. Dehydrogenase activity was estimated according to Kittock & Law (1968), grinding the exposed embryos in acetone and read at 485 nm. α-amylase activity (EC 3.2.1.1) was estimated from 24-hour imbibed seeds using the standard starch-DNSA assay (Sadasivam & Manickam, 1996).

Seed damage indices

The levels of advanced oxidative protein products (AOPP) were estimated by homogenizing control and aged seeds in chilled tris-HCl buffer (pH 8.1) with 0.1% Triton X100. About 100 µL extract, following cooling centrifugation, was diluted to 1-mL and reacted with 1.16 M potassium iodide (10 µL) and glacial acetic acid (20 µL) at an incubation gap of 5 minutes; followed by absorbance recorded immediately at 340 nm (Kalousova et al., 2002). Soluble proteins were also estimated from the same extraction as per Lowry et al. (1951) and the AOPP levels were expressed as µmol of chloramine-T. mg^{-1} protein.

Lipid peroxidation in onion seeds was estimated *via* the thiobarbituric acid assay and calculated using the Eq. 5 (Heath & Packer, 1968).

$$\text{Lipid peroxidation (µmol malondialdehyde g}^{-1}\text{ seeds)} = 6.45\,(A_{532} - A_{600}) - (0.56 \times A_{450}) \quad (5)$$

Seed antioxidative potential

DPPH radical scavenging activity was measured by reacting 100 µL methanolic extract, diluted to 1-mL, with 4 mL of 0.1 mM DPPH reagent. After 20 minutes incubation, the absorbance of reaction mixture (A_{sample}) was recorded at 517 nm against absolute methanol (blank) and DPPH in methanol ($A_{control}$) using the Eq. 6 and expressed in % (Mensor et al., 2001).

$$\text{DPPH radical scavenging activity}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (6)$$

Seed enzymatic antioxidants

Control and aged seeds from all biological replicates were crushed in 0.05 M sodium phosphate extraction buffer (pH 7.5) containing 1% PVP and 1-mM EDTA in chilled pestle mortars on ice baths. The supernatant obtained following cooling centrifugation at 4°C and 10,000 rpm for 20 minutes was used for estimation of antioxidative enzymes *viz*., CAT (E.C. 1.11.1.6) (Aebi, 1983), POD (E.C. 1.11.1.7) (Claiborne & Fridovic, 1979) and SOD (E.C. 1.15.1.1) (Marklund & Marklund, 1974). Specific activity was obtained after dividing the enzymatic activity by the soluble proteins in the enzymatic extract.

Isozyme profiling

0.5 g seeds were crushed in 50 mM sodium phosphate buffer (pH 7.5) with 1% PVP and 1-mM EDTA. Chilled pestle mortars and ice baths were used for crushing. All proteins were resolved on a 10% resolving gel and 4% stacking gel prepared as per standard procedure (Laemmli, 1970). Total soluble proteins in the extractions were estimated to ensure that 90 µg protein was loaded per well. Esterase isozymes were visualized by incubating the gels in freshly prepared staining solution with 10 mL of 1% napthyl acetate in 70% ethanol and 0.5 g of fast blue RR salt dissolved in 200 mL of 50 mM sodium phosphate buffer (pH 7.5) at 37°C till the reddish-brown bands appear (Brewbaker et al., 1968).

Statistical analysis

The standard error of the mean average value was calculated using Microsoft Excel (2016). Data was subjected to one way ANOVA followed by Tukey's HSD post hoc test at $p \leq 0.05$ for the comparison analysis using Minitab (2017). Pearson's correlation analysis was conducted at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ using OriginPro 2026 statistical software.

Results

Analysis of variance and Tukey's HSD test comparison revealed significant impact of saturated sodium chloride salt induced accelerated ageing treatments especially prolonged exposure period on the seed germination, seedling vigour and biochemical characteristics in onion seeds.

Changes in seed moisture content during accelerated ageing

Onion seeds subjected to high temperature and humidity conditions for durations longer than 24 hours showed a notable increase in the seed moisture (Table 1). A 3.6% point increase in the seed moisture content in 144-hour-aged seeds corresponded to a reduction of 75% point germination (%) in the treated seeds. A 100% reduction in the germination ability of the onion seeds was recorded after 288 hours of ageing treatment. Additionally, a 5.6% point increase was recorded in the seed moisture content following the 288-hours ageing, resulting in the presence of 99% physiologically dead seeds in comparison to the untreated seeds.

Changes in seed germination, vigour and germination speed during accelerated ageing

Seed ageing for 12 hours resulted in 6.7% point increase in germination, followed by a continuous decline with longer ageing (Table 1; Figure 1a). A similar trend was observed for other *in-vitro* germination parameters *viz*., seedling length, vigour indices and germination speed index (GSI) (Table 1; Figure 1b). The increase in seedling length recorded after 12-hours ageing could chiefly be attributed to 30.2% higher root growth following ageing while only a minor 3% increment was recorded in the shoot length. Root length maintained 21.5% increase even in 24-hours-aged seeds contributing to a longer seedling length despite the

Table 1: Impact of saturated NaCl salt-induced accelerated ageing on the germination and associated vigour parameters in onion (*Allium cepa* L.)

Duration of accelerated ageing (hours)	*Moisture content (%)*	*Germination (%)*	*Shoot length (cm)*	*Root length (cm)*	*Seedling length (cm)*	*SVI-I*	*Fresh weight of ten seedlings (g)*	*SVI-II*
0	$7.02^{e} \pm 0.35$	$77.3^{ab} \pm 1.3$	$7.4^{a} \pm 0.20$	$3.9^{b} \pm 0.18$	$11.3^{b} \pm 0.16$	$875.8^{b} \pm 27.5$	$0.49^{a} \pm 0.01$	$37.5^{ab} \pm 1.14$
12	$8.14^{de} \pm 0.19$	$84.0^{a} \pm 2.6$	$7.6^{a} \pm 0.32$	$5.1^{a} \pm 0.29$	$12.7^{a} \pm 0.23$	$1070.1^{a} \pm 48.8$	$0.46^{b} \pm 0.01$	$38.4^{a} \pm 1.73$
24	$9.17^{bcd} \pm 0.18$	$73.3^{b} \pm 2.8$	$6.7^{ab} \pm 0.38$	$4.8^{a} \pm 0.24$	$11.5^{ab} \pm 0.58$	$845.5^{b} \pm 62.7$	$0.44^{ab} \pm 0.03$	$32.0^{bc} \pm 2.37$
48	$9.03^{cd} \pm 0.19$	$60.0^{c} \pm 1.5$	$6.9^{ab} \pm 0.11$	$3.8^{b} \pm 0.18$	$10.7^{bc} \pm 0.11$	$640.6^{c} \pm 20.6$	$0.43^{ab} \pm 0.01$	$26.0^{c} \pm 1.05$
72	$9.76^{bc} \pm 0.59$	$48.7^{d} \pm 2.6$	$5.9^{b} \pm 0.16$	$3.6^{b} \pm 0.09$	$9.5^{c} \pm 0.22$	$462.4^{d} \pm 32.0$	$0.39^{b} \pm 0.01$	$19.1^{d} \pm 0.61$
144	$10.57^{b} \pm 0.08$	$2.7^{e} \pm 0.9$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{d} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{e} \pm 0.0$
288	$12.65^{a} \pm 0.21$	$0.0^{e} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{d} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{e} \pm 0.0$

*Means carrying the similar superscript are statistically at par with each other, as calculated based on Tukey's HSD test at $p \leq 0.05$.

4 per cent point recorded decline in the germination. These enhancements in seedling length, although statistically significant, were still minor and unlikely to be of critical biological importance for long-term vigour and seedling establishment. Overall, short term accelerated ageing appeared to provide transient metabolic stimulation, but still insufficient to offset the seed quality deterioration associated with prolonged ageing.

Mean germination time (MGT) and GSI were also significantly affected by accelerated ageing (Figure 1b). MGT recorded no significant variation up to 72 hours of ageing, but 144-hours-aged seeds recorded a markedly reduced GSI of 0.31 seeds day^{-1} with concomitant increase in MGT than control seeds. Seeds aged for 288 hours failed to sprout, a finding consistent with 99% mortality observed in Figure 1a and further corroborated with 100% unstained embryos obtained in the TTC test (Figure 2a).

Prolonged ageing increased the proportion of dead, non-germinating seeds and induced severe seedling abnormalities that failed to establish successfully. Initial ageing for 12 hours resulted in 6.7% rise in germination,

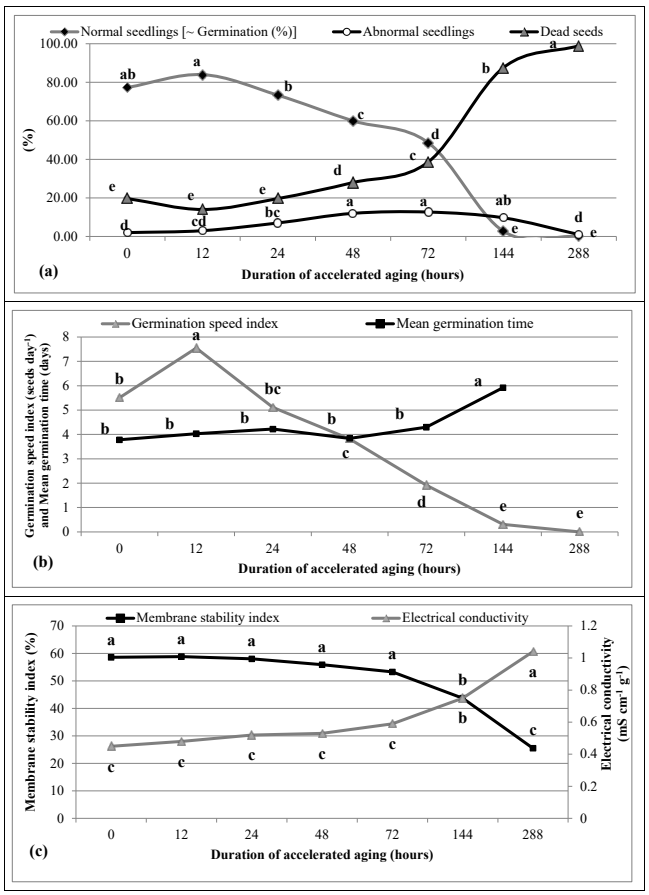

Figure 1: Impact of saturated NaCl salt-induced accelerated ageing on the (a) presence of normal germinated seedlings, abnormal seedlings and dead seeds (b) germination speed index and mean germination time (c) electrolyte leakage and membrane stability index in onion (*Allium cepa* L.).

Table 2: Impact of saturated NaCl salt-induced accelerated ageing on the antioxidant profile of onion (*A. cepa* L.) seeds

Duration of accelerated ageing (hours)	*AOPP levels (µM chloramine-T mg^{-1} protein)*	*Lipid peroxidation (µmoles g^{-1} seeds)*	*DPPH radical scavenging activity (%)*
0	2.00^{e} ± 0.06	0.217^{e} ± 0.05	65.56^{a} ± 0.51
12	1.08^{e} ± 0.19	0.297^{de} ± 0.02	68.77^{a} ± 0.90
24	7.05^{d} ± 0.29	0.396^{cd} ± 0.01	59.06^{ab} ± 0.93
48	11.42^{c} ± 0.37	0.414^{cd} ± 0.03	53.36^{b} ± 1.10
72	13.88^{c} ± 0.48	0.466^{c} ± 0.07	52.85^{b} ± 1.01
144	25.87^{b} ± 0.90	0.638^{b} ± 0.01	25.83^{c} ± 0.20
288	53.81^{a} ± 1.51	1.386^{a} ± 0.05	14.07^{d} ± 0.18

*Means carrying the similar superscript are statistically at par with each other, as calculated based on Tukey's HSD test at $p \leq 0.05$.

statistically comparable to the control, while reducing dead seeds by 5.7% (Figure 1a). Further ageing led to a progressive decline in germination with increase in abnormal seedlings and dead seeds. The emergence of abnormal seedlings followed a parabolic trend, rising up to 72-hours and then declining due to the predominance of dead seeds at later durations (Figure 1a).

Electrolyte leakage and membrane damage during accelerated ageing

Electrical conductivity (EC) of aged onion seeds increased significantly ($p \leq 0.05$) while the membrane stability index (MSI) declined correspondingly (Figure 1c). Electrolyte leakage rose progressively with prolonged exposure to high temperature and humidity. Untreated seeds recorded the lowest EC (0.45 mS cm^{-1} g^{-1}) with 58.6% MSI whereas the 288-hours-aged seeds exhibited a stark rise of 130.1% in EC and a 33.1%% point reduction in MSI relative to control.

Changes in seed viability during accelerated ageing

Tetrazolium staining and the activities of enzymes dehydrogenase, α-amylase and esterase were used as seed viability indices under ageing treatments. Stained embryos were dominant in untreated and 12-hours-aged seeds, consistent with their higher germination, whereas prolonged ageing led to dominance of shrivelled embryos with a sharp rise in partially stained and unstained seeds (Figure 2a). Unstained embryos reached 80 and 100% after 144- and 288-hours of ageing, respectively.

Dehydrogenase activity was significantly ($p \leq 0.05$) enhanced in 12-hours-aged seeds, exhibiting 55.3% increase over control. However, prolonged ageing caused significant diminution in the enzyme activity, with reductions of 88.4 and 99.4% after 144- and 288-hours ageing, respectively (Figure 2a). Similarly, α-amylase activity increased modestly by 2.7% in 12-hours-aged seeds followed by a progressive decline with prolonged ageing. The strongest decline in the α-amylase activity was observed in 144- and 288-hours-aged

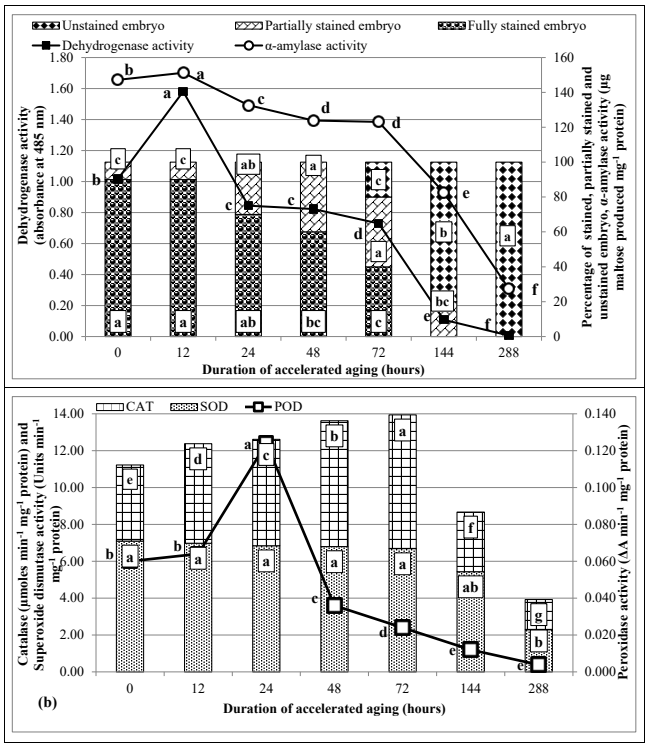

Figure 2: Impact of saturated NaCl salt-induced accelerated ageing on the (a) seed embryo viability percentage and the activity of seed viability enzymes – dehydrogenase and α-amylase and (b) activities of the enzymatic antioxidants in onion (*A. cepa* L.).

seeds recording a 43.9 and 81.4% decrease, which resulted in a mere 2.67% and nil germination in 144- and 288-hours-aged seeds, respectively (Figure 2a; Table 1).

Esterase isozyme profiling revealed a potential association between seed germination capacity and three prominent esterase bands with Rf values 0.39, 0.45 and 0.51, corresponding to band 1, 2 and 3, respectively in Figure 3a and b. The highest band intensity was observed in control and 12-hours-aged seeds but declined progressively with

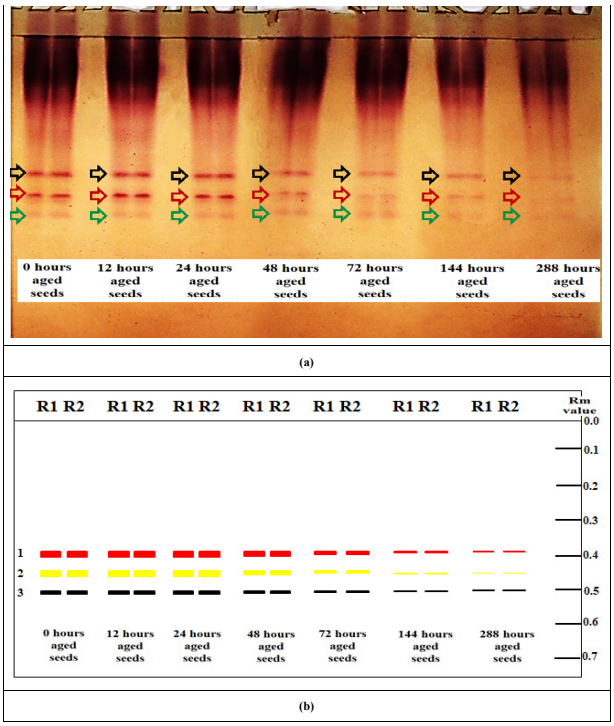

Figure 3: Esterase isozymic patterning (a) and its diagrammatic representation (b) in control and saturated NaCl salt-induced accelerated aged onion (*A. cepa* L.) seeds.

ageing, particularly at Rf 0.39 and 0.45. A marked loss of band intensity in 144- and 288-hours-aged seeds coincided with a 99% loss in germination and viability (Figure 1a), indicating grave physiological damage under prolonged ageing.

These results highlight esterase profiling as a promising biochemical marker for onion seed vigour and viability. However, further densitometric and gene expression analyses, along with cross-validation in other seeds with short storage life, are needed to confirm its reliability relative to other markers such as amylase, lipase and catalase.

Oxidative damage indicators during accelerated ageing

Parameters such as lipid peroxidation and AOPP levels were quantified to assess damage in onion seeds subjected to saturated salt-mediated accelerated ageing. Both MDA and AOPP levels increased significantly ($p \leq 0.05$) with ageing, with sharp rises evident following 24 hours of ageing. The highest degree of damage occurred in 288-hours-aged seeds, showing a 6.39-fold and 26.91-fold increase in MDA and AOPP levels, respectively (Table 2). These trends were closely associated with declines in germination, seedling vigour and viability, reflecting the progressive intensification of oxidative stress induced seed damage (Table 1; Figures 1 and 2a). Elevated AOPP and MDA levels indicate severe oxidative modification of lipids and proteins which in conjunction with other deteriorative changes impaired seed quality.

Antioxidant responses during accelerated ageing

Table 2 presents the non-enzymatic antioxidant profile of control and artificially aged onion seeds. DPPH radical scavenging activity recorded an initial increase by 4.9% after 12 hours of accelerated ageing, followed by a steady decline with prolonged ageing. The lowest values were recorded in seeds aged for 288 hours, showing a stark 78.5% reduction as compared to 0-hours control seeds, suggesting that prolonged ageing adversely impacts antioxidant capacity (Table 2; Figure 2b).

Enzymatic antioxidants showed differential responses to ageing. SOD activity remained statistically similar up to 72 hours, after which it declined steadily, reaching a 67.8% reduction in 288-hour-aged seeds (Figure 2b). CAT and POD activities initially increased, peaking at 72 and 24 hours, respectively, before declining with further ageing. POD activity decreased sharply after 48 hours, whereas CAT and SOD recorded pronounced declines following 144 hours of ageing. The lowest activities of CAT (1.64 µmoles min^{-1} mg^{-1} protein), POD (0.004 ΔA min^{-1} mg^{-1} protein) and SOD (2.29 Units min^{-1} mg^{-1} protein) were observed in seeds accelerated aged for 288 hours.

Correlation analysis

A bivariate Pearson correlation analysis was performed to examine associations between seed germination and various biochemical attributes in accelerated aged onion seeds (Figure 4). Germination percentage showed strong and statistically significant positive correlation with DPPH activity (r = 0.98), α-amylase activity (r = 0.93) and dehydrogenase activity (r = 0.94) apart from the germination and vigour traits. Conversely, negative correlations were observed with electrical conductivity (r = -0.91) and lipid peroxidation

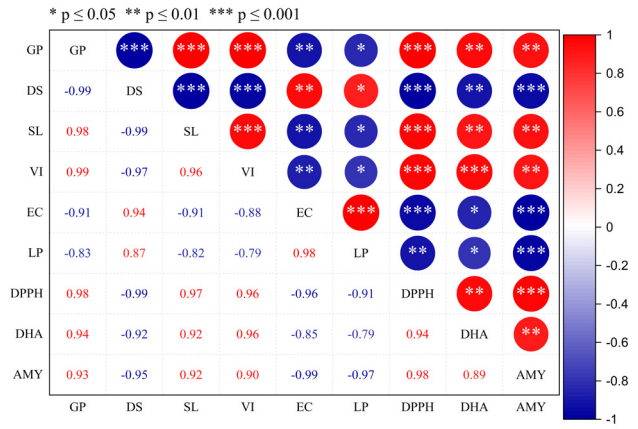

Figure 4: Pearson's correlation analysis in the onion seeds as impacted by the saturated NaCl salt-induced accelerated ageing. GP - germination percentage; DS - percentage of dead seeds; SL – seedling length; VI – seedling vigour index-I; EC – Electrical conductivity of seeds; LP – lipid peroxidation; DPPH – DPPH radical scavenging activity; DHA – dehydrogenase activity; AMY – α-amylase activity

(r = -0.83), suggesting their role in suppression of germination. As illustrated in Figure 4, DPPH radical scavenging activity also exhibited strong positive correlation ($p \leq 0.001$) with germination percentage (r = 0.98), seedling length (r = 0.97), seedling vigour index (r = 0.96), α-amylase activity (r = 0.98) and dehydrogenase activity (r = 0.94). Negative correlations of the DPPH radical scavenging activity were found with the percentage of dead seeds (r = -0.99), electrical conductivity (r = -0.96) and lipid peroxidation (r = -0.91). These correlation studies suggest that seeds exhibiting higher antioxidant capacity tend to display better physiological performance during ageing. Additionally, electrical conductivity and lipid peroxidation levels were inversely correlated with germination and seedling vigour, implying that elevated membrane leakage and MDA accumulation are associated with reduced seed quality parameters. Collectively, these correlation results underscore significant linear associations among seed germination, viability and antioxidant potential.

Discussion

Saturated salt-induced accelerated ageing effectively simulates natural ageing by inducing oxidative and moisture stress to assess seed longevity, viability and vigour, especially in small-seeded vegetables such as onion that deteriorate rapidly due to water uptake (Singh et al., 2015). In the present study, exposure to elevated temperature and relative humidity (42 ± 2°C and 75 ± 1% RH) progressively increased seed moisture, which served as the physiological driver of ageing. Seed moisture is a major physiological indicator of seed health and commercial quality. A 5.6% rise in seed moisture after 288 hours coincided with complete loss of germination (Table 1; Figure 1a), establishing a critical threshold beyond which repair mechanisms failed.

A slight increase in germination and vigour indices was noted after 12 hours of ageing (Table 1), likely reflecting the seed resilience to sub-lethal stress priming. It seems that 12-hours ageing likely provided transitory stimulation, altering the seed reserve mobilisation due to minor stress-induced metabolic priming which, though inadequate to mitigate the steady deterioration of seed quality associated with extended ageing, could indicate a hypothetical inference for the parallel transient stress or sub-optimal conditions experienced during post-harvest seed handling and transport. However, deterioration followed progressively, with slowed germination, reduced vigour and longer mean germination time between 24 to 72-hours of ageing (Table 1; Figure 1b). This is particularly relevant given seedling vigour is a critical trait influencing crop establishment, growth and yield, with vigorous seeds translated into robust viable seedlings capable of withstanding environmental stresses and contributing to crop productivity and profitability. Seeds aged for 144- and 288-hours exhibited a rapid loss of germination capacity due to higher proportions of dead seeds (Figure 1a). Vigour decline was inversely linked to protein deterioration and rapid moisture uptake, similar to aged radish seeds (Jain et al., 2006).

Membrane integrity also declined with rising seed moisture, which disrupted the seed's cellular machinery and ultrastructure integrity and accelerated the rate of seed deterioration. Literature perusal of relevant studies similarly highlighted a physiological association of germination decline to elevated seed moisture content and structural changes such as swollen mitochondria, folded cell walls and indistinct nuclear membranes paralleled with loss of viability markers – dehydrogenase, esterase and α-amylase (Bhanuprakash et al., 2010; Xia et al., 2015), as also evidenced in the current study (Figures 2a and 3). Increased electrolyte leakage further evidenced membrane disintegration, with progressively higher electrical conductivity values (Figure 1c), in line with similar findings in radish and oats (Jain et al., 2006; Xia et al., 2015).

Esterases are hydrolytic enzymes that convert esters, derived from lipid degradation, into alcohols and acids. Their activities are known to increase with the rise in germination capacity of seed lots by facilitating the degradation of seed reserves and thereby releasing materials essential for embryo growth and development (Varier and Dadlani, 1992; Subramani et al., 2011). Similar age-dependent alterations in esterase isozyme profiles were reported by Varier and Dadlani (1992) in pearl millet seeds, where loss of esterase activity was associated with reduced seed quality. Esterase isozyme profiling revealed two prominent bands (Rf 0.39 and 0.45) strongly linked with seed germination and seedling vigour (Figure 3). Control and 12-hour-aged seeds expressed bands with exceedingly high intensity while 288-hours-aged seeds documented a significant intensity loss in its band profile, in alignment with their observed respective germination and seedling vigour trends (Table 1; Figure 1a). Bhanuprakash et al. (2010) also highlighted a similar correlation between esterase activity and seed viability, as in our study. This supports esterase as a potential biochemical marker of seed viability, though further quantitative validation, enzyme localization and gene expression studies are essential. Moreover, esterase activity should be viewed as one of several potential markers, alongside α-amylase and antioxidant systems such as catalase activity, rather than the definitive marker of viability.

Serious moisture-induced oxidative damage to the seed's cellular machinery in terms of higher MDA accumulation and AOPP levels coupled with elevated ROS generation exacerbated lipid peroxidation, further deteriorating membrane integrity and seed metabolism, thus accelerating cellular ageing. Overall, the 6.39-fold increase in MDA and 26.91-fold rise in AOPP levels in 288-hours-aged seeds demonstrated the extensive oxidative damage. These

indices coincided with declining antioxidant enzyme activities (Table 2).

Initial rise in CAT and POD up to 72 and 24 hours, respectively, suggested a transient upregulation in the seed's defensive response machinery to mitigate the ROS overproduction, following which the activities fell sharply thereafter (Table 2; Figure 2b), indicating irreversible cellular damage due to ROS overexpression surpassing the cell's detoxification capacity. The POD activity fluctuations were in contrast to the previous reports of unaltered POD expression for up to 6 days in accelerated aged seeds, after which a consistent reduction was noted in aged seeds for up to 15 days (Bhanuprakash et al., 2010).

Collectively, these results highlight moisture-driven deterioration, ROS-induced oxidative damage, protein oxidation and impaired enzymatic activity as hallmarks of onion seed ageing. The irrecoverable damage by these underlying mechanisms is expressed physiologically as membrane disruption, reduced germination and seedling vigour, higher proportions of dead seeds and abnormal seedlings. A considerable deterioration in the band profile of esterase isozymes documented in seeds aged for 144 and 288 hours could be strongly linking their efficient activities for effective germination, vigour and viability.

Short-term ageing proved to be non-deteriorating and rather capable of inducing reversible metabolic priming, which could positively impact root growth. These results could be extrapolated as sub-optimal conditions experienced in real time during seed transportation and the gap between seed harvesting/sorting and packaging. Ageing for 72 hours was recorded as an ideal ageing duration for producing low-vigour seed (with less than 50% germination) from a high-germination seed lot, mainly required by seed researchers for various vigour enhancement and stress-ameliorating studies and by physiologists for understanding the underlying physio-biochemical and molecular mechanisms responsible for various metabolic and proteomic modulations in the seeds.

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सारांश

प्याज के बीजों की जीवनीयता एवं ओज में शीघ्र गिरावट आती है, जिस कारण उनकी भंडारण क्षमता भी कम होती है। प्रस्तुत अध्ययन का उद्देश्य त्वरित वृद्धावस्था के दौरान प्याज के बीजों में होने वाले जैव-रसायनिक परिवर्तनों तथा उनके अंकुरण पर प्रभाव का अध्ययन करना था। बीजों को संतृप्त NaCl-आधारित त्वरित वृद्धावस्था परीक्षण के अंतर्गत 42 ± 2°C तापमान एवं 75 ± 1% सापेक्ष आर्द्रता पर 0, 12, 24, 48, 72, 144 तथा 288 घंटे तक रखा गया। 72 घंटे की वृद्धावस्था से अंकुरण लगभग 50% तक घट गया, जबकि 288 घंटे की वृद्धावस्था के पश्चात बीज पूर्णतः अजीवित हो गए। वृद्धावस्था के साथ बीजों में नमी की मात्रा, मैलोनडायएल्डिहाइड तथा उन्नत ऑक्सीकरण प्रोटीन उत्पादों (AOPP) का संचय बढ़ा, जबकि झिल्ली स्थिरता, बीज अंकुरण तथा ओज में निरन्तर कमी दर्ज की गई। डिहाइड्रोजनेज़ एवं α-एमाइलेज एंजाइमों की गतिविधियों में कमी के कारण भंडारित खाद्य पदार्थों के उपयोग एवं अंकुरण क्षमता पर प्रतिकूल प्रभाव पड़ा। साथ ही, कैटालेज़ एवं पेरॉक्सीडेज जैसे एंटीऑक्सीडेंट एंजाइमों की गतिविधियों में गिरावट तथा ऑक्सीडेटिव क्षति सूचकों में वृद्धि ने वृद्धीकृत बीजों में सक्रिय ऑक्सीजन प्रजातियों (ROS) से होने वाली क्षति को स्पष्ट किया। आइसोजाइम विश्लेषण में एस्टरेज़ बैंड (Rf 0.39 एवं 0.45) की तीव्र क्षीणता देखी गई। 288 घंटे वृद्धीकृत बीजों में MDA की मात्रा 6.39 गुना तथा AOPP का संचय 26.91 गुना अधिक पाया गया। अध्ययन से यह निष्कर्ष निकला कि विशेषकर Rf 0.39 एवं 0.45 वाले एस्टरेज़ आइसोजाइम का बीज जीवनीयता तथा उनके अंकुरण में 99–100% कमी के साथ सम्बद्ध था। अतः एस्टरेज़ आइसोजाइम प्रोफाइलिंग प्याज के बीजों की जीवनीयता एवं ओज के आकलन हेतु एक उपयोगी जैव-रसायनिक सूचक के रूप में प्रयुक्त की जा सकती है।