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A STUDY ON STORABILITY OF HYDRO AND HALO-PRIMED UPLAND RICE SEED

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BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

GRADUATE PROGRAM

DEPARTMENT OF PLANT SCIENCES

M.Sc. PROGRAM IN SEED SCIENCE AND TECHNOLOGY

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M.Sc. Thesis

By

Yilikal Melak Assaye

January 2021

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCES (M.Sc.) IN SEED SCIENCE AND TECHNOLOGY

January 2021

Bahir Dar

THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by Mr. Yilikal Melak Assaye entitled **"A STUDY ON STORABILITY OF HYDRO AND HALO-PRIMED UPLAND RICE SEED".** We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (M.Sc.) in Seed Science and Technology.

Board of Examiners

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Name of Internal Examiner	Signature	Date
_____	_____	_____
Name of Chair Person	Signature	Date

DECLARATION

This is to certify that this thesis entitled **"A STUDY ON STORABILITY OF HYDRO AND HALO-PRIMED UPLAND RICE SEED",** submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in "**Seed Science and Technology**" to the Graduate Program of College of Agriculture and Environmental Sciences Bahir Dar University by Mr. Yilikal Melak Assaye (ID. No. BDU1100656), is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

Yilikal Melak _____ _____

Name of the Student Signature Date

Karta Kaske (PhD**)** _____ _____

Major Advisor Signature Date

Wossen Tarekegne (PhD) _____ _____

Co-Advisor Signature Date

STATEMENT OF THE AUTHOR

I declare and affirm that this thesis is my own independent work with frequent guidance of advisory board, and that all reference sources used for the thesis have been properly acknowledged. This thesis has been submitted to Department Graduate Council of Plant Science, College of Agriculture and Environmental Sciences Bahir Dar University by Yilikal Melak Assaye in partial fulfillment of the requirement for degree of Master of Science in "**Seed Science and Technology**". Further, I can give my consent that this thesis can be reserved at Bahir Dar University College of Agriculture and Environmental Sciences library to be made available to photocopying, interlibrary loan, and for the title and summary to be made available to outside institutions.

Name of the Student Yilikal Melak Assaye

Signature_____

Date of submission: January / 2021

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DEDICATION

This thesis is dedicated to my first child Mahider Yilikal who was born at the beginning of the thesis write up session.

A STUDY ON STORABILITY HYDRO AND HALO-PRIMED UPLAND RICE SEED

BY

Yilikal Melak Assaye

Major Advisor Karta Kaske Kalse (PhD)

Co-Advisor Wossen Tarekegne (PhD)

ABSTRACT

Seed priming enhances seed performance in many crop species including rice. But, storing primed seed for longer period of time is the challenge in commercializing of the technology. This study was conducted to investigate the storability of halo-primed and re-dried seed of upland rice under laboratory conditions. Rice seeds were hydro-primed (distilled water for 24 hours) and halo-primed using different concentrations of calcium chloride in 0.25, 0.5, 0.75% and 1.00% (w/w) $CaCl_2$ solutions for 24 hours and re-dried. The primed seeds were compared with dry seeds. Both primed and dry seeds were stored in low-density polyethylene and high-density polyethylene plastic bags at 25± 3^oC for 0, 15, 30, 45, 60, and 75 days. Data on germination percentage, seedling fresh and dry weights, speed of germination, root and shoot lengths, vigor index and thousand seed weight were collected every fifteen days. It was observed that storing rice seed hydro-primed (distilled water) and halo-primed (@1.00% $CaCl_2$), re-dried, and stored in high-density polyethylene plastic bags could readily maintain seed germination and seedling growth parameters until 45 days whereas low-density polyethylene bags could maintain physiological quality until 30 days. Therefore, it is recommended that rice seed primed with 1.00% $CaCl_2$, re-dried and stored in high-density polyethylene plastic bags could be sold within 45 days after priming.

***Key words*: Halo Priming, Hydro Priming, Storability, Upland Rice**

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ABBREVIATION / ACRONYMS

CSA	Central Statistical Agency
CV	Coefficient of Variation
SEM	Standard Error of Mean
DAP	Days after Priming
DiW	Distilled Water
DSR	Direct Seeded Rice
EIAR	Ethiopian Institute of Agricultural Research
FcDiS	Food Crop Diversification Support Project
HDPE	High Density Polyethylene
HSD	Honestly Significant Difference
IRRI	International Rice Research Institute
LDPE	Low Density Polyethylene
LEA	Late Embryogenesis Abundant
MoA	Ministry of Agriculture
NERICA	New Rice for Africa
NSD	Nations Statistical Database
PEG	Polyethylene Glycol
PICS	Purdue Improved Crop Storage
RH	Relative Humidity
SMC	Seed Moisture Content
SNNPR	South Nations Nationalities and Peoples Region
WARDA	West African Rice Development Association
JICA	Japan International Cooperation Agency

CHAPTER 1. INTRODUCTION

1.1 Background and Justification

Rice production was introduced to Ethiopian during early 1970s and currently it is a strategic food security commodity with a significant potential of change in the livelihoods of farmers of Ethiopia. Currently, rice is grown in the country on 57,576 ha of land with about 1.7 million quintals of annual production supporting about 178,185 households (CSA, 2020). Major rice production areas of Ethiopia include Western Tigray (Tsegede, Tselemt); Amhara (Fogera, Dera, Libo Kemkem, Metema, North Achefer); Oromia (Jimma, Shebe, Illu Ababora, Chewaqa); Benishangul Gumuz (Metekel, Pawe, Assosa); SNNPR (Kaffa, Gimbo, Bench Maji, Gura Farda, Sheka, Yeki and Gamo Gofa) areas are to mention some (EIAR, 2013). The domestic market for local rice seems to be huge considering the high proportion of population of Ethiopia in the low-income market. There is an increasing trend of using rice in mixture with teff to bake Injera hence greatly increasing the demand (Tesfaye Getnet, 2019).

Good quality seed is among critical inputs for increasing yield of crops. However, limited availability of quality seed of rice has been a challenge for smallholder farmers in enhancing rice production in Ethiopia. It is well recognized that boosting rice production depends heavily on planting of high-quality seeds which provide rapid and uniform emergence and stand establishment. Thus, several seed invigoration techniques including priming have been used to improve seed performance and alleviate from detrimental effects of environmental stresses. Priming methods such as hydro-priming (Jisha and Puthur, 2014), osmo-priming (Yacoubi *et al*., 2013), halo-priming, priming with salt solutions (Paparella *et al*., 2015)**,** and bio-priming (Reddy and Reddy, 2012) have been studied to improve seed performance. The beneficial effects of seed priming include faster emergence, better stands, and lower incidence of re-sowing, more vigorous plants. Seed priming in known concentrations of salt solutions is a low-cost solution to poor stand establishment in direct seeded rice (DSR) by significantly enhancing rice seed quality characteristics (Farooq *et al.*, 2006).

In a series of studies, priming with $CaCl_2$, KCl and ascorbate was more pragmatic than other techniques employed to improve the seedling emergence of rice, maize and wheat (Farooq *et al*., 2010). However, research results showed that priming and re-drying with

$CaCl_2$ and water solution is the most effective (Farooq *et al*., 2009). Several reports exist on different seed priming methods in rice (Anwar *et al*., 2013), but there is a limited information on the germination and seedling growth of rice seed after certain period of storage of primed and re-dried seeds. However, Goswami (2019) affirmed that unequal plant stand, and poor germination are major constraints in seeds sown under areas which receive erratic and low rainfall which can be enhanced by sowing primed seeds, ultimately improving plant stand in the field.

For routine handling and trading of primed seeds, re-drying and storage for some periods of time is required, however, the longevity of primed seed is affected by many factors such as relative air humidity, seed moisture content, and temperature (Farooq *et al*., 2006). Despite the benefits of seed priming, one of the major problems in priming technologies is the limited storability of primed seeds (Hussain *et al*., 2015). Therefore, combinations of the right method of priming methods and seed packaging techniques need to be developed to store primed seeds. Such techniques will revolutionize farming in moisture stress areas where farmers have low access to technologies (Sivasubramaniam, 2011) because seed priming is found a doable technology for poor and erratic crop stand in rice (Rehman, *et al*., 2011).

Therefore, this research was conducted to evaluate the longevity of halo-primed and re-dried seed with different optimized concentrations of calcium chloride in low density polypropylene and high-density polypropylene (HDPE) bags. Therefore, the determination of maximum storage time for primed and re-dried seed of rice was conducted in combination with different seed packaging materials and priming solution.

1.2 **Statement of the Problem**

NERICA-4 is commonly sown or provided for farmers or seed producing companies after six months of storage duration which leads to deteriorate physiological its physiological quality to a certain level under Fogera National Rice Research and Training Center which in turn brings about erratic crop emergence after sowing.

Seed priming is a commercially successful practice, but reduced longevity of primed seeds during storage may limit its application. One of the major problems in priming technologies is the limited storability of primed seeds. Beneficial effects of seed priming

were maintained only for 15 days of storage at room temperature, beyond which the performance of primed seeds was worse even than non-primed seeds (Hussain *et al*., 2015).

1.2 Objectives

1.2.1 General objective

- To investigate storability of hydro- and halo-primed rice seed, within different $CaCl_2$ concentrations, storage duration and packaging types under optimized priming conditions.

1.2.2 Specific objectives

- To evaluate the effects of different concentrations of $CaCl_2$ priming on physiological quality of upland rice,
- To optimize packaging methods for primed upland rice seed for rice seed physiological quality characteristics,
- To determine optimum storage time with primed rice seed physiological quality.

CHAPTER 2. LITERATURE REVIEW

2.1. Origin and Distribution of Rice Crop

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the worlds such as Asia, Africa, South America and Australia (Bahmanyar and Ranjbar, 2007). The crop has great importance in terms of food, area and production (Bakhsh *et al.,* 2011). The domestication of rice began centuries ago, and currently *Oryza glaberrima* and *Oryza sativa* are the two species grown globally (Roychoudhury, 2020). The history of the domestication of rice can be traced back to 10,000 years ago when the *Oryza sp.* was a mere wild grass and artificial continuous selection by humans has led to the development of a stable amenable cultivar (Wei and Huang, 2019). The Asian rice, *O. sativa* and African rice, *O. glaberrima* were the two most cultivated species in the world. In recognition to the importance of rice sector to the country economy, the Government of Ethiopia has developed a National Rice Research and Development Strategy in Ethiopia (NRRDSE) to guide the integrated and focused promotion of the rice sector in the effort to ensure food security in the country (MoA, 2010).

Upland rice known as New Rice for Africa (NERICA) was developed by West African Rice Development Association (WARDA) in the late 1990s (African Development Bank, 2014). NERICA was developed by crossing the indigenous African rice (*O. glaberrima* Steud.) with exotic Asian rice (*O. sativa* L) (FcDiS, 2010). Released upland rice varieties have been disseminated and expanded to different agro-ecologies of the country (Tamirat Belayneh and Jember Tekle, 2017).

2.2. Botanical Description

Botanical name of rice mainly includes *Oryza sativa* L. (Asia rice), *Oryza glaberrima* Steud (Africa rice). *Oryza sativa* L. has 3 types namely indica, japonica and tropical japonica (JICA, 2010). Cultivated rice is an annual crop but some of its wild relatives are perennial in nature. The rice plant, typically a grass, consists of root, stem (culm), tillers, leaves and panicles at the terminal part of productive tillers. The plant height varies by variety and environmental conditions, ranging from 0.4 m in dwarf varieties to 5 m in some deep-water floating rice (Dunna and Roy, 2013). Rice plants tiller, i.e., develop multiple shoots, depending on the variety, spacing, and soil fertility. The grass has long,

slender leaves 50–100 cm long and 2–2.5 cm broad. The small wind-pollinated flowers are produced on a branched arching to pendulous inflorescence 30–50 cm long. The inflorescence is an open panicle. Flowers are distinct in having six anthers as opposed to the commonly seen three anthers in other grasses. Spikelet's have a single floret, lemma, and palea enclosing a grain (caryopsis) 5–12 mm long and 2–3 mm thick that can be yellow, red, brown, or black. The lemmas may be awnless, partly or fully awned. The rice kernel has four primary components: the hull or the husk, the seed coat or bran, the embryo or germ, and the endosperm (Emani *et al*., 2008).

2.3. Seed Deterioration and Moisture Content

Seed moisture is a critical factor influencing seed quality, as seed shelf life is highly dependent upon its moisture contents (Bradford *et al*., 2018). Preserving seed at optimum seed moisture content both increases shelf life and reduces contamination by storage fungi (Bradford *et al*., 2018). Controlling seed moisture content is possible using hermetic containers and saturated salt solutions (Smith, 1992). Rate of seed respiration increased at high seed moisture contents and heat generated by the respiring seeds is enough to kill the seeds (Duarte *et al*., 2004). Storage of maize at high moisture contents (15%) resulted in low germination percentage, dry matter losses (up to 35%) along with fungal growth (Afzal *et al*., 2017). High seed moisture content is the major reason that can speed up the process of seed deterioration (Bradford *et al*., 2018). High germination in super bag at 8% initial seed moisture content (SMC) was the outcome of dry chain that was continuously maintained by the hermetic nature of super bag (Bradford *et al*., 2018). Reduction of seed moisture by initial drying and packaging in moisture proof containers can reduce the deterioration of commodities by fungi, storage losses due to insects and thus increase the shelf life seeds (Bradford *et al*., 2018). Fluctuations in relative humidity of the storage environment affect seed moisture contents (Mbofung *et al*., 2013). Seed absorbs moisture under conditions of high ambient relative humidity and quickly loses its germination due to the deteriorative process occurring at faster rate (Mbofung *et al*., 2013).

Maintenance of high seed germination and vigor from harvest until planting is critical for improvement of quality in seed production (Majumder *et al*., 2016). Even though seed storage is vital for preserving seed for next generation, several studies reported that crop production showed losses during longer storages (Majumder *et al*., 2016). As a result, simple hydration, with or without chemicals, midway during storage followed by

immediate drying back, would considerably extend seed viability (Pramila, 2008). Similarly, Khan *et al.* (2016) demonstrated that numerous cereal species have been primed successfully for improving seed quality and longevity. Seed invigoration can significantly contribute to the rapid stand establishment (Farooq *et al.,* 2006). Furthermore, other studies revealed that seed priming significantly improved seed vigor (Farooq *et al*., 2010) Similarly, characteristics produced during the seed priming are transferred to the next generation (Lal *et al*., 2018).

2.4. Seed Priming and Its Benefits

Seed priming is a method in which seeds are hydrated and dried to original moisture content but the actual emergence of the radicle is prevented (Goswami, 2019). Basically, seed priming is physiological advancement of seeds which involves physiological metabolism of seeds through soaking of seeds in water or solution of other conventional priming agents under controlled condition (Duta, 2018). Research results showed that rate of water uptake by seeds found higher in osmo-primed than untreated seeds of wheat and barely regardless of the water potential level (Al-Karaki, 1998). Seeds osmo-priming increased germination percentage and increased speed of germination at high water potentials, whereas the germination at low water potentials was not affected by osmo-priming treatments (Al-Karaki, 1998). In addition, seed priming could enhance the performance of dry direct-seeded rice, and promote early vigor and better establishment (Mahajan *et al*., 2011).

Unequal plant stand and poor germination is a major constraint in seeds under areas which receive erratic and low rainfall (Goswami, 2019). This problem of poor germination can be overcome by sowing primed seeds (Goswami, 2019). Seed priming improves the germination ability of seed and ultimately improves plant stand in the field (Goswami, 2019). Thereby, several cereals as well have been primed effectively for better seed quality and durability (Khan *et al*., 2016). Fast and homogenous crop emergence is vital for improving crop quality (Khan *et al*., 2016). Final quality of direct seeded crops is mainly affected by homogeny and percentage of seedling emergence (Khan *et al*., 2016). Furthermore, other studies indicated that seed priming is taken as a pre-sowing partial hydration of seeds and enables the seeds to realize a germinating state without actual radicle protrusion (Rehman *et al*., 2011). Correspondingly, improved germination rates, germination percentage, and synchrony enable the primed seeds to perform better with

healthy stand establishment (Rehman *et al*., 2011). Primed seeds usually exhibit increased germination rate, greater germination uniformity, and sometimes greater total germination percentage (Farooq *et al*., 2006). Similarly, Harris *et al.* (1999) concluded that primed seed emerges faster and more completely, producing more vigorous seedlings than non-primed crops. Similarly, Tilahun Taddesse *et al*. (2013) showed that sowing hydro-primed seeds soaked and dried for 24 hrs. was found efficient for rice production in Fogera plains in northwestern Ethiopia.

2.5. Priming Techniques and Rice Seed Longevity

Although the negative logarithmic relation between longevity and moisture content did not differ significantly, *glaberrima* rice showed marginally greater longevity than *japonica* rice (Bam *et al*., 2009). Nevertheless, viability losses of primed seeds during storage are a major limiting factor to wide adoption of seed priming technique (Wang *et al*., 2018). Seeds are usually re-dried near to their original weight to permit routine handling (Farooq *et al.*, 2010). However, adjusting of the priming protocols by accurate timing to stop the treatment followed by rapid drying is of major importance to overcome the problem of seed storability due to prolonged treatment (Duta, 2018). Priming of seeds is generally intended to reduce time to germination, often leading to improved emergence. However, as a negative side effect, priming reduces longevity of seeds. For several species tested we found that the desired longevity could be obtained by keeping the seeds, after a priming treatment, under a mild water and / or temperature stress for a period of several hours to days (Bruggink *et al*., 1999). Post-priming benefits can vary with seed priming type, re-drying, seed lot vigour and storage conditions, including temperature, relative humidity and oxygen. Priming strategies involve rapid re-drying after hydration; therefore, dehydration may be beneficial for extending the longevity and retaining the benefits of primed seeds (Butler *et al*., 2009). Prolonged storage can reduce germination and seedling growth of primed seeds, particularly when stored at 25^{o}C due to restricted starch metabolism (Hussain *et al*., 2015). However, storability of the primed seed per se is either improved or adversely affected, depending upon the initial physiological status of the seed (Sivasubramaniam, 2011). For instance, rice seed storage after priming and re-drying was evaluated within 0, 15, 30, 45 and 60 days after priming (DAP) to ascertain how long a primed rice seed can maintain improvements induced by priming when stored at 25^{o}C (near ambient temperature) without losing its viability (Hussain *et al*., 2015). As

compared with non-primed seeds, priming had a positive effect up till 15 days after storage, beyond which germination attributes were lower than non-priming treatments (Hussain *et al*., 2015). However, evaluation for storage duration of 60 DAP showed significant reduction of germination percentage, germination index and vigor index throughout different priming treatments and cultivars (Hussian *et al*., 2015). Storage of seeds under different conditions for 15–60 days did not influence the longevity of non-primed rice seeds (Wang *et al*., 2018). Storage of primed seeds under aerobic environment did not reduce seed longevity during 60 days of storage low RH and room temperature (Wang *et al*., 2018). Nevertheless, increase of RH significantly reduced the viability of primed seeds stored for 15–60 days (Wang *et al*., 2018).

Careful seed drying process should be conducted for safe storage and appropriate packaging material to maintain dryness and viability after re-drying (Bradford *et al*., 2018). Because most farmers cannot afford or have access to cold storage facilities, maintaining seed dryness after re-drying by lowering its moisture content throughout the supply chain is required (Bradford *et al*., 2018). The high viscosity of protoplasm with reduced molecular mobility in the cytoplasm, limits the deteriorative process at low moisture content of seed (Gurusinghe and Bradford 2001). Recently, Bakhtavar *et al*. (2019) observed high germination percentage and starch and crude protein content after 4 months' storage with an initial seed moisture content of 8% and 10% in Super bags. Rice seed stored in super bag retained high germination performance.

2.6. Influence of Halo-priming on Orthodox Seed Physiological Attributes

In rice, it was reported that seed priming treatments reduced the time taken to initiate the germination process, improves the rate of germination and synchronization, moreover enhances the lengths of shoots and roots and thus increases the fresh and dry mass of the seedlings (Farooq *et al*., 2006). Halo-priming was found to be effective in bringing about a prominent increase in shoot length of seedlings (Rehman *et al*., 2011). Halo-priming was found to be more efficient than hydro-priming in enhancing the seedling vigor, uniform growth (Jisha *et al*., 2014). Osmo-priming with re-drying effectively improved quality attributes in direct seeded rice as compared to respective non-primed seed (Rehman *et al*., 2011). Halo-primed rice seed with $CaCl_2$ enhances seed vigour with improved

germination, seedling emergence. Rapid and uniform germination occurs due to elevated α-amylase activity and enhanced starch hydrolysis there by more sugar are available for embryo and seedling growth that leads to improvement in seed quality (Farooq *et al*., 2006). Kurdikeri *et al*. (1995) reported improved field emergence in maize seeds planted after soaking in water and with the 0.5% $CaCl_2$ than dry seeds, which resulted in erratic crop emergence. These findings illustrated that seed priming enhanced seed field performance in direct seeded rice with improved seedling emergence and vigor (Kurdikeri *et al*., 1995). Reduction in the lag time of imbibition may be accounted for in part by some germination rate enhancement in the osmo-priming treatments (Al-Karaki, 1998). Seedling fresh weight, shoot length and root length were enhanced in osmo-primed seeds in comparison to untreated seeds (Al-Karaki, 1998). Seed halo-priming could improve germination, emergence and growth of wheat (Pirasteh-Anosheh *et al*., 2013). Seed quality tests including mean germination time, shoot length, shoot dry mass, root length and root dry mass showed improvement during rice seed osmo-priming (Hasan *et al*., 2016) and Yari, *et al*. (2012) found seed priming duration of 24 hrs. with $CaCl_2$ suitable for all rice cultivars. Farooq *et al*. (2009) demonstrated that although surface drying with $CaCl_2$ was effective, re-drying was more efficient and preferred technique in achieving better germination and seedling growth attributes, as well as in saving labor, cost, energy and for prolonged storage before sowing or selling.

2.7. Influence of Hydro-priming on Orthodox Seed Physiological Quality Attributes

The upland system for rice production is considered as one of the most sustainable alternatives, in which impact of seed priming might have great relevance (Mondo *et al*., 2016). A promising strategy that may permit direct field sowing is the use of primed seeds, providing enhancement of the physiological performance of seeds and helping to resolve the challenge of poor stand establishment (Farooq *et al*., 2011). Therefore, hydro-priming can reduce time between seed sowing and seedling emergence, resulting in rapid and uniform seedlings emergence, high seed vigor, and better and uniform stand establishment (Mondo *et al*., 2016). Further, Aynadis Lijalem (2011) also reported that NERICA-4 rice seed quality parameters like root length, total and standard germination (%) were significantly different from the dry seed when seeds were soaked for 24 hours (hrs.) and incubated for 24 hrs. and highly significant differences in seedling shoot length, emergence index and vigor Index-I.

2.8. Storage Atmosphere and Packaging Materials on Rice Seed Physiological Quality

Many enzymatic changes, oxidation and respiration occur in rice during storage (Naik and Chetti, 2018). If the viability and vigour is not maintained properly during storage period, it will be difficult to sell it as a seed material for the next season. As seed is hygroscopic in nature, seed quality is affected by variation in moisture content, relative humidity, and temperature (Naik and Chetti, 2018). A reliable technique to combat these factors is the use of sealed packaging materials or moisture vapor proof containers like polythene bag, aluminum foil, or any sealed container to maintain the quality for longer period (Naik and Chetti, 2018). It was found that polythene containers experience lesser moisture fluctuation, reduced depletion of food reserves, hence less decline in all the seedling vigour parameters (Naik and Chetti, 2018). Post-harvest storage life of rice largely depends on the genotypes/varieties, treatment, packaging material and storage conditions (Naik and Chetti, 2018). Seed quality is judged by seedling vigour parameters like root and shoots length and seedling dry weight (Naik and Chetti, 2018). Generally, higher the seedling length, vigour index and seedling dry weight; higher is the seed quality (Naik and Chetti, 2018).

During storage, seed quality greatly deteriorates, and this may be associated with factors such as temperature, moisture content of seeds, relative humidity, insect infestation, disease development and other physio-chemical changes like weight loss or gain and nutrient depletion (Neelesh *et al*., 2011). Most of these factors are influenced by packaging type and storage duration (Tang and Ngome, 2015). Low density polyethylene bags showed significant decrease in germination percentage of upland rice seed (Tang and Ngome, 2015). However, significant increase in thousand seed weight was obtained (Tang and Ngome, 2015). Low density polypropylene bags also showed significant increase in moisture content of rice seeds and the percentage of colored grains, all at one-month storage (Tang and Ngome, 2015). On the other side, other studies demonstrated that PICS bags prevented moisture penetration over the three-month storage period during maize seed preservation. Contrarily, seed borne pathogens were associated along with the seed and insect attack was severe in easily permeable containers (Chowdhury *et al*., 2014). The triple bag consists of two inner high-density polyethylene bags acting as oxygen barriers, which in turn are encased in an outer woven polypropylene bag that serves primarily for

mechanical strength (Baoua, 2012). Azad *et al.* (2014) found that higher vigour when wheat seeds dried to 12 percent moisture content and preserved in polythene bags stored for nine months.

2.9. Effects of Seed Hydration and Dehydration on Seed Longevity and Deterioration Repair Mechanisms

All seeds stored under air dry conditions will have suffered a degree of deterioration. This may have been incurred, for example, during delays between collection and processing if seeds are held under inappropriate conditions, during processing or whilst in storage. Indeed, even in the natural environment, macromolecules within the seed tissues will incur some damage through normal metabolism. If the damage accumulated is not too severe, repair will be possible (Butler, 2009). For this reason, dehydration after priming by removing a large quantity of moisture from the seed is a key to successful long-term seed storage, so the seeds should be dehydrated to their original moisture level (Gurusinghe and Bradford 2001). A rapid dehydration after seed priming leads to loss of the advancement obtained by priming (Gurusinghe and Bradford 2001). It may alter the soluble carbohydrate content, which in turn reduces desiccation tolerance and longevity of storage (Gurusinghe and Bradford 2001). On the contrary, a slow drying-back may increase the accumulation of late embryogenesis abundant proteins that offer a beneficial mechanism to improve seed longevity (Gurusinghe *et al*., 2002). However, fast re-drying at high temperatures may prompt the synthesis of heat shock proteins that are valuable to seed longevity (Gurusinghe *et al*., 2002). Gurusinghe and Bradford (2001) noted an improvement in longevity for seeds given a slow-drying, high temperature treatment after priming. Drying seeds slowly after priming may induce the synthesis of LEA (late embryogenesis abundant) proteins, while incubating seeds at high temperatures may induce heat shock proteins, both of which may provide protective mechanisms that are beneficial to seed longevity (Gurusinghe *et al*., 2002). On the other side, hermetic methods reduce temperature variation which stabilizes grain respiration in turn prevents enhanced degradation of the reserves necessary for germination (Harrington, 1973). Karta Kaske *et al*. (2019) demonstrated that hermetic seed packaging techniques like PICS bag and grain pro-super bag could be able to maintain germination and other physiological parameters of wheat seed for 6 months as compared to polypropylene bag, Furthermore, Essien *et al*. (2010) explained long-term storage of rice could be possible through hermetic storage

techniques which could preserve germination potential and vigor in with generation of oxygen depleted, carbon dioxide-enriched atmosphere caused by the respiration of the living organisms in the ecological system of a sealed storage even though Wang *et al*. (2018) showed that oxygen did not influence the longevity of primed rice seeds stored under low RH. Therefore, low RH or low temperature conditions could be able to extend rice seed viability within 60 days of storage. Farooq *et al*. (2010) demonstrated that re-drying could achieve better germination and growth attributes where it is desirable for prolonged storage of seeds. Similarly, Ibrahim *et al*. (2013) outlined that hydrated upland rice seeds can be re-dried for four hours without loss of physiological advancement obtained from hydration phase even if the effect of extended re-drying was recommended for further research. Higher seedling length is an indication of maintenance of vigour in the seeds preserved in cold storage. Type of package can preserve or alter physiological potential of a seed, however, there are unverified claims that use of hermetic bags can prolong storability of primed rice seeds.

2.10. Basic Seed Physiological Characters Induced by Priming

Seed quality is considered as a major uptake barrier of improved rice production (Alam *et al*., 2009). Deterioration of dry seeds in storage results from complex interactions among many physical and biochemical factors, and multiple mechanisms to resist these changes are also likely to be presented (Walters, 1998).Therefore, improved seed priming techniques can reduce time between seed sowing and seedling emergence, resulting in rapid and uniform seedlings emergence, high seed vigor, better and uniform stand establishment, in many field crops (Mondo *et al*., 2016). Some major aspects during assessing the seed quality are the percent of germination, which translates the potential for a seed lot to produce normal seedlings under controlled temperature, and percent of seedlings emergence, considered as a vigor test, that approximate the results to field conditions (Mondo *et al*., 2016).

2.10.1. Effect of seed priming on germination and vigor

Seed priming is a simple, low-cost, low-risk intervention and powerful technique to improve seedling emergence and vigor of several crops (Somasundaram and Bhaskaran,

2017). In general, high longevity rice seeds responded well to the priming treatments compared to low longevity seeds (Somasundaram and Bhaskaran, 2017). In rice, it was reported that seed priming treatments reduced the time taken to initiate the germination process, improves the rate of germination and synchronization, moreover, enhances the lengths of shoots and roots and thus increases the fresh and dry mass of the seedlings (Farooq *et al*., 2006). Halo-priming was found to be effective in bringing about a prominent increase in shoot length of seedlings particularly in the tolerant varieties subjected to the stressed conditions, for which the variety is known to be tolerant. Halo-priming was found to be more efficient than hydro-priming in enhancing the seedling vigor, uniform growth, and stress tolerance potential of rice varieties (Jisha *et al*., 2014). Among the osmopriming agents Calcium chloride ($CaCl_2$) showed the best activity in regard to seed germination (%), germination energy (%), germination speed (%) and germination index (Islam *et al*., 2012). Germination showed no statistical difference under hermetic conditions, while under woven storage techniques, germination decreased meaningfully after three and six months, suggesting a corresponding decrease of seed vigor associated with woven bags rice storage (Guenha *et al*., 2014). Root length is one of the important parameters to justify the vigorous nature of seed (Rani, 2013). Similarly, Seed vigor comprises those properties that determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions (Association of Official Seed Analysts, 1983).

Vigor index-I was calculated by the formula developed by (Abdul-Baki and Anderson (1973) as standard germination multiplied by seedling length. Vigor index II**:** Vigor index II was calculated by multiplying the normal germination percentage with seedling dry weight. At the end of the standard germination test, the same 10 normal seedlings used for measuring root and shoot length were put in iron container and dried in hot air oven maintained at 80 ± 1°C for 24 hours. After drying, seedlings were weighed and expressed in milligrams (Kata *et al*., 2014). The weight of thousand whole rice seeds counted from each bag was weighed with the sensitive balance and recorded (Tang and Ngome, 2015). Furthermore, rice seed moisture content was measured using a rice moisture meter. For each of the two bags per bag type, moisture content of seeds randomly collected was measured three times and only the average value recorded (Tang and Ngome, 2015).

CHAPTER 3. MATERIALS AND METHODS

3.1 Description of the Study Area

The experiment was conducted at Fogera National Rice Research and Training Center from 2019 to 2020. Fogera is located at 11°58'N and 37°41'E and at an altitude of 1811 m above sea level. It attains unimodal rainfall pattern from June to mid-September, with a mean annual precipitation of 1200 mm and mean annual minimum and maximum temperatures of 13°C and 25°C, respectively (Tilahun Tadesse *et al*., 2013).

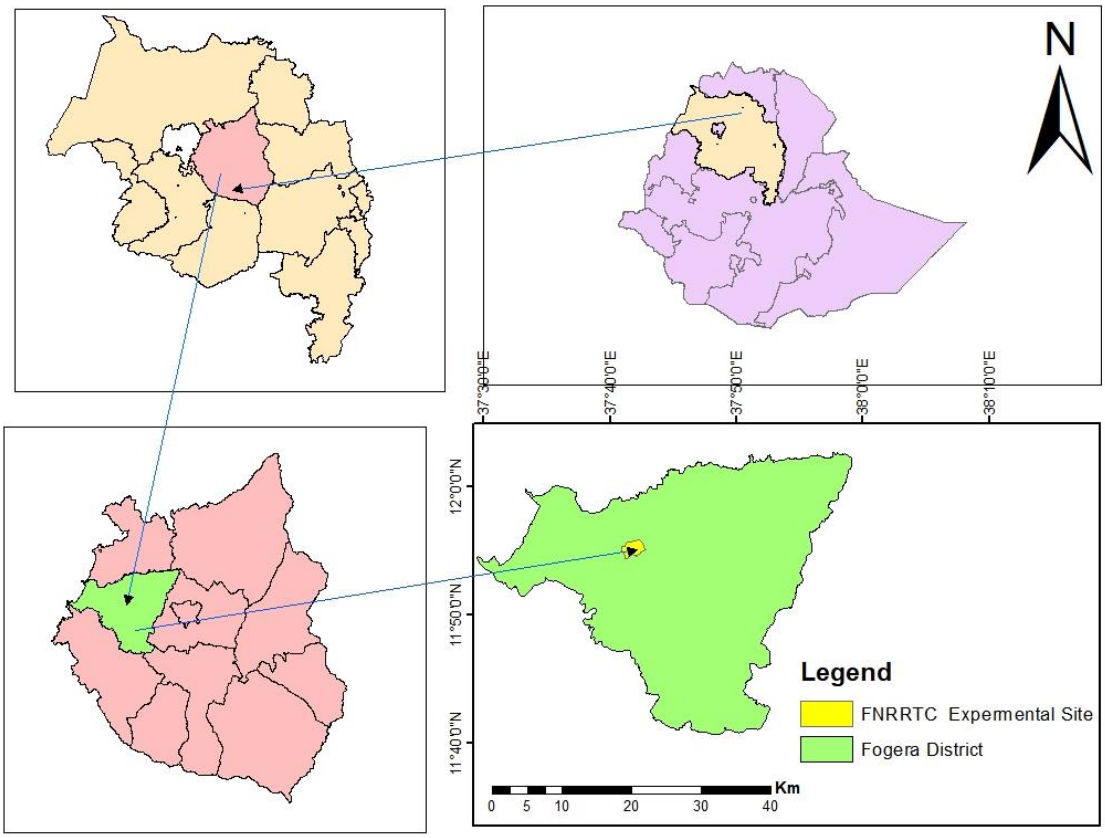

Figure. 1. Study Area

3.2 Rice Variety and Seed Source

A newly harvested and two weeks-stored 14.4 kg of basic seed of a popular upland rice variety NERICA-4 was obtained from Fogera National Research and Training Center. Seed was manually cleaned and made free from extraneous materials and empty hulls and

randomly partitioned to a working sample of 50 grams using seed divider, in accordance with (ISTA, 2014). The initial seed moisture content of the seed was 12% on dry weight basis.

3.3 Priming solutions, Treatments and Experimental Design

Two priming treatments were prepared from $CaCl_{2,}$ and distilled water. Then optimization by studying the different salt: water ratio was performed to select the best condition. The ratio of seed weight to solution volume was 1:5 (Farooq *et al*., 2006). The dry seed was used as control. The treatments were dry seed (control), hydro-primed seed (0% $CaCl_2$), and halo-primed seed at four (0.25%, 0.5%, 0.75%, and 1%) $CaCl_2$ optimized concentrations.

3.4 Laboratory Materials

Data loggers, sealer, oven, sensitive balance, distilled water and other minute lab materials were used from Bahir Dar Institute of Technology, Self-bought, Bahir Dar Soil Laboratory and Bahir Dar Seed and Other Inputs Quality Control and Quarantine Laboratory.

3.5 Experimental Procedures

3.5.1 Priming treatments for storage experiment

Distilled water was prepared by Bahir Dar Soil Laboratory, chemistry department and poured to sixty independent beakers of 100ml capacity with different concentrations of distilled water and calcium chloride scales. These are 100ml (hydro-primed), 99.75ml DiW (distilled water) (0.25% $CaCl_2$), 99.5ml DiW (0.5% $CaCl_2$), 99.5ml DiW (0.5% $CaCl_2$), 99.25ml DiW (0.75% $CaCl_2$) and 99ml DiW (1% $CaCl_2$) rates. Treatments were arranged in four replications. Uniform-sized rice seeds were selected and immersed into distilled water containing different concentrations of calcium chloride ($CaCl_2$) in a proportion of (0, 0.25%, 0.5%, 0.75%, 1%) and dry seed as control. Then the seeds were primed for 24 h with respective priming solution treatments in a germination box at 25^oC (room temperature). After 24 hrs. of priming treatment, the seeds were washed with distilled water for 2 min (Jisha *et al*., 2014) and surface dried on paper towel for 24 hours at a room temperature slow drying at 25 ± 3^oC. Surface dried seed from 70 bags were

exposed electric light source by spreading the seeds on A4 sized envelopes on a doubled table to re-dry to their original moisture methodology adopted from Farooq *et al*. (2009), with modification on re-drying technique. Then, 50 gram of working sample of basic seed of rice (var. NERICA-4) were sealed in each of 72 low density polyethylene (LDPE) bags and high-density polypropylene bags (HDPE) double layered bags and stored under ambient laboratory conditions of Amhara Region Seed and Other Inputs Quarantine and Quality Control Authority Laboratory, Bahir Dar. These seed samples were preserved for six respective storage periods at room temperature. Seed in 14 of the bags were maintained untreated (dry control). Seed in the remaining sixty 70 bags were primed in distilled water (0% $CaCl_2$), or $CaCl_2$ at different concentrations. Temperature and relative humidity in the storage room was monitored using data loggers (Table 1).

Table 1. Average temperature and relative humidity

Days of storage	Temperature	Relative humidity
0	21.9	69.6
15	22.3	59.16
30	21.92	58.4
45	21.65	58.65
60	21.8	59.56
75	22.3	59.16

The storage periods were 0 month (baseline), 15 days, 30 days, 45 days, 60 days, and 75 days after priming as per (Hussain *et al*., 2015). Through completion of each storage periods, one hundred seeds were sown on a germination box distributed equally with four replications for standard germination testing and other physiological seed quality tests were assessed. Average protein content of rice seed in HDPE bags were found 7% and for those in LDPE bags were found to be 6.8%. The storage experiment was laid out in a randomized complete block design (RCBD) nested within each of the storage periods. RCBD design was used to control error caused by moisture spray for germination testing perpendicular to the arrangement of treatments. There was a total of twelve (12) treatment combinations consisting of 5 priming concentrations of $CaCl_2$, and untreated (dry) seed sealed in each of two packaging materials (LDPE and HDPE bags) throughout six storage periods.

3.6 Data Collection

3.6.1 Germination percentage

After the primed seeds were dried back to the original seed moisture content (12%), samples were subjected to germination tests (top of paper method) using 100 seeds per replication (with four replications). Seeds were germinated on moist medium filter paper in a germination box placed in room temperature (25±3°C) in open condition and sprayed with distilled water every two days. The first count was done after 5 days and final count after 14 days. The special treatment applied during the test was soaking of seeds in water for 24 hours and incubating for 24 hours then re-drying to the original moisture content at room temperature supported with electric lamp before germination. Normal and abnormal seedlings and dead seeds were determined following the rice seed testing rules prescribed in ISTA (2014). Germination was computed using the formula adopted by ISTA (2014). Germination (%) = Sn/Gn , where, *Sn* is the sown number of seeds, and *Gn* is the total number of normal germinated seeds.

3.6.2 Speed of germination

Daily germination counts were performed until no further germination was observed for seven days. An index of the speed of germination was then calculated by adding the quotients of the daily counts divided by the number of days of germination (Maguire, 1962).

Speed of germination = $\sum$ (n/t); where, n = number of seeds newly germinating at time 't', t = days from sowing

3.6.3 Seedling vigour index

Seedling vigour index was calculated as per the formula of Abdul-Baki and Anderson (1973).

3.6.4 Seedling vigour index – I (Shoot and root length basis)

At 14 days after sowing (DAS), 10 normal seedlings were randomly sampled from germination test of each treatment and each replication. The root length and shoot length were separately measured using a ruler.

Vigor index II = seed germination (%) × seedling length (cm); where in Seedling length = shoot length (cm) + root length (cm).

3.6.5 Root length

Ten normal seedlings were taken randomly from each replication for measuring the root length. The length between the collar region and the tip of the primary root was measured and mean value was recorded in cm (Maguire, 1962).

3.6.6 Shoot length

The same ten normal seedlings used for root length measurement were also used for shoot length measurement. The length between the collar region and the tip of the shoot was measured and the mean value was recorded in cm (Maguire, 1962).

3.6.7 Seedling vigour index – II

The seedling vigour index –II was computed using the formula Vigor index II = Seed germination (%) × seedling dry weight (mg); seedling dry weight measurement is described under topic 3.6.9 (Maguire, 1962).

3.6.8 Fresh weight of seedlings

Ten seedlings selected for measuring root and shoot length were weighed and weight was recorded in milligrams (mg) (Maguire, 1962).

3.6.9 Seedling dry weight

After the germination test i.e., on 14^{th} day, 10 seedlings from each replication were selected at random, placed in an iron container and seedling fresh weight weighed then dried for 24 hours in a hot air oven maintained at 80°C. The dried seedlings were removed from the oven and cooled at room temperature for 30 minutes and then the dry weight of

seedling was recorded using an electronic balance. The average weight of seedling was computed and expressed in milligram per ten seedlings (Maguire, 1962).

3.6.10 Thousand seed weight

Thousand seed weight was measured at the end of each of the storage period. A thousand seed was machine counted and the weight was measured using 0.01g precision balance.

3.6.11 Moisture content

Initial moisture content for each treatment was checked until it reaches 12 percent using moisture tester machine (Quick moisture tester, Poland) and moisture content was evaluated in subsequent storage periods.

3.7 Data Aanalysis

Data from storage experiments were subjected to statistical analysis using SAS 9.4. The effects of priming treatments and storage methods on seed quality parameters were assessed using a three-way analysis of variance throughout the storage period. Percentage data were arcsine transformed and arithmetic means were used for presentation in the text. Mean comparison was carried out using Tukey HSD (Honestly Significance Difference) method at 5% level of significance. Tukey HSD was used because it is good for post-hoc multiple comparison and ease of calculation. Mean (±SEM) of germination percentage and speed of germination were plotted using Sigma Plot Version 12.5.

CHAPTER 4. RESULTS AND DISCUSSIONS

4.1 Seed Germination Percentage

Total germination was significantly affected by duration of storage ($F_=$ 66.94, F_{df}=5, F_{dfe} =213, P<0.01), $CaCl_2$ (F=5.65, F_{df}=5, P<0.01), their interaction (F=1.60, F_{df}=25, P<0.05), and the three-way interaction (F=1.77, F_{df}=25, P<0.01), but not between bag types, interaction between bags and duration, and interaction between bags and $CaCl_2$. Significant effect of duration of storage (F=61.22, F_{df}=5, P<0.01), $CaCl_2$ (F=11.00, F_{df}=5, P<0.01), their interaction (F=2.59, F_{df}=25, P<0.01), and the three-way interaction (interaction between calcium chloride levels, bag type and storage period). (F=1.68, F_{df}=25, P<0.05) was detected on standard germination percentage. Furthermore, significant effect of duration of storage (F=51.60, F_{df}=5, P<0.01), bag (F=6.23, F_{df}=1, P<0.05), their interaction (F=2.71, F_{df}=5, P<0.05), $CaCl_2$ (F=4.17, F_{df}=5, P<0.05), and the three-way interaction (F=1.70, F_{df}=25, P<0.05) was detected on standard percentage of dead seeds. There was no significant effect of bag types, interaction between bags and duration, and interaction between bags and $CaCl_2$.

Mean comparison using Tukey HSD method for germination percentage of rice seed at the outset of storage and subsequent intervals of storage in HDPE and PE bags revealed that total germination percentage and standard germination percentage showed non-significant reduction up until 75 days duration of storage, in all of the priming conditions and dry seed (control). Therefore, standard germination percentage of primed NERICA-4 seeds in varying conditions of temperature (21.9-22.57) and relative humidity (59.6-58.54) throughout every storage period could be maintained for 75 days at 5% level of significance. This result was associated with lower percentage of abnormal seedlings and dead seeds in subsequent storage periods. NERICA-4 rice variety showed poor performance with distilled water priming of 48 hours whereas germination percentage was increased by osmo-priming and re-drying (Mamun *et al*., 2018).

HDPE bags experienced relatively better germination performance. Similar study by Bakhtavar *et al*. (2019) showed that rice seed stored in super bag retained high germination percentage. This could be due to decreased oxygen levels and increased carbon dioxide levels in HDPE bags (Baoua *et al*., 2012), which in turn reduces respiration

rate. The energy that is saved from loss for respiration might help to enhance germination. This result also agreed with Chowdhury *et al*. (2014) who reported studied impermeable storage containers had higher germination percentage. However, this result is in contrast with Hussain *et al*. (2015), who demonstrated seed germination for primed rice was maintained only for 15 days.

One percent (1%) treated NERICA-4 rice seeds showed significantly greater total germination and standard germination compared with dry seed (control) and hydro-primed rice seed, in both packaging materials in every storage period. This is probably due to the positive contribution of halo-priming over dry seed and hydro-priming. Maximum mean value of standard germination was obtained in 1% $CaCl_2$ primed seeds in HDPE bags. This result corresponds with Shivanisingh *et al*. (2017) who demonstrated that green gram priming with 1.00 % calcium chloride with distilled water solution resulted significant improvement in germination. Besides, Yari *et al*. (2012) showed that maximum seed germination percentage was found when rice seeds primed by one percent (1%) $CaCl_2$. Further, all priming conditions were found slightly greater in standard germination percentage throughout every storage periods. This result goes in line with previous output that explains seed priming to bring about some biochemical alterations in the metabolism within seeds, which ultimately favors germination (Jisha and Puthur, 2014).

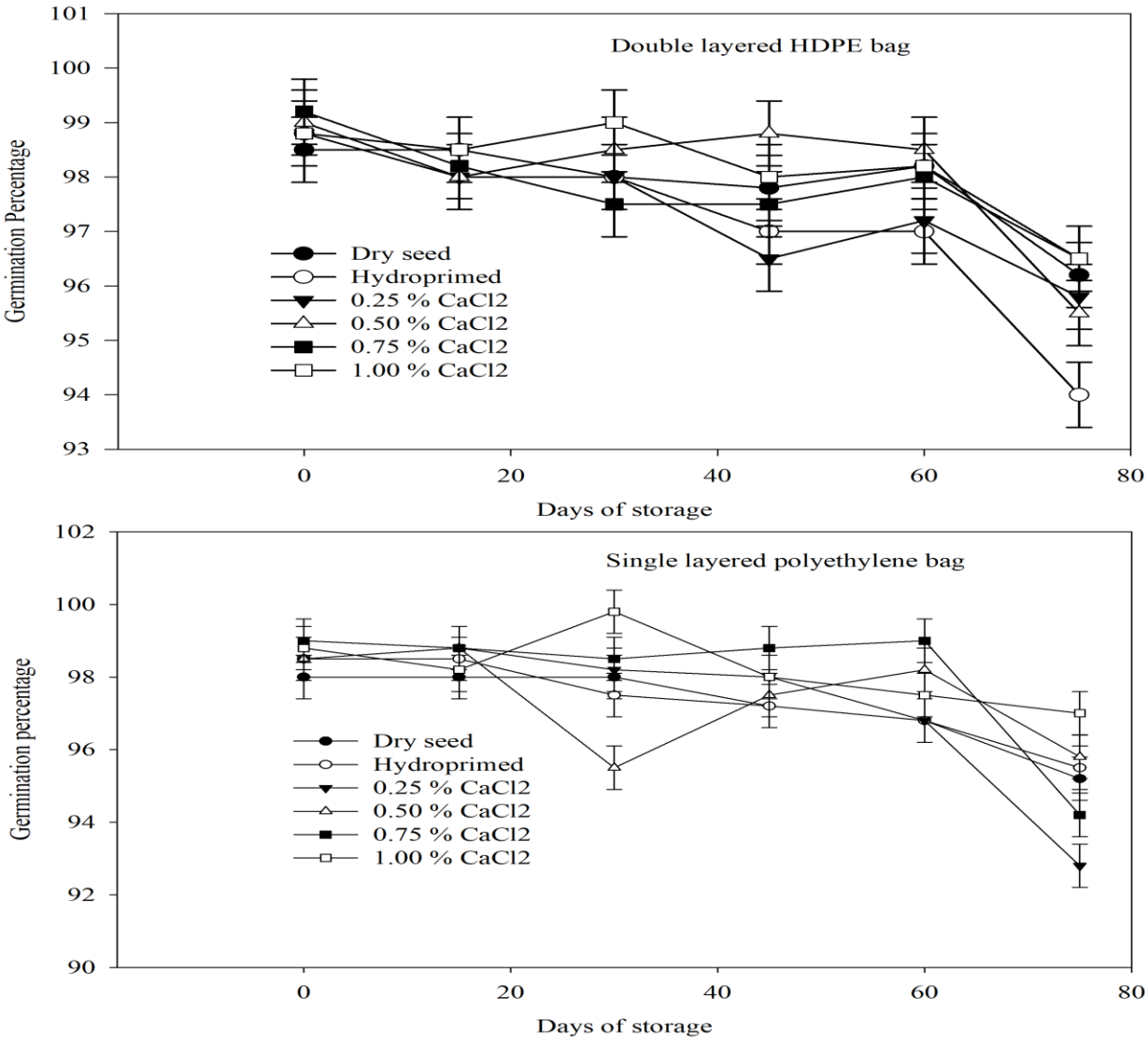

Figure.2. Standard germination percentage of primed and re-dried seeds stored in high density polyethylene bags (upper) and low-density polyethylene bags (bottom) after 0 to 75 days of storage

NERICA-4 rice seed that received 1.00% $CaCl_2$ priming treatment exhibited better total germination percentage, standard germination percentage, vigor index-I, and lower percentage of dead seeds compared to dry seed (negative control) and hydro-primed (@0.00% $CaCl_2$ concentration). Therefore, 1.00% $CaCl_2$ concentration has the optimum for in-vitro improvement of physiological potential of NERICA-4 rice seed under 59% to 60% relative humidity and temperature of 22^{o}C-23^{o}C.

Table 2. Effect of storage period, storage bags and priming on dead seeds percentage (%) *

Bag type	Storage time (days)	Priming (CaCl2) (Mean ± SEM)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	1.50±0.28^{e-g}	1.25±0.40^{fg}	1.25±0.25^{fg}	1.50±0.28^{e-g}	1.25±0.25^{fg}	1.00±0.00^{fg}
	15	1.75±0.25^{d-g}	1.50±0.28^{e-g}	1.25±0.25^{fg}	1.50±0.28^{e-g}	1.75±0.25^{d-g}	1.25±0.25^{fg}
	30	2.00±0.81^{c-g}	2.50±0.64^{b-g}	1.75±0.47^{d-g}	1.50±0.28^{e-g}	0.25±0.25^{g}	1.25±0.25^{fg}
	45	2.75±0.47^{b-g}	2.75±0.47^{b-g}	2.00±0.70^{c-g}	2.50±0.28^{b-g}	2.00±0.40^{c-g}	1.25±0.25^{fg}
	60	2.75±0.25^{b-g}	2.75±0.25^{b-g}	3.25±0.47^{a-f}	1.25±0.47^{fg}	2.25±0.25^{b-g}	1.00±0.00^{fg}
	75	4.25±0.47^{a-d}	4.50±0.50^{a-c}	3.25±0.47^{a-f}	4.25±0.47^{a-d}	3.00±0.70^{b-f}	1.00±0.00^{fg}
HDPE	0	1.50±0.28^{e-g}	1.25±0.25^{fg}	0.75±0.25^{fg}	1±0.40^{fg}	1.50±0.50^{e-g}	1.25±0.47^{fg}
	15	1.50±0.25^{e-g}	1.50±0.28^{e-g}	1.75±0.25^{d-g}	1.75±0.25^{d-g}	1.75±0.25^{d-g}	1.50±0.28^{e-g}
	30	1.25±0.75^{fg}	2.00±0.40^{c-g}	2.00±0.40^{c-g}	1.50±0.64^{e-g}	0.25±0.25^{g}	1.00±0.00^{fg}
	45	2.25±0.25^{b-g}	2.00±0.70^{c-g}	2.75±0.25^{b-g}	1.25±0.75^{fg}	2.50±0.28^{b-g}	1.25±0.47^{fg}
	60	1.75±0.47^{d-g}	2.50±0.28^{b-g}	2.75±0.25^{b-g}	1.50±0.50^{e-g}	2.00±0.00^{c-g}	1.75±0.25^{d-g}
	75	3.25±0.75^{a-f}	4.00±1.08^{a-e}	2.25±0.75^{b-g}	1.50±0.50^{e-g}	3.00±0.00^{b-f}	1.75±0.62^{d-g}

*Means followed by the same letters are not significantly different at 5% level of significance

4.2 Seedling Fresh Weight

The overall effect of independent variables was significant on seedling fresh weight ($F=5.14$, $P<0.01$). Duration of storage ($F=50.48$, $F_{df}=5$, $P<0.01$) concentrations of $CaCl_2$ ($F=4.55$, $F_{df}=5$, $P<0.01$), their interaction ($F=1.71$, $F_{df}=25$, $P<0.05$) and the three-way interactions ($F=1.63$, $F_{df}=25$, $P<0.05$) posed significant effect on seedling fresh weight. However, the effects of bag types, interaction between bags and duration, interaction between bags and $CaCl_2$ were not statistically significant.

Mean comparison using Tukey HSD method between means of seedling fresh weight showed non-significant variation with gradual decrease in seedling fresh weight through the advance in storage time from pre-storage treatments to 75 days of storage in every priming conditions and dry seed (control) (Table 3). In line with this result, Vimala *et al*. (2002) showed that seedling fresh weight, decreased with the increase in storage period. HDPE bags showed increased fresh weight mean values compared with LDPE bags in all priming conditions and dry seed (control). NERICA-4 rice seeds primed with 1.00% $CaCl_2$ showed maximum seedling fresh weight mean values, in both packaging materials. Minimum mean value of seedling fresh weight was obtained in every storage period from dry seed (control). This finding goes in agreement with Rehman *et al*. (2015) found the highest seedling fresh weight using $CaCl_2$ osmo-priming treatments. Furthermore, Rani, (2013) showed that fresh weight of seedlings has positive effect on seed quality, as it represents the vigour of seed. A healthy and vigorous seed record high value for fresh weight (mg) which is an indication of good quality seed.

Table 3. Mean (±SEM) of effect of storage period, storage bags and priming on seedling fresh weight (mg)

Bag type	Storage time (days)	Priming (CaCl2)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	$0.60\pm0.02^{a-n}$	$0.65\pm0.01^{a-l}$	$0.69\pm0.02^{a-h}$	$0.63\pm0.02^{a-k}$	$0.74\pm0.01^{a-d}$	0.74 ± 0.02^{ab}
	15	$0.60\pm0.03^{a-n}$	$0.56\pm0.02^{a-n}$	$0.58\pm0.03^{a-n}$	$0.49\pm0.06^{b-n}$	$0.64\pm0.04^{a-m}$	$0.52\pm0.03^{a-n}$
	30	$0.54\pm0.03^{a-n}$	$0.49\pm0.03^{b-n}$	$0.55\pm0.03^{a-n}$	$0.55\pm0.02^{a-n}$	$0.64\pm0.04^{a-m}$	$0.52\pm0.06^{a-n}$
	45	$0.51\pm0.02^{b-n}$	$0.49\pm0.03^{b-n}$	$0.58\pm0.03^{a-n}$	$0.49\pm0.02^{b-n}$	$0.63\pm0.03^{a-n}$	$0.48\pm0.07^{a-n}$
	60	$0.51\pm0.02^{b-n}$	$0.46\pm0.02^{f-n}$	$0.52\pm0.02^{a-n}$	$0.46\pm0.02^{f-n}$	$0.56\pm0.03^{a-n}$	$0.46\pm0.02^{b-n}$
	75	$0.48\pm0.02^{e-n}$	$0.45\pm0.06^{f-n}$	$0.47\pm0.02^{a-n}$	$0.43\pm0.07^{i-n}$	$0.4\pm0.06^{l-n}$	$0.55\pm0.02^{a-n}$
HDPE	0	$0.7\pm0.04^{a-g}$	$0.57\pm0.04^{a-n}$	$0.67\pm0.14^{a-j}$	$0.68\pm0.018^{a-i}$	$0.74\pm0.04^{a-c}$	$0.71\pm0.04^{a-f}$
	15	$0.5\pm0.06^{b-n}$	$0.53\pm0.06^{f-n}$	$0.48\pm0.01^{e-n}$	$0.68\pm0.018^{a-i}$	$0.72\pm0.05^{a-c}$	$0.67\pm0.08^{a-k}$
	30	$0.49\pm0.02^{b-n}$	$0.57\pm0.02^{a-n}$	$0.47\pm0.03^{e-n}$	$0.44\pm0.04^{h-n}$	$0.51\pm0.03^{b-n}$	$0.52\pm0.05^{a-n}$
	45	$0.49\pm0.02^{a-n}$	$0.48\pm0.02^{b-n}$	$0.44\pm0.04^{h-n}$	$0.54\pm0.007^{a-n}$	$0.48\pm0.01^{e-n}$	$0.48\pm0.02^{e-n}$
	60	$0.55\pm0.03^{a-n}$	$0.44\pm0.01^{h-n}$	$0.44\pm0.03^{h-n}$	$0.44\pm0.02^{g-n}$	$0.52\pm0.01^{a-n}$	$0.43\pm0.02^{h-n}$
	75	$0.39\pm0.03^{m-n}$	0.38 ± 0.02^{n}	$0.43\pm0.03^{h-n}$	$0.41\pm0.01^{k-n}$	$0.42\pm0.06^{b-n}$	$0.52\pm0.05^{a-n}$

*Means followed by the same letters are not significantly different at 5% level of significance

4.3 Seedling Dry Weight

The overall ANOVA revealed that duration of storage ($F=61.22$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=11.00$, $F_{df}=5$, $P<0.01$), their interaction ($F=2.59$, $F_{df}=25$, $P<0.01$) and three-way interaction posed significant effect on ($F=1.68$, $F_{df}=25$, $P<0.05$) seedling dry weight. However, interaction between bag type and duration, and interaction between bag type and $CaCl_2$ was non-significant.

Seedling dry weight was maintained until 75 days of storage with gradual decrease in all priming conditions including dry seed (control) (Table 4). In line with this result, Vimala *et al*. (2002) reported that characters like seedling dry weight decreased with the increase in storage period. Seedling dry weight is positively correlated with quality of seed, because it is the key parameter which reflects on seedling vigor index-II. Results on seedling dry weight showed that with the advance of the storage period, the seedling dry weight showed gradual decrease (Rani, 2013). Maximum seedling dry weight was obtained from seed priming with $CaCl_2$ (Farooq *et al*., 2010).

Figure. 3. NERICA-4 seedlings oven dried and removed after 24 hrs (Kata *et al*., 2014).

Table 4. Mean (± SEM) of effect of storage period, storage bags and priming on seedling dry weight (g)*

Bag type	Storage time (days)	Priming ($CaCl_2$)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	0.04±0.01^{c-f}	0.04±0.01^{ef}	0.04±0.00^{d-f}	0.03±0.01^{d-f}	0.04±0.00^{c-f}	0.04±0.00^{a-f}
	15	0.04±0.00 $^{c-f}$	0.04±0.01^{c-f}	0.04±0.00^{b-f}	0.05±0.01^{a-f}	0.05±0.00^{a-e}	0.06±0.002^{a-c}
	30	0.04±0.00^{b-f}	0.04±0.00^{e-f}	0.05±0.00^{a-f}	0.05±0.00^{a-d}	0.05±0.00 $^{a-d}$	0.06±0.00^{ab}
	45	0.05±0.00^{a-f}	0.04±0.00^{c-f}	0.04±0.00^{b-f}	0.05±0.00^{a-f}	0.05±0.00^{a-f}	0.05±0.00^{a-e}
	60	0.04±0.00^{b-f}	0.04±0.00^{d-f}	0.04±0.00^{c-f}	0.05±0.00^{c-f}	0.04±0.00^{a-f}	0.04±0.00^{a-f}
	75	0.05±0.00^{a-f}	0.03±0.00^{ef}	0.04±0.00 $^{c-f}$	0.04±0.00 $^{c-f}$	0.04±0.00^{b-f}	0.04±0.00 $^{a-e}$
HDPE	0	0.05±0.00 $^{a-f}$	0.03±0.00^{f}	0.03±0.00^{ef}	0.04±0.00^{d-f}	0.04±0.00 $^{c-f}$	0.04±0.00^{b-f}
	15	0.05±0.01^{a-f}	0.05±0.01^{a-f}	0.05±0.00^{a-f}	0.05±0.01^{a-e}	0.05±0.00^{a-d}	0.05±0.02^{a-d}
	30	0.04±0.00^{b-f}	0.05±0.00^{a-e}	0.05±0.00^{a-e}	0.06±0.00^{a-c}	0.05±0.01^{a-d}	0.05±0.01^{a-d}
	45	0.04±0.00 $^{b-f}$	0.05±0.01^{a-e}	0.04±0.00^{b-f}	0.05±0.00 $^{a-f}$	0.05±0.00 $^{a-e}$	0.06±0.00^{a-c}
	60	0.04±0.00^{d-f}	0.04±0.00^{b-f}	0.04±0.00 $^{c-f}$	0.04±0.00^{c-f}	0.04±0.00 $^{a-f}$	0.04±0.00^{b-f}
	75	0.03±0.00^{ef}	0.04±0.00 $^{c-f}$	0.04±0.00 $^{c-f}$	0.04±0.00^{c-f}	0.04±0.00 $^{c-f}$	0.05±0.00^{a-f}

*Means followed by the same letters are not significantly different at 5% level of significance

4.4 Shoot Length

Seedling shoot length was significantly affected by bag types ($F=17.96$, $F_{df}=1$, $P<0.01$), durations ($F=297.13$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=2.40$, $F_{df}=5$, $P<0.05$), interaction between duration of storage and bag type ($F=3.14$, $F_{df}=5$, $P<0.05$), and the three-way interaction ($F=2.21$, $F_{df}=25$, $P<0.05$) but not between interaction between bag type and calcium chloride, interaction between $CaCl_2$ and duration, from effect tests.

Seedling shoot length of dry seeds stored in HDPE bags maintained higher until 30 days (Table 5). In hydro-primed seeds, HDPE bags maintained relatively higher seedling shoot length until 30 days of storage. Seeds which were treated with 1.00% $CaCl_2$ exhibited longer shoots up to 30 days of storage. This could be due to the preservation capacity of hermetic bags, and relatively higher amount calcium chloride which might trigger seedling shoot length. This result is supported with findings that explore halo-priming as effective means of bringing about a prominent increase in shoot length of seedlings (Jisha *et al*., 2014). Maximum shoot length was obtained from seed priming with $CaCl_2$ previously (Farooq *et al*., 2009). Halo-priming with $CaCl_2$ (1.0%) significantly affected shoot length of forage sorghum (Fu *et al*., 1988).

Table 5. Mean (±SEM) of effects of storage period, storage bags and priming on seedling shoot length (cm)*

Bag	Days	Priming ($CaCl_2$)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	$8.62\pm0.3^{a-e}$	$8.07\pm0.25^{a-h}$	$8.07\pm0.25^{a-h}$	$7.8\pm0.3^{a-i}$	$8.45\pm0.58^{a-f}$	$8.17\pm0.32^{a-g}$
	15	$7.42\pm0.64^{a-n}$	$6.65\pm0.23^{e-q}$	$7.52\pm0.3^{a-m}$	$7.55\pm0.5^{a-l}$	$7.02\pm0.10^{c-p}$	$8.05\pm0.7^{a-h}$
	30	$6.57\pm0.39^{e-q}$	$5.7\pm0.36^{i-u}$	$5.97\pm0.4^{h-u}$	$7.5\pm0.24^{a-m}$	$7.52\pm0.17^{a-m}$	$7.3\pm0.26^{b-o}$
	45	$6.57\pm0.11^{e-r}$	$5.45\pm0.29^{l-w}$	$5.65\pm0.42^{k-v}$	$6.00\pm0.46^{h-u}$	$7.2\pm0.8^{b-o}$	$6.2\pm0.23^{g-t}$
	60	$5.01\pm0.21^{p-x}$	$5.32\pm0.06^{n-x}$	$5.65\pm0.25^{k-v}$	$4.96\pm0.31^{p-x}$	$4.67\pm0.09^{q-x}$	$5.3\pm0.4^{o-x}$
	75	$3.96\pm0.46^{u-x}$	3.48 ± 0.12^{wx}	$4.42\pm0.48^{r-x}$	3.26 ± 0.12^{x}	3.26 ± 0.12^{x}	$4.25\pm0.34^{s-x}$
HDPE	0	$8.30\pm0.14^{a-e}$	$9.00\pm0.10^{a-c}$	9.15 ± 0.13^{ab}	$8.87\pm0.3^{a-d}$	9.50 ± 0.35^{a}	9.20 ± 0.18^{ab}
	15	$7.67\pm0.17^{a-k}$	$8.50\pm0.93^{a-f}$	$7.47\pm0.34^{a-k}$	$8.00\pm0.15^{a-h}$	$8.6\pm0.40^{a-f}$	$7.97\pm0.45^{a-h}$
	30	$6.97\pm0.14^{c-p}$	$7.17\pm0.18^{b-o}$	$7.57\pm0.21^{a-k}$	$6.67\pm0.19^{e-q}$	$6.5\pm0.31^{f-r}$	$7.77\pm0.52^{a-j}$
	45	$6.82\pm0.62^{d-p}$	$6.6\pm0.41^{e-q}$	$6.32\pm0.11^{g-r}$	$7.2\pm0.41^{b-o}$	$6.90\pm0.3^{d-p}$	$6.00\pm0.30^{h-u}$
	60	$5.66\pm0.35^{m-w}$	$5.22\pm0.16^{o-x}$	$4.91\pm0.15^{p-x}$	$5\pm0.18^{p-x}$	$4.95\pm0.45^{p-x}$	$5.7\pm0.41^{j-v}$
	75	3.40 ± 0.09^{wx}	3.33 ± 0.03^{wx}	3.37 ± 0.06^{wx}	$3.65\pm0.09^{v-x}$	$3.96\pm0.16^{v-x}$	$4.1\pm0.30^{t-x}$

*Means followed by the same letters are not significantly different at 5% level of significance

4.5 Root Length

The overall ANOVA revealed that seedling root length was significantly different between independent variables (F=16.21, P<0.01). Seedling root length was significantly different among durations (F=214.04, F_{df}=5, P<0.01), $CaCl_2$ (F=3.27, F_{df}=5, P<0.05), interaction between bags and duration (F=4.47, F_{df}=5, P<0.05), interaction between duration and calcium chloride (F=1.69, F_{df}=25, P<0.05), interaction between bags and $CaCl_2$ (F=2.50, F_{df}=5, P<0.05) but not between bag types, and the three-way interaction, from effect tests.

Seedling root length exhibited statistically non-significant decline until 45 days of storage though rapid decrease was observed during 60 and 75 days of storage regardless of types of storage bags (Table 6). Before storage, both packaging types showed significantly higher seedling length. On the other hand, the three-way interaction showed non-significant difference in root length throughout all storage periods. Seedling root length showed non-significant differences between storage containers, storage conditions and their interaction, up to 2 months of storage (Naik and Chetti, 2018). Similar study by Aynadis Lijalem (2011) demonstrated that NERICA-4 rice seedling root length was significantly different from the dry seed when seeds were soaked for 24 hrs. and incubated for 24 hours. Maximum root length was obtained from seed priming with $CaCl_2$ (Farooq *et al*., 2009).

Table 6. Mean of seedling length as affected by storage duration and bag type*

Storage Duration (Days)	Bag type	
	HDPE	PE
0	10.78 ± 0.19^{a}	10.97 ± 0.19^{a}
15	10.12 ± 0.21^{a}	9.55 ± 0.17^{b}
30	9.5 ± 0.21^{b}	9.46 ± 0.15^{b}
45	8.59 ± 0.32^{cd}	8.51 ± 0.22^{d}
60	6.59 ± 0.22^{e}	6.21 ± 0.30^{ef}
75	5.78 ± 0.12^{ef}	5.36 ± 0.09^{f}

*Means followed by the same letters are not significantly different at 5% level of significance

Maximum root length of NERICA-4 seedlings was observed for seeds which were primed with 1.00% $CaCl_2$ showed the highest mean root length in HDPE bag and in PE bag (Table 7). One percent $CaCl_2$ in HDPE bags significantly differed from that of dry seed.

Table 7. Mean of seedling length as affected by bag type and $CaCl_2$ concentration*

Bag type	Priming ($CaCl_2$)					
	Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
HDPE	7.73±0.45^{b}	8.18±0.42^{ab}	8.21±0.42^{ab}	8.85±0.45^{a}	8.85±0.45^{a}	8.86±0.43^{a}
PE	8.54±0.45^{ab}	8.36±0.44^{ab}	8.43±0.44^{ab}	8.47±0.45^{ab}	8.33±0.41^{ab}	8.57±0.42^{ab}

*Means followed by the same letters are not significantly different at 5% level of significance

Tukey method mean separation of the interaction effect of duration of storage and $CaCl_2$ levels in every priming condition revealed that NERICA-4 seedling root length showed gradual decrease in every subsequent storage period but did not change significantly for 45 days of storage, whereas significantly declined during 60 and 75 days of storage (Table 8).

Table 8. Means of root length as affected by duration of storage and $CaCl_2$ *

Duration	Priming ($CaCl_2$)					
	Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
0	11.01 ± 0.32^{ab}	$10.66\pm0.4^{a-d}$	11.4 ± 0.21^{a}	$10.60\pm0.29^{a-d}$	$10.81\pm0.35^{a-c}$	$10.78\pm0.37^{a-c}$
15	$9.7\pm0.16^{a-f}$	$8.90\pm0.30^{d-g}$	$9.13\pm0.28^{b-g}$	$9.60\pm0.26^{a-f}$	$9.78\pm0.27^{a-f}$	$9.92\pm0.3^{a-f}$
30	$10.01\pm0.25^{a-f}$	$9.78\pm0.33^{a-f}$	$8.62\pm0.4^{a-g}$	$9.51\pm0.37^{b-f}$	$10.22\pm0.4^{a-e}$	$10.61\pm0.1^{a-d}$
45	$9.43\pm0.44^{b-f}$	$8.15\pm0.56^{f-i}$	$9.07\pm0.33^{c-g}$	$7.48\pm0.63^{g-j}$	$8.55\pm0.37^{e-h}$	$8.62\pm0.12^{e-g}$
60	5.87 ± 0.33^{jk}	6.11 ± 0.30^{jk}	5.91 ± 0.17^{jk}	6.14 ± 0.32^{jk}	$6.41\pm0.48^{i-k}$	$6.69\pm0.36^{h-k}$
75	6.20 ± 0.4^{jk}	6.01 ± 0.56^{jk}	5.79 ± 0.18^{jk}	5.30 ± 0.35^{jk}	5.78 ± 0.49^{jk}	5.63 ± 0.42^{jk}

*Means followed by the same letters are not significantly different at 5% level of significance

4.6 Seedling Length

The overall ANOVA revealed that seedling shoot length was significantly different between independent variables ($F=28.15$, $P<0.01$). Seedling shoot length was significantly affected by bag types ($F=10.17$, $F_{df}=1$, $P<0.01$), durations ($F=392.87$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=3.84$, $F_{df}=5$, $P<0.05$), interaction between $CaCl_2$ and duration ($F=1.71$, $F_{df}=25$, $P<0.05$), but not between interaction between bag type and calcium chloride, interaction between duration of storage and bag type and the three-way interaction, from effect tests. Hence, the analysis output on bag type showed that HDPE bags (15.10±0.11 cm) showed significantly higher mean value of seedling length compared to PE bags (14.60±0.11cm). The possible reason for greater seedling length in HDPE bags could be reserved energy due to reduced respiration in HDPE bags which could be additional source of energy for seedling length. The output on the effect of duration and calcium chloride on seedling length showed that 1% $CaCl_2$ before storage showed maximum mean seedling length whereas dry seed showed the minimum. Further, before storage treatments showed maximum mean values of seedling length in all priming conditions and dry seed then gradual decrease in seedling length in subsequent storage periods, and then become declined at 75 days of storage. Similar study demonstrated that maximum shoot was obtained from seed priming with $CaCl_2$ (Farooq *et al*., 2009).

Figure. 4. Preparing NERICA-4 seedlings for shoot and root length measurement (Kata *et al*., 2014).

Table 9. Mean (± SEM) seedling length (cm) as affected by bag type, duration of storage, and $CaCl_2$ concentrations*

Bag type	Days	Priming (CaCl2)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	18.27±0.85^{a-i}	19.2±0.53^{a-e}	19.62±0.43^{a-d}	18.30±0.49^{a-i}	18.67±1.06^{a-h}	19.85±0.57^{a-e}
	15	17.40±0.66^{a-j}	15.72±0.62^{d-q}	17.10±0.43^{a-k}	17.00±0.75^{a-l}	17.12±0.51^{a-k}	17.72±1.03^{a-j}
	30	16.30±0.14^{c-m}	15.37±0.61^{e-r}	15.2±0.84^{f-s}	16.02±0.15^{c-o}	16.25±0.4^{c-n}	17.62±0.29^{q-j}
	45	16.42±0.00^{c-m}	13.17±1.25^{k-u}	14.25±0.81^{j-t}	13.10±1.45^{l-u}	15.82±0.43^{d-p}	14.87±0.40^{h-s}
	60	12.22±0.00^{o-x}	12.3±0.87^{n-w}	11.83±0.43^{q-x}	10.27±1.02^{u-x}	10.21±1.07^{u-x}	11.82±1.05^{q-x}
	75	8.95±0.28^{v-x}	9.26±0.25^{u-x}	8.80±0.11^{wx}	8.91±0.16^{v-x}	9.96±0.17^{u-x}	10.88±0.27^{t-x}
HDPE	0	19.10±0.58^{a-e}	19.2±0.81^{a-f}	19.52±0.9^{a-d}	20.40±0.43^{ab}	20.67±0.34^{a}	20.90±0.47^{a}
	15	17.32±0.28^{a-j}	17.22±0.87^{a-j}	16.22±0.71^{c-o}	17.75±0.2^{a-j}	18.9±0.42^{a-g}	18.50±0.8^{a-j}
	30	17.02±0.67^{a-l}	17.12±0.70^{a-k}	16.55±0.80^{b-m}	17.17±0.24^{a-j}	17.07±1.02^{a-k}	18.17±0.55^{a-j}
	45	15.85±0.76^{d-p}	15.17±0.91^{f-s}	14.92±0.40^{h-s}	15.07±0.67^{g-s}	15.30±0.65^{e-r}	14.57±0.24^{i-t}
	60	11.75±0.92^{r-x}	11.90±0.60^{p-x}	11.25±0.18^{s-x}	11.78±0.68^{q-x}	10.80±1.19^{t-x}	12.83±1.04^{m-v}
	75	8.58±0.14^{wx}	8.30±0.14^{wx}	8.82±0.13^{wx}	8.80±0.18^{wx}	10.00±0.40^{u-x}	9.48±0.45^{u-x}

*Means followed by the same letters are not significantly different at 5% level of significance

4.7 Vigor Index-I

The overall ANOVA revealed that seedling vigor index-I was significantly affected by independent variables (F=25.74, df= P<0.01). Seedling vigor index-I was significantly affected by durations (F=357.94, F_{df}=5, P<0.01), bag types (F=6.35, F_{df}=1, P<0.01) and $CaCl_2$ (F=4.48, F_{df}=5, P<0.01), but not by interaction between duration and calcium chloride, interaction between bags and duration, interaction between bags and $CaCl_2$ and the three-way interaction, from effect tests (Figure 2).

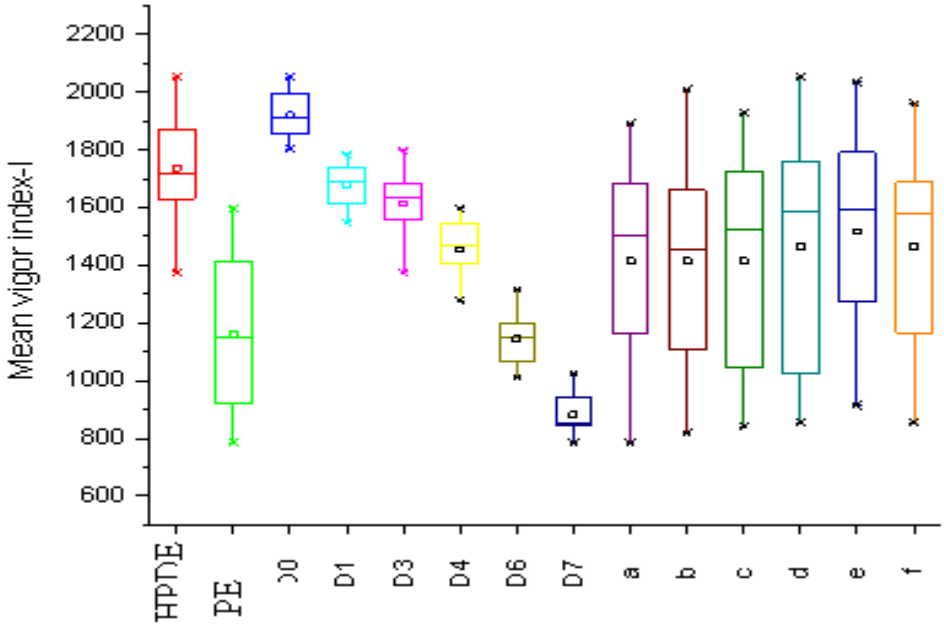

Figure. 5. Box plot of interactions of duration, packaging type and halo-($CaCl_2$) priming

Key: a= 0.00%; b=0.25%; c=0.5%; d= 0.75%; e=1.00% $CaCl_2$ priming and f = dry seed

The analysis in three-way interaction showed non-significant effect and that seedling vigor index-I could be maintained until 60 days of storage. However, significantly highest mean value of seedling vigor index-I was found before storage treatments and minimum mean value of seedling vigor index-I was found after 75 days of storage. This result is related with the finding that halo-priming was found to be more efficient than hydro-priming in enhancing the seedling vigor of rice varieties (Jisha *et al.*, 2014). Similarly, NERICA-4 rice variety showed poor performance with distilled water priming of48 hours whereas seed germination and seedling growth parameters particularly germination percentages,

germination energy, vigor index, shoot and root lengths were increased by the treatments osmo-priming and re-drying (Mamun *et al*., 2018).

4.8 Vigor Index-II

The overall ANOVA revealed that seedling vigor index-II was significantly affected by independent variables ($F=5.69$, $P<0.01$). Seedling vigor index-II was significantly different between durations ($F=33.14$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=34.56$, $F_{df}=5$, $P<0.01$), interaction between duration and calcium chloride ($F=1.75$, $F_{df}=25$, $P<0.01$), but not interaction between bags and duration, between bag types, interaction between bags and $CaCl_2$ and the three-way interaction, from effect tests.

Tukey mean separation method revealed that vigor index-II could be highly be enhanced until 15 days of storage (Table 10). In line with this result, Vimala *et al*. (2002) showed that character vigour index, decreased with the increase in storage period. This result is related with the findings of Jisha *et al*. (2014) who reported halo-priming was found to be more efficient than hydro-priming in enhancing the seedling vigor of rice varieties. Similarly, Mamun *et al*. (2018) reported poor performance of NERICA-4 rice variety with distilled water priming of 48 hours whereas seed germination was increased by the treatments osmo-hardening. Osmo-priming using $CaCl_2$ with re-drying effectively improved seedling vigor in direct seeded rice as compared to control (dry seed) (Rehman *et al*., 2011).

Table 10. Mean (± SEM) of effect of storage duration and calcium chloride levels on vigor index-II*

Bag type	Storage time (days)	Priming ($CaCl_2$)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	3.99±0.70^{t-w}	3.41±0.58^{v-w}	3.33±0.35^{vw}	2.99±0.76^{w}	4.43±0.4^{r-w}	3.29±0.47^{vw}
	15	4.06±0.26^{s-w}	3.72±0.16^{u-w}	4.59±0.16^{r-w}	4.78±0.63^{q-w}	5.11±0.09^{o-w}	4.57±0.22^{r-w}
	30	17.41±0.82^{b-f}	18.17±0.71^{a-d}	16.64±1.05^{b-h}	18.13±2.01^{a-d}	18.13±1.68^{a-d}	17.83±2.97^{b-e}
	45	15.26±0.71^{c}	14.96±1.32^{c-i}	12.86±2.54^{d-l}	13.91±0.62^{d-k}	12.32±2.16^{d-l}	15.35±0.46^{c-i}
	60	9.49±0.71^{i-v}	10.37±0.70^{h-s}	9.39±1.07^{i-v}	11.04±0.31^{g-q}	10.11±0.35^{i-t}	11.270.69^{i-o}
	75	9.46±0.44^{i-v}	8.22±0.78^{k-w}	11.51±0.75^{f-n}	11.31±0.33^{f-o}	13.49±0.73d-l	12.19±0.74^{d-l}
HDPE	0	4.75±0.29^{q-w}	3.3±0.13^{vw}	3.34±0.42^{vw}	3.33±0.19^{vw}	4.1±0.14^{s-w}	3.38±0.18^{vw}
	15	4.49±1.09^{r-w}	3.24±1.27^{vw}	5.52±1.20^{m-w}	4.92±0.46^{m-w}	5.23±0.54^{n-w}	4.45±0.24^{r-n}
	30	15.21±1.97^{c-i}	17.34±1.14^{b-g}	14.96±0.92^{c-i}	24.44±0.41^{a}	22.73±1.70^{ab}	20.48±2.66^{a-c}
	45	11.61±0.95^{e-m}	10.69±0.91^{h-r}	10.39±1.01^{h-s}	12.1±1.24^{d-l}	11.19±1.76^{f-p}	12.03±1.08^{d-l}
	60	11.61±0.79^{e-m}	10.69±0.49^{h-r}	10.39±0.64^{h-s}	12.1±0.22^{d-l}	11.19±0.69^{f-p}	12.03±1.41^{d-l}
	75	8.49±0.84^{j-w}	7.45±0.67^{l-w}	10.38±1.93^{h-s}	8.47±0.49^{j-w}	9.91±0.51^{i-u}	11.78±1.09^{e-l}

*Means followed by the same letters are not significantly different at 5% level of significance according to Tukey Honestly Significant Difference test at α=0.05.

4.9 Speed of Germination

Thousand seed weight was significantly affected by bag types ($F=7.88$, $F_{df=}1$, $P<0.01$), durations ($F=435.53$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=17.07$, $F_{df}=5$, $P<0.01$), interaction between bags and duration ($F=6.68$, $F_{df}=5$, $P<0.01$), interaction between duration and calcium chloride ($F=1.92$, $F_{df}=25$, $P<0.01$), interaction between bags and $CaCl_2$ ($F=3.58$, $F_{df}=5$, $P<0.01$), and the three-way interaction ($F=3.53$, $P<0.01$), from effect tests. Tukey method analysis of means of speed of germination during initial and subsequent days of storage revealed that speed of germination was maintained for forty-five days of storage in all conditions of priming and both packaging types (Figure 3).

The three-way interaction ANOVA revealed that speed of germination declined as the storage time was advanced from 0 to 75 days. Speed of germination tended to be quicker with at 1% calcium chloride level and from initial to duration of 45 days, in both packaging materials. This could be due to combined effects of osmotic substance calcium chloride and re-drying which makes the seed embryo imbibe water rapidly thereby enhanced speed of germination. Similar experiment by Simon (1972) revealed that pea embryos placed in water solutes leakage decreases rapidly at initial then slowly increased. For this reason, when pea embryos were dried over calcium chloride and set to imbibe water again, then embryos tended to leak as before. Further, NERICA-4 rice seeds which were sealed with HDPE bags revealed enhanced speed of germination compared to PE bags. This result is similar to the findings of Silva *et al*. (2018) who affirmed the type of packaging used while storing the seeds is important to preserve the physiological quality of the seeds. Hence, plastic bottle seed packaging influenced the results of the germination speed index after storage of 90 and 180 days, the plastic bottle package presented higher values, since this type of packaging can preserve or alter the minimum physiological characteristics of the seeds during storage.

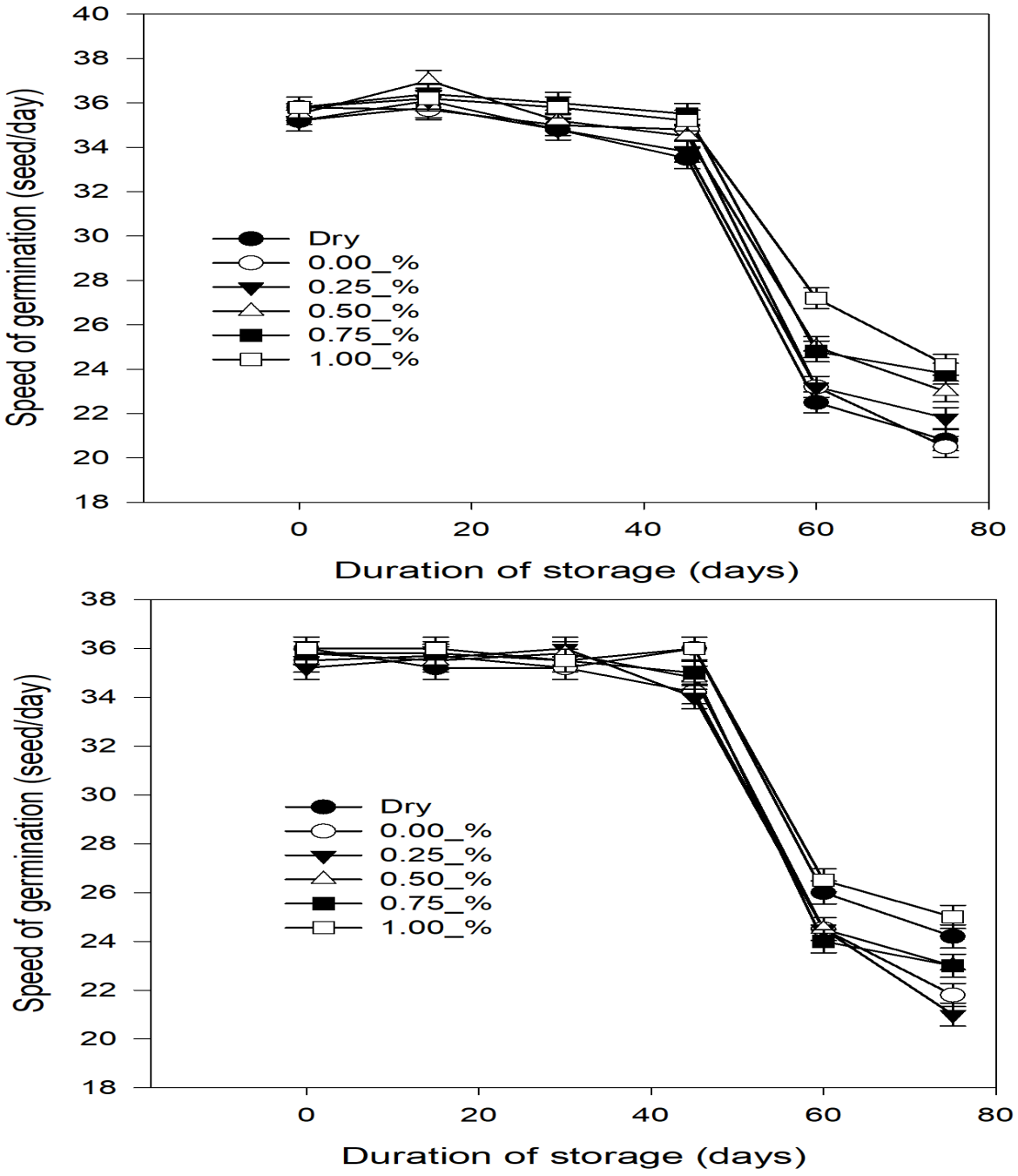

Figure. 6. Mean (± SEM) of effect of storage duration and calcium chloride levels, and bag type on speed of germination

4.10 Thousand Seed Weight

Thousand seed weight was significantly affected by bag types (F=981.11, F_{df}=1, P<0.01), durations (F=80.19, F_{df}=5, P<0.01), $CaCl_2$ (F=45.33, F_{df}=5, P<0.01), interaction between bags and duration (F=78.60, F_{df}=5, P<0.01), interaction between duration and calcium chloride (F=2.96, F_{df}=25, P<0.01), interaction between bags and $CaCl_2$ (F=3.58, F_{df}=5, P<0.01), and the three-way interaction (F=1.68, F_{df}=5, P<0.05), from effect tests.

Three-way analysis of variance for thousand seed weight indicated that initial or before storage priming treatments did not show any significant difference among different concentrations of calcium chloride and dry seed, in both packaging materials (Table 11). However, as storage time advanced from 15 to 75, there existed a slight decrease in thousand seed weight in NERICA-4 seeds packaged in the two of packaging materials, through every of the priming treatments. This result is in line with Tang and Ngome (2015) demonstrated that slight changes in upland rice thousand seed weight when evaluated during different storage periods and package types. Osmo-priming with higher levels of calcium chloride particularly 1% $CaCl_2$ showed increased thousand seed weight compared dry seed in both PE and HDPE bags through subsequent storage periods. Dry seed (control) showed the lowest thousand seed weight in the two of packaging materials, but HDPE bags exhibited maximum mean values of thousand seed weight compared to PE bags starting from 15 days to 75 days of storage.

Table 11. Mean (±SEM) of effect of storage period, storage bags and priming on thousand seed weight (g)*

Bag type	Duration (Days)	Priming (CaCl2)					
		Dry seed	0.00%	0.25%	0.50%	0.75%	1.00%
PE	0	30.64±0.11^{a-l}	30.74±0.08^{a-j}	30.86±0.05^{a-h}	30.94±0.04 $^{a-g}$	31.03±0.04^{a-f}	31.28±0.016^{a}
	15	30.42±0.13^{e-p}	30.55±0.04^{c-m}	30.62±0.04^{a-l}	30.68±0.025^{a-k}	30.83±0.06^{a-i}	31.12±0.10^{a-d}
	30	29.71±0.06^{q-w}	29.80±0.06^{o-w}	29.87±0.04^{m-w}	30.04±0.06^{k-u}	30.16±0.09^{i-s}	30.56±0.13^{c-l}
	45	29.34±0.05^{u-x}	29.35±0.05^{u-x}	29.5±0.09^{s-w}	29.74±0.07^{q-w}	29.85±0.06^{n-w}	30.28±0.33^{g-r}
	60	28.72±0.16^{xy}	27.66±0.25^{z}	27.72±0.27^{z}	27.95±0.20^{z}	28.04±0.20^{yz}	28.28±0.21^{yz}
	75	28.19±0.05^{yz}	27.59±0.02^{z}	27.63±0.02^{z}	27.97±0.006^{z}	28.04±0.01^{yz}	28.12±0.06^{yz}
HDPE	0	30.65±0.09^{a-l}	30.74±0.078^{a-j}	30.83±0.08^{a-i}	30.93±0.038^{a-g}	31.09±0.04^{a-e}	31.25±0.019^{ab}
	15	30.63±0.10^{a-l}	30.56±0.18^{c-l}	30.72±0.11^{a-k}	30.93±0.22^{a-g}	31.15±0.15^{a-d}	31.17±0.18^{a-c}
	30	30.15±0.04^{i-s}	30.37±0.11^{f-q}	30.47±0.09^{d-o}	30.57±0.09^{b-l}	30.70±0.07^{a-k}	31.035±0.07^{a-f}
	45	29.97±0.03^{l-v}	30.07±0.00^{j-t}	30.09±0.00^{j-t}	30.31±0.04^{g-r}	30.5±0.04^{c-n}	30.96±0.00^{a-g}
	60	30.08±0.17 $^{j-t}$	30.20±0.13^{h-r}	30.33±0.18^{g-r}	30.49±0.13^{d-o}	30.67±0.12^{a-k}	31.02±0.06 $^{a-f}$
	75	29.19±0.07^{wx}	29.30±0.05^{v-x}	29.45±0.12^{t-w}	29.68±0.10^{r-w}	29.76±0.18^{p-w}	30.22±0.08^{h-r}

*Means followed by the same letters are not significantly different at 5% level of significance

4.11. Moisture Content

Moisture content significantly affected by bag types ($F=448.99$, $F_{df}=1$, $P<0.01$), durations ($F=82.22$, $F_{df}=5$, $P<0.01$), $CaCl_2$ ($F=13.22$, $F_{df}=5$, $P<0.01$), interaction between bags and duration ($F=61.43$, $F_{df}=5$, $P<0.01$), interaction between duration and calcium chloride ($F=2.31$, $F_{df}=25$, $P<0.01$), interaction between bags and $CaCl_2$ ($F=14.03$, $F_{df}=5$, $P<0.01$), and the three-way interaction ($F=2.17$, $F_{df}=25$, $P<0.01$), from effect tests.

Tukey HSD method showed that moisture content of Nerica-4 rice seed at the onset of the experiment showed nearly 12% in both packaging materials. However, gradual increase in polypropylene bags in subsequent storage periods whereas almost uniformly continued in every storage period in HDPE bags. This result goes in line with Choundary *et al*. (2014) who affirmed almost moisture content increased to some without exceeding the standard in almost impermeable containers. Similarly, Tang and Ngome (2015) demonstrated that slight changes in moisture content evaluated during different storage periods and package type.

Table 12 Mean (±SEM) for interaction effect of storage period, storage bags and priming on Nerica-4 initial and subsequent seed moisture content (g)*

Bag type	Duration (Days)	Priming ($CaCl_2$)					
		Dry seed	**0.00%**	**0.25%**	**0.50%**	**0.75%**	**1.00%**
PE	0	$11.90\pm0.02^{j-l}$	11.89 ± 0.02^{l}	$11.87\pm0.04^{h-l}$	11.93 ± 0.02^{l}	11.88 ± 0.04^{l}	$11.98\pm0.01^{k-l}$
	15	$12.03\pm0.05^{f-l}$	$12.16\pm0.13^{j-l}$	$12.21\pm0.17^{i-l}$	$11.96\pm0.02^{d-s}$	$11.96\pm0.02^{d-s}$	$12.13\pm0.12^{i-l}$
	30	$12.4\pm0.02^{a-f}$	$12.07\pm0.04^{b-h}$	$12.52\pm0.04^{c-i}$	$12.27\pm0.11^{a-c}$	$12.3\pm0.05^{a-d}$	$12.4\pm0.04^{a-f}$
	45	$12.07\pm0.04^{a-j}$	$12.22\pm0.02^{h-l}$	$12.41\pm0.01^{h-l}$	$12.05\pm0.02^{a-e}$	$12.22\pm0.12^{d-j}$	$12.07\pm0.05^{j-l}$
	60	$11.98\pm0.01^{a-g}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.45\pm0.00^{a-d}$	$12.35\pm0.00^{a-h}$	$11.98\pm0.00^{j-l}$
	75	$12.42\pm0.04^{a-d}$	12.57 ± 0.02^{ab}	$12.56\pm0.0.05^{a-c}$	12.52 ± 0.07^{ab}	12.56 ± 0.05^{a}	$12.45\pm0.02^{a-e}$
HDPE	0	11.89 ± 0.02^{l}	$11.92\pm0.02^{k-l}$	$11.97\pm0.02^{h-l}$	12.04 ± 0.08^{j}	11.92 ± 0.03^{kl}	11.90 ± 0.03^{l}
	15	$12.00\pm00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$
	30	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$
	45	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$
	60	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$
	75	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$	$12.00\pm0.00^{j-l}$

*Means followed by the same letters are not significantly different at 5% level of significance

CHAPTER 5. CONCLUSION AND RECOMMENDATION

Conclusion

Results indicated that halo-priming improved rice seed germination and vigor performance. NERICA-4 rice seeds halo-primed with 1% concentration of $CaCl_2$ and re-dried and stored in HDPE bags exhibited superior seed germination and vigor characteristics. Mean germination percentage, vigor index, seedling length, seedling shoot length, seedling root length, seedling dry weight, speed of germination and thousand seed weight were significantly influenced by the type of storage bag and storage periods.

Recommendation

From the analysis of variance, it is recommended that NERICA-4 rice seeds primed with 1% concentrations of $CaCl_2$ and sealed in HDPE bags commenced primed seeds seed storability 45 days. On the other side, NERICA-4 rice seeds primed with 1% concentrations of $CaCl_2$ and PE bags-maintained storability for 30 days. Further studies are required using controlled storage humidity and temperature, and biochemical and field experiments to strengthen the recommendation.

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APPENDIX

Table 13. Annex table on F-ratio for effect of independent variables on NERICA-4 rice seed

Independent variables	DF	TG	SG	DS	FW	DW	SL	VI	VII	Sp. G	Sh. L	RL	TSW	Mc
Duration	5	66.94 **	61.22 **	51.60 **	50.48 **	33.26 **	392.87 **	357.94 **	33.14 **	435.52 **	297.13 **	214.04 **	396.77 **	82.23 **
Bag	1	2.64 **	0.27 ns	6.23 *	2.69 ns	0.15 ns	10.17 **	6.35 *	0.46 ns	7.88 **	17.96 **	1.43 ns	661.06 **	448.99 **
$CaCl_2$	5	5.65 ns	11.00 **	4.17 **	4.55 **	32.85 **	3.84 **	4.48 **	34.56 **	17.07 **	2.40 *	3.27 **	39.67 **	13.23 **
Rep	3	0.94 ns	0.63 ns	0.76 ns	0.41 ns	2.48 ns	0.33 ns	0.49 ns	1.83 ns	1.58 ns	0.08 ns	0.64 ns	2.77 ns	1.21 ns
Duration*Bag	5	0.94 ns	2.06 ns	2.71 *	1.81 ns	1.80 ns	2.07 ns	1.74 ns	1.69 ns	6.68 **	3.14 **	4.47 **	109.00 **	61.43 **
Bag*$CaCl_2$	5	0.49 ns	1.66 ns	0.88 ns	1.72 ns	0.40 ns	1.41 ns	1.29 ns	0.33 ns	3.58 **	1.74 ns	2.50 *	2.81 *	14.04 **
Duration*$CaCl_2$	25	1.60 *	2.59 **	1.28 ns	1.71 *	1.62 *	1.72 *	1.52 ns	1.75 *	1.92 **	1.26 ns	1.69 *	1.14 ns	2.32 **
Duration*Bag*$CaCl_2$	25	1.77 *	1.68 *	1.70 *	1.63 *	0.99 ns	1.13 ns	1.25 ns	0.90 ns	3.53 **	2.21 **	1.29 ns	1.14 ns	2.18 **

*= Significantly different at 5% level of significance and **= significantly different at 1% level of significance

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Mr. Yilikal Melak Assaye, was born on November 29, 1977 E.C in Dega-Damot Woreda, West Gojam Zone, Amhara Regional State, Ethiopia. He attended his primary and Junior education in Tame Elementary School from 1987 to 1992 E.C. He attended his secondary school education at Feres bet from 1993 to 1994 E C. He had Diploma in Plant Science, from Woreta College of Agriculture in 1997E C.

Then he joined in Amhara region, Degadamot Agricultural Office in 1998 E.C, as an expert in crop production. In 2002 E.C Yilikal joined Bahir Dar University, College of Agriculture and Environmental Sciences for his BSc. From 2004 to 2006 E.C, in Mecha Woreda Koga irrigation development office and from 2007 to 2009 E.C, he worked in Ethiopian Institute of Industrial Development, Cotton Development Directorate, Addis Ababa. Then he joined Ethiopian Institute of Agricultural Research in 2009 E.C, as a junior researcher. He got opportunities of short-term training in the areas of rice seed, entitled 'Rice seed production and Extension methods training' facilitated by International Rice Research Institute at PhilRice in Philippines for two months and 'Quality Rice Variety maintenance and variety development' at Japan International Cooperation Agency Tsukuba, in Japan. Finally, he attained long-term training opportunity in Seed Science and Technology (M. Sc program) at the same University sponsored by Ethiopian Institute of Agricultural Research. Yilikal has done his M. Sc thesis entitled as 'Study on Storability of hydro and halo-primed upland rice seed'.