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Biochemical basis of seed deterioration during accelerated ageing in wheat cultivars

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Abstract

Nearly 25 percent of global wheat production is lost to poor seed storage, and biochemical deterioration during storage remains a leading cause. This research examined the biochemical changes associated with seed ageing in four wheat cultivars subjected to accelerated ageing (40 °C, 100% RH) for 0, 3, 6, 9, and 12 days at Seoul National University, South Korea. Germination percentage, seedling vigor index, malondialdehyde (MDA) content, superoxide dismutase (SOD) and catalase (CAT) activities, and electrical conductivity were measured. Germination declined from 96.8 to 43.2 percent after 12 days of ageing. MDA content rose by 147 percent while SOD activity dropped 58.3 percent. Cultivar Keumkang retained the highest germination (61.7%) after 12 days, linked to greater residual antioxidant enzyme activity. Lipid peroxidation and membrane damage were the primary drivers of viability loss.

Keywords: Seed deterioration, accelerated ageing, wheat cultivars, lipid peroxidation, malondialdehyde, antioxidant enzymes, germination, superoxide dismutase, membrane integrity, seed vigor

Introduction

Nearly 25 percent of harvested wheat seeds lose viability during storage in tropical and subtropical regions, making seed deterioration a persistent challenge for food security ^[1]. The biochemical mechanisms behind this loss involve a cascade of oxidative events that damage cellular structures, but the relative contribution of each pathway varies across genotypes and storage conditions ^[2].

During storage, reactive oxygen species (ROS) accumulate in seed tissues, triggering lipid peroxidation of membrane phospholipids. This produces malondialdehyde (MDA), a reliable marker of oxidative membrane damage ^[3]. Antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)-normally scavenge ROS, but their activity declines progressively with seed age ^[4]. The resulting imbalance between ROS production and scavenging capacity accelerates cellular breakdown.

Accelerated ageing tests, which expose seeds to high temperature and humidity, compress the natural ageing timeline into days rather than months, and correlate well with field storage outcomes ^[5]. Different wheat cultivars display varying tolerance to ageing stress, suggesting genetic control over antioxidant defense systems ^[6]. This research investigated the biochemical changes during accelerated ageing in four Korean wheat cultivars to identify the key deterioration pathways and rank cultivars for storage tolerance.

Research area description

The laboratory experiment was conducted at the Seed Science Laboratory, Seoul National University, Seoul, South Korea (37.46°N, 126.95°E) from March to June 2023. The accelerated ageing chamber (Model AAC-200, Daihan Scientific, Korea) maintained 40±0.5 °C and 100±2% relative humidity. All biochemical analyses were performed at the same facility using freshly harvested seeds from the 2022 winter wheat crop grown at the university's experimental farm in Suwon, Gyeonggi Province.

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Material and Methods

Material

Four Korean wheat cultivars-Keumkang, Jokyung, Baekjoong, and Uri-were used. Seeds were harvested in June 2022, cleaned, and stored at 4 °C until the experiment began. Initial seed moisture was adjusted to 12±0.5 percent. Chemical reagents including thiobarbituric acid, nitroblue tetrazolium, and hydrogen peroxide were purchased from Sigma-Aldrich Korea. All chemicals were analytical grade.

Methods

Seeds of each cultivar were placed in mesh bags (50 seeds per bag, four replications) and subjected to accelerated ageing at 40 °C and 100 percent relative humidity for 0, 3, 6, 9, and 12 days.

After each ageing interval, germination was tested on moist filter paper at 20 °C for 8 days following ISTA protocols [7]. Seedling vigor index was calculated as germination percentage multiplied by mean seedling length. MDA content was determined by the thiobarbituric acid reactive substances method [8]. SOD activity was measured by its ability to inhibit nitroblue tetrazolium photoreduction, and CAT activity was assayed by monitoring H₂O₂ decomposition at 240 nm [9]. Electrical conductivity of seed leachates was measured after 24 hours of soaking in deionized water at 25 °C using a conductivity meter (Orion Star A215). Protein carbonyl content was estimated using the dinitrophenylhydrazine method. Data were analyzed by factorial ANOVA in R 4.2.1 with Tukey's HSD at *p*≤0.05.

Results

Table 1: Germination percentage and seedling vigor index of wheat cultivars during accelerated ageing

Cultivar	0 d	3 d	6 d	9 d	12 d	SVI-0d	SVI-12d	Decline %
Keumkang	97.3	91.7	82.4	71.6	61.7	1824	783	57.1
Jokyung	96.8	87.2	73.9	58.3	44.6	1791	512	71.4
Baekjoong	95.6	84.1	69.7	52.8	38.4	1753	421	76.0
Uri	97.4	82.9	66.3	49.1	28.2	1809	287	84.1

SVI = seedling vigor index. Tukey's HSD (*P*≤0.05): Cultivar = 4.8; Duration = 3.7; Interaction = 7.2

Germination declined progressively across all cultivars with ageing duration (Table 1). Mean germination fell from 96.8 to 43.2 percent after 12 days. Keumkang was the most tolerant, retaining 61.7 percent germination at day 12, while Uri suffered the sharpest decline to 28.2 percent. Seedling vigor index dropped 57.1 to 84.1 percent depending on cultivar.

Table 2: MDA content (nmol/g FW) and antioxidant enzyme activities at 0 and 12 days of ageing

Cultivar	MDA-0d	MDA-12d	SOD-0d	SOD-12d	CAT-0d	CAT-12d
Keumkang	4.21	8.73	18.4	10.6	7.82	4.31
Jokyung	4.38	10.91	17.6	7.83	7.41	3.12
Baekjoong	4.52	11.87	16.9	6.41	7.13	2.67
Uri	4.47	13.24	17.1	5.22	7.28	2.14

SOD in U/mg protein; CAT in μmol H₂O₂/min/mg protein. All differences significant at *p*≤0.05

MDA content increased sharply during ageing, rising on average by 147 percent from day 0 to day 12 (Table 2). Uri showed the highest MDA accumulation (13.24 nmol/g), while Keumkang had the lowest (8.73 nmol/g). SOD activity fell by 42.4 to 69.5 percent, and CAT declined 44.9 to 70.6 percent across cultivars. Keumkang retained the highest residual enzyme activities at day 12.

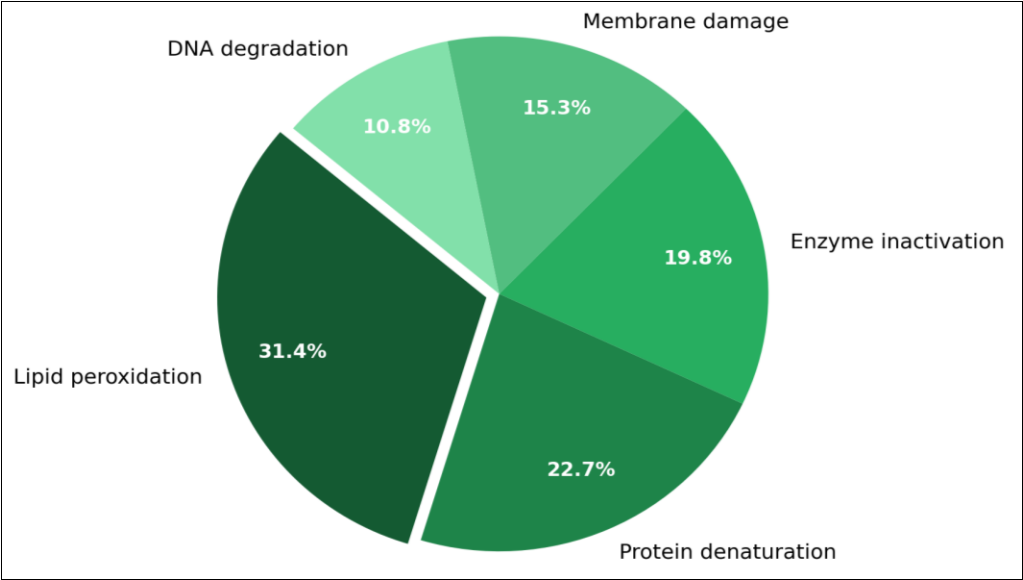


Fig 1: Relative contribution of biochemical factors to seed deterioration across four wheat cultivars.

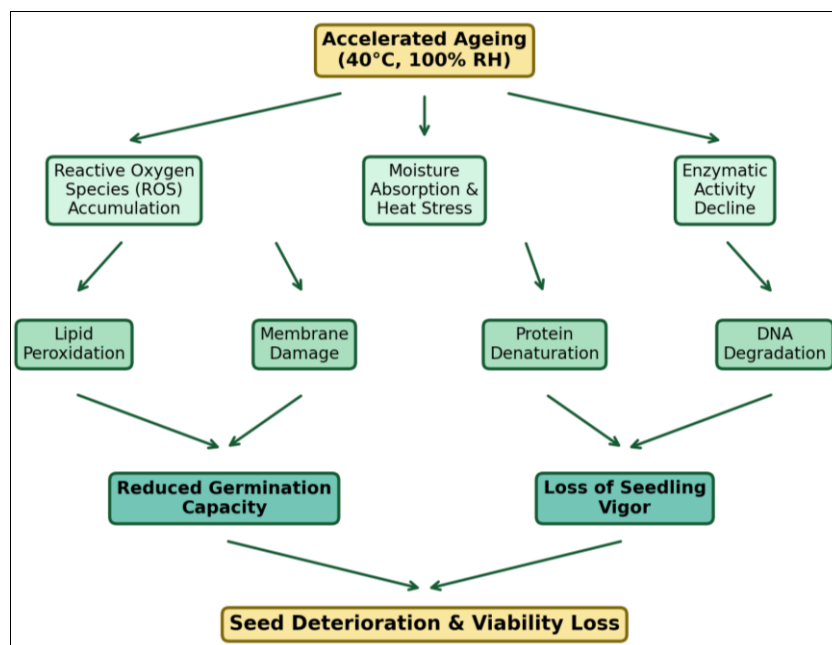


Fig 2: Schematic pathway of biochemical events leading to seed deterioration under accelerated ageing

Electrical conductivity of seed leachates increased from 14.3 to 38.7 $\mu\text{S}/\text{cm}/\text{g}$ across the ageing period, confirming progressive membrane breakdown. Protein carbonyl content rose by 89 percent in Uri but only 41 percent in Keumkang, further supporting the role of oxidative protein damage in cultivar-specific deterioration rates.

Discussion

The sharp decline in germination accompanied by rising MDA content confirms lipid peroxidation as a primary driver of seed deterioration during accelerated ageing. This aligns with findings by Lehner *et al.* ^[10] who reported similar oxidative cascades in wheat stored under high humidity. The 147 percent increase in MDA reflects extensive membrane phospholipid damage, which was corroborated by the parallel rise in electrical conductivity ^[11].

Cultivar differences in ageing tolerance appear linked to antioxidant defense capacity. Keumkang retained 57.6 percent of its initial SOD activity at day 12, compared to just 30.5 percent in Uri. This matches reports by Yin *et al.* ^[12] showing that genotypes with higher residual antioxidant enzyme pools maintain seed viability longer. The correlation between MDA accumulation and germination loss was strong ($r = -0.91$), suggesting MDA may serve as an early screening marker for storage tolerance in breeding programs ^[13]. Protein carbonylation, though less studied than lipid peroxidation in seed ageing, contributed meaningfully to the deterioration process, particularly in susceptible cultivars ^[14].

Conclusion

Lipid peroxidation and antioxidant enzyme decline were the main biochemical mechanisms behind seed deterioration during accelerated ageing in wheat. MDA content and electrical conductivity served as reliable indicators of membrane damage and viability loss. Among the four cultivars, Keumkang showed the greatest ageing tolerance, retaining 61.7 percent germination after 12 days-linked to its superior residual SOD and CAT activities.

These results suggest that screening wheat germplasm for antioxidant enzyme stability could identify lines with better storage potential. MDA content at early ageing stages may be a

practical predictor for ranking cultivar storability without prolonged storage trials.

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