

## Research article

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# Effects of Storage Period, Storage Conditions and Packaging Type on Maize Inbred Line Seed Qualities

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## Abstract

### Keywords

maize seed;  
storage;  
packaging;  
germination;  
vigor

The aim of this research was to study the effects of storage period, storage condition and packaging type on maize inbred seed qualities over 12 months storage. The experimental design was split-split plot with four replications. The study manipulated storage period (0, 3, 6, 9, and 12 months) as the main plot, uncontrolled storage condition (USC) and controlled storage condition (CSC) as the sub-plot, and packaging types including woven polypropylene (WPP), high density polyethylene (HDPE), polyamide (PA) with linear-low density polyethylene (LLDPE) and vacuum packing (PA + LLDPE + VACUUM, PLV) as the sub-sub plot. The results showed that the storage period did not affect seed germination; however, seed vigor decreased progressively with increased storage period. Seeds stored under USC exhibited lower moisture content, seed germination and vigor compared to those in CSC. Notably, seed vigor declined after the sixth month in USC storage. Among the packaging types, seeds in WPP had the lowest moisture content. Packaging type did not significantly influence seed germination throughout the storage period. However, different packaging types were able to maintain seed vigor for varying duration, especially for 6 months in PLV and 3 months in WPP packaging.

## 1. Introduction

Maize inbred seeds were important as the parents of maize hybrids in hybrid seeds production. Inbred seeds were shown to be susceptible to inbreeding depression in the breeding program which resulted in decreased plant vigor, yield, and physiological seed quality [1]. Seed quality had an impact on crop yield as measured by percentage of germination, plant stand establishment, and grain yield [2].

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Deterioration of seed quality can be attributed to various factors. In particular, the period of storage, seed moisture content, storage conditions related to temperature and humidity, packaging types, including the combination of all factors mentioned. The seeds deteriorated gradually throughout the entire storage period, resulting in permanent damage. This deterioration compromises the integrity of their membranes and can lead to macromolecular oxidation. As a result, the seeds lose their ability to germinate due to metabolic degradation [3]. Seed germination was related to storage time with the seeds deteriorated as the storage period progressed. Bhattacharya and Raha [4] reported that after 12 months of storage, maize seed germination rates decreased from 89% to 7%. Additionally, the combination of storage duration, storage temperature, and seed moisture content during storage are important factors that can cause seeds to lose viability [5]. Seeds are hygroscopic and can exchange moisture with the surrounding air until there is an equilibrium between the moisture content of the seeds and the relative humidity of the air. Seed moisture content increased as relative humidity increased. This led to an increase in metabolic activities, particularly seed respiration, which increased the deterioration rate of sorghum seed [6]. Increasing storage temperature and moisture resulted in increased seed respiration and 2-3% increase in final seed moisture at 30-40°C, resulting in reduced germination [7]. However, even with low seed moisture content, storing seeds at high storage temperature can cause seed deterioration because the high temperatures stimulate reactions inside the seeds. The deterioration of soybean seeds is accelerated when stored at room temperature (20-25°C, 65-70% RH) compared to cold storage conditions (10°C, 80-85% RH). This effect is observed in seeds with both high moisture content (12%) and low moisture content (less than 5%), with a more pronounced impact on seeds with a moisture content of 12% [8].

Seeds are generally kept in plastic packaging. Moisture and gas protection properties are critical considerations when choosing packaging [9]. Since the seed moisture content in packaging was determined by the packaging's water vapor permeability, the seed moisture content varied as the relative humidity around the packaging changed [10]. According to Naik and Chetti [11], when paddy seeds were stored in gunny bags for 18 months storage at both ambient (25±2°C) and cold storage (4±1°C), their moisture content and fungal growth were significantly higher than in vacuum packed bags. Primed sweet corn seed that had an initial germination rate of 85% was stored for 12 months under vacuum storage. After storage, the seeds retained a germination rate of 84%. It is likely that the preservation of seed vigor was related to the decreased peroxidation of free radicals in the absence of oxygen [12]. Each type of seed requires different storage conditions and packaging. Seeds with high moisture content should be stored in open bag, whereas seeds with a low moisture content should be stored in a hermetic bag [13]. Therefore, this research aimed to study the effects of storage conditions and packaging type on the seed quality of a maize inbred line over 12 months of storage. Selecting the proper storage conditions and packaging types will assist in the preservation and improvement of inbred seed quality, which will be the benefit of maize seed production.

## 2. Materials and Methods

The experimental design was a split-split plot design with four replications. The main plot comprised storage periods (0, 3, 6, 9 and 12 months), while the sub-plot was divided into uncontrolled storage condition (USC) and controlled storage condition (CSC) at 15°C with 50% RH. The sub-sub plot involved various packaging types; woven polypropylene (WPP), high density polyethylene (HDPE), and polyamide (PA) combined with linear-low density polyethylene (LLDPE) and vacuum packing (PA + LLDPE + VACUUM, abbreviated as PLV).

## 2.1 Seed materials

Maize inbred seed KWSTL6001 with a moisture content of 10% was packed into three types of packaging. There were 40 bags of each type of packaging. Each filled bag weighed 500 g. Twenty bags were then stored in uncontrolled storage condition and 20 bags in controlled storage condition.

## 2.2 Storage conditions

Throughout the storage period, a Mini data logger 174H (Testo SE & Co. KGaA, Germany) was used to record storage temperature and relative humidity, from which seed qualities were determined. Seed qualities were examined at intervals of three months.

## 2.3 Seed quality determination

Seed moisture content was determined by the hot air oven method. Initially,  $4.5 \pm 0.5$  g of ground seeds were weighed, with two sub-samples taken. These sub-samples were then dried at  $130^{\circ}\text{C}$  for a duration of 4 h. After drying, the seeds were re-weighed to determine their final weight, from which the seed moisture content could be calculated [14]. Seed germination was determined by the between paper method with 4 replications, each of 50 seeds, and germination percentages at 4 and 7 days were determined [14]. Seed vigor was evaluated using four different methods. The first was an electrical conductivity (EC) test. For each sample, four lots of 50 seeds were weighed and soaked in 75 mL of distilled water for 24 h. EC was then assayed by measuring electrolyte leakage using a PC 510 Bench pH/Conductivity Meter (Eutech Instruments Pte Ltd., Singapore) [15]. Second, a radicle emergence test was conducted. Eight replications (each of 25 seeds) were subjected to the seed germination test procedure, and the number of seeds that produced a radicle at 66 h were recorded for each replication [14]. Third, a seed germination index test was performed with 4 replications, each of 50 seeds. The germination test procedure was followed and the number of seeds that produced shoot  $\geq 2$  cm were measured everyday over 7 days. Fourth, an accelerated aging test was performed by placing  $40 \pm 1$  mL distilled water in each plastic box and weighing out  $40 \text{ g} \pm 1\%$  of seeds. Each batch of seeds was divided into two sub-samples, which were then placed in the plastic boxes within an aging chamber at  $43^{\circ}\text{C}$  for a duration of 72 h. The samples in the AA boxes were then removed from the aging chamber and a germination test was performed following the seed germination procedure [15].

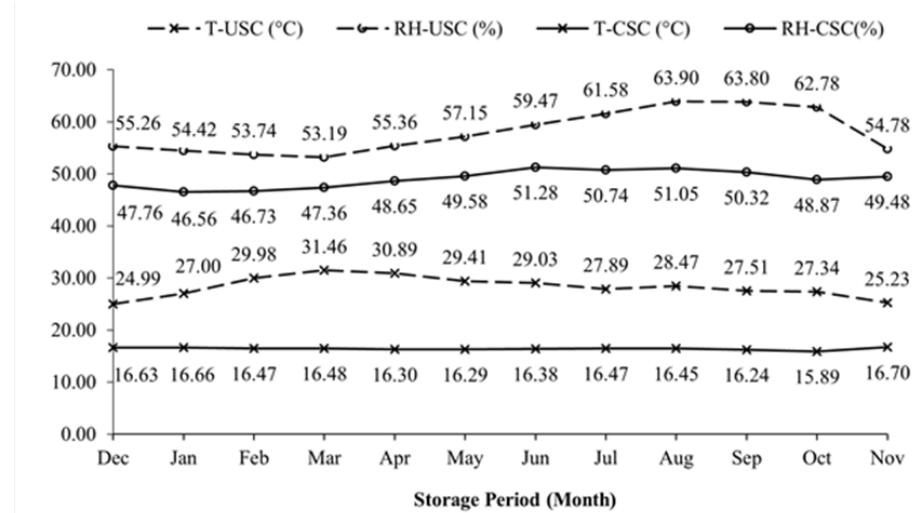
## 2.4 Statistical analysis

Analysis of Variance (ANOVA) was employed to compare the differences between the means of data using the Least Significant Difference (LSD) method. Correlation analysis was performed at the 95% confidence level using the statistical software Statistix 8 (Analytical Software, USA).

## 3. Results and Discussion

### 3.1 Effects of storage period, storage condition and packaging type on the seed moisture content

The temperature and relative humidity (RH) data for seeds packed in USC and CSC for 12 months storage are presented in Figure 1. In the case of USC, temperatures ranged between  $24.99\text{--}31.46^{\circ}\text{C}$



**Figure 1.** Temperature (°C) and relative humidity (RH) at uncontrolled storage conditions and controlled storage conditions for 12 months of storage (T = temperature, RH = relative humidity, USC = uncontrolled storage condition, CSC = controlled storage condition)

and RH fluctuated from 53.19-63.90% RH whereas the CSC maintained fairly stable conditions of  $16 \pm 0.2^{\circ}\text{C}$  and  $49 \pm 1.7\%$  RH. The RH inside the CSC was lower than that of the USC due to the control of temperature and RH.

Storage period, storage condition and packaging type all influenced the seed moisture content (SMC). The initial moisture content (MC) of seeds was 10.65% at 0<sup>th</sup> month of storage (before storage) and declined to its lowest in the 3<sup>rd</sup> month, which was 10.24%. This was due to it being the summer season which had the lowest RH. Following that, the SMC increased again in the 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> months, since RH increased to reach its equilibrium moisture content (EMC). This result corresponded to the grain EMC calculated by Sadaka and Bautista [16] (Figures 2A and 2B). Meanwhile, the SMC of seeds stored in CSC was 10.45% which was higher than the SMC of seeds stored in USC, which was 10.36%. Furthermore, the results of SMC correlated with packaging type ( $r = 0.4696^{**}$ ), with WPP-packed seeds having the lowest SMC at 10.58 when compared with seeds packed in HDPE and PLV (Table 1).

The relationship between temperature and RH is inverted. As the temperature increases, the RH decreases because temperature is the main factor that affects RH [17]. The SMC of maize inbred seeds varied in response to the relative humidity (RH) of the surrounding atmosphere, which was the main factor influencing the SMC of maize seeds [10]. Mettananda [18] reported that paddy seeds stored in ambient storage absorbed more moisture due to high RH. Because seeds are hygroscopic, they absorb and desorb moisture from the environment [19]. As a result, the moisture content (MC) of seeds fluctuates based on the moisture levels in the environment. Even if the seeds have been dried to a low moisture content, moisture exchange between the seeds and the surrounding environment continues in an uncontrolled environment until equilibrium is reached [20]. Seeds packed in WPP had the lowest MC because pores from the manufacturing process enabled moisture to easily permeate and seeds moisture changes could occur quickly depending on the equilibration with environmental RH [21, 22]. When the RH in the atmosphere was low, the seeds desorbed MC into the atmosphere to reach EMC [17]. Consistent with research by Ng'ang'a *et al.* [23] and Baributsa *et al.* [24], the MC of maize in this study decreased as a consequence of warm and dry environment during storage.

### 3.2 Effects of storage period, storage condition, and packaging type on the seed electrical conductivity

During the first 6 months of storage, the EC of the seeds did not significantly vary and was in the range of 13.52-13.71  $\mu\Omega\text{.g}^{-1}$ . There was a significant increase in EC to its highest value of 30.83  $\mu\Omega\text{.g}^{-1}$  at the 9<sup>th</sup> month (Figure 2C) and this correlated with the AA test results ( $r = -0.5259^{**}$ ). Seeds stored in USC showed a deterioration of EC to 20.93  $\mu\Omega\text{.g}^{-1}$ , which was greater than the EC of seeds stored in CSC which had an EC of 18.98  $\mu\Omega\text{.g}^{-1}$  (Table 1). Meanwhile, seeds packed in WPP and HDPE with seed EC of 20.39  $\mu\Omega\text{.g}^{-1}$  and 20.12  $\mu\Omega\text{.g}^{-1}$ , respectively, deteriorated significantly faster than seeds packed in PLV, which had a seed EC of 19.36  $\mu\Omega\text{.g}^{-1}$ .

After 6 months, it is evident that the EC values doubled from its initial measurement when assessed in the 9<sup>th</sup> and 12<sup>th</sup> months. However, conversely, during the same time intervals of 0, 3, and 6 months after storage, the seeds still maintained consistently high germination rates with no significant differences observed. The EC values of the seeds increased as the storage period increased due to membrane degradation. This caused electrolyte leakage from the seeds. As a result, the conductivity of the seeds increased [15]. The same was true for Arief *et al.* [25], who found that the EC values of maize seeds stored for shorter periods were lower than those stored for longer periods. For the storage condition, RH and temperature in USC were the main factors which led to seed deterioration. The higher RH in the atmosphere caused higher SMC, resulting in a decrease in phospholipids and proteins in the cell membranes. As a result, cell membrane permeability decreased, allowing the electrolytes in the seeds to leak out [26]. Furthermore, a higher temperature was the main factor that caused the higher value of EC [27]. According to Fessel *et al.* [28], the EC of maize seeds increases when stored at high temperatures (30°C), resulting in higher leakage and indicating a decline of seed vigor due to loss of membrane integrity. Differences in packaging types affected EC values due to gas and moisture permeability from the environment. It was noticed that PLV has less volatility for O<sub>2</sub> and SMC during the storage periods than WPP and HDPE, resulting in low seed activity and maintenance of seed vigor. This result was similar to the finding of Abreu *et al.* [29] in sunflower seeds and Meena *et al.* [30] in soybean seeds.

### 3.3 Effects of storage period, storage condition, and packaging type on the seed germination

Seed germination was not affected by either storage period or packaging type. The seeds packed in WPP, HDPE, and PLV had an average germination percentage of 94% after 12 months of storage. Only storage conditions resulted in a statistically significant difference in the germination percentage (Figure 2D). The seeds stored in the CSC had a germination of 96% which was higher than USC, which had a germination of 94% and the germination of seeds correlated with the storage conditions ( $r = 0.3472^{**}$ ).

The germination percentage for each type of packaging consistently exceeded the standard 85% threshold required for basic maize seeds in Thailand [31]. This suggests that the seeds maintained a high germination rate even when stored for over a year in all types of packaging. However, seed germination was affected by storage conditions. The seeds packed in different packaging types had fluctuated seed moisture content, especially those packed in WPP which had pores from the manufacturing process that allowed easier moisture exchange with the environment when compared with HDPE and PLV. The fluctuations led to the degradation of proteins and carbohydrates, resulting in the loss of seed viability [32]. Additionally, fluctuations in temperature and RH in USC conditions affected seed respiration; seed respiration increased with increasing temperature and RH [33]. Moreover, the fluctuation of temperature and RH caused the grain moisture to fluctuate, resulting in reduced protein digestion [34]. Gill and Delouche [35] found that

maize seeds stored at high temperature and RH (30°C, 75% RH) lost viability after 4 months of storage which was faster than seeds stored at 7°C, 50% RH, which showed declined viability after 16 months. Similar to Mbafung *et al.* [36], the germination of seeds stored in a warehouse with uncontrolled temperature and RH significantly decreased when compared to seeds stored under controlled low temperature and relative humidity.

### 3.4 Effects of storage period, storage condition, and packaging type on the radicle emergence, seed germination index and seeds exposed to accelerated aging

Throughout the 12-month experimental period, storage period and packaging type had no effect on radicle emergence (RE). The RE of the seeds was in the range from 87.60%-92.35% from the beginning to the end of storage and correlated with germination percentage ( $r = 0.2754^{**}$ ). However, RE was negatively correlated with the storage period ( $r = -0.4021$ ), which tended to decrease as storage period increased (Table 2). Only the storage conditions influenced the RE value. Seeds stored in CSC had RE of 92.74%, which was higher than those stored in USC, which was at 86.89% (Figure 2E).

The seed germination index (GI) of maize seeds declined as the storage period increased ( $r = -0.4021^{**}$ ). The initial GI was 10.28 and was significantly reduced to 9.45 at the 9<sup>th</sup> month (Figure 2F). The GI of seeds stored in CSC was 10.33, which was higher than GI of seeds stored in USC, which was 9.25. For seeds packed in PLV, the GI was 10.02, which was higher than those packed in other packaging types (Table 1). In addition, the GI values correlated with the conductivity of the seeds ( $r = -0.3728^{**}$ ) and AA test ( $r = 0.4699^{**}$ ).

Before storage (0<sup>th</sup> month of storage), the initial accelerated aging (AA) germination percentage of seeds was 94% and significantly decreased at the 6<sup>th</sup> month to 74%. At the 12<sup>th</sup> month, the seeds showed the lowest AA germination percentage of 48% (Table 1) with longer storage times reducing the vigor of the seeds ( $r = -0.5922^{**}$ ). Seed vigor was also affected by storage conditions ( $r = 0.4555^{**}$ ), and following 12 months of storage, seeds stored in CSC had more than 80% AA germination percentage compared to 60% for the seeds stored in USC (Figure 2G). Packaging types also influenced the seed vigor. Seeds packed in WPP and PLV had higher AA germination percentage than seeds packed in HDPE (74, 74 and 68%, respectively).

Low RE seeds had poor vigor and germination [37]. This was consistent with the results for GI. Seeds with lower GI had deteriorated, resulting in a delay in seed metabolism and germination [38]. The GI of seeds decreased with increasing storage time due to natural aging which led to a decrease in vigor. This result was in accordance with Garoma *et al.* [39] for maize parental line seeds. Seeds stored in USC had a lower GI than those stored in CSC. According to Azadi and Younesi [40], sorghum seeds stored at room temperature showed reduced GI as the temperature and RH increased. Consistent with study of Meena *et al.* [30], vacuum-packing groundnut seeds had a minimum decline in the seedling vigor index when compared with gunny bags and HDPE.

Fluctuating temperature and humidity conditions led to seed deterioration and especially when the temperature and humidity increased, which affected the compositions of the seed. Seeds stored at 30°C and 75±5%RH showed increased protein degradation and respiration. As a result, food reserves and seed vigor decreased with increasing temperature and SMC [41, 42]. Furthermore, the lower seed vigor of seeds packed in HDPE was probably the result of moisture flux in packaging as the seeds volume was less than packaging volume [43]. In addition, the fluctuation of RH due to temperature changes made the seeds more responsive, resulting in deterioration [44].

**Table 1.** Effect of storage period, storage condition, and packaging type on seed moisture content (SMC), oxygen content in packaging (O<sub>2</sub>), electrical conductivity (EC), germination (G), radicle emergence (RE), germination index (GI) and accelerated aging (AA) of maize inbred seeds throughout 12 months of storage.

Factor	SMC (%)	EC ( $\mu\text{O} \cdot \text{g}^{-1}$ )	G (%)	RE (%)	GI	AA (%)
Storage period (M)						
0 month	10.65 <sup>a</sup>	13.66 <sup>c</sup>	95	91.06	10.28 <sup>a</sup>	94 <sup>a</sup>
3 months	10.24 <sup>d</sup>	13.52 <sup>c</sup>	96	92.35	10.04 <sup>ab</sup>	83 <sup>ab</sup>
6 months	10.30 <sup>cd</sup>	13.71 <sup>c</sup>	94	90.31	10.09 <sup>ab</sup>	74 <sup>bc</sup>
9 months	10.38 <sup>bc</sup>	30.83 <sup>a</sup>	95	87.60	9.45 <sup>bc</sup>	64 <sup>cd</sup>
12 months	10.45 <sup>b</sup>	28.07 <sup>b</sup>	94	87.75	9.09 <sup>c</sup>	48 <sup>d</sup>
Storage condition (C)						
USC	10.36 <sup>b</sup>	20.93 <sup>a</sup>	94 <sup>b</sup>	86.89 <sup>b</sup>	9.25 <sup>b</sup>	60 <sup>b</sup>
CSC	10.45 <sup>a</sup>	18.98 <sup>b</sup>	96 <sup>a</sup>	92.74 <sup>a</sup>	10.33 <sup>a</sup>	84 <sup>a</sup>
Packaging type (P)						
WPP	10.23 <sup>c</sup>	20.39 <sup>a</sup>	95	90.96	9.70 <sup>b</sup>	74 <sup>a</sup>
HDPE	10.40 <sup>b</sup>	20.12 <sup>a</sup>	94	90.80	9.64 <sup>b</sup>	68 <sup>b</sup>
PLV	10.58 <sup>a</sup>	19.36 <sup>b</sup>	95	87.69	10.02 <sup>a</sup>	74 <sup>a</sup>
F-test (M)	**	**	ns	ns	*	**
F-test (C)	**	**	**	**	**	**
F-test (P)	**	**	ns	ns	**	**
CV (%)	1.33	6.40	2.91	8.82	4.94	12.51

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively, by the F test.

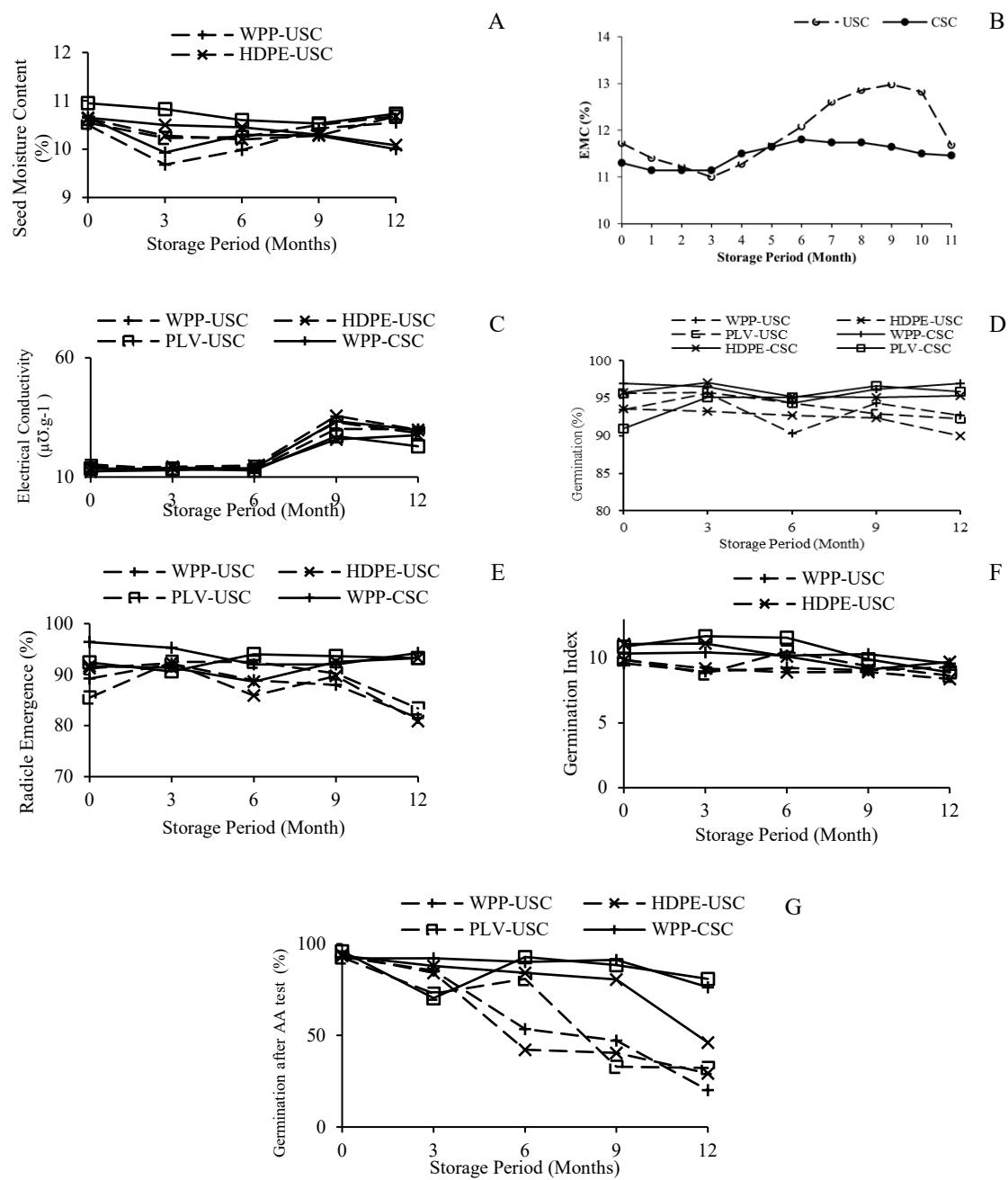
In each column, different superscripts represent significant differences ( $p \geq 0.05$ ). ns represents non-significant difference ( $p \geq 0.05$ )

WPP = woven polypropylene, HDPE = high density polyethylene, PLV = polyamide + linear-low density polyethylene + vacuum, USC = uncontrolled storage condition, CSC = controlled storage condition

**Table 2.** Correlations of storage period, storage condition, and packaging type on seed moisture content (SMC), oxygen content in packaging (O<sub>2</sub>), electrical conductivity (EC), germination (G), radicle emergence (RE), germination index (GI) and accelerated aging (AA) of maize inbred seeds throughout 12 months storage.

Storage Period	SMC	O <sub>2</sub>	EC	G	RE	GI
SMC	0.1203-ns					
O <sub>2</sub>	0.2112*	-0.3121**				
EC	0.7998**	0.0095 ns	0.0495 ns			
G	0.0800- ns	-0.1281 ns	-0.0822 ns	-0.0923 ns		
RE	-0.1806*	-0.0428 ns	0.1926*	-0.2696**	0.2754**	
GI	-0.4021**	0.2655**	-0.0774 ns	-0.3728**	0.1524 ns	0.3254**
AA	-0.5922**	0.0936 ns	0.1445- ns	**0.5259-	0.2878**	0.4667**
						0.4699**

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively, by the F test



**Figure 2.** Seed moisture content (A), equilibrium moisture content (B), electrical conductivity (C), germination percentage (D), radicle emergence (E), germination index (F), and accelerated aging test (G) of maize inbred seeds during 12 months of storage under different storage conditions and packaging types. (WPP = woven polypropylene, HDPE = high density polyethylene, PLV = polyamide+linear-low density polyethylene+vacuum, USC = uncontrolled storage condition, CSC = controlled storage condition).

#### 4. Conclusions

The findings in this study indicates that longer storage periods lead to reduced seed vigor. Controlled storage conditions (CSCs) gave better seed quality retention over 12 months than uncontrolled storage conditions (USCs). All packaging types tested allowed for moisture exchange with the environment. Polyethylene vacuum (PLV) and woven polypropylene (WPP) packaging maintained seed vigor for 6 and 3 months, respectively.

For storing maize inbred seeds for 6 months under uncontrolled conditions, PLV packaging is recommended. For year-long storage, seeds should be kept under controlled conditions and WPP packaging is suggested due to its cost-effectiveness while maintaining seed vigor and germination levels comparable to PLV packaging.

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