



# Evaluation of Seed Quality of Grain Corn Varieties through Accelerated Ageing

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

In an accelerated ageing test carried out to evaluate seed quality two hybrid grain corn varieties, 4546 and 888, were subjected to ageing conditions to assess their tolerance. The objective was to identify any differences between the varieties in their ability to maintain seed quality under accelerated ageing conditions. These tests simulate and hasten the natural ageing process of seeds, providing insight into their performance during storage over time and under adverse conditions. Following the ageing process, factors such as germination rate, vigour, and overall seed quality were assessed. The seeds of hybrid grain corn varieties 4546 and 888 were exposed to accelerated ageing by maintaining them at 40°C and 100% relative humidity in a growth chamber.

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Evaluations were conducted at intervals of 0, 48, 96, and 144 hours. The overall results indicated that seed quality in grain corn deteriorates following accelerated ageing treatment. Variety 4546 showed a rapid decrease in germination, germination rate, and seedling vigour throughout the testing period. Both varieties experienced an increase in moisture content from 11% to 20% during the ageing process. Additionally, the electrical conductivity of seed leachate increased for both varieties as the testing progressed. The experiment concluded that the 888 grain corn variety outperformed 4546 in all evaluated parameters. The 4546 variety was found to be highly sensitive to accelerated ageing.

*Keywords: Grain corn; germination; accelerated ageing test.*

## 1. INTRODUCTION

Grain corn constitutes an essential agronomic commodity that significantly contributes to the food, feed, and seed sectors. In the Malaysian context, it functions as a fundamental element in the formulation of animal feed. The surge in demand for grain corn has precipitated a dramatic escalation in imports, exceeding 3 million metric tons in the year 2018 [1]. The assurance of seed supply security has emerged as a pivotal aim under the National Agro-Food Policy (DAN) spanning 2011 to 2020. Consequently, it is imperative to engage in research endeavours that focus on augmenting seed productivity and quality. The quality of seeds is a vital determinant that affects the productivity of crops utilised for animal feed and grain production. One of the principal evaluations of seed quality pertains to seed germination. Metrics such as germination velocity, germination index (GI), and the ratio of normal to abnormal seedlings yield valuable insights into the growth potential of plants prior to their transplantation into the field. Currently, there exists a paucity of information regarding the quality of grain corn seeds within Malaysia. As a result, this investigation was initiated to assess the seed quality of various grain corn cultivars and their implications for growth performance and agricultural enhancement. To address the escalating demand for food grains, spurred by demographic expansion, it is crucial to mitigate seed losses during and subsequent to the harvest period. Seeds are preserved for varying durations to guarantee a consistent and balanced supply throughout the calendar year. Analysing the physical, physiological, and biochemical transformations in seeds under conditions of accelerated ageing can deepen our comprehension of the seed deterioration phenomenon. The relative storability of a seed batch can be forecasted through accelerated ageing, which entails subjecting seeds to elevated temperatures (40°C) and high relative

humidity (100%). This methodology is instrumental in determining whether to retain or discard a specific seed variety or batch. Accelerated ageing has been established as a self-ageing process, and it is extensively applied to evaluate seed vigour and deterioration during storage [2]. Seed deterioration is an unavoidable phenomenon that impacts all seeds, resulting in a gradual reduction in seed viability. The deterioration rate is predominantly influenced by storage temperature and seed moisture content, which are critical determinants of seed longevity. The capacity of seeds to endure degradation varies across species [3]. Processing and storage obstacles are particularly pronounced in tropical nations such as India, where elevated temperatures and humidity levels, in conjunction with variable temperature and humidity, hinder effective seed preservation. Seed deterioration is conventionally characterised by diminished seedling growth, germination potential, and viability [4]. The current study endeavours to evaluate the relative longevity of two corn varieties by exposing them to deterioration assessments involving extended incubation periods at 40°C (48, 96, and 144 hours) and augmented moisture content of 20%. Furthermore, this research aims to identify physiological alterations in deteriorated seeds that could act as indicators of seed quality.

## 2. MATERIALS AND METHODS

### 2.1 Seed Materials

The experiment was executed at the Malaysian Agriculture Research and Development Institute (MARDI) situated in Serdang, Selangor, employing two distinct varieties of grain corn seeds, specifically 4546 and 888. The hybrid cultivar 4546, engineered by Pioneer/Corteva Agriscience, is the product of sophisticated hybridization methodologies aimed at augmenting yield and enhancing resistance to environmental stresses. Manufactured in

Thailand, this cultivar is particularly suitable for tropical ecosystems, thereby promoting agricultural productivity. Conversely, cultivar 888 was developed by Green World Genetics (GWG), a seed production enterprise based in Malaysia. This cultivar was specifically tailored for the region's tropical climate, providing enhanced resistance to indigenous pests and diseases, thereby improving yield stability while simultaneously minimizing the necessity for chemical inputs.

## 2.2 Accelerated Ageing Test

The accelerated ageing (AA) assessment was conducted at a temperature of 40°C across three distinct time durations: 48, 96, and 144 hours. This assessment incorporated three replicates consisting of 50 seeds each. The seeds were systematically arranged within plastic germination containers filled with deionized water, positioned in a single layer on a mesh to avert direct contact with the aqueous medium. For each treatment condition, subsamples of 50 seeds were encapsulated within laminated foil packets, subsequently incubated at 40°C, and withdrawn after intervals of 48, 96, and 144 hours. Seed lots that were not subjected to the accelerated ageing assessment functioned as control groups. The moisture content of the seeds was quantified employing the high constant temperature oven technique.

## 2.3 Moisture Content Test

Seed moisture content constitutes a critical determinant of seed quality and its capacity for storage. Consequently, its assessment during the evaluation of seed quality is of paramount importance. The seed moisture content was quantified utilising high constant temperature oven drying conducted at 130°C for a duration of two hours [5]. Prior to the drying process, the seeds were subjected to grinding. The calculations were performed based on a wet basis. Typically, seed moisture content is articulated in terms of fresh weight or wet basis, which can be computed utilising the subsequent formula.

The formula is delineated as follows:

$$\text{Moisture content (\%)} = \frac{[(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100.}$$

This methodological approach guarantees precise measurement, thereby facilitating a

dependable evaluation of seed viability and potential germination rates.

## 2.4 Germination Test

Grain corn seeds underwent a comprehensive germination assessment, which was executed utilising four replicates comprising 50 seeds each. Pristine plastic germination containers were filled with a sand-based substrate, and this substrate was adequately moistened with distilled water. In each container, fifty seeds were positioned atop the sand, and the lids were firmly secured to mitigate moisture evaporation. The substrate was rehydrated whenever it exhibited signs of desiccation. The evaluation of germination occurred on the eighth day post-sowing, with outcomes articulated as the percentage of viable seedlings. Both the germination percentage and germination rate were meticulously calculated and documented. The calculation of germination percentage was performed employing the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated} \times 100}{\text{Total number of seeds}}$$

In order to ascertain the rate of germination, quantified as the mean germination time (MGT), a total of 50 seeds were employed for the germination process. The counts of germinated seeds were meticulously documented on a daily basis over a period of seven days. The MGT was derived utilising the subsequent formula:

Mean germination time (MGT) =  $\sum nd / \sum n$ , where n represents the number of seeds that exhibited germination on day d, and d denotes the number of days elapsed since the initiation of the germination experiment.

## 2.5 Seed Leachate

A cohort of 30 seeds was immersed in 250 ml of deionised water maintained at a temperature of 25°C for a duration of 24 hours within an incubator environment. The electrical conductivity (EC) of the leachate derived from the seeds was quantified utilising a conductivity meter (EUTECH Instruments CON 510). The conductivity measurements were articulated in terms of  $\mu\text{S cm}^{-1} \text{g}^{-1}$  of seed.

## 2.6 Tetrazolium (TZ) Test

The tetrazolium assay (TZ) for assessing seed viability was performed on two distinct cultivars,

4546 and 888, both prior to and subsequent to the ageing treatment. In preparation for this assay, the seeds underwent a preconditioning process involving immersion in distilled water at a temperature of 20°C for a duration of 36 hours. Subsequently, the seeds were longitudinally dissected through the central axis of the embryo and at a quarter of the length of the endosperm. Each seed was then submerged in a 1.0% solution of 2,3,5-triphenyltetrazolium chloride and incubated in a dark environment at 30°C for a period of 3 hours. The assay was executed using 20 seeds placed within plastic receptacles. Following the staining duration, the solution was discarded, and the seeds were thoroughly rinsed under continuous running water. The seeds were assessed based on the uniformity, spatial distribution, and intensity of the staining observed in the embryonic tissues, resulting in classification into two categories: viable and non-viable. The staining intensity and morphological characteristics of the embryo were meticulously examined under microscopy.

## 2.7 Statistical Analysis

The empirical investigations were conducted utilising a Complete Randomized Design (CRD) framework with four independent replications. The Statistical Analysis System (SAS) software was employed for the execution of analysis of variance (ANOVA). The means of the treatments were subjected to comparative analysis via Tukey's test ( $p \leq 0.05$ ).

## 3. RESULTS AND DISCUSSION

### 3.1 Germination Percentage, Germination Index and Mean Germination Time

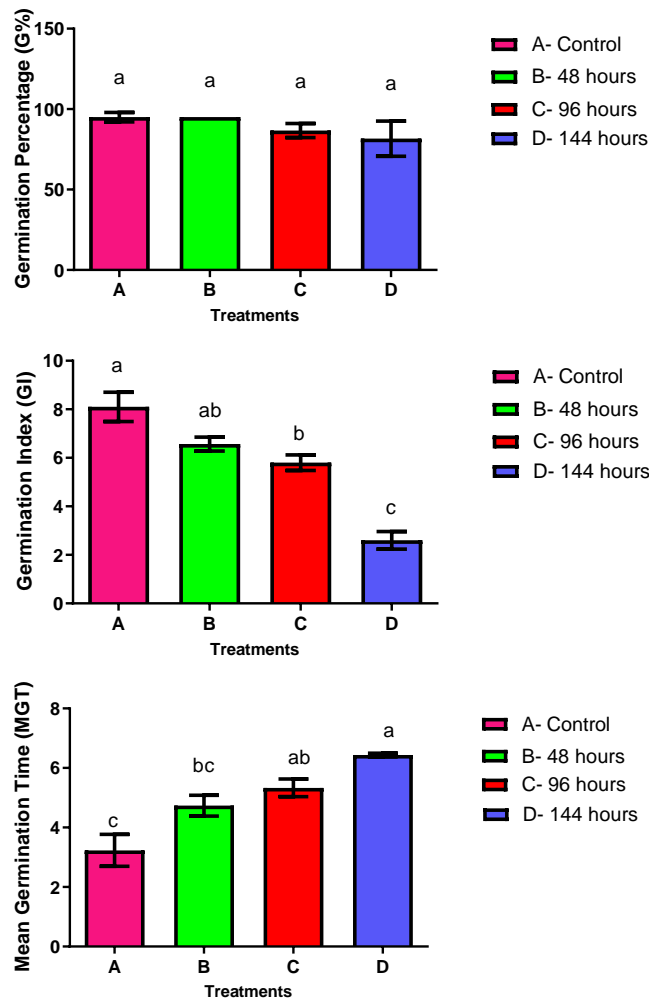
The results indicated that the grain corn seeds initially had low moisture content, with 11.42% for variety 888, which increased to 21.45% after 6 days, and 10.98% for variety 4546, which rose to 18.02% (Table 1). This increase in moisture content could be attributed to the continuous and gradual absorption of moisture by the seeds, given their hydrophilic nature. In this study, seed quality deterioration was linked to a decrease in germination percentage (G%), germination index (GI), and mean germination time (MGT) as storage time progressed under accelerated ageing conditions (Fig. 1 and Fig. 2). Similar reductions in physiological parameters during ageing have been reported by Vijay [6] in soybean and Godakahriz [7] in safflower. For the cultivar 4546,

the quality of seeds exhibited a decline with ageing, as evidenced by the germination percentage (G%) of control seeds, which was initially recorded at 76.7% (Fig. 2). This value diminished to 42.1% by the fourth day and further decreased to 15% by the sixth day of accelerated ageing. Conversely, the cultivar 888 displayed superior resilience, beginning with an initial G% of 95%, which sustained at 81.6% after six days of accelerated ageing (Fig. 1). The observed variability in the response to both natural and accelerated ageing may be attributed to genetic determinants and the intrinsic capacity of each cultivar to endure stress. The findings indicate that cultivar 888 preserved the highest quality regarding germination, germination index, and mean germination time. The observed increases in G%, germination index (GI), and mean germination time (MGT) following 48 hours of controlled deterioration in this cultivar suggest that robust metabolic repair processes likely occurred, thereby enhancing seed invigouration due to the elevated moisture content and temperature present during the assessment. The Germination Index (GI) for both cultivars exhibited a decline as the experimental duration progressed (refer to Fig. 1). The cultivar 888 commenced with a GI of 8.1 (control), which subsequently decreased to 6.5 (48 hours), 5.8 (96 hours), and 2.6 (144 hours) (Fig. 1). In contrast, cultivar 4546 started with a lower GI of 4.76 (control), which diminished to 0.4 by the conclusion of the experiment at 144 hours (Fig. 2). The deterioration of seeds in cultivar 4546 was marginally more pronounced than that observed in cultivar 888. The Mean Germination Time (MGT) for control seeds of cultivar 888 varied from 3.2 to 6.4 (refer to Fig. 1), whereas for cultivar 4546, it ranged from 3.4 to 7.6 (refer to Fig. 2). The peak MGT was noted at 96 hours for cultivar 888 (5.3) and for cultivar 4546 (4.3) (refer to Fig. 2). As the ageing duration extended to 144 hours, all cultivars demonstrated a significant increase in MGT (refer to Fig. 1 and Fig. 2). The highest MGT was documented for cultivar 4546 (7.6) and for cultivar 888 (6.4), while the lowest MGT was observed for cultivar 4546 (3.9) and for cultivar 888 (3.2). Disruption of seed metabolism initially impedes the germination process, thereby resulting in delayed seedling emergence. This delay subsequently affects critical processes such as water absorption, enzyme activation, and the production of growth hormones. Consequently, seedling emergence is prolonged as the seed encounters difficulties in transitioning to active growth [8].

**Table 1. The average moisture content following the accelerated ageing of grain corn seeds.**

| Treatment | Moisture content |              |
|-----------|------------------|--------------|
|           | Variety 888      | Variety 4546 |
| Control   | 11.42 c          | 10.98 b      |
| 48 hours  | 16.26 b          | 15.02 a      |
| 96 hours  | 19.83 b          | 16.45 a      |
| 144 hours | 21.45 a          | 18.02 a      |

Means with different letters indicate significant differences at the  $P \leq 0.05$  level according to Tukey's HSD test.



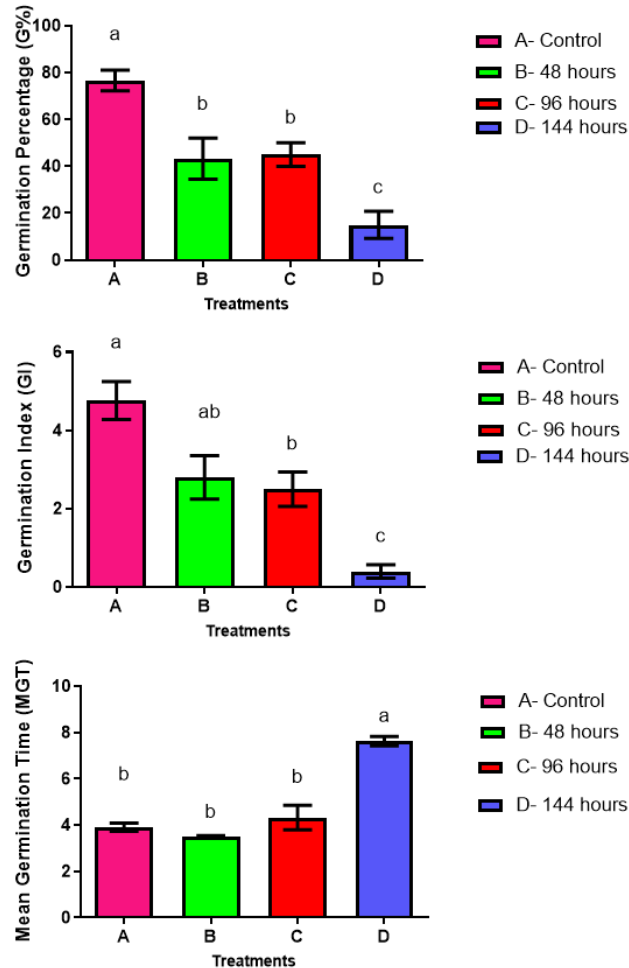
**Fig. 1. Average percentage of germination, germination index, and mean germination time of germination as a result of accelerated ageing on grain corn variety 888**

The electrical conductivity of seed leachate functions as a dependable metric for evaluating seed deterioration. The degree of membrane impairment during storage can be quantified through the assessment of the electrical conductivity of the seed leachate [9]. Within the confines of this investigation, an increase in electrical conductivity was observed concomitant

with the prolongation of accelerated ageing (Table 2). For grain corn, the control seeds demonstrated a comparatively lower electrical conductivity, with variety 888 measuring  $11.4 \mu\text{S cm}^{-1} \text{g}^{-1}$  and variety 4546 at  $10.9 \mu\text{S cm}^{-1} \text{g}^{-1}$ . Conversely, under conditions of accelerated ageing, the conductivity values escalated, attaining  $21.5 \mu\text{S cm}^{-1} \text{g}^{-1}$  for variety 888 and

18.2  $\mu\text{S cm}^{-1} \text{g}^{-1}$  for variety 4546 by the sixth day (144 hours) of ageing (Table 2). Gupta [10] elucidated that the enhancement in electrical conductivity following accelerated ageing is ascribed to the deterioration of membranes and the metabolic alterations occurring within the seed. Seeds characterised by minimal electrolyte leakage are regarded as

possessing high vigour, whereas those exhibiting significant leakage are classified as having low vigour. The elevation in seed leachate may be attributable to the compromised capacity of seed cellular membranes to reorganize and ameliorate damage sustained during imbibition, preceding germination [11].

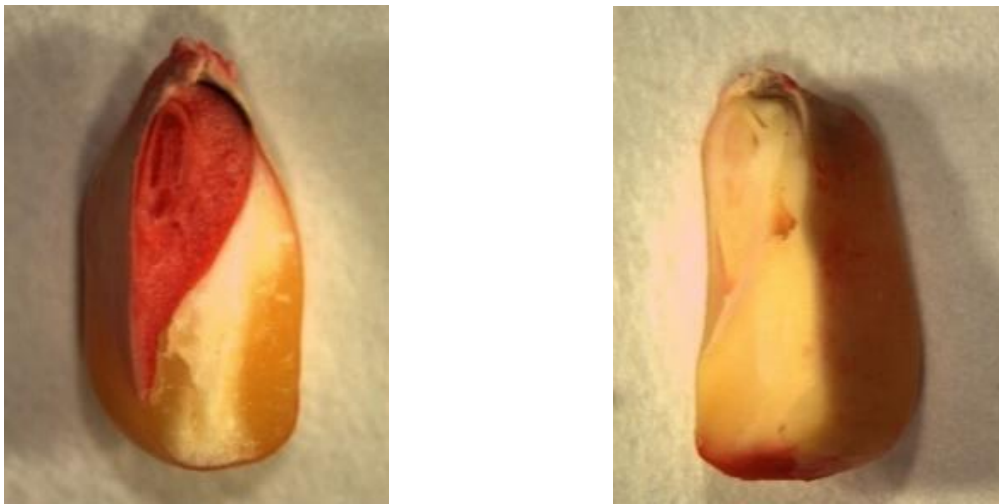


**Fig. 2. Average percentage of germination, germination index, and mean germination time of germination as a result of accelerated ageing on grain corn variety 4546**

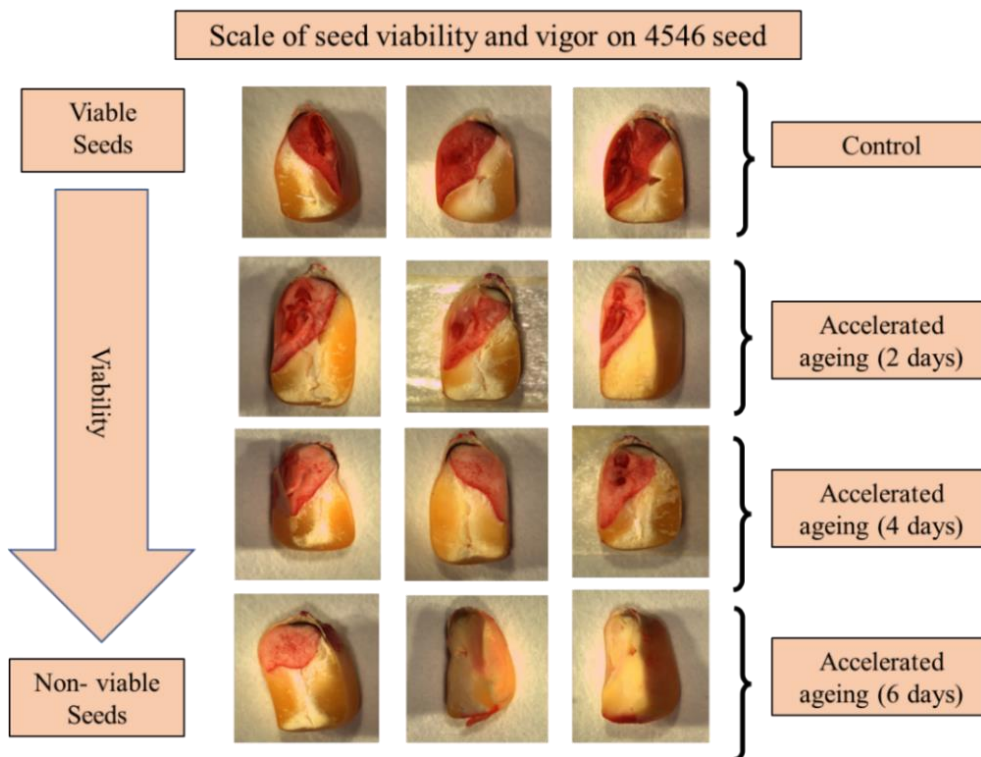
**Table 2. Electrical conductivity resulting from the accelerated ageing process of grain corn seeds**

| Treatment | Electrical conductivity ( $\mu\text{S cm}^{-1}\text{g}^{-1}$ ) |              |
|-----------|--|--------------|
|           | Variety 888  | Variety 4546 |
| Control   | 11.4 c   | 10.9 b       |
| 48 hours  | 16.3 b   | 16.5 a       |
| 96 hours  | 19.8 ab  | 16.2 a       |
| 144 hours | 21.5 a   | 18.2 a       |

Means with different letters indicate significant differences at the  $P \leq 0.05$  level according to Tukey's HSD test.



**Fig. 3. The tetrazolium staining pattern observed in grain corn seeds (Left - Seeds were determined to be viable when the living embryonic tissues exhibited a red coloration; Right - Seeds were classified as non-viable when they remained unstained or displayed a white appearance)**



**Fig. 4. Assessment of seed viability and vigour for grain corn evaluated across a sample of 4546 seeds**

### 3.2 Seed Viability

The viability of seeds was assessed utilising tetrazolium (TZ) staining techniques on cultivar 888 and cultivar 4546. The ensuing results from the TZ staining indicated that a substantial

majority of the seeds retained their viability. The interpretation of seed viability was based on the distinct topographical staining patterns observed in the embryo, as well as the degree of coloration intensity present within the tissues of the grain corn seeds.

From the TZ assay, a classification system delineating seed viability and vigour is established based on their staining characteristics. The construction of this classification system commences with seeds exhibiting high viability and vigour positioned at the apex, subsequently followed by seeds of intermediate viability, and culminating with non-viable seeds situated at the base of the classification (Fig. 4). This classification framework will subsequently be employed to evaluate additional maize seeds subjected to the TZ assay. Seeds deemed viable are those for which the embryo displays a uniformly intense red hue, possesses normal tissue morphology, exhibits firmness, and features both the embryonic axis and cotyledon node region exhibiting coloration, alongside cotyledons that encompass more than 50% of their surface area in colour (Fig. 3). Conversely, seeds categorized as non-viable are characterised by a completely white or partially stained appearance, alongside soft tissues indicative of necrotic conditions.

#### 4. CONCLUSION

The experimental analysis revealed that the 888 grain corn variety exhibited superior performance compared to the 4546 variety across all assessed parameters. The 4546 variety demonstrated a pronounced sensitivity to accelerated ageing. This investigation elucidates that distinct grain corn varieties exhibit differential rates of deterioration when subjected to comparable environmental conditions. Furthermore, it emphasizes that initial vigour, as opposed to the initial germination percentage, serves as a more dependable predictor of a seed lot's efficacy under adverse environmental circumstances. Moreover, the findings accentuate the necessity of meticulous processing and storage for varieties possessing lower vigour potential, owing to their heightened vulnerability to accelerated degradation.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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