Alleviating delayed field curing-induced deterioration in rice seeds by cathodic water invigoration: A comparison of seed and seedling responses in two upland rice species and their interspecific hybrid

by

RAPHAEL KWAME BAM

Submitted in partial fulfilment of the academic requirements of

Doctor of Philosophy

in Biology
School of Life Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Westville
South Africa

AUGUST 2021

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Biology, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa, under the supervision of Dr Boby Varghese and co-supervision of Professor Sershen Naidoo and Professor N.W. Pammenter. The research was financially supported by a National Research Foundation, South Africa grant.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Dr Boby Varghese

Date: 25 – 08 – 2021

DECLARATION 1: PLAGIARISM

I, Raphael Kwame Bam, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed: Raphael Kwame Bam

Date: 25 – 08 – 2021

DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 2

Raphael Kwame Bam, Sershen, Boby Varghese, and Norman Pammenter (2016). Impacts of delayed field curing of rice in a humid tropical environment on subsequent seed germinability and vigour. 2nd Global Summit on Plant Science. Hotel Crowne Plaza, Heathrow, London, United Kingdom, 06-08 October, 2016.

Raphael Kwame Bam, Sershen, Boby Varghese, B.M. Dzomeku and Norman Pammenter (2017). Impacts of delayed field curing of rice in a tropical environment on rice seed quality. 12th Triennial Conference of the International Society of Seed Science, Monterey, California, USA, 10-14 September, 2017.

Raphael Kwame Bam, Sershen, Boby Varghese, B.M. Dzomeku, Z. Appiah-Kubi and Norman Pammenter* (2019). Impacts of delayed field curing on rice seed quality in a tropical environment. Experimental Agriculture 55: 412-427.

Chapter 5

Raphael Kwame Bam, Sershen, Boby Varghese, Nelisha Murugan and Norman Pammenter (2017). The effects of cathodic invigoration of field cured and stored rice seeds on plant establishment and growth in three rice species. 12th Triennial Conference of the International Society of Seed Science, Monterey, California, USA, 10-14 September, 2017.

Author's contribution: Raphael Kwame Bam did all the experimental works, analysed the data and wrote the original manuscripts.

Signed: Raphael Kwame Bam

Date: 25 – 08 – 2021

ABSTRACT

Rice constitutes the major source of the world's food supply. A number of varieties are grown in many parts of the world, all which produce orthodox seeds that are usually stored between growing seasons. As in other crop species, storage-induced loss of seed quality (viability and vigour) is inevitable but considerable research effort has been invested in optimising seed bank storage protocols for rice to ensure acceptably high levels of seed germinability and seedling emergence. However, poor post-harvest practices such as delayed field curing before threshing in developing countries in the humid tropics, such as Ghana, result in curing-induced seed deterioration in the field prior to *ex situ* storage. Given that many rice growing regions are likely to experience significant levels of climate change, seed processing and storage induced declines in rice seed quality could exacerbate the crop losses incurred by rice farmers in these regions in the future.

This motivated the present study which was designed around three aims: 1) to investigate how environmental conditions and the duration of field curing influenced seed macro-structural integrity, susceptibility to microbial infection, and seed germinability and vigour; 2) to identify macro-structural and ultrastructural indicators/ biomarkers of field curing and associated storage-induced stress/ damage; 3) to assess whether the invigoration of field-cured seeds with cathodic water (CW), an established antioxidant-based seed invigoration medium, and de-ionized water (DW) can alleviate the deteriorative effects of delayed field curing on rice seed cellular integrity, germination and subsequent seedling growth and biomass. The specific objectives of this comparative study, which involved an Asian (*Oryza sativa* L.) and African (*Oryza glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*), were as follows: a) to compare the impacts of delayed field curing in wet and dry environments on seed physical, physiological and pathological quality; b) to identify potential ultrastructural biomarkers of seed sensitivity to delayed field curing-induced stress/ damage in embryonic root meristematic cells using transmission electron microscopy (TEM); c) to assess whether CW invigoration alleviates cellular stress/ damage induced by delayed field curing using selected ultrastructural biomarkers of seed sensitivity to such curing-induced stress/ damage; and d) to assess whether invigoration with CW and DW improves seed germinability and emergence, and subsequent plant growth in field cured seeds, relative to non-invigorated (NI) dry seeds.

For objective (a), seeds of both species and their interspecific hybrid were grown in Ghana, harvested, field cured in open (wet) and within ventilated rainproof containers (dry) for five weeks. Harvested panicles were sampled from the wet and dry cured environments at weekly intervals for 5 weeks, hand threshed, and depending on the moisture content (MC) at sampling, seed samples were further dried to 12% and stored hermetically at 4°C until used. Sub-samples of both species and the hybrid from the weekly samples were used for seed microflora studies and germination assay. Environmental parameters (temperature and relative humidity) were measured in wet (weather station) and dry (tiny tag data loggers) cured environments. Seed samples of both species and hybrid were taken daily from wet and dry cured environments at 08h00 and 15h00 during the 5 week period, equilibrated overnight at 20°C for seed MC and water activity measurements. Sub-samples of seed samples of both species and hybrid taken daily at 08h00 from the wet and dry cured environments were used to measure percentage of seeds with cracked endosperm. Results revealed that dry field curing delayed endospermic cracking and reduced crack frequency (10% in *O. sativa*; 40% in *O. glaberrima*; 36% in the hybrid), enhanced subsequent seed germinability (2%), and reduced subsequent seedling vigour (radicle length, 37%; seedling dry weight, 11%) relative to wet cured seeds across both species and hybrid. However, fungal infection was higher (22%) in dry than wet cured seeds: seeds infected with fungal species (e.g., *Aspergillus flavus*, *Bipolaris oryzae*, *Curvularia lunata, Fusarium moniliforme* and *Phoma sp*) was higher in dry cured *O. sativa* (22%) and the hybrid (31%) than the wet cured seeds. Seed germinability and seedling vigour declined with delayed field curing, but the decline was higher in the hybrid than in both species.

For objective (b), seeds of both species and their interspecific hybrid field cured for different durations (0, 2 and 5 weeks) and hermetically stored at 4°C for 20 months were removed from storage, equilibrated overnight at 25°C and invigorated with DW for 18 h. Untreated dry (non-imbibed seeds, cured for different durations (0, 2 and 5 weeks) were used as the controls for this study. These controls were used for two reasons: 1) to establish whether ultrastructural abnormalities induced by curing are permanent or can be reversed/ repaired during imbibition; 2) to establish whether seed deterioration during curing leads to imbibitional damage. The severity of ultrastructural lesions related to cellular stress/ damage differed across species and the hybrid and progressed at a rate in agreement with the viability and vigour loss brought about by delayed field curing. These microscopy studies also served to identify useful ultrastructural

biomarkers of field curing induced cellular stress/ damage in both rice species and the hybrid. These include marked abnormalities in cell wall and key organelle [nucleus (N), nucleolus (nu), mitochondria (M), lipids, vacuoles, plastids and amyloplasts] ultrastructural integrity with delayed field curing. This study suggests that these biomarkers may serve as useful screening tools for rice breeders looking to identify species/ varieties that produce seeds that are more resistant to field curing-induced deterioration.

For objective (c), seeds of both species and their interspecific hybrid were field cured for different durations (0, 2 and 5 weeks), stored hermetically and removed from storage and equilibrated as in the experiment for objective (b). However, in the experiment designed to address this objective, seeds of both species and hybrid cured for different durations (0, 2 and 5 weeks) were invigorated with CW and DW for 18 h before comparing their ability to alleviate the effects of field curing induced cellular stress/ damage in terms of the ultrastructural biomarkers of such stress/ damage identified via objective (b). Seed germinability was significantly higher in CW invigorated 5 week cured seeds of both species and 2 and 5 week cured seeds of the hybrid than DW invigorated seeds suggesting that CW invigoration was more effective in alleviating curing-induced stress/ damage. Ultrastructural studies showed that CW invigoration of cured seeds of both species and hybrid appears to have enhanced the repair of damaged cellular components and/ or reduced the damage that ensues during imbibition in the deteriorated seed. More importantly, seeds subjected to CW invigoration exhibited more prominent signs [e.g., M exhibited elongated profile, homogenous matrix, well-differentiated cristae (Cr), and well-defined outer mitochondrial membrane (OM) and inner mitochondrial membrane (IM), and highly developed Golgi bodies (G)] of repair and germinative metabolism than those imbibed with DW. Interestingly, the degree of mitochondrial and G development in CW invigorated seeds was greater in the hybrid than the two species. The results obtained for these biomarkers indicated that CW invigoration can alleviate the cellular stress/ damaged induced by field curing and also point to superior restorative effects of CW on viability, vigour and cellular integrity and metabolism in cured seeds compared with DW.

For objective (d), endosperm integrity (i.e., nature/severity of physical damage to endosperm, caryopsis coat thickness, and presence of seed microflora) of stored (4°C for 20 months) NI seeds of both species and their interspecific was assessed using scanning electron microscopy (SEM). Seeds of both species and the hybrid stored for the same duration as in the SEM studies

were also equilibrated overnight at 25°C and invigorated for 18 h in CW and DW before being sown in individual pots under greenhouse conditions. Untreated dry seeds, cured for different durations were used as the controls for this study. The uncured seeds (0 week) of both species and the hybrid did not exhibit macro-structural damage; however, 5 week-cured seeds exhibited deep cracks which exposed the aleurone cells (grains and lipid bodies) and facilitated fungal infection and insect damage which may have contributed to viability and vigour loss in cured seeds. Cathodic water invigoration significantly enhanced seedling emergence (17% in 5 week cured *O. sativa*; 23% in 5 week cured *O. glaberrima*; 16 and 19% in 2 and 5 week cured hybrid, respectively) in field cured seeds of both species and the hybrid than DW invigoration. Also, CW invigoration significantly produced taller seedlings in CW invigorated cured (2 and 5 weeks) seeds of both species and the hybrid than DW invigorated seeds. Across both species and hybrid, CW enhanced panicle biomass, although differences between CW and DW were not always significant. The invigorative effect of CW on plant growth from cured seeds was generally not observed in terms of total biomass yield and changes in biomass allocation to stem, leaves, and roots.

Overall, the study has deepened our understanding of the physical, pathological, physiological, and ultrastructural lesions that contribute to loss of rice seed quality (seed viability and vigour) during delayed field curing. It has also served to identify a number of seed physiological, ultrastructural, and macro-structural biomarkers/ indicators that can be used by breeders for screening rice varieties for sensitivity/ tolerance to field curing. The results have also provided a basis for many new opportunities for research on alleviating seed processing- and storage - induced deterioration in crop seed quality through seed soaking/ invigoration treatments (particularly those involving CW and other antioxidant-based solutions), which is going to represent an increasingly important research area as the effects of climate change and developmental challenges threaten food security in many parts of the world. However, future studies must screen a larger number of rice genotypes to assess the plasticity of the biomarkers identified to be reliable indicators of curing-induced stress and stress recovery before they are adopted by the seed industry. Additionally, future studies must use biochemical, physiological, molecular, omic and biophysical approaches to fully understand the mechanism(s) of action of CW invigoration in deteriorated rice seed in order to extend this method to other crop species that experience a decline in seed quality during seed processing and or storage.

ACKNOWLEDGMENTS

I am very grateful to God for His super hyperabundant grace which has made it possible for me to complete my studies.

To my supervisor, Dr Boby Varghese, thank you very much for agreeing to step in to complete the supervision of my thesis write up. Also, I am very grateful to you for ensuring that I settled well into life in Durban and for opening your home to me. Thank you for your manifold support throughout my studies.

I owe a debt of gratitude to my co-supervisor, Professor Sershen Naidoo, for his dedicated and meticulous supervision. You have always made yourself available to me whenever I needed your help. Words cannot express how grateful I am to you for your encouragement, kindness, thoughtfulness, support, sacrifices, new opportunities and for working hard to the completion of this work.

To Professor Norman W. Pammenter, thank you for your invaluable contributions to my thesis work. Thank you for supporting my studies and for the opportunity to acquire new skills at Kew Gardens, Wakehurst, Sussex, UK.

To my first supervisor, the late Professor Patricia Berjak, thank you for giving me the opportunity to study at University of KwaZulu-Natal (UKZN).

To Dr Wynston Woodenberg, Mr. Subashen Naidu and Ms Nelisha Murugan, I am very grateful for your assistance with microscopy which contributed to the results in Chapters 3 to 5.

Mr Dominic Gondwe, my late office mate, friend and a brother was instrumental in finding accommodation for me at Westville campus and always facilitated my registration at the beginning of each semester. Thank you and rest in peace.

Dr Ademola Emmanuel Adetunji, thank you for providing me with assistance in formatting the table of content, table of tables and table of figures.

To Andrea Kristine Naidoo, Cherisse Naidoo, Rineshia Naidoo, Nombali Gumede, Philani Ndlovu, Prince Sandile Khuzwayo and Mr Malibongwe Sithole, thank you all for assisting me with data collection at various stages of my research work.

To my wonderful wife Professor Victoria Bam and my children Joseph, Abigail and Emmanuel Bam, thank you all for your understanding, moral and prayer support.

TABLE OF CONTENTS

Contents Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

4-HNE:	4-hydroxy-2-nonenal
ANOVA:	Analysis of Variance
ATP:	Adenosine triphosphate
Ca:	Calcium
CAT:	Catalase
Cr:	Cristae
CW:	Cathodic water
CWL:	Cell wall
DAI:	Days after initiation
DAS:	Days after seeding
DHAR:	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
DW:	De-ionized water
FEGSEM:	Field Emission Gun Scanning Electron Microscope
fmb:	Fresh mass basis
G:	Golgi body/ bodies
GR:	Glutathione reductase
H:	hour(s)
IM:	Inner mitochondrial membrane
MC:	Moisture content
M:	Mitochondria
min:	Minutes
MDA:	Malondialdehyde
MDHAR:	Monodehydroascorbate reductase
Mg:	Magnesium
MGT:	Mean germination time
MM:	Mitochondrial membrane
N:	Nucleus/ Nuclei
NI:	Non-imbibed/non-invigorated
NM:	Nuclear membrane
NOR:	Nucleolar organizer

NS:	Not significant
nu:	Nucleolus/nucleoli
NV:	Nucleolus vacuole
OM:	Outer mitochondrial membrane
PLD:	Phospholipase D
RH:	Relative humidity
RNA:	Ribonucleic acid
ROS:	Reactive oxygen species
RSR:	Root: shoot ratio
SEM:	Scanning electron microscope
SOD:	Superoxide dismutase
SSA:	Sub-Saharan Africa (SSA)
TEM:	Transmission electron microscopy

CHAPTER 1: INTRODUCTION

1.1 *Ex situ* seed storage and food security

Seeds are classified into three categories based on their storage behaviour, namely orthodox, recalcitrant, and intermediate (Hong *et al*., 1996). Cereal crops such as rice (*Oryza sp*), wheat (*Triticum aestivum*), maize (*Zea mays),* barley (*Hordeum vulgare*), and sorghum (*Sorghum bicolor*) produce orthodox seeds, and constitute the major source of the world's food supply (Singh *et al.,* 2013; Ebone *et al*., 2019), but they are also the most vulnerable to climate change (Singh *et al*., 2013). Rice is an important cereal crop that provides the food needs of over 50% of the world's population (Rao and Jackson, 1996; Londo *et al.,* 2006). The seed industry plays a critical role in ensuring food security as the sustained, and increased production of these crops is dependent on the availability of high-quality seeds. Therefore, maintenance of seed longevity in storage is of critical importance in the conservation of genetic resources and for ensuring high seedling establishment and crop yield (Righetti *et al*., 2015; Groot *et al*., 2015; Rao *et al*., 2017; Solberg *et al*., 2020).

Seed longevity or storability is an important seed quality trait in rice (Walters *et al*., 2005; Lin *et al*., 2015) because rice seeds are known to have shorter longevity in storage compared with seeds of many other crop species (Walters *et al*., 2005). Seed longevity is influenced by the storage environment [moisture content (MC), relative humidity (RH), and storage temperature] and, therefore crucial in ensuring high seed quality. Seed longevity is influenced by the initial seed quality of the seed lot, which is also affected by such factors as cultivar, pre-harvest and harvest seed production environment, harvest, and post-harvest drying, handling, and processing practices (Ellis *et al*., 1992; Kochanek *et al*., 2011; Rao *et al.*, 2017; Kumar and Kalita, 2017). Pre-zygotic high temperature seed production environment results in heat stress and leads to morpho-anatomical, physiological, and biochemical changes in plants and reduces the yield of crops (Kaushal *et al*., 2016) and potential longevity of seeds (Kochanek *et al*., 2011). Kochanek *et al*. (2011) have shown that high temperature pre-zygotic growth environment reduced potential seed longevity (initial seed quality) in *Plantago cunningghamii* relative to plants grown in a cooler pre-zygotic growth environment. They also observed that the parental pre-zygotic growth environment modulated seed longevity through a parental effect. Post-zygotic effects of heat stress on developing seeds have been reported to cause a

decrease in seed germination, delayed germination, or loss in vigour leading to a reduction in seedling emergence and establishment (Piramila *et al*., 2012; Kaushal *et al*., 2016). These findings suggest the worrying impact of climate change on food security. Poor post-harvest practices, e.g., late harvesting and delayed field curing prior to threshing in the humid tropics, result in field weathering (TeKrony *et al*., 1980; Nagata *et al*., 2013; Rao *et al*., 2017) and cause increased microflora activity in seeds (Mousa *et al.*, 2011) leading to reduction in seed quality and storage longevity.

1.2 Factors affecting seed quality

Seed development is made up of two main phases, namely embryogenesis, and maturation (Manfre *et al*., 2009; Bewley *et al*., 2013). The maturation phase, which is also termed desiccation (Angelovici *et al*., 2010), is further categorized into early and late maturation phases (Chatelain *et al*., 2012; Dekkers *et al*., 2015): developing seeds acquire desiccation tolerance considerably and accumulate storage compounds (e.g., carbohydrates, proteins, and oils) and acquire germination capacity and desiccation tolerance during the early maturation phase while seeds dry out leading to the acquisition of dormancy and quiescent state during the late maturation stage (Vertucci and Farrant, 1995; Hoekstra *et al.,* 2001; Manfre *et al*., 2009; Angelovici *et al*., 2010; Chatelain *et al*., 2012; Leprince *et al*., 2017). Therefore, acquisition of seed vigour during seed development, and seed deterioration during desiccation and storage is controlled by cellular, biochemical and molecular mechanisms (Corbineau 2012; Dekkers *et al*. 2015; Zhang *et al*., 2021). Understanding the causes of viability and vigour loss during storage is key to successful *ex situ* conservation and increased agricultural productivity (Chen *et al*., 2013). Therefore, seed quality and storage longevity are important for plant adaptation to survival in changing environments, biodiversity conservation and agricultural production (Sano *et al*., 2016).

Furthermore, depending on species, cultivar, and environment, seed longevity increases gradually to between 30- and 50- fold to reach maximum during the late maturation stage (Hay *et al*., 2015; Righetti *et al*., 2015). These physiological traits are essential to high seed vigour and germinability and successful seedling establishment in the field (Righetti *et al*., 2015). The late seed maturation phase varies among species. For example, the percentage of the late maturation phase relative to the total seed development time for rice is about 78% compared with 60% and 30% for *H. vulgare* and *T. aestivum*, respectively (Leprince *et al*., 2017).

Therefore, environmental conditions during this phase of seed development will significantly influence the initial seed quality and may be exacerbated by poor post-harvest drying practices.

Orthodox seeds enter into a glassy state; thus, the components of their cells become an amorphous solid when dried and stored at the typical low temperatures of seed banks (Walters *et al*., 2010). At such state, at which ageing rates are reduced, and longevity is maximized (e.g., Ellis *et al*., 1990; Walters, 1998), seeds inexorably continue deterioration and ageing up to their death (Murthy *et al*., 2003; Walters *et al*., 2004; 2005; Xia *et al*., 2015a). Therefore, maintaining seed quality (seed vigour and viability) during storage is of paramount importance to agricultural productivity and food security.

Several pre- and post-harvest environments and management practices affect the initial quality of seeds prior to storage. Therefore, potential seed longevity is influenced by the initial seed quality of the seed lot, which is also affected by such factors as cultivar, pre-harvest and harvest seed production environments, harvest, and post-harvest drying, handling, and processing practices (Ellis *et al*., 1992; Kochanek *et al*., 2011; Rao *et al.*, 2017).

Seed quality, which includes longevity, is acquired during seed development and maturation phase of seed growth (Ellis et al., 1993; Ellis and Hong 1994; Leprince *et al*., 2017), but environmental conditions during seed development and maturation could impact negatively on seed quality (Ellis et al., 1993; Hay and Probert, 1995; Probert *et al*., 2007). Furthermore, post-harvest drying environment and poor post-harvest drying practices could also negatively impact seed quality then and in storage (Rao and Jackson, 1996; Whitehouse *et al*., 2015; Kumar and Kalita, 2017). These factors can influence the longevity of species and cultivars within species and between cultivars through their initial seed quality or what is termed seed lot constant or potential longevity in the improved seed viability equation (Ellis and Roberts, 1981).

1.2 Rationale and motivation for this study

Efficient seed germinability, seedling emergence, and enhancement of seedling performance are of fundamental relevance to crop production in these times of global climatic change and world population. Rapid and uniform seedling emergence determines a successful plant establishment and crop productivity (Harris *et al*., 2001). However, poor seed quality after seed storage is the primary cause of poor seedling establishment, seedling abnormalities, or even

failure of emergence (Groot *et al*., 2012). Climatic change may exacerbate abiotic stresses such as drought and may compromise seed germination and seedling growth, particularly in crops such as rice (Fernandez-Pascual *et al*., 2015; Kaushal *et al*., 2016).

Rice is grown across the six continents of the world (Africa, Asia, Europe, North, and South America) under four main growing ecologies (upland, rainfed lowland, irrigated, and flood - prone) worldwide (Prasad *et al*., 2017). In Africa, rice is produced in 40 countries under about 10 million hectares of land, with most of the production coming from the humid tropical sub-Saharan Africa (SSA) region (Zenna *et al*., 2017), including Ghana. Apart from rice being a major staple food to many in Africa, including Ghana, it is a cash crop to about 70% of the population (Zenna *et al*., 2017). In Ghana, rice is grown in all 16 administrative regions. About 84% of the total production comes from the rainfed upland rice production system, which forms about 91% of the total rice area (Ministry of Food and Agriculture, 2012). One of the major constraints to rice production in Ghana is the absence of quality seed production and marketing system, making farmers use farmer-saved seeds (Bam *et al.*, 2019). Poor agricultural production practices by farmers in harvesting, threshing, drying, and storage account for poor rice seed quality in SSA and many developing countries, including Ghana (Bam *et al*., 2007; Seck *et al*., 2010; Kumar and Kalita 2017). In Ghana, for example, the unavoidable practice of delaying the threshing of harvested rice panicles in the field for weeks to months (Nyaaba, 2015; Kumar and Kalita, 2017) leads to seeds being exposed to alternate wetting and drying and subsequent deterioration (Bam *et al.*, 2019).

Understanding the factors which affect rice seed quality in the humid tropical environments (e.g., many parts of Africa) during seed development, maturation, harvest, and post-harvest practices and drying will, therefore, be key to improving seed germinability and seedling establishment. Mature orthodox seeds can dry to moisture content levels of 5 - 10% (fresh weight basis) during desiccation and can be dried further to water content levels of 1-5% with or without no loss of viability (Manfre *et al*., 2009). During the desiccation phase of seed development, orthodox seeds become hygroscopic, with their moisture status being determined by the atmospheric conditions (Bewley and Black, 1994; Ellis and Hong, 1994; Hay and Probert, 1995; Leprince *et al*., 2017). Further drying of orthodox seeds at the end of the desiccation phase, will subsequently minimize the rate of seed deterioration and hence improve seed viability and longevity in storage (Hay and Probert, 1995; Probert *et al*., 2007; Manfre *et*

al., 2009). Although adjusting seed water content is an important factor in maintaining seed viability (Kibinza *et al*., 2006; Kong *et al*., 2014; Xia *et al*. 2015a), studies have shown that there are limits to the beneficial effects of drying, such as drying below critical moisture content will not improve longevity (Ellis *et al*., 1995; Hong and Ellis, 1996; Ellis and Hong, 2006) and may even have detrimental effects on seed survival in storage (Vertucci and Roos, 1990; Vertucci *et al*., 1994). Depending on the species, excessive drying to low water contents may or may not have harmful effects on seed quality, e.g., excessive drying did not exhibit a harmful effect on seed quality of oil-rich seeds, whereas damaging effect was observed in rice when dried to 4% water content (Zeng *et al*., 1998). Furthermore, seed storability decreased as seed desiccation rate increased in field-desiccated beans (*Phaseolus vulgaris*) due to increased vapour pressure deficit resulting from high temperature and relative humidity (Moreau-Valancogne *et al*., 2007). Therefore, seed deterioration is related to seed desiccation rate in the field (Hay and Probert, 1995; Walters *et al*., 1998; Moreau-Valancogne *et al*., 2007) and pre- and post-harvest seed drying environments (TeKrony *et al*., 1980; Sanhewe and Ellis, 1996).

Climate change impacts negatively on many biological-climate interactions (Fernandez-Pascual *et al*., 2015; Kaushal *et al*., 2016 and the references therein) and renders food production systems vulnerable, particularly in SSA (Agnolucci *et al*., 2020). High temperatures as a consequence of climate change have resulted in increased heat stress in various agricultural crops, thereby limiting agricultural productivity (Singh *et al*., 2013; Shinohara *et al*., 2006; Kaushal *et al*., 2016). Seeds subjected to heat stress may result in membrane damage and excessive reactive oxygen species (ROS) production (Kaushal *et al*. 2016; Kijowska-Oberc *et al*., 2021), leading to oxidative stress/ damage and or inadequate antioxidant production (reviewed by Bailly *et al*., 2008; Rajjou and Debeaujon, 2008 and references therein). Consequently, mitochondrial efficiency would decline, the release of peroxidative enzymes may be triggered and cause cellular damage during imbibition (McDonald 1999; Neya *et al*., 2004). Therefore, seed development and maturation environments in tropical environments can negatively impact seed longevity by reducing seed quality, particularly seed vigour (Egli *et al*., 2005; Shinohara *et al*., 2006; Zhou *et al*., 2020) and viability (Kijowska-Oberc *et al*., 2021). For example, embryos of early germinating *T. aestivum* seeds produced from high temperature stress environment during seed development and maturation (i.e., from 10 days after anthesis to maturity) resulted in mitochondrial degeneration, reduced rate of adenosine triphosphate (ATP) accumulation, lowered energy levels, and rates of oxygen uptake than embryos from low

temperature seed production environments (Grass and Burris, 1995). These findings might explain the poorer seed longevities reported in *O. sativa* seeds produced in high temperature environments when compared with seeds produced from cooler low temperature environments (Chang 1991; Ellis *et al*., 1992, 1993; Rao and Jackson 1996). These authors also observed that seeds of *O. sativa* subspecies (subsp) *japonica* had poorer seed longevities than *O. sativa* subsp *indica* produced in high temperature environments. Also, Rao and Jackson (1996) reported poorer longevities in *O. glaberrima* than *O. sativa* subsp *indica*. These findings suggest differences in seed longevity between species and between cultivars within species in high temperature seed production environments and highlight the importance of identifying rice species and cultivars/genotypes that are capable of maintaining high seed qualities (viability and vigour) in stressful environments.

Seed deterioration can occur between physiological maturity and harvest maturity, especially if harvesting is delayed (TeKrony *et al*., 1980; Anderson and Baker, 1983; Seck *et al*., 2010; Leprince *et al*., 2017), especially in wet tropical environments. Prevailing weather conditions, especially high temperature and relative humidity and/or rainfall, will determine the extent of field weathering and hence, the quality of harvested seed (Morita *et al*., 2016; Nagata *et al*., 2013; Moreano *et al*., 2011; Changrong *et al*., 2007; Ellis *et al*., 1993; Sassou and Kueneman, 1984; TeKrony *et al*., 1980) then, and in storage. Apart from the seed production environment, which can impact seed quality negatively, unfavourable climatic conditions such as rainfall, high temperature, and RH during harvesting or post-harvest field curing will further impact negatively on the physiological quality (germinability and vigour) of seeds (TeKrony *et al*., 1980; Moreano *et al*., 2011) and hence seed storage longevity (Ellis, 2011). Moreau-Valancogne *et al*. (2007) investigated the effect of microclimate on desiccation rate and seed storability of *P. vulgaris* and observed a high correlation between seed storability, desiccation rate, and vapour pressure deficit (combined effect of high temperature and RH) in a field environment. Moreover, in rice, high temperatures (particularly during daytime) during grain filling caused endospermic cracking of grains (Morita *et al*., 2016; Nagata *et al*., 2004; Takahashi *et al*., 2002). Alternate drying and wetting cycles experienced during maturation drying and field curing, especially during unfavourable climatic conditions, could also lead to the development of additional cracks (Lan and Kunze, 1996; Kunze, 2008). Additionally, high post-harvest drying temperatures also caused multiple severe cracks in rice and reduced normal percentage germination while levels of abnormal seedlings and seed death increased (Menezes

et al., 2012). Both embryo and endosperm of cereals age during storage, but their rates of deterioration may be non-synchronous. Although the embryo is the basic determinant of seed viability, the integrity of the endosperm determines the capacity of a seed to produce a normal healthy seedling (Mandal and Basu, 1981; Menezes *et al*., 2012). Germination and seedling growth of cereals, such as rice, is dependent on the mobilization of storage reserves from the endosperm by the embryo and endosperm tissues (Ritchie *et al*., 2000; He and Yang, 2013; Yan *et al*., 2014). Therefore, structural deformation of the endosperm of the seed evident by the formation of cracks in the endosperm may compromise its integrity and hence affect the quality of the seed.

Furthermore, poor agricultural production practices such as delayed field curing and late threshing account for poor rice seed quality in SSA and many developing countries (Seck *et al*., 2010; Nyaaba, 2015; Kumar and Kalita, 2017). In Ghana, for example, harvested rice could be delayed in the field for up to 5 weeks before threshing (Nyaaba, 2015), mainly due to lack of mechanization (Kumar and Kalita, 2017) and competition for labour to harvest other field crops (Bam *et al*., 2019). High temperature and humidity are characteristic of humid tropical seed production environments and promote fungal sporulation and accumulation of mycotoxins in seeds (Rossi *et al*., 2009; Lahouar *et al*., 2016; Bradford *et al*., 2018 and the references therein) during delayed field curing. In *S. bicolor*, for example, heavy sporulation and grain colonization of fungi were associated with increased temperature and RH, leading to a decline in seed germinability, dehydrogenase, and ά-amylase activity, field emergence potential, and seedling vigour (Topani *et al*., 2007). According to Mousa *et al*. (2011), fungal invasion and subsequent contamination of *O. sativa* with mycotoxins begins in the field and is exacerbated by poor post-harvest practices. Although fungi can develop under a wide range of temperatures (5^{o}C – 45^{o}C) (Rossi *et al*., 2009), humid tropical environments favour their growth. Fungal sporulation and mycotoxins can also negatively impact seed storage longevity by affecting cell organelles. Harman and Granett (1972) reported a reduction in pea (*Pisum sativum)* germination from 80% in non-inoculated to 30% in *Aspergillus ruber* inoculated seeds after two weeks of hermetic storage. Comparing the ultrastructure of the embryonic axes of the inoculated with non-inoculated *P. sativum* seeds, the authors observed significant levels of plasmalemma damage, withdrawal of cytoplasm away from the cell wall (CWL), and mitochondrial damage of the inoculated compared with the non-inoculated seeds. These findings suggest the devastating effect fungal activity can have on seed quality and storage longevity.

As alluded to above, oxidative stress/ damage is a consequence of uncontrolled production of ROS and/ or inadequate antioxidant capacity during dehydration, seed deterioration/ageing during storage and germination (reviewed by Bailly 2004; Bailly *et al*., 2008; Rajjou and Debeaujon, 2008; Corbineau, 2012, and references therein). Reactive oxygen species such as superoxide ion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl (•OH) radicals, and hydrogen peroxide (H_2O_2) molecules accumulate during ageing and damage essential macromolecules such as lipids, proteins, and nucleic acids, resulting in cellular dysfunction (reviewed by Bailly 2004; Bailly *et al*., 2008; Rajjou and Debeaujon, 2008, and references therein) and cell ultrastructural damage (Powell and Matthews, 2012; Kong *et al*., 2014; Xia *et al*., 2015a; Yin *et al*., 2016). To alleviate the harmful effects of ROS accumulation during ageing and germination will require the exogenous application of antioxidants to scavenge ROS and improve endogenous antioxidant capacity and/or employ treatments that will minimize or inhibit ROS production (Berjak 1978; Basra *et al*., 1994; Berjak *et al*., 2011; Gondwe *et al*., 2016; Adetunji *et al*., 2020; Fatokun *et al*., 2020a)**.** In deteriorated or aged seeds, the process of imbibition or rehydration will lead to imbibitional damage (Neya *et al*., 2004). Several methods of seed invigoration (e.g., hydropriming, osmopriming, antioxidants, etc.) have been used to repair damaged DNA and cellular membranes and also activate antioxidant mechanisms to detoxify ROS and improve vigour and germinability of aged seeds (reviewed by McDonald 1999; Farooq *et al*., 2009; Paparella *et al*., 2015 and references therein; Adetunji *et al*., 2020). Seed invigoration involves controlled hydration (priming) in which seeds are hydrated and dried back to the original moisture content and uncontrolled hydration (soaking) in which seeds are soaked in water or salt solution and surface-dried to enhance the quality of seeds (Thornton and Powell, 1992; Basra *et al*., 1994; Farooq *et al*., 2009). Studies have shown that soaking crop seeds and surface drying rather than drying back to original moisture content was more effective in enhancing seed quality in onion (*Allium cepa*) (Basra et al., 1994) and rice (Farooq *et al*., 2010).

The principle of electrochemistry was also used to extend the viability of aged *Z. mays* (Pammenter *et al*., 1974; Berjak, 1978). In their work, a negative electric charge applied to aged seeds provided cathodic protection to aged seeds. For example, aged *Z. mays* seeds provided with cathodic protection by placing them in a cathodic field of -300 V potential extended *Z. mays* seed viability compared with the control (aged seeds without cathodic protection) (Pammenter *et al*., 1974; Berjak, 1978), enhanced seedling vigour (Berjak.1978), reduced

conductivity of seed steep water, reduced number of chromosomal aberrations (Pammenter *et al*., 1974) and improved subcellular ultrastructure (Berjak, 1978) than the untreated seeds. Following these studies, several other studies (e.g., Shirahata *et al*., 1997; Hanaoka, 2001; Hanaoka *et al*., 2004; Berjak *et al*., 2011) have used electrolyzed $CaCl_2/MgCl_2$ solution (termed cathodic water; Berjak *et al*., 2011) to demonstrate how CW can provide cathodic protection against ROS attack. Cathodic water is strongly reducing (Hanaoka, 2001; Hanaoka *et al*., 2004; Berjak *et al*., 2011), has strong antioxidant properties (Hanaoka, 2001; Hanaoka *et al*., 2004), and even protect DNA from ROS damage (Shirahata *et al*., 1997). Furthermore, depending on the species, CW has been shown to increase DNA quantity and purity (Fatokun *et al*., 2020a), increase ROS detoxifying antioxidant enzyme activities, and reduce lipid peroxidation products in artificially aged orthodox seeds (Adetunji *et al*., 2020; Fatokun *et al*., 2020 b) and enhanced seed germinability and vigour in naturally aged (Gondwe *et al*., 2016) and artificially aged orthodox seeds (Adetunji *et al*., 2020, 2021; Fatokun *et al*., 2020a, b).

This link between post-harvest seed treatment and deterioration in seed quality and the potential benefits offered by seed invigoration and priming treatments served as the motivation for the present study on alleviating delayed field curing-induced deterioration in rice seeds by CW invigoration, as poor seed germinability and enhancement of seedling performance still persist in the humid tropics due to poor seed quality. The impact of global climate change on seed development, post-harvest drying and drying practices, storage and germination on seed, and seedling responses have led to renewed interest in the assessment of seed quality, especially in the humid tropics where the impact of climate change is greatest. Despite renewed interest in identifying markers of seed quality (seed deterioration/ageing), most of the studies have focused on accelerated ageing- and/or controlled deterioration-induced damage/stress. As a consequence, much work has not focused on characterizing markers of quality, including ultrastructural biomarkers of field curing- and associated storage-induced stress/ damage in the humid tropics. Identifying species/varieties of staple food crops that are less susceptible to seed deterioration during practices such as field curing can aid breeding programs and increase agricultural productivity. Several invigoration methods and different priming agents have been used in an attempt to repair deteriorated/aged seeds but with variable results. Cathodic water has long been shown to effectively scavenge ROS and repair damaged DNA, and recently it has been shown to improve seed germinability and vigour of accelerated aged orthodox seeds than non-electrolyzed salt solutions. However, to date, its use as a seed invigoration/priming

treatment to enhance seed performance has received little attention.

1.3 Aims

1) to investigate how environmental conditions and the duration of field curing influenced seed macro-structural integrity, susceptibility to microbial infection, and seed germinability and vigour; 2) to identify macro-structural and ultrastructural indicators/ biomarkers of field curing and associated storage-induced stress/ damage; 3) to assess whether the invigoration of field-cured seeds with cathodic water (CW), an established antioxidant-based seed invigoration medium, and de-ionized water (DW) can alleviate the deteriorative effects of delayed field curing on rice seed cellular integrity, germination and subsequent seedling growth and biomass.

1.4 Objectives

The specific objectives of this comparative study, which involved an Asian (*Oryza sativa* L.) and African (*Oryza glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*), were as follows: a) to compare the impacts of delayed field curing in wet and dry environments on seed physical, physiological and pathological quality; b) to identify potential ultrastructural biomarkers of seed sensitivity to delayed field curing-induced stress/ damage in embryonic root meristematic cells using transmission electron microscopy (TEM); c) to assess whether CW invigoration alleviates cellular stress/ damage induced by delayed field curing using selected ultrastructural biomarkers of seed sensitivity to such curing-induced stress/ damage; and d) to assess whether invigoration with CW and DW improves seed germinability and emergence, and subsequent plant growth in field cured seeds, relative to non-invigorated (NI) dry seeds.

1.5. Outline of thesis structure

The remainder of this thesis is presented as a series of research articles, each containing an Abstract, Introduction, Materials and Methods, Results, Discussion, and Concluding Remarks. These research articles are from four research chapters (Chapters 2 to 5), which are followed by a Concluding chapter (Chapter 6).

In Chapter 2, the responses of harvested samples of Asian (*O. sativa* L.) and African (*O. glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*) to field curing for different durations in dry (ventilated rainproof containers) and wet (open)

humid tropical field environments in Ghana on physical, physiological and pathological quality are assessed and compared

In Chapter 3, Transmission Electron Microscopy (TEM) was used to identify ultrastructural biomarkers of seed-deterioration sensitivity induced by delayed field curing in an Asian (*O. sativa* L.) and African (*O. glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*) in a humid tropical environment in Ghana. Relationships between seed vigour, germinability, and ultrastructural biomarkers are made within each species in relation to field curing and among the species.

In Chapter 4, TEM was used to assess the alleviatory effects of CW on each of the ultrastructural biomarkers [CWL morphology, cell area, nuclear and nucleolar morphology, mitochondrial morphology, Golgi bodies (G), lipids, cellular vacuolation, plastids, and amyloplasts] of curing-induced stress/ damage identified in Asian (*O. sativa* L.) and African (*O. glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*) in Chapter 3.

In Chapter 5, the effectiveness of CW in ameliorating the effects of delayed field curing on rice seed and subsequent seedling performance was compared among an Asian (*O. sativa* L.) and African (*O. glaberrima* Steud) upland rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*). The species and hybrid were assessed for endosperm integrity, germinability, seedling emergence and vigour, and plant growth following imbibition with CW, DW, and NI. The results were related to caryopsis thickness and endosperm responses to field curing

The final chapter, Chapter 6, integrates the major findings of the various research chapters to provide the major conclusions of the study and make recommendations for future research on alleviating delayed field curing-induced deterioration in rice grown in the humid tropics.

1.6 The study species and the hybrid

Rice is an important cereal crop that provides the food needs for over 50% of the world's population (Rao and Jackson, 1996; Londo *et al*., 2006). Rice is grown globally because of its economic importance as a cash crop and its use as a global food security crop (Prasad *et al*., 2017). Two species of rice are cultivated across the world: An Asian species (*Oryza sativa* L) comprising three subspecies namely *indica*, *japonica*, and *javanica*, and an African species (*O. glaberrima* Steud) native to West Africa (Rao and Jackson, 1996; Kush, 1997; Linares, 2002;

Semon *et al*., 2005; Vaughan *et al*., 2008; Wang *et al*., 2014). These species are grown in a wide range of environments such as upland, lowland/hydromorphic, mangrove swamps, irrigated, and deep water/floating rice environments (WARDA, 1993). Whereas *O. sativa* is widely cultivated across the rice producing regions of the world, the cultivation of *O. glaberrima* is mostly limited to West Africa (Vaughan, 2008). Also, whereas *O. sativa* is known to be high yielding and less tolerant to biotic and abiotic stresses, *O. glaberrima* is known to be low yielding but tolerant to biotic and abiotic stresses (Jones *et al*., 1997; Linares, 2002). Due to the tolerance of *O. glaberrima* to biotic and abiotic stresses, farmers in SSA continued to grow it despite its poor yields. This motivated plant breeders at West Africa Rice Development Association (WARDA, now known as AfricaRice) to combine the high yielding traits of *O. sativa* with the attributes of weed suppressive ability of *O. glaberrima* acquired through morphological plasticity in canopy development by making crosses between the two species (Jones *et al*., , 1997). These crosses or interspecific hybrid (*O. sativa* x *O. glaberrima*), which is called New Rice for Rice (NERICA) (Jones *et al*., 1997; Semon *et al*., 2005).

Rainfall amount and distribution are the most important constraint to upland rice production in Ghana and in other parts of Africa where the ecology exists. In Ghana, the upland ecology is characterized by unpredictable and variable rainfall, increasing temperatures, and longer dry periods (Chemura *et al*., 2020). Rice is produced in this ecology without supplemental irrigation (Zenna *et al*., 2017) and is, therefore, climate sensitive. In Ghana, only less than 2% of its agricultural land is under irrigation, making the country's farmers over-reliant on rainfed production and drought vulnerability (Chemura *et al*., 2020).

The species and the hybrid differ in a number of traits. In terms of grain length and shape (length-to-width ratio) characteristics, *O. sativa* is long and slender (Fig. 1.1a), the hybrid (Fig. 1.1c) is of medium grain length and medium shape, while *O. glaberrima* is short and bold (Fig. 1.1b) (IRRI, 2002). *O. sativa* (Fig. 1.1a) and the interspecific hybrid (Fig. 1.1c) have white pericarp whereas *O. glaberrima* Steud (Fig. 1.1b) has a brown pericarp. The structure of cross-sections of the brown rice (Fig. 1.2) revealed a thicker caryopsis coat in *O. glaberrima* (Fig. 1.2b) than *O. sativa* (Fig. 1.2a) and the hybrid (Fig. 1.2c). Furthermore, the aleurone layer varied from two layers in *O. sativa* (Fig. 1.2a; AL) and *O. glaberrima* (Fig. 1.2b; AL) to four in the interspecific hybrid (Fig. 1.2c; AL). Previous studies (Bam *et al*., 2007; Bam *et al*., 2008) have also shown that *O. glaberrima* had marginally greater longevity than *O. sativa*.

1.7 Significance of the study

The study will deepen the understanding of the physical, pathological, physiological, and ultrastructural lesions that contribute to the loss of rice seed quality (seed viability and vigour) during delayed field curing. The study will also identify a number of seed physiological, ultrastructural, and macro-structural biomarkers/ indicators that can be used by breeders for screening rice varieties for sensitivity/ tolerance to field curing. Although delayed field curing may represent a regional practice, the impact of climate change on seed quality is a global challenge. The results from this study will provide information on CW's ability to repair curing-induced stress/ damage, improve seed vigour, and subsequent seedling growth challenges that threaten food security in many parts of the world.

Figure 1.1 Scanning electron micrographs of (a) *O. sativa*, (b) *O. glaberrima,* and (c) interspecific hybrid (bar = 1 mm in each case) uncured seeds

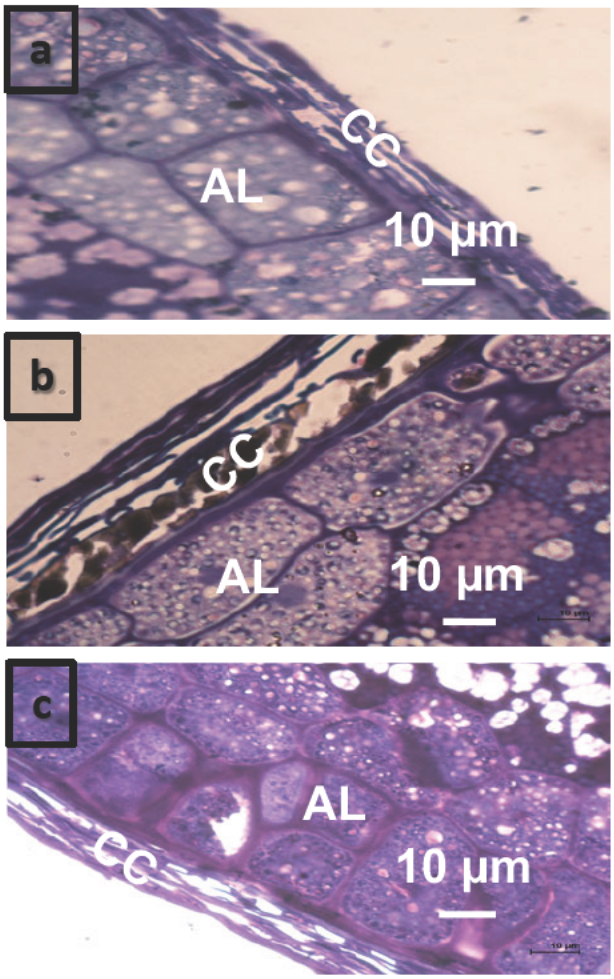

Figure 1.2 Structure of cross section of endosperm of (a) *O. sativa*, (b) *O. glaberrima* and (c) interspecific hybrid: caryopsis coat (CC), aleurone layer (AL). Sections were taken from the dorsal side of the endosperm

CHAPTER 2: Impacts of delayed field curing on rice seed quality in a tropical environment

R.K. Bam, Sershen, B. Varghese, B.M. Dzomeku, Z. Appiah-Kubi, And N.W. Pammenter

2.0 Abstract

Rice farmers in tropical SSA and other developing countries in the tropics prolong curing of harvested panicles in the field before threshing. This practice is believed to compromise the subsequent quality of the seeds as a consequence of being exposed to repeated wetting and drying events. Three upland rice species, viz. *O. sativa*, *O. glaberrima* and an *O. sativa* × *O. glaberrima* interspecific hybrid, were grown in Ghana, harvested, and field cured under open (wet) and within ventilated but rainproof containers (dry) conditions for five weeks. Seeds from both environments were assessed weekly for physical, physiological and pathological quality. The relationships between air temperature and RH, and MC and water activity differed between curing environments but within curing environments, relationships between seed MC and water activity were comparable among species. Prolonged field curing resulted in structural damage evidenced by the formation of multiple cracks in the endosperm; these were more frequent in wet cured seeds. Diverse fungal species were isolated from wet (22 species) and dry (23 species) cured seeds with a number of soil borne species in the wet environment. Curing environment did not influence levels of fungal infection in *O. glaberrima* seeds but dry curing was associated with higher levels of fungal infection in *O. sativa* and *O. sativa* × *O. glaberrima*. Seed germinability in all three species was higher in the dry cured seeds although vigour was relatively lower than wet cured seeds. Field curing seeds of these three species within a dry environment could potentially improve subsequent seed viability, aid in moisture management and minimize structural damage to the endosperm; however, research on how fungal infection could be curbed under such conditions is needed.

2.1 Introduction

Poor seedling establishment is a major constraint to production and productivity in direct seeded rice production systems (Harris *et al*., 2001). Poor seed quality is a major cause of poor seedling establishment, seedling abnormalities or even failure of emergence (Groot *et al*., 2012). Then, farmers can ensure sufficient production by planting high-quality seed or sowing seed of poor quality at high rates to compensate for seed deaths and seedling abnormality. However, since sowing poor quality seed at increasing rates has cost implications for rice production and weed control, the use of high quality seed remains a more viable option. The absence of a quality rice seed production and distribution system in Ghana has led to the use of farmer-saved seeds from previous harvests as the main seed source among farmers. However, such seed is stored in uncontrolled storage environments in which they are exposed to air humidity ranging between 29 and 95% (Bam *et al.,* 2007). Under such fluctuating environmental conditions, rice seeds deteriorate at a faster rate than those stored in controlled environments, for example at -20^{o}C and 2 to 4^{o}C (Hay *et al*., 2013).

Seed storage and potential longevities are affected by amongst other things, seed production environment, genotype and crop maturity (Rao and Jackson, 1996). Prevailing weather conditions during maturity determine the extent of field weathering and hence, the quality of harvested seed (TeKrony *et al*., 1980; Nagata *et al*., 2013) and its subsequent storage potential. Rainy or humid and warm weather conditions have been suggested to favour field weathering and physiological deterioration of seeds (TeKrony *et al*., 1980), which is the case for seed production in tropical rice producing countries including Ghana. This is largely a consequence of crack formation during the early ripening stage in response to high temperatures (Nagata *et al*., 2013). Varietal differences in endospermic cracking propensity exist (Nagata *et al*., 2013) and can benefit hybridization programs if cracking is successfully reduced in high yielding cultivars. Also, additional cracks may develop due to alternating drying and wetting cycles of paddy in the field (Kunze, 2008), which may reduce vigour and viability of seeds.

A common post-harvest practice among rice farmers in Ghana is to leave (cure) harvested rice in the field for a number of weeks (Bam *et al*., 2007), up to five weeks, before threshing (Nyaaba, 2015), mainly due to competition with harvesting of other crops for labour. As commented previously, exposure to diurnal changes in air temperature and RH and the wet and dry cycles typical of a humid tropical environment during field curing of harvested panicles

could influence seed quality and storage longevity by promoting fissure and crack development. Furthermore, this is likely to be accompanied by seed microflora activity, particularly fungi since fungal sporulation and grain mold severity in many crop species are greatly influenced by seed water activity and storage temperature (Topani *et al*., 2007; Mousa *et al*., 2011). Mycotoxigenic fungal invasion and subsequent contamination of rice with mycotoxins begins in the field and is exacerbated by poor post-harvest practices (Mousa *et al*., 2011).

The seed moisture content (MC) of newly harvested grain can compromise the allowable storage time of seeds, with dryer seeds keeping longer. Farmers generally achieve this reduction in MC via field dry down. During this field drying, commonly termed 'natural curing', biochemical processes continue within a seed until the simple sugars are converted into starch and other components such as oil and protein. Water is released during some of these processes and farmers prefer this natural water loss to high heat drying, which compromises seed quality (Menezes *et al*., 2012). Though moisture release from curing is not reversible, increases of seed MC can occur with increases of air humidity. For these reasons, we have termed as 'field curing' the farmers practice of maintaining harvested rice panicles in the field for weeks before threshing, where they are exposed to drying-wetting cycles.

Delayed field curing is an unavoidable practice in the developing countries due to lack of mechanization. At the time of this study there were no published reports on how controlling seed MC more precisely in the field (e.g., by reducing water uptake) can influence seed longevity and subsequent survival. Furthermore, the exact mechanism(s) via which seed vigour and viability are lost during delayed field curing (Nyaaba, 2015) are unclear. Given that delayed field curing is likely to persist in many developing countries it would be prudent to identify strategies that can alleviate the potential negative effects of this practice on seed vigour and viability.

Given the above, the present study tests the following hypotheses: (1) the seeds exposed to wetting and drying (wet cured seeds) cycles during field curing develop more fissures and cracks than those that are field cured within a dry environment (dry cured seeds); (2) wet cured seeds exhibit higher levels of physiological deterioration of both embryo and endosperm prior to, and during storage than dry cured seeds; (3) wet cured seeds exhibit higher levels of microbial infection than dry cured seeds; (4) species differ in terms of their susceptibility to

curing induced cracking, embryo and endosperm physiological deterioration and microbial infection. The study was conducted on three contrasting rice species *Oryza sativa* L., *O. glaberrima* Steud and an *O. sativa* × *O. glaberrima* interspecific hybrid, in a humid tropical environment in Ghana. Comparisons of seed moisture relations, endosperm integrity, microflora activity, germinability and vigour are made within and across species, between wet and dry cured seeds.

2.2 Materials and Methods

2.2.1 Plant material

Three upland rice species, namely *O. sativa* L. subsps. *japonica*, *O. glaberrima* Steud. and an interspecific hybrid between *O. glaberrima* Steud. and *O. sativa* L. were planted at the CSIR-Crops Research Institute, Kumasi, Ghana (06º43.018'N, 01º989'W) on 8 September 2014 and harvested at maturity on 3 February 2015.

2.2.2 Field curing

Harvested panicles of each species were heaped in the field in six approximately equal amounts and a heap of each species was immediately threshed while the remaining five were left in the open field to cure (referred to as 'wet cured', henceforth). The hand threshed, winnowed and cleaned seed of each species were placed in sealed plastic containers and immediately transported to the laboratory for further drying; these seeds served as the controls. Also, panicles of each species were placed in transparent plastic containers (50 cm long, 33 cm high and 32 cm wide) with lids in place and incubated in the field. Holes of approximately 2 x 2 cm were cut into all four sides of the bowls to facilitate gaseous exchange with the atmosphere. The rainproof, yet ventilated containers prevented the seeds from being exposed to the wetting (i.e., rain or dew) and drying cycles typical of tropical environments; this treatment is referred to as 'dry cured', henceforth. The containers were fixed to a wooden pole at about 1.3 m above ground to safeguard them and their contents from tropical windstorms. Thereafter, panicles of the three species were taken from both wet and dry cured environments at weekly intervals (for five weeks), hand threshed, air dried to about 12% MC (fresh mass basis [fmb]) and stored hermetically at 4 ˚C.

2.2.3 Measurement of environmental parameters

Air temperature and RH were logged hourly for five weeks using Tiny tag data loggers (Gemini Data Loggers, UK) placed within each container. For comparison purposes, data on air temperature and RH (from February 3, 2015, to March 10, 2015) was obtained for the wet cured environment from the CSIR-Crops Research Institute's weather station (Davis instruments, France), located c. 200 m from the study site. To minimize the confounding effects of increased temperatures within the containers used for dry curing, these were fixed in the field prior to harvesting, and temperature and RH monitored within them for a month using Tiny tag data loggers. These data were compared to data from the weather station at weekly intervals for four weeks. After each measurement interval, additional holes were inserted into the containers until the temperature ($t = 0.77$, d.f. = 135; $P = 0.442$); and RH ($t = 1.63$, d.f. = 230; $P = 0.105$) were statistically comparable between the wet and dry cured environments.

2.2.4 Seed moisture content and water activity

Seed samples from wet (n = 648) and dry (n = 648) cured environments were taken at 08h00 and 15h00 daily during the five-week curing period for MC determination. Seed MC was first estimated using a grain moisture tester (Kett Electric Laboratory, Tokyo, Japan): MC was determined on seed samples with MC < 17% on a three 5 g milled samples instead of two 5 g samples at 130^{o}C for 2h but for seed samples with MC ≥ 17%, the two-stage drying method was used (International Seed Testing Association, 2005).

Sub-samples of seeds taken for MC determinations (n = 1296) were also used for water activity measurements. Water activity was determined at 20^{o}C on triplicates of milled seed samples using the AquaLab 3TE (Decagon Devices Inc., Pullman, Washington, USA), after seeds were incubated in sealed containers and left overnight in the laboratory to equilibrate.

2.2.5 Assessment of damage to endosperm

At harvest, a batch of panicles of each of the three species were hand threshed and hand de-hulled, non-fissured seeds (of each species) were placed in the wet (n = 2,500 seeds/specie) and dry (n = 2,500 seeds/species/bowl) cured environments. Dry curing was achieved by placing seeds within ventilated rainproof bowls as described earlier. The wet cured seeds were placed in muslin bags and tied onto 2 m high poles in the field to avoid rodent attack. Four replicates

of 20 seeds of each species were randomly drawn daily at 08h00 from the wet and dry cured environments to determine the number of cracked seeds using a Grainscope TX-200 (Kett Electric Laboratory, Tokyo, Japan).

2.2.6 Seed microflora studies

Seed microflora were characterized *in vitro* on seed samples taken weekly from wet and dry cured environments using the blotter method (International Seed Testing Association, 2001). Two hundred seeds (eight replicates of 25 seeds) per species, per environment, per week were randomly selected and surface sterilized with 1% NaOCl for 3-5 min, washed three times with distilled water, and placed on wet blotters within plates (n = 25 seeds per plate) and incubated for seven days at 28±2ºC under a 12 h near ultraviolet light and 12 h darkness cycle (Mathur and Kongsdal, 2003). Habit characteristics of fungal colonies found on the infected seeds were examined under the stereoscopic microscope (Leica MS 5). A compound microscope (Leica DMLS) was then used to identify fungal species based on morphological characteristics (Mathur and Kongsdal, 2003).

2.2.7 Seed germination and vigour

Thirty-six seed lots of the three species (18 each from wet and dry environments) were sampled for MC after field curing for five weeks and the remaining seed were stored at 4ºC (for close to 21 days) until they were withdrawn from storage and held in sealed bags overnight at 25ºC to equilibrate before germination assessments. Four replicates of 50 seeds of each wet cured seed sample and 25 seeds (because of limited quantity of seeds from the dry cured environment) of each dry cured seed sample for each sampling time were tested for germination between moist rolled anchor seed germination paper towels measuring 10" x 15" (Anchor paper Co., USA) at alternating temperatures of 34/1˚C (16 h day/8 h night) (Ellis *et al*., 1983) for 14 days. The first germination counts were made on day 7 and all hard, non-germinated seeds were de-hulled to remove any effect of seed coat dormancy. Final germination counts were made on day 14 of germination. The criterion for germination was normal seedling development (International Seed Testing Association, 2005).

Seed vigour was based on radicle length and seedling dry weight. Seedlings were selected for radicle length and dry weight measurements using randomized allocation and selection of numbers. The lengths of five randomly selected seedlings per replicate, per treatment (n=20)

were measured 7 days after initiation (DAI). Seedling dry mass for each replicate of a treatment was measured on 10 randomly selected seedlings at 14 DAI. Seedlings were dried at 80ºC for 72 h before being weighed to four decimals.

2.2.8 Data analyses

For both wet and dry cured environments, weather data collected at 08h00 and 15h00 from the weather station and Tiny tag data loggers for the wet and dry cured environments were used. Relationships between air temperature and RH were compared within and between the wet and dry cured environments using Simple Linear Regression with Groups (GenStat Release 17, VSI International, UK). The relationship between seed MC and water activity of the three species was also compared between the wet and dry cured environments. Differences in the relationships between seed MC and water activity in the wet and dry cured environments were determined using a comparison of regressions. Cubic models were fitted to the data to quantify the relationships. For each curing environment, the data for MC and water activity for the three species were pooled for the analysis.

The number of seeds infected with fungi was compared within each isolated fungal species between the curing environments using a chi-square test of independence (with data for the various sampling times being pooled). Where the number of isolates was less than 5, the CHIPERMTEST procedure was followed to calculate the chi-square value and probability. Data on total number of seeds infected per species was also compared between curing environments using chi-square test of independence (GenStat Release 17, VSI International, UK). Germination and seed vigour (radicle length and seedling dry weight) data were compared within and across species, wet cured and dry cured, and within sampling times using analysis of variance (ANOVA; GLM Multivariate) (IBM SPSS version 24). Germination data were normalized using arcsine transformation while seedling length and dry weight data were normalized using the common logarithm ($\log_{10}$) before analysis. The Bonferroni post-hoc test was used to separate means at the 5% level of significance. Standard error (SE) was calculated for all means shown.

2.3 Results

2.3.1 Field curing environments

The mean air temperature for the wet cured environment was 28.8±0.6°C and 31.1±1.1°C for the dry cured environment. The mean air RH was 71.8±7.5% and 61.5±6.9% for the wet and dry cured environments, respectively. Air temperature and RH were significantly and positively related in both environments (Fig. 2.1). Comparison of regressions also showed significant (r^2 = 0.75; $p < 0.001$) differences between the wet and dry cured environments.

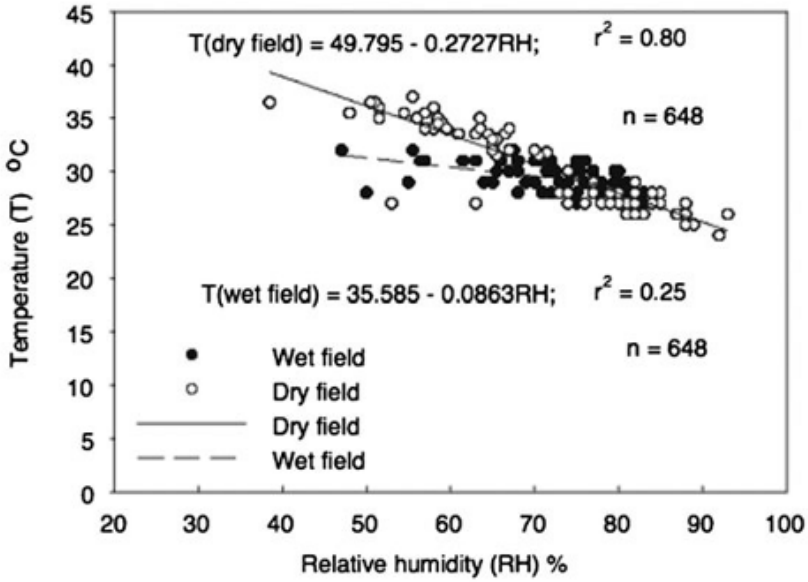

Figure 2.1 Relationships between air temperature and relative humidity of three rice species (*O. sativa*, *O. glaberrima* and *O. sativa* × *O. glaberrima*) dry cured (n = 648) (open symbols) and wet cured (n = 648) (filled symbols) over 5 weeks. Comparison of regressions showed significant ($r^2 = 0.75 \pm 0.146$; $p < 0.001$, n = 1296) differences between wet and dry field cured environments.

2.3.2 Water activity and seed moisture content

Seed water activity at 08h00 and 15h00 was similar between curing environments for all species (Fig. 2.2A and B). A similar trend was observed for seed MC in both environments (Fig. 2.2C and D). Water activity in dry cured seeds was significantly ($p < 0.001$) lower than in wet cured seeds (Fig. 2.2A and B). The mean water activity ranged from 0.55 ± 0.15 in the dry cured to 0.67 ± 0.23 in the wet cured seeds. The mean MC was 11.32 ± 1.98% and 14.23 ± 5.50% for

dry and wet cured environments, respectively. The data also showed that the wet cured seeds were subjected to a more pronounced daily drying (day) and wetting (night) cycles over the five weeks (Fig. 2.2).

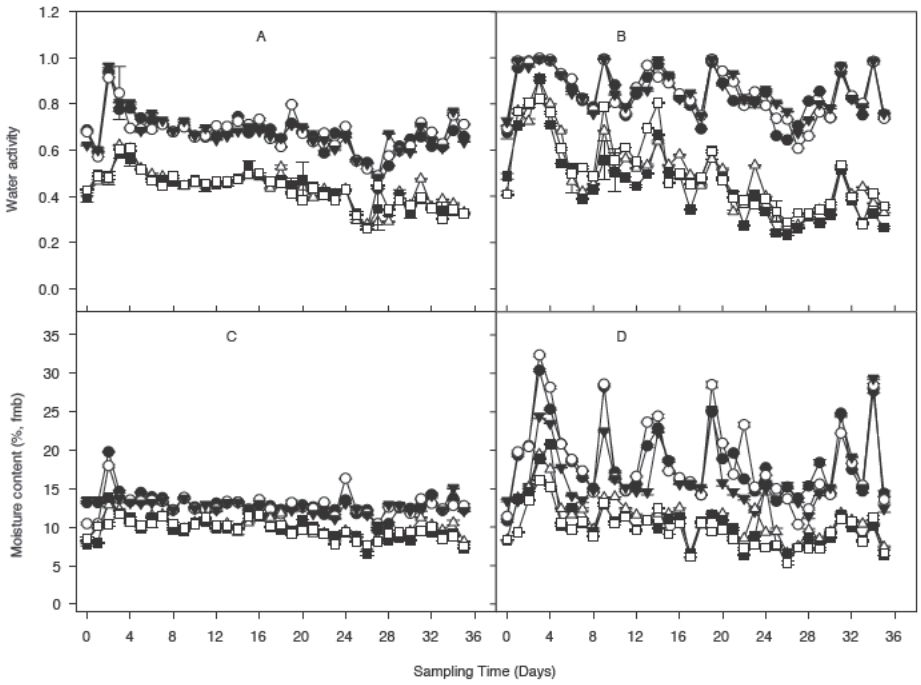

Figure 2.2 **Diurnal changes in water activity and seed moisture content of *O. sativa* (open circles at 08h00; filled squares at 15h00), *O. glaberrima* filled inverted triangles at 08h00; open squares at 15h00 and *O. sativa* × *O. glaberrima* (filled circles at 08h00; open inverted triangles at 15h00) seeds dry and wet cured for 5 weeks. A: Water activity of seeds sampled from dry cured environment, n = 648; B: Water activity of seeds sampled from wet cured environment, n = 648; C: Moisture content of seeds sampled from dry cured environment, n = 648; D: Moisture content of seeds sampled from wet cured environment, n = 648. Values represent mean of three replicates ± SD.**

2.3.3 Relationships between seed moisture content and water activity

The relation between seed MC and water activity of the three species was sigmoidal and described by a cubic model in both wet and dry cured field environments (Fig. 2.3; Table 2.1). The sorption characteristics in the wet and dry cured environments could not show the inflection point (water activity values between 0 and 0.20) but showed linear (water activity values between 0.20 and 0.85) and reflection points (water activity > 0.85) similar to an isotherm. The

reflection point was more pronounced in the wet than dry cured environment. Comparison of the relationships between seed MC and water activity in the dry and the wet field cured environments showed significant ($r^2 = 0.82$; $p < 0.001$) differences between environments. To test if there were differences in the relationships between seed MC and water activity between the dry and wet cured environments at water activity below 0.85, water activity values 0.85 and above were removed from data sets for both dry and wet cured seeds before analysis. Comparison of relationships between seed MC and water activity showed significant ($r^2 = 0.77$; $p < 0.001$, n = 1124) differences between both environments. Within both environments, differences among species in their sorption characteristics were not significant ($p > 0.05$).

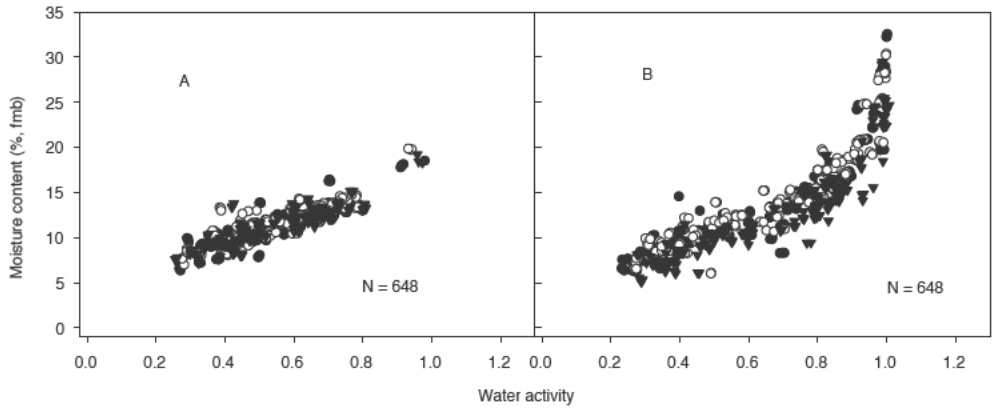

Figure 2.3 Relationships between seed moisture content and water activity of seed samples of *O. sativa*, *O. glaberrima* and *O. sativa* × *O. glaberrima* taken from dry cured (A) (n = 648) and wet cured (B) (n = 648) at 08h00 and 15h00. The relationship is described by cubic model in the dry (A) and wet (B) field cured environments. Comparison of regressions showed significant ($r^2 = 0.822 \pm 0.185$; $p < 0.001$, n = 1296) differences in the sorption characteristics of the three species among the dry and wet field cured environments.

Table 2.1 The relationship is described by a cubic model ($y = a + bx + cx^2 + dx^3$) for wet and dry field cured environments: *y* = moisture content (%, fmb) and x is water activity of seed

Curing Environment	*a* (s.e.)	*b* (s.e.)	*c* (s.e.)	*d* (s.e.)	Adjusted r^2
Wet cured	-4.63	0.687	-0.0113	0.0000720	0.87
	(1.615)	(0.087)	(0.0010)	(0.0000010)	
Dry cured	5.94	0.024	0.0022	0.0000018	0.76
	(1.793)	(0.002)	(0.0002)	(0.0000001)	

Harvested panicles of three rice species were field cured in wet and dry field environments for five weeks. Samples of seed were taken at 08h00 and 15h00 each day in sealed bottles. Cubic models were fitted to the data from both curing environments. Values represent mean ± SE. Comparison of regressions showed significant differences in the relationship between dry and wet field cured environments ($r^2 = 0.822 \pm 0.185$; $p < 0.001$, n = 1296).

2.3.4 Endosperm crack frequency

The effect of field curing on the percentage seed exhibiting endosperm cracking (labelled '% cracking' henceforth) in the three rice species, in wet and dry cured environments is shown in Fig. 2.4. In all three species % cracking increased with curing time, irrespective of the environment. Percentage cracking in the dry cured environment was initially below the wet cured environment in all species but seeds in both curing environments eventually reached 100% (Fig. 2.4). At harvest crack frequency was initially low (4.0±0.7%) for all the three species, however, after just three days of curing in the wet environment this reached 40.4±4.5% in all the three species and by two weeks of curing percentage cracking reached 100% in all three species. Within the wet cured environment, cracking percentage reached 100% in nine days in *O. sativa* and 10 days in *O. sativa* × *O. glaberrima* and *O. glaberrima*. However, within the dry cured environment, percentage of cracks reached 100% in 11 days in *O. sativa*, 12 days in *O. sativa* × *O. glaberrima* and 14 days in *O. glaberrima* (Fig. 2.4).

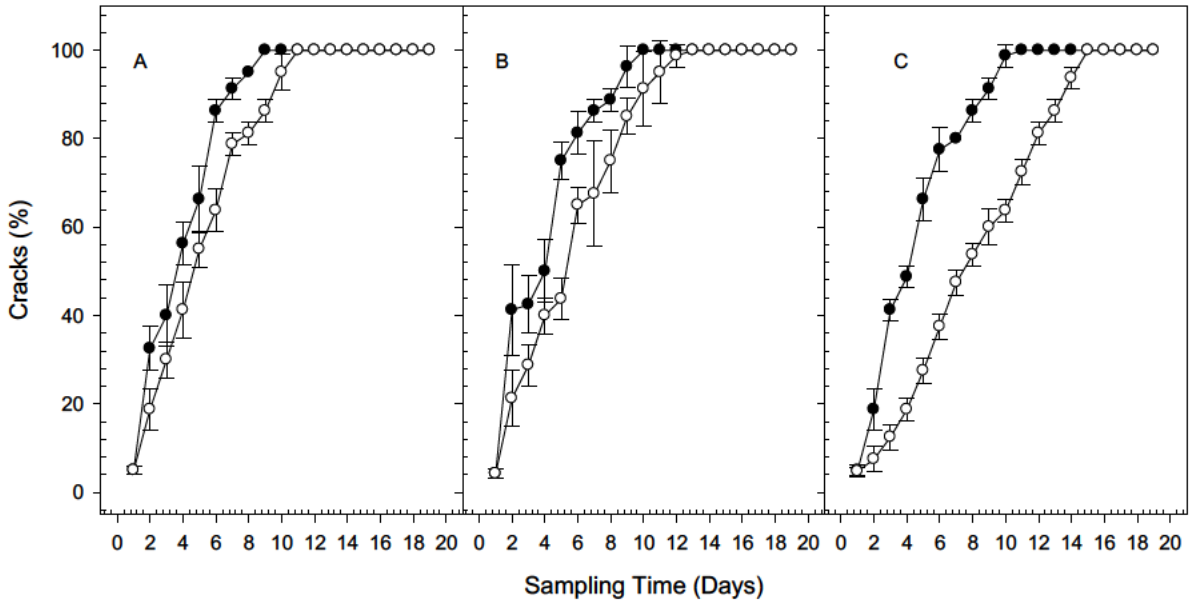

Figure 2.4 Time course of crack development in harvested seed samples of *O. sativa* (A), *O. sativa* × *O. glaberrima* (B) and *O. glaberrima* (C) dry cured (open symbols) and wet cured (filled symbols). Values represent mean of four replicates ± SD.

2.3.5 Seed microflora

A number of fungal species were isolated from wet (22) and dry (23) cured environments (Table 2.2A, B, C). The frequency of occurrence of some of the fungal species such as *Aspergillus niger, Curvularia pallenses, Curvularia oryzae, Fusarium equisetti, F. oxysporium, F. solani*, *Nigrospora oryzae*, *Penicillium* sp and *Myrothecium* sp was, however, low and thus all subsequent analyses of the occurrence of fungal species was limited to 13 species.

Two categories of fungi were isolated from the samples taken from wet and dry cured environments: saprophytic and potential pathogenic colonizers. The saprophytic colonizers were *Rhizopus* sp, *Botrytis* sp, *Phoma* sp and *Trichoderma* sp while the remaining isolates were potential pathogenic colonizers (Table 2.2A, B, C). The most frequently occurring fungal species across the three species were *Rhizopus* sp, *A. flavus, B. oryzae, C. lunata, F. pallidoroseum, F. monilliforme, Melanospora* sp, *Phoma* sp, *Sclerotium rolfsii* and *Verticillium* sp. Except for *Botrytis* sp, *F. pallidoroseum*, *Melanospora* sp, *S. rolfsii* and *Verticillium* sp, which preferentially colonized the wet cured seeds, the remaining species preferentially colonized the dry cured seeds of all three species (Table 2.2A, B, C). *Botrytis* sp and *S. rolfsii*

were confined to wet cured samples. Except for the fourth week of sampling when *Verticillium* sp was isolated from dry cured *O. sativa* seeds, these species were not isolated in dry cured *O. glaberrima* and *O. sativa* × *O. glaberrima* seeds. Across the three species, *A. flavus, B. oryzae*, *C. lunata*, *F. molliniforme* and *Phoma* sp were associated with dry cured seeds. The number of seeds in which *Alternaria* sp, *S. rolfsii*, *Trichoderma* sp and *Verticillium* sp occurred in the wet and dry cured samples were low, and generally associated with wet cured seeds and where they occurred in the dry cured environment, they were only isolated from four and five week cured seeds. Except for *O. glaberrima* where fungal infection was comparable between curing environments, a significantly higher number of seeds infected with fungi occurred in dry cured *O. sativa* ($P = 0.016$) and *O. sativa* × *O. glaberrima* seeds ($P < 0.001$; Table 2.3). Across species, significantly ($P < 0.001$) higher fungal infection was associated with the dry cured environment (Table 2.3).

Table 2.2A Fungal species isolated and number of seeds infected with each fungal species isolated from three rice species cured in wet (W) and dry (D) cured environments for various sampling times (weeks).

Species	Sampling Time	Fungal species isolated from wet (W) and dry (D) cured rice seeds																							
		Rhizopus sp		*Aspergilus flavus*		*Bipolaris oryzae*		*Botryti s sp*		*Curvularia lunata*		*Fusarium pallidoroseum*		*F. moniliforme*		*Melanospora sp*		*Phoma sp*		*Alternaria sp*		*Sclerotium rolfsii*		*Trichoderma sp*		*Verticilliu m sp*	
	Week	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
	0	0	0	16	16	45	45	0	0	23	23	4	4	6	6	0	0	17	17	0	0	0	0	0	0	0	0
O. s	1	0	0	6	31	1	15	3	0	9	14	13	4	7	19	12	2	6	44	2	0	0	0	1	0	18	0
	2	0	0	0	16	12	13	1	0	3	14	2	17	2	7	27	6	6	23	2	0	0	0	1	0	51	0
	3	3	1	1	15	16	36	0	0	14	21	0	4	4	30	6	0	8	46	0	0	0	0	2	0	50	0
	4	0	90	0	13	23	14	7	0	19	10	17	2	7	25	4	9	12	14	0	0	41	0	13	1	0	7
	5	0	55	1	15	23	17	0	0	11	8	5	0	21	27	11	9	26	25	0	2	25	0	2	1	14	0
Total		**3**	**146**	**24**	**106**	**120**	**130**	**11**	**0**	**79**	**90**	**41**	**31**	**47**	**114**	**60**	**26**	**75**	**169**	**4**	**2**	**66**	**0**	**17**	**2**	**133**	**7**
χ^2 *(5 d.f., Wet vs Dry)*		110.98; *P*<0.001		31.00; *P*<0.001		21.66; *P*<0.001		–		12.21; *P*= 0.032		36.77; *P*<0.001		13.54; *P*=0.019		18.00; *P*=0.001		34.70; *P*<0.001		6.00; *P* =0.197		–		2.49; *P*=0.569		140.00; *P*<0.001	

Seed samples of *O. sativa* (Table 2.2A), *O. glaberrima* (Table 2.2B) and *O. sativa* × *O. glaberrima* (Table 2.2C) were assessed from the day of harvest (week 0) and thereafter weekly for 5 weeks from wet and dry cured panicles for the occurrence of seed mycoflora. At each sampling, 200 seeds (eight replicates of 25 seeds per replicate) were randomly sampled from each species from each curing environment. The totals for the various fungal species isolated from wet and dry cured environments represented the number of seeds from which the fungal species were isolated for five weeks. Chi-square values were not calculated where fungal species were isolated from only one curing environment. O. s = *O. sativa*

Table 2.2B Fungal species isolated and number of seeds infected with each fungal species isolated from three rice species cured in wet (W) and dry (D) cured environments for various sampling times (weeks).

Species	Sampling Time	Fungal species isolated from wet (W) and dry (D) cured rice seeds																									
		Rhizopus sp		*Aspergilus flavus*		*Bipolaris oryzae*		*Botryti s sp*		*Curvulari a lunata*		*Fusarium pallidorose um*		*F. moniliforme*		*Melanosp ora sp*		*Phoma sp*		*Alternaria sp*		*Sclerotiu m rolfsii*		*Trichoderma sp*		*Verticilliu m sp*	
	Week	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
	0	29	29	4	4	27	27	0	0	65	65	7	7	6	6	1	1	23	23	0	0	0	0	0	0	0	0
O. g	1	0	36	0	16	0	15	24	0	12	11	10	2	8	3	0	0	15	21	0	0	0	0	3	0	0	0
	2	1	50	0	8	5	16	18	0	18	8	27	1	8	7	2	1	9	25	0	0	0	0	3	0	0	0
	3	38	0	1	3	4	35	10	0	19	26	0	0	0	13	3	3	14	17	0	1	0	0	0	0	7	0
	4	0	47	2	18	9	1	0	0	2	15	0	5	1	15	12	0	11	5	4	0	48	0	3	0	24	0
	5	0	61	0	16	20	6	5	0	11	7	5	1	18	15	0	2	17	5	2	1	0	0	10	3	10	0
Total		**68**	**223**	**7**	**65**	**65**	**100**	**57**	**0**	**127**	**132**	**49**	**16**	**41**	**59**	**18**	**7**	**89**	**96**	**6**	**2**	**48**	**0**	**19**	**3**	**41**	**0**
χ^2 *(5 d.f., W et vs Dry)*		204.55; *P*<0.001		20.06; *P*=0.002		54.36; *P*<0.001		–		15.72; *P*=0.008		27.47; *P*<0.001		25.45; *P*<0.001		11.77; *P*=0.015		17.38; *P*=0.004		4.44; *P*=0.217		–		2.40; *P*=0.533		–	

O. g = *O. glaberrima*

Table 2.2C Fungal species isolated and number of seeds infected with each fungal species isolated from three rice species cured in wet (W) and dry (D) cured environments for various sampling times (weeks).

Species	Sampling Time	Fungal species isolated from wet (W) and dry (D) cured rice seeds																									
		Rhizopus sp		*Aspergilus flavus*		*Bipolaris oryzae*		*Botryti s sp*		*Curvulari a lunata*		*Fusarium pallidoroseu m*		*F. moniliforme*		*Melanosp ora sp*		*Phoma sp*		*Alternaria sp*		*Sclerotiu m rolfsii*		*Trichoderma sp*		*Verticillium sp*	
	Week	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
O. s × O. g	0	0	0	2	2	37	37	0	0	31	31	12	12	8	8	3	3	16	16	3	3	0	0	0	0	0	0
	1	0	0	7	14	0	47	8	0	25	23	24	18	15	58	0	0	20	22	0	0	0	0	0	0	12	0
	2	0	0	0	15	7	27	2	0	17	42	21	3	16	4	7	1	13	37	3	0	0	0	10	0	2	0
	3	7	0	1	20	6	24	2	0	7	53	3	11	3	15	3	0	4	17	0	2	36	0	0	0	5	0
	4	0	2	0	8	9	26	0	0	5	20	0	0	3	43	1	0	11	28	2	1	44	0	2	4	9	0
	5	0	1	2	17	36	35	0	0	11	18	5	1	21	51	11	1	26	16	4	3	25	0	2	2	14	0
Total		**7**	**3**	**12**	**76**	**95**	**196**	**12**	**0**	**96**	**187**	**65**	**45**	**66**	**179**	**25**	**5**	**90**	**136**	**12**	**9**	**105**	**0**	**14**	**6**	**42**	**0**
χ^2 *(5 d.f., Wet vs Dry)*		10.00; *P*=0.075		16.60; *P*=0.007		48.64; *P*<0.001		–		30.53; *P*<0.001		18.57; *P*=0.006		45.34; *P*<0.001		6.30; *P*=0.260		20.96; *P*<0.001		5.15; *P*=0.356		–		8.89; *P*=0.009		–	

O. s × O. g = *O. sativa* × *O. glaberrima*

Table 2.3 Total number of seed samples from three rice species infected with fungal species isolated in wet and dry cured environments during various sampling times (weeks).

Sampling Times	*O. sativa*		*O. glaberrima*		*O. sativa* × *O. glaberrima*		*Across species	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
0	111	111	162	162	112	112	385	385
1	78	129	72	104	111	182	261	415
2	106	96	91	116	98	129	295	341
3	104	153	96	98	77	142	277	393
4	143	185	116	106	86	132	345	423
5	139	159	98	117	157	145	394	421
Total	681	833	635	703	641	842	1,957	2,378
χ^2 (5 d.f., Wet vs Dry)	14.00; $p = 0.016$		7.55; $p = 0.183$ **NS**		24.11; $p <$ 0.001		26.68; $p < 0.001$	

O. sativa, *O. glaberrima* and *O. sativa* × *O. glaberrima* were assessed from the day of harvest (week 0) and thereafter weekly for 5 weeks from wet and dry cure panicles for the occurrence of seed mycoflora. Total number of seeds from which fungal species were isolated from each rice species were pooled for wet and dry curing environments. Identical number of seeds of *O. glaberrima* from wet and dry cured environments were infected with fungi. Significantly higher number of seeds of *O. sativa* ($p = 0.016$) and *O. sativa* × *O. glaberrima* ($p < 0.001$) from dry cured environment were infected with fungi.

*Total number of seeds from which fungal species were isolated from the three rice species in wet and dry cured environments was pooled for each sampling time. NS = Not significant

2.3.6 Seed germination and vigour

Differences in seed quality measured as normal seedling production (radicle and shoot production) differed significantly between species ($p < 0.001$), sampling time ($p < 0.001$) and curing environments ($p = 0.023$) (Table 2.4). *O. glaberrima* and *O. sativa* generally exhibited greater seed germinability than *O. sativa* × *O. glaberrima*, being higher in the dry than wet cured environment. Compared with the control, seed germinability started declining from week 2. By week 5, percentage germination had declined significantly from 91% in the control to 76% (Table 2.5). Seed germinability was significantly influenced by species × sampling time and species × curing environment interactions. There were, however, no significant interactions among species × sampling time × curing environment (Table 2.4).

Seedling vigour was evaluated in terms of radicle length and seedling dry weight. Significant differences in radicle length ($p < 0.0001$), and dry weight ($p < 0.0001$) was observed between species, sampling times and field cured environments (Table 2.5). Within the species, *O. sativa* produced the longest radicles followed by *O. glaberrima* and then *O. sativa* × *O. glaberrima*. Dry cured seeds produced seedlings with shorter radicles and lower dry weight compared with wet cured ones. Compared with the control, radicle length began declining in cured seeds from week 3 onwards. In contrast, seedling dry weight declined relative to the control from week 2 onwards (Table 2.5). There was significant species × sampling time ($p = 0.027$) and sampling time × curing environment interactions. No significant species × curing environment interactions was observed for radicle length and seedling dry weight. There was also no significant interaction between species × sampling time × curing environment (Table 2.4).

Table 2.4 Results of the analysis of variance (ANOVA) for germination (%) and radicle length (cm) and seedling dry weight (mg) of three rice species field cured in wet and dry environments.

Factor	*d.f.*	F_G-value	Germination Probability (*P*)	F_R-value	Radicle length Probability (*P*)	F_S-value	Seedling dry weight Probability (*P*)
Species (S)	2	10.807	< 0.001	152.798	< 0.001	74.990	< 0.001
Sampling Time (T)	5	25.299	< 0.001	21.069	< 0.001	13.317	< 0.001
Environment (E)	1	5.307	0.023	365.194	< 0.001	61.199	< 0.001
S x T	10	2.235	0.022	0.330	0.971 NS	2.140	0.027
S x E	2	3.891	0.024	1.563	0.214 NS	2.872	0.061 NS
T x E	5	1.229	0.302NS	1.110	0.360 NS	4.071	0.002
S x T x E	10	1.035	0.421NS	0.674	0.746 NS	0.519	0.873 NS
Error	95			*		**	

d.f. = degrees of freedom; F_G is the F-value for germination (%), F_R is the F-value for radicle length and F_S is the F-value for seedling dry weight.
*Error *d.f.* for radicle length = 101; **Error *d.f.* for seedling dry weight = 104. NS = Not significant ($P > 0.05$)

Table 2.5 Main effects of species, environment and sampling time (weeks) on seed germinability (%) and vigour (radicle length (cm) and seedling dry weight (mg)) of three rice species during delayed field curing in wet and dry cured environments.

Species				Environment				Sampling Time			
	Germination	Radicle length	Seedling dry weight		Germination	Radicle length (cm)	Seedling dry weight		Germination	Radicle length	Seedling dry weight
O. sativa	86[a]	5.92[a]	23.52[a]	Wet cured	83[a]	5.68[a]	23. 23[a]	0	91a	5.47[a]	24.21[a]
								1	87[ab]	5.14[af]	22.70[ab]
O. sativa × *O. glaberrima*	81[b]	4.20[b]	19.59[b]	Dry cured	85[b]	4.16[b]	20.89[b]	2	86[bc]	5.05[ab]	22.03[b]
								3	82[cd]	4.82[bcfg]	21.93[bc]
O. glaberrima	85[a]	4.62[c]	23.61[a]					4	79[de]	4.45[dgh]	21.14[bdf]
								5	76[e]	4.34[eh]	20.37[ef]

Different lower case letters in a column show significant differences in germination, radicle length and seedling dry weight across species, curing environment and sampling time.

2.4 Discussion

Though delayed field curing of rice has been, and is very likely to continue to be, an unavoidable practice in many developing countries (e.g., Ghana), no published reports on how changes in seed MC during curing influences seed vigour and viability are available at the time of this study. Herein, we showed how field curing in a dry environment could potentially improve subsequent seed viability by improving MC management. Furthermore, the results show that the benefits of dry field curing may be largely based on the minimization of structural damage to the endosperm, which is promoted during wet field curing.

Much work has explored the theory of hygroscopic behaviour of seeds under controlled environments (Hogan and Karon, 1955; Hay and Timple, 2016), but fewer report the effect in the field, particularly from a large sample as in our study. However, in the field environment in which seeds are most often cured in tropical rice growing regions air temperature and RH do not remain constant. The present study evaluated the sorption behaviour of rice using climatic data during field curing in the wet and dry environments for five weeks. The relationship between MC and water activity for the three rice species was similar and sigmoidal in the wet and dry cured environments (Fig. 2.3). However, the reflection point or multi-molecular (water-to-water molecules) water binding sites (Vertucci and Leopold, 1987) dominated the sigmoid curve more in the wet cured than the dry cured environment. The difference in the shape of the relationships between the wet and dry cured environments might be due to inter-environment differences in temperature and hydration levels. When compared with dry cured environment, lower temperatures in the wet cured environment could be due to evaporation from soil and plant tissues. In contrast, heat produced from respiratory activities of seed and fungi within the dry cured environment could have led to higher temperatures. Differences in hydration levels suggested hydration induced structural changes in cellular components due to fewer number of multilayer of water molecules (water-to-water molecules) or tighter molecular packing of polymer chains resulting from the higher temperatures in the dry cured environment (Vertucci and Leopold, 1987). The dominance of multi-molecular water binding sites in the wet cured environment would have implications for seed storage as high water activity could result in deteriorative processes leading to vigour and germination decline. The similarity of the sorption

characteristics for the species in the wet and dry curing environments (Fig. 2.3) suggested that these species had similar chemical composition (Vertucci and Leopold, 1987).

At harvest, 4% of the seeds exhibited endospermic cracks in all three species (Fig. 2.4), which probably occurred during grain filling or maturation. Alternate drying and wetting cycles during field curing, especially during unfavourable climatic conditions, resulted in the development of additional cracks (Kunze, 2008). Higher percentage cracks observed in the wet cured seeds might be as a result of higher magnitudes of diurnal moisture change resulting from moisture desorption and re-adsorption and large moisture differentials between the internal and surface of seeds in the wet cured environment. Such moisture differentials can induce tensile stresses at the centre of the grain as accumulated moisture induces seed surface layer expansion (Kunze and Choudhury, 1972). In contrast, within the dry cured environment (where diurnal changes in seed MC remained almost constant) (Fig. 2.2C), lower rate of moisture adsorption presumably allowed for moisture to diffuse deeper and at a much slower rate into the grain thereby reducing the intensity of tensile stresses at the centre of the grain (Kunze, 2008). This difference may also have accounted for the displacement of the time course for cracking in the dry cured below the wet cured environment until they all reached 100% (Fig. 2.4).

B. oryzae and *C. lunata* are two seed borne fungi which were most frequently isolated across the three species at harvest (Table 2.2A, B, C) and suggested that infection occurred during crop growth or after maturation, before harvest. These two fungal pathogens were also significantly more predominant in the dry cured than wet cured seeds (Table 2.2A, B, C). The total number of seeds infected with specific fungal species in the wet cured environment was lower than the dry cured environment (Table 2.3) probably due to the release of anti-fungal proteins and hydrolytic enzymes, which accumulate in dead seed coats or pericarps, upon hydration of the wet cured seeds (Raviv *et al*., 2017). The water activity of wet cured seeds was also more frequently above 0.85 (Fig. 2.3B), and this may have facilitated higher activity of such anti-fungal enzymes, if present. Rossi *et al.* (2009) also suggested that reduction in number of seeds infected with fungi in environments subjected to precipitation might be due to washing off of conidia by rain or heavy dew or by dispersal of conidia by wind. They showed that sampling stalk residues of *Z. mays* from the field after rainfall decreased the number of fungal spores in field exposed stalk residues compared with *Z. mays* stalks kept in a dry condition. In contrast, wide temperature range within the dry cured environment (Fig. 2.1) probably

presented a more conducive environment for fungal sporulation and hence higher fungal infection from seeds cured in this environment.

Soil-borne fungal species, e.g., *Botrytis* sp, *Alternaria* sp, *S. rolfsii*, *Trichoderma* sp, and *Verticillium* sp (Bonner *et al*., 1994; Notteghem *et al*., 1997), were mostly isolated from wet cured seed samples (Table 2.2A, B, C). The absence of these fungal species in the control seed samples at harvest, their complete absence or occurrence in weeks four and five samples only in the dry cured environment, and occurrence mainly in wet cured seeds (Table 2.2A, B, C) demonstrated the impact of prolonged field curing on the invasion of soil-borne fungal species. *Pyricularia oryzae* was not isolated in this study, despite its reputation as a seed borne pathogen of rice, and this is consistent with the findings of Fisher and Petrini (1992), who did not also isolate *P. oryzae* from rice seeds. The work of Ou (1985) suggests that this may be because the fungus requires rice plant extracts in culture media to grow.

Germination, radicle length, and dry weight all declined with delayed curing (Table 2.5). This was to be expected based on the increased endosperm cracking, and fungal infection with delayed curing. Fungal activity has a deleterious effect on seed germination and vigour (Nghiep and Gaur, 2004). Unfavourable climatic conditions such as high temperature, RH, and rainfall during post-harvest field curing impacted negatively on the physiological quality of seeds (TeKrony *et al*., 1980). The effect of deteriorated or poor quality seed is manifested in vigour loss, slower, uneven rate of germination, reduced seedling vigour, and seedling abnormalities (Groot *et al*., 2012).

Seed germination was higher in the dry cured compared with the wet cured environment (Table 2.5). In contrast, radicle length and dry weight were lower in the dry cured compared with the wet cured environment (Table 2.5). Germination count was made 7 DAI of germination whereas measurement of seedling root and dry weight determination were made 14 DAI. By 14 DAI, there were mycelia growth mostly observed on seedlings from the dry cured environment and may explain the observed trend. Despite lower MC and water activity in the dry cured environment, high temperature (Fig. 2.1) was probably supportive of fungal sporulation and mycotoxin contamination of seeds, and this may explain why higher numbers of seeds were infected with fungi in this environment. Decline in seed germinability, and seedling vigour, fungal sporulation and grain colonization have been reported as a consequence

of increasing temperature and RH levels (Topani *et al*., 2007). Some seed-borne pathogens in the germinating seeds resulted in seedling decay, thereby affecting the seedling survival. *B. oryzae*, *A. padwickii* and *C. lunata* were isolated from rice seedlings with decayed roots and shoots while *B. oryzae, C. curvularia* and *Aspergillus* sp, were also isolated from seedlings with short roots in previous studies (Nghiep and Gaur, 2004). In our study, *B. oryzae*, *C. lunata* and *Aspergillus* sp preferentially colonised dry cured seeds and might have also been responsible for the observed decline in seedling weight and radicle length observed in seedlings arising from dry cured seeds (Tables 2.2 and 2.5).

In this study, prolonged field curing compromised the integrity of the endosperm by promoting multiple cracks in the endosperm. The dry cured environment reduced seed MC and the amount of active water in seeds, and delayed cracking, implying that it has greater potential to minimize seed deterioration during curing and subsequent storage than wet curing. Curing environment did not influence the number of seeds with fungal infection in *O. glaberrima* but dry curing was associated with higher fungal infection in *O. sativa* and *O. sativa* × *O. glaberrima*. Fungal infection of seeds in the dry cured environment was higher compared with wet cured seeds. Prolonged field curing also increased the vulnerability of wet cured seeds to soil borne fungal infection. Species, curing environment, and delayed field curing significantly influenced seed germination and seedling vigour. Germination and seedling vigour declines with prolonged field curing have implications not only for seed quality but also subsequent seedling establishment and grain yield. These findings suggest that dry curing could be an option for improving farmers' current post-harvest practices due to its positive effect on the management of MC, seed germinability, and its ability to delay endosperm cracking however, research on how fungal infection could be curbed under such conditions is needed.

CHAPTER3: Ultrastructural biomarkers of rice seed deterioration-sensitivity to field curing in a tropical production environment: a comparison of an Asian and African species and their interspecific hybrid

R.K. Bam, B. Varghese, S. Naidu, W.R. Woodenberg, N.W. Pammenter and Sershen

3.1 Abstract

Seed quality is of fundamental importance to food production and identifying species/varieties of staple food crops that are less susceptible to seed deterioration during practices such as field curing can aid breeding programmes and increase agricultural productivity. This study aimed to identify potential ultrastructural biomarkers of seed sensitivity to delayed field curing-induced stress/ damage in embryonic root meristematic cells using TEM. Seeds of one rice species of Asian (*O. sativa* L.) origin, one of West African (*O. glaberrima* Steud) origin and their interspecific hybrid (*O. sativa* × *O. glaberrima*) were exposed to delayed field curing in a humid tropical environment in Ghana. Seeds were subjected to different durations of field curing (0, 2 and 5 weeks), hermetically stored at 4°C for 20 months and subsequently fixed for microscopy after imbibition with DW for 18 h. Untreated dry seeds, cured for different durations (0, 2 and 5 weeks) were used as the controls for two reasons: 1) to establish whether ultrastructural abnormalities induced by curing are permanent or can be reversed/repaired during imbibition; 2) to establish whether seed deterioration during curing leads to imbibitional damage. Cell area, nuclear and nucleolar sizes, and number of some key organelles (e.g. mitochondria, lipid bodies, and amyloplasts) differed between imbibed and NI seeds of uncured and cured in both species and the hybrid but these differences were not always statistically significant. The frequency and/severity of ultrastructural abnormalities increased with delayed field curing in parallel with viability and vigour loss. Marked CWL folding observed only in cured *O. glaberrima* seeds was reversed/repaired by imbibition. However, this was not the case for the slight CWL folding observed in NI 5 week-cured *O. sativa* seeds. In contrast, there was no CWL folding in NI cured hybrid seeds. Ultrastructural abnormalities such as separation of the CWL and plasmalemma was observed in NI and imbibed, cured *O. sativa* and hybrid seeds. Field curing induced abnormalities in cell, nuclear and nucleolar morphology, number of vacuoles or inclusions of organelle debris, and morphology and number of lipid bodies in both species and the hybrid prior to imbibition. However, they differed in terms of their responses to imbibition. Mitochondria in cured seeds (of both species and the hybrid) also exhibited

internal derangement or a distorted shape. Electron density of the mitochondrial matrix, greater differentiation of Cr and recovery of mitochondrial membrane integrity increased after imbibition in line with germinative metabolism, but these features decreased in prominence with delayed field curing. There were signs of mitochondrial fission in 5 week-cured seeds which is thought to reflect oxidative damage and was most pronounced in the hybrid. We conclude that the severity of ultrastructural lesions related to cellular stress/ damage differed across species and the hybrid and progressed at a rate in parallel with the viability and vigour loss caused by delayed field curing. The present study has therefore served to identify a number of ultrastructural biomarkers of delayed field curing induced stress/ damage in rice seeds. These biomarkers may serve as useful screening tools for rice breeders looking to identify species/varieties that produce seeds that are more resistant to field curing-induced deterioration and potentially to assess the utility of seed invigoration/priming treatments in improving vigour and viability in deteriorated rice seeds.

Keywords: Transmission electron microscopy, mitochondria, lipid bodies, nucleus, ultrastructure

3.2 Introduction

Climate change impacts negatively on many interactions between biology and climate (Fernandez-Pascual *et al*., 2015) and renders food production systems vulnerable (Agnolucci *et al*., 2020). Sub-Saharan Africa has been reported as one of the regions with the greatest impacts of climate change on food productivity and security (Agnolucci *et al*., 2020).

Seed maturation environment (Ellis *et al*., 1993; 1994), environmental conditions during seed maturation drying and post-harvest drying practices (Probert *et al*., 2007; Whitehouse *et al*., 2015) are important factors that can influence rice seed quality and storage longevity. During seed desiccation or maturation drying, cells shrink, and tissues become highly compressed and form glasses within the cytomatrix to protect seeds in the dry state (Koster, 1991; Walters and Koster, 2007; Manfre *et al*., 2009; Sano *et al*., 2016). Further drying of seeds at the end of the desiccation phase is required to reduce the rate of seed ageing and improve subsequent seed viability and longevity in storage (Hay and Probert, 1995; Probert *et al*., 2007; Whitehouse *et al*., 2015). Drying seeds at the end of the seed desiccation phase is important because the moisture content of mature seeds is still high and will affect the subsequent rate of viability loss

if seeds are not dried ex-planta (Leprince *et al*., 2017; Whitehouse *et al*., 2017). Therefore, in developed countries, especially in the temperate regions, seeds are harvested during the onset of the maturation phase for artificial drying (Ellis *et al*., 1987), a practice that ensures high quality seed production (Ellis *et al*., 1993; Whitehouse *et al*., 2015, 2017) among commercial seed producers. On the contrary, in almost all countries in SSA, including Ghana, about 90-95% of seed production is in the informal seed supply system in smallholder farming systems (Balasubramanian *et al*., 2007; Dorward *et al*., 2007; Marfo *et. al*., 2008; Seck *et al*., 2010). Notwithstanding, legislation governing seed production, distribution and sale of seeds are non-existent in about 75% of the countries in SSA and in countries including Ghana where seed acts have been enacted, seed quality standards are not being enforced due to logistical constraints (Balasubramanian *et al*., 2007). Consequently, seed quality continues to be a major constraint to rice production in SSA.

Delayed field curing in these tropical environments may disturb the metabolically and physically quiescent state of the seeds and subsequently compromise seed quality by allowing for deteriorative processes such as unbalanced metabolism at intermediate water contents, which could lead to oxidative stress (Walters and Koster, 2007; Kranner *et al*., 2010) and damage cellular ultrastructure and biological macromolecules (e.g., lipids, proteins, DNA and RNA) (Powell and Matthews, 2012). Cellular membranes may serve as a major site of damage to cell ultrastructure during desiccation (Leprince *et al*., 1993; Walters *et a*l., 2002; Benamar *et al*., 2003; Woodenberg *et al*., 2018) as cells shrink and cause tensions to be created within cell membranes leading to the shearing of membrane connections to the CWL (reviewed in Walters *et al*., 2002; Woodenberg *et al*., 2018) and loss of membrane material through vesiculations (reviewed in Walters *et al*., 2002; Corbineau *et al.* 2004). Therefore, the ability of a protoplasm to minimize damage from desiccation and/or rehydration cycles, maintain cellular and ultrastructure integrity in the dry state and mobilize repair mechanisms on rehydration are key to establishing desiccation tolerance in plants and plant tissues (Bewley, 1997; Walters and Koster, 2007) and seed viability (Webb and Arnott, 1982). These ultrastructural biomarkers of seed deterioration could potentially be used to compare species and varieties for seed sensitivity to environmentally-induced seed deterioration. Ultrastructural abnormalities in the nuclei, mitochondria and plastids of stored *Z. mays* seeds (Berjak 1986), coalescence of lipid bodies into lipidic mass or larger units and withdrawal of plasmalemma from CWL of naturally aged pine (*Pinus pinea*) seeds (Castro and Martinez-Honduvilla 1984) and in accelerated aged oats

(*Avena sativa*) and *O. sativa* (Kong *et al*., 2014; Xia *et al*., 2015a; Yin *et al*., 2016), mitochondria exhibiting loss of Cr and vacuole-like structure in accelerated aged *A. sativa* and *O. sativa* seeds (Kong *et al*., 2014; Xia *et al*., 2015a,b; Yin *et al*., 2016) and loss of mitochondrial membrane in accelerated aged or desiccated *P. sativum* (Benamar *et al*., 2003; Wang *et al*., 2012a) and aged *O. sativa* seeds (Yin *et al*., 2016) have been reported as ultrastructural biomarkers of seed deterioration. Identifying species/varieties of staple food crops that are less susceptible to environmentally-induced seed deterioration can aid breeding programmes and increase agricultural productivity.

A number of studies have been reported on the relationships between biochemical and ultrastructure changes of embryo cells during desiccation and accelerated ageing in the literature but there is dearth of information on ultrastructural responses to environmentally induced seed deterioration especially in tropical crop production systems. In this regard, we used transmission electron microscopy (TEM) to investigate the ultrastructural indicators of seed deterioration-sensitivity to field curing-induced damage in two rice species and their interspecific hybrid. The species investigated were from an Asian origin (*O. sativa* L.), West African origin (*O. glaberrima* Steud) and their interspecific hybrid, were produced in a tropical environment in Ghana. Comparisons are made within and across species and the hybrid between NI and DW imbibed uncured and cured seeds, and NI across field curing period.

3.3 Materials and Methods

3.3.1 Plant material and field curing

The methods used for this aspect of the study follow those described for wet cured seeds in sections 2.3.1 and 2.3.2 of Chapter 2.

3.3.2 Seed moisture content determination

Seeds that were stored for 20 months after 0, 2, and 5 weeks of curing (both species and hybrid) were withdrawn from storage (4°C), equilibrated overnight at 25°C and seed MC was thereafter determined gravimetrically. Seed MC was determined on five replicates of five seeds each, per treatment. Seeds were oven-dried at 130°C for 2h (International Seed Testing Association, 2005), cooled on silica gel and weighed using a five-place electronic balance (Mettler, MT5; Germany). Moisture content was then calculated on a fmb.

3.3.3 Seed imbibition

Samples of 20 months stored 0, 2 and 5 week cured seeds (both species and hybrid) were removed from storage, equilibrated overnight at 25°C, imbibed with DW for 18 h and used to assay germination. Two hundred and sixty seeds each of both species and the hybrid cured for 0, 2 and 5 weeks were imbibed with DW by placement on two layers of germination towel moistened with 5 ml DW and covered with another layer of germination towel moistened with 5 ml DW within 9 cm Petri dishes (250 seeds per dish, with treatments being placed in dedicated dishes), sealed with parafilm. The Petri dishes containing the seeds were slowly agitated on an orbital shaker (Labcon, SPO-MP 15, Laboratory Marketing Services, Maraisburg, South Africa) for 18 h at 25°C. Excess moisture on the seed surface was then dried with blotting paper (Farooq *et al*., 2010) before germinating the seeds in DW or processing seeds for TEM. Untreated dry seeds cured for 0, 2 and 5 weeks were used as the controls for this study.

3.3.4 Seed germination and vigour

Four replicates of 50 seeds each, per treatment combination (total of 18 treatment combinations) comprising DW (9) and NI (9) were used for this study. Methods for seed germination and vigour (radicle length) measurements follow those described in section 2.27 of Chapter 2.

3.3.5 Sample preparation for transmission electron microscopy

The method of sample preparation for TEM was modified after Bechtel and Pomeranz (1978). Five randomly selected seed samples (i.e., five replicates) (Sershen *et al*., 2016) of each of the treatment combinations (DW and NI seeds of *O. sativa, O. glaberrima* and *O. sativa* × *O. glaberrima* cured for 0, 2 and 5 weeks) were prepared for TEM. Each seed was de-husked after which the epithelial tissue surrounding the embryo of each seed was removed to enhance tissue fixation. The embryo of each seed sample was excised from the endosperm and fixed in 4.5% glutaraldehyde with 3.0% paraformaldehyde in 0.05 M phosphate buffer at pH 6.7 and then held at 4°C for 24 h. The embryos were washed three times in a 0.1 M phosphate buffer for 5 min each, and post-fixed in 0.5% osmium tetroxide for 2 h at room temperature. The samples were washed again three times in 0.1 M phosphate buffer for 5 min each, dehydrated in graded acetone [30%, 50%, 75%, for 2 × 10 min each, and 100% for 2 × 20 min each] followed by

infiltration, first in a 1:1 mixture of epoxy resin:100% acetone for 6 h and then in whole epoxy resin for 24 h, and embedded in fresh low-viscosity resin and polymerized at 70°C for 8 h.

3.3.6 Microtomy and microscopy

Three randomly selected embryos out of the five replicates fixed per treatment combination were sectioned using a Reichert-Jung Ultracut E microtome (Leica, Vienna, Austria). Survey sections measuring 1 μm were cut, stained with 1% toluidine blue and viewed with a light microscope to reach the embryonic root meristematic region, after which ultrathin sections of the embryonic root meristems were then collected on copper grids and stained with 2% aqueous uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963). The ultrastructural changes of the embryonic root meristems were examined using a Joel JEM 1010 electron transmission microscope (JEOL, Tokyo, Japan) at 100 kV using iTEM Soft Imaging System GmbH imaging software. The ultrastructure of the embryonic root meristem cells of the three randomly selected embryos per treatment combination including the dry samples were viewed and images of normal and abnormal features of the cells were captured using the iTEM Soft Imaging System GmbH imaging software for further analysis. ImageJ version 1.50i (Wayne Rasband National Institutes of Health, USA) was subsequently used for measuring cell area, and nuclear and nucleolar sizes (using images captured at a constant magnification of ×10,000) while the ultrastructure of a number of discernible cell organelles (lipids, vacuoles, mitochondria, plastids and amyloplasts) was observed using images captured at ×25,000 in each of six cells in the three replicates viewed from each of the DW and NI treatments.

3.3.7 Statistical analysis

Data generated in the study were tested for normality using a Shapiro-Wilks Test prior to analysis. Percentage germination was normalized using arcsine transformation before analysis. Data on seed germination (14 days DAI) and vigour (measured as radicle length at 7 DAI), cell area, nuclei size, nucleolar size, and subcellular organelles (number of lipids, vacuoles, mitochondria, amyloplasts and plastids) were compared within species, across field curing periods, and within field curing periods between seed treatments. Data were analysed using analysis of variance (ANOVA; GLM; three-way and two-way ANOVA) using SPSS 25 (IBM, SPSS Statistics, Chicago, IL, USA): Two-way ANOVA was used for within species response to field curing and seed imbibition treatment while three-way ANOVA was used when data for

both species and the hybrid were pooled for analysis. Means were separated using the Bonferroni post-hoc test at P = 0.05.

3.4 Results

3.4.1 Seed germination and vigour

Seed germinability of NI seeds declined significantly by 36% in *O. sativa*, 30% in *O. glaberrima* and 50% in *O. sativa* × *O. glaberrima* after 5 weeks of field curing (Fig. 3.1). Similarly, seed vigour measured as radicle length 7DAI also declined significantly by 35% in *O. sativa*, 28% in *O. glaberrima* and 30% in *O. sativa* × *O. glaberrima* after 5 weeks of field curing (Figs. 3.2).

In *O. sativa*, seed germinability did not differ between DW and NI after 0, 2 and 5 weeks of curing (Fig. 3.1A). In contrast, *O. glaberrima* seed germinability differed significantly between DW and NI seeds after 0 (P = 0.023), 2 (P = 0.014) and 5 (P = 0.012) weeks of curing (Fig. 3.1B). For the hybrid, it was only after 2 weeks curing that DW exhibited significantly (P = 0.022) higher germination than the NI seeds (Fig. 3.1C). When the two species and hybrid was compared, irrespective of imbibition treatment, the hybrid (94 ± 3%) had significantly higher percentage seed germination than *O. glaberrima* (91 ± 4%) and *O. sativa* (88 ± 5%) when the seeds were not cured. For 2 week cured seeds, *O. glaberrima* (82 ± 5%) and *O. sativa* (76 ± 9%) had significantly higher germination than *O. sativa* × *O. glaberrima* (69 ± 7%). However, after 5 weeks of curing, *O. glaberrima* (67 ± 7%) had significantly higher germination than *O. sativa* (58 ± 6%) and *O. sativa* × *O. glaberrima* (52 ± 8%).

In *O. sativa,* after 0, 2 and 5 weeks of curing, DW produced significantly [5% (P = 0.013), 11% (P = 0.018) and 15% (P = 0.018), respectively] longer radicles at 7 DAI than the NI (Fig. 3.2A). In *O. glaberrima,* the difference in radicle length between DW and NI after 0 week of curing was negligible. On the contrary, radicle length between DW and NI at 7 DAI was 13% and 20% significantly longer after 2 and 5 weeks of curing, respectively (Fig. 3.2B). For the hybrid, the difference between DW and NI after 0 week of curing was negligible. In contrast, radicle length between DW and NI was 7 and 9% significantly greater after 2 and 5 weeks curing, respectively (Fig.3. 2C). When the two species and hybrid were compared, irrespective of imbibition treatment, *O. sativa* radicle length was significantly greater in 0 (82.0 ± 2.8mm) and 2 (72.6 ±

5.5mm) week cured seeds than the hybrid (0 week cured, 72.8 ± 4.0 mm; 2 week cured, 60.8 ± 5.1 mm) and *O. glaberrima* (0 week cured, 70.4 ± 5.6 mm; 2 week cured, 63.6 ± 6.3 mm) but these differences across the species and hybrid were not observed in 5 week cured seeds.

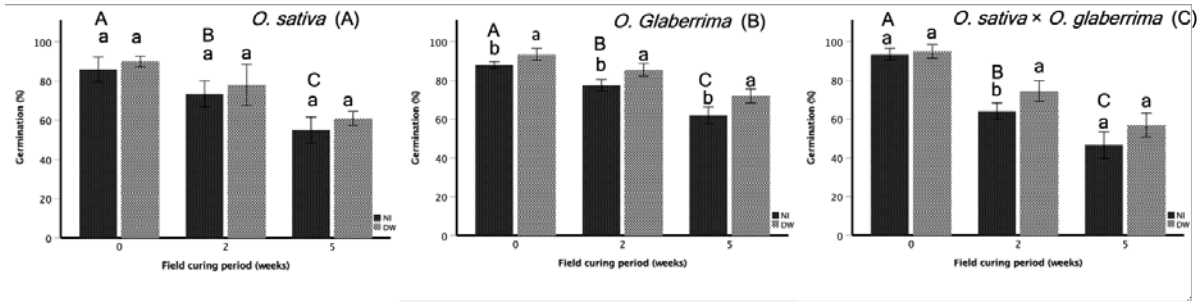

Figure 3.1 Percentage seed germination of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* from each post-storage imbibition treatment [non-imbibed (NI); imbibed with deionized water (DW) for 18 h] of seeds field cured for 0, 2 and 5 weeks. Values represent mean (±SE of mean) (n = 50 seeds for each of four replicates). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across seed imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

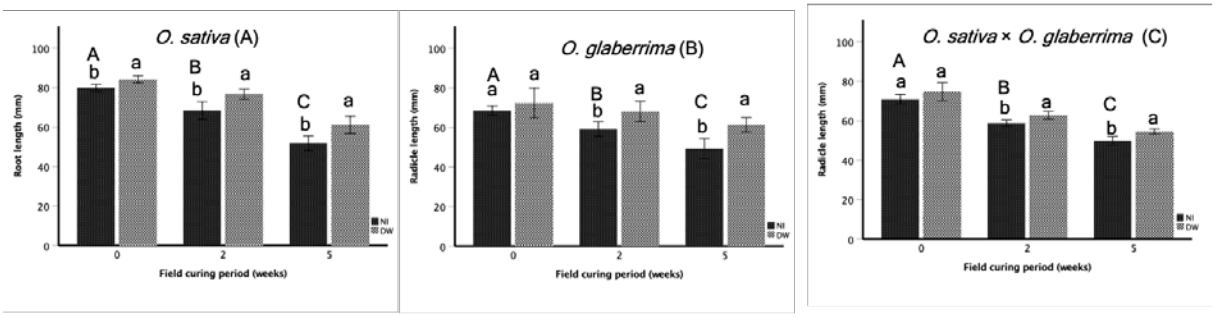

Figure 3.2 Radicle length at 7 DAS of seedlings of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* from each imbibition treatment (NI, DW) of seeds field cured for 0, 2 and 5 weeks and stored for 20 months. Values represent mean (± SE of mean) (n = 10 seedlings for each of four replicates). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.2 Ultrastructure and subcellular organelle response to delayed field curing and imbibition

All the ultrastructural observations reported on below were based on observations of embryonic root meristematic cells. Given that one of the main objectives of the study was to identify ultrastructural biomarkers of rice seed deterioration-sensitivity to field curing, we have focused on seven ultrastructural biomarkers traditionally used to assess seed response to stress (Webb and Arnott, 1982; Wang *et al*., 2012a; Xia *et al*., 2015a,b; Sershen *et al*., 2016).

3.4.3 Changes in cell wall morphology

Plasmalemma was appressed to the CWLs of the cells in NI uncured (0 week) seeds of both species and the hybrid (Figs. 3.3a, 3.4a, 3.5a). 0 week cured seeds of *O. sativa* (Fig 3.3a), *O. glaberrima* (Fig. 3.4a) and the hybrid (Fig. 3.5a) displayed no CWL convolution or folding. When 0 week cured seeds of both species and the hybrid were imbibed with DW for 18 h, the plasmalemma remained appressed to the CWLs (Figs. 3.3b, 3.4b, 3.5b). Cell wall folding was not observed in NI 2 week cured seeds of both *O. sativa* (Fig. 3.3c) and the hybrid (Fig. 3.5c) but there appeared to be the withdrawal of plasmalemma from the CWL and marked separation of adjacent CWLs in 2 week cured *O. sativa* seeds imbibed with DW (Fig. 3.3d, insert). Marked CWL folding was apparent in the NI seeds of 2 weeks cured *O. glaberrima* seeds (Fig. 3.4c, arrowhead), but this was reversed when the seeds were imbibed with DW for 18 h (Fig. 3.4d). Unlike *O. sativa* and *O. glaberrima*, numerous vesicles appeared to be closely associated with the CWL of the hybrid suggesting leakage of cytoplasmic content into the CWL (Fig. 3.5c) (Corbineau *et al*., 2004). The cells of DW imbibed 5 week cured seeds of both species and the hybrid exhibited greater ultrastructural damage than either 0 or 2 week cured seeds but this damage was more severe in 5 week cured *O. sativa* (Fig. 3.3e) and hybrid (Fig. 3.5e) than *O. glaberrima* (Fig. 4e) seeds. In NI 5 week cured *O. sativa* seeds, slight CWL folding (CWL; Fig. 3.3e, insert), withdrawal of the plasmalemma from the CWL in cells in some parts of the embryonic root meristem cells (Fig. 3.3e, insert, asterisks) and what appeared to be separation of adjacent CWLs was seen (Fig. 3.3e). There appeared to be co-signs of plasmalemma withdrawal from the CWL and marked separation of adjacent CWLs in the hybrid (Fig. 3.5e). In both *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f), the cells appeared to be plasmolysed to some extent after the seeds were imbibed in DW for 18 h, even though these seeds exhibited 57 ± 3% and 56 ± 5% viability, respectively. Also, both *O. sativa* and the hybrid exhibited

separation of adjacent CWLs in some parts of the embryonic root meristem. In contrast, *O. glaberrima* (Fig. 3.4e, arrowhead) exhibited a marked CWL folding after 5 weeks of curing but the plasmalemma remained appressed to the CWL. In *O. glaberrima* (Fig. 3.4f), the marked CWL folding observed in the NI 5 week cured seeds was completely reversed by DW imbibition although there appeared to be separation of adjacent CWLs in some parts of the embryonic root meristem. There was no apparent signs of plasmolysis in *O. glaberrima* (Fig. 3.4f) cells as seen in *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f).

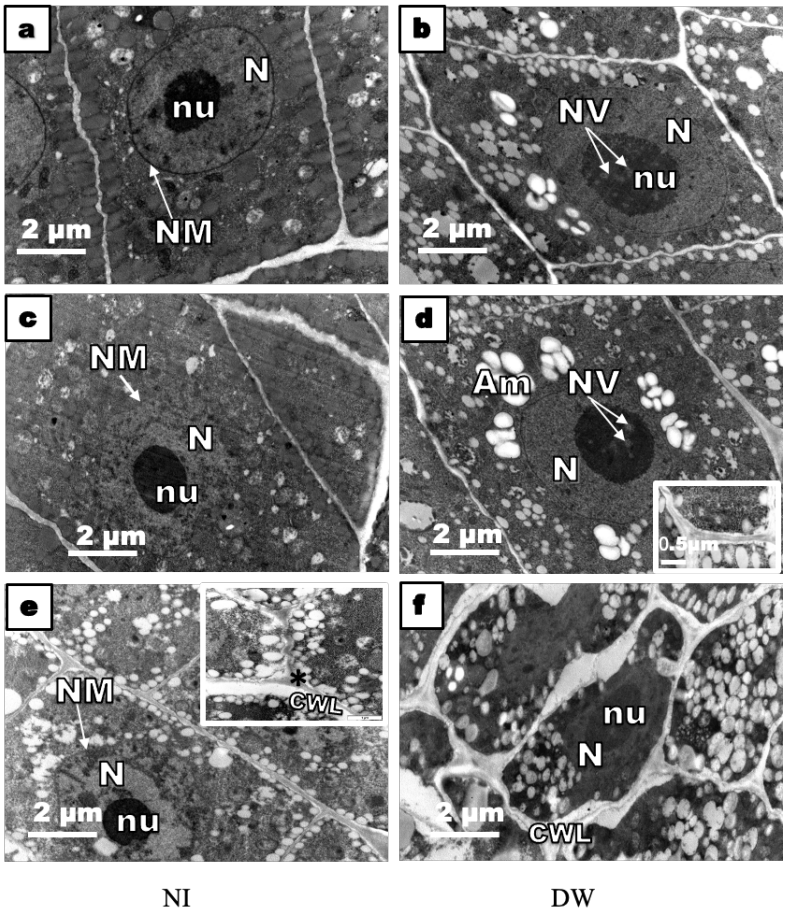

Figure 3.3 Changes in ultrastructure of embryonic root meristem cells of 20-month stored *O. sativa* field cured for 0, 2 and 5 weeks in dry (NI) and imbibed (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibition; N nucleus, nu nucleolus, NM nuclear membrane, NV nucleolus vacuole, CWL cell wall, Am amyloplast

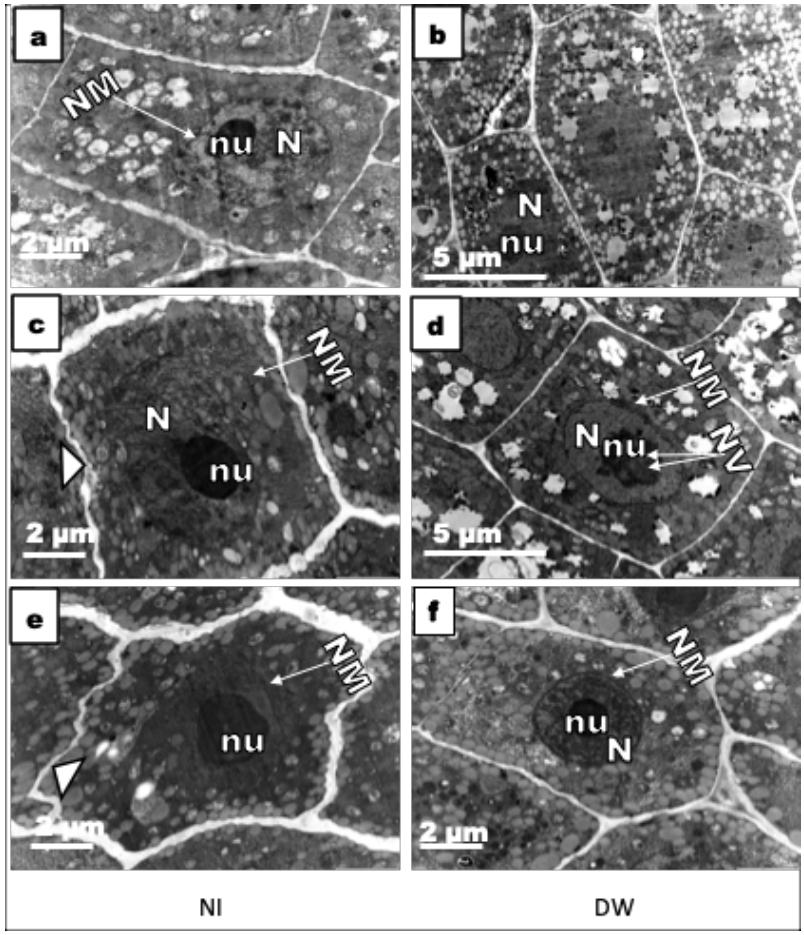

Figure 3.4 Changes in ultrastructure of embryonic root meristem cells of 20-month stored *O. glaberrima* field cured for 0, 2 and 5 weeks in dry (NI) and imbibed (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibition; N nucleus, nu nucleolus, NM nuclear membrane, NV nucleolus vacuole, arrowhead marked CWL folding

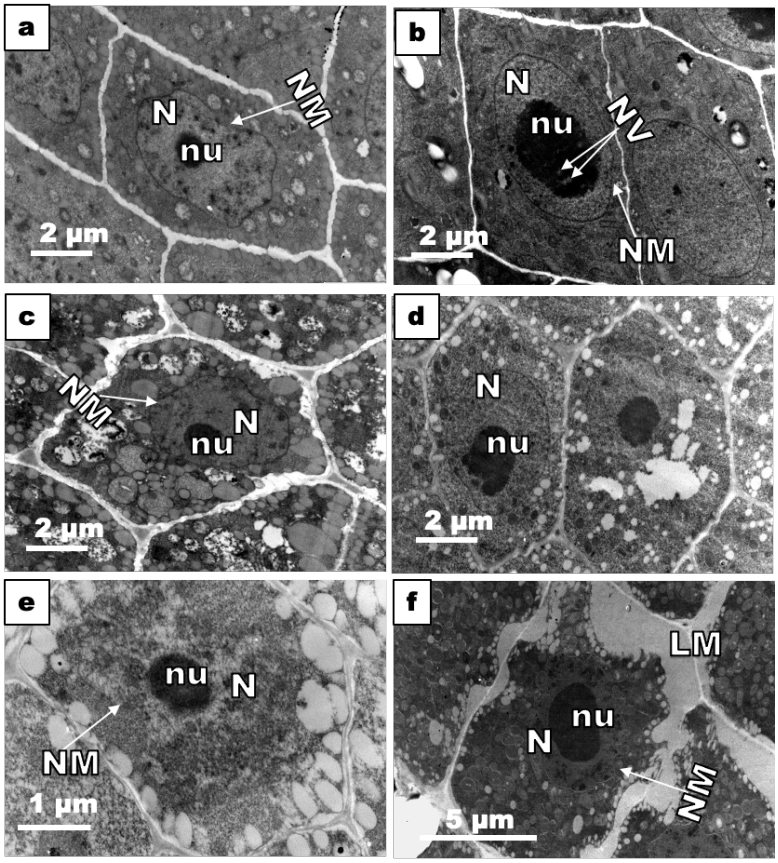

Figure 3.5 Changes in ultrastructure of embryonic root meristem cells of 20-month stored hybrid (*O. sativa* × *O. glaberrima*) field cured for 0, 2 and 5 weeks in dry (NI) and imbibed (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibition; N nucleus, nu nucleolus, NM nuclear membrane, LM lipid mass, NV nucleolus vacuole.

3.4.4 Changes in cell area

Delayed field curing resulted in significant cell shrinkage in NI cells in both species and the hybrid (Fig. 3.6). Cell areas of the NI seeds shrank significantly: 48% in *O. sativa* (Fig. 3.6A), 31% in *O. glaberrima* (Fig. 3.6B) and 50% in the hybrid (Fig. 3.6C) with 5 weeks of curing. In *O. sativa*, cell area in DW imbibed 2 week cured embryos was significantly ($P = 0.023$) larger than NI 2 week cured seeds (Fig. 3.6A). In contrast, cell area was comparable between DW imbibed and the NI 0 and 5 week cured *O. sativa* seeds (Fig. 3.6A). In *O. glaberrima* (Fig. 3.6B), cell areas were significantly larger in DW imbibed 0 ($P = 0.036$) and 2 ($P = 0.017$) week cured seeds when compared with the NI seeds whereas the cell area in DW and NI 5 week cured seeds was comparable ($P = 0.407$). In the hybrid (Fig. 3.6C), apart from the DW imbibed 0 week cured seeds which had significantly ($P = 0.038$) larger cell area than the NI seeds, cell area in DW imbibed and NI 2 ($P = 0.101$) and 5 ($P = 0.821$) week cured seeds were comparable. When the two species and hybrid were compared, irrespective of the imbibition treatment, the cell area was significantly larger ($P = 0.001$) in *O. sativa* (90.20 ± 7.09 µm2) than both *O. glaberrima* (76.08 ± 13.81 μm^2) and the hybrid (70.24 ± 11.48 μm^2) in 0 week cured seeds. For the 2 week cured seeds, significantly ($P < 0.001$) larger cell area was observed in both *O. sativa* (73.74 ± 11.16 μm^2) and *O. glaberrima* (66.03 ± 8.51μm^2) than in the hybrid (51.40 ± 9.99 μm^2). When seeds were cured for 5 weeks, *O. glaberrima* (48.58 ± 9.32 μm^2) and *O. sativa* (44.04 ± 12.89 μm^2) had comparable but significantly ($P = 0.015$) larger cell area than the hybrid (29.62 ± 7.78 μm^2).

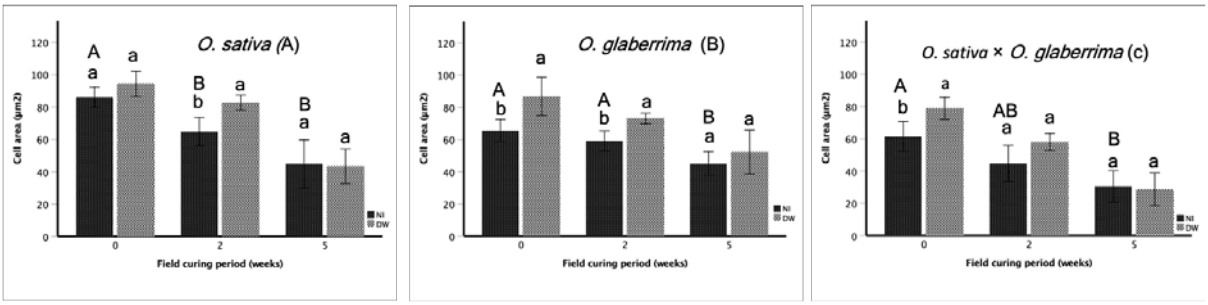

Figure 3.6 Cell area of NI and DW treated embryonic root meristem of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds field cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.5 Changes in nuclear and nucleolar morphology

Stored uncured (0 week) and NI seeds of both species and hybrid exhibited a spherical nuclear (N) profile surrounded by distinct double membrane (Fig. 3.3a, 3.4a, 3.5a). The nuclear membranes (NM) of *O. sativa* (Fig. 3.3a) and *O. glaberrima* (Fig. 3.4a) were regular while that of the hybrid (Fig. 3.5a) was irregular. In both species and the hybrid, the nucleoplasm displayed a heterogenous chromatin matrix. The nucleoli (nu) were dense, compact and appeared to be closely associated with nucleolar organizer (NOR) (Fig 3.3a, 3.4a, 3.5a). When uncured seeds of both species and the hybrid were imbibed with DW for 18 h, their N presented a regular conformation with a regular and distinct NM surrounding the nucleus (Figs. 3.3b, 3.4b, 3.5b). In contrast to the NI uncured seeds of both species and hybrid, the cells presented a sparsely but homogeneously dispersed chromatin in the nucleoplasm in cells of DW imbibed 0 week cured seeds (Figs. 3.3b, 3.4b, 3.5b). The nu ultrastructure of DW imbibed 0 week cured seeds of both species and the hybrid revealed numerous electron transparent areas which appeared to be nucleolar vacuoles (NVs) (Figs. 3.3b, 3.4b, 3.5b). Although the nu were compact in both species and the hybrid, they were not as dense in DW imbibed 0 week cured seeds as in the NI non-imbibed seeds.

For 2 week cured NI *O. sativa* seeds, the nuclear profile was somewhat lobed in conformation and was surrounded by a less distinct NM than NI 0 week cured seeds and irregular NM (Fig.

3.3c). In *O. glaberrima*, the N profile appeared to be essentially spherical in conformation and was surrounded by a less distinct and irregular NM compared with the NI 0 week cured seeds (Fig. 3.4c). In 2 week cured hybrid seeds, the N profile was somewhat spherical in conformation and was surrounded by distinct but irregular NM (Fig. 3.5c). The nucleoplasm of the 2 week cured seeds of both species and the hybrid presented clumped chromatin, interspersed with electron transparent areas similar to 0 week cured seeds. Cells in the 2 week cured seeds of both species and the hybrid presented a dense and compact nucleoli which lacked NOR unlike the 0 week cured seeds (Fig. 3.3c, 3.4c, 3.5c). When 2 week cured seeds of both species and the hybrid were imbibed with DW for 18 h, their N were spherical in conformation (Figs. 3.3d, 3.4d, 3.5d), the NM surrounding the N regular and distinct in *O. sativa* (Fig. 3.3d) and the hybrid (Figs. 3.5d) cells, but NM appeared to be disrupted in some sections of the DW imbibed 2 week cured *O. glaberrima* (Fig. 3.4d) cells. In contrast to the NI cured seeds, both species and the hybrid presented a sparsely but homogeneously dispersed chromatin in the nucleoplasm in DW imbibed 2 week cured seeds (Figs. 3.3d, 3.4d, 3.5d). The nu ultrastructure of DW imbibed 2 week cured seeds revealed several electron transparent areas which appeared to be NVs in *O. sativa* (Fig. 3.3d) and *O. glaberrima* (Fig. 3.4d), but NV was absent in the hybrid (Fig. 3.5d). While the nu in DW imbibed 2 week cured *O. sativa* cells (Fig. 3.3d) was compact, nu appeared to be disorganized in *O. glaberrima* cells (Fig. 3.4d) and was compact but irregular and appeared to be associated with NOR in the hybrid (Fig. 3.5d).

The N cells of NI 5 week cured seeds of *O. sativa* presented a more deteriorated ultrastructure than the 2 week cured seeds. The nucleus appeared lobed whilst the NM surrounding the N displayed signs of loss of integrity (Fig. 3.3e). In both *O. glaberrima* (Fig. 3.4e) and the hybrid (Fig. 3.5e), the N profiles and NM surrounding the nuclei were less distinguishable. The nucleoplasm, clearly distinguishable, in the 5 week cured seeds of *O. sativa* presented a much denser clumped chromatin than NI 2 week cured seeds and appeared to have withdrawn from the nu and was most often associated with the NM (Fig. 3.3e). On the contrary, the nucleoplasm in both *O. glaberrima* (Fig. 3.4e) and the hybrid (Fig. 3.5e) cells were less distinguishable. The nu of both *O. glaberrima* and the hybrid were dense, compact and appeared not to be associated with NOR as in 0 week cured seeds. When 5 week cured seeds of both species and the hybrid were imbibed in DW for 18 h, the N profile presented an elliptical conformation in *O. sativa* (Fig. 3.3f), and a spherical in *O. glaberrima* (Fig. 3.4f) and the hybrid (Fig. 3.5f). The NM in DW imbibed 5 week cured *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f) cells were less

distinguishable when compared with DW imbibed 2 week cured *O. sativa* (Fig. 3.3d) and the hybrid (Fig. 3.5d). While the NM was regular in *O. sativa* (Fig. 3.3f), it was irregular in the hybrid (Fig. 3.5f) cells. In contrast, the NM in the cells in DW imbibed 5 week cured *O. glaberrima* was regular and distinguishable. The n in the DW imbibed 5 week cured *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f) were dense and elliptical in conformation, their nucleoplasm was characterized by chromatin condensation. For DW imbibed 5 week cured *O. glaberrima* seeds, the nu was dense and presented a spherical conformation, and the nucleoplasm was characterized by chromatin condensation (Figs. 3.4f). The degree of chromatin condensation in DW imbibed *O. glaberrima* (Fig. 3.4f) cells was greater than DW imbibed 5 week cured *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f). Nucleolar vacuoles were absent in the DW imbibed 5 week cured seeds of both species and the hybrid suggesting lesser nucleolar activity in relation to DW imbibed 0 and 2 week cured seeds.

The nuclear area of the NI seeds declined significantly with field curing in *O. sativa* (Fig. 3.7A), *O. glaberrima* (Fig. 3.7B) and the hybrid (Fig. 3.7C). In *O. sativa*, the nuclear area shrank by 50% compared with 48% in *O. glaberrima* and 42% in the hybrid after 5 weeks of curing. When seed s of *O. sativa* were imbibed with DW for 18 h, differences in nuclear area between DW imbibed and NI 0, 2 and 5 week cured seeds were not significant, although there was a trend for the DW imbibed 0 and 2 week cured seeds to have larger nuclear areas when compared with NI seeds (Fig. 3.7A). In *O. glaberrima*, the nuclear area of embryonic root meristem cells were comparable between DW imbibed and NI 0 week cured seeds. However, for 2 ($P = 0.01$) and 5 ($P = 0.036$) week cured seeds DW imbibed seeds had significantly larger nuclear areas than the NI seeds (Fig. 3.7B). When data for both species and hybrid were compared, irrespective of imbibition treatment, the hybrid had significantly smaller nuclear area (14.83 ± 1.92 μm^2) when compared with either *O. glaberrima* (19.29 ± 2.20 μm^2) or *O. sativa* (20.48 ± 2.79 μm^2). For 2 week cured seeds, there were no significant differences in nuclear area among the species and hybrid. However, N area in *O. glaberrima* (11.67 ± 2.82 μm^2) and the hybrid (11.90 ± 2.06 μm^2) were not significantly different but both *O. glaberrima* and the hybrid had significantly larger N area than *O. sativa* (9.12 ± 1.86 μm^2) in 5 week cured seeds.

In *O. sativa*, field curing resulted in the significant (72%) contraction in nucleolar area of cells after 5 weeks of curing (Fig. 3.8A). The nucleolar area of *O. glaberrima* contracted significantly (48%) after 5 weeks of curing (Fig. 3.8B). There was also a 29% contraction in nucleolar area

of the embryonic root meristem cells of the hybrid after 5 weeks of curing but this was not significantly different (Fig. 3.8C). When 0, 2 and 5 week cured seeds of *O. sativa* (Fig. 3.8A) and the hybrid (Fig. 3.8C) were imbibed in DW for 18 h, the differences in nucleolar sizes between the DW imbibed and the NI seeds were comparable. In contrast, DW imbibed 0 (P = 0.002), 2 (P = 0.008) and 5 (P = 0.016) week cured *O. glaberrima* seeds had significantly larger nucleolar areas than the NI seeds (Fig.3.8B). When data for the species and hybrid were compared, irrespective of imbibition treatment, nucleolar areas in 0 and 2 week cured seeds were comparable among the species and the hybrid. On the contrary, nucleolar area was significantly smaller in *O. sativa* (1.12 ± 0.40 μm^2) than *O. glaberrima* (2.13 ± 0.81 μm^2) and the hybrid (2.12 ± 0.44 μm^2).

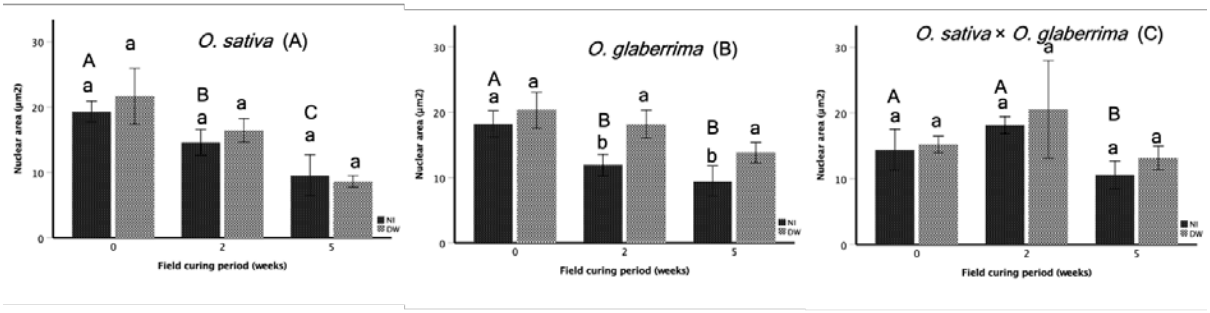

Figure 3.7 Nuclear area of NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (P < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

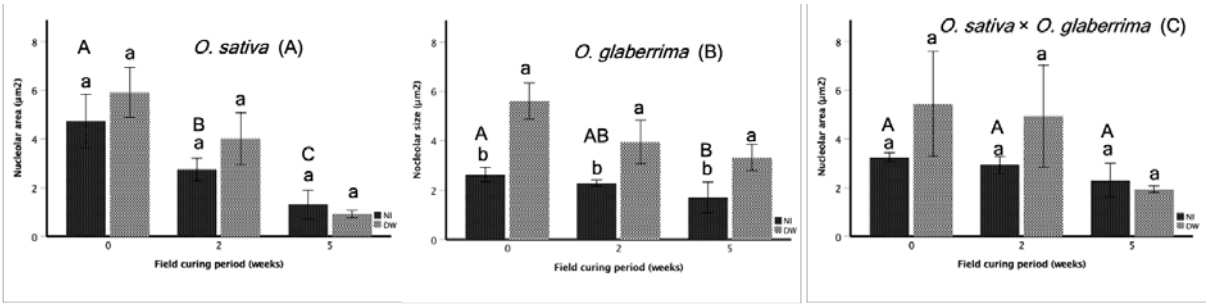

Figure 3.8 Nucleolar area of NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.6 Mitochondrial morphology

In NI and DW imbibed *O. glaberrima* and the hybrid seeds, the mitochondria in meristematic cells generally presented circular profiles. Cells in NI 0 week cured seeds of both species and the hybrid exhibited mitochondria (M) with a clearly distinguishable outer membrane (OM) and inner membrane (IM), electron translucent patchy matrix and poorly differentiated internal structure (Figs. 3.9a, 3.10a, 3.11a). After 18 h of imbibition with DW, M showed signs of repair in all the 0 week cured seeds of both species and the hybrid (Fig. 3.9b, 3.10b, 3.11b). The hybrid (Fig. 3.11b) appeared to have the most well-developed (mature) M among the two species and the hybrid; the OM and the IM were well distinguished, there was greater differentiation of the cristae (Cr) and the matrix was more homogenous when compared with *O. sativa* (Fig. 3.9b) and *O. glaberrima* (Fig. 3.10b). Some of the M in 0 week cured seeds of *O. sativa* appeared to have developed vacuolar structures (Fig.3.9b; M').

For NI 2 week cured seeds, the OM and IM were distinguishable in *O. sativa* (Fig. 3.9c) but in *O. glaberimma* (Fig. 10c) and the hybrid (Fig. 3.11c), some parts of individual M exhibited signs of damage to the OM and IM. Mitochondria showing limited signs of activity with poorly differentiated internal structures and heterogenous matrices were apparent in 2 week cured *O. sativa* (Fig. 3.9c), *O. glaberrima* (Fig. 3.10c) and hybrid (Fig. 3.11c) seeds. The cells in NI 2 week cured *O. glaberrima* (Fig. 3.10c) seeds presented distorted M (arrow). In DW imbibed 2

week cured *O. sativa* (Fig. 3.9d) seeds, some M' had an elongated profile and were characterized by a lack of internal homogeneity and poorly developed Cr when compared with *O. glaberrima*. These elongated M' appeared to be in the process of division (Fig. 3.9d). The OM and IM were quite distinguishable in the 2 week cured *O. sativa* seeds (Fig. 3.9d), although some parts appeared to be damaged. The cells of the 2 week cured *O. glaberrima* (Fig. 10d) seeds exhibited elongated cup-shaped M characterized but lack of internal homogeneity despite evidence of numerous developing Cr (Fig. 3.10d). Both OM and IM were very distinguishable in some parts of the mitochondrion and in some parts, OM lacked definition. In DW 2 week cured hybrid (Fig. 3.11d) seeds, M had circular profiles, the electron density of the matrix within them appeared to be similar to that of DW 0 week (Fig. 3.9b) cured seed. The mitochondrial matrix was also markedly more homogenous than in M within *O. sativa* and *O. glaberrima* cells and the internal structure appeared to show less differentiation of Cr than in DW 0 week cured seeds. The cells of the 2 week cured hybrid seeds showed a mixture of M with well-defined OM and IM and those (M') showing signs of damage to the OM and IM (Fig. 3.11d). In DW 2 week cured hybrid (Fig. 3.11d) seeds, M had circular profiles, and exhibited an electron density similar to that in DW 0 week (Fig.3.9b) cured seed; the mitochondrial matrix was also markedly more homogenous than in M within *O. sativa* and *O. glaberrima* cells, and the internal structure appeared to show less differentiation of Cr than in cells of DW 0 week cured seeds. The M in cells of 2 week cured hybrid were a mixture of M with well-defined OM and IM and others (M') that exhibited signs of damage to the OM and IM (Fig. 3.11d).

In cells of NI 5 week cured *O. sativa* (Fig. 3.9e) seeds, M were swollen and showed little signs of activity with poorly differentiated internal structures which also showed signs of derangement (dr) (Fig. 3.9e). The cells in NI 5 week cured seeds of *O. glaberrima* presented M with an inactive appearance, with matrices that were heterogenous and showed undifferentiated internal membrane structure (Fig. 3.10e). In the cells of NI 5 week cured seeds of the hybrid, M appears to be inactive with undistinguishable OM and IM in some parts and non-homogenous matrices (with electron translucent patches) (Fig. 3.11e).

After imbibing 5 week cured *O. sativa* seeds with DW for 18 h, some of the M exhibited vacuolar structures internally (Fig. 9f; insert), evidence that they had deteriorated or aged. Some of these M also exhibited an elongated profile (M, asterisk) which showed signs of division. An

illustrative example of this is shown in Figure 3.9f. In DW imbibed 5 week cured *O. glaberrima* (Fig. 3.10f) seeds, the M were largely deranged and contained an electron dense mitochondrial matrix without clearly defined structure and poorly differentiated Cr (Fig. 3.10f; M''; M'''). Mitochondria were spherical and lacked well developed OM and IM (Fig. 3.10f; M''). In DW imbibed 5 week cured hybrid seeds, M were circular with an electron dense matrix, clearly defined OM, and IM in some parts of the M and contained Cr that were well developed and extensive. An illustrative example of this is shown in Fig. 3.11f: a pair of dividing M (arrows) and another pair of recently divided M (asterisk) in cells from DW 5 week cured seeds.

Field curing did not significantly reduce the number of discernible M /cell in the cells of NI seeds of both species and the hybrid (Fig. 3.12A, B, C). In *O. sativa*, although relatively more M were observed per cell in the 0 and 2 week cured seeds imbibed with DW than the NI seeds, the number of discernible M/cell in cells was not significantly different between seeds imbibed with DW and NI seeds (Fig. 3.12A). The absence of significant difference in number of M/cell between NI and DW imbibed seeds of 0 and 2 week cured seeds might be as a result of the wider range in the number of M/cell for the DW imbibed 0 and 2 week cured seeds relative to NI 0 and 2 week cured seeds. In contrast to the 0 and 2 week cured seeds, the number of M/cell declined significantly in DW imbibed 5 week cured seeds relative to NI seeds (Fig. 3.12A). In *O. glaberrima*, the number of M/cell in the embryonic root meristem cells of 0, 2 and 5 week cured seeds was not significantly different between DW and NI (Fig. 3.12B). In contrast, except for 0 week cured seeds, the number of discernible M/cell increased significantly in 2 and 5 week cured hybrid DW relative to NI seeds (Fig. 3.12C). When the two species and the hybrid were compared, irrespective of imbibition treatment, differences in the number of M/cell in 0 and 2 week cured seeds was comparable. In contrast, differences in the number of M/cell in the 5 week cured seeds differed significantly ($P < 0.001$) across the species and the hybrid: it was highest in *O. sativa* × *O. glaberrima* (4.83 ± 3.87) followed by *O. glaberrima* (3.33 ± 0.82) and lowest in *O. sativa* (1.33 ± 0.52).

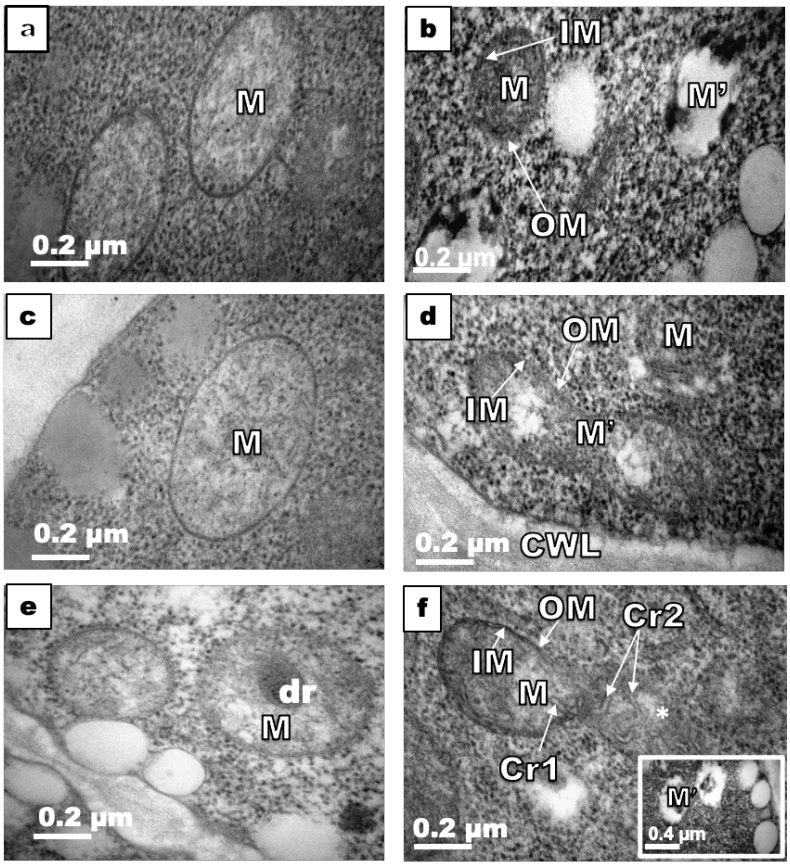

Figure 3.9 Changes in mitochondrial ultrastructure of embryonic root meristem cells of 20-month stored *O. sativa* field cured for 0, 2 and 5 weeks in dry (NI) and imbibition (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibed; M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, Cr Cristae, dr deranged mitochondria, asterisks(*) mitochondrial fission

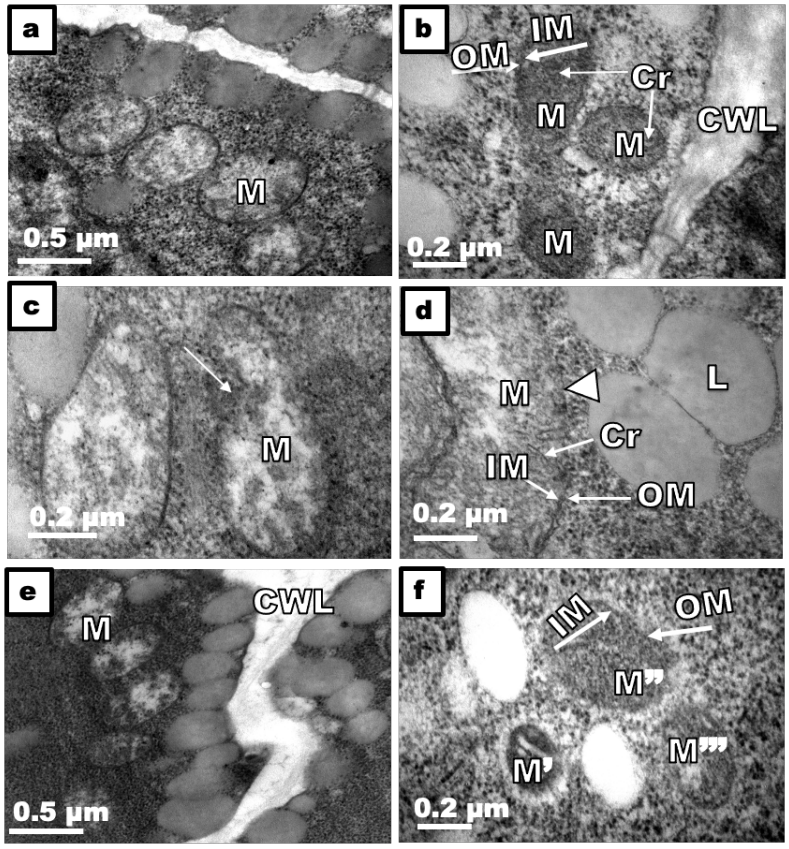

Figure 3.10 Changes in mitochondrial ultrastructure of embryonic root meristem cells of 20-month stored *O. glaberrima* field cured for 0, 2 and 5 weeks in dry (NI) and imbibed (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibition; Cr Cristae, M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, M' mitochondria with vacuolar structure, M'' and M''' mitochondrial fission.

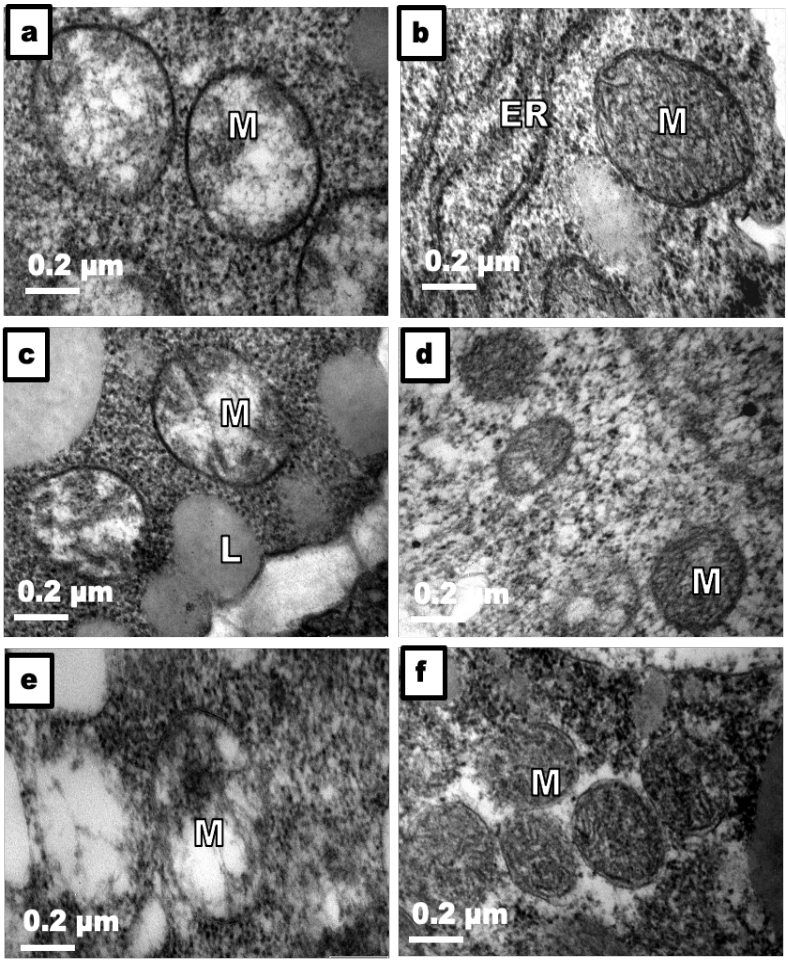

Figure 3.11 Changes in mitochondrial ultrastructure of embryonic root meristem cells of 20-month stored hybrid (*O. sativa* × *O. glaberrima*) field cured for 0, 2 and 5 weeks in dry (NI) and imbibed (DW) conditions: a. 0 (uncured) week cured and non-imbibed, b. 0 week cured and 18 h imbibition, c. 2 week cured and non-imbibed, d. 2 week cured and 18 h imbibition, e. 5 week cured and non-imbibed f. 5 week cured and 18 h imbibition; M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, Cr Cristae, M' mitochondria with damaged membrane, arrows and asterisks show mitochondrial fission, ER endoplasmic reticulum, L lipid bodies.

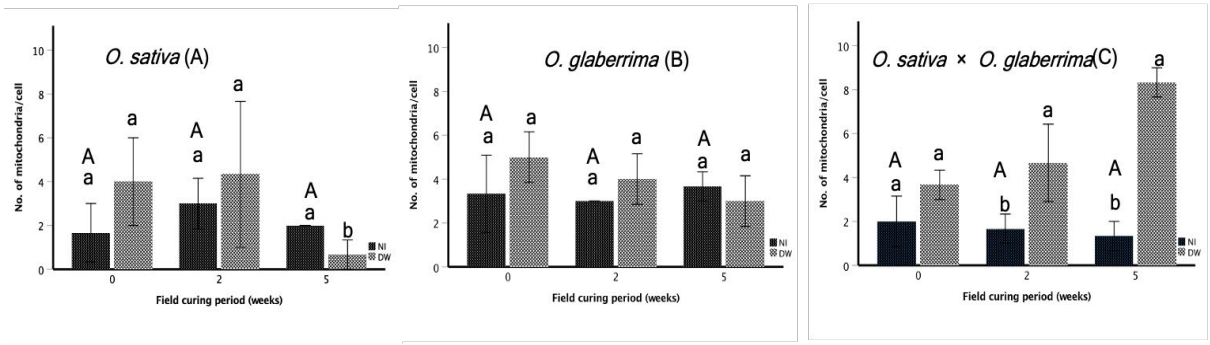

Figure 3.12 Number of mitochondria in NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.7 Lipid bodies

Lipid bodies present in NI 0 (Figs. 3.3a, 3.4a, 3.5a) and 2 (Figs. 3.3c, 3.4c, 3.5c) week cured seeds of both species and the hybrid were largely found near the plasmalemma. One of the obvious changes observed in NI 2 week hybrid (Fig. 3.5c) was the coalescence of lipid bodies. When 0 (Figs. 3.3b, 3.4b, 3.5b) and 2 (Figs. 3.3d, 3.4d, 3.5d) week cured seeds were imbibed for 18 h, lipid bodies were distributed throughout the cytomatrix in both species and the hybrid although some lipid bodies were seen to coalesce into larger units in the DW 2 week cured hybrid (Fig. 3.5d) cells. Except for *O. glaberrima*, some lipid bodies in NI 5 week cured seeds of *O. sativa* (Fig. 3.3e) and the hybrid (Fig. 3.5e) appeared to have coalesced. In DW imbibed 5 week cured *O. sativa* (Fig. 3.3f) and the hybrid (Fig. 3.5f) seeds, lipid bodies coalesced and formed large confluent masses probably due to damage to membranes bounding the lipid bodies.

The number of lipid bodies/cell declined significantly ($P = 0.019$) over time in *O. sativa* and in *O. glaberrima* ($P = 0.013$) cells during field curing (Figs. 3.13A, B). In contrast, the number of lipid bodies/cell did not decline significantly ($P = 0.770$) in the hybrid over time during field curing (Fig. 3.13C). Except for 5 week cured *O. sativa* seeds, the number of lipid bodies/cell in DW imbibed 0 and 2 week cured seeds was significantly lower than NI 0 and 2 week cured seeds (Fig. 3.13A). The number of lipid bodies/cell in 0 week cured *O. glaberrima* seeds

declined (24%) significantly ($P = 0.05$) while 2 week cured *O. glaberrima* seeds declined (41%) significantly ($P = 0.044$) in the DW relative to the NI cells (Fig. 3.13B). DW imbibed 0 and 5 week cured hybrid seeds resulted in significant reduction in the number of lipid bodies/cell; 53% and 29%, respectively in DW relative to NI seeds (Fig. 3.13C). On the contrary, the number of lipid bodies/cell did not differ significantly between DW and NI 2 week cured seeds (Fig. 3.13C). When the two species and hybrid were compared, irrespective of the imbibition treatment, the number of lipid bodies/cell were comparable across the species and the hybrid in 0 and 5 week cured cells. In contrast, *O. glaberrima* (44 ± 15) had significantly greater number of lipid bodies/cell when compared with the hybrid (34 ± 3) but the number of lipid bodies/cell in *O. sativa* (39 ± 6) was comparable to *O. glaberrima* and the hybrid in 2 week cured seeds.

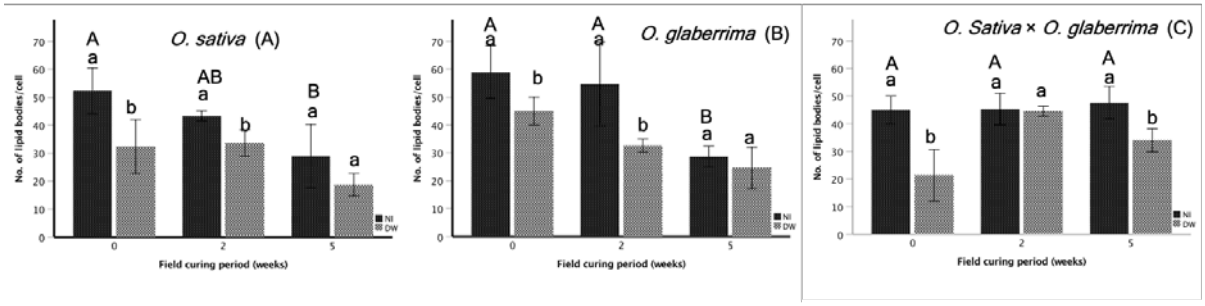

Figure 3.13 Number of lipid bodies/cell in NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly ($p < 0.05$) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.8 Cellular vacuolation

In the embryonic root meristem cells of the NI 2, and 5 week cured seeds of both species and the hybrid, the vacuoles showed evidence of autophagic activity involving plastids engulfing M and lipid inclusions (e.g. Fig. 3.4e; 3.5c). In some cells of the DW imbibed seeds of both species and the hybrid, vacuoles coalesced and showed inclusions of damaged vesicles (e.g. Fig. 3.4b, d).

Field curing ($P > 0.05$) and imbibition did not significantly ($P > 0.05$) influence the degree of cellular vacuolation in both species and hybrid. Differences in the number of vacuoles/cell

between DW and NI cells of 0, 2 and 5 week cured seeds were not significantly different in both species and the hybrid (Fig. 3.14). Although there was no significant difference in the number of vacuoles/cell between DW (9) and NI (7) 5 week cured hybrid seeds, the range for DW treated seeds was wider (20) than the range for NI seeds (7). Also, when data for both species and the hybrid were compared, irrespective of imbibition treatment, the degree of vacuolation in the cells in 0, 2 and 5 week cured seeds was comparable across both species and hybrid.

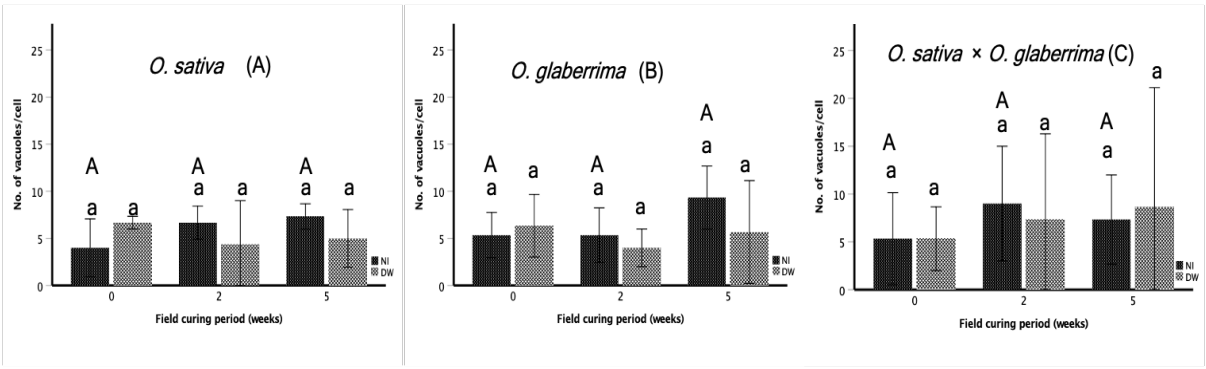

Figure 3.14 Number of vacuoles/cell in NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly ($p < 0.05$) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.4.9 Plastids and Amyloplasts

Plastids were mostly seen in the NI uncured and cured seeds while amyloplasts were mostly observed in DW imbibed seeds. In both species and the hybrid, differences in the number of plastids/cell in cells were not significantly different between DW imbibed and NI 0, 2 and 5 week cured seeds (Fig. 3.15). Except for 0 week cured *O. sativa* and 2 week cured *O. glaberrima*, differences in the number of amyloplasts/cell in cells were not significantly different between DW and NI cured seeds (Fig. 3.16). In the hybrid, the number of amyloplasts/cell in the cells of 0, 2 and 5 week cured seeds were not significantly different between DW and NI cells (Fig. 3.16C). When the two species and hybrid were compared,

irrespective of the imbibition treatment, the number of plastids and amyloplasts in the cells of 0, 2 and 5 week cured seeds were comparable across the two species and the hybrid.

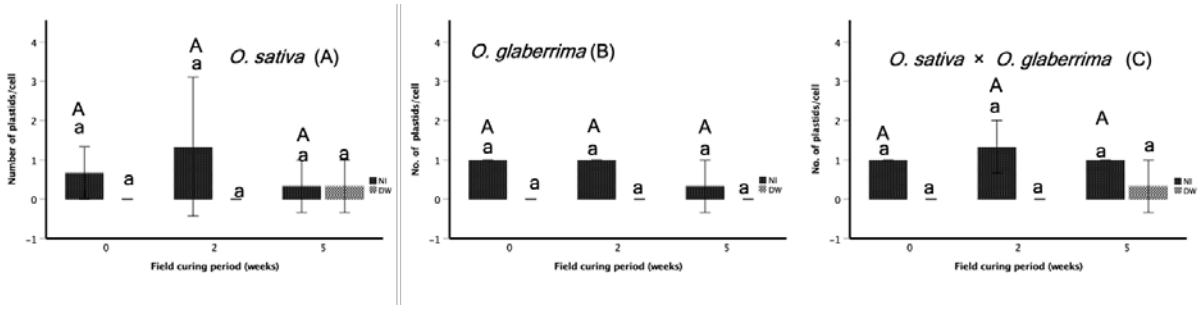

Figure 3.15 Number of plastids in NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

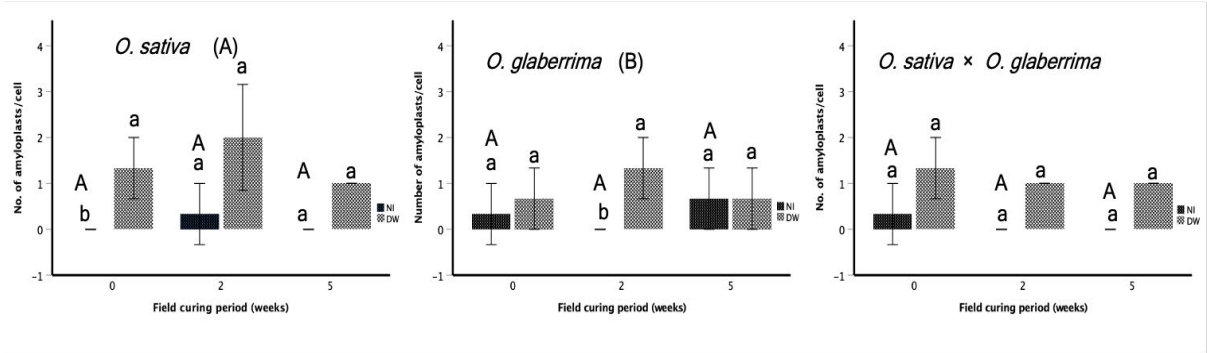

Figure 3.16 Number of amyloplasts in NI and DW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments; uppercase letters represent comparisons among NI seed treatment across field curing period.

3.5 Discussion

Seed germinability and vigour in both species and hybrid declined with delayed field curing; an established consequence of seed aging (Garcia de Castro and Martinez-Honduvilla, 1984; Bhatia *et al*., 2010; Leprince *et al*., 2017). Although differences in seed germinability between NI and DW imbibed 0, 2 and 5 week cured *O. sativa* and 0 week cured hybrid seeds were comparable, we observed the development of significantly longer radicle length 7 DAI in DW treated seeds than the NI seeds. As seed quality is lost, seed vigour decreases first without affecting germination; as vigour is further reduced, seed germination decreases (ISTA, 1995). The increase in germination capacity with DW imbibition in 0, 2 and 5 week cured seeds of *O. glaberrima* and 2 week cured hybrid suggest an alleviation of deterioration associated with aging. Reduction in seed germination and vigour (radicle length) with increasing duration of field curing may also be attributed to the reduction in the recovery of mitochondrial structure and function (Bewley, 1997; Benamar *et al*., 2003; Wang *et al*., 2012a) due to seed aging. We observed in our study that the recovery of OM and IM integrity was either decreased or delayed with delayed field curing in both species and the hybrid (Figs. 3.9 - 3.11) upon imbibition.

The present study revealed differences in the response of the CWLs of the embryo root meristematic cells among the two species and an interspecific hybrid of rice to delayed field curing in a humid tropical environment. The CWLs of the embryonic root meristematic cells of the dry seeds of both *O. sativa* and *O. glaberrima* folded during delayed field curing; the severity of folding increased with delayed field curing especially in the *O. glaberrima* seeds (Fig. 3.4c, 3.e; arrowheads). The CWLs of the embryonic root meristematic cells in the dry seeds exhibited two characteristic patterns of folding: the CWL folding observed in *O. sativa* (Fig. 3.3e; insert) was slightly undulating with the plasmalemma appearing to have separated from the CWLs while the CWL folding in the dry embryo root meristem cells of *O. glaberrima* was irregular and marked with the plasmalemma remaining appressed to the CWL (Fig. 3.4e). Differences in the observed CWL morphology between *O. sativa* and *O. glaberrima* may be due to differences in the extent of shrinkage of the protoplast in comparison to the CWL (Webb and Arnott, 1982). The marked CWL folding of the embryonic root meristematic cells of dry *O. glaberrima* seeds suggested a greater shrinkage in the protoplast relative to the CWL thereby causing the CWL to conform to the shrunken protoplast and hence the observed marked structural alteration. On the contrary, the slightly undulating CWL in *O. sativa* may suggest

equal and uniform shrinkage of the protoplast and the CWL. Maintaining structural integrity of plant cells requires coordination of water loss among the protoplasmic components and between protoplasm and CWL (Bewley 1979; Webb and Arnott, 1982). Differences in CWL folding between *O. glaberrima* and *O. sativa* or the hybrid may also be a result of differences in their CWL properties (Oliver, 2007). Several factors including species, tissues within a seed, size of CWL, lipids, ratios of proteins, and starches in a seed, and proportion of celluloses, hemicelluloses, pectins in the CWL may influence the water retention capacity of the protoplast and determine the degree of shrinkage upon water loss (Webb and Arnott, 1982; Woodenberg *et al*., 2018). For example, accumulation of lipids during desiccation offers better intracellular mechanical stabilization because of their ability to occupy more subcellular space and minimize cell shrinkage (Farrant 2000; Woodernberg *et al*., 2018). The ability of dry seeds to retain viability is related to the stability of the CWL conformation during desiccation (Webb and Arnott, 1982). In seeds with highly folded CWLs, rapid expansion of the CWL occurs without synthesis of new CWL material during germination (Webb and Arnott, 1982) thereby resulting in rapid germination. The marked CWL folding in 5 week cured *O. glaberrima* (Fig. 13B) embryonic root meristem cells may be due to the high rate of lipid loss compared with *O. sativa* (Fig. 3.13A) and the hybrid (Fig. 3.13C). The marked folding in the *O. glaberrima* seeds may have led to a higher percentage germination in the 5 week cured seeds (67.7 ± 7 %) compared with *O. sativa* (58.6 ± 6 %) or the hybrid (52 ± 8 %).

Cell area declined significantly in both species and the hybrid with delayed field curing (Fig. 3.6). During seed desiccation or maturation drying, cells shrink, and tissues become highly compressed and form glasses within the cytomatrix to protect seeds in the dry state (Koster, 1991; Walters and Koster, 2007; Manfre *et al*., 2009; Sano *et al*., 2016). When seeds were imbibed with DW, only 2 week cured *O. sativa*, 0 and 2 week cured *O. glaberrima* and 0 week cured hybrid had significantly larger cell area than the NI seeds suggesting their ability to better recover from the shrinkage or CWL folding. Also, the lack of significant differences in cell area between DW and NI imbibed field cured seeds suggest their inability to completely recover due to small shrinkage or slightly undulated walls in *O. sativa* (Fig. 3.3a, 3.5d) or CWL folding and collapse (Fig. 3.3f) or due to cytoplasmic lysis (Fig. 3.4f).

Delayed harvesting of dried, physically and metabolically quiescent seeds or leaving already dried harvested seeds in their panicles in the field for weeks in the humid tropics where cycles

of drying and wetting are experienced could result in field weathering, followed by a faster rate of seed deterioration during storage (TeKrony *et al*., 1980; Egli *et al*., 2005; Bhatia *et al*., 2010). Rapid accumulation of free radicals or reactive oxygen species (ROS) are triggered during field weathering in tropical environments and creates an imbalance between de-novo synthesized antioxidants which are required to counteract the free radicals thereby resulting in increased lipid peroxidation, loss of membrane integrity and eventually germination and vigour loss (Bhatia *et al*., 2010). Therefore, field weathering compromises seed quality as a result of accumulated damage caused by ROS to macromolecules (e.g. lipids, proteins, and nucleic acids) (Powell and Mathews, 2012; Waterworth *et al*., 2019), cellular ultrastructure and reduced cellular maintenance activities (Waterworth *et al*., 2019). The significant decline in the number of lipid bodies/cell in the NI *O. sativa* (Fig. 3.13A) and *O. glaberrima* (Fig. 3.13B) relative to the uncured seeds with delayed field curing may suggest lipid peroxidation and hydrolysis of membrane lipids or coalescence of lipid bodies because of ageing. Wang *et al*. (2012b) studied lipid degradation in *O. sativa* during accelerated ageing and suggested that phospholipid hydrolysis followed by lipid peroxidation may contribute to the aging process. In *Arabidopsis thaliana*, phospholipase D (PLD) mediated hydrolysis of membrane lipids have been reported to destroy lipid bodies in seed embryos during both natural and accelerated ageing (Devaiah *et al*., 2007).

We observed a significant reduction in number of lipid bodies in DW imbibed 0 and 2 week cured seeds of *O. sativa* and *O. glaberrima*, and 0 and 5 week cured *O. sativa* × *O. glaberrima* relative to their NI seeds (Fig. 3.13). Except for the 5 week cured *O. sativa* × *O. glaberrima*, reduction in the number of lipid bodies in the DW imbibed seeds in relation to their NI seeds might have resulted from the lipid bodies being used for respiration or converted into sugars during the germination process. This observation is supported by the findings of Leonova *et al*. (2010) who reported that degradation of oil bodies in the embryo of *A. sativa* during the first day of germination was either instantly respired or converted into sugars. In contrast, a decline in the number of lipid bodies in DW imbibed 5 week cured hybrid relative to the NI seeds might be due to coalescence of lipid bodies upon imbibition leading to large confluent masses of lipidic material probably due to disintegration of the membrane bounding the lipid bodies (Fig. 3.5f). This phenomenon was also reported in naturally aged *P. pinea* seeds by Castro and Martinez-Honduvilla (1984).

Although we did not observe a significant change in the number of vacuoles between the NI and DW imbibed seeds within each curing period in *O. sativa*, *O. glaberrima* and the hybrid, we observed a wider range in the number of vacuoles/cell for the DW treated 5 week cured hybrid seeds relative to the NI seeds (Fig. 3.14C). Also, although there were no significant differences in cellular vacuolation among the species and hybrid, there appeared to be a trend for the DW treated hybrid meristematic cells to develop more vacuoles than both species (Fig. 3.14) and this might suggest repair processes during imbibition or germination (Wesley-Smith *et al*., 2001; Sershen *et al*., 2016) because of increased capacity for autophagic elimination of damaged cells (Sershen *et al*. 2016; Czernekova´ *et al*., 2018).

Nucleolar size is influenced by the environment (Ma *et al*., 2016; Weeks *et al*., 2019). This may explain the significant reduction in the nucleolar size in *O. sativa* and *O. glaberrima* (Fig. 8A, B) with delayed field curing. The significant increases in nucleolar sizes in DW imbibed 0, 2 and 5 weeks cured seeds of *O. glaberrima* (Fig. 8B) when compared with the NI seeds may suggest increased ribosomal biogenesis and DNA synthesis (Khrolenko *et al*., 2012; Ma *et al*., 2016; Kalinina *et al*., 2018).

The presence of NV in DW imbibed 0 and 2 week cured and their absence in 5 week cured seeds of both species and the hybrid may suggest higher nucleolar activity in the 0 and 2 weeks cured compared with the 5 week cured seeds (Figs 3.3 - 3.5). Deltour and Barsy (1985) also reported nucleoli vacuolation in cortical radicle cells of *Z. mays* during germination: nucleoli vacuolation increased during the early stages of germination but declined with the progression of germination. They reported 32% vacuolated nucleoli 8 h compared with 16% vacuolated nuclei between 24 h and 72 h of imbibition/germination. Although we did not determine the number of vacuoles/nu, NV are a major characteristic of plant cells and represent high nucleolar activity (RNA synthesis) (Lewis 1943; Johnson and Jones, 1967; Stępiński, 2014; Kalinina *et al*., 2018) and may be well connected with mitosis (Jennane *et al*., 2000). Nucleolar vacuole development during imbibition/germination may increase the external nucleolar exchange surface for fluxing of ribonucleoproteins (RNPs) to the cytoplasm (Deltour, 1985).

During desiccation and/or rehydration cycles, it is imperative that plant tissues minimize damage, maintain cellular and ultrastructural integrity, and be able to repair damage upon imbibition (Bewley 1979). Mitochondria are the most important organelle in embryo cells but

are also the source of ROS generation, therefore, the recovery of mitochondrial ultrastructure integrity and the retention of mitochondrial functionality following imbibition of aged seeds is key to their ability to germinate and establish (Bewley 1997; Wang *et al*., 2012a; Xia *et al*., 2015b; Mao *et al*., 2018; Ratajczak *et al*., 2019). The present study revealed that the inner structure of M lacked internal homogeneity and Cr and undistinguishable outer and inner membranes. However, following imbibition, the electron dense areas of M increased when compared to the dry seeds, albeit it decreased with delayed field curing suggesting mitochondrial repairs or biogenesis. The decrease in electron density, level of Cr differentiation and recovery of outer and inner mitochondrial membrane integrity after imbibition may suggest increased mitochondrial deterioration with delayed field curing or seed ageing. Yin *et al*., (2016) reported that respiratory capacity and enzyme activity in *O. sativa* were compromised in aged seeds that exhibited a loss of mitochondrial ultrastructural integrity. The lack of definition for outer and inner mitochondrial membranes (Fig. 3.9d, f; 3.10f, 3.11d, f) in aged seeds in the present study, supports previous findings that the outer and inner mitochondrial membranes are the main targets of stress damage during desiccation and ageing (Benamar *et al*., 2003; Xia *et al*., 2015a; Yin *et al*., 2016). Studies in *P. sativum* (Wang *et al*., 2012a) and *O. sativa* (Yin *et al*., 2016) have further showed that inner mitochondrial membrane integrity and its recovery was more sensitive to desiccation and ageing than the outer mitochondrial membrane as seen here in week 2 and 5 cured seeds (Fig. 3.9f, asterisks; Fig. 3.10d, arrowhead). In the DW imbibed 5 week cured *O. sativa* (Fig. 3.9f, insert) and *O. glaberrima* (Fig. 3.11f, M'), M presented vacuole-like structures suggesting their inability to be repaired and become functional after 18 h of imbibition. Kong *et al*., (2014) and Xia *et al*., (2015a) also reported vacuolar structures of M and the disappearance of mitochondrial membranes and Cr in aged *A. sativa* seeds. Mitochondrial fission also appeared to have been evident in the 5 week cured embryonic root meristem cells of both species and the hybrid especially. We also observed that irrespective of the imbibition treatment, differences in the number of M/cell at 0 and 2 weeks cured seeds were not significant among the species and hybrid, however, the hybrid (5 ± 4) had the highest number of M/cell followed by *O. glaberrima* (4 ± 1) and *O. sativa* (2 ± 1) in 5 week cured seeds suggesting that the hybrid seeds may have been subject to the highest degree of oxidative stress. Our findings are consistent with those of Berjak and Villiers (1973) who also reported increased number of M/cell upon imbibition of aged *Z. mays* embryos than those from non-aged germinating embryos.

3.6 Concluding remarks

This study has shown that delaying of harvested panicles *of O. sativa*, *O. glaberrima* and their hybrid in the field for weeks in tropical environments before threshing has negative consequences on seed quality which can have far-reaching consequences on crop production and hence food security. The findings support other studies by showing that loss of CWL and meristematic cell ultrastructural integrity can lead to seed ageing (Webb and Arnott, 1982; Wu *et al*., 2011; Xi *et al*., 2016; Woodernberg *et al*., 2018).

A major objective of this study was to identify ultrastructural biomarkers of seed sensitivity to field curing induced stress/ damage across two upland *Oryza* sp and their interspecific hybrid. Untreated dry NI seeds, cured for different durations (0, 2 and 5 weeks) were used as the controls to establish whether ultrastructural abnormalities induced by curing are permanent or can be reversed/ repaired during imbibition and whether seed deterioration during curing leads to imbibitional damage. Importantly, the results showed differences in the impact of delayed field curing on CWL morphology and ultrastructural integrity of key organelles (nucleus, nucleolus, mitochondria, lipids, and vacuoles) involved in germinative metabolism among the species and the hybrid. A major difference was that there was no apparent CWL folding in cured hybrid seeds but slight CWL folding in 5 week-cured *O. sativa* which do not appear to be reversed after imbibition, while in cured *O. glaberrima* seeds, marked CWL folding was reversed after imbibition. Furthermore, CWL abnormalities such as separation of the CWL from the plasmalemma was a common feature in NI and DW imbibed cured *O. sativa* and hybrid seeds. Upon imbibition, mitochondrial repair was evident in both species and hybrid, but the degree of recovery decreased with delayed field curing. Moreover, mitochondrial fission was associated with 5 week-cured seeds in both species and hybrid, with the highest level of fission occurring in the hybrid. The severity of ultrastructural lesions related to cellular stress/ damage differed across species and the hybrid and progressed at a rate in parallel with the viability and vigour loss caused by delayed field curing. The present study has identified several ultrastructural biomarkers that can be used by plant breeders for screening rice varieties for sensitivity/tolerance to field curing deterioration and potentially to assess the utility of seed invigoration/priming treatments in improving vigour and viability in deteriorated rice seeds.

CHAPTER 4: Alleviatory effects of cathodic invigoration on curing-induced stress as assessed through ultrastructural biomarkers: a comparison across an Asian and African rice species and their interspecific hybrid

R.K. Bam, B. Varghese, S. Naidu, W.R. Woodenberg, N. W. Pammenter, Sershen

4.1 Abstract

Structural changes and loss of ultrastructural cellular integrity represent a major cause of loss of seed germinability and vigour during post-harvest processing and storage. Delayed harvest and/or threshing of dried and metabolically quiescent seeds in tropical environments, where temperature and RH are high, could promote the ageing process. Identifying species/varieties of staple food crops that are less susceptible to in-field and in-storage deterioration can increase agricultural productivity and even aid breeding programmes. In this study, TEM was used to assess the use of CW treatment in alleviating the ultrastructural stress/ damage induced by delayed field curing in *O. sativa*, *O. glaberrima* rice species and their interspecific hybrid. Several previously established ultrastructural biomarkers of curing-induced stress/ damage (CWL morphology, cell area, nuclear and nucleolar morphology, mitochondrial morphology, G, lipids, cellular vacuolation, plastids and amyloplasts) were assessed in embryonic root meristem cells of seeds exposed to different durations of field curing (0, 2 and 5 weeks) in a humid tropical environment in Ghana. Cured and uncured seeds of both species and the hybrid were hermetically stored at 4°C for 20 months, imbibed with CW or DW (control), and assessed after 18 h. The ultrastructural biomarkers were compared between 1) DW-uncured and CW-uncured and 2) DW-cured and CW-cured imbibed seeds of both species and the hybrid. Cathodic water invigoration significantly enhanced seed germinability in cured seeds of both species and hybrid (*O. sativa* and *O. glaberrima*, 5 week cured seeds; *O. sativa* × *O. glaberrima*, 2 and 5 week cured seeds) and vigour in both uncured and cured seeds relative to DW invigorated seeds. Slight CWL folding which was apparent only in DW imbibed 5 week cured *O. sativa* seeds appeared to be repaired by CW invigoration although plasmalemma withdrawal from CWL was observed in both DW and CW imbibed seeds. Cell area, nuclear and nucleolar sizes were comparable between CW and DW imbibed uncured and cured seeds of both species and the hybrid. The number of lipids/cell in both cured and uncured seeds were significantly higher in CW relative to DW invigorated seeds but the differences between CW and DW invigorated cured seeds were not always statistically significant. Golgi bodies showed

apparently greater hypersecretory activity with numerous G derived vesicles in CW than DW imbibed cured and non-cured seeds. Marked differences in mitochondrial morphology between CW and DW were apparent. More importantly, cured and uncured seed subjected to CW invigoration exhibited more prominent signs (e.g., M which exhibited elongated profile, homogenous matrix, well differentiated Cr, and well-defined OM and IM, and highly developed G) of germinative metabolism than those invigorated with DW. We conclude that the alleviatory effects of CW was greater than DW in terms of its effects on the biomarkers of stress/ damage. In terms of seed germinability, CW was more effective in the cured seeds than the uncured seeds. Although CW was more effective across the two species and the hybrid, the hybrid appeared to have responded best to CW invigoration in terms of repair of damaged cellular components and/or reduced the damage that ensues during imbibition in deteriorated seed. Our results suggest the effectiveness of CW in alleviating curing-induced ultrastructural damage and loss of vigour and viability in rice seed and provides impetus for future studies on the application of this invigoration technique to other crop species susceptible to deterioration during processing and storage.

Keywords: Cathodic water, Golgi bodies, mitochondria, mitochondrial membrane, transmission electron microscopy, ultrastructure

4.2 Introduction

Climate change impacts many interactions between organismal biology and climate (Fernandez-Pascual *et al*., 2015) and poor agricultural practices could exacerbate its adverse impacts, rendering food production systems vulnerable leading to food insecurity (Agnolucci *et al*., 2020). Thus, changing climate conditions impact the seed maturation process, reducing seed quality, seed viability and storage longevity, and inducing a faster ageing process (Kijowska-Oberc *et al*., 2020). Furthermore, during delayed field curing, alternate desiccation and rehydration cycles may predispose the seeds to more rapid ageing (Bhatia *et al*., 2010; Wiebach *et al*., 2020), which may adversely affect ultrastructural integrity (Berjak and Pammenter, 2000) and seed vigour and viability (Berjak and Pammenter, 2000; Xia *et al*., 2015a, b).

Imbibition can repair the damage/ stress incurred in seeds during maturation drying and post-harvest (including storage) (Bewley, 1979). Seed priming/ invigoration of deteriorated or low

vigour seeds is designed to take advantage of this possibility (McDonald, 1999). Seed priming is a pre-sowing treatment that has been used to invigorate low vigour or deteriorated seeds (Powell *et al*., 2000; Butler *et al*., 2009; Paparella *et al*., 2015) of a number of crop species (Powell and Matthews, 2000; Butler *et al*., 2009; Paparella *et al*., 2015). During priming, the seeds are imbibed in osmotic solution or solid matrix carriers that partially hydrate seed close to but prevent radicle emergence and followed up by drying back to original MC to trigger pre-germination metabolism, which includes repair events, e.g. antioxidant, DNA (Bradford, 1986; Farooq *et al.*, 2010; Forti *et al*., 2020). An adaptation of the priming technique is termed seed invigoration/seed soaking where seeds are soaked in water (hydropriming) or various salt solutions (e.g., $CaCl_2$, $MgCl_2$, KCl, $MgSO_4$, K_3PO_4, KH_2PO_4, NaCl) or antioxidants/reducing agents (Adetunji *et al*., 2020; Fatokun *et al*., 2020b) and then planted out without them dried back as in traditional priming (Basra *et al*., 1994; Farooq *et al*., 2009). Studies on both seed priming and soaking have incorporated antioxidants such as ascorbic acid (Farooq *et al*., 2010; Farooq *et al*., 2012; Ye *et al*., 2012; Gondwe *et al*., 2016), α-tocopherol and glutathione (Draganic and Lekic, 2012) to invigorate seeds. Most recently, two studies (Adetunji *et al*., 2020; Fatokun *et al*., 2020b) have shown how the reduced cathodic fraction of an electrolyzed $CaCl_2/MgCl_2$ solution (CW) can be used to reinvigorate aged seeds of certain species. However, both studies worked on artificially aged seeds. While CW alleviated the effects of seed ageing to some extent, they did not assess whether these beneficial effects promoted the repair of ultrastructural damage and metabolic stress that manifest during imbibition in deteriorated seeds. This motivated the present study which used TEM to assess the use of CW soaking in alleviating the ultrastructural stress/ damage induced by delayed field curing in *O. sativa* and *O. glaberrima* rice species and their interspecific hybrid (*O. sativa* × *O. glaberrima*). The selection of the ultrastructural biomarkers of curing-induced stress/ damage to be assessed was based on the results of a previous study (see Chapter 3).

Irrespective of whether the seeds are to be primed or 'invigorated' the hydration process initiates the synthesis of many proteins and antioxidant enzymes (e.g., ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GSHPx), and glutathione reductase (GR) that are associated with repair and scavenging of ROS during the early stages of imbibition (Bailly, 2004; Bailly *et al*., 2008; Long *et al*., 2015). Seed invigoration also leads to increased levels of ascorbic acid and tocopherols necessary for

counteracting ROS (Basra *et al*., 1994). The use of reducing agents or exogenous application of antioxidants that can quench ROS could further alleviate the negative impacts of ROS. Berjak *et al*., (2011) used CW to reduce oxidative stress in plant tissues exposed to cryopreservation procedures, while studies (Hanaoka, 2001; Hanaoka *et al*., 2004) have shown that CW has strong antioxidant properties, can scavenge ROS and even protect DNA from ROS damage (Shirahata *et al*., 1997). Furthermore, seed soaking in CW was shown to enhance DNA quantity and purity (Fatokun *et al*., 2020a), increase ROS detoxifying antioxidant enzyme activities (e.g. CAT, SOD and glutathione reductase (GR)) (Adetunji *et al*., 2020; Fatokun *et al*., 2020b), and reduce lipid peroxidation products, i.e. malondialdehyde (MDA) (Fatokun *et al*., 2020b) and 4-hydroxy-2-nonenal (4-HNE) (Adetunji *et al*., 2020; Fatokun *et al*., 2020b) production in aged orthodox species.

4.3 Materials and Methods

4.3.1 Plant material and field curing

The methods used for this aspect of the study follow those described for wet cured seeds in sections 2.3.1 and 2.3.2 of Chapter 2.

4.3.2 Seed invigoration

Seeds of both species and the hybrid, cured for 0, 2 and 5 weeks, and stored at 4°C for 20 months were withdrawn from storage and held overnight at 25°C to equilibrate before invigoration. These seeds were invigorated with either CW or DW (DW served as control for the invigoration treatment) by placing 50 seeds per replicate/treatment (for cured) and for the control (non-cured) on germination towel (two layers) within a 9 cm Petri dish. The towel was moistened with 5 ml of CW or DW and covered with another layer of germination towel moistened with 5 ml CW or DW, respectively. Cathodic water was generated from an aqueous solution of 1 µM $CaCl_2$ and 1 mM $MgCl_2$ according to Berjak *et al*., (2011). The CW and DW treated seeds were slowly agitated in the dark by gently shaking the Petri dish on a Labcon orbital shaking platform SPO-MP 15 (Laboratory Marketing Services, Maraisburg, South Africa) for 18 h at 25°C, removed, and surface dried with blotting paper immediately (Farooq *et al*., 2010) before sowing and ultrastructural analysis.

4.3.3 Seed germination and vigour

Seed germination and vigour were determined as described in section 2.2.8 of Chapter 2

4.3.4 Sample preparation for transmission electron microscopy

Seeds (5 each) of both species and the hybrid cured for different durations (0, 2 and 5 weeks) and invigorated with CW and DW for 18 h were randomly selected for processing for TEM examination. The methods used for sample preparation followed those described in section 3.3.5 of Chapter 3.

4.3.5 Microtomy and microscopy

The methods used for this aspect of the study followed those described in section 3.3.6 of Chapter 3.

4.3.6 Statistical analysis

The methods used for data analysis in this study follow those described in section 3.3.7 of Chapter 3. Two-way ANOVA was used to compare CW and DW imbibition within each field curing period within each species and hybrid while three-way ANOVA was used when data for both species and the hybrid were pooled for analysis.

4.4 Results

4.4.1 Seed germination and vigour

In both *O. sativa* (Fig. 4.1A) and *O. glaberrima* (Fig. 4.1B), seed germinability was comparable between CW and DW after 0 and 2 weeks of curing. In contrast, CW imbibed 5 week cured *O. sativa* and *O. glaberrima* seeds exhibited significantly higher germinability than DW imbibed seeds. For the hybrid, differences in germinability between CW and DW imbibed 0 week cured seeds were not significant. However, CW imbibed 2 and 5 week cured hybrid seeds had significantly higher germinability than the DW imbibed seeds (Fig. 4.1C).

In both species and the hybrid, 0, 2 and 5 week cured seeds imbibed with CW produced significantly longer radicles than DW imbibed seeds (Fig. 4.2). In *O. sativa*, radicles of CW imbibed seeds were longer than DW imbibed seeds after 0 (7%), 2 (9%) and 5 (20%) weeks of curing (Fig. 4.2A). In *O. glaberima*, radicles of CW imbibed seeds were also significantly

longer than DW imbibed seeds after 0 (18%), 2 (17%) and 5 (8%) weeks of curing (Fig. 4.2B). Similarly, for the hybrid, radicles of CW imbibed seeds were longer than DW imbibed seeds after 0 week (12%), 2 (14%) and 5 (15%) weeks of curing (Fig. 4.2C).

When 0 week cured *O. sativa* seeds were imbibed with CW, seedling dry mass increased significantly (10%; $P = 0.012$) relative to seedlings generated from DW imbibed seeds. After 2 (10%; $P = 0.049$) and 5 (9%; $P = 0.016$) weeks of curing, CW imbibed seeds also produced seedlings with a significantly higher dry mass compared with seedlings produced by DW imbibed seeds (Fig. 4.3A). In *O. glaberrima*, CW imbibed 0, 2 and 5 week cured seeds enhanced subsequent seedling dry mass significantly (7%, 6% and 9%, respectively) relative to DW imbibed seeds (Fig. 4.3B). For the hybrid, seedling dry mass was also significantly enhanced in CW imbibed 0, 2 and 5 week cured seeds by 6%, 7% and 9%, respectively, when compared with DW imbibed seeds (Fig. 4.3C).

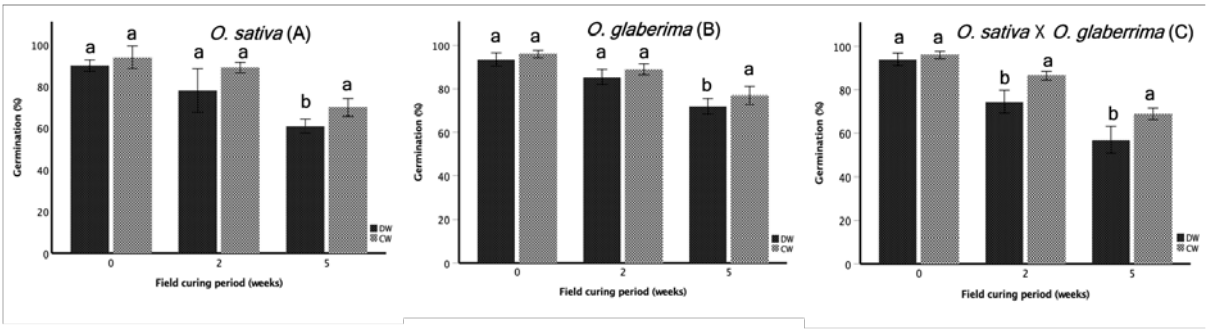

Figure 4.1. Percentage seed germination of *O. sativa*, *O. glaberrima*, and hybrid from each post-storage imbibition treatment [imbibed with de-ionized water (DW) and cathodic water (CW) for 18 h] of seeds field cured for 0, 2 and 5 weeks. Values represent mean (±SE of mean) (n = 50 seeds for each of four replicates). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across seed hydration treatments.

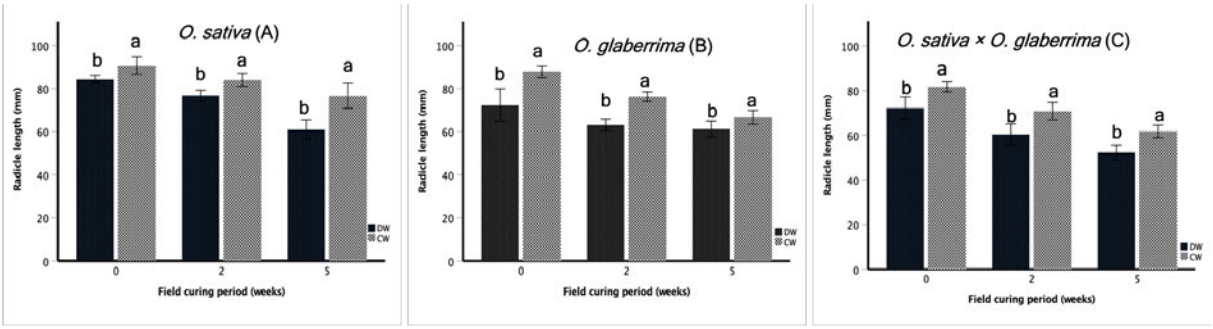

Figure 4.2. Radicle length at 7 DAS of seedlings of *O. sativa*, *O. glaberrima*, and hybrid from each imbibition treatment (DW, CW) of seeds field cured for 0, 2 and 5 weeks and stored for 20 months. Values represent mean (± SE of mean) (n = 10 seedlings for each of four replicates). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across imbibition treatments.

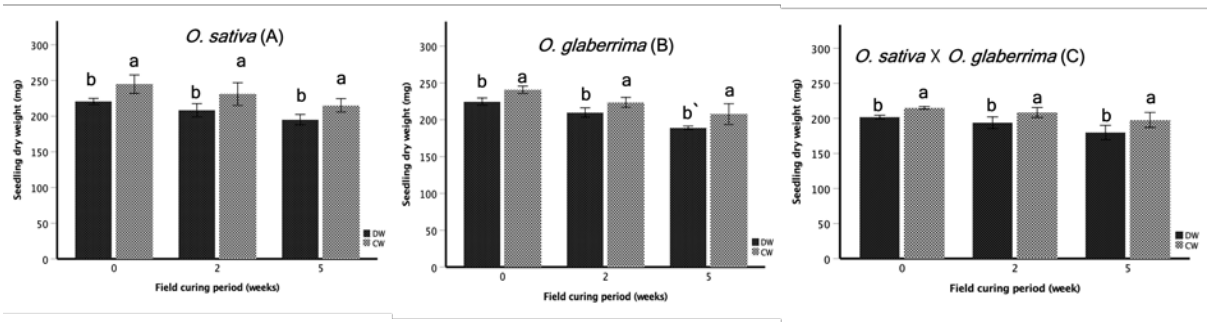

Figure 4.3. Seedling dry weight at 14 DAS of seedlings of *O. sativa*, *O. glaberrima*, and hybrid from each imbibition treatment (DW, CW) of seeds field cured for 0, 2 and 5 weeks and stored for 20 months. Values represent mean (± SE of mean) (n = 10 seedlings for each of four replicates). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across hydration treatments.

4.4.2 Curing-induced stress biomarkers response to cathodic invigoration

Given that the main objective of this study was to assess the alleviatory effect of CW on curing-induced stress ultrastructural biomarkers, we have focused on the seven ultrastructural biomarkers identified in Chapter 3. In addition to these seven biomarkers, we also focused on G which are traditionally used to assess physiological activity of imbibed/ germinating seeds

(Webb and Arnott, 1982; Berjak and Pammenter, 2000; Wang *et al*., 2012a; Sershen *et al*., 2016). For the ultrastructural studies, we focused on embryonic root meristematic cells.

4.4.2.1 Cell wall morphology

The withdrawal of plasmalemma from CWL observed in DW imbibed 2 week cured O. sativa (Fig. 4.4c) seeds appeared to be repaired by CW invigoration (Fig. 4.4d). The slight CWL folding which was apparent in DW imbibed 5 week cured *O. sativa* seeds (Fig. 4.4e) was not observed in CW imbibed 5 week cured *O. sativa* (Fig. 4.4f) seeds. However, withdrawal of plasmalemma from the CWL was apparent in both DW (Fig. 4.4e) and CW (Fig. 4.4f) imbibed seeds. In *O. sativa*, the cells appeared to be plasmolysed to some extent after the 5 week cured seeds were imbibed in DW (Fig. 4.4e) and CW (Fig. 4.4f) for 18 h. In 5 week cured *O. glaberrima* seeds, there appeared to be no apparent CWL folding in both DW (Fig. 4.5e) and CW imbibed seeds (Fig. 4.5f). Also, 5 week cured hybrid (Fig. 4.6e) seeds imbibed with DW (Fig. 4.6e) and CW (Fig. 4.6f) exhibited no apparent CWL folding. However, the cells appeared to be plasmolysed to some extent after 5 weeks of field curing in the DW imbibed hybrid seeds (Fig. 4.6e) but there appeared to be no plasmolysis in the CW imbibed seeds (Fig. 4.6f).

4.4.2.2 Cell area

In both species and the hybrid, there were no significant differences in cell area between CW and DW imbibed 0, 2 and 5 week cured seeds. When the two species and hybrid were compared, irrespective of the imbibition treatment, the cell area in 0 and 2 week cured seeds was comparable across both species and the hybrid. In contrast, there were significant ($P = 0.004$) differences in cell area across the species and the hybrid in 5 week cured seeds: it was highest in *O. glaberrima* (53. 26 ± 11.19 μm^2) followed by *O. sativa* (40.16 ± 4.83 μm^2) and the hybrid (40.09 ± 5.92 μm^2).

4.4.2.3 Nuclear and nucleolar morphology

When uncured (0 week cured) seeds of both species and the hybrid were imbibed with DW (Figs. 4.4a, 4.5a, 4.6a) and CW (Figs. 4.4b, 4.5b, 4.6b) for 18 h, their nuclei (N) presented a regular conformation with a distinct nuclear membrane (NM) surrounding their N. The cells presented a homogeneously dispersed chromatin in the nucleoplasm in cells of DW (Figs. 4.4a, 4.5a, 4.6a) and CW (Figs 4.4b, 4.5b, 4.6b) imbibed 0 week cured seeds. Numerous NV appeared

to be apparent in the nucleolus (nu) of DW (Figs. 4.4a, 4.5a, 4.6a) and CW (Figs. 4.4b, 4.5b, 4.6b) imbibed 0 week cured seeds of both species and the hybrid.

When 2 week cured seeds of both species and the hybrid were imbibed with DW (Figs. 4.4c, 4.5c, 4.6c) and CW (4.4d, 45d, 4.6d) for 18 h, their N were spherical in conformation, and the NM was regular and distinct in *O. sativa* (Figs. 4.4c, 4.4d), *O. glaberimma* (Fig. 4.5c) and the hybrid (Figs. 4.5c, 4.5d) seeds. In contrast, disruption of NM was apparent in some parts of N in the cells of DW (Fig. 4.5c) imbibed 2 week cured *O. glaberrima* seeds. Both species and the hybrid presented a homogeneously dispersed chromatin in the nucleoplasm in the cells of DW and CW imbibed 2 week cured seeds. Nucleolar vacuoles appeared to be apparent in the nu of DW and CW imbibed 2 week cured seeds of both species and hybrid. Whereas the nu in DW (Fig. 4.5c) imbibed 2 week cured *O. glaberrima* seeds looked disrupted, the nu in CW (Fig. 4.5d) imbibed 2 week cured *O. glaberrima* seeds was compact and spherical in conformation.

When 5 week cured seeds of both species and the hybrid were imbibed with DW for 18 h, the N profile presented an elliptical conformation in *O. sativa* (Fig. 4.4e), and a spherical conformation in *O. glaberrima* (Fig. 4.5e) and the hybrid (Fig. 4.6e). When 5 week cured seeds of both species and the hybrid were imbibed with CW, N presented a spherical conformation in *O. sativa* (Fig. 4.4f) and the hybrid (Fig. 4.6f) seeds but in CW imbibed *O. glaberrima* (Fig. 4.5f), the nuclear profile was lobed. The NM in DW imbibed *O. sativa* (Fig. 4.4e) and hybrid (Fig. 4.6e) seeds was less distinguishable than in CW imbibed 5 week cured *O. sativa* (Fig. 4.4f) and hybrid (Fig. 4.6f) seeds. In contrast, the NM in the cells in DW (Fig. 4.5e) and CW (Fig. 4.5f) imbibed 5 week cured *O. glaberrima* seeds was clearly defined and distinguishable. Generally, the nucleoplasm in the DW imbibed 5 week cured seeds of both species and the hybrid appeared to be characterized by chromatin condensation more than the CW imbibed seeds. The degree of chromatin condensation in DW (Fig. 4.5e) imbibed *O. glaberrima* seeds was greater than CW imbibed 5 week cured *O. glaberrima* (Fig. 4.5f) seeds. Except for CW imbibed 5 week cured *O. glaberrima* (Fig. 4.5f) seeds, NV appeared to be absent in the DW and CW imbibed 5 week cured seeds of both species and the hybrid.

In both species and the hybrid, the nuclear and nucleolar sizes in the cells did not differ significantly between DW and CW imbibed 0, 2 and 5 week cured seeds. When the two species and hybrid were compared, irrespective of the imbibition treatment, the nuclear and nucleolar sizes in 0, 2 and 5 week cured seeds were comparable across both species and the hybrid.

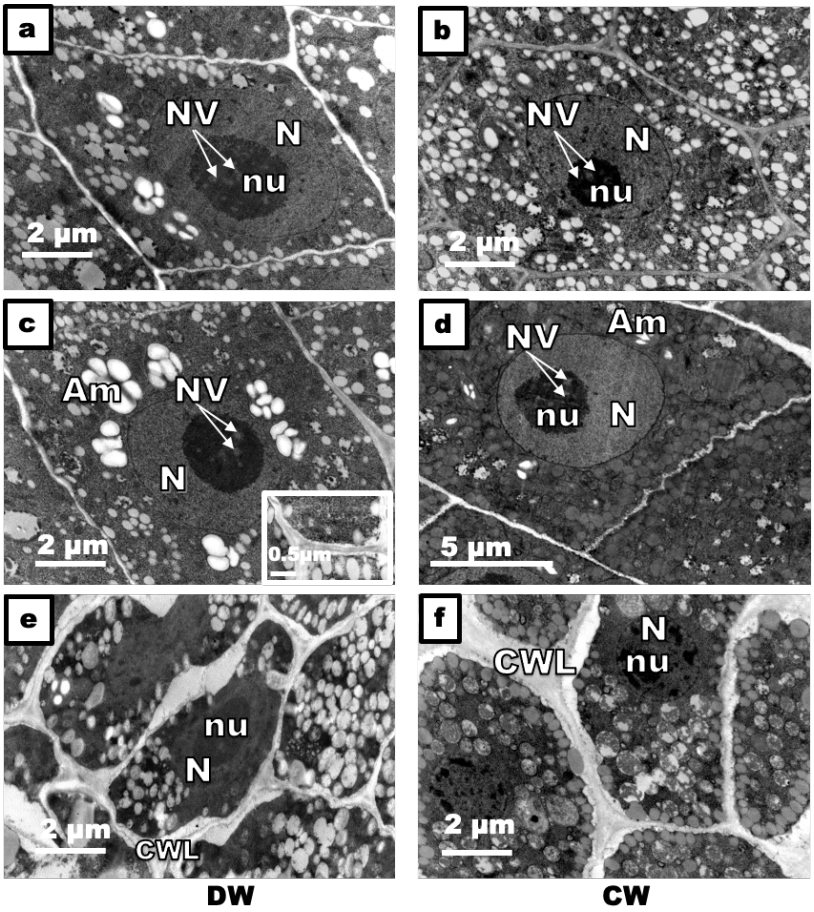

Figure 4.4 Changes in ultrastructure of embryonic root meristem cells of 20-month stored *O. sativa* seeds field cured for 0, 2 and 5 weeks and imbibed with deionized water (DW) and cathodic water (CW) for 18 h: a. 0 (non-cured) week cured seeds imbibed with DW, b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; N nucleus, nu nucleolus, NM nuclear membrane, NV nucleolus vacuole, CWL cell wall, Am amyloplast.

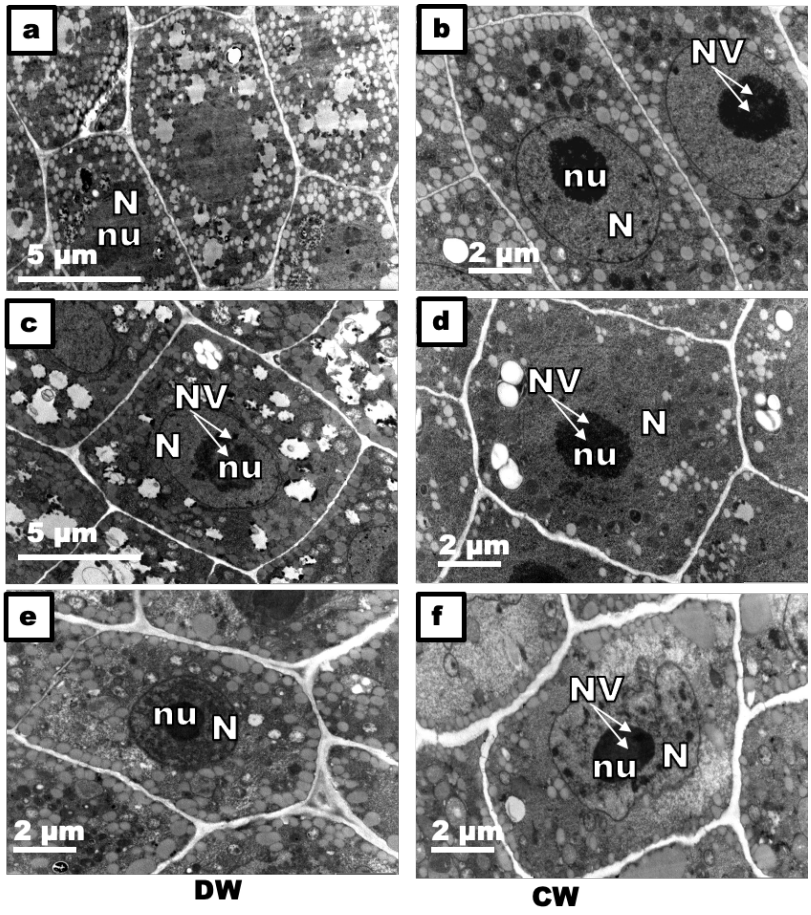

Figure 4.5 Changes in ultrastructure of embryonic root meristem cells of 20-month stored *O. glaberrima* seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; N nucleus, n nucleolus, NM nuclear membrane, NV nucleolus vacuole, Am amyloplast, arrow head marked cell wall folding.

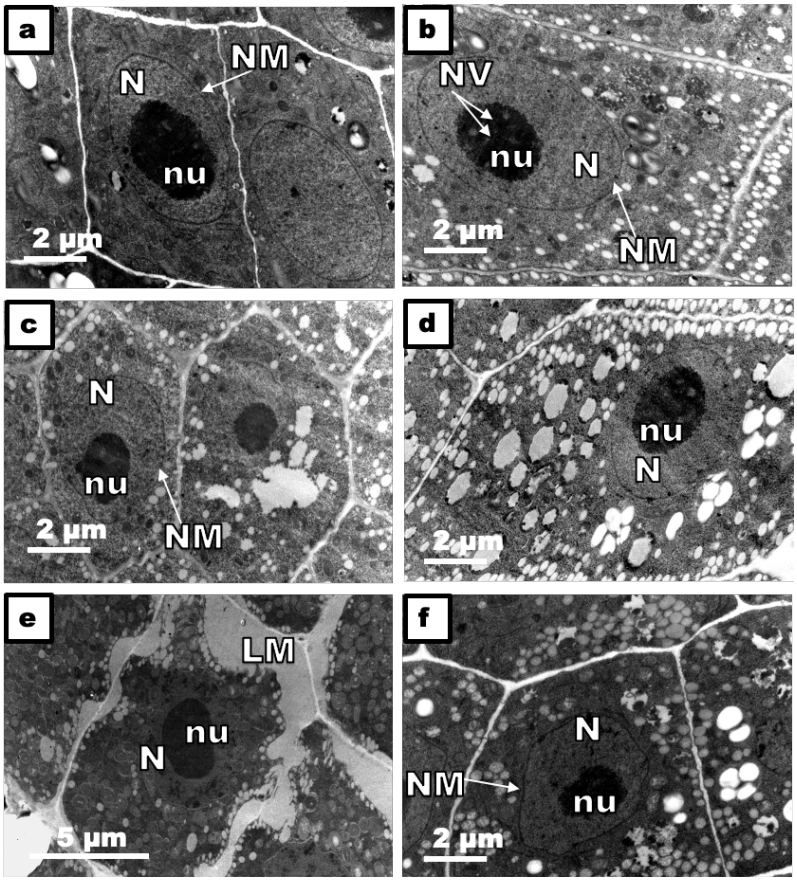

Figure 4.6 Changes in ultrastructure of embryonic root meristem cells of 20-month stored hybrid (*O. sativa* × *O. glaberrima*) seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; N nucleus, n nucleolus, NM nuclear membrane, LM lipid mass, NV nucleolus vacuole.

4.4.2.4 Mitochondrial morphology

In 0 week cured *O. sativa* seeds, the M in the meristematic cells presented circular (Fig. 4.7a) and elongated (Fig. 4.7b) profiles when the seeds were imbibed in DW and CW, respectively. In DW imbibed 0 week cured seeds, the cells exhibited M with OM and IM which lacked clearer definition (Fig. 4.7a). In contrast, OM and IM were well-defined and clearly distinguishable in CW imbibed 0 week cured seeds (Fig. 4.7b). Mitochondria in cells of CW (Fig. 4.7b) imbibed

0 week cured seeds showed ample signs of activity with greater differentiated internal structures and more homogenous matrices than the cells in DW (Fig. 4.7a) imbibed 0 week cured seeds. When 2 week cured *O. sativa* seeds were imbibed with DW or CW, some of the M in both DW (Fig. 4.7c) and CW (Fig. 4.7d) imbibed seeds presented elongated profiles. Whereas M showed heterogenous matrices with poorly differentiated Cr in the DW imbibed seeds, M in CW imbibed seeds exhibited a more homogenous and more differentiated internal structures than the DW imbibed seeds. The OM and IM were quite distinguishable, although some parts appeared to be damaged in the DW imbibed 2 week cured *O. sativa* seeds (Fig. 4.7c). In contrast, OM and IM were well defined in the CW imbibed 2 week cured seeds (Fig. 4.7d). In DW imbibed 5 week cured *O. sativa* seeds, some of the M exhibited an elongated profile with poorly differentiated internal structures and showed signs of division (Fig. 4.7e; M, arrowhead), while some of the M had exhibited vacuolar structures internally (Fig. 4.7e; insert). Both OM and IM were quite distinguishable in some parts of the mitochondrion but in some regions OM and IM appeared to be damaged (Fig. 4.7e; asterisk). On the contrary, in CW imbibed 5 week cured *O. sativa* (Fig. 4.7f) seeds, M in the meristematic cells exhibited circular profiles, a mixture of M with well-defined OM and IM and others (M'; arrowhead) exhibited signs of damage to the OM and IM. Those M with well-defined OM and MI showed ample signs of activity with greater differentiation of internal structures and homogenous matrices, and others (M' and M") exhibited limited signs of activity with poorly differentiated internal structures and heterogenous matrices (Fig. 4.7f).

In DW imbibed 0 week cured *O. glaberrima* seeds (Fig. 4.8a), M in the meristematic cells exhibited circular profiles characterized by homogenous matrices and poorly developed Cr. The M in cells of the 0 week cured seeds were a mixture of M with quite well-defined OM and IM, and others (M') that exhibited signs of damage to OM and IM (Fig. 4.8a; arrowhead). On the contrary, M in cells of CW imbibed 0 week cured *O. glaberrima* seeds (Fig. 4.8b) were a mixture of M with elongated (M) and circular (M') profiles which were characterized by homogenous matrices and greater differentiation of Cr when compared with DW imbibed 0 week cured seeds (Fig. 4.8a). The M in the cells of CW imbibed 0 week cured seeds (Fig. 4.8b) exhibited clearly defined OM and IM than DW imbibed 0 week cured seeds (Fig. 4.8a). The cells in DW imbibed 2 week cured *O. glaberrima* seeds (Fig. 4.8c) presented an elongated M characterized by lack of internal homogeneity and poorly differentiated internal structures. Both OM and IM were very distinguishable in some parts of the M and in other parts, M exhibited

signs of damage to the OM and IM in the DW imbibed 2 week cured seeds (Fig. 4.8c). On the contrary, the cells in CW imbibed 2 week cured *O. glaberrima* seeds (Fig. 4.8d) presented an elongated M profile with an active appearance and matrices that were homogenous and showed well-differentiated internal structures when compared with DW imbibed 2 week cured seeds. Both OM and IM were clearly defined in all the parts of the M in CW imbibed 2 week cured seeds (Fig. 4.8d) whereas in DW imbibed 2 week cured seeds (Fig. 4.8c), parts of the M exhibited signs of damage to the OM and IM. In DW imbibed 5 week cured *O. glaberrima* (Fig. 4.8e) seeds, M presented circular profiles and lacked clearly defined OM and IM, with poorly differentiated Cr being apparent (Fig. 4.8e; M"; M"'). Some M were deranged (M') and contained electron dense mitochondrial matrix without a clearly defined structure. In contrast, in CW imbibed 5 week cured *O. glaberrima* (Fig. 4.8f) seeds, M presented elongated profiles which exhibited signs of activity with greater differentiation of internal structures and clearly defined OM and IM than those (M"; M"') in DW imbibed 5 week cured seeds.

In DW imbibed 0, 2 and 5 week cured hybrid (Figs. 4.9a, c, e) seeds, M in the meristematic cells generally presented circular profiles. In contrast, M in the meristematic cells of CW imbibed 0, 2 and 5 week cured hybrid (Figs. 4.9b, d, f) seeds generally presented elongated profiles. Mitochondria showing signs of activity but without a clearly differentiated internal structures and homogenous matrices were apparent in the DW imbibed 0 week cured hybrid (Fig. 4.9a) seeds. On the contrary, M in the cells of CW imbibed 0 week cured hybrid (Fig. 4.9b) seeds exhibited fewer but well-differentiated internal structures and a slightly electron transparent patchy matrix. The M in the cells of both DW and CW imbibed 0 week cured seeds exhibited clearly defined OM and IM. In DW imbibed 2 week cured hybrid (Fig. 4.9c) seeds, the M in the cells contained electron dense mitochondrial matrix without a clearly differentiated internal structures. The M in cells of DW imbibed 2 week cured hybrid seeds were a mixture of M with well-defined OM and IM and others (M') that exhibited signs of damage to the OM and IM (Fig. 4.9c). In CW imbibed 2 week cured hybrid (Fig. 4.9d) seeds, M exhibited less dense mitochondrial matrix compared with DW imbibed seeds but M in the CW imbibed seeds showed greater signs of activity with well-differentiated internal structures and clearly defined OM and IM. In DW imbibed 5 week cured hybrid (Fig. 4.9e) seeds, M contained electron dense matrices, clearly defined OM and IM in some parts of the M, and Cr. In CW imbibed 5 week cured hybrid (Fig. 4.9f) seeds, M exhibited a homogenous matrix, clearly defined OM and IM and contained well-differentiated internal structures. Dividing M (arrows) and recently divided

M (asterisk) were present in some cells from DW 5 week cured hybrid (Fig. 4.9e) seeds, while some of the M in the CW imbibed 5 week cured hybrid (Fig. 4.9f) seeds appeared to have undergone fusion.

Differences in number of M/cell between CW and DW imbibed 0, 2 and 5 week cured seeds of both species and the hybrid were not significant. The range in the number of M/cell might explain the lack of differences between CW and DW in some of the treatments (Figs. 4.10A, B, C). When the two species and the hybrid were compared, irrespective of the imbibition treatment, differences in the number of M/cell in 0 and 2 week cured seeds was comparable. In contrast, differences in the number of M/cell in the 5 week cured seeds differed significantly ($P < 0.007$) across the species and the hybrid: it was highest in the hybrid (6.33 ± 2.88) followed by *O. glaberrima* (3.50 ± 1.87) and *O. sativa* (2.00 ± 2.10).

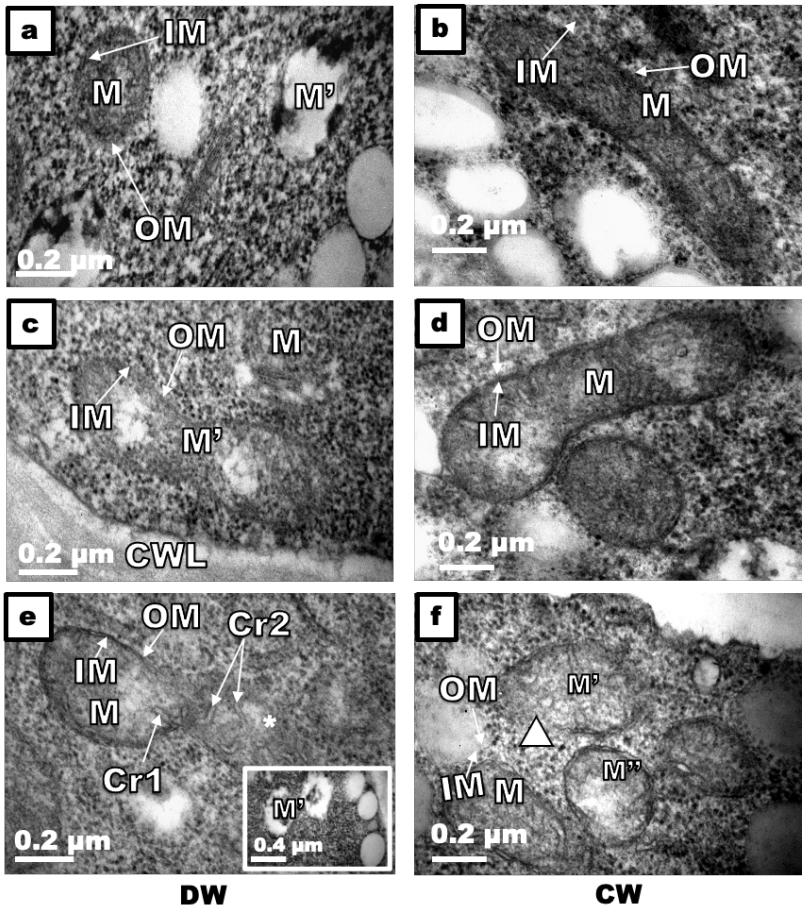

Figure 4.7 Changes in mitochondrial structure of embryonic root meristem cells of 20-month stored *O. sativa* seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, Cr Cristae, dr deranged mitochondria, asterisks(*) mitochondrial fission.

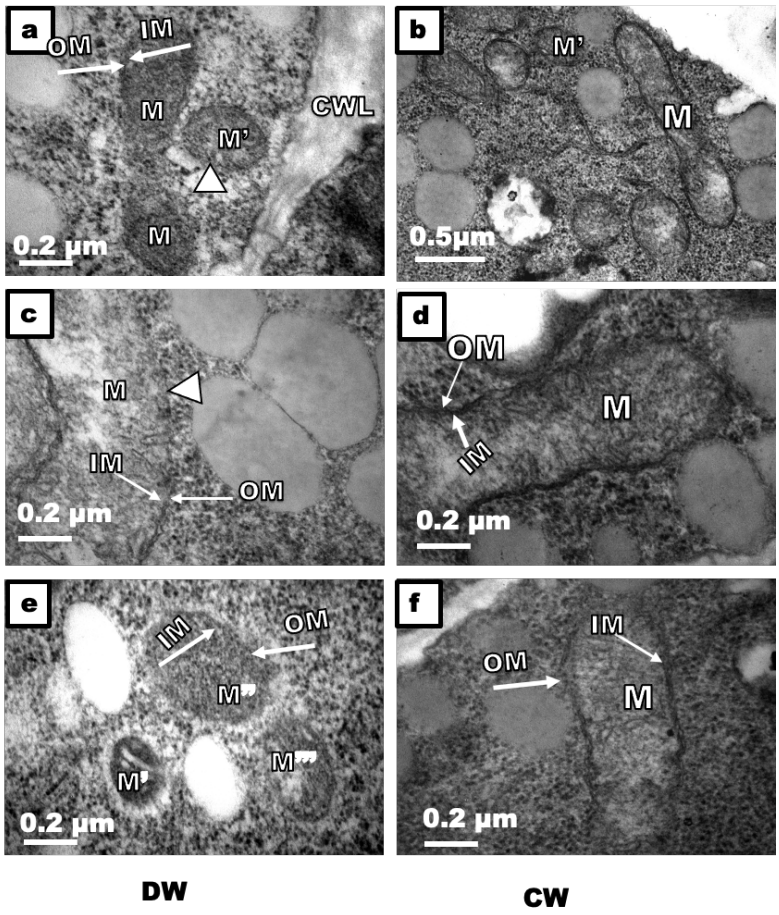

Figure 4.8 Changes in mitochondrial structure of embryonic root meristem cells of 20-month stored *O. glaberrima* seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, Cr Cristae, M' mitochondria with vacuolar structure, M'' and M''' mitochondrial fission.

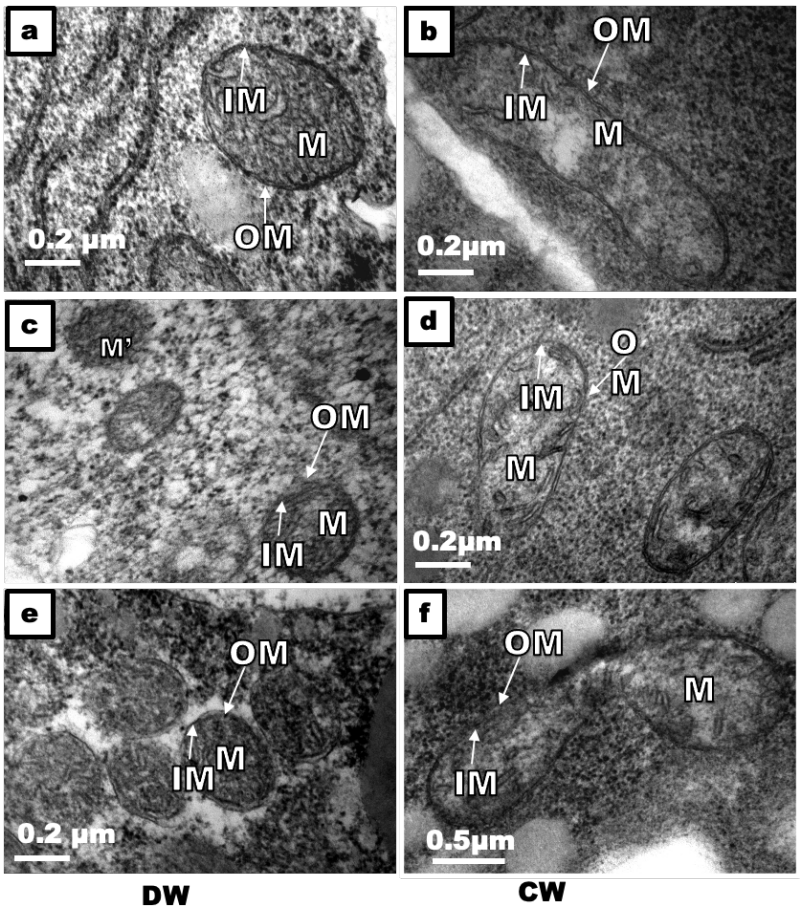

Figure 4.9 Changes in mitochondrial structure of embryonic root meristem cells of 20-month stored hybrid seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; M mitochondria, IM inner mitochondria membrane, OM outer mitochondria membrane, Cr Cristae, M' mitochondria with blurry membrane, arrows and asterisks show mitochondrial fission, ER endoplasmic reticulum, L lipid bodies.

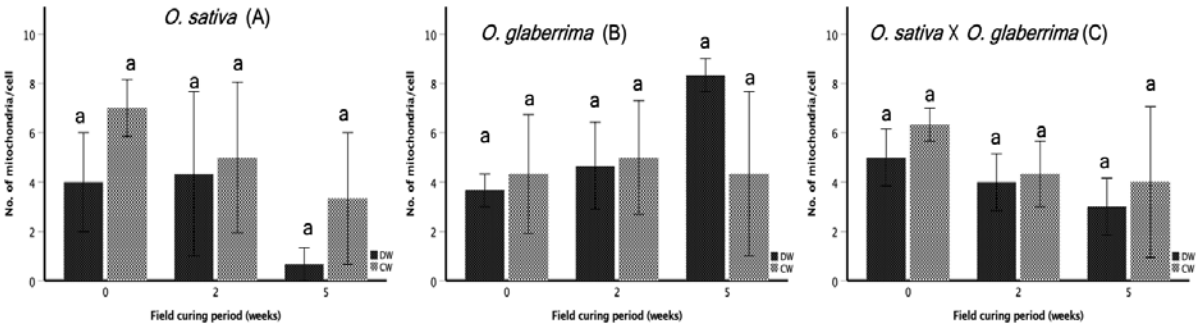

Figure 4.10. Number of mitochondria in DW and CW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and hybrid embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly ($P < 0.05$) different: lowercase letters represent comparisons within field curing period across imbibition treatments.

4.4.2.5 Golgi bodies

The degree of development and activity of G in the cells were greater in CW imbibed seeds than in the DW imbibed seeds in both species and the hybrid (Figs. 4.11 – 4.13).

In DW imbibed 0 cured *O. sativa* (Fig. 4.11a) seeds, G were disorganized and consisted of about four loose cisternae in the cytomatrix. In the CW imbibed 0 week cured *O. sativa* (Fig. 4.11b) seeds, the developing assembly of G appeared to be normal and active than observed in DW imbibed 0 week cured seeds. In both DW imbibed 2 (Fig. 4.11c) and 5 (Fig. 4.11e) week cured *O. sativa* seeds, the developing assemblies of G were normal and showed hypersecretory activities (arrowheads) with numerous G derived vesicles. In contrast, in CW imbibed 2 (Fig. 4.11d) and 5 (Fig. 4.11f) week cured *O. sativa* seeds, G were more developed and apparently more active than in cells in DW imbibed seeds; G showed apparently greater hypersecretory activity with numerous G derived vesicles (arrowheads) in the CW imbibed than DW imbibed seeds.

Cells in DW imbibed 0, 2 and 5 week cured *O. glaberrima* (Figs. 4.12a, c, e) seeds generally exhibited less developed G, sometimes with loose cisternae and appeared to have lacked much activity. In contrast, the cells in CW imbibed 0, 2 and 5 week cured *O. glaberrima* (Figs. 4.12b, d, f) seeds presented G which were normal, more developed and apparently more active than

DW imbibed seeds. However, G were more developed and apparently more active in *O. sativa* (Fig. 4.11; arrowheads) than in *O. glaberrima* (Fig. 4.12; arrowheads) seeds.

In the hybrid, the cells in the CW imbibed 0 (Fig. 4.13b), 2 (Fig. 4.13d) and 5 (Fig. 4.13f) week cured seeds presented many highly developed G with apparently very active G relative to DW imbibed 0 (Fig. 4.13a), 2 (Fig. 4.13c) and 5 (Fig. 4.13e) week cured seeds. Golgi bodies showed apparently greater hypersecretory activity with numerous G derived vesicles (arrowheads) in the CW imbibed than DW imbibed seeds. There were many highly developed and very active G in the hybrid than *O. sativa* (Fig. 11) and *O. glaberimma* (Fig. 4.12) seeds.

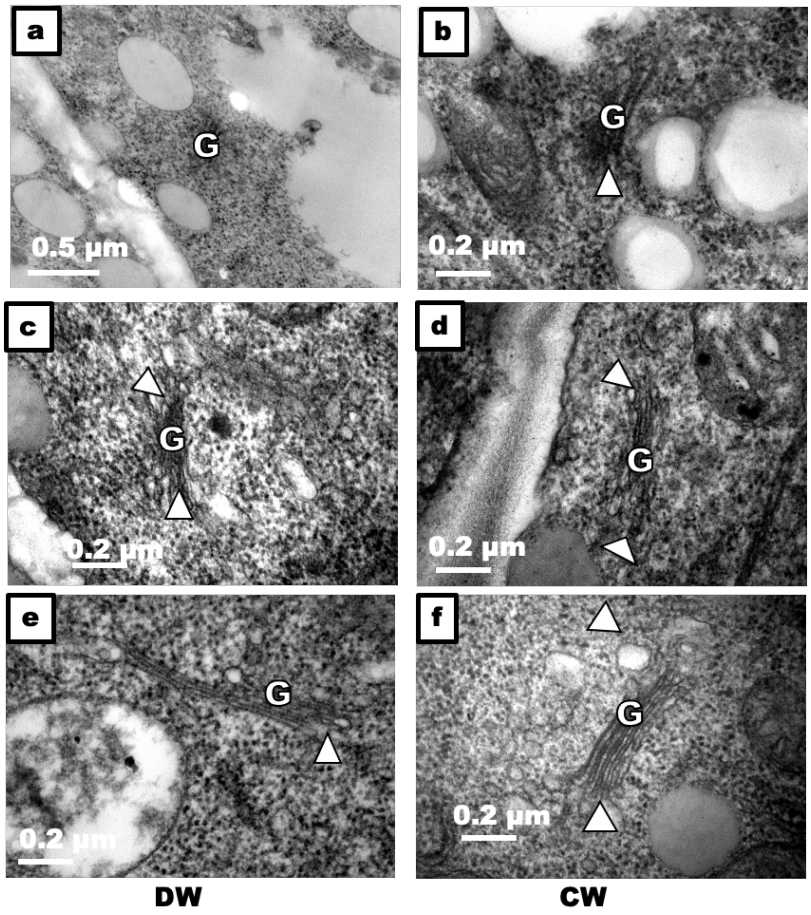

Figure 4.11 Development of Golgi bodies in embryonic root meristem cells of 20-month stored *O. sativa* seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; G Golgi bodies, arrowhead Golgi derived vesicles.

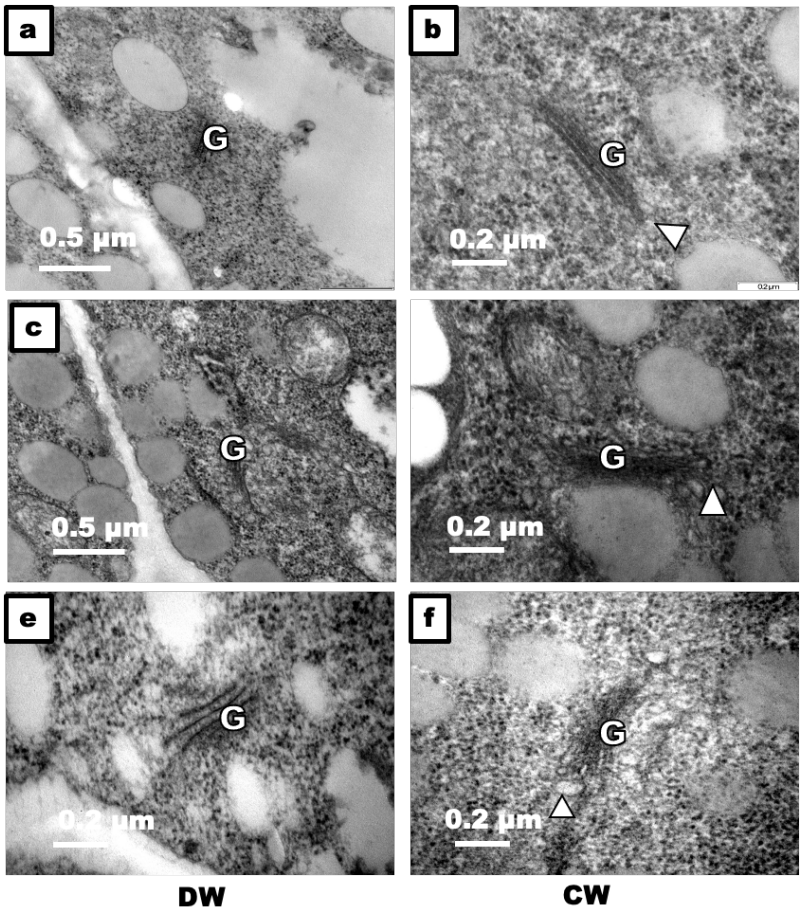

Figure 4.12 Development of Golgi bodies in embryonic root meristem cells of 20-month stored *O. glaberrima* seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; G Golgi bodies, arrowhead Golgi derived vesicles.

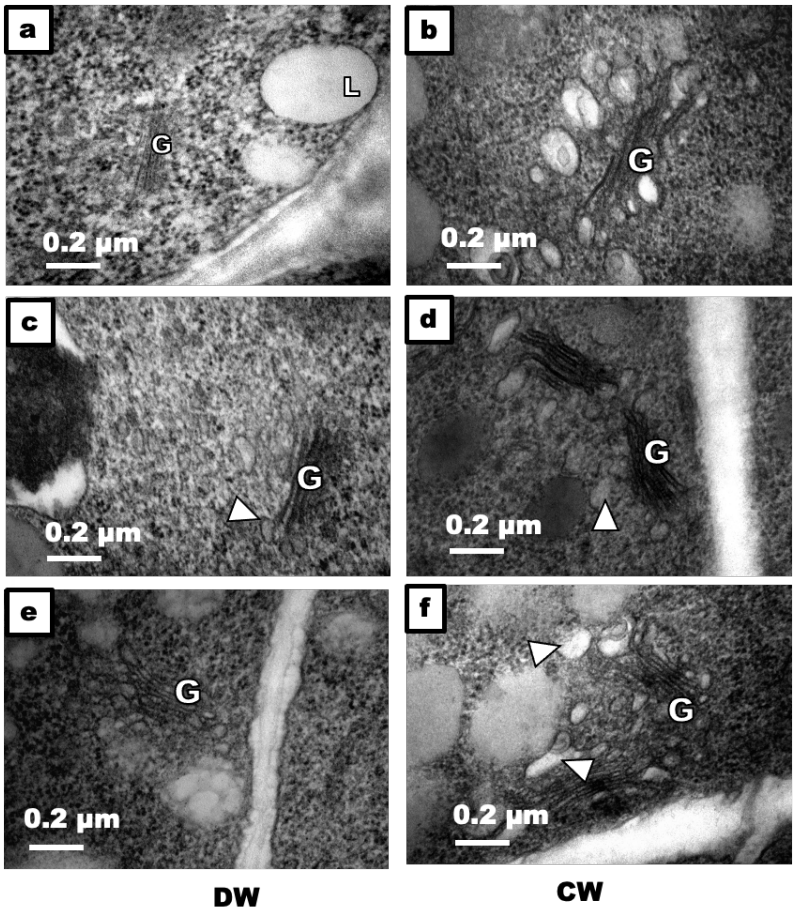

Figure 4.13 Development of Golgi bodies in embryonic root meristem cells of 20-month stored hybrid seeds field cured for 0, 2 and 5 weeks and imbibed with DW and CW for 18 h: a. 0 week cured seeds imbibed with DW; b. 0 week cured seeds imbibed with CW; c. 2 week cured seeds imbibed with DW; d. 2 week cured seeds imbibed with CW; e. 5 week cured seeds imbibed with DW; f. 5 week cured seeds imbibed with CW; G Golgi bodies, arrowhead Golgi derived vesicles.

4.4.2.6 Lipid bodies

In *O. sativa*, the number of lipid bodies/cell in CW and DW imbibed 0, 2 and 5 cured seeds were comparable (Fig. 4.14A). In 0 week cured *O. glaberrima*, the number of lipids/cell was significantly higher in CW than DW imbibed seeds. On the contrary, the number of lipid bodies/cell was comparable in CW and DW after 2 and 5 weeks of curing (Fig. 4.14B). In 0 week cured hybrid, the number of lipids/cell was significantly higher in CW than DW imbibed seeds. However, differences in the number of lipid bodies/cell between CW and DW imbibed 2 and 5 week cured hybrid seeds was comparable (Fig. 4.14C). The large variations in the

number of lipid bodies/cell in some of the CW imbibed treatments may explain the lack of differences between CW and DW. When both species and the hybrid were compared, irrespective of the imbibition treatment, differences in the number of lipid bodies/cell in 0 week cured seeds were significant (P = 0.001) across both species and the hybrid: the number of lipids/cell was higher in *O. glaberrima* (52.50 ± 9.61) than *O. sativa* (39.00 ± 10.06) and the hybrid (33.50 ± 14.31). In contrast, differences in the number of lipid bodies/cell in the 2 and 5 week cured seeds were comparable across both species and the hybrid.

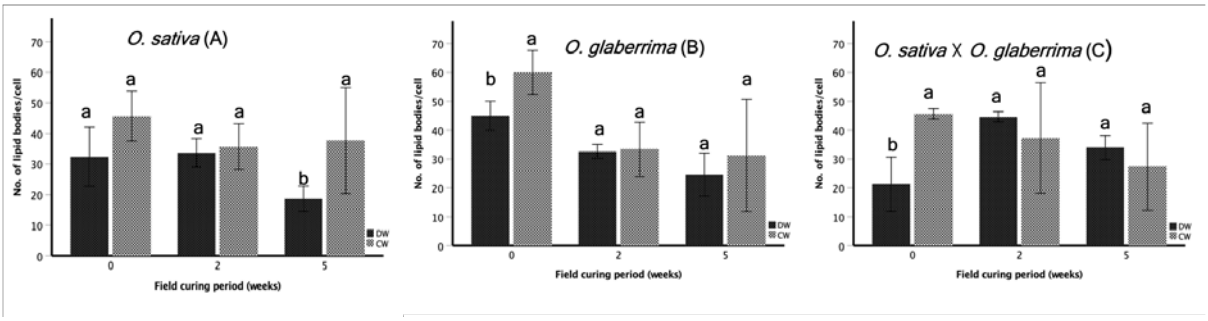

Figure 4.14. Number of lipid bodies in DW and CW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and hybrid embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (P < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments.

4.4.2.7 Cellular vacuolation

Except for 2 week cured *O. sativa* seeds, differences in the number of vacuoles/cell between CW and DW imbibed 0 and 5 week cured seeds was not significant (Fig. 4.15A). In *O. glaberrima,* differences in the number of vacuoles/cell of 0, 2 and 5 week cured seeds imbibed with CW and DW was not significant (Fig. 4.15B). For the hybrid, when 0 week cured seeds was imbibed with CW and DW, the number of vacuoles/cell was comparable between them. On the contrary, CW imbibed 2 and 5 week cured hybrid (Fig. 4.15C) seeds had significantly lower number of vacuoles/cell than the DW imbibed seeds. When both species and the hybrid were compared, irrespective of the imbibition treatment, the number of vacuoles/cell was comparable across the species and hybrid in 0 and 5 week cured seeds. In contrast, differences in the number of vacuoles/cell in the 2week cured seeds differed significantly (P = 0.026) across

the species and hybrid: the number of vacuoles/cell was higher in the hybrid (11.17 ± 6.05) than *O. sativa* (6.00 ±4.52) and *O. glaberrima* (5.33 ± 2.79) seeds.

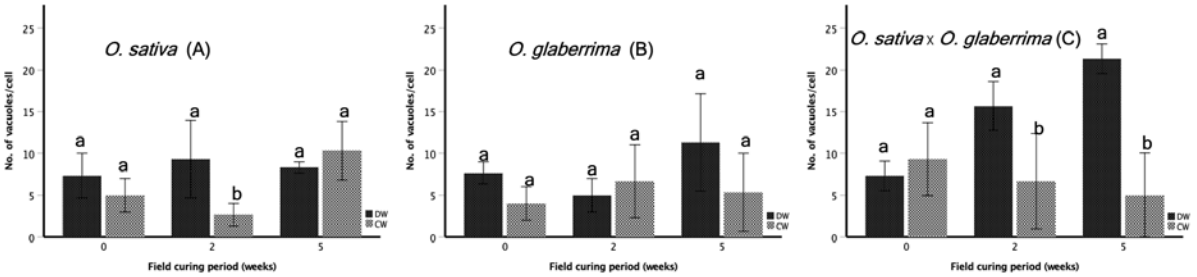

Figure 4.15. Number of vacuoles in DW and CW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and hybrid embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across imbibition treatments.

4.4.2.8 Amyloplasts

In both species and the hybrid, differences in the number of amyloplasts/cell were not significantly different between CW and DW imbibed 0, 2 and 5 week cured seeds (Fig. 4.16A, B, C). When both species and the hybrid were compared, irrespective of the imbibition treatment, the number of amyloplasts/cell was comparable across the species and hybrid in 0, 2 and 5 week cured seeds.

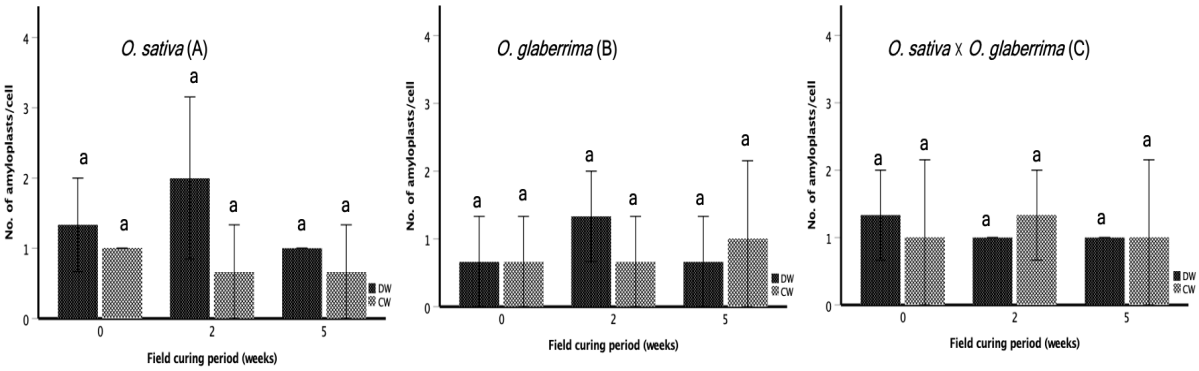

Figure 4.16. Number of amyloplasts in DW and CW treated embryonic root meristem cells of *O. sativa*, *O. glaberrima*, and hybrid embryos of seeds cured for 0, 2 and 5 weeks and stored for 20 months. Bars represent mean (± SE of mean) (n = 3). Values followed by different letters are significantly (*P* < 0.05) different: lowercase letters represent comparisons within field curing period across hydration treatments.

4.5 Discussion

Accumulation of ROS is considered a major cause of seed ageing (Bailly *et al*., 2002; Ratajczak *et al*., 2019; Wiebach *et al*., 2020) with the reduction in seed vigour and/or viability being an inevitable consequence (McDonald, 1999; Kong *et al*., 2014; Xia *et al*., 2015b). A variety of seed invigoration treatments have been used to enhance the vigour of aged seeds (Harris *et al*., 2002; Farooq *et al*., 2006; reviewed by Paparella *et al.*, 2015 and references therein). Some of these treatments include the use of antioxidants and salts that have the ability to act as reducing agents (Basra *et al*., 1994; Gondwe *et al*., 2016; Adetunji *et al*., 2020; Fatokun *et al*., 2020).

The study has shown that DW and CW invigoration differed in terms of alleviating curing induced seed deterioration in rice. This was evidenced by the fact that imbibition with CW compared with DW enhanced germination capacity in 5 week cured *O. sativa* and *O. glaberrima* seeds and 2 and 5 week cured hybrid seeds. Additionally, CW compared with DW invigoration enhanced seedling vigour (radicle length and seedling dry weight) in 0, 2 and 5 week cured *O. sativa*, *O. glaberrima* and hybrid seeds. These results suggest that CW, which is enriched in Ca^{2+} and Mg^{2+} ions, was more effective in alleviating curing induced deterioration

of both species and the hybrid; based on the results of previous studies this was very likely a consequence of the antioxidant potential of CW (Hanaoka *et al*., 2004; Adetunji *et al*., 2020; Fatokun *et al*., 2020b). Furthermore, CW has been reported to enhance levels of germination associated and antioxidant enzymes and reduce levels of lipid peroxidation products (Adetunji *et al*., 2020) and maintain DNA integrity (Fatokun *et al*., 2020b) in aged orthodox crop seeds. Studies have also shown Ca^{2+} to act as a second messenger to improve plant stress responses (Sanders *et al*., 1999; Kudla *et al*., 2018).

The beneficial effects of CW imbibition in enhancing seed germinability and seedling vigour in both species and the hybrid could also be attributed to the effects of these metal ions (Ca^{2+} and Mg^{2+}) on seed germinability and growth. Several reports (Meharg *et al*., 2008; Farooq *et al*., 2009; Hochmuth *et al*., 2012; Adetunji *et al*., 2020; Fatokun *et al*., 2020b) have shown that Ca^{2+} and Mg^{2+} are both essential cations which are required for seed germination and plant growth. In the present study this possibility is corroborated by the increased cellular and physiological activities in terms of strong development of G and mitochondrial differentiation (Berjak and Pammenter, 2000) and elongation (Chen *et al*., 2005; Chen and Chan, 2010; Gomes and Scorrano, 2011) induced in CW compared with DW imbibed seeds.

Delayed field curing of quiescent crop seeds in the humid tropics exposes these seeds to cycles of drying and wetting/imbibition which bring about the shrinking and re-expanding of cells, respectively. This environmentally induced repeated shrinking and expanding of cells can influence seed meristematic cellular ultrastructure (e.g. loss of CWL-plasmalemma connectivity or damaged plasmalemma (Webb and Arnott, 1982) and metabolism (Sacandé *et al.*, 2001; Walters and Koster, 2007; Long *et al*., 2011). Our findings have shown that CW invigoration appeared to have reversed the plasmalemma withdrawal observed in DW imbibed 2 week cured seeds (Fig. 4.4c) and the slight CWL folding observed in the embryonic root meristem cells of *O. sativa* (Figs. 4.4e) and may suggest the ability of CW invigoration to bring about the repair of CWL ultrastructural abnormalities induced by curing.

While nuclear and nucleolar sizes were comparable between DW and CW imbibed 0, 2 and 5 week cured seeds of both species and hybrid, there were no observed differences in nuclear and nucleolar profiles between DW and CW imbibed 0 and 2 week cured seeds of both species and the hybrid. The major structural change was found in the N of the 5 week cured seeds of both

species and the hybrid. For example, the degree of chromatin condensation in the nucleoplasm in the DW imbibed 5 week cured *O. glaberrima* (Fig. 4.5e) seeds appeared to be higher than in the CW imbibed *O. glaberrima* (Fig. 4.5f) seeds which based on the work of Petruzzelli *et al*. (1992) may be indicative of the suppression of nucleic acid biosynthesis in DW (as opposed to CW) imbibed seeds.

Mitochondria are a major target stress induced damage during seed storage due to their large turnover of ROS (Macherel *et al*., 2007; Møller *et al*., 2007; Ratajczak *et al*., 2019). Repair of M in deteriorated seeds is of fundamental importance for seed germination to progress. The degree of M differentiation (Berjak and Pammenter, 2000) and elongation (Chen *et al*., 2005; Chen and Chan, 2010; Gomes and Scorrano, 2011) are, therefore, important physiological indicators of meristematic cell capacity to participate in metabolic activities related to growth/developmental processes (Berjak and Pammenter, 2000; Wang *et al*., 2012a). Although we did not observe significant differences in the number of M/cell between DW and CW imbibed 0, 2 and 5 week cured seeds of both species and the hybrid (Fig. 4.10), there were marked differences in mitochondrial morphology between DW and CW invigorated seeds (Figs. 4.7 – 4.9). For example, M in DW invigorated seeds were mostly circular in profile while those in CW imbibed seeds exhibited elongated profiles (Figs. 4.7 – 4.9). Furthermore, in DW imbibed seeds of both species and the hybrid, M exhibited an electron dense mitochondrial matrix, undifferentiated Cr and poorly-defined OM and IM: these features decreased in prominence with delayed field curing but were still present. In contrast, in CW invigorated seeds, M exhibited homogenous matrix, well differentiated Cr, well-defined OM and IM in line with germinative metabolism irrespective of the curing period in both species and hybrid (Figs. 4.7 – 4.9). Longer M are associated with more Cr (Gomes and Scorrano, 2011) and this serves to maintain MM potential, increase ATP production and respiratory capacity (Srivastava, 2017), reduce ROS generation (Liesa and Shirihai, 2013) and protect M from degradation (Gomes and Scorrano, 2011). On the contrary, shorter M are less efficient in ATP production, displays latent dysfunction, and contribute to faster cell death (Gomes and Scorrano, 2011). This might explain why CW imbibed seeds which had longer and more differentiated M than the DW imbibed seeds exhibited enhanced germinability and vigour relative to the DW imbibed seeds.

Cellular membranes are a major site of ultrastructural damage during desiccation (Leprince *et al*., 1993; Walters *et al*., 2002; Benamar *et al*., 2003; Woodenberg *et al*., 2018) and their ability to repair upon rehydration is key prerequisite of desiccation tolerance in plant cells and tissues (Webb and Arnott, 1982; Bewley, 1997; Walters and Koster, 2007). Mitochondrial membranes are the primary targets for ageing- and desiccation induced damage (Benamar *et al*., 2003; Wang *et al*., 2012a). Seed survivability after imbibition-dehydration increases with increased recovery of mitochondrial structure and function during seed germination (Wang *et al*., 2012a). Both OM and IMs of M in the cells of CW imbibed 0, 2 and 5 week cured seeds of both species and hybrid were clearly defined and distinguishable unlike those in the cells of DW imbibed seeds. Furthermore, in some cases some parts of some M in DW imbibed seeds exhibited signs of damage to OM and IM. Ca^{2+} is very vital for the structure and function of plant cells and membranes (White and Broadley, 2003; Wang *et al*., 2012a), and influences the stability and function of M (Wang *et al*., 2012a). Therefore, Ca^{2+} ions in CW and the strong reducing properties of CW relative to DW (Berjak *et al*., 2011; Adetunji *et al*., 2020; Fatokun *et al*., 2020b) might have resulted in a more effective repair and protection of MMs (OM and IM) in CW imbibed 0, 2 and 5 week cured seeds.

One of the important ultrastructural biomarkers associated with seed deterioration is the disappearance of G (Smith and Berjak, 1995). Golgi bodies are responsible for synthesizing and trafficking complex carbohydrates for plant CWL and transport proteins to vacuoles (Matheson *et al.*, 2006). Therefore, the development of G in cells during imbibition is an important indicator of intracellular activity and physiological status (Berjak and Pammenter, 2000; Sershen *et al*. 2016). The highly developed G (indicative of high levels of activity) observed in CW compared with DW imbibed seeds of both species and hybrid cured seeds suggest higher levels of intracellular metabolic activity in CW imbibed seeds. Furthermore, the highly developed and apparently very active G observed in the hybrid (Fig. 4.13) relative to *O. sativa* (Fig. 4.11) and *O. glaberimma* (Fig. 4.12) cured seeds also suggest a more vigorous cellular activity in the hybrid than both species.

In uncured *O. glaberrima* and the hybrid and cured *O. sativa* seeds, CW invigoration significantly enhanced the number of lipid bodies/cell compared with DW imbibed seeds (Fig. 4.14). Though there was no statistical difference between CW and DW in the number of lipid bodies/cell in the 2 and 5 week cured *O. glaberrima* and hybrid seeds, there was a trend for CW

imbibed seeds to have higher number of lipid bodies/cell. This observation including the significantly higher number of lipid bodies/cell in the CW invigorated cured *O. sativa* seeds may suggest the effect of CW in alleviating curing-induced deterioration. Lipid peroxidation and hydrolysis of membrane lipids by PLD may destroy lipids in embryo cells and contribute to the aging process in both natural and accelerated aged seeds (Devaiah *et al*., 2007; Wang *et al*., 2012b).

Increases in vacuolation and fusion of vacuoles prior to autophagy in response to desiccation stress had been observed in the embryonic root meristematic cells of recalcitrant seeds of some species (Berjak *et al*., 1999; Wesley-Smith *et al*., 2001; Sershen *et al*., 2016). The number of vacuoles/cell in 2 week cured *O. sativa* and 2 and 5 week cured hybrid seeds was significantly higher when they were invigorated with DW as opposed to CW (Fig. 4.15). This may be an indication of higher levels of autophagic elimination of damaged organelles in DW compared with CW invigorated seeds. This further supports the fact that CW may have reduced oxidative stress induced damage during imbibition to a greater degree than DW. Similarly, in *Brassica napus* seeds, osmopriming with PEG led to the reduction in the number of vacuoles (Lechowska *et al*., 2019).

4.6 Concluding remarks

The main aim of this study was to assess whether curing-induced stress/ damage subjected to rice seeds could be alleviated by CW invigoration in relation to eight ultrastructural biomarkers of stress/ damage The study has shown that imbibing uncured and cured seeds of both species and hybrid with CW appears to have enhanced the repair of damaged cellular components and/or reduced the damage that ensues during imbibition in deteriorated seed. More importantly, cured and uncured seed subjected to CW invigoration exhibited more prominent signs (e.g., M which exhibited elongated profile, homogenous matrix, well differentiated Cr, and well-defined OM and IM, and highly developed G) of germinative metabolism than those invigorated with DW. In fact DW invigorated seeds exhibited several ultrastructural abnormalities (e.g., M were often swollen with poorly differentiated Cr). These findings may explain the enhanced seed germinability in cured seeds and vigour in CW in both uncured and cured seeds relative to DW invigorated seeds. The hybrid appeared to have responded best to CW imbibition, particularly in terms of M and G development and activity, which appears to

have translated into higher germinability of cured seeds relative to the two species. Our results suggest the effectiveness of CW in alleviating curing-induced ultrastructural damage and loss of vigour and viability in rice seed and provides impetus for future studies on the application of this invigoration technique to other crop species susceptible to deterioration during processing and storage.

CHAPTER 5: Ameliorating the effects of field curing on rice seed and seedling performance through cathodic invigoration: A comparison among *Oryza sativa* L., *Oryza glaberrima* Steud and their interspecific hybrid

R.K. Bam, B. Varghese, N. Murugan, N.W. Pammenter and Sershen

5.1 Abstract

Seeds of two upland rice species (*O. sativa* and *O. glaberrima)* and their interspecific hybrid (*O. sativa* × *O. glaberrima*) grown in subtropical Ghana were harvested at maturity (non-cured) and after 2 and 5 weeks of curing in the field. These seeds were then stored at 4℃ for 20 months, after which they were assessed for endosperm integrity, germinability, seedling emergence and vigour and plant growth following invigoration with cathodic water (CW) and deionized water (DW). The non-cured seeds of both species and the hybrid exhibited no evident structural damage, while 5 weeks cured seeds incurred severe post-storage structural damage to the endosperm. The caryopsis coat of both species was comparably thicker than the hybrid before and after curing; caryopsis coat thickness did, however, decrease in all three seed types with curing. Seedling emergence and vigour declined in both species and the hybrid after 5 weeks of curing. While post-storage invigoration with CW and DW did not significantly enhance seedling emergence of non-cured seeds, seedling emergence was enhanced in 2 week cured seeds of *O. sativa* and *O. glaberrima* by CW and DW comparably. However, CW was more effective for the invigoration of 2 week cured hybrid and 5 week cured seeds of both species and the hybrid. Both CW and DW enhanced seedling vigour of 0 week cured *O. sativa* and the hybrid comparably, but CW was more effective in enhancing seedling vigour in 0 week cured *O. glaberrima* and 2 and 5 week cured seeds of both species and hybrid. In general, the subsequent growth in terms of total, stem, leaf, root and panicle biomass at anthesis was not affected by field curing in both species and the hybrid. Cathodic invigoration enhanced panicle biomass, but differences between CW and DW was not always significant. The degree to which field curing compromises rice seed and seedling emergence and vigour differed across species. But cathodic invigoration may ameliorate these adverse effects in rice grown in the humid tropics.

Keywords: Aleurone grains, caryopsis coat, cathodic water, rice, seed storage, vigour

5.2 Introduction

Seedling emergence and enhancement of seedling performance is of fundamental relevance, especially in current times of unprecedented global climate change. Poor plant establishment resulting from poor germination and uneven plant stand has been a major challenge to rice production in direct-seeded rice production systems in tropical and sub-tropical regions (Harris *et al*., 2001, 2002). Loss of seed vigour and viability in storage before sowing is one of the major factors accounting for poor plant establishment (Finch-Savage and Bassel, 2016). Reduction in seed vigour results in slower and less uniform seedling emergence which can subsequently affect dry matter accumulation and yield (Tekrony and Egli, 1991). For crop species, plant establishment and early vegetative growth are critical for out-competing agricultural weeds (Asch *et al.*, 1999). For most crops, improving seed storage longevity and subsequent plant establishment begins with reducing physical (field weathering) and physiological deterioration of seeds (Tekrony *et al*., 1980; Anderson and Baker, 1983). A previous study by Bam *et al*. (2019) showed that prolonged field curing of harvested rice panicles before threshing compromises subsequent seed quality as a consequence of repeated exposure to drying and wetting events. This exposes the rice panicles to repeated drying-wetting cycles, and in the humid tropics, this results in moisture re-absorption, which can cause endosperm cracking. Loss of endosperm integrity can compromise rice seed embryo growth (Menezes *et al*., 2012) and subsequent plant establishment. Hence, it is imperative to guard against and/or ameliorate the effects of post-harvest practices that promote *in situ* weathering and physiological deterioration of seeds. Such measures can improve post-harvest seed quality and storage longevity and plant establishment and performance (Tekrony *et al*., 1980; Anderson and Baker, 1983).

Seed quality, which includes longevity during storage, is acquired during seed development and maturation (Leprince *et al*., 2017), but pre-harvest environmental conditions and poor post-harvest drying practices can negatively impact seed quality and storage longevity (Whitehouse *et al*., 2015; Kumar and Kalita, 2017). Seed quality is susceptible to high temperatures prior to the end of the seed-filling phase. In rice, when such temperatures accompany the early ripening stage (maturation drying), endospermic cracking is promoted (Hayashi *et al*., 2015). This cracking can compromise rice seed quality, storage longevity and subsequent plant establishment (Menezes *et al.*, 2012). Additionally, while curing in the field, seeds can be exposed to high temperatures and RH, typical of the tropics, which can accelerate seed

deterioration after seeds have lost their free water (Roberts, 1973; Leprince *et al*., 2017), the basis for accelerated ageing (Delouche and Baskin, 1973) and controlled deterioration (Matthews, 1980).

Alternate drying-wetting events associated with delayed field curing in tropical environments (Bam *et al*., 2019) are thought to disturb this metabolically and physically quiescent phase in seeds, compromising seed quality by allowing for unbalanced metabolism at intermediate water contents, which can lead to oxidative stress (Kranner *et al*., 2010). Uncontrolled production of ROS in aged seeds (reviewed by Bailly 2008; Rajjou and Debeaujon, 2008) has spurred several studies on post-harvest treatment (mostly priming) of seeds with antioxidants such as ascorbic acid (Farooq *et al*., 2010; Ye *et al*., 2012; Farooq *et al*., 2012; Gondwe *et al*., 2016), α-tocopherol and glutathione (Draganic and Lekic, 2012; Adetunji *et al*., 2020) etc. Invigorating aged or deteriorated seeds with a reducing agent that can react with free radicals and sowing them without drying them back to shedding MC (as is done in priming) has been shown to be more beneficial than drying-back in rice (Basra *et al*., 1994; Farooq *et al*., 2010, Wang *et al*., 2012a). Seed invigoration treatments enhance seed germination totality and rate and improve seedling vigour and growth in rice (Harris *et al*., 2002; Farooq *et al*., 2006, 2010).

Whilst a wide range of inorganic salts and synthetic and organic antioxidants have been used to prime and/ or invigorate aged and deteriorated seeds, a novel electrochemical approach to invigorating seeds involving cathodic protection has also been described by Berjak *et al*. (2011). The authors demonstrated that CW ameliorated oxidative stress and enhanced normal seedling production (root and shoot development) and growth (biomass yield) from cryopreserved *Strychnos gerrardii* axes. Gondwe *et al*. (2016) were the first to have shown that CW invigorated seeds improved seed germinability and vigour (viz., *P. sativum*, *Cucurbita maxima* and *Lycopersicon esculentum*) of naturally aged orthodox seeds. Also, Adetunji *et al*. (2020, 2021) and Fatokun *et al*. (2020a, b) have shown the ability of CW to scavenge ROS and enhance endogenous antioxidant capacity more efficiently than DW controlled deteriorated seeds. Their findings also highlight differences in the species response to CW and the level of controlled deterioration ageing. Thus, CW may have the ability to ameliorate the adverse effects of environmentally-induced seed deterioration and enhance seedling performance and crop yield.

Therefore, the present study assessed whether invigoration with CW and DW improves seed germinability and emergence, and subsequent plant growth in field cured seeds of *O. sativa*, *O. glaberrima* and their interspecific hybrid. The results of the invigoration experiments were also related to caryopsis thickness and endosperm responses to field curing as part of this inter-species comparison.

5.3 Materials and Methods

5.3.1 Plant material

The methods used for this aspect of the study follow those described for wet cured seeds in sections 2.3.1 and 2.3.2 of Chapter 2. After 20 months of storage at 4 °C, non-field cured and field cured seed samples were assessed for physical deterioration (as described below), germination, seedling establishment and growth studies in the greenhouse.

5.3.2 Physical deterioration assessment

5.3.2.1 Endosperm integrity

To assess the endosperm integrity of the seeds, ten seeds per species were randomly drawn from each of the non-cured (week 0) and cured (week 2 and 5) seed batches, de-hulled, and assessed for endosperm integrity using a SEM. Images of whole seeds were first captured using a Nikon AZ100 stereo microscope mounted with a Nikon DXM 12000C camera (Nikon, Japan) to determine the presence (and nature) or absence of cracks. Seeds with cracks from the field-cured treatments were then mounted on aluminium stubs with tape, coated with gold and scanned using a Field Emission Gun Scanning Electron Microscope (FEGSEM) (Ultra Plus, Zeiss, Germany) to determine the nature/severity of the physical damage to the endosperm. FEGSEM photomicrographs were captured between 5 and 12 kV.

5.3.2.2 Measurement of caryopsis coat thickness

Randomly selected seed samples (n = 3) of each species cured for 0, 2 and 5 weeks were de-hulled and measured for seed coat thickness. About 2 mm of seed material was excised from the embryo end of each seed before fixing the remaining sample immediately, according to Bechtel and Pomeranz (1978). This method, which was originally developed for fixing dry rice caryopsis for TEM was modified slightly: samples were embedded in resin, sectioned, and etched in potassium methoxide; blocks were sonicated to loosen the resin, after which they were

coated with gold and viewed using a Zeiss Leo 1450 SEM (Zeiss, Germany). Measurements of caryopsis coat thickness were taken at approximately the same locations (n=6 per sample, at different places along the caryopsis) across samples.

5.3.3 Seed invigoration treatments, seed germination and seedling emergence and plant growth trials in the greenhouse

Seeds of both species and the hybrid, cured for 0, 2 and 5 weeks, were withdrawn from storage and held overnight at 25 °C to equilibrate before invigoration. These seeds were invigorated with either CW or DW by placing 50 seeds per replicate/treatment (for cured) and for the control (non-cured) on a germination towel (two layers) within a 9 cm Petri dish. The towel was moistened with 5 ml of CW or DW and covered with another layer of germination towel moistened with 5 ml CW or DW, respectively. Cathodic water was generated from an aqueous solution of 1 µM $CaCl_2$ and 1 mM $MgCl_2$, according to Berjak *et al*. (2011). The CW and DW treated seeds were slowly agitated by gently shaking the Petri dish on a Labcon orbital shaking platform SPO-MP 15 (Laboratory Marketing Services, Maraisburg, South Africa) for 18 h at 25°C, removed, and surfaced dried with blotting paper immediately (Farooq *et al*., 2010) before sowing. Non-invigorated seeds were used as the control for this experiment.

5.3.3.1 Laboratory study

Four replicates of 50 seeds from each treatment combination (species × curing duration × imbibition solution) and their NI controls were tested for germination between moist rolled paper towels measuring 10 in. × 15 in. (Anchor paper Co., USA) at 34/11 °C (16 h day/ 8 h night) for 14 days (Ellis *et al*., 1983). Germination counts were conducted daily until there was no further germination. The criterion for germination was 2 mm radicle elongation. Seed vigour was based on mean germination time (MGT). Mean Germination Time in days was calculated for each replicate of treatment following the formula of Ellis and Roberts (1980):

$$MGT\ (days) = \Sigma(nD)/\Sigma n$$

where n is the number of seeds newly germinated on day D, D is the number of days after the beginning of the experiment and Σn is the final germination. MGT measures the lag period between the start of imbibition during germination and the mean germination time when the radicle reaches 2 mm (Matthews and Khajeh-Hosseini, 2007).

5.3.3.2 Greenhouse study

Polyvinyl chloride (PVC) pipes (20 cm in diameter and 60 cm long) with 0.5 cm holes along their length and closed-off at the base with porous plastic membrane were filled with soil, compacted with an iron bar and watered to field capacity a day before sowing. Seeds of each treatment combination (species × curing duration × imbibition solution) and their NI controls were sown (10 seeds in each of 27 pipes, per treatment combination) at about 2 cm beneath the soil surface. This experiment was carried out in a temperature-controlled polycarbonate greenhouse under ambient light: 32°C/19°C (day/night), 70% RH, 1200 μmol m^{-2} s^{-1} at midday. Five trials, with 27 pipes in each, were run concurrently, and treatments and controls were arranged in a randomized complete block design.

5.3.4 Plant establishment and growth measurements

Plant establishment (referred to as seedling emergence, henceforth) was assessed 14 days after seeding (DAS), after which seedlings were thinned to one per pipe. Seedling vigour was assessed as the seedling height at 28 DAS. Seedling height for each treatment was measured from the surface of the soil to the base of the youngest leaf using a steel ruler. The plants were watered to field capacity as and when necessary after thinning until harvesting. The position of the pipes was randomly rearranged once a week. At harvest (i.e. 112 DAS), plant height for each treatment was measured from the surface of the soil to the tip of the main stem panicle. The number of tillers in each treatment were counted to obtain the total number of tillers per plant. The plants were then removed from the pipes by gentle immersion in a tank filled with tap water. The roots were gently but thoroughly washed until all the soil particles were removed. The total length of the plant was measured from the tip of the root to the tip of the panicle using a steel ruler. The roots were then separated from the plant from the crown region of the root. Each plant was then separated into panicles, leaves, stem and roots, and oven-dried at 80^{o}C for 72 h. Dry weights of these plant organs were weighed to five decimal places using an electronic balance (MT5; Mettler, Giessen, Germany). These data were used to calculate total dry weight, root: shoot ratio (RSR) and biomass allocation patterns to panicles, leaves, stems and roots (Poorter *et al*., 2012).

5.3.5 Data analyses

The methods used in analysing data in this study follow those described in sections 2.2.8 of Chapter 2 and 3.3.7 of Chapter 3. Correlation coefficients were determined between curing time and seedling emergence and MGT.

5.4 Results

5.4.1 Physical deterioration assessment

5.4.1.1 Endosperm integrity

There was absence of endosperm cracks in non-field cured (week 0; control) seeds stored at 4ºC for 20 months (Fig. 5.1A). However, the endosperms in 5-week field cured seeds of both species and the hybrid exhibited multiple cracks (Fig. 5.1B), in the direction of both the short and long axis. The 5-week field cured seeds showed deep cracks, which exposed the aleurone cells (Fig. 5.1C, insert). Closer inspection of the aleurone cells revealed exposed aleurone grains and lipid bodies (Fig. 5.1D). The cracks also appeared to have facilitated fungal infection (Fig. 5.1E) and insect damage (Fig. 5.1F).

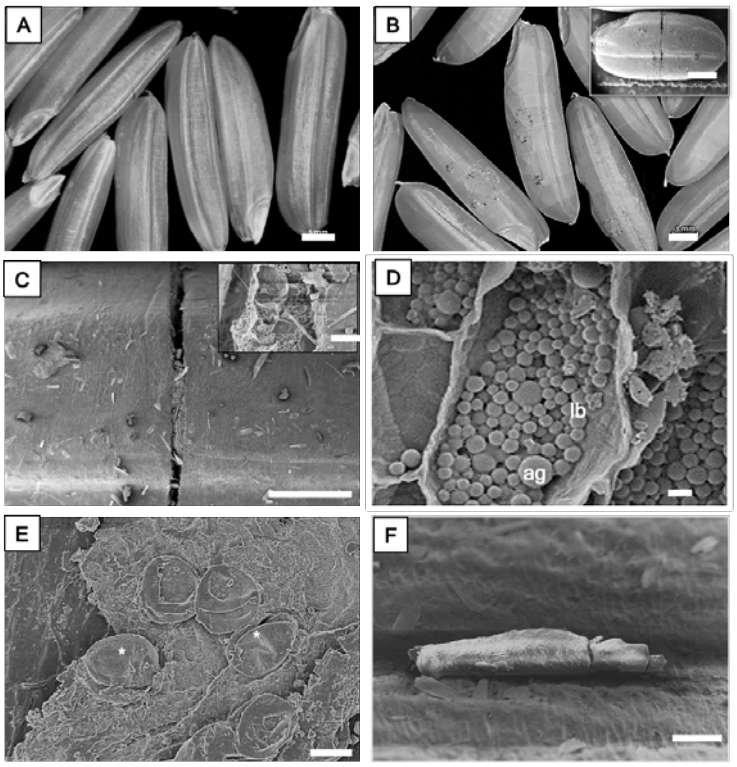

Figure 5.1 A: Stereo micrograph of seed samples at harvest (control) showing intact and smooth surfaces (bar =1 mm); B: stereo micrograph of seed samples after 5 weeks of field curing with multiple cracks on the surface (bar=1 mm), insert shows SEM image of seed with a large crack (bar =1 mm); C: deep crack going through the endosperm (bar = 200 μm), inset shows higher magnification of the crack showing exposed aleurone cells (bar = 20μm); D: higher magnification of the aleurone cell shown in C exposed aleurone grains (ag) and lipid bodies (lb) (bar =3 μm); E: fungal spores (*) on seed surface (bar =20 μm); F: Presence of dead insect in the endosperm (bar=20μm).

5.4.1.2 Caryopsis coat thickness

Seed coat thickness differed significantly among the species and hybrid within the field curing period and within species and hybrid across the curing period (Fig. 5.2). There was also a significant species × field curing period (p = 0.046) interaction effect on seed coat thickness. Regardless of the curing period, *O. sativa* and *O. glaberrima* had significantly thicker seed coats than the hybrid, even though there was no significant difference in this parameter between *O. sativa* and *O. glaberrima* (Fig. 5.2). In both species and the hybrid, seed coat thickness was lowest in 5-week cured seeds compared with non-cured seeds (Fig. 5.2). Except for *O.*

glaberrima, there was a significant reduction in seed coat thickness between 0- and 2-week cured seeds (Fig. 5.2).

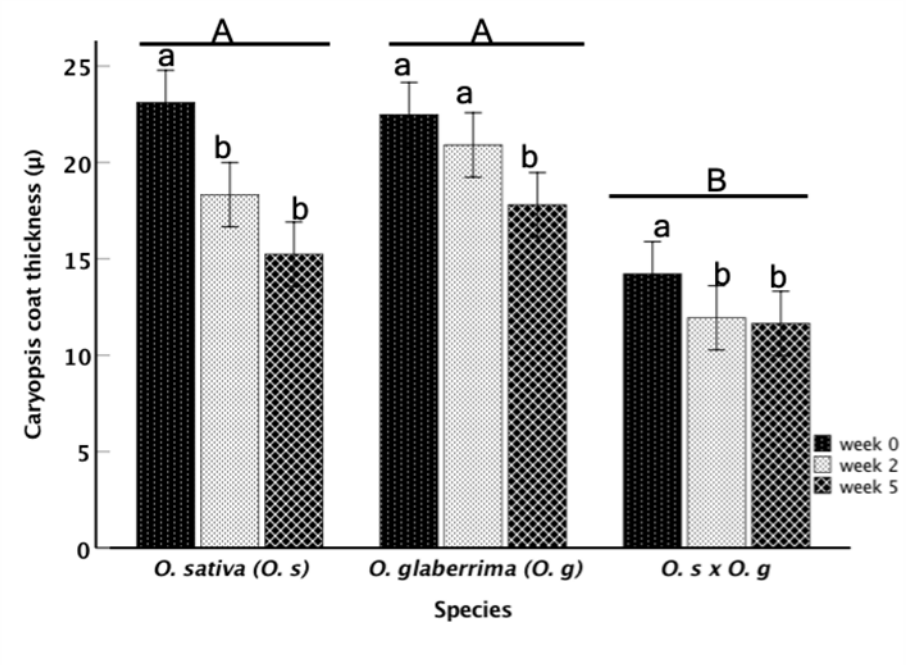

Figure 5.2 Caryopsis coat thickness of *O. sativa*, *O. glaberrima* and *O. sativa* × *O. glaberrima* seeds field cured for 0, 2 and 5 weeks and stored at 4°C for 20 weeks. Values represent mean ± SD (n = 3). Values labelled with different lowercase letters are significantly different when compared within species across field curing period (n = 3; ANOVA; *P* = 0.006 for *O. sativa, P* = 0.015 for *O. glaberrima*, and *P* = 0.030 for *O. sativa* × *O. glaberrima*). Different uppercase letters indicate significant (*P* < 0.05) differences among species within field curing period.

5.4.2 Plant establishment and growth

5.4.2.1 Seedling emergence

Percentage seedling emergence of NI seeds declined significantly with delayed field curing in both species and the hybrid (Fig. 5.3). In NI seeds, seedling emergence in *O. sativa* (Fig. 5.3A) and *O. glaberrima* (Fig. 5.3B) declined by c. 58% compared with c. 64% in the hybrid (Fig. 5.3C) after 5 weeks of curing (Fig. 5.3). There were significant negative correlations between curing time and seedling emergence in *O. sativa* ($r = -0.851$; $p < 0.001$), *O. glaberrima* ($r = -0.889$; $p < 0.001$) and the hybrid ($r = -0.886$; $p < 0.001$).

In both species and the hybrid, post-storage invigoration treatments (CW and DW) did not significantly enhance seedling emergence of non-cured seeds (Fig. 5.3). There was no significant difference in seedling emergence between CW and DW in 2 week cured *O. sativa* (Fig. 5.3A). However, CW and DW significantly enhanced seedling emergence by 31 and 19%, respectively, relative to NI in the 2 week cured *O. sativa* (Fig. 5.3A). Cathodic water invigoration significantly increased seedling emergence by 17 and 33% relative to DW and NI, respectively, in 5 week cured *O. sativa* (Fig. 5.3A). DW also significantly enhanced seedling emergence by 20% relative to NI (Fig. 5.3A). Seedling emergence was comparable between CW and DW invigorated 2 week cured *O. glaberrima*, but both CW and DW significantly enhanced emergence relative to NI by 23 and 19%, respectively (Fig. 5.3B). For 5 week cured *O. glaberrima*, CW significantly enhanced seedling emergence relative to DW and NI by 24 and 39%, respectively (Fig. 5.3B). The beneficial effect of CW was apparent in the hybrid: significantly higher seedlings emerged in CW invigorated 2 and 5 week cured seeds than DW and NI (Fig. 5.3C). CW significantly increased seedling emergence by 16% in 2 and 19% in 5 week cured hybrid than DW (Fig. 5.3C). When the two species and the hybrid were compared, irrespective of the post-storage invigoration treatments, seedling emergence was comparable across the species and hybrid when the seeds were not cured. For 2 week cured seeds, *O. glaberrima* (71 ± 9%) had significantly higher emergence than *O. sativa* (61 ± 13%) and the hybrid (55 ± 8%). For 5 week cured seeds, *O. glaberrima* (52 ± 13%) again had significantly higher emergence than the hybrid (42 ±9%), but differences in emergence were comparable between *O. glaberrima* and *O. sativa* (50 ± 10%).

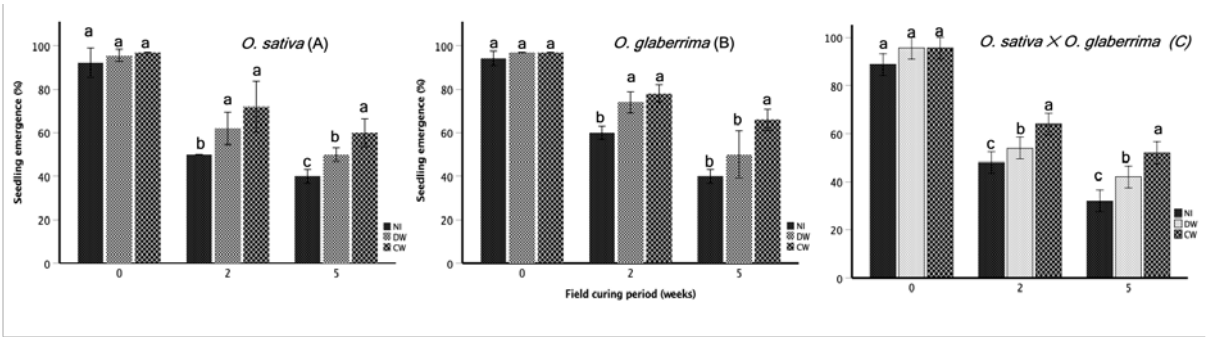

Figure 5.3 Percentage seedling emergence at 14 days after seeding (DAS) of seedlings of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* from each post-storage invigoration seed treatment (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Values represent mean ±SD (n = 10 seeds for each of five trials). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across post-storage invigoration seed treatments; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.2 Mean germination time

Mean germination time or lag period of NI seeds increased with delayed field curing in both species and the hybrid (Fig. 5.4). There were significant positive correlations between field curing time and MGT in *O. sativa* (r = 0.574; $P = 0.05$), *O. glaberrima* (r = 0.883; $P < 0.001$) and *O. sativa* × *O. glaberrima* (r = 0.760; $P = 0.004$). Cathodic water invigorated seeds had the lowest MGT within both species and the hybrid, except for 0 week cured *O. glaberrima* seeds (Fig. 5.4) where there was no significant difference among the invigoration treatments. Also, within each species and hybrid, MGT was significantly lower in CW than DW invigorated seeds except for 0 and 2 week cured *O. glaberrima* (Fig. 4B) seeds. MGT was highest in *O. sativa* × *O. glaberrima* (4.2 days) followed by *O. sativa* (3.6 days) and lowest (3.2 days) in *O. glaberrima*, irrespective of curing or post-storage treatment (Fig.5. 4).

5.4.2.3 Seedling vigour

Seedling vigour was based on seedling height at 28 DAS. The seedling height of NI seeds declined significantly after 5 weeks of curing in both species and the hybrid (Fig. 5.5). When seeds of the species and hybrid were not invigorated, subsequent seedling height declined

significantly by 18% for *O. sativa* (Fig 5A), 14% for *O. glaberrima* (Fig. 5.5B) and 28% for the hybrid (Fig. 5.5C) after 5 weeks of curing. Significant negative correlations were obtained between curing time and seedling vigour (height) in *O. sativa* ($r = -0.630$; $p = 0.012$), *O. glaberrima* ($r = -0.679$; $p = 0.005$) and the hybrid ($r = -0.858$; $p < 0.001$).

Cathodic water treatment produced significantly taller seedlings in 0, 2 and 5 week cured seeds than the NI seeds for both species and the hybrid (Fig. 5.5). In *O. sativa*, seedling height was not significantly different between CW and DW invigorated 0 week cured seeds. In contrast, seedling height differed significantly between DW and NI in the 0 week cured seeds (Fig. 5.5A). For 2 and 5 week cured *O. sativa*, CW produced significantly taller seedlings than DW invigorated seeds (Fig. 5.5A). DW invigorated 2 and 5 week cured seeds of *O. sativa* also produced significantly taller seedlings than NI seeds (Fig. 5.5A). The stimulatory effect of invigoration on seedling height was significantly higher in CW invigorated 0, 2, and 5 week cured *O. glaberrima* seeds than DW invigorated seeds (Fig. 5B). DW invigorated 0 and 2 week cured seeds were also significantly taller than NI seeds (Fig. 5B). On the contrary, differences in seedling height between DW and NI invigorated 5 week cured *O. glaberrima* seeds were insignificant (Fig. 5.5B). The stimulatory effect of invigoration on seedling height was comparable between CW and DW in 0 week cured hybrid (Fig. 5.5C). In contrast, differences in seedling height between DW and NI in the 0 week cured hybrid seeds was significant (Fig. 5C). The stimulatory effect of invigoration on seedling height was significantly higher in CW invigorated 2 and 5 week cured hybrid than DW and in DW invigorated seeds than NI 2 and 5 week cured hybrid seeds (Fig. 5.5C). When the two species and hybrid were compared, irrespective of the invigoration treatment, the seedling height in 0 week cured *O. sativa* (51.6 ± 5.0 cm) and *O. glaberrima* (49.6 ± 5.3 cm) was comparable between the two species, but both species were significantly taller than the hybrid (44.6 ± 2.8cm). Also, for 2 week cured seeds, *O. sativa* (45.9 ± 5.1 cm) and *O. glaberrima* (44.5 ± 4.3 cm) had comparable heights and both species were again significantly taller than the 2 week cured hybrid (41.8 ± 4.4 cm). However, after 5 weeks of curing, seedlings of *O. sativa* (43.2 ± 4.7 cm) were significantly taller than *O. glaberrima* (40.4 ± 3.4 cm) and the hybrid (36.0 ± 5.1 cm).

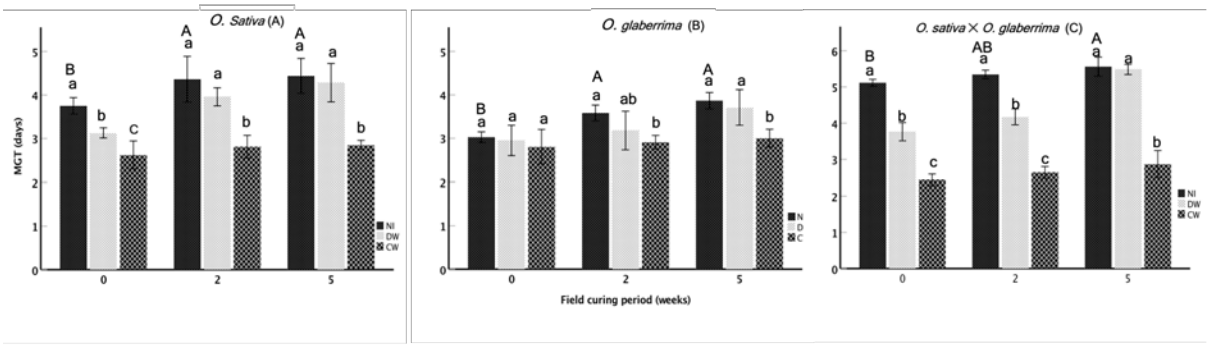

Figure 5.4 Mean germination time of *O. sativa*, *O. glaberrima* and *O. sativa* × *O. glaberrima* from each post-storage invigoration treatment (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Values represent mean ± SD (n = 50 seeds per replicate of 4). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across post-storage invigoration seed treatments; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

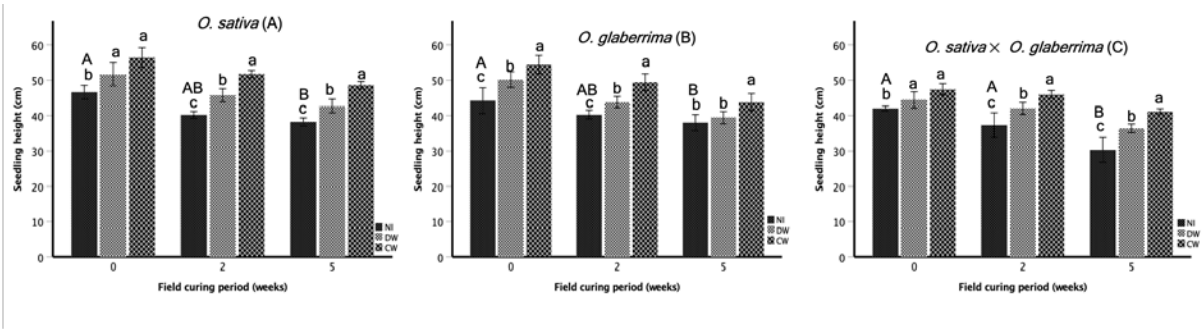

Figure 5.5 Seedling height at 28 DAS of seedlings of *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* from each post-storage invigoration seed treatment (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Values represent mean ± SD (n = 10 seedlings for each of five trials). Values followed by different letters are significantly different ($P < 0.05$): lowercase letters represent comparisons within field curing period across post-storage invigoration seed treatments; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4 Biomass production and allocation

5.4.2.4.1 Total biomass

Total biomass accumulation at anthesis declined significantly by 35% after 5 weeks of field curing in *O. sativa* (Fig. 5.6A). In contrast, biomass accumulation was not influenced significantly after 5 weeks of field curing in *O. glaberrima* (Fig. 5.6B) and the hybrid (Fig. 5.6C). The total biomass of plants produced from non-invigorated seeds across field curing periods was comparable among the species and hybrid (Fig. 5.6).

In *O. sativa*, non-cured (0 week) seeds invigorated with CW produced plants with significantly greater total biomass than DW by 21% and NI by 32%. However, total biomass of plants produced from 2 and 5 week cured *O. sativa* seeds was unaffected by invigoration treatments (Fig. 5.6A). In *O. glaberrima*, the total biomass of plants produced from CW invigorated 0 week cured seeds was not significantly different from DW imbibed plants. Total biomass was comparable between DW imbibed and NI 0 week cured *O. glaberrima* (Fig. 5.6B). Cathodic water invigoration did not significantly enhance total biomass relative to DW in 2 and 5 week cured seeds. De-ionized water invigoration did not also significantly enhance total biomass relative to NI in 2 and 5 week cured *O. glaberrima* (Fig. 5.6B). Cathodic water invigoration of 2 weeks cured hybrid seeds had significantly enhanced total biomass relative to DW and NI; plants produced from 0 and 5 weeks cured seeds were unaffected by invigoration treatments (Fig. 5.6C). When both species and the hybrid were compared, irrespective of the invigoration treatment, 0 week cured *O. sativa* (33.1 ± 7.0 g/plant) produced significantly greater total biomass than the hybrid (25.2 ± 4.6 g/plant), but total biomass was comparable between *O. sativa* and *O. glaberrima* (27.9 ± 7.8 g/plant) and between *O. glaberrima* and the hybrid (25.2 ± 4.6 g/plant). For 2 week cured seeds, *O. sativa* (30.8 ± 4.5 g/plant) yielded significantly greater total biomass than *O. glaberrima* (24.3 ± 5.9 g/plant) and the hybrid (24.6 ± 2.9 g/plant). On the contrary, differences in total biomass yield across the two species and the hybrid were comparable after 5 weeks of curing.

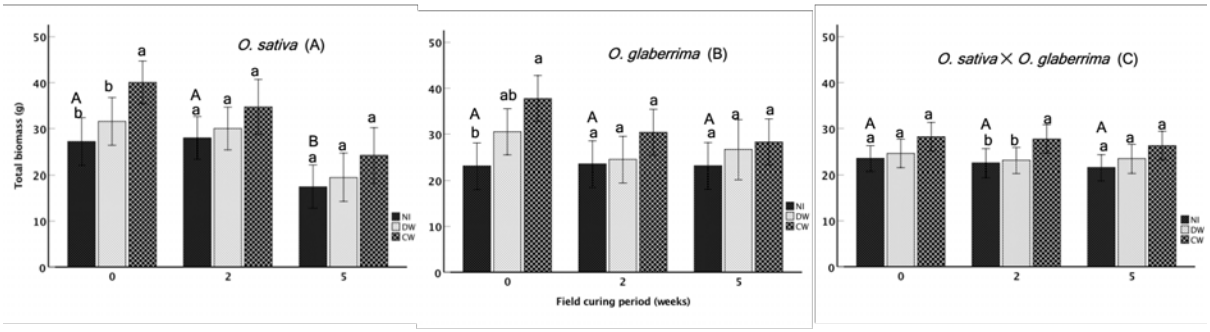

Figure 5.6 Total biomass production at flowering in *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* plants from each post-storage invigoration seed treatments (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Bars represent mean (+SD) (n = 5 for five trials). Values followed by different letters are significantly ($P < 0.05$) different: lowercase letters represent comparisons within field curing period across post-storage invigoration seed treatments; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4.2 Panicle biomass

Panicle biomass was unaffected by delayed field curing in both species and the hybrid (Fig. 5.7). Panicle biomass in plants from 0 week cured *O. sativa* seeds was unaffected by invigoration treatments (Fig. 5.7A). However, CW invigoration significantly enhanced panicle biomass relative to DW invigoration and NI 2 week cured *O. sativa* seeds. For 5 week cured *O. sativa* seeds, CW invigoration significantly enhanced panicle biomass than DW invigoration. Panicle biomass was, however, not significantly different between DW and NI invigoration treatments (Fig. 5.7A). In 0 week cured *O. glaberrima,* CW invigoration significantly enhanced panicle biomass relative to DW and NI. On the contrary, invigoration treatments did not significantly enhance panicle biomass in 2 and 5 week cured *O. glaberrima* (Fig. 5.7B). Plants produced from 0, 2 and 5 week cured hybrid seeds invigorated with CW yielded significantly higher panicle biomass than NI seeds (Fig. 5.7C). While panicle biomass from CW invigorated 0, 2 and 5 week cured hybrid seeds were not significantly different from DW invigorated 0, 2 and 5 week cured seeds, there was always a trend for plants produced from CW invigorated seeds to produce relatively higher panicle biomass (Fig. 5.7C). When both species and the hybrid were compared, irrespective of the invigoration treatment, 0 week cured hybrid (7.6 ± 2.0 g/plant) yielded significantly greater panicle than *O. sativa* (1.4 ± 1.0 g/plant) and *O. glaberrima* (0.9 ± 1.9 g/plant). For 2 week cured seeds, the hybrid (6.6 ± 2.2 g/plant) again

yielded significantly greater panicle biomass than *O. sativa* (2.7 ± 2.1 g/plant) and *O. glaberrima* (0.7 ± 1.3 g/plant). Significantly greater panicle biomass was observed in the 5 week cured hybrid (5.8 ± 2.4 g/plant) than both *O. sativa* (1.9 ± 1.4 g/plant) and *O. glaberrima* (1.1 ± 1.7 g/plant).

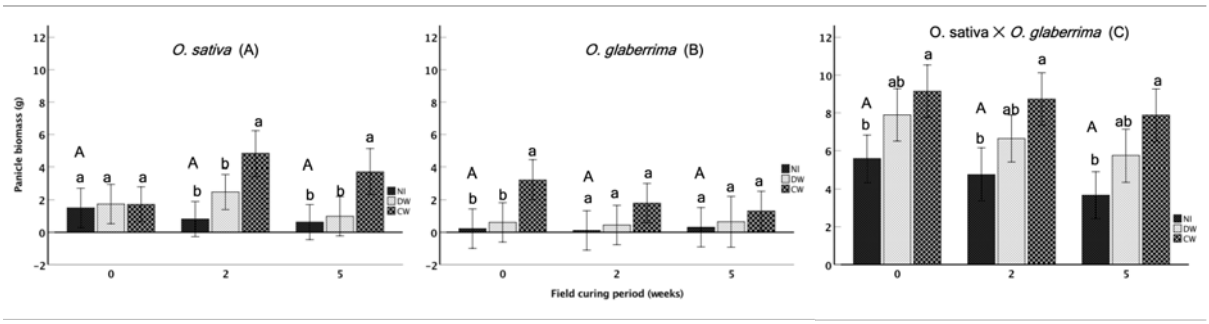

Figure 5.7 Panicle biomass at flowering in *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* plants from each post-storage invigoration seed treatments (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Bars represent mean (+SD) (n = 5 for five trials). Post-storage invigoration seed treatments labelled with different letters are significantly (*P* < 0.05) different when compared within a species and field curing period; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4.3 Leaf biomass

Leaf biomass was unaffected after 5 weeks of field curing in *O. sativa* (Fig. 5.8A) and *O. glaberrima* (fig. 5.8B). However, leaf biomass in plants produced from hybrid NI seeds significantly declined by 41% after 5 weeks of curing (Fig. 5.8C). Leaf biomass of plants produced from 0, 2 and 5 week cured *O. sativa* seeds were unaffected by invigoration treatments (Fig. 8A). Seed invigoration treatment did not significantly influence leaf biomass in 0 and 2 week cured *O. glaberrima* (Fig. 5.8B) as well. But, CW invigoration of 5 week cured *O. glaberrima* significantly enhanced leaf biomass than NI seeds. Leaf biomass in this species was comparable between CW and DW and between DW and NI in the 5 weeks cured seeds (Fig. 5.8B). In the hybrid, leaf biomass was not enhanced by invigoration treatments in 0 and 2 weeks cured seeds (Fig. 5.8C). For the 5 week cured hybrid, leaf biomass was comparable between CW and DW invigorated plants. However, both CW and DW invigoration significantly

enhanced greater leaf biomass than the plants from NI seeds (Fig. 5.8C). When both species and hybrid were compared, irrespective of the invigoration treatment, leaf biomass in 0 week cured seeds was comparable across both species and hybrid. In contrast, for the 2 week cured seeds, *O. glaberrima* (7.6 ± 2.5 g/plant) and *O. sativa* (7.4 ± 1.4 g/plant) yielded significantly greater leaf biomass than the hybrid (4.3 ± 1.2 g/plant). Similar to the 2 week cured seeds, *O. glaberrima* (6.7 ± 2.4 g/plant) and *O. sativa* (6.6 ± 1.8 g/plant) yielded significantly greater leaf biomass than the hybrid (4.6 ± 1.8 g/plant) in 5 week cured seeds.

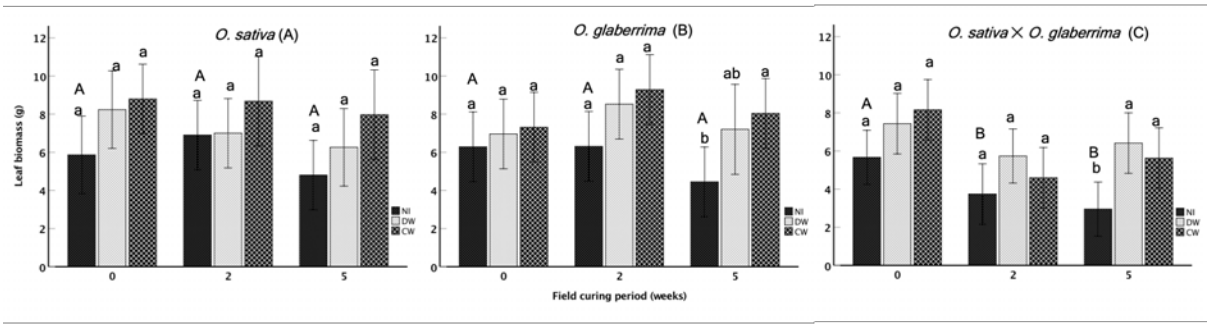

Figure 5.8 Leaf biomass at flowering in *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* plants from each post-storage invigoration seed treatments (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Bars represent mean (+SD) (n = 5 for five trials). Post-storage invigoration seed treatments labelled with different letters are significantly (*P* < 0.05) different when compared within a species and field curing period; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4.4 Stem biomass

Stem biomass was unaffected after 5 weeks of field curing in both species and the hybrid (Fig. 5.9). Seed invigoration treatment did not significantly enhance stem biomass in 0, 2 and 5 week cured *O. sativa* seeds (Fig. 5.9A). In 0 week cured *O. glaberrima*, CW invigoration significantly enhanced stem biomass than DW invigoration and NI but stem biomass was comparable between CW and DW (Fig. 5.9B). For 2 week cured seeds, invigoration treatments did not enhance stem biomass yield. Stem biomass was significantly enhanced by CW invigoration relative to DW invigoration and NI in 5 week cured *O. glaberrima* (Fig. 5.9B). For the 0, 2 and 5 week cured hybrid, invigoration treatments did not significantly enhance stem biomass yield

(Fig. 5.9C). When both species and hybrid were compared, irrespective of the invigoration treatments, 0 week cured *O. sativa* (12.3 ± 4.4 g/plant) and *O. glaberrima* (10.1 ± 5.3 g/plant) yielded significantly greater stem biomass than the hybrid (6.8 ± 1.7 g/plant). For 2 week cured seeds, *O. sativa* (12.9 ± 2.5 g/plant) yielded significantly greater stem biomass than *O. glaberrima* (7.8 ± 3.2 g/plant) and the hybrid (7.8 ± 1.0 g/plant). On the contrary, stem biomass was comparable across both species and hybrid after 5 weeks of field curing.

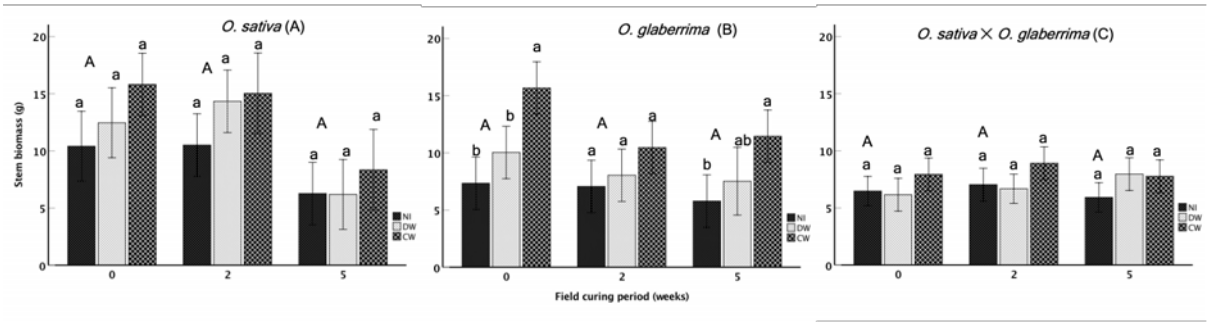

Figure 5.9 Stem biomass production at flowering in *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* plants from each post-storage invigoration seed treatments (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Bars represent mean (+SD) (n = 5 for five trials). Post-storage invigoration seed treatments labelled with different letters are significantly ($P < 0.05$) different when compared within a species and field curing period; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4.5 Root biomass

Root biomass yield was not significantly impacted in *O. sativa* (Fig. 5.10A) and *O. glaberrima* (Fig. 5.10B) after 5 weeks of field curing. In contrast, the root biomass of the hybrid increased significantly by 36% after 5 weeks of field curing (Fig. 5.10C). Root biomass yield was significantly enhanced by CW than DW invigoration in 0 week cured *O. sativa*. In contrast, root biomass yield was comparable between DW and NI (Fig. 5.10A). In both 2 and 5 week cured *O. sativa*, invigoration treatments did not significantly influence root biomass yield (Fig. 5.10A). Root biomass was comparable across invigoration treatments in both 0 and 2 week cured *O. glaberrima* seeds (Fig. 5.10B), surprisingly, CW invigoration resulted in a reduction in root biomass relative to NI in 5 week cured *O. glaberrima*. Root biomass yield was, however, comparable between CW and DW (Fig. 5.10B). Root biomass did not respond to invigoration

treatments in 0 week cured hybrid seeds (Fig. 5.10C). Root biomass in 2 and 5 week cured hybrid did not differ significantly between CW and DW invigoration. However, there was a significant reduction in root biomass in DW relative to NI, and CW and DW relative to NI in 2 week and 5 week cured hybrid, respectively (Fig. 5.10C). When the species and the hybrid were compared, irrespective of the invigoration treatment, 0 week cured *O. sativa* (10.8 ± 2.5 g/plant) and *O. glaberrima* (10.6 ± 3.5 g/plant) yielded significantly greater root biomass than the hybrid (3.8 ± 2.2 g/plant). For 2 week cured seeds, *O. glaberrima* (8.2 ± g/plant) yielded significantly greater biomass than the hybrid (5.9 ± g/plant) but *O. sativa* (7.9 ± g/plant) was comparable with *O. glaberrim*a and the hybrid. For 5 week cured seeds, root biomass yield was significantly higher in *O. glaberrima* (10.3 ± 2.7 g/plant) than *O. sativa* (5.7 ± 2.8 g/plant) and the hybrid (5.7 ± 2.5 g/plant).

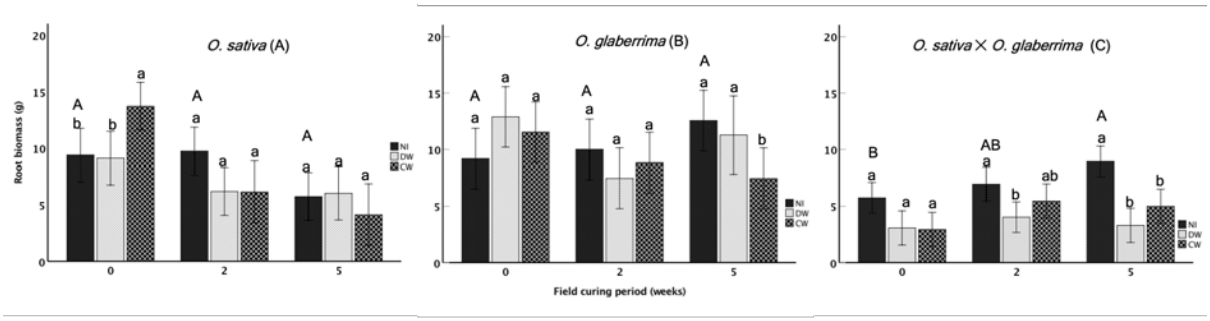

Figure 5.10 Root biomass at flowering in *O. sativa*, *O. glaberrima*, and *O. sativa* × *O. glaberrima* plants from each post-storage invigoration seed treatments (NI, DW and CW) of seeds field cured for 0, 2 and 5 weeks. Bars represent mean (+SD) (n = 5 for five trials). Post-storage invigoration seed treatments labelled with different letters are significantly (*P* < 0.05) different when compared within a species and field curing period; uppercase letters represent comparisons within a species and non-invigorated (NI) seed treatment, across field curing period.

5.4.2.4.6 Root to shoot ratio and biomass allocation

In *O. sativa*, only 2 week cured seeds invigorated with CW and DW significantly reduced RSR than NI seeds (Table 5.1); CW and DW invigoration reduced RSR comparably. In *O. glaberrima*, CW invigoration significantly reduced RSR relative to DW and NI in 5 week cured seeds only (Table 5.1). In *O. sativa* × *O. glaberrima*, while RSR was comparable across post-storage invigoration treatments for 0 week cured seeds, CW and DW invigoration of 2 and 5

week cured seeds significantly reduced RSR than NI (Table 5.1); CW and DW invigoration reduced RSR comparably.

No stimulatory effect of invigoration treatments on biomass allocation to root, stem, leaves and panicles was observed in 0 week cured *O. sativa* (Table 5.1). In contrast, CW and DW invigoration of 2 week cured *O. sativa* seeds comparably reduced biomass allocation to roots and enhanced biomass allocation to panicles relative to NI (Table 5.1). Similarly, CW invigoration of 5 week cured *O. sativa* seeds significantly decreased biomass allocation to roots and significantly enhanced biomass allocation to panicles than DW (Table 5.1). In 0 week cured *O. glaberrima*, CW invigoration significantly enhanced biomass allocation to panicles relative to DW and NI. However, there was no invigoration treatment effect on biomass allocation to root, stem and leaves in the 0 week cured seeds (Table 5.1). No significant stimulatory effect of invigoration treatments on biomass allocation to root, stem, leaves and panicles was observed in 2 week cured *O. glaberrima* (Table 5.1). On the contrary, for 5 week cured *O. glaberrima* seeds, CW invigoration significantly reduced biomass allocation to roots and increased biomass allocation to stem at the expense of panicles. CW and DW invigoration however increased biomass allocation to leaves comparably (Table 5.1).

In 0 week cured hybrid seeds, CW and DW invigoration significantly reduced biomass allocation to the roots but this did not result in increased allocation to stem, leaves or panicles. In contrast, 2 week cured CW and DW decreased biomass allocation to roots to the benefit of the panicles. Furthermore, 5 week cured CW and DW invigorated seeds also significantly reduced biomass allocation to roots and increased allocation to leaves and panicles (Table 5.1). Although differences in biomass allocation between CW and DW were comparable, there was a trend for biomass allocated to panicles from CW treated seeds to be relatively greater than those from DW treated seeds.

Table 5.1 A comparison of panicle, leaf, stem, root mass, and root: shoot ratio at flowering in plants from post-storage invigorated *O. sativa, O. glaberrima* and *O. sativa × O. glaberrima* seeds which were field cured for 0, 2 and 5 weeks and stored for 20 weeks at 4°C.

Species	Curing period (Weeks)	Post-storage invigoration treatment														
		Panicle mass fraction			Leaf mass fraction			Stem mass fraction			Root mass fraction			Root: shoot ratio		
		NI	DW	CW	NI	DW	CW	NI	DW	CW	NI	DW	CW	NI	DW	CW
O. sativa	0	0.06^{a}	0.06^{a}	0.04^{a}	0.21^{a}	0.26^{a}	0.22^{a}	0.38^{a}	0.39^{a}	0.39^{a}	0.35^{a}	0.29^{a}	0.34^{a}	0.56^{a}	0.41^{a}	0.53^{a}
(O. s)	2	0.03^{b}	0.09^{ab}	0.14^{a}	0.25^{a}	0.23^{a}	0.25^{a}	0.38^{a}	0.47^{a}	0.43^{a}	0.34^{a}	0.21^{b}	0.18^{b}	0.53^{a}	0.26^{b}	0.22^{b}
	5	0.04^{b}	0.06^{b}	0.16^{a}	0.28^{a}	0.32^{a}	0.33^{a}	0.32^{a}	0.32^{a}	0.34^{a}	0.30^{a}	0.30^{a}	0.17^{b}	0.45^{a}	0.44^{a}	0.21^{a}
O. glaberrima	0	0.01^{b}	0.01^{b}	0.09^{a}	0.26^{a}	0.23^{a}	0.20^{a}	0.31^{a}	0.34^{a}	0.42^{a}	0.42^{a}	0.41^{a}	0.30^{a}	0.83^{a}	0.73^{a}	0.44^{a}
	2	0.01^{a}	0.02^{a}	0.06^{a}	0.26^{a}	0.35^{a}	0.31^{a}	0.29^{a}	0.32^{a}	0.35^{a}	0.44^{a}	0.31^{a}	0.29^{a}	0.91^{a}	0.46^{a}	0.41^{a}
(O. g)	5	0.01^{a}	0.03^{a}	0.04^{a}	0.19^{b}	0.27^{a}	0.28^{a}	0.25^{b}	0.28^{b}	0.40^{a}	0.55^{a}	0.43^{b}	0.27^{c}	1.23^{a}	0.75^{b}	0.38^{c}
O. s × O. g	0	0.24^{a}	0.33^{a}	0.33^{a}	0.24^{a}	0.30^{a}	0.33^{a}	0.28^{a}	0.25^{a}	0.28^{a}	0.24^{a}	0.12^{b}	0.10^{b}	0.34^{a}	0.14^{a}	0.12^{a}
	2	0.21^{b}	0.29^{ab}	0.32^{a}	0.17^{a}	0.25^{a}	0.17^{a}	0.31^{a}	0.29^{a}	0.32^{a}	0.31^{a}	0.17^{b}	0.20^{b}	0.46^{a}	0.22^{b}	0.25^{b}
	5	0.17^{b}	0.25^{ab}	0.30^{a}	0.14^{b}	0.27^{a}	0.21^{ab}	0.28^{a}	0.34^{a}	0.30^{a}	0.42^{a}	0.14^{b}	0.19^{b}	0.73^{a}	0.17^{b}	0.24^{b}

Different lowercase letters in rows show significant differences when compared within field curing period ($p < 0.001$) across post-storage invigoration treatments ($p < 0.001$) within each species ($p < 0.001$). (n = 3-5 for *O. sativa*, 4 -5 for *O. glaberrima* and 5 for *O. sativa × O. glaberrima*). NI= Non-invigoration; DW = De-ionized water; CW = Cathodic water

5.5 Discussion

The present study used CW and DW to invigorate seeds of two rice species and their hybrid, field cured for 5 weeks in a tropical environment to alleviate the adverse effects of environmentally induced deterioration. Herein, we showed that invigorating field cured seeds with CW and DW improved seed germinability and seedling emergence and seedling vigour of both species and the hybrid. Furthermore, the results showed that CW was more effective in alleviating the environmentally induced seed deterioration by enhancing seedling emergence and vigour of both species and the hybrid.

The current study revealed severe structural damages to the endosperms of *O. sativa*, *O. glaberrima* and their interspecific hybrid after 5 weeks of field curing. The damage to the endosperms compromised the quality of the seeds produced and accelerated their deterioration in storage. Delayed field curing compromised seed endosperm integrity, reduced seed coat thickness, seedling emergence and vigour, and to some extent, growth in terms of biomass accumulation in both species and hybrid. A decline in seedling emergence and vigour associated with delayed field curing were more pronounced in the hybrid, suggesting that the hybrid was more susceptible to field curing deterioration (Figs. 5.2 – 5.6).

As alluded to above, seeds of both species and the hybrid that were cured for 2 and 5 weeks exhibited severe structural damages to the endosperm (Fig. 5.1). For both species and the hybrid, the severity of this damage was greatest in 5 week cured seeds, which displayed multiple, deep cracks through the endosperm (Figs. 5.1). Cycles of moisture absorption and release that were previously shown to occur in field cured rice seeds (Bam *et al*., 2019) may have weakened the caryopsis coat (Long *et al*., 2015) and the endosperm, causing deep cracks which exposed the aleurone cells (aleurone grains and lipid bodies) (Figs. 5.1C, D). In addition, there were indications that this curing-induced deterioration of the endosperm may have promoted fungal (Fig. 5.1E) and insect infestation (Fig. 5.1F). The degree of endosperm deterioration increased with curing time and appears to be consequential to a decline in caryopsis coat thickness with increased curing time (Fig. 5.2). Although seeds of both species and the hybrid exhibited a decline in caryopsis coat thickness with curing time, the hybrid exhibited significantly lower caryopsis coat thickness in both the non-field cured and in the

week 5 cured seeds (Fig. 5.2). The thinner caryopsis coat exhibited by the hybrid may have contributed to the severity of deterioration in the hybrid as manifested in the hybrid having the largest reduction in seedling emergence (64% compared with 58% in *O. sativa* and *O. glaberrima*) (Fig. 5.3) and height (hybrid = 28%; *O. sativa* = 18%; *O. glaberrima* = 14%) (Fig. 5.5) after 5 weeks of field curing. Caryopsis coat thickness influences seedling emergence and vigour (De Souza and Marcos-Filho, 2001). In the present study, the decline in caryopsis coat thickness (Fig. 5.2) with curing may have been a consequence of species characteristics and alternate drying-wetting cycles associated with field curing, weakening the seed coat (Probert, 2003; Long *et al*., 2015), causing it to collapse and shrink. This may also prevent sufficient drying (Probert, 2003), which promotes unbalanced oxidative metabolism (reviewed by Bailly, 2008) and may subsequently increase rates of seed deterioration. These may explain the decline in percentage seedling emergence (Fig. 5.3) and seedling vigour (Figs. 5.4, 5.5) after 5 weeks of field curing observed in the present study.

In a previous study by Menezes *et al*. (2012), non-severely cracked rice endosperms produced abnormal seedlings or normal but weak seedlings while seeds with severely cracked endosperms failed to germinate. As in the present study, those authors attributed decline in seed germination, seedling emergence and establishment, and plant growth and productivity to endosperm cracks (multiple and deep) in the direction of both short and long axis (Menezes *et al*., 2012). The cracks and fissures may have also influenced the seed coat permeability during germination since intact seed coats (e.g., Fig. 5.1A) may better regulate the rate of water uptake and thus protect the embryo from imbibitional damage (De Souza and Marcos-Filho, 2001), which is commonly reported in dry seed tissues. Therefore, the rate of water uptake during germination of non-severely cracked endosperms may be lower than that of severely cracked endosperms, and therefore, the degree of embryo damage may be lower in non-severely cracked than severely cracked seeds. Thus, the decline in seedling emergence and vigour after 2 and 5 weeks of curing of both species and the hybrid (Fig. 5.3) may suggest differences in water uptake during germination, possibly due to differences in the level of damage to the embryos. Studies have also shown that damage to aleurone cells through ageing during storage or artificial removal reduced seed germination and seedling vigour in *O. sativa* (Mandal and Basu, 1981) and *H. vulgare* (Ritchie *et al*., 2000). This was ascribed to the reduced ability of these cells to synthesize hydrolases such as α- amylase to break down the endosperm for the release of proteins, lipids and minerals required for seed germination and early seedling growth.

Damage or removal of aleurone cells can also result in the loss of phytic acid-rich granules and Ca and Mg ions, which are stored in the aleurone cells and are essential for seed germination and growth (Meharg *et al*., 2008). As alluded to above, damage to the endosperm may have also increased the vulnerability of the seeds investigated here to invasion by fungi (Fig. 5.1E), and insects (Fig. 5.1F, see insert) that can in turn carry fungal spores into storage. Bam *et al.* (2019) also isolated saprophytic (*Botrytis* sp and *Trichoderma* sp) and potential pathogenic (*Alternaria* sp, *S. rolfsii*, and *Verticillium* sp) soil-borne fungal species from 5 week cured seeds of *O. sativa*, *O. glaberrima* and the hybrid in a previous study. This infestation by fungi and insects may have also contributed to the decline in seed quality observed in the field cured seeds investigated in the present study.

The results discussed thus far suggest that harvested seeds/panicles were exposed to multiple drying-wetting cycles for weeks in the field, which led to physical deterioration and possibly compromised germinative metabolism and oxidative damage (not reported here) prior to storage for 20 months at 4°C. Depending on the extent of damage prior to rehydration after storage, this kind of damage may or may not be reversed in a crop (Gurusinghe and Bradford, 2001) and other plant species (e.g., *Digitalis purpurea*; Butler *et al*., 2009). While three drying-wetting cycles (priming-redrying cycles) improved seed longevity in *Digitalis purpurea* (Butler *et al*., 2009), eight drying-wetting cycles decreased seed germinability and vigour in *A. sativa* (Berrie and Drennan, 1971). In this study, seeds of both species and the hybrid experienced 14 drying-wetting cycles after 2 weeks of curing and 35 such cycles after 5 weeks of curing (data shown in Bam *et al*., 2019). This may explain the significant negative correlation observed between field curing time and seedling emergence and vigour [(seedling emergence: *O. sativa*, $r = -0.851$; $p < 0.001$; *O. glaberrima*, $r = -0.889$; $p < 0.001$; and the hybrid: $r = -0.886$; $p < 0.001$); (seedling vigour: *O. sativa*, $r = -0.630$; $p = 0.012$; *O. glaberrima,* $r = -0.679$; $p = 0.005$; and the hybrid $r = -0.858$; $p < 0.001$)] in both species and hybrid. Sustained oxidative stress can eventually lead to a breakdown in antioxidant recycling pathways (reviewed by Bailly *et al.*, 2008; reviewed by Kranner *et al*., 2010). Insufficient antioxidant control can lead to oxidative damage to macromolecules, which may have resulted in seed deterioration (reviewed by Kranner *et al*., 2010) with the resultant loss in vigour and viability (Berrie and Drennan, 1971; Butler *et al*., 2009). Seed quality also decreases during storage, and when seed quality is lost, seed vigour decreases first without affecting

germination. As vigour is further reduced, both germination and seedling growth declines (ISTA, 1995).

Damage to natural antioxidant systems in stored, aged or deteriorated orthodox seeds can be ameliorated through the exogenous application of antioxidants, for e.g., CW (Gondwe *et al*., 2016; Adetunji *et al*., 2020; Fatokun *et al*., 2020) and or the use of priming agents such as water (Harris *et al*., 2001, 2002; Farooq *et al*., 2010). Seed invigoration or priming stimulates repair processes and/or re-configures membranes, increases the activity of scavenging enzymes and improves mobilization of reserves resulting in enhanced seed germination and seedling emergence (Powell *et al*., 2000; Hussain *et al*., 2016). Seedling emergence was comparable across post-storage invigoration treatments (CW and DW) and NI for non-cured (0 week; control) seeds in both species and the hybrid (Fig. 5.3), suggesting that if curing is avoided, 20 months of storage did not compromise seedling emergence rate; seedling emergence was compromised significantly in field cured seeds of both species and the hybrid (Fig. 5.3). While invigoration of 2 week cured seeds with CW and DW improved germinability and seedling emergence in *O. sativa* and *O.glaberrima* comparably, seed germinability and seedling emergence was significantly enhanced by CW invigoration relative to DW in 5 week cured *O. sativa* (Fig. 5.3A) and *O. glaberrima* (Fig. 5.3B), and in both 2 and 5 week cured hybrid seeds (Fig. 5.3C). Our finding is consistent with other findings from earlier priming works which showed that low vigour seeds benefited from priming because they had to be repaired before germination advancement occurred (Powell *et al*., 2000; Adetunji *et al*., 2020; Fatokun *et al*., 2020a, b). Higher seedling emergence in 5 week cured *O. sativa* (Fig. 5.3A) and *O. glaberrima* (Fig. 5.3B) and 2 and 5 week cured hybrid (Fig. 5.3C) following CW invigoration suggests that the accumulated damage, which may have occurred during extended field curing and storage, were best ameliorated by CW invigoration, and may be related to the ability of CW to scavenge ROS (Adetunji *et al*., 2020, 2021; Fatokun *et al*. 2020a, b) and enhance endogenous antioxidant capacity better than DW. Findings from previous researchers that CW invigoration of controlled deteriorated aged seeds enhanced germination enzyme activities (GR, CAT and SOD) and increased endogenous antioxidant capacity thereby reducing electrolyte leakage, lipid peroxidation products (MDA and 4-HNE), conjugated dienes and protein carbonylation better than DW (Adetunji *et al*., 2020; Fatokun *et al*., 2020a, b) may explain the positive invigorative effect of CW in our present study. Although invigoration with DW was beneficial to some extent (e.g., 2 and 5 week cured *O. sativa*, Fig. 5.3A; 2 week cured *O. glaberrima*, Fig.

5.3B; 5 week cured hybrid, Fig. 5.3C), the superior invigorating ability of CW in this and other studies (Gondwe *et al.*, 2016; Adetunji *et al*., 2020; Fatokun *et al*., 2020a, b) may be due to its stronger reducing power than DW. Hanaoka *et al*. (2004) also reported that electrolysis of water produces reduced water as a cathodic fraction with higher pH (pH = 9.0 -10.0), lower oxidation potential, lower dissolved oxygen, and higher dissolved hydrogen than non-electrolyzed water (pH = 7.0) due to the higher dissociation activity at the cathode. This suggests that DW is a better priming agent than tap water. However, the CW used in this study was generated from the electrolysis of 1 µM $CaCl_2$ and 1 mM $MgCl_2$, which has a higher pH (pH=11.2) (Berjak *et al*., 2011; Gondwe *et al*., 2016; Adetunji *et al*., 2020) than that generated from the electrolysis of tap water (pH = 9.0–10.0). This suggests a higher dissociation activity at the cathode, making the CW used in this study a stronger reducing agent than electrolyzed tap water. Also, Ca^{2+} and Mg^{2+} are essential cations required for seed germination and growth (Meharg *et al*., 2008; Fatokun *et al*., 2020b). Studies have shown high remobilization of Ca^{2+} during rice seed germination to meet its large requirement in highly metabolically active, differentiating cells (Lu *et al*., 2013). Moreover, Ca^{2+} regulates the production of Gibberellic acid (GA) and the release of hydrolases in the scutellum and their transport to the endosperm for mobilization to the embryo in cereal grains (Ritchie *et al.*, 2000), thereby triggering germination. These findings suggest that apart from Ca^{2+} and Mg^{2+} ameliorating the adverse effects of lipid peroxidation, these bivalent ions may also provide an initial source of nutrients for germination and early seedling growth. These factors may have also contributed to the benefits offered by CW invigoration reported here.

Unlike seedling emergence, CW and DW invigoration enhanced seedling vigour (i.e. seedling height) in non-cured and cured seeds of both species and the hybrid (Fig. 5.5). While seedling vigour was comparable between CW and DW in 0 week cured *O. sativa* and the hybrid, seedling vigour was significantly enhanced by CW relative to DW in 0 week cured *O. glaberrima* (Fig. 5.5). Cathodic water significantly enhanced seedling vigour in 2 and 5 week cured seeds than DW in both species and the hybrid (Fig. 5.5A, B, C). The beneficial effect of CW invigoration on seedling vigour seen in both species and the hybrid (Fig. 5.5A, B, C) might be attributed to the beneficial effects of Ca^{2+} and Mg^{2+}. Ca^{2+} and Mg^{2+} are both essential cations that are not only required for seed germination but are also required for plant growth (Meharg *et al*., 2008; Hochmuth *et al*., 2012; Adetunji *et al*., 2020; Fatokun *et al*., 2020b). For example, priming with $CaCl_2$ increased the concentration of calcium in the shoot of plants (e.g., *O.*

sativa, Farooq *et al*., 2006; 2009; *P. sativum*, Fatokun *et al*. 2020b) and enhanced plant height in *O. sativa* (Farooq *et al*., 2006; 2009) than hydropriming and dry control seeds. Higher seedling vigour in CW treated seeds may have also been the consequence of faster amplification of repair genes (Sharma and Maheshwari, 2015) and increased DNA concentration and purity of DNA (Fatokun *et al*., 2020a). These may have led to rapid and synchronous germination and emergence in CW treated seeds compared with DW and NI (Fig. 5.5) resulting in early autotrophic growth of seedlings (Harris *et al*., 2002) due to early resource capture (Asch *et al*., 1999).

Except in few instances, the positive invigorative effect of CW or DW on seedling vigour was not carried forward into subsequent plant growth measured in terms of biomass accumulation and partitioning in both species and the hybrid (Figs. 5.6 – 5.10; Table 5.1), suggesting that the effect of CW invigoration waned with ontogeny in both species and the hybrid. Although priming with CW or non-electrolysed calcium/magnesium (Ca/Mg) solution led to increased Ca and Mg concentration in *P. sativum* relative to hydropriming (Fatokun *et al*., 2020b), the concentrations of Ca and Mg accumulated in the plants as a result of priming are too low to meet the plant's growth requirement (Hochmuth *et al*., 2012). Enhanced seedling vigour in non-cured and cured seeds of both species and hybrid invigorated with CW did not enhance growth than DW except in few cases, for example, total (Fig. 5.6A, C) and panicle (Fig. 5.7A, B) biomass yield. Except for root biomass (Fig. 5.10), there was always a trend for plants from CW invigorated seeds to yield greater biomass (Figs. 5.6-5.9), although differences in biomass yield between CW and DW were not always significant. Generally, CW and DW invigoration reduced RSR and biomass allocation to the roots in both species and the hybrid (Table 5.1). Invigoration of 5 week cured *O. sativa* seeds with CW significantly decreased biomass allocation to roots and significantly enhanced biomass allocation to panicles than DW (Table 5.1). In 0 week cured *O. glaberrima*, CW invigoration also significantly enhanced biomass allocation to panicles relative to DW and NI. Furthermore, invigoration of 5 week cured *O. glaberrima* seeds with CW significantly reduced biomass allocation to the roots and increased biomass allocation to stem at the expense of panicles (Table 5.1). The increased above-ground biomass appeared to have been the consequence of altered biomass allocation patterns, i.e., reduced allocation to roots (Table 5.1). The trends in biomass allocation for *O. sativa* were similar to that of the hybrid, except that biomass allocation to panicles of *O. sativa* was about 50% less than the hybrid. Although there was no difference between CW and DW in the

biomass allocation to the panicles, there was a trend for CW invigorated hybrid to allocate higher biomass to panicles (Table 5.1). The invigorative effect of CW points to the ability of CW to produce some long-term effects on growth attributes. Invigoration resulted in the acceleration of phenological events (Farooq *et al*., 2006) and allowed plants to reach reproductive maturity earlier (Rice and Dyer, 2001). Seed invigoration also reduced vegetative and reproductive phases of growth and increased rice yield (Harris *et al.,* 2001; Farooq *et al*., 2006, 2009). The higher biomass allocation to panicles in CW treatments suggests that they have reached the reproductive phase earlier than DW and NI even though differences between the invigoration treatments were not always statistically significant (Fig. 5.7; Table 5.1).

5.6 Concluding remarks

The implications of this study are relevant for post-harvest practices and management, which are critical for food productivity and security, especially in tropical agricultural production systems. Herein, we showed that although post-storage invigoration of field curing induced deteriorated seeds of *O. sativa*, *O. glaberrima* and the hybrid with both CW and DW in general enhanced seedling emergence and vigour, CW invigoration was more effective in enhancing seed emergence and seedling vigour than DW. This suggests that CW was more effective in ameliorating the environmentally induced deterioration damage in the seeds. Even though CW invigoration was more effective in enhancing seedling vigour than DW, the invigorative effect was not generally carried forward to maturity (anthesis), explaining why total biomass yield and biomass allocation to stem, leaves, and roots were generally comparable between them. These results suggest that the negative effects of field curing were best ameliorated by CW invigoration and may be related to the ability of CW to enhance endogenous antioxidant capacity to scavenge ROS (Berjak *et al*., 2011; Adetunji *et al*., 2020), which is a major cause of seed deterioration. Further studies are required to characterize the molecular and biochemical mechanisms by which CW invigoration enhanced seed germinability and vigour in curing induced deteriorated rice seeds. Such studies would be useful in explaining the mechanisms by which CW ameliorates the negative effects of field curing, the mechanism of DNA repair and quantify the endogenous antioxidant production during CW invigoration. The present study validates the use of CW invigoration for field cured seeds of rice species typically grown in the humid tropics.

CHAPTER 6: Concluding Remarks and Recommendations

6.1 Introduction

Delayed field curing before storage is an inevitable part of post-harvest seed handling and technology in many tropical countries. Such field curing invariably results in increased deterioration and reduced quality and vigour in seeds. The purpose of the present study was to firstly characterize the morpho-physiological extent of this damage and the environmental determinants thereof, and secondly, to assess whether the invigoration of field-cured seeds with CW can alleviate the deteriorative effects of delayed field curing on rice seed ultrastructural integrity, seed germination and subsequent seedling growth. The results deepen our understanding of the physical, pathological, physiological, and ultrastructural lesions that contribute to loss of seed quality (seed viability and vigour) during delayed field curing**.** Additionally, the findings shed light on the possible mechanisms via which seed soaking/ invigoration treatments, with CW in particular, can alleviate the effects of field curing seed germinative development and seedling growth. The two rice species used in this study, namely, an Asian (*O. sativa* L.) and of African (*O. glaberrima* Steud) origins, and their interspecific hybrid are grown across the world. In Africa, especially in Ghana, they are widely grown to provide food to the ever-increasing population and also serve as a cash crop. The significance of the study's major findings, limitations of the study, and recommendations for future studies form the focus of this chapter.

6.2 Significance of findings, limitations and recommendations for future studies

Seed germinability and seedling vigour differed significantly across the species and the hybrid, curing environment (wet cured field environment where panicles were exposed to the weather and dry cured environment accomplished by placing panicles within ventilated rainproof containers in the field) and sampling time (curing period). The two species had significantly higher percentage germination and seedling vigour than the hybrid. The present work revealed some possible explanations for the differences in seed and seedling responses of both species and their interspecies hybrid in both environments. For example, structural damage to endosperm and fungal infection appeared to be significant contributors to field curing-induced deterioration and differed between species and curing environments. Dry field curing environment delayed endospermic cracking, reduced endospermic crack frequency, and enhanced subsequent seed viability than the wet cured environment where seeds were subjected

to numerous wet and dry cycles. It made dry field curing environment a potential field curing option for reducing field curing-induced deterioration in rice seeds. However, a reduction in subsequent seed vigour in dry-cured seeds due to a higher number of seeds infected with fungal species argues against its potential use.

This study substantiates previous studies in which identical fungal species were also isolated from stunted rice seedling roots (Nghiep and Gaur, 2004) and may have been responsible for the low vigour in the dry-cured seeds. In this regard, further research must be conducted on how fungal infection could be curtailed in dry field-cured rice seeds and whether certain varieties/hybrids are more or less susceptible to such infection. Although wet cured seeds revealed a lower number of fungal infected seeds and initial high seed vigour possibly due to the release of anti-fungal proteins and hydrolytic enzymes from dead seed coats or pericarps (Raviv *et al*., 2017), the high water activity of wet cured seeds were shown to exacerbate seed deterioration in storage. Relative humidity is related to water activity and MC (Walters, 1998), and as RH increased, MC also increased thereby increasing seed deterioration during storage (Kong *et al*., 2014; Xia *et al*., 2015a; Rao *et al*. 2017). Increased MC very likely promoted enzymatic oxidations and rapid cellular damage and decreased antioxidant enzyme activities (Sarvajeet and Narendra, 2010; Kong *et al.*, 2014; Xia *et al*., 2015a,b). Future studies should look at whether dressing/treating panicles with a surfactant fungicide in the field could reduce levels of infection and decrease the rate of seed deterioration.

The microscopy studies carried out here have revealed useful ultrastructural biomarkers of field curing induced viability and vigour loss in both species and the hybrid. These include CWL and key organelle (nucleus, nucleolus, mitochondria, lipids, vacuoles, plastids and amyloplasts) ultrastructural integrity and these biomarkers may serve as useful screening tools for rice breeders looking to identify species/ varieties that produce seed that are more resistant to curing-induced deterioration. In this regard, the ultrastructural observations made here suggest inter-species differences in curing-induced stress/ damage. For example, while marked CWL folding in embryo root meristematic cells of non-invigorated cured *O. glaberrima* reversed upon imbibition, slight CWL folding was evident in cured *O. sativa* whereas no CWL folding was apparent in the hybrid seeds. However, CWL abnormalities such as separation of adjacent CWLs and plasmalemma separation were evident in *O. sativa* and hybrid probably due to differences in CWL anatomical and/or chemical properties (based on observation

reported for other species by Webb and Arnott 1982; Oliver, 2007; Woodenberg *et al*., 2018). This is definitely a potential useful area of investigation for future studies. Also, the efficiency of mitochondrial repair or biogenesis decreased with curing duration across both species and the hybrid following imbibition, but the mitochondrial improvement was more marked in the hybrid than the two species. These findings provide strong support for previous findings that mitochondria are the main target of stress damage during seed desiccation and ageing (Benamar *et al*., 2003; Xia *et al*., 2015a,b; Yin *et al*., 2016). It must also be noted that the current study evaluated only two rice species and their interspecific hybrid, and it is not clear how much phenotypic plasticity exists for these biomarkers and their responses to stress in rice varieties. Such variation, if present, could be exploited by breeders in rice breeding programs to develop varieties that are more resilient to climate change scenarios predicted for the humid tropics. Therefore, it is recommended that a large number of rice varieties be screened to assess the plasticity of the biomarkers identified to be reliable indicators of stress and stress recovery.

Significantly higher seed germinability (viability) in CW invigorated field-cured seeds, and vigour (radicle length and seedling dry weight) in both CW invigorated non-cured and field-cured seeds relative to DW invigorated seeds suggest the effectiveness of CW in alleviating curing-induced viability and vigour loss. Considering the beneficial effects of CW invigoration in terms of seed germinability (i.e., capacity) and vigour in cured seeds, the most significant alleviatory effect on germinability was observed in the hybrid seeds, whereas the greatest alleviatory effect on vigour was observed in *O. sativa* and the hybrid. A possible basis for these differences is the fact that the decline in MM integrity with curing time was more pronounced in *O. sativa* and *O. glaberrima* than the hybrid. Most importantly, CW invigoration revealed the development of marked active G and a greater degree of mitochondrial improvement (structural development and elongation) than DW in both non-cured and cured seeds of the hybrid than *O. sativa* and *O. glaberrima*. These results also suggest that the mechanisms underlying alleviation of curing induced stress/ damage by CW may differ across species based on the extent of mitochondrial improvement and elongation, and G development and could be used by rice breeders to select varieties/cultivars that are less susceptible to curing-induced stress/ damaged in the humid tropics. These findings corroborate earlier findings (Berjak and Pammenter, 2000; Chen *et al*., 2005; Chen and Chan, 2010; Gomes and Scorrano, 2011) that G development, degree of mitochondrial repair and/ or mitochondrial biogenesis and mitochondrial elongation are important physiological indicators of recovery following imbibition in aged seeds. Observation made in this study of these biomarkers also point to

superior restorative effects of CW on viability, vigour and ultrastructure in cured seeds compared with DW.

These results provide an impetus for future studies on the application of this invigoration technique to other crop species susceptible to deterioration during processing and storage. However, a shortcoming of the present study is that nucleolar activity [determined by the number and sizes of NVs; Stępiński (2014)] could not be quantitatively measured; nucleolar activity is an important indicator of plant cell functionality and is indicative of high RNA synthesis (Stępiński, 2014; Kalinina *et al*., 2018). In this regard, future studies should measure the effect of CW invigoration on nucleolar activity in embryo meristematic cells. It should also be mentioned that the ultrastructural studies described in Chapters 3 and 4 required lengthy and laborious preparative procedures: de-husking seeds after invigoration and removal of epithelial tissue covering the seed embryo to enhance tissue fixation. While these methods were adequate for the purposes of this study, if ultrastructural studies are to be used as a screening tool in breeding programmes, more effort is needed to reduce the labour-intensive nature of the sample preparation and fixation methods used here.

The uncured seeds of both species and the hybrid used for the greenhouse study did not exhibit macro-structural damage, however, the 5 week cured seeds exhibited severe post-storage structural damage to the endosperm. The 5-week field cured seeds showed deep cracks and exposed the aleurone cell with its grains and lipid bodies. The cracks also facilitated fungal infection and insect damage. Caryopsis coat thickness decreased with delayed field curing in both species and the hybrid. However, caryopsis coat thickness before and after field curing was thicker in the two species than the hybrid. The observed physical deterioration in the delayed cured seeds of both species and hybrid might explain the decline in germinability and vigour. In the greenhouse seedling growth study, seedling emergence and vigour were enhanced in cured seeds of both species and the hybrid invigorated with CW to a greater extent than with DW. Across both species and hybrid, CW enhanced panicle biomass although differences between CW and DW were not always significant. Both CW and DW generally enhanced seedling emergence and vigour of cured seeds compared with NI seeds. However, cured hybrid seeds responded better to CW than the two species. Even though CW invigoration was more effective in enhancing seedling vigour than DW, the invigorative effect was generally not observed in terms of total biomass yield and biomass allocation to stem, leaves, and roots. Cathodic water invigoration may be useful to rice growers, especially in the humid

tropics, because of its effectiveness as an ameliorative method for alleviating field curing-induced stress/ damaged seeds in the field environment. These findings suggest that CW invigoration of field curing-induced deteriorated seeds can be used to alleviate poor seedling establishment in direct-seeded rice production systems, but adequate nutrition will have to be applied to the plants later so that the benefits can be carried forward to maturity. Biochemical (e.g., electrolyte leakage, lipid peroxidation products, protein oxidation, antioxidant contents, antioxidant defense enzymes and germination associated enzymes), physiological (e.g., chlorophyll fluorescence, carbon dioxide assimilation rate, leaf gas exchange etc.), molecular (e.g., DNA repair, DNA replication, specific protein levels etc.), omic (e.g., transcriptomic, proteomic, and metabolomic analysis) and biophysical (thermal properties of lipids) approaches should be applied to fully understand the response of the species and the hybrid to CW. Such studies are essential for understanding the mechanisms by which CW ameliorates the negative effects of field curing, given the increasing number of studies that are now reporting on the use of CW for invigorating aged/ stressed seeds (Gondwe *et al*., 2016; Adetunji *et al*., 2020, 2021; Fatokun *et al*., 2020a, b).

6.3 Concluding remarks

In conclusion, this study adopted a multidisciplinary approach to understanding the phenomenon of field curing-induced seed deterioration in rice in the humid tropics. While field curing may represent a regional practise, deterioration in rice seed quality is a global challenge and the study's investigations on how seed soaking/invigoration treatments, particularly the use of CW, can be used to improve seed vigour and subsequent seedling growth open the door for future studies on the use of these treatments in rice and other crop species. Importantly, the study has identified a number of ultrastructural biomarkers and macro-morphological indicators that can be used for screening rice varieties for sensitivity/ tolerance to field curing-induced deterioration and potentially to assess the utility of seed invigoration/ priming treatments in improving vigour and viability in deteriorated rice seeds. The results have also provided a basis for many new opportunities for research on alleviating seed processing and storage -induced deterioration in crop seed quality, which is going to represent an increasingly important research area as the effects of climate change and developmental challenges threaten food security in many parts of the world.

REFERENCES

Adetunji EA., Sershen, Varghese B., and Pammenter N. (2020). Effects of inorganic salt solutions on vigour, viability, oxidative metabolism and germination enzymes in aged cabbage and lettuce seeds. Plants 9: Article 1164. DOI: 10.3390/plants9091164

Adetunji AE., Sershen, Varghese B. and Pammenter N.W. (2021). Effects of exogenous application of five antioxidants on vigour, viability, oxidative metabolism and germination enzymes in aged cabbage and lettuce seeds. South African Journal of Botany 137, 85–97. DOI: 10.1016/j.sajb.2020.10.001

Anderson JD. and Baker JE. (1983). Deterioration of seeds during aging. Phytopathology 73:321-325. DOI: 10.1094/Phyto-73-321

Angelovici R., Galili G., Fernie, AR. and Fait A. (2010). Seed desiccation: a bridge between maturation and germination. Trends in Plant Science 15: 211-218

Agnolucci P., Rapti C., Alexander P., Lipsis VD., Holland RA., Eigenbrod, F. and Ekins P. (2020). Impacts of rising temperatures and farm management practices on global yields of 18 crops. Nature Food 1: 562-571. DOI: 10.5522/04/12768425

Asch F., Sow A. and Dingkuhn M. (1999). Reverse mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigour. Field Crops Research 62: 191-202. DOI: 10.1016/S0378-4290(99)00020-9

Bailly C., El-Maarouf-Bureau H. and Corbineau F. (2008). From intracellular signalling network to cell death: the dual role of reactive oxygen species in seed physiology. Comptes Rendus Biologies 331: 806–81. DOI:10.1016/j.crvi.2008.07.022

Bailly C. (2004). Active oxygen species and antioxidants in seed biology. Seed Science Research, 14: 93–107. DOI: 10.1079/SSR2004159

Bailly C., Bogatek-Leszczynska R., Côme D. and Corbineau F. (2002). Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. Seed Science Research 12: 47-55. DOI: 10.1079/SSR200197

Balasubramanian V., Sie M., Hijmans RJ. and Otsuka K. (2007). Increasing rice production in sub-Saharan Africa: Challenges and opportunities. Advances in Agronomy 94: 55-133. DOI: 10.1016/S0065-2113(06)94002-4

Balasubramanian V. and Hill J.E. (2002). Direct seeding of rice in Asia: emerging issues and strategic research needs for 21st century. In Pandey S., Mortimer M., Wade L., Tuong TP., Lopes K., Hardy B. (eds) Direct seeding: research strategies and opportunities. International Rice Research Institute, Manila, Philippines, pp 15-19.

Bam RK., Sershen, Varghese B., Dzomeku BM., Appiah-Kubi Z. and Pammenter N.W. (2019). Impacts of delayed field curing on rice seed quality in a tropical environment. Experimental Agriculture 55: 412-427. DOI: 10.1017/S001447971800008X

Bam RK., Hong TD., Ellis RH., Kumaga FK., Ofori K. and Asiedu EA. (2008). Storage behaviour of two contrasting upland rice genotypes. Ghana Journal of Agricultural Science 41: 113-120.

Bam RK., Craufurd PQ., Dorward PT., Asiedu EA., Kumaga FK. and Ofori K. (2007). Introducing improved cultivars: Understanding farmers' drying and storage practices in Central Ghana. Experimental Agriculture 43: 301-317.

Basra AS., Singh B. and Malik CP. (1994). Amelioration of the effects of ageing in onion seeds by osmotic priming and associated changes in oxidative metabolism. Biologia Plantarum 36: 365-371. DOI: 10.1007/BF02920933

Bechtel DB. and Pomeranz Y. (1978). Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis. The gem. American Journal of Botany 65: 75-85. DOI: 10.1002/j.1537-2197.1977.tb11941.x

Benamar A., Tallon C., Macherel D. (2003). Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. Seed Science Research 13: 35–45

Berjak P., Sershen, Varghese B. and Pammenter NW. (2011). Cathodic amelioration of the adverse effects of oxidative stress accompanying procedures necessary for cryopreservation of embryonic axes of recalcitrant-seeded species. Seed Science Research 21: 187-203. DOI:10.1017/S0960258511000110

Berjak P. and Pammenter NW. (2000). What ultrastructure has told us about recalcitrant seeds. Revista Brasileira de Fisiologia Vegetal 12: 22-55.

Berjak P., Walker M., Watt MP. and Mycock DJ. (1999). Experimental parameters underlying failure or success in plant germplasm cryopreservation: a case study on zygotic axes of *Quercus robur* L. Cryo-Letters 20: 252–262.

Berjak P., Dini M., and Givers HO. (1986). Deteriorative changes in embryos of long-stored uninfected maize caryopses. South African Journal of Botany 52: 109-116.

Berjak P. (1978). Viability extension and improvement of stored seeds. South African Journal of Science 74: 365-368.

Berjak P. and Villiers TA. (1973). Ageing in plant embryos. II Age-induced damage and its repair during early germination. New Phytologist 71: 135-144.

Berrie AMM. and Drennan DSH. (1971). The effect of hydration-dehydration on seed germination. New Phytologist ,70: 135-142. DOI: 10.1111/j.1469-8137.1971.tb02518.x

Bewley JD, Bradford KJ, Hilhorst HWM. and Nonogaki H. (2013) Seeds: physiology of development, germination and dormancy. Springer, New York

Bewley JD. (1997). Seed germination and dormancy. Plant Cell 9: 1055–1066 .

Bewley JD., and Black M. (1994). Seeds. Physiology of development and germination, 2nd. ed. New York, Plenum Press.

Bewley JD. (1979). Physiological aspects of desiccation tolerance. Annual Review of Plant Physiology 30: 195-238.

Bhatia VS., Yadav S., Jumrani K. and Guruprasad KN. (2010). Field deterioration of soyabean seed: Role of oxidative stresses and antioxidant defense mechanism. Journal of Plant Biology, 37(2): 179-190.

Bonner FT., Vozzo JA., Elam WW. and Land Jr. SB. (1994). Tree seed technology training course. Instructors manual. General Technical Report SO-106. New Orleans, L.A.: US Department of Agriculture, Forest Service, Southern Forest Experiment Station. 160p

Bradford KJ., Dahal P., Van Asbrouck J., Kunusoth K., Bello P., Thompson J. and Wu F. (2018). The dry chain: Reducing post-harvest losses and improving food safety in humid climates. Trends in Food Science and Technology, 71: 84-93. DOI: 10.1016/j.tifs.2017.11.002

Bradford KJ. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions Horticultural Science 21: 1105-1112.

Butler LH., Hay FR., Ellis RH., Smith RD. and Murray TB. (2009). Priming and re-drying improve the survival of mature seeds of Digitalis purpurea during storage. Annals of Botany 103: 1261–1270. DOI:10.1093/aob/mcp059

Castro MFG. and Martinez-Honduvilla CJ. (1984). Ultrastructural changes in naturally aged *Pinus pinea* seeds. Physiologia Plantarum 62: 581-588.

Chang TT. (1991). Findings from a 28-yr seed viability experiment. International Rice Research Newsletter (IRRN): 16: 5-6.

Changrong Y., Sripichitt P., Juntakool S., Hongtrakul V. and Sripichitt A. (2007). Modifying controlled deterioration for evaluating field weathering resistance of soybean. Kasetsart Journal (Natural Science) 41: 232-241.

Chatelain E., Hundertmark M., Leprince O., Le Gall S., Satour P., Deligny-penninck S., Rogniaux H and Buitink J. (2012). Temporal profiling of the heat-stable proteome during late maturation of *Medicago truncatula* seeds identifies a restricted subset of late embryogenesis abundant proteins associated with longevity. Plant Cell and Environment 35: 1440-1455. DOI: 10.1111/j.1365-3040.2012.02501.x

Chemura A., Schauberger B. and Gornott C. (2020). Impacts of climate change on agro-climatic suitability of major food crops in Ghana. PLOS ONE 15(6): e0229881. DOI: 10.1371/journal. pone.0229881

Chen H. and Chan C.H. (2010). Physiological functions of mitochondrial fusion. Annals of the New York Academy of Sciences 1201: 21-25. DOI: 10.1111/j.1749-6632.2010.05615.x

Chen H., Chomyn A. and Chan DC. (2005). Disruption of fusion results in mitochondrial heterogeneity and dysfunction. The Journal of Biological Chemistry, 280: 26185-26192. DOI: 10.1074/jbc.M503062200

Chen H., Osuna D., Colville L., Lorenzo O., Graeber K., Küster H., Leubner-Metzger G. and Kranner I. (2013). Transcriptome-wide mapping of pea seed ageing reveals a pivotal role for genes related to oxidative stress and programmed cell death. DOI: 10.1371/journal.pone.0078471

Corbineau F. (2012). Markers of seed quality: from present to future. Seed Science Research 22: S61-S68. DOI: 10.1017/S0960258511000419

Corbineau F., Berjak P., Pammenter N., Vinel D., Picard MA. and Côme D. (2004). Reversible cellular and metabolic changes induced by dehydration in desiccation-tolerant wheat seedling shoots. Physiologia Plantarum 122: 28-38.

Czernekova´ M., Janelt K., Student S., Jo¨nsson KI. and Poprawa I. (2018). A comparative ultrastructure study of storage cells in the eutardigrade *Richtersius coronifer* in the hydrated state and after desiccation and heating stress. PLoS ONE 13(8): e0201430. DOI: 10.1371/journal.pone.0201430

Day G., Oldham P., Acheampong J., Opoku-Apau A. and Langyintuo A. (1997). Improving the competitiveness and marketability of locally produced rice in Ghana: Rice marketing in Ghana. DfID Crop Post Harvest Programme Project No.R6688 Project Report.

De Souza FD. and Marcos-Filho J. (2001). The seed coat as a modulator of seed environment relationships in Fabaceae. Brazilian Journal of Botany 24: 365-375. DOI: 10.1590/S0100-84042001000400002

Dekkers BJW., Costa MCD., Maia J., Bentsink L., Ligterink W. and Hilhorst HWM. (2015). Acquisition and loss of desiccation tolerance in seeds: from experimental model to biological relevance. Planta 241: 563-577. DOI: 10.1007/s00425-014-2240-x

Delouche JC. and Baskin CC. (1973). Accelerated ageing techniques for predicting the relative storability of seed lots. Seed Science and Technology 1:427–452.

Deltour R. and Barsy TD. (1985). Nucleolar activation and vacuolation in embryo radicle cells during early germination. Journal Cell Science 76: 67-83.

Deltour R. (1985). Nuclear activation during early germination of the higher plant embryo. Journal of Cell Science 75: 43-83.

Devaiah SP., Pan X., Hong Y., Roth M., Welti R. and Wang X. (2007). Enhancing seed quality and viability by suppressing phospholipase D in Arabidopsis. The Plant Journal, 50: 950–957

Dorward P., Craufurd P., Marfo K. Dogbe W and Bam R. (2007). Improving participatory varietal selection and the role of informal seed diffusion mechanisms for upland rice in Ghana. Euphytica 155: 315-327. DOI 10.1007/s10681-006-9333-y

Draganic I. and Lekic I. (2012). Seed priming with antioxidants improves sunflower seed germination and seedling growth under favourable germination conditions. Turkish Journal of Agriculture and Forestry 36: 421-428. DOI: 10.3906/tar-1110-16.

Ebone L.A., Caverman A. and Chavarria G. (2019). Physiologic alterations in orthodox seeds due to deterioration processes. Plant Physiology and Biochemistry 145: 34-42. DOI: 10.1016/j.plaphy.2019.10.028

Egli DB., TeKrony DM., Heitholt JJ. and Rupe J. (2005). Air temperature during seed filling and soybean seed germination and vigour. Crop Science 45: 1329-1335. DOI:10.2135/cropsci2004.0029

Ellis RH. (2011). Rice seed quality development and temperature during late development and maturation. Seed Science Research, 21: 95-101. DOI: 10.1017/S0960258510000425

Ellis RH. and Hong TD. (2006). Temperature sensitivity of the low-moisture content relations in hermetic storage. Annals of Botany 97: 785-791.

Ellis RH., Hong TD. and Roberts EH. (1995). Survival and vigour of lettuce and sunflower seeds stored at low and very low moisture contents. Annals of Botany 76: 521-534.

Ellis RH. and Hong TD. (1994). Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). Annals of Botany 73: 501-506. DOI: 10.1006/anbo.1994.1062

Ellis RH., Hong TD. and Jackson MT. (1993). Seed production environment, time of harvest, and the potential longevity of seeds of three cultivars or rice (*Oryza sativa* L.). Annals of Botany 72: 583-590.

Ellis RH., Hong TD. and Roberts EH. (1992). The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. Annals of Botany 69: 53-58.

Ellis RH., Hong TD., Roberts EH. and Tao KL. (1990). Low moisture content limits to relations between seed longevity and moisture. Annals of Botany 65: 493-504.

Ellis RH. (1988). The viability equation, seed viability nomographs, and practical advice on seed storage. Seed Science and Technology 16: 29-50.

Ellis RH., Hong TD and Roberts EH. (1987). The development of desiccation tolerance and maximum seed quality during seed maturation. In six grain legumes. Annals of Botany 59: 23-29. DOI: 10.1093/oxfordjournals.aob.a087280

Ellis RH., Hong TD., and Roberts EH. (1983). Procedures for removal of dormancy from rice seed. Seed Science and Technology 11: 77 - 112.

Ellis RH., and Roberts EH. (1981). The quantification of ageing and survival in orthodox seeds. Seed Science and Technology 9: 373-409.

Ellis RH. and Roberts EH. (1980). Towards a rational basis for testing seed quality. In Seed Production (ed. P.D. Hebblethwaite), pp. 605-635, Butterworths, London.

Farooq M., Irfan M., Aziz T., Ahmad I. and Cheema SA. (2012). Seed priming with ascorbic acid improves drought resistance in wheat. Journal of Agronomy and Crop Science 199: 12-22. DOI:10.1111/i.1439-037X.2012.00521.x

Farooq M., Wahid A., Ahmed N. and Asad SA. (2010). Comparative efficacy of surface drying and re-drying and priming in rice: changes in emergence, seedling growth and associated metabolic events. Paddy Water Environment 8: 15-22. DOI: 10.1007/s10333-009-0170-1

Farooq M., Basra SMA., Wahid A., Khaliq A. and Kobayashi N. (2009). Rice seed invigoration: A review. In: E. Lichtfouse (eds.), Organic Farming, Pest Control and Remediation of soil pollutants, Sustainable Agriculture Reviews 1: 137 – 175. DOI: 10.1007/978-1-4020-9654-9-9

Farooq M., Basra SMA. and Wahid A. (2006). Priming of field-sown rice enhances germination, seedling establishment, allometry and yield. Plant Growth Regulation 49: 285-294. DOI 10.1007/s10725-006-9138-y

Farrant J. (2000). A comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. Plant Ecology 151: 29-39.

Fatokun K., Beckett RP., Varghese B., Cloete J. and Pammenter NW. (2020a). Influence of cathodic invigoration on the emergence and subsequent growth of controlled deteriorated pea and pumpkin seeds. Plants 9: Article 955. DOI:10.3390/plants9080955

Fatokun K., Beckett RP., Varghese B., Sershen and Pammenter NW. (2020b). Germination indices of orthodox seeds as influenced by controlled deterioration and cathodic water seed invigoration. Journal of Environmental Biology 41: 1105-1111

Fernandez-Pascual E., Seal CE. and Pritchard HW. (2015). Simulating the germination response to diurnally alternating temperatures under climate change scenarios: comparative studies on *Carex diandra* seeds. Annals of Botany 115: 201-209.

Forti C., Ottobrino V., Bassolino L., Toppino L., Rotino GL., Pagano A., Macovei A. and Balestrazzi A. (2020). Molecular dynamics of pre-germinative metabolism in primed eggplant (*Solanum melongena* L.) seeds. Horticulture Research 7: Article 87. DOI: 10.1038/s41438-020-0310-8

Finch-Savage W.E and Bassel GW. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. Journal of Experimental Botany 67: 567-591. DOI: 10.1093/jxb/erv490.

Fisher PJ. and Petrini O. (1992). Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). New Phytologist 120: 137-143.

Gomes LC. and Scorrano L. (2011). Mitochondrial elongation during autophagy. Autophagy 7: 1251-1253. DOI: 10.4161/auto.7.10.16771

Gondwe DSB., Berjak P., Pammenter NW. Sershen and Varghese B. (2016). Effect of priming with cathodic water and subsequent storage on invigoration of *Pisum sativum*, *Cucurbita maxima* and *Lycopersicon esculentum* seeds. Seed Science and Technology 44: 1-12. DOI: 10.15258/sst.2016.44.2.09

Grass L. and Burris JS. (1995). Effect of heat stress during seed development and maturation on wheat (*Triticum durum*) seed quality. II. Mitochondrial respiration and nucleotide pools during early germination. Canadian Journal of Plant Science 75: 831-839

Groot SPC., de Groot L., Kodde J. and van Treuren R. (2015). Prolonging the longevity of ex-situ conserved seeds by storage under anoxia (2015). Plant Genetic Resources: Characterization and Utilization 13: 18-26. DOI:10.1017/S1479262114000586

Groot S.P.C., Surki, AA., de Vos, RCH. and Kodde J. (2012). Seed storage at elevated partial pressure of oxygen, a fast method for analysing seed ageing under dry conditions. Annals of Botany 110: 1149-1159.

Gurusinghe S, and Bradford KJ. (2001). Galactosyl-sucrose oligosaccharides and potential longevity of primed seeds. Seed Science Research 11: 121 – 133. DOI: 10.1079/SSR200167

Hanaoka K. (2001). Antioxidant effects of reduced water produced by electrolysis of sodium chloride solutions. Journal of Applied Electrochemistry 31: 1307-1313. DOI: 10.1023/A:1013825009701

Hanaoka K., Sun D., Lawrence R., Kamitani Y. and Fernandes G. (2004). The mechanism of enhanced antioxidant effects against superoxide anion radicals of reduced water produced by electrolysis. Biophysical Chemistry 107: 71–82. DOI:10.1016/j.bpc.2003.08.007

Harman GE. and Granett AL. (1972). Deterioration of stored pea seed: changes in germination, membrane permeability and ultrastructure resulting from infection by *Aspergillus ruber* and from aging. Physiological Plant Pathology 2: 271-278.

Harris D., Tripathi RS. and Joshi A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. In: Pandey S., Mortimer M., Wade L., Tuong T.P., Lopez K. and Hardy B. (Eds). Proceedings of the International Workshop on "Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities", 25–28 January 2000, Bangkok, Thailand.

Harris D., Pathan AK., Gothkar P., Joshi A., Chivasa W. and Nyamudeza P. (2001). On-farm seed priming: using participatory methods to revive and refine a key technology. Agricultural Systems 69: 151-164.

Hay FR. and Timple S. (2016). The longevity of desorbing and adsorbing rice seeds. Seed Science Research 26: 306-316. DOI:10.1017/S0960258516000222

Hay FR., de Guzman F. and Sackville Hamilton NR. (2015). Viability monitoring intervals for genebank samples of *Oryza sativa*. Seed Science and Technology 43: 218–237.

Hayashi T., Kobayashi A., Tomita K. and Shimizu T. (2015). A new method for evaluation of the resistance to rice kernel cracking based on moisture absorption in brown rice under controlled conditions. Breeding Science 65: 381-387. DOI: 10.1270/jsbbs.65.381

Hay FR., de Guzman F., Ellis D., Makahiya H., Borromeo T. and Hamilton NRS. (2013). Viability of *Oryza sativa* L. seeds stored under genebank conditions for up to 30 years. Genetic Resource and Crop Evolution 60: 275-296.

Hay FR. and Probert RJ. (1995). Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in foxglove (*Digitalis purpura* L.). Annals of Botany 76: 639-647. DOI: 10.1006/anbo.1995.1142.

He D. and Yang P. (2013). Proteomics of rice seed germination. Frontiers in Plant Science 4: Article 246. DOI: 10.3389/fpls.2013.00246

Hochmuth G., Maynard D., Vavrina C., Hanlon E. and Simonne E. (2012). Plant Tissue Analysis and Interpretation for Vegetable Crops in Florida; University of Florida: Gainesville, FL, USA.

Hoekstra FA., Golovina EA. and Buitink J. (2001). Mechanisms of plant desiccation tolerance. Trends in Plant Science 6: 43-438.

Hogan JT. and Karon ML. (1955). Hygroscopic equilibria of rough rice at elevated temperatures. Journal of Agricultural and Food Chemistry 3: 855-860.

Hong TD. and Ellis RH. (1996). A protocol to determine seed storage behaviour. IPGRI Technical Bulletin No. 1. Rome. International Plant Genetic Resources Institute..

Hong TD., Linington S. and Ellis RH. (1996). Seed storage behaviour: a Compendium. Handbooks for Genebanks No. 4. International Plant Genetic Resources Institute, Rome.

Hussain S., Khan F., Cao W., Wu L. and Geng M. (2016). Seed priming alters the production and detoxification of reactive oxygen intermediates in rice seedlings grown under sub-optimal temperature and nutrient supply. Frontiers in Plant Science 7: Article 439. DOI: 10.3389/fpls.2016.00439

International Rice Research Institute (IRRI) (2002). Standard evaluation system for rice (SES). 56 pp

International Seed Testing Association (ISTA). (2005). International rules for seed testing. Edition 2005. Switzerland, The International Seed Testing Association.

ISTA (2001). International rules for seed testing. Rules Amendments. Seed Science and Technology 29: 1-127.

ISTA (1995). Handbook of vigour test methods. Third edition. International Seed Testing Association, Zurich.

Jennane A., Thiry M., Diouri M. and Goessens G. (2000). Protoplasma 210: 172-178.

Johnson JM. and Jones LE. (1967). Behaviour of nucleoli and contracting nucleolar vacuoles in tobacco cells growing microculture. American Journal of Botany 54: 189-198.

Jones M.P., Dingkuhn M., Aluko G.K. and Semon M. (1997). Interspecific *O. sativa* × *O. glaberrima* progenies in upland rice improvement. Euphytica 92: 237-246.

Kalinina NO., Makarova S., Makhotenko A., Love AJ. and Taliansky M. (2018). The multiple functions of nucleolus in plant development, disease and stress responses. Frontiers in Plant Science, 9: 132. DOI: 10.3389/fpls.2018.00132

Kaushal N., Bhandari K., Siddique KHM and Nayyar H. (2016). Food crops face rising temperatures: An overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. Cogent Food and Agriculture 2:1, 1134380. DOI: 10.1080/23311932.2015.1134380

Khrolenko YA., Burundukova OL., Lauve LS., Muzarok TI., Makhan'kov VV., and Zhuravlev YN. (2012). Characterization of the variability of nucleoli in the cells of Panax ginseng Meyer in vivo and in vitro. Journal of Ginseng Research 36: 322-326. DOI: 10.5142/jgr.2012.36.3.322

Kibinza S., Vine D., Côme D, Bailly C. and Corbineau F. (2006). Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active species scavenging. Physiologia Plantarum 122: 496-506. DOI: 10.1111/j.1399-3054.2006.00771.x

Kijowska-Oberc J., Staszak AM and Ratajczak E. (2021). Climate change affects seeds ageing? Initiation mechanism and consequences of loss of forest tree seed viability. Trees. DOI: 10.1007/s00468-020-02072-w

Kochanek J., Steadman KJ., Probert RJ. and Adkins SW. (2011). Parental effects modulate seed longevity: exploring parental and offspring phenotypes to elucidate pre-zygotic environmental influences. New Phytologist 191: 223-233. DOI: 10.1111/j.1469-8137.2011.03681.x

Kong LQ., Mao PS., Yu XD. and Xia FS. (2014). Physiological changes in oat seeds aged at different moisture contents. Seed Science and Technology 42: 190-201. DOI:10.152158/sst.2014.42.2.08

Koster KL. (1991). Glass formation and desiccation tolerance in seeds. Plant Physiology, 96: 302-304. DOI: 10.1104/pp.96.1.302

Kranner I., Minibayev FV., Beckett RP. and Seal. CE. (2010). What is stress? Concepts, definitions and applications in seed science. New Phytologist 188: 655-673. DOI: 10.1111/j.1469-8137.2010.03461.x

Kudla J., Becker D., Grill E., Hedrich M., Kummer U., Parniske M., Romeis T. and Schumacher K. (2018). New Phytologist 218: 414-431. DOI: 10.1111/nph.14966

Kumar D. and Kalita P. (2017). Reducing post-harvest losses during storage of grain crops to strengthen food security in developing countries. Foods 6 (8). DOI:10.3390/foods6010008

Kunze OR. and Choudhury MSU. (1972). Moisture adsorption related to tensile strength of rice. Cereal Chemistry 49: 684-696.

Kunze OR. (2008). Effect of drying on grain quality: moisture re-adsorption causes fissured grains. Agricultural Engineering International: the CIGR Ejournal. Invited Overview No. 1. Volume X. 17pp

Khush GS. (1997). Origin, dispersal, cultivation and variation of rice. Plant Molecular Biology 35: 25-34.

Lahouar A., Marin S., Crespo-Sempere A., Saïd S. and Sanchis V. (2016). Effects of temperature, water activity, and incubation time on fungal growth and aflatoxin B1 production by toxinogenic Aspergillus flavus isolates on sorghum seeds. Revista Argentina De Microbiología 48(1):78-85. DOI: 10.1016/j.ram.2015.10.001

Lan, Y. and Kunze, OR. (1996). Relative humidity effects on the development of fissures in rice. Cereal Chemistry 73: 222-224.

Lechowska K., Kubala S., Wojtyla L., Nowaczyk G., Quinet M., Lutts S. and Garnczarska M. (2019). New insight on water status in germinating Brassica napus seeds in relation to priming-improved germination. International Journal of Molecular Sciences 20(3): 540. DOI: 10.3390/ijms20030540

Leonova S., Grimberg A., Martilla S., Stymne S. and Carlsson A. (2010). Mobilization of lipid reserves during germination of *Avena sativa*, a cereal rich in endosperm oil. Journal of Experimental Biology 61: 3089-3099.

Leprince O., Pellizzaro A., Berriri S. and Buitink J. (2017). Late seed maturation: drying without drying. Journal of Experimental Agriculture 68: 827-841. DOI: 10.1093/jxb/erw363

Leprince O., Hendry GAF., McKersie BD. (1993) The mechanisms of desiccation tolerance in developing seeds. Seed Science Research 3: 231–246.

Lewis WH. (1943). Nucleolar vacuoles in living normal and malignant fibroblasts. Cancer Research 531-536.

Liesa M. and Shirihai OS. (2013). Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. Cell Metabolism 17: 491–506. DOI: 10.1016/j.cmet.2013.03.002

Lin Q., Wang W., Ren Y., Jiang Y., Sun A., Qian Y., He N., Hang NT., Liu Z., Li L., Liu L., Jiang L. and Wan J. (2015). Genetic dissection of seed storability using two different populations with a same parent rice cultivar N22. Breeding Science 65: 411-419.

Linares OF. (2002). African rice (*O. glaberrima*): History and future potential. Proceedings of the National Academy of Sciences, USA 99: 16 360–16 365.

Londo JP., Chiang Y-C., Hung K-H., Chiang T-Y. and Schaal BA. (2006). Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. Proceedings of National Academy of Science USA 103: 9578-9583.

Long LR., Kranner I., Panetta DF., Birtic S., Adkins SW. and Steadman KJ. (2011). Wet-dry cycling extends seed persistence by re-instating antioxidant capacity. Plant Soil 338: 511-519. Doi: 10.1007/s11104-010-0564-2

Long RL., Gorecki MJ., Renton M., Scott JK., Colville L., Goggin DE., Commander LE., Westcott DA., Cherry H. and Finch-Savage WE. (2015). The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. Biological Reviews 90: 31-59. DOI: 10.1111/brv.12095

Lu L., Tian S., Liao H., Zhang J., Yang X., Labavitch JM. and Chen W. (2013). Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-Ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. PLoS ONE 8: Article e57360. DOI:10.1371/journal.pone.0057360.

Ma T-H., Lee L-W., Lee C-C., Yi Y-H., Chan S-P., Tan BC-M. and Lo SJ. (2016). Genetic control of nucleolar size: An evolutionary perspective. Nucleus 7: 112-120. DOI: 10.1080%2F19491034.2016.1166322

Macherel D., Benamar A., Avelange-Macherel M-H. and Tolleter D. (2007). Function and stress tolerance of seed mitochondria. Physiologia Plantarum 129: 233-241. DOI: 10.1111/j.1399-3054.2006.00807.x

Mandal K. and Basu RN. (1981). Role of embryo and endosperm in rice seed deterioration. Proceedings of Indian National Science Academy B47 No.1 109-114.

Manfre AJ., LaHatte GA., Climer CR. and Marcotte Jr. WR. (2009). Seed dehydration and the establishment of desiccation tolerance during seed maturation is altered in the Arabidopsis thaliana mutant atem6-1. Plant Cell Physiology 50: 243-253. DOI: 10.1093/pcp/pcn185

Mao C., Zhu Y., Cheng H., Yan H., Zhao L., Tang J., Ma X. and Mao P. (2018). Nitric oxide regulates seedling growth and mitochondrial responses in aged oat seeds. International Journal of Molecular Science 19: article 1052.

Marfo KA., Dorward PT., Craufurd PQ., Ansere-Bioh F., Haleegoah J. and Bam R. (2008). Identifying seed uptake pathways: The spread of Agya Amoah cultivar in Southwestern Ghana. Experimental Agriculture 44: 257-269. DOI:10.1017/S0014479708006170

Matheson LA., Hanton SL. and Brandizzi F. (2006) Traffic between the plant endoplasmic reticulum and Golgi apparatus: to the Golgi and beyond. Current Opinion in Plant Biology 9: 601–609.

Matthews S. (1980). Controlled deterioration: a new vigour test for seeds. In Seed Production (Ed P. D. Hebblethwaite), 647–660. London: Butterworths.

Mathur SB. and Kongsdal O. (2003). Common laboratory seed health testing methods for detecting fungi. Second Edition. International Seed Testing Association, Switzerland.

Matthews S. and Khajeh-Hosseini M. (2007). Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize (Zea mays). Seed Science and Technology 35: 200-212. DOI: 10.15258/sst.2007.35.1.18

McDonald MB. (1999). Seed deterioration: Physiology, repair and assessment. Seed Science and Technology, 27: 177-237.

Meharg AA., Lombi E., Williams PN., Scheckel KG., Feldmann J., Raab A., Zhu Y. and Islam R. (2008). Speciation and localization of Arsenic in white and brown rice grains. Environmental Science & Technology 42: 1051-1057. DOI: 10.1021/es702212p

Menezes NL., Cicero SM., Villela FA. and Bortolotto RP. (2012). Using X-rays to evaluate fissures in rice seeds dried artificially. Revista Brasileira de Sementes 34: 70-77. DOI: 10.1590/S0101-31222012000100009

Ministry of Food and Agriculture (MoFA) (2012). Facts and figures. Statistics, Research, and Information Directorate (SRID), Accra, Ghana.

Møller IM., Jensen PE. and Hansson A. (2007). Oxidative modifications to cellular components in plants. Annual Plant Biology 58: 459-481.

Moreano T., Braccini AL., Scapim CA, Krzyzanowski FC., Neto F. and Marques OJ. (2011). Changes in the effects of weathering and mechanical damage on soybean seed during storage. Seed Science and Technology 39: 604-611.

Moreau-Valancogne P., Coste F., Vandewalleb P., Wagner MH., Ladonne F. and Crozat Y. (2007). Modelling the effects of microclimate on bean seed desiccation rate and seed storage ability. Annals of Applied Biology 150: 41-51.

Morita S., Wada H. and Matsue Y. (2016). Countermeasures for heat damage in rice grain quality under climate change. Plant Production Science19: 1-11. DOI: 10.1080/1343943X.2015.1128114 .

Mousa W., Ghazali FM., Jinap S., Ghazali HM. and Radu S. (2011). Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. Journal of Applied Microbiology 111: 1262-1274.

Murthy UMN., Kumar PP. and Sun WQ. (2003). Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, malliard reactions and their relationship to glass state transition. Journal of Experimental Botany 54: 1057-1067. DOI: 10.1093/jxb/erg092

Nagata K., Sasaki R. and Ohdaira Y. (2013). Cultivar differences in the grain cracking of rice under the high air temperature conditions during grain filling. Japanese Journal of Crop Science 82: 42-48. DOI: 10.1626/jcs.82.42

Nagata K., Takita T., Yoshinaga S., Terashima K., and Fukuda A. (2004). effect of air temperature during the early grain-filling stage on grain fissuring in rice. Japanese Journal of Crop Science 73: 336–342.

Neya O., Golovina EA., Nijsse J. and Hoekstra FA. (2004). Ageing increases the sensitivity of neem (*Azadirachta indica*) seeds to imbibitional stress. Seed Science Research 14: 205-217. DOI: 10.1079/SSR2004170

Nghiep HV. and Gaur A. (2004). Role of *Bipolaris oryzae* in producing abnormal seedlings of rice (*Oryza sativa*). Omonrice 12: 102-108.

Notteghem JL., Roux-Cuvelier M., André F. and Roumen E. (1997). Rice diseases in the Carmargue., France. In: Chataigner J. (ed.). Maladies du riz en region médditerranéenne et les possibilités d'amélioration de sa résistance. Montepellier: Cashiers Options Méditerranéennes 15: 41-44.

Nyaaba SA. (2015). Effects of storage material on the seed quality characteristics of four rice varieties in Ghana. M. Phil dissertation submitted to Kwame Nkrumah University of Science and Technology, Ghana. Unpublished. 75pp

Oliver M. (2007). Lessons on dehydration tolerance from desiccation-tolerant plants. pp 11-50. In: M.A. Jenks and A.J. Woods eds. Plant desiccation tolerance. DOI: 10.1002/9780470376881.ch9

Ou, SH. (1985). Rice diseases. Second edition. Commonwealth Agricultural Bureaux, Commonwealth Mycological Institute, Kew, England.

Pammenter NW., Adamson JH., and Berjak P. (1974). Viability of stored seed: Extension by cathodic protection. Science 186: 1124-1125.

Paparella S., Araújo SS., Rossi G., Wijayasinghe M., Carbonera D. and Balestrazzi A. (2015). Seed priming: state of the art and new perspectives. Plant Cell Reports 34: 1281-1293. DOI: 10.1007/s00299-015-1784-y.

Petruzzelli L., Melillo MT., Zacheo TB. and Taranto G. (1992). Physiological and ultrastructural changes in isolated wheat embryos during salt and osmotic shock. Annals of Botany 69: 25-31.

Piramila BHM., Prabha AL., Nandagopalan V., and Stanley AL. (2012). Effect of heat treatment on germination, seedling growth and some biochemical parameters of dry seeds of black gram. International Journal of Pharmaceutical and Phytopharmacological Research, 1, 194–202.

Poorter H., Niklas KJ., Reich PB., Oleksyn J., Poot P., and Mommer L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193: 30-50. DOI: 10.1111/j.1469-8137.2011.03952.x

Powell, AA., and Matthews, S. (2012). Seed aging/repair hypothesis leads to new testing methods. Seed Technology 34: 15–25.

Powell AA., Yule LJ, Jing H., Groot, SPC., Bino RJ. and Pritchard HW. (2000). The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equations. Journal of Experimental Botany 51: 2031–2043. DOI: 10.1093/jexbot/51.353.2031

Prasad R., Shivay YS. and Kumar D. (2017). Current status, challenges, and opportunities in rice production. In: Chauhan BS., Jabran K. and Mahajan G. (eds). Rice production worldwide. Pp1-32

Probert R., Adams J., Coneybeer J., Crawford A. and Hay F. (2007). Seed quality for conservation is critically affected by pre-storage factors. Australian Journal of Botany 55: 326-335. DOI: 10.1071/BT06046

Probert RJ. (2003). Seed viability under ambient conditions, and the importance of drying. In: R.D. Smith, J.D. Dickie, S.H. Linington, H.W. Pritchard, R.J. Probert (eds). Seed conservation: Turning science into practice. Royal Botanic Gardens, Kew, UK. . pp 339-365

Rajjou L. and Debeaujon I. (2008). Seed longevity: survival and maintenance of high germination ability of dry seeds. Comptes Rendus Biologies 331(10):796-805. DOI: 10.1016/j.crvi.2008.07.021.

Rao NK., Dulloo ME. and Engels JMM. (2017). A review of factors that influence the production of quality seed for long-term conservation in genebanks. Genetic Resources and Crop Evolution 64: 1061-1074. DOI: 10.1007/s10722-016-0425-9

Rao NK. and Jackson MT. (1996). Seed longevity of rice cultivars and strategies for their conservation in genebanks. Annals of Botany 77: 251-260. DOI: 10.1006/anbo.1996.0029

Ratajczak E., Małecka A., Bagniewska-Zadworna A. and Kalemba EM. (2019). The production, localization and spreading of reactive oxygen species contributes to the low vitality of long-term stored common beech (*Fagus sylvatica* L.) seeds. Journal of Plant Physiology 174: 147–156.

Raviv B., Aghajanyan L., Granot G., Maover V., Frenkel O., Gutterman Y. and Grafi G. (2017). The dead seed coat functions as a long-term storage for active hydrolytic enzymes. PLoS One 12(7): e0181102.

Reynolds ES (1963) The use of lead citrate at high pH as an electron- opaque stain in electron microscopy. Journal of Cell Biology 17: 208–211

Rice KJ. and Dyer AR. (2001). Seed ageing, delayed germination and reduced competitive ability in *Bromus tectorum*. Plant Ecology 155: 237-243. DOI: 10.1023/A:1013257407909

Righetti K., Vu JL., Pelletier S., Vu BL., Glaab E., Lalanne D., Pasha A., Patel RV., Provart NJ., Verdier J., Leprince O. and Buitink (2015). Inference of longevity-related genes from a robust coexpression network of seed maturation identifies regulators linking seed storability to biotic defence-related pathways. The Plant Cell 27: 2692-2708. DOI: 10.1105/tpc.15.00632

Ritchie S., Swanson SJ. and Gilroy S. (2000). Physiology of the aleurone layer and starchy endosperm during grain development and early seedling growth: new insights from cell and molecular biology. Seed Science Research 10: 193-212. DOI: 10.1017/S0960258500000234

Roberts E.H. (1973). Predicting the storage life of seeds. Seed Science and Technology 1: 499-514.

Rossi V., Scandolara A. and Battilani P. (2009). Effect of environmental conditions on spore production by *Fusarium verticillioides*, the causal agent of maize ear rot. European Journal of Pathology 123: 159-169.

Sacandé M., Golovina EA., van Aelst AC., and Hoekstra FA. (2001) Viability loss of neem (*Azadirachta indica*) seeds associated with membrane phase behaviour. Journal of Experimental Botany 52: 919–931.

Sanders D., Brownlee C. and Harper JF. (1999). Communicating with calcium. Plant Cell 11 691–706.

Sanhewe AJ. and Ellis RH. (1996). Seed development and maturation in *Phaseolus vulgaris*. II. Post-harvest longevity in air-dry storage. Journal of Experimental Botany 47: 959-965.

Sano N., Rajjou L., North HM., Debeaujon I., Marion-Poll A. and Seo M. (2016). Staying alive: Molecular aspects of seed longevity. Plant Cell Physiology 57(4): 660-674. DOI:10.1093/pcp/pcv186

Seck PA., Tollens E., Wopereis MCS., Diagne A. and Bamba I. (2010). Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. Food Policy 35: 403-411

Semon M., Nielsen R., Jones MP. and McCouch SR. (2005). The population structure of African cultivated rice *Oryza glaberrima* (Steud.): Evidence for elevated levels of linkage disequilibrium caused by admixture with *O. sativa* and ecological adaptation. Genetics 169: 1639-1647. DOI: 10.1534/genetics.104.033175

Sershen, Varghese B., Naidoo C. and Pammenter NW. (2016). The use of plant stress biomarkers in assessing the effects of desiccation in zygotic embryos from recalcitrant seeds: challenges and considerations. Plant Biology 18: 433-444.

Sharma SN. and Maheshwari A. (2015). Expression patterns of DNA repair genes associated with priming small and large chickpea (*Cicer arietinum*) seeds. Seed Science and Technology 43(2): 1-12. DOI:10.15258/sst.2015.43.2.11

Shinohara T., Hampton JG. and Hill MJ. (2006). Location of deterioration within garden pea (*Psium sativum*) cotyledons is associated with the timing of exposure to high temperature. New Zealand Journal of Crop and Horticultural Science 34: 299-309.

Shirahata S., Kabayama S., Nakano M., Miura T., Kusumoto K., Gotoh M., Hayashi H., Otsubo K., Morisawa S. and Katakura Y. (1997). Electrolyzed–Reduced Water Scavenges Active Oxygen Species and Protects DNA from Oxidative Damage. Biochemical and Biophysical Research Communications 234: 269–274. DOI: 10.1006/bbrc.1997.6622

Singh RP., Prasad PVV. and Reddy KR. (2013). Impacts of changing climate and climate variability on seed production and seed industry. Advances in Agronomy 118:49-110. DOI: 10.1016/B978-0-12-405942-9.00002-5

Smith MT. and Berjak P. (1995). Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. In: Jaime, J., Galili, G. (Eds.), Seed Development and Germination. Marcel Dekker Inc., New York, pp. 701–746.

Srivastava S. (2017). The mitochondrial basis of aging and age related deterioration. Genes Genes 8, 398; DOI:10.3390/genes8120398

Stępiński D. (2014). Functional ultrastructure of the plant nucleolus. Protoplasma 251: 1285-1306. DOI: 10.1007/s00709-014-0648-6

Solberg SØ., Yndgaard F., Andreasen C., Bothmer R., Loskutov IG. and Asdal Å. (2020). Long-term storage and longevity of orthodox seeds: A systematic review. Frontiers in Plant Science 11: Article 1007. DOI: 10.3389/fpls.2020.01007

Takahashi W., Ojima T., Nomura M. and Nabeshima M. (2002). Development of a model for predicting cracked rice kernel of rice cultivar “Koshihikari”. Hokuriku Crop Sci. 37: 48–51. DOI: 10.1270/jsbbs.65.381

TeKrony DM. and Egli DB. (1991). Relationship of seed vigour to crop yield: A review. Crop Sci. 31: 816-822. DOI:10.2135/cropsci1991.0011183X003100030054x

TeKrony DM., Egli DB. and Phillips AD. (1979). Effect of field weathering on viability and vigour of soybean seed. Agronomy Journal 72: 749 – 753. DOI:10.2134/agronj1980.00021962007200050014x

Thornton J.M. and Powell A.A. (1992). Short term aerated hydration for the improvement of seed quality in *Brassica oleracea* L. Seed Science Research 2: 41-49.

Topani VA., Rachana MR., Navi SS., Thakur PP., Bandyopadhyay R., Varanavasiappan, S. and Seetharama, N. (2007). Effect of temperature and relative humidity regimes on grain mould sporulation and seed quality in sorghum (*Sorghum bicolor* (L) Moench). Archives of Phytopathology and Plant Protection 40: 113-127.

Vaughan D.A., Lu B-R. and Tomooka N. (2008). The evolving story of rice evolution. Plant Science 174: 394-408.

Vertucci CW. and Farrant JM. (1995). Acquisition and loss of desiccation tolerance. In seed development and germination1, J. Kigel and G. Galili, eds (Marcel Dekker: New York, USA, pp. 237-271.

Vertucci, CW., Roos, E.E. and Crane, J. (1994). Theoretical basis of protocols for seed storage. III. Optimum moisture contents for pea seeds stored at different temperatures. Annals of Botany 74: 531-540.

Vertucci, C. W., and Roos, E.E. (1990). Theoretical basis of protocols for seed storage. Plant Physiology 94: 1019-1023.

Vertucci CW. and Leopold AC. (1987). Water binding in legume seeds. Plant Physiology 85: 224 - 231.

Walters C., Ballesteros D. and Vertucci VA. (2010). Structural mechanics of seed deterioration: Standing the test of time. Plant Science 179: 565-573. DOI: 10.1016/j.plantsci.2010.06.01

Walters C. and Koster KL. (2007). Structural dynamics and desiccation tolerance in plant reproductive organs. pp 251-280. In: Jenks MA and Woods JA. eds. Plant desiccation tolerance. DOI: 10.1002/9780470376881.ch9

Walters C., Wheeler L. and Grotenhuis JM. (2005). Longevity of seeds stored in a genebank: species characteristics. Seed Science Research 15: 1–20. DOI: 10.1079/SSR2004195

Walters C., Wheeler L. and Stanwood PC. (2004). Longevity of cryogenically stored seeds. Cryobiology 48: 229-244.

Walters C., Farrant JM., Pammenter NW. and Berjak P. (2002). Desiccation stress and damage. In Black M. and Pritchard HW (eds.): Desiccation and plant survival. CABI Publishing, Wallingford, pp. 263-291.

Walters C. (1998). Understanding the mechanisms and kinetics of seed aging. Seed Science Research 8(2): 223-244. DOI:10.1017/S096025850000413X

Walters C., Rao KN. and Hu X. (1998). Optimizing seed water content to improve longevity in ex situ genebanks. Seed Science Research (Supplement No.1): 15-22.

Wang WQ, Cheng HY., Møller IM. and Song SQ. (2012a). The role of recovery of mitochondrial structure and function in desiccation tolerance of pea seeds. Physiologia Plantarum 144: 20 – 34. DOI 10.1111/j1399-3054.2011.01518.x

Wang F., Wang R., Jing W. and Zhang W. (2012b). Quantitative dissection of lipid degradation in rice seeds during accelerated aging. Plant Growth Regulation 66: 49-58. DOI: 10.1007/s10725-011-9628-4

Wang Y., Yu Y., Haberer G., Marri PR., Fan C., Goicoechea J.L., Zuccolo A., Xiang Song X., Kudrna D., Jetty SS., Ammiraju J.S., Cossu RM., Maldonado C., Jinfeng CJ., Seunghee LS., Sisneros N., Baynast KD., Golser W., Wissotski M., Kim W., Sanchez P., Ndjiondjop M-N, Sanni K., Long M., Carney J., Panaud O., Wicker T., Machado C.A., Chen M., Mayer KFX., Rounsley S., and Wing RA. (2014). The genome sequence of African rice (*Oryza glaberrima*) and evidence for independent domestication. Nature Genetics 46: 982-988. DOI:10.1038/ng.3044

WARDA. 1993. West Africa Rice Development Association Annual Report 1992. Mbé, Côte d'Ivoire

Watson ML. (1958). Staining of tissue sections for electron microscopy with heavy metals. Journal of Biochemical and Biophysical Cytolology 4: 475-478.

Waterworth WM., Bray CM. and West CE. (2019). Seeds and the art of genome maintenance. Frontiers in Plant Science 10: 706. DOI: 10.3389/fpls.2019.00706

Webb MA. and Arnott HJ (1982). Cell wall conformation in dry seeds in relation to the preservation of structural integrity during desiccation. American Journal of Botany 69:1657–1668

Weeks SE, Metge BJ. and Samant RS. (2019). The nucleolus: a central response hub for the stressors that drive cancer progression. Cellular and Molecular Life Sciences 76: 4511-4524. DOI: 10.1007/s00018-019-03231-0

Wesley-Smith, J., Pammenter, N.W., Berjak, P., Walters, C. (2001). The effects of two drying rates on the desiccation tolerance of embryonic axes of recalcitrant jackfruit (Artocarpus heterophyllus Lamk.) seeds. Annals of Botany 88, 653-664. DOI: 10.1006/anbo.2001.1519

White PJ. and Broadley MR. (2003). Calcium in plants. Annals of Botany 92(4): 487-511. DOI: 10.1093/aob/mcg164

Whitehouse KJ., Hay FR. and Ellis RH. (2017). High-temperature stress during drying improves subsequent rice (*Oryza sativa* L.) seed longevity. Seed Science Research 27: 281-291. DOI: 10.1017/S0960258517000277

Whitehouse, K.J., Hay, F.R. and Ellis, R.H. (2015). Increases in the longevity of desiccation-phase developing rice seeds: response to high-temperature drying depends on harvest moisture content. Annals of Botany 116, 245–259. DOI: 10.1093/aob/mcv091

Wiebach J., Nagel M., Börner A., Altmann T. and Riewe D. (2020). Age-dependent loss of seed viability is associated with increased lipid oxidation and hydrolysis. Plant Cell Environment 43(2): 303-314. DOI:10.1111/pce.13651

Woodenberg WR., Sershen, Varghese B. and Pammenter NW. (2018). Zygotic embryo cell wall responses to drying in three gymnosperm species differing in seed desiccation sensitivity. Protoplasma. 255: 1461-1475. DOI: 10.1007/s00709-018-1243-z

Wu S., Zhou F., Zhang Z., and Zhing D. (2011). Mitochondrial oxidative stress causes mitochondrial fragmentation via differential modulation of fission-fusion proteins. FEBS Journal 278: 941-954. DOI:10.1111/j.1742-4658.2011.08010.x

Xia F., Chen L., Sun Y. and Mao P. (2015a). Relationships between ultrastructure of embryo cells and biochemical variations during ageing of oat (*Avena sativa* L.) seeds with different moisture content. Acta Physiologiae Plantarum 37:89. DOI: 10.1007/s11738-015-8

Xia F., Wang X., Li M. and Mao P. (2015b). Mitochondrial structural and antioxidant system responses to aging in oat (*Avena sativa* L.) seeds with different moisture contents. Plant Physiology and Biochemistry 94: 122-129. DOI: 10.1016/j.plaphy.2015.06.002

Yan D., Duermeyer L., Leoveanu C. and Nambara E. (2014). The functions of endosperm during seed germination. Plant and Cell Physiology 55: 1521-1533. DOI: 10.1093/pcp/pcu089

Ye N., Zhu G., Liu Y., Zhang A., Li Y., Lui R., Shi L., Jia L. and Zhang J. (2012). Ascorbic acid and reactive oxygen species are involved in the inhibition of seed germination by abscisic acid in rice seeds. Journal of Experimental Botany 63: 1809-1822. DOI: 10.1093/jxb/err336

Yin G., Whelan J., Wu S., Zhou J., Chen B., Chen X., Zhang J., He J., Xin X. and Lu X. (2016). Comprehensive mitochondrial metabolic shift during the critical node of seed ageing of rice. PLOS ONE | DOI:10.1371/journal.pone.0148013

Yin GK., Whelan J., Wu SH., Zhou J., Chen B., Chen XL., Zhang JM., He X., Xin JJ. and Lu XX. (2016). Comprehensive mitochondrial metabolic shift during the critical node of seed aging in rice. PLoS ONE 11: 1–19.

Zeng, X.-Y., Chen, R-Z., Fu, J-R. and Zhang, X-W. (1998). The effects of water content during storage on physiological activity of cucumber seeds. Seed Science Research 8(Supplement No.1): 65-68.

Zenna N., Senthilkumar K., and Sie M. (2017). Rice production in Africa. In: Chauhan BS., Jabran K. and Mahajan G. (eds). Rice production worldwide. Pp117-136

Zhang K., Zhang Y., Sun J., Meng J. and Tao J. (2021). Deterioration of orthodox seeds during ageing: influencing factors, physiological alterations and the role of reactive oxygen species. Plant Physiology and Biochemistry 158: 475-485. DOI: 10.1016/j.plaphy2020.11.031

Zhou W., Chen F., Luo X., Dai Y., Yang Y., Zheng C., Yang W. and Shu K. (2020). A matter of life and death: Molecular, physiological and environmental regulation of seed longevity. Plant Cell Environment 43: 293-302