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Special Issue

Tropical Biotechnology

Edited by

Dr. Javier Linares-Pastén



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Featured Application: Seed longevity is an attribute that deserves consideration in the adoption and in informing the assessment of seed priming. Seed survival after priming is higher for varieties that can withstand damage and ageing better even in adverse conditions.

Abstract: Agriculture depends on the ability of seeds to survive until the next planting season under ambient conditions that may be averse to seed quality even when the seed is in a quiescent state. Seed priming invigorates seeds, but the impact on the longevity of seeds has limited its adoption. This study investigated the effect of the storage of primed rice seed on seed viability, vigor, and longevity. Three seed priming methods were employed on the rice cultivar New Rice for Africa (NERICA 4) seeds. Subsequently, the seeds were stored for 120 days at 25 °C and 65% relative humidity, simulating the ambient seed storage conditions of the tropics and sub-tropics. The primed seed recorded increased vigor compared to the non-primed seed until 90 days of storage. However, seed storage for 120 days reduced seed vigor and viability for all the seeds. The results indicated a significant reduction in seed vigor, increased solute leakage, generation of hydrogen peroxide, and accumulation of malondialdehyde after storage. Priming enhances cell membrane integrity and maintains seed vigor in storage at near ambient conditions long enough before reversal of its performance by the storage conditions. This assures that primed seed can either be stored until the following planting season or remain viable in the soil during delayed germination.

Keywords: imbibition damage; NERICA; seed aging; seed longevity; seed priming; seed storage; solute leakage



Citation: Bore, E.K.; Ishikawa, E.; Libron, J.A.M.A.; Goto, K.; Odama, E.; Nakao, Y.; Yabuta, S.; Sakagami, J.-I. Primed Seeds of NERICA 4 Stored for Long Periods under High Temperature and Humidity Conditions Maintain Germination Rates. *Appl. Sci.* **2023**, *13*, 2869. <https://doi.org/10.3390/app13052869>

Academic Editors: Gerhard Soja and Javier Linares-Pastén

Received: 20 December 2022

Revised: 10 February 2023

Accepted: 21 February 2023

Published: 23 February 2023



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1. Introduction

Low-input agriculture in marginal areas of developing regions of the world faces considerable challenges. The crucial stage in crop development is seed germination, which is frequently constrained by abiotic factors, such as low soil water potential, high temperatures, low soil fertility, salinity [1], and declining land productivity [2], which ultimately result in poor crop establishment and reduced yields. Low-cost seed priming techniques have been implemented successfully [1] to address such challenges. Tackling yield reductions requires low-cost and sustainable strategies [1,3–6]. Seed priming has been demonstrated as a valid approach to closing yield gaps and improving plant performance. However, there is a low adoption rate of market primed seeds by rice farmers and the seed industry, which could be partly attributed to storage, which presents the risk of extensive losses [7] due to viability loss.

Agriculture depends on the ability of seeds to survive until the following sowing season under ambient conditions. In the tropics and sub-tropics, the grain temperatures typically remain between 25 °C and 35 °C [8,9]. The ambient temperature is 25 °C [10], with 65% relative humidity [11], which is usually sufficiently adverse for seed storage [12] and accelerates aging [13]. However, seeds must be stored for a period in readiness for use by farmers as a general practice while preserving the initial seed quality [14]. Delouche [12] suggested that good-quality seed of the major sub-tropical and tropical crops could be stored satisfactorily from harvest to the next planting season for up to 9 months at 20 °C and 60% relative humidity for cereal seed, with seed moisture contents ranging to a maximum of about 13%. Furthermore, rice was arbitrarily classified as a good storer under ambient conditions in warm-humid areas, maintaining good germination even up to 3 years under favorable conditions [11].

Various seed-priming techniques have been applied to rice [6,15–18] to improve seed vigor, uniform emergence, persistence, and performance over a range of abiotic stresses [19–21]. The technique involves partial hydration of seeds to initiate metabolic processes necessary for germination without the emergence of the radicle [3]. It stimulates seeds and alleviates environmental stresses and has been attributed to various mechanisms. Hydropriming makes water available for metabolic activation [22]; partial seed rehydration initiates activation of early germination events in the seed, including activation of the reactive oxygen species (ROS) antioxidant mechanism [21]. Furthermore, nitrates in potassium nitrate (KNO₃) stimulate seed germination [23] by reducing seed dormancy, promoting antioxidant defenses, and increasing water uptake [24]. In addition, polyethylene glycol (PEG) delays water entry [25], limits ROS-mediated oxidative injury; increases the activities of antioxidant enzymes; increases the accumulation of free amino acids, proline, and soluble sugars; reduces malondialdehyde (MDA) accumulation; and stabilizes the cell [26,27]. These result in increased stress tolerance under drought or excessive soil moisture conditions.

Despite the documented seed invigoration, seed priming tends to reduce the longevity of high-vigor seeds [16,22,28,29], which usually deteriorate quickly, losing the beneficial effects of priming during subsequent storage [7,29]. Therefore, this is a critical aspect for consideration by farmers and industries interested in seed processing and commercial seed storage [30]. Furthermore, primed rice seed deterioration has been correlated with hampered starch metabolism or depletion of energy reserves [30–32], impaired antioxidative systems [33], and damaged cell structures [34], which destabilize selective permeability during storage. In addition, Wang [30] suggested that primed rice seeds were more sensitive to high relative humidity and elevated temperatures, environmental factors that directly influence seed deterioration, than non-primed seeds [35]. Moreover, one of the limitations of most studies is that experiments are commonly performed on artificially aged seeds using short-term exposure to elevated temperature and humidity, which may fall short of a real aging process [22].

Several studies on rice have indicated variation in seed longevity between lines [36,37] and varieties [10] and that priming efficiency is influenced by genetic diversity [38]. The aging of seeds leads to the loss of viability and a decrease in resistance to stresses during storage [7], including between sowing and germination in the soil [39]. In these conditions, seeds are subjected to stresses, including humidity and temperature fluctuations, which could affect the seed persistence and accelerate the deterioration of the seed [30] or result in seed death [39]. There is a need to understand the storability of primed seed for cultivar NERICA 4, one of the newly adopted varieties cultivated under the common farming conditions in Sub-Saharan Africa due to its superior comparability to local landraces in tolerance to harsh climatic conditions, tillering ability, and yield [2,40]. The objective of the present study was to assess the vigor and longevity of rice cv. NERICA 4 primed seeds during short-term storage (120 days) in ambient conditions simulating the tropics and sub-tropics. Upscaling studies on understanding the longevity of primed seeds under

storage would help in expanding their application under field conditions [41] and improve the predictability of primed seeds in storage.

2. Materials and Methods

Seed preparation, priming, and storage treatments: NERICA 4, an interspecific hybrid rice between *Oryza sativa* L. and *O. glaberrima* Steud, was used. The seeds were soaked and selected in salt water with a specific gravity of 1.13 and rinsed with water. Later, the seeds were dried to a moisture content of 13.6% measured using a grain moisture tester (Riceter, Kett Electric Laboratory, Tokyo, Japan). A weighed quantity of the seeds was soaked by submerging in the priming liquid, including distilled water for hydro priming, PEG 6000 (10% *w/v*) solution for osmopriming, and KNO₃ (0.5% *w/v*) solution for halopriming for 24 h at 30 °C in the dark. Subsequently, the seeds were removed from the solution, rinsed with distilled water, and dried to the initial weight in a natural convection incubator (SIB 35, Sansyo, Tokyo, Japan) at 30 °C. The primed and non-primed seeds were placed in mesh bags and stored in a plant growth chamber (LPH 241-S, Biotron NK System Nippon Medical and Chemical Instruments Co., Ltd., Osaka, Japan) set at 25 °C, 65% relative humidity for 120 days.

Germination test: A germination test was performed after priming at 30-day intervals up to 120 days of storage. The test was carried out in three replicates, each consisting of 25 seeds. The seeds were placed in a Petri dish (Ø 90 mm × 15 mm) lined with germination paper moistened with 8 mL distilled water and incubated in a low-temperature incubator (Yamato Scientific Co., Tokyo, Japan) set at 30 °C for 7 days. The petri dishes were left open on the third day to allow shoot growth, and an equal volume of water was added daily. The seed was considered germinated when the radicle had protruded through the seed coat. The germinated seeds were counted daily for 7 days after sowing. Subsequently, 10 randomly selected best-developed seedlings from each replicate were sampled, and the shoot and maximum root length were measured. Later, the seedlings were separated from the seed remains and dried in an oven at 80 °C to constant weight to obtain the dry weight. Germination percentage (*Gp*), germination index (*GI*), mean germination time (*MGT*), seedling vigor index (*SVI*) [27], mean daily germination [42] and Speed of germination *T*50 [43] were calculated according to the following formulae:

$$Gp = \frac{\text{Germinated Seeds}}{\text{Total No. of seeds}} \times 100 \quad (1)$$

$$GI = \sum (Gt / Tt) \quad (2)$$

$$MGT = \sum (Gt * Tt) / \sum Gt \quad (3)$$

where *Gt* is the number of germinated seeds on Day *t*, *Tt* is the time corresponding to *Gt* in days.

$$SVI\ 1 = Gp \times l \quad (4)$$

$$SVI\ 2 = Gp \times DM \quad (5)$$

where *l* is the total seedling length and *DM* is the dry weight of 10 seedlings.

$$T50 = ti + \left(\frac{\left(\frac{N+1}{2-ni} \right)}{(nj - ni)} \right) * (tj - ti) \quad (6)$$

where, *N* is the final number of seeds that germinated, *ni* and *nj* are cumulative numbers of seeds germinated by adjacent counts at times when *ni* < *N*/2 < *nj* [38].

$$MDG = \frac{\text{Final germination \%}}{\text{No of days to final germination \%}} \quad (7)$$

2.1. Electrical Conductivity

Seed electrical conductivity (EC): In total, 75 seeds were weighed and soaked in 20 mL of distilled water at 30 °C and kept in the dark for 24 h. The EC of the solution was measured using a conductivity meter (EZDO 7200 Sceto Shouji Inc., Taipei, Taiwan) after gentle swirling and expressed in $\mu\text{S}\cdot\text{cm}^{-1}\text{ g}^{-1}\text{ FW}$ [44–46] by dividing by the weight of the tested seeds.

2.2. Estimation of Hydrogen Peroxide and Malondialdehyde

Hydrogen peroxide (H_2O_2) was extracted by homogenizing 10 seeds of known weight with 3 mL of phosphate buffer (50 mM, pH 6.8). The homogenate was centrifuged at $6006\times g$ for 25 min. The extracted solution was mixed with 1 mL of 0.1% titanium III chloride in 20% (v/v) sulfuric acid, and the mixture was centrifuged for 15 min at $6006\times g$. The intensity of the yellow color of the supernatant was measured at 410 nm [47,48]. H_2O_2 level was calculated using the extinction coefficient of $0.28\text{ }\mu\text{mol}^{-1}\text{ cm}^{-1}$ and expressed in $\mu\text{mol g}^{-1}\text{ FW}$ [48].

Malondialdehyde content was determined through the thiobarbituric acid (TBA) reaction following the methodology described previously [30,32,39,49]. Briefly, 10 seeds weighing approximately 0.26 g fresh seed weight were ground in 4 mL of 50 mM phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at $15,000\times g$ for 20 min at 4 °C. Thereafter, 1.5 mL of the extract was mixed with 1.5 mL 0.5% (w/v) TBA containing 20% (w/v) trichloroacetic acid (TCA). After mixing, the reaction mixture was incubated in an oven at 95 °C for 30 min. The reaction was stopped through cooling in an ice bath and later centrifuged at $9998\times g$ for 10 min. The absorbance of the supernatant was measured at 532 nm for maximum absorbance and 600 nm for the correction of nonspecific turbidity [50] in a 1.5 mL cuvette. MDA equivalents (MDA-TBA complex) in $\text{nmol g}^{-1}\text{ FW}$ [51] were calculated using an extinction coefficient of $155,000\text{ }\mu\text{mol}^{-1}\text{ cm}^{-1}$ [52,53] and the formula [50]:

$$\text{MDA equivalents } (\text{nmol g}^{-1}) = [(A_{532} - A_{600})/155,000]10^6 \quad (8)$$

2.3. Data Analysis

Analysis of variance of the means and comparison using post hoc Tukey's honestly significant difference test was performed using the Statistical Tool for Agricultural Research Version: 2.0.1, International Rice Research Institute (IRRI) 2013–2020, Los Baños, Philippines and Microsoft Excel for Microsoft 365 MSO Version 2301, Microsoft Corp., Washington, DC, USA.

3. Results

3.1. Change in Germination

Table 1 shows the initial germination percentage on Day 1 after sowing was significantly reduced for all the stored seeds. After 30 days of storage, non-primed and halo-primed seeds reduced by about 17% and 20%, respectively. Furthermore, after 90 days of storage, hydroprimed and osmoprimed seeds reduced by about 21% and 17%, respectively. The results indicate a delay in the germination recorded on the first day of germination after storage. Furthermore, there was a significant difference between treatments in the initial germination percentage for primed seed, i.e., 85%, 97%, and 88% for hydropriming, halopriming, and osmopriming, respectively, compared to the non-primed 69% at 0 days of storage (Table 1). However, the benefit conferred by seed priming was insignificant after 90 days of storage compared to the non-primed seed (Table 1).

The time course of germination percentage over 7 days after sowing for non-primed and primed rice seeds stored for 0–120 days is shown in Figure 1. There was a significant reduction in the germination capacity measured by the final germination percentage on day 7 after sowing for hydroprimed and osmoprimed seeds from 100% recorded for each treatment at 0 days of storage to 93% and 91%, respectively, after 120 days of storage

(Figure 1). The reduction in the final germination percentage for haloprimered seeds from 100% to 95% was not statistically different. Moreover, the changes in the final germination for non-primed seeds through the storage period were also not statistically significant.

Table 1. Initial germination (first count on day 1 after sowing) of non-primed and primed seed stored for 120 days after priming.

Treatment	Storage Period (Days)					<i>p</i> Value
	0	30	60	90	120	
Non-primed	69 ± 5 ^{c A}	52 ± 4 ^{b B}	53 ± 5 ^{b B}	51 ± 2 ^B	41 ± 10 ^B	<0.001
Hydropriming	85 ± 2 ^{b A}	75 ± 6 ^{a AB}	73 ± 6 ^{a AB}	64 ± 11 ^{BC}	48 ± 8 ^C	<0.001
Haloprimering	97 ± 5 ^{a A}	77 ± 6 ^{a B}	81 ± 5 ^{a AB}	67 ± 8 ^{BC}	53 ± 9 ^C	<0.001
Osmoprimering	88 ± 4 ^{ab A}	72 ± 4 ^{a AB}	76 ± 7 ^{a AB}	71 ± 8 ^B	40 ± 7 ^C	<0.001
<i>p</i> value	<0.001	0.001	0.001	0.007	0.277	

The data shown are the means of the germination percentages ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$) as determined via Tukey's HSD post hoc test. Lowercase letters compare means along columns between treatments within a specific storage period; uppercase letters compare means along rows between different storage period within the same treatment.

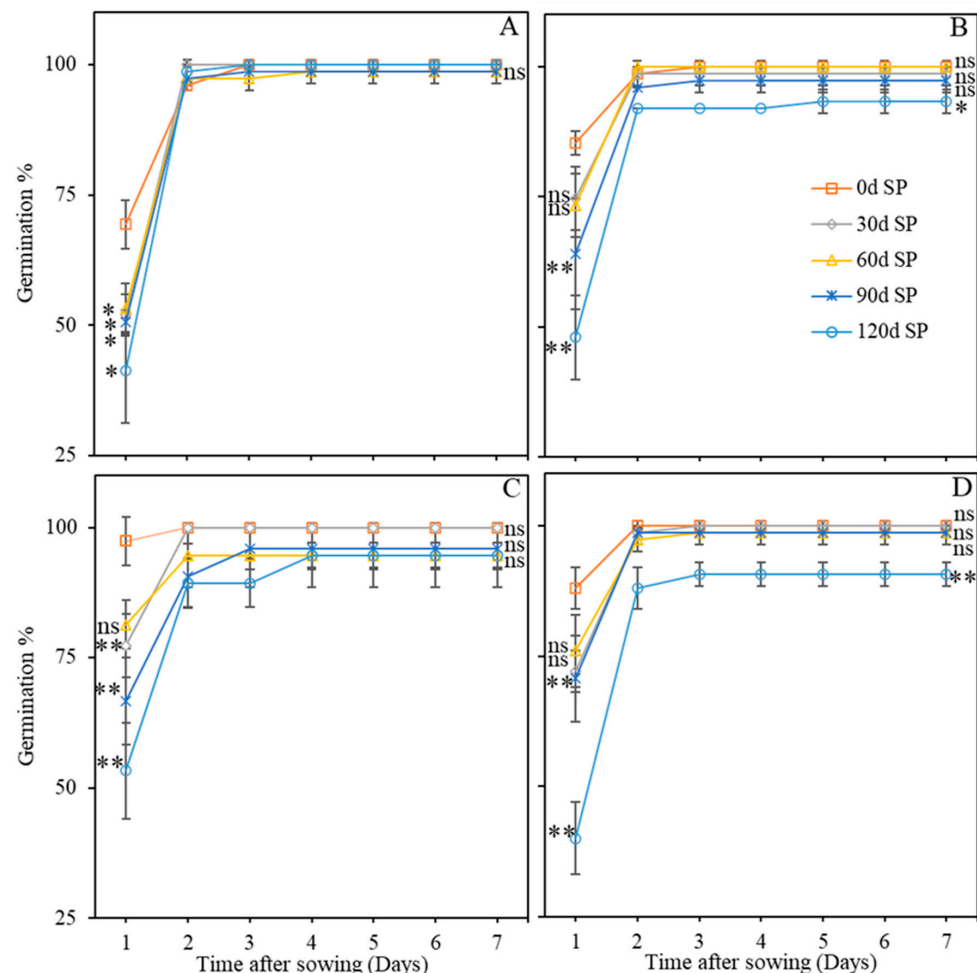


Figure 1. Time course of germination as a percentage over a period of 7 days after sowing for non-primed (A), hydroprimed (B), haloprimered (C), and osmoprimered (D) rice seeds stored at 25 °C/65% RH for 0–120 days. Error bars on the means indicate standard deviation ($n = 3$), while * and ** indicate significant difference ($p < 0.05$ and $p = 0.01$, respectively) between germination percentage at 0 and 120 days of storage for the initial and final germination as determined via Tukey's HSD post hoc test; "ns" indicates non-significant differences.

Furthermore, there was a decline in the *GI* for the non-primed and primed seeds due to storage. Figure 2 shows that the *GI* was significantly lower on day 30 for non-primed and day 90 for primed seeds as compared to un-stored seeds (day 0 of storage) (Figure 2). *MGT* and the time to 50% germination were significantly increased, indicating a reduction in the seed vigor for both primed and non-primed seed (Figure 2). The *SVI* of haloprimed and osmoprimed seeds significantly reduced after 60 days and 120 days of storage, respectively. In addition, there was a general reduction in the hundred-seed weight for the primed and non-primed seeds after 120 days of storage compared to the un-stored seeds. The percentage reductions in hundred-seed weight were 1.1%, 2.6%, 2.7%, and 3.1% for non-primed, osmopriming, hydropriming, and halopriming, respectively.

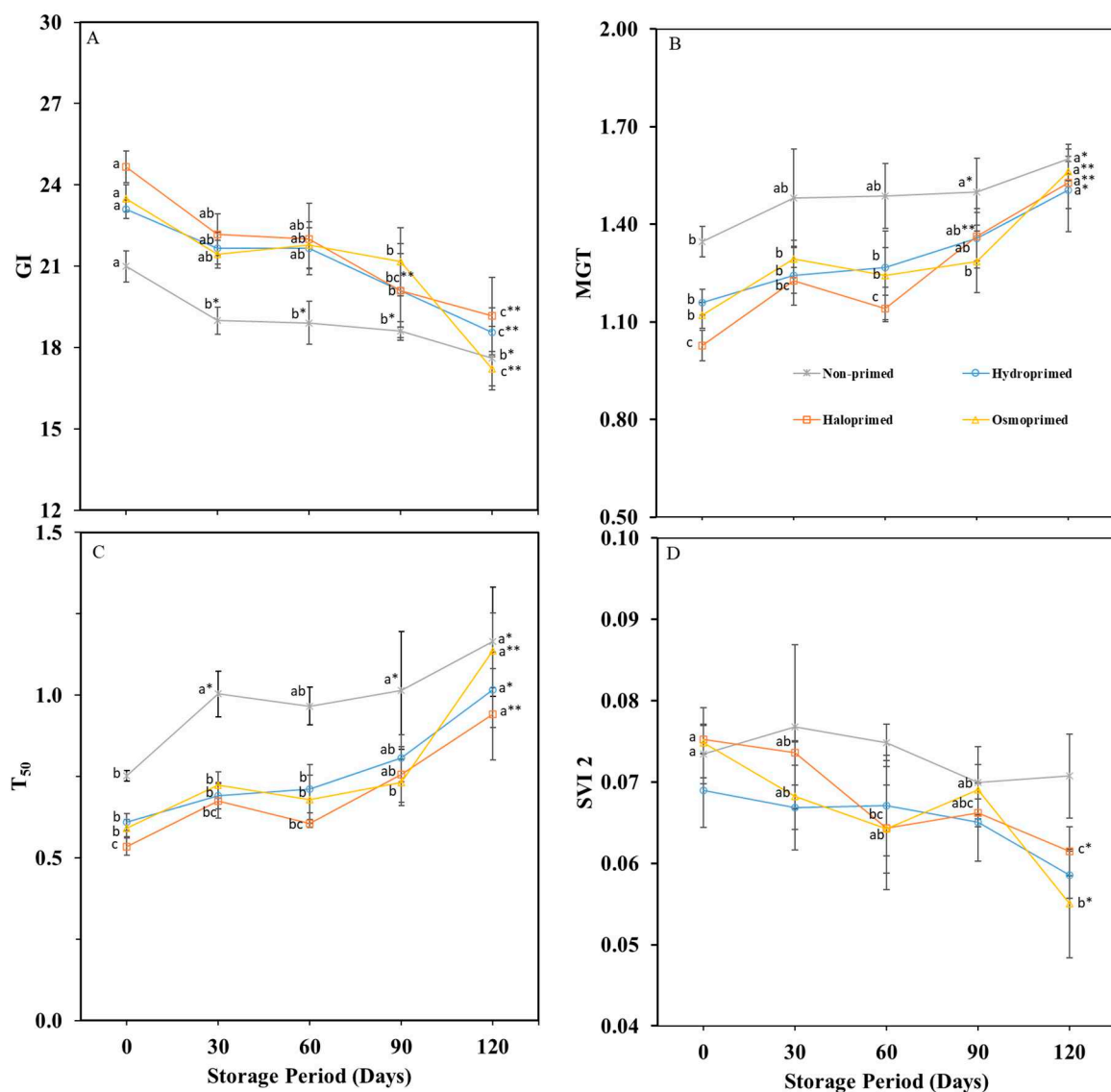


Figure 2. Germination parameters: germination index (A), mean germination time (B), time to 50% germination (C), seedling vigor index (D) for non-primed and primed seed stored for 0–120 days at 25 °C/65% RH. Error bars on the means indicate standard deviation and different letters indicate significantly different at $p < 0.05$ and $p < 0.01$ —denoted by * and **, respectively—within treatments across the storage period.

3.2. Seed EC and the Relationship with Germination Parameters

Primed and non-primed seeds displayed a significant increase in the EC of seed leachates all through the storage period due to imbibition damage and seed aging. Figure 3 shows the trend in solute leakage through the storage period. The rate of change of EC obtained from the slope of the trend has also been considered and was highest for the non-primed seed at $6.07 \mu\text{S cm}^{-1} \text{g}^{-1} \text{FW}$ per month, followed by the haloprimed seed at $4.73 \mu\text{S cm}^{-1} \text{g}^{-1} \text{FW}$ per month and hydroprimed seed at $4.36 \mu\text{S cm}^{-1} \text{g}^{-1} \text{FW}$ per month, and least for osmoprimed seed at $1.76 \mu\text{S cm}^{-1} \text{g}^{-1} \text{FW}$ per month.

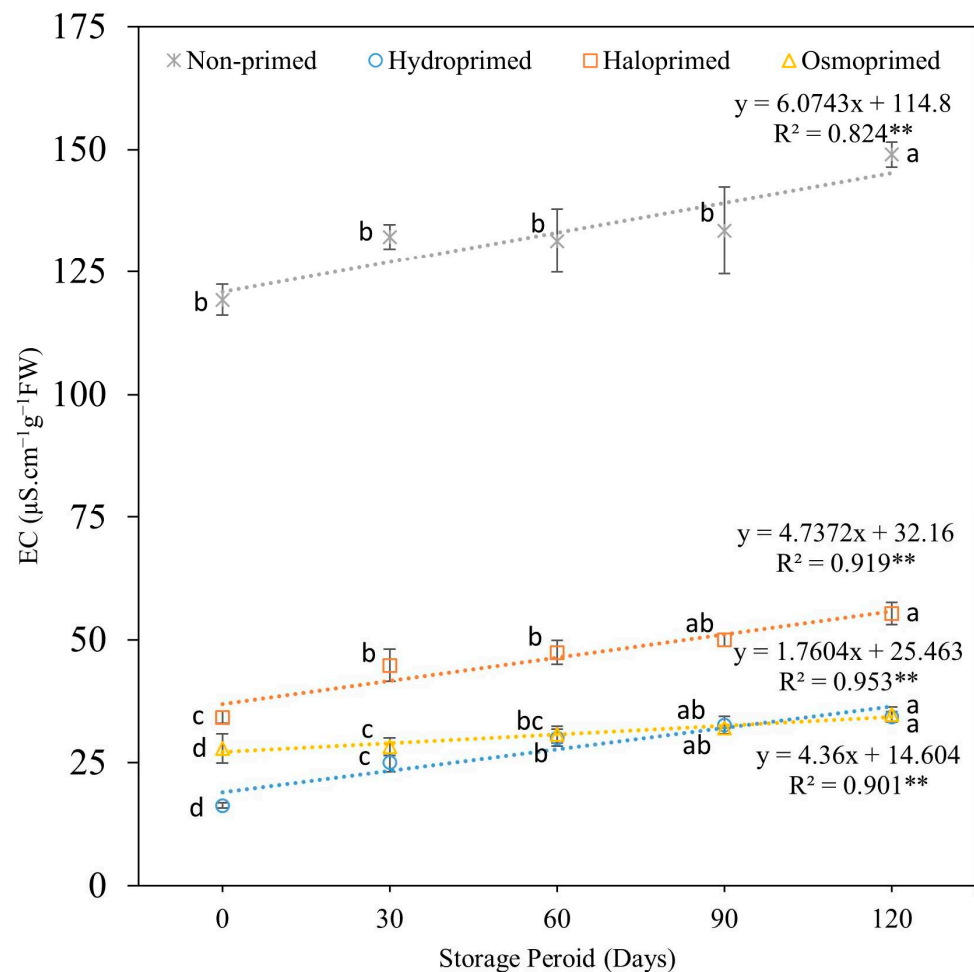


Figure 3. The trend of seed electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1} \text{FW}$) for non-primed and primed seed after 0–120 days of storage. Error bars on the means indicate standard deviation ($n = 3$) and different letters on error bars indicate significantly different ($p < 0.01$) means denoted by ** within the same treatment as determined using Tukey's HSD test.

There was a negative correlation (Table 2) between seed EC and the germination parameters GI and $SVI 2$, except the $SVI 2$ for the non-primed seeds, for which the reduction was not significant and also nonsignificant for hydropriming and osmopriming. The correlation was strong and significant for the haloprimed seed for both the parameters.

Table 2. Linear correlations between germination parameters and biochemical processes as determined using the Pearson product–moment correlation.

Non-Primed												
	EC 24	Germination	MGT	GI	T50	SVI 1	SVI 2	H ₂ O ₂	MDA	100 Seed Weight	Seedling Length	DM (g)
EC 24	1.00											
Germination	0.05	1.00										
MGT	0.98 **	−0.11	1.00									
GI	−0.95 *	0.20	−0.99 **	1.00								
T50	0.97 **	−0.06	0.99 **	−0.99 **	1.00							
SVI 1	−0.74	−0.53	−0.68	0.65	−0.75	1.00						
SVI 2	−0.41	0.24	−0.37	0.36	−0.33	0.09	1.00					
H ₂ O ₂	0.81	0.36	0.69	−0.62	0.67	−0.63	−0.66	1.00				
MDA	0.58	0.69	0.42	−0.33	0.42	−0.67	−0.47	0.91 **	1.00			
100 Seed Weight	−0.30	−0.92 *	−0.12	0.03	−0.15	0.64	0.14	−0.66	−0.91 *	1.00		
Seedling Length	−0.62	−0.71	−0.53	0.49	−0.60	0.97 **	0.01	−0.62	−0.74	0.78	1.00	
DM (g)	−0.44	0.05	−0.36	0.33	−0.33	0.20	0.98 **	−0.76	−0.63	0.33	0.15	1.00
Hydropriming												
	EC 24	Germination	MGT	GI	T50	SVI 1	SVI 2	H ₂ O ₂	MDA	100 Seed Weight	Seedling Length	DM (g)
EC 24	1.00											
Germination	−0.71	1.00										
MGT	0.87	−0.83	1.00									
GI	−0.89 *	0.88 *	−0.99 **	1.00								
T50	0.82	−0.80	1.00 **	−0.98 **	1.00							
SVI 1	−0.65	0.78	−0.94	0.92 *	−0.97 **	1.00						
SVI 2	−0.75	0.77	−0.97 **	0.95 *	−0.99 **	0.98	1.00					
H ₂ O ₂	0.34	−0.55	0.76	−0.70	0.82	−0.93	−0.87	1.00				
MDA	−0.06	−0.35	0.43	−0.38	0.52	−0.70	−0.62	0.89 *	1.00			
100 Seed Weight	−0.53	0.92 *	−0.77	0.80	−0.77	0.83	0.75	−0.69	−0.51	1.00		
Seedling Length	−0.63	0.75	−0.93 *	0.90 *	−0.96 **	1.00 **	0.97 **	−0.94 *	−0.70	0.81	1.00	
DM (g)	−0.77	0.69	−0.96 **	0.93	−0.98 **	0.95	0.99 **	−0.84	−0.56	0.65	0.95 *	1.00

Table 2. Cont.

Halopriming												
	EC 24	Germination	MGT	GI	T50	SVI 1	SVI 2	H ₂ O ₂	MDA	100 Seed Weight	Seedling Length	DM (g)
EC 24	1.00											
Germination	−0.68	1.00										
MGT	0.91 *	−0.39	1.00									
GI	−0.98 **	0.60	−0.96 *	1.00								
T50	0.88 *	−0.35	0.99 **	−0.93 *	1.00							
SVI 1	−0.78	0.14	−0.80	0.76	−0.80	1.00						
SVI 2	−0.89 *	0.90 *	−0.72	0.84	−0.71	0.45	1.00					
H ₂ O ₂	0.21	0.06	0.51	−0.30	0.58	−0.12	−0.27	1.00				
MDA	0.53	−0.06	0.69	−0.52	0.77	−0.63	−0.46	0.77	1.00			
100 Seed weight	−0.65	0.42	−0.82	0.75	−0.82	0.31	0.69	−0.78	−0.60	1.00		
Seedling Length	−0.59	−0.14	−0.68	0.58	−0.68	0.96 **	0.20	−0.11	−0.59	0.17	1.00	
DM (g)	−0.91 *	0.85	−0.78	0.87	−0.77	0.53	0.99 **	−0.33	−0.54	0.71	0.28	1.00
Osmopriming												
	EC 24	Germination	MGT	GI	T50	SVI 1	SVI 2	H ₂ O ₂	MDA	100 Seed Weight	Seedling Length	DM (g)
EC 24	1.00											
Germination	−0.94 *	1.00										
MGT	0.85	−0.91 *	1.00									
GI	−0.89 *	0.94 *	−1.00 **	1.00								
T50	0.86	−0.95 *	0.98 **	−0.99 **	1.00							
SVI 1	−0.63	0.80	−0.93 *	0.91 *	−0.94 *	1.00						
SVI 2	−0.85	0.92 *	−0.93 *	0.94 *	−0.91 *	0.82	1.00					
H ₂ O ₂	0.56	−0.69	0.52	−0.56	0.66	−0.57	−0.38	1.00				
MDA	0.53	−0.76	0.80	−0.79	0.87	−0.93 *	−0.66	0.79	1.00			
100 Seed Weight	−0.72	0.84	−0.67	0.71	−0.79	0.67	0.57	−0.97 **	−0.82	1.00		
Seedling Length	−0.41	0.61	−0.82	0.78	−0.81	0.96 **	0.68	−0.41	−0.88 *	0.48	1.00	
DM (g)	−0.46	0.41	−0.44	0.44	−0.35	0.26	0.70	0.34	0.03	−0.12	0.17	1.00

* and ** indicate that the correlation coefficient is significant at ($p < 0.05$) and ($p < 0.01$), respectively.

3.3. Biochemical Processes and Their Interaction with Germination Parameters

The variation in the concentration of MDA during storage was not significant for 90 days for haloprimed and osmoprimed seeds and 30 days for non-primed and hydroprimed seeds, followed by a significant reduction at 60 days and a subsequent increase for all the treatments to the highest level at 120 days. This was significantly higher as compared to 60 days for non-primed and 90 days for the primed seed (Figure 4). Between priming treatments, MDA levels were only significantly different at 0 and 60 days of storage, where the non-primed seed recorded significantly higher than halopriming and hydroprimed seed at 0 days and 60 days, respectively (Table 3). MDA accumulation by primed seed was lower and more significant for hydroprimed and haloprimed as compared to the non-primed seed.

There was a medium to a strong positive correlation between H_2O_2 produced and MDA accumulated by the seed in storage (Table 2). The correlation was significant for non-primed and hydroprimed seeds, while nonsignificant for haloprimed and osmoprimed seeds. There was a moderate to strong curvilinear relationship between the MDA accumulated in the seed with the germination parameters, i.e., *GI*, *MGT*, *T50*, and *SVI 2*, except for the *SVI 2* for the non-primed seed described by the equation $y = mx^2 + bx + c$. The correlation coefficients were high, ranging from 0.461 to 0.934.

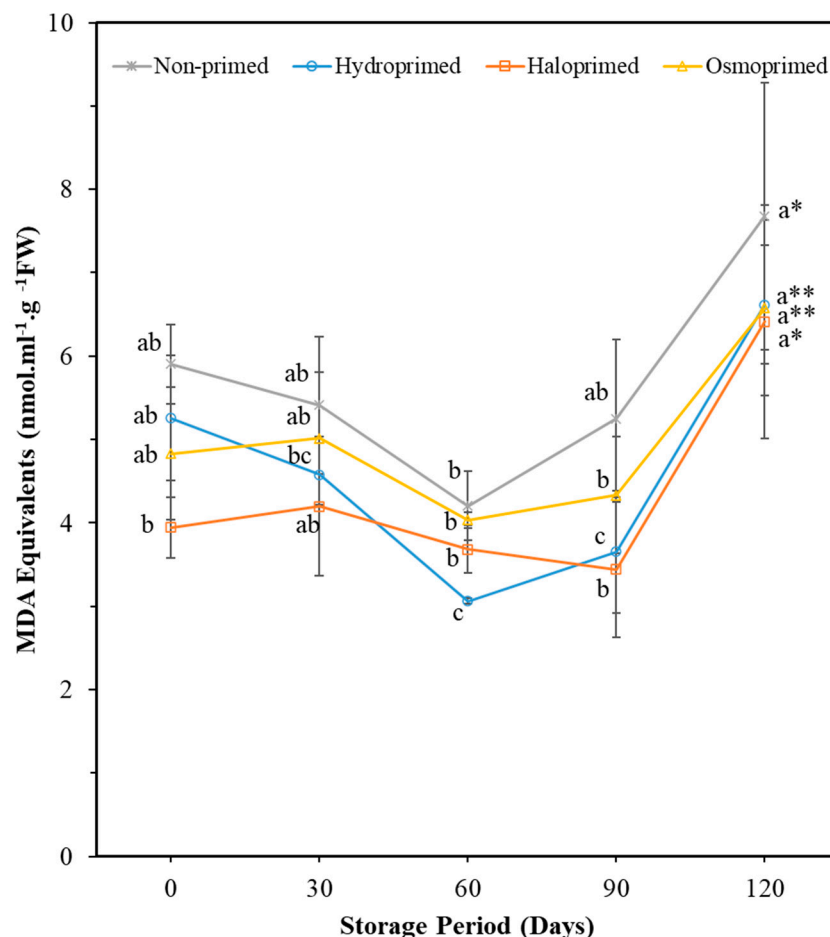


Figure 4. MDA equivalents ($\text{nmol} \cdot \text{mL}^{-1} \cdot \text{g}^{-1} \text{FW}$) for non-primed and primed seed stored for 0–120 days at $25^\circ\text{C}/65\%\text{RH}$. Error bars on the means indicate standard deviation and different letters indicate significantly different at $p < 0.05$ and at $p < 0.01$ denoted by * and **, respectively, within treatments across the storage period as determined via post hoc Tukey's HSD test.

Table 3. MDA accumulation (nmol/g FW) of non-primed and primed seed stored for 0–120 days after priming.

Treatment	Storage Period (Days)				
	0	30	60	90	120
Non-primed	5.90 ± 0.5 a	5.41 ± 0.8	4.2 ± 0.4 a	5.25 ± 0.9	7.68 ± 1.6
Hydropriming	5.26 ± 0.7 a	4.58 ± 0.4	3.06 ± 0.03 b	3.65 ± 0.7	6.62 ± 0.7
Halopriming	3.95 ± 0.4 b	4.20 ± 0.8	3.68 ± 0.3 ab	3.44 ± 0.8	6.41 ± 1.4
Osmopriming	4.83 ± 0.8 ab	5.02 ± 0.8	4.04 ± 0.1 a	4.34 ± 0.7	6.58 ± 1
<i>p</i> value	0.028	0.1783	<0.01	0.0889	0.599

The data shown are the means of the measurements ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$) as determined via Tukey's HSD test between treatments in a particular storage period.

The germination percentages of the non-primed and primed seeds were relatively high even after 120 days of storage (Table 4). Non-primed seeds showed a stable final germination trend. However, the first-count germination after 30 days of storage and the seed vigor (*GI* and *T50*) of the seed dropped. Just like a non-primed seed, the changes in final germination for halopriming were not significant, while hydropriming and osmopriming had a significant reduction in the final germination percentage. However, the initial germination percentages were sustained higher for longer—90 days for hydropriming and osmopriming and 60 days for halopriming. Seed viability was reduced for the primed seed and was also insignificant until after 90 days. At 120 days, germination was lower and slower (Figure 1), shorter and more spread in time (Figure 2), and more varied (Table 4) for the primed seed compared to the non-primed.

Table 4. Germination parameters (final germination, seedling vigour index and Seedling length) Biochemical activities (Hydrogen peroxide and Malondialdehyde) of non-primed and primed seed stored for 120 days after priming.

Storage Period (Days)	Gp (%)	SVI 1	Seedling Length (cm)	H ₂ O ₂ (μmol·g ^{−1} FW)	MDA (nmol·g ^{−1} FW)
Non-Primed					
0	100 ± 0	17.2 ± 0.7	17.19 ± 0.7	1.473 ± 0.49	5.903 ± 0.5 ^{ab}
30	100 ± 0	16.5 ± 1.8	16.49 ± 1.8	1.419 ± 0.67	5.411 ± 0.8 ^{ab}
60	99 ± 0.02	17.3 ± 0.4	17.47 ± 0.1	1.366 ± 0.66	4.204 ± 0.4 ^b
90	99 ± 0.02	16.9 ± 0.3	17.12 ± 0.2	1.566 ± 0.27	5.252 ± 0.9 ^{ab}
120	100 ± 0	16.4 ± 0.4	16.41 ± 0.4	2.036 ± 0.98	7.662 ± 1.6 ^a
<i>p</i> value	0.58	0.713	0.534	0.621	0.014
Hydropriming					
0	100 ± 0.02 ^a	16.4 ± 0.3 ^a	16.39 ± 0.3 ^a	0.823 ± 0.19 ^b	5.281 ± 0.7 ^{ab}
30	98.67 ± 0 ^a	16.3 ± 1.3 ^a	16.49 ± 1 ^a	0.554 ± 0.21 ^b	4.580 ± 0.4 ^{bc}
60	100 ± 0 ^a	16.5 ± 0.3 ^a	16.48 ± 0.3 ^a	0.475 ± 0.09 ^b	3.063 ± 0.03 ^c
90	97 ± 0.02 ^a	15.0 ± 0.6 ^a	15.41 ± 0.3 ^{ab}	0.646 ± 0.34 ^b	3.652 ± 0.7 ^c
120	93 ± 0.02 ^b	12.3 ± 1.6 ^b	13.16 ± 1.6 ^b	1.608 ± 0.98 ^a	6.615 ± 0.7 ^a
<i>p</i> value	0.005	0.002	0.003	<0.001	<0.001
Halopriming					
0	100 ± 0	17.3 ± 1.1 ^a	17.34 ± 1.1	1.282 ± 0.54 ^{ab}	3.946 ± 0.4 ^b
30	100 ± 0	13.8 ± 2.6 ^{ab}	13.79 ± 2.6	0.312 ± 0.44 ^c	4.200 ± 0.8 ^{ab}
60	95 ± 0.06	15.7 ± 0.5 ^{ab}	16.65 ± 0.6	0.226 ± 0.19 ^c	3.685 ± 0.3 ^b
90	96 ± 0.04	15.3 ± 0.2 ^{ab}	15.95 ± 0.8	0.802 ± 0.44 ^{bc}	3.443 ± 0.8 ^b
120	95 ± 0.02	13.4 ± 1.3 ^b	14.19 ± 1.1	1.949 ± 0.18 ^a	6.413 ± 1.4 ^a
<i>p</i> value	0.185	0.041	0.052	<0.001	<0.001

Table 4. Cont.

Storage Period (Days)	Gp (%)	SVI 1	Seedling Length (cm)	H ₂ O ₂ (μmol·g ⁻¹ FW)	MDA (nmol·g ⁻¹ FW)
Osmopriming					
0	100 ± 0 ^a	16.7 ± 1.4 ^a	16.74 ± 1.4	1.678 ± 0.51 ^{ab}	4.833 ± 0.8 ^{ab}
30	100 ± 0 ^a	15.2 ± 2.0 ^{ab}	15.16 ± 2	0.282 ± 0.02 ^{bc}	4.335 ± 0.8 ^{ab}
60	99 ± 0.02 ^a	16.6 ± 0.3 ^a	16.82 ± 0.4	0.164 ± 0.04 ^c	4.038 ± 0.1 ^b
90	99 ± 0.02 ^a	16.5 ± 0.5 ^{ab}	16.73 ± 0.1	1.115 ± 0.49 ^{bc}	4.335 ± 0.7 ^b
120	91 ± 0.02 ^b	13.2 ± 1.3 ^b	14.54 ± 1.7	2.555 ± 0.28 ^a	6.579 ± 1 ^a
<i>p</i> value	<0.001	0.028	0.188	<0.001	0.008

Data shown are the means of the measurements ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$) as determined via post hoc Tukey's HSD test within each treatment across the storage period.

4. Discussion

4.1. Effect of Storage Period on Germination Behavior

There was a reduction in the final germination percentage for all the primed seeds and it was significant for hydroprimed and osmoprimed seeds after 120 days of storage (Figure 1). These results were similar to previous studies [10,30,31], where a general decline in the *GI* for the non-primed and primed seeds due to storage was observed. The reduction in seed vigor for non-primed seeds was earliest at 30 days for all the germination parameters but remained almost constant thereafter. At 120 days of storage, the final germination percentage and *GI* was lower for the primed seed compared to the non-primed seed. Furthermore, after the initial 30 days of storage, non-primed seeds were less sensitive to the storage conditions than the primed seeds, an observation made by Hussain et al. [31]. However, the higher germination rate and vigor for the primed seeds were maintained in storage for up to 90 days based on the initial germination, *T50*, *MGT*, and *GI*. Seed vigor was negatively correlated with electrical conductivity, and solute leakage detected by EC was associated with dead or damaged tissue after imbibition damage and seed aging—or the interaction of the two factors—and has been used to monitor the cell contents' leakage due to loss of integrity of cellular systems.

4.2. Membrane Stability

EC recorded in this study was lower for primed seeds as compared to the non-primed seeds similar to a previous study [45]. However, the rate of change of EC of seed leachates has also been considered. Imbibition damage often results from both the physical and metabolic impact of rapid water uptake by dry seed and ROS attack on macromolecules like lipids, which are a main component of the cell membrane. Physical damage probably occurs during imbibition and redrying, which together make up the process of priming [45]. Woodstock and Tao [54] suggested that PEG prevents injury in low-vigor seeds by reducing the rate of water uptake during initial imbibition. The delay provides time for membrane reorganization necessary for compartmentalization before tissue hydration causes mixing and loss of cellular contents. Studies on chickpea [55], onion [56], cucumber [57], barley [58], pepper [59], and palisade grass [23], recorded low EC of seed leachate for primed seed compared to control. Lamichaney et al. [55] opined that the priming process leads to the repair mechanism of membranes, enhancing their stability. The different levels of EC recorded in this study could probably have also been contributed by the priming process as noted by Xu et al. [45] due to electrolyte loss during seed washing or the seed priming material used; PEG-primed seed, for example, recorded low EC [58] and reduced leakage of electrolytes [60]. Additionally, it is non-ionic, unlike KNO₃ [56], and [45] noted the discrepancy and the difficulty of interpreting cell membrane integrity based on EC levels alone. However, an increase in conductivity with aging [61] is expected in terms of membrane disruption and can only be explained by the trend. Priming reduced leakage of electrolytes, possibly via reduction of imbibition damage, and improved the ability to repair after imbibition. It also maintained better seed vigor induced by priming during the initial

period of storage. Membrane stabilization and priming-controlled water uptake would only be beneficial within the period in which the seed performs better than the non-primed seed, but there is also a possibility of leakage from physical damage [45] because of the hydration and rehydration cycle during priming. In this study, MDA was only statistically lower for halopriming and hydropriming at 0 days and 60 days, respectively, but overall, MDA accumulation was lower for the primed seed and highest for all after 120 days of storage.

Cell membrane integrity is stable in dry seed [45] due to the glassy state of cell membranes, and damage due to imbibition and redrying results in leakage of intracellular soluble constituents into extracellular space. Even though the cell membrane integrity in hydrated seeds may be greater than in dry seeds due to repairs after imbibition, the losses of electrolytes before hydration-induced cell membrane integrity is attained, indicating the extent of the damage and loss of integrity of cellular systems. Membrane injury at the beginning of imbibition is critical, and PEG or similar osmotic regulators have been used to reduce imbibitional injury in seeds known to have deteriorated through aging [54]. Membrane stability for the primed seed in this study was influenced by treatment and storage (Table 5), and the germination indices for the primed seed were lower at 120 days of storage. Therefore, priming seems to safeguard only against the metabolic impact of imbibition damage and not against physical damage.

Table 5. The effect of the interaction between treatment and storage on germination parameters and biochemical activities of NERICA 4 rice seed stored at 25 °C/65%RH.

Source of Variation	EC	Initial Germination	Final Germination	MGTGI	T50	SVI 1	SVI 2	MDA	Seed Weight	Length	Seedling DM	MDG	H ₂ O ₂	MDA
Treatment	**	**	*	**	**	**	**	**	ns	**	**	ns	**	**
Storage	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Treatment × Storage	*	ns	**	**	**	ns	ns	ns	ns	ns	ns	**	**	ns

Two-way ANOVA results; * and ** indicate statistical significance at $p < 0.05$ and $p < 0.01$, respectively. ns indicates non-significant differences.

4.3. ROS Production and Lipid Peroxidation

Aging during storage is a major cause of decreased seed quality [22,62] in terms of germinability and longevity. During seed imbibition, lipids are engaged in membrane reorganization while facing free-radical-mediated oxidative injury [63]. Lipid peroxidation causes imbibition damage [64] when dry seeds rapidly uptake water, affecting seed quality [65]. An increasing number of hydration–rehydration cycles also results in decreased seed viability [66]. Furthermore, biochemical and cellular events triggered by water uptake are accompanied by the generation of ROS, especially H₂O₂ [67], one of the primary ROS [68] present in plant tissues within certain normal limits [69]. It has two roles, i.e., beneficial [68] and deleterious [51,67,70]. Synthesis of H₂O₂ results in an oxidative burst upon application of related elicitors such as high temperature or mechanical damage [68]. At high levels, H₂O₂ may attack a variety of susceptible enzymes and ultrastructural cell components with detrimental effects [71,72], which may affect the growth axis potential and eventually lead to cell death.

In this study, H₂O₂ may have possibly been stimulated by water imbibition during seed priming resulting from increased respiration. In a similar study, Wojtyla et al. [67] indicated that an intense increase in respiratory activity spurs H₂O₂ production, which continues due to the storage condition stresses and the reduction in the antioxidative mechanisms. Furthermore, there was a positive correlation between the production of H₂O₂ and MDA accumulated in the seed, which was highest after 120 days of storage. H₂O₂-producing and consuming processes are active early in germination [73]. Predominantly, catalase and other H₂O₂-consuming enzymes, peroxidases, superoxide dismutase, and glutathione peroxidase have been recorded as active in the early stages of the imbibition phase. Bailly et al. [74] observed a high decrease in catalase and glutathione reductase activity on the first day of accelerated aging and a slight transient increase in the activity of

the enzymes SOD, CAT, and GR. Moreover, genes associated with cell wall synthesis and degradation were also activated during the process of imbibition, and most cell-wall-related genes were upregulated under H_2O_2 treatment [70]. The increased antioxidant capacity of the primed seed maintained the H_2O_2 at levels comparable to the non-primed seed (Table 4), and the reduction in the antioxidant capacity may have led to the increasing levels of H_2O_2 . In this study, for hydropriming, the changes in MDA were insignificant until after 60 days and 90 days for halopriming and osmopriming. A significant change occurred for the non-primed seeds at 120 days of storage, but the levels were higher than the primed seeds. Similarly, MDA content was enhanced in primed Chinese cabbage seeds [29] and sunflowers [74] with increased storage duration and temperature. Moreover, in the non-primed seeds of barley exposed to stress [42] and in pepper [59], this was accompanied by increased EC and a decrease in ROS antioxidants and seed vigor. Additionally, MDA is often employed as an indicator of lipid peroxidation, an end-product that easily penetrates biological membranes and affects other cellular components.

This study documented a decline in seed vigor and viability in storage after 120 days. In a similar study, conventional or traditional storage of rice seed at 25 °C recorded a reduction in germination parameters [10]. Furthermore, high temperature and relative air humidity during storage periods [35,75] affect seed quality and are considered critical environmental factors that directly influence deterioration. The relationship between rice seed survival and storage temperature is negative [76]. Higher temperatures cause an increased rate of chemical reactions in the seed, thereby initiating seed deterioration. Moreover, in a study on indica-type rice, in ideal conditions, the primed seed could not be stored beyond 60 days [30], and in conditions similar to the present study (30 °C–31 °C and 57–67% relative humidity), decreased vigor in primed seed beyond 15 days.

The RH directly affects seed moisture content during storage because seed moisture reaches an equilibrium with the level of water vapor surrounding the seeds [35,77]. Moreover, even small increments in moisture content are expected to influence the storability of seed significantly at an elevated temperature. Increased moisture content results in increased respiration and a drastic loss of viability. At 25 °C, the approximate equilibrium moisture contents for rice are 12.8% and 14.6% at 60% and 75% RH, respectively [11,14,78]. In this experiment, the seed moisture content was at 13.6% and kept at 65% RH, indicating that the seed was kept within the approximate range of its equilibrating moisture.

High-vigor seed was used in this experiment and was considered to be at the resistant phase of the seed survival curve [79], characterized by high germination. The storage conditions stress was not severe enough to take the seed through to the lethal aging stage characterized by a rapid drop in final germination. The lethal aging phase of the seed survival curve was not attained at the end of the storage period, but seed vigor was lower. This could be due to the oxidative damages initiated by imbibition during priming and storage condition stresses, but primed seed could maintain relatively low oxidative damage for up to 90 days in storage, a period in which the seed would still be safeguarded from the metabolic impact of imbibition damage by priming. An important survival strategy to keep cells alive in limited water conditions is to reduce their metabolic activity to a quiescent state [80]. Moreover, long storage in unsuitable conditions leads to the accumulation of cellular oxidative damage progressively inducing loss of seed vigor and germination capacity until the irreversible death of the embryo.

Seeds require efficient antioxidant systems as a protective mechanism to promote their longevity [81]. The antioxidative mechanisms, which were assumed to be better in primed seeds, had a hysteresis effect on the accumulation of MDA after exposure to stress in storage conditions. After priming, the seed never returns to a truly quiescent state; it gradually accumulates MDA and declines in vigor and viability, probably due to a decline in antioxidant systems. Membrane integrity was enhanced during priming, but the additional hydration–rehydration cycles make the seed prone to the effects of temperature and relative humidity in storage. The susceptibility of the primed seed to the storage conditions was, however, evident after 120 days, when its seed vigor was less than that of

the non-primed seed. NERICA 4 showed a slower and gradual vigor loss at 25 °C and 65% relative humidity and presents adequate time between priming and sowing or germination before any marked deterioration, which may be better than other reported varieties.

5. Conclusions

Primed seeds have the advantage of cell membrane stability, quicker repair, and probably an effective antioxidative mechanism that protects the seed from damage and is gradually worn out in storage. Storing primed seeds in near-ambient tropical to sub-tropical seed storage conditions has a negative effect on seed vigor and longevity. However, primed high vigor NERICA 4 rice seed could be stored for 90 days before any marked deterioration. After priming, the seed fails to re-establish a truly quiescent state after dehydration that cascades until lethal aging occurs. The ambient conditions are not severe enough to result in a drastic reduction of seed vigor and viability, which is an assurance that primed NERICA 4 seed could be either stored until the next planting season or remain viable in the soil during delayed germination. This study can contribute to the adoption of seed priming and can also inform the assessment of seed priming methods that sustain the storability and longevity of primed seeds in tropical and sub-tropical conditions in the future.

Author Contributions: Conceptualization, E.K.B. and Y.N.; methodology, E.K.B. and E.I.; formal analysis, E.K.B., J.A.M.A.L. and K.G.; investigation, E.K.B.; resources, J.-I.S.; data curation, J.-I.S.; writing—original draft preparation, E.K.B.; writing—review and editing, E.O. and S.Y.; visualization, E.K.B.; supervision, J.-I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ABE Initiative Program.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: I thank JICA for their support through the African Business Education (ABE) Initiative for Youth Scholarship Program and the Kenya Plant Health Inspectorate Service for the opportunity.

Conflicts of Interest: The authors declare no conflict of interest.

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