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PhD

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Seed drying regime and subsequent longevity in rice (*Oryza sativa* L.) genebank accessions

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ABSTRACT

Maximising potential seed longevity during harvesting and drying will minimise later testing and regeneration, accumulation of genetic damage, and depletion of seed accessions in genebanks. Experiments with freshly-harvested seeds at the International Rice Genebank determined the effect of drying environment on subsequent longevity in contrasting cultivars of rice (*Oryza sativa* L.).

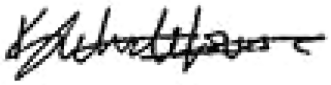
Genotypes varied considerably in differences in subsequent longevity from initial drying after harvest in a flat-bed dryer at 45°C compared with 15°C/15% RH (standard genebank drying room): from similar longevity up to a 3-fold increase with 45°C. The variation amongst accessions was associated with harvest moisture content: up to 16.2-16.7% longevity was similar, with a progressively greater benefit to subsequent longevity from drying at 45°C the greater the harvest moisture content above this value. Longevity improvement did not appear to be associated with duration of seed development (days after 50% anthesis; DAA), or by total period of exposure to 45°C. Improvement in longevity compared with drying at 15°C/15% RH was also detected when high temperature exposure was delayed after harvest. Drying seeds at 45°C with different relative humidities, revealed a similar beneficial effect to drying for seeds when harvested at a moisture content ≥16.5%.

Seeds harvested at a moisture content where they are still metabolically active (≥16.5%) are considered to remain in the desiccation phase of seed development and therefore able to continue to improve longevity *ex planta* when exposed to drying at 45°C. The consistent relationship between relative improvement in longevity and harvest MC when seeds from different harvest seasons and at different stages of maturity were dried at different durations under different regimes at 45°C confirmed that the temperature of drying is the most important factor which enables seeds to continue to accrue longevity *ex planta*.

Declaration

I hereby declare that the work presented is entirely my own and where i have consulted the work of others, this has been fully acknowledged.

The paper: **Whitehouse KJ, Hay FR, Ellis RH. 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 has been incorporated into Chapter 2.



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ABBREVIATIONS

°C	Degrees Celsius
σ	The standard deviation of the normal distribution of seed deaths in time
B_1	The probit rate of loss in dormancy
BD	Batch dryer
BIL	Backcross inbred line
CAT	Catalase
CBD	Convention on Biological Diversity
cf.	Compared with
CF	Chlorophyll fluorescence
Con	Continuous
CSSL	Chromosome substitution line
DAA	Days after 50% anthesis
DAP	Days after pollination
DAT	Days after transplanting
DH	Double hybrid
DNA	Deoxyribonucleic acid
DR	Dryroom
DS	Dry season
DW	Dry weight
eMC	Estimated moisture content
eRH	Equilibrium relative humidity
ES	Experimental station
Exp.	Experiment
f.wt.	Fresh weight

FAO	Food and Agriculture Organisation
GRC	Genetic Resources Center
H ×W × D	height × width × depth
HSPs	Heat shock proteins
In	Intermittent
IPGRI	International Plant Genetic Resources Institute
IRG	International Rice Genebank
IRGC	International Rice Genebank Code
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
K	Potassium
K_d	Initial proportion of non-dormant seeds
kDa	Kilo Dalton
K_i	Initial seed viability
LEA	Late embryogenesis abundant
LiCl	Lithium chloride
M	Molar
mAmp	Mega ampere
MAS	Marker assisted selection
MC	Moisture content
$MgCl_2$	Magnesium chloride
MM	Mass maturity
N	Nitrogen
NED	Normal equivalent deviates
NIL	Near isogenic line
P	Phosphorus

P	Probability
p_{50}	Time of viability to fall 50%
PD	Pre-drying
PSI	Pounds per square inch
QTL	Quantitative trait loci
R	Rehydrate
RAPD	Random amplified polymorphic DNA
Rep.	Replicate
RFO	Raffinose family oligosaccharide
RH	Relative humidity
ROS	Reactive oxygen species
RNA	Ribonucleic acid
rpm	Rounds per minute
s.e.	Standard error
T	Total
T_g	Glass transition temperature
V	Volts
WC	Water content
WS	Wet season

EQUATIONS

$$v = K_i - p/\sigma \quad [1]$$

$$\log_{10}\sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \quad [2]$$

$$v = K_i - p/10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2} \quad [3]$$

$$\log_{10}\sigma = K - C_W \log_{10} m \quad [4]$$

$$K = K_E - C_H t - C_Q t^2 \quad [5]$$

v is the probit percentage viability, *p* is storage period (days), *t* is temperature (°C), and *m* is moisture content (%). K_i is the percentage viability at the beginning of storage and σ is the standard deviation of seed deaths over time (days). *K*, K_E, C_W, C_H and C_Q are species specific constants (Ellis and Roberts, 1980a).

$$WC_t = (WC_i - WC_e)e^{-kt^a} + WC_e \quad [6]$$

WC is water content (g g^{-1} dry weight) and so WC_t is water content at time t, WC_i is the initial water content and WC_e is the equilibrium water content. t is drying time (days) and k, a are equation constants (Raj *et al*., 2010).

$$g = (K_{\mathrm{d}} + \beta_1 p) \times (K_{\mathrm{i}} - ({}^{p}/_{\sigma}))$$

[7]

g is the ability to germinate (probits), p, K_{i} and σ are as explained in equations [1] to [5], K_{d} is the initial proportion of non-dormant seeds and β_1 is the probit rate of loss in dormancy (Kebreab and Murdoch, 1999).

$$\mathrm{WC} = y + c\,(\mathrm{eRH}/100) + \frac{k'k(\mathrm{eRH}/100)}{1 + k(\mathrm{eRH}/100)}$$

[8]

$$y = \frac{K'K(\mathrm{eRH}/100)}{1 + K(\mathrm{eRH}/100)}$$

[9]

WC is water content (g g^{-1}) and c, k, k', K, K' are parameters that relate to the number and strength of weak and multi-molecular water-binding sites (D'Arcy and Watt, 1970).

CONTENTS

CHAPTER 1

INTRODUCTION, OBJECTIVES AND OVERVIEW OF THESIS

Crop genetic diversity comprising samples of landraces, modern and obsolete varieties, and their wild relatives are the biological basis of food security (FAO, 2013), and as such they are given high conservation priority (Maxted *et al*., 1997). Cultivated Asian rice (*Oryza sativa* L.) is the most important food crop of the developing world, being a staple for more than half of the global population (FAO, 2013). It produces orthodox seeds: the seeds can be dried and stored at a low temperature and low moisture content in genebanks – a form of *ex situ* conservation – to ensure the long-term preservation of genetic diversity (Ellis and Hong, 2007; Hay *et al*., 2013). Although orthodox seeds remain viable for many decades under genebank storage conditions, over time their viability declines and regeneration is necessary in order to maintain the genetic integrity (Cromarty *et al*., 1982; Rao *et al*., 2006). Optimising seed storage longevity will maximise the regeneration interval, reduce economic costs and limit the loss of genetic diversity. This thesis presents research which evaluates the current pre- and post-harvest practices followed by the genebank at the International Rice Research Institute (IRRI) in terms of how they affect the potential storage life of seeds.

1.1. Germplasm Conservation

With increasing concern over the predicted impact of climate change on global biodiversity and food security, coupled with the growing world population, taking action to conserve biodiversity and to secure the availability of their resources has become an urgent priority (Groom *et al*., 2006). Methods of conservation exist in the form of *in situ* i.e. creation of genetic reserves, and *ex situ* conservation where germplasm (seeds, living specimens, tissue culture or vegetative propagules) is conserved through cryopreservation, seedbanking or genebanking (Thormann *et al*., 2006). Genebanks are a safe, efficient and relatively inexpensive method of conserving germplasm (genetic resources) outside the natural environment (FAO, 2010). They complement *in situ* conservation efforts and therefore act as an insurance policy should other conservation

methods fail (Thormann *et al*., 2006; Vetelainen *et al*., 2009). The recommendation to duplicate material in other national/international genebanks (Rao *et al*., 2006) provides added security should the original material be lost e.g. natural disaster or war. Furthermore the ease of access and distribution of material facilitates breeding programmes during times of agricultural instability as well as aiding species reintroductions (Thormann *et al*., 2012). Effective maintenance and management of the current diversity represented in genebanks is paramount in reducing the frequency of retest intervals and regeneration, and therefore limiting the waste of resources and unnecessary loss of genetic material.

1.2. Water in Seeds

Water is a ubiquitous component of living tissues, biological macromolecules and macromolecular complexes and participates in intracellular activity as well as being the basis of cellular organisation and structural integrity (Priestley, 1986). Seeds of many species are hygroscopic and exchange water with their surroundings until they reach equilibrium. The tendency of water to move into the tissues from the outside is dependent upon the relative humidity (RH) of the atmosphere and the moisture content (MC), or water content, of the seed. It will also depend on the seed's chemical composition (oil content), size and seed coat (Owen, 1956; Priestley, 1986). The moisture content of the seed can be determined by measuring the relative humidity of the air in equilibrium with the seed (eRH).

1.2.1. Moisture sorption isotherms

How a seed interacts with water can be explained by sorption isotherms which show a reverse sigmoid relationship between seed moisture content and eRH at a certain temperature (Cromarty *et al*., 1982; Ellis *et al*., 1991b). An isotherm can be separated into three distinct phases which correspond to the different levels of water binding (Figure 1.1; Vertucci and Leopold, 1986; Vertucci, 1989). Region I (<15% RH) consists of water that is strongly bound at ionic sites and is imperative to the working function of the cell whereas in region II (between 15-85% RH), the water that is adsorbed is less tightly bound. Region III (>85% RH) consists of predominantly "free" or "freezable" water which will form ice

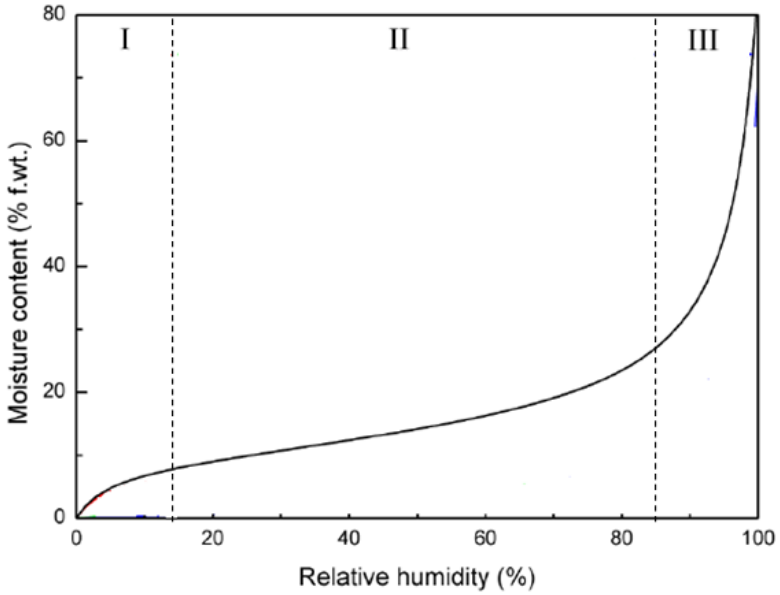


Figure 1.1. A schematic seed water sorption isotherm showing the three hydration regions corresponding to the relative proportions of strong, weak, and loosely bound water (regions I, II and III, respectively; adapted from Vertucci, 1989).

crystals when the seed is exposed to sub-zero temperatures causing cellular damage (Vertucci and Leopold, 1984; Vertucci, 1989; Leopold and Vertucci, 1989). The shape of the isotherm whether adsorption (hydrating) or desorption (dehydrating) depends on temperature and the composition of the seed. At higher temperatures the atmosphere has a greater affinity for water at any given RH and so less water is absorbed (Vertucci and Leopold, 1987). This explains why desorption isotherms have a slightly higher water content at any given eRH compared with adsorption isotherms. This difference between desorption and adsorption isotherms is known as hysteresis.

1.2.2. Seed storage behaviour categories

Not all seeds are amenable to be stored in genebanks as they differ in response to dehydration. Based on this, seeds can be divided into two major categories (Roberts, 1973). “Orthodox” seeds are desiccation-tolerant and can be dried to moisture contents in the water sorption regions I and II (typically 15-20% RH; Figure 1.1) without damage (Roberts and Ellis, 1989), whereas “recalcitrant” seeds are desiccation-intolerant and can

only partially tolerate the removal of water in region III, down to approximately 85% RH (Figure 1.1; Roberts, 1973) . They therefore cannot be stored at sub-zero temperatures without damage from cellular ice formation. Seeds of some particular species do not conform to either of these storage categories and are termed “intermediate” They have a much more limited desiccation tolerance compared with orthodox seeds and lose viability more rapidly at low temperatures (Ellis *et al.*, 1990, 1991a). Typically they can survive (to varying degrees) the removal of water in sorption zone II (20-50% RH; Figure 1.1) and are capable of maintaining high viability over medium-term storage in appropriate environments (Ellis *et al.*, 1991c).

Desiccation tolerance is acquired during seed development (section 1.3.1) and although desiccation sensitivity is reduced during development of intermediate (Ellis *et al.,* 1991b) and recalcitrant seeds (Hong and Ellis, 1990), it is not reduced to the extent that occurs in orthodox seeds. All species discussed in this thesis show orthodox seed storage behaviour.

1.3. Orthodox seed development

Post-fertilization, orthodox seed development can be divided into three distinct phases. Histo-differentiation, or embryogenesis, is the first phase and is when the embryonic tissues develop. This is followed by seed filling where reserves are deposited and the seed dry weight increases. At the end of this phase, the seeds have reached their maximum dry weight and are termed to be at “mass maturity” (MM) (Figure 1.2; Ellis and Pieta-Filho, 1992). An abscission layer is deposited forming a barrier between the seed and the mother plant. As a result the moisture status of the seeds is now determined by the ambient conditions; the seeds have become hygroscopic (Ellis and Hong, 1994). The seeds undergo a maturation drying phase where they lose water (decline in fresh weight) until they are at equilibrium with the ambient RH, their moisture content will fluctuate slightly thereafter in response to changes in the ambient conditions (Figure 1.2). The result is a mature dry seed with a reduced metabolism (Bewley and Black, 1994).

1.3.1. The acquisition of physiological traits in Oryza

A seed acquires physiological traits (ability to germinate, desiccation tolerance and potential longevity) during development and ideally orthodox seeds should be collected when these traits have peaked to maximise seed quality (Kermode and Bewley, 1985; Ellis *et al*., 1987; Pieta-Filho and Ellis, 1991). Since this thesis focuses specifically on *Oryza sativa* L. seeds, the relative timings of such physiological traits during development has been reviewed and compiled from a subsection of studies on rice seed quality development (Ellis *et al*., 1993b; Ellis and Hong, 1994; Kameswara Rao and Jackson, 1996a, b, c; Kameswara Rao and Jackson, 1997) and will be stated accordingly in this section.

Harrington (1972) claimed seed quality peaks at the end of the seed filling phase and declines thereafter, however in light of more recent research such a hypothesis has since been refuted.

The estimated time to reach mass maturity varied between varieties and varieties × environment (to be discussed further in section 1.5) ranging from 14.2 to 23.1 days after 50% anthesis (DAA) (mean 19.3; Figure 1.2). Mass maturity coincides with the end of seed filling and it is around this time (approximately 21 DAA) that seeds reach their maximum dry weight. The increase in dry weight occurs from approximately 7 DAA at a rate which varies between varieties. The final weight depends on seed size and composition. Once the abscission layer is formed (the time when seeds achieve maximum dry weight) the moisture content of the seeds naturally starts to decline until equilibrium is approached. It is during this maturation drying phase when seeds acquire desiccation tolerance, which continues to increase thereafter, until the seeds have reached equilibrium with the environment (Figure 1.2). Although rice seeds acquire desiccation tolerance relatively early in development (before the end of seed filling), tolerance to desiccation to very low (approximately 5% and below) moisture contents does not develop until between 14 and 22 days after mass maturity (mean 18 days; Ellis and Hong, 1994). Although seeds can be collected for storage as soon as they have acquired desiccation tolerance, potential longevity does not reach its maximum until between 25 and 38 DAA (mean 34 DAA; Figure 1.2), around the same time maximum germination – particularly after desiccation –

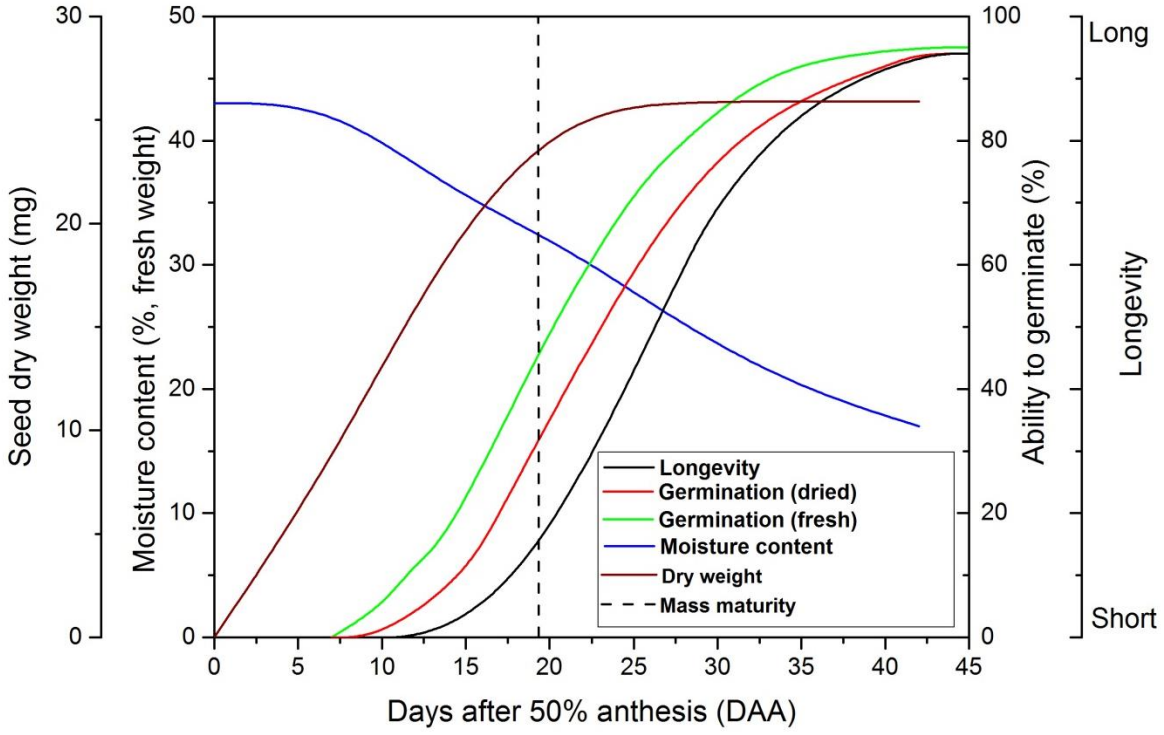


Figure 1.2. Schematic showing the physiological changes which occur during rice seed development. The vertical dashed line symbolises mass maturity (MM), when the seed has reached its maximum dry weight (brown line) and begins to decline in moisture content (blue) as a result of maturation drying. Seeds start to acquire the ability to germinate (green), desiccation tolerance (red) and longevity (black) before mass maturity and increases thereafter. Optimum collection time would be when all three of these physiological traits have begun to plateau (a compilation of data from: Ellis *et al*., 1993b; Ellis and Hong, 1994; Kameswara Rao and Jackson, 1996a, b, c, 1997).

is attained (between 21 and 37 DAA; mean 33.5; Figure 1.2). The timing of events suggests desiccation tolerance to very low moisture contents and the ability to survive air-dry storage may have a common cause (Hong and Ellis, 1992a; Ellis and Hong, 1994).

1.4. Seed longevity, viability and vigour

The period of time in storage where the seed is in a state of quiescence but is still viable i.e. still able to germinate, is referred to as its longevity (Roberts, 1972). Orthodox seeds not only survive drying to low moisture contents and exposure to low temperatures but their longevity increases in a predictable manor when stored under these conditions (Roberts, 1973). However, even under optimum storage conditions, deterioration cannot be prevented and the seeds gradually lose their ability to germinate (section 1.9). This loss in quality is expressed as a loss in vigour and eventually it will reach a point where seeds are no longer viable (Roberts, 1972; Priestley, 1986). Seed vigour is defined as "the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (ISTA, 1995). Within a population of seeds, individual seeds show subtle differences in vigour which affect the seed's ability to carry out all the physiological functions that allow them to perform. High vigour seeds are able to produce normal, rapidly growing seedlings which show little sensitivity to external factors (Corbineau and Côme, 2006). Artificial stresses (e.g. temperature and water stress) are used to show signs of weakness, such as slow germination, which is characteristic of a deteriorated seed lot. A low quality seed lot will lose viability in storage much sooner than a high quality seed lot.

1.4.1. The improved viability equations

The improved viability equations (Ellis and Roberts, 1980a, b) were developed to make accurate predictions from controlled storage experiments of the percentage viability of a seed lot after a certain period of time at a given constant temperature and moisture content. They are fitted using probit analysis under the assumption that seed deaths follow a normal distribution with respect to time. Therefore, plotting the percentage viability against time produces seed survival curves (Figure 1.3A) which are cumulative normal distributions of negative slope. Transforming the percentage viability to normal

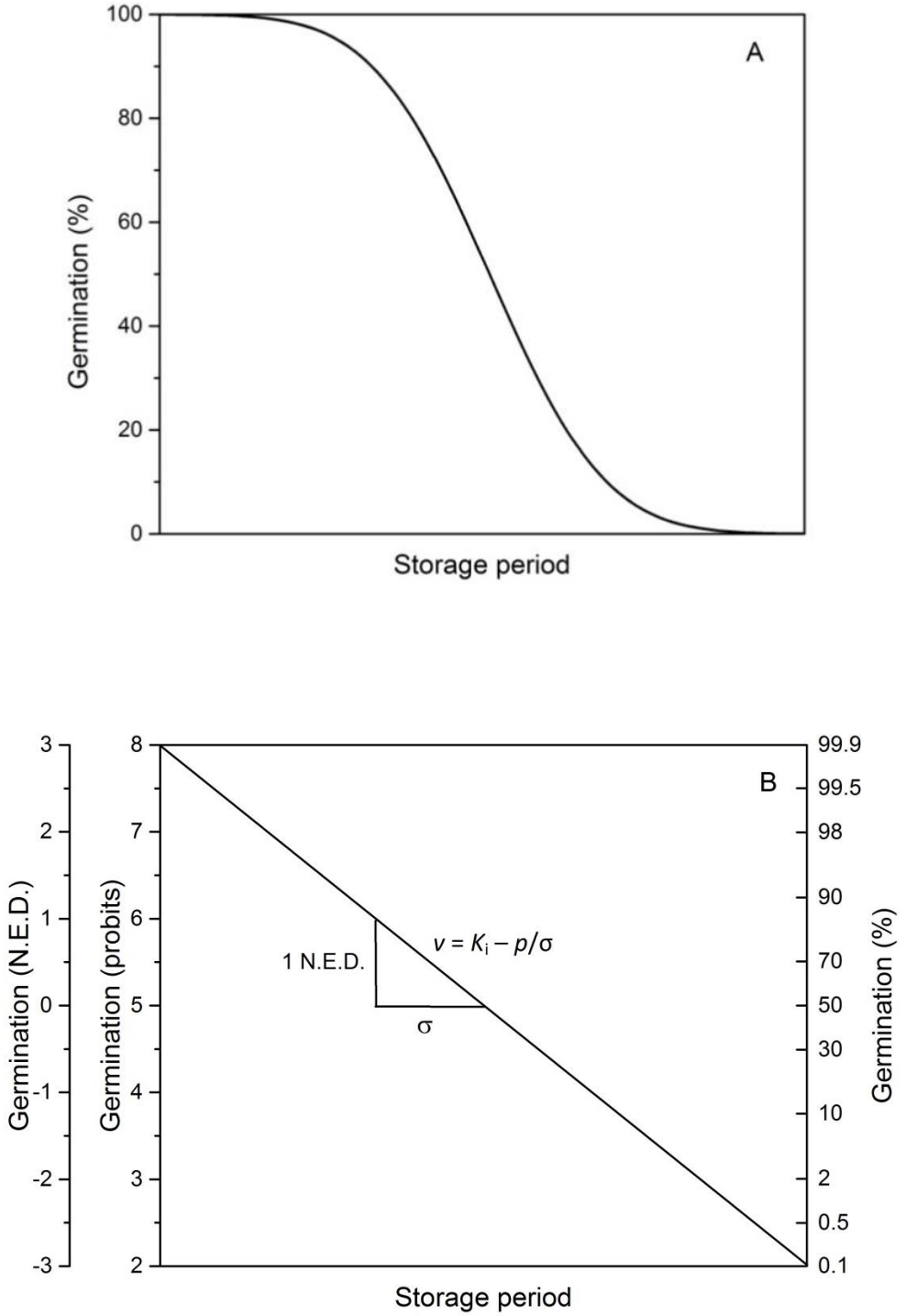


Figure 1.3. Seed survival curves. **A.** The changes in % germination (viability) over time. The frequency of seed deaths over time follows a normal frequency distribution. **B.** Germination (%) transformed to probits or normal equivalent deviates (NED). The viability, *v* of the seed lot after *p* days in storage depends on the slope (1/σ; is the slope of the transformed survival curve) (Hay, 1997).

equivalent deviates (NED), or probits (by adding 5), produces a straight line from which the p_{50} value, or half viability period, can be deduced (Figure 1.3B). The equation of the line is (Ellis and Roberts, 1980a):

$$v = K_i - p/\sigma \quad [1]$$

where v is the viability after p days in storage. K_i is the intercept and represents the initial viability of the seeds. The slope of the line is the value of σ (time to fall by one NED/probit; Figure 1.3B) and is measured in days. The viability model, as originally developed, assumed that the value of σ is constant between seed lots of the same species stored under identical conditions. Hence K_i, which is dependent on genotype and the pre-storage environment (see section 1.5) is the parameter which determines the longevity of a particular seed lot in a given storage environment and may therefore also be referred to as "potential longevity" (Demir and Ellis, 1992b). The effects of temperature, t and moisture content, m on seed longevity are species specific, according to:

$$\log_{10}\sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \quad [2]$$

Combining equations [1] and [2] produces the full viability model, equation [3]:

$$v = K_i - p/10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2} \quad [3]$$

In equations [2] and [3], K_E and C_W are species specific moisture constant contents and C_H and C_Q are species specific temperature constants which are predicted to have the same value within a species. The relative benefit of reducing temperature on seed longevity becomes less at lower temperatures due to the instability of the temperature co-efficient, Q_{10} (Ellis, 1991) but appears to be the same for all orthodox species, at least between the temperatures -13°C and +90°C (Dickie *et al*., 1990), with temperature coefficients C_H and C_Q, taking universal values of 0.0329 and 0.000478, respectively (Dickie *et al*., 1990). Similar values of 0.0322 (C_H) and 0.000454 (C_Q) have been provided for 12 different

species (Ellis and Hong, 2007). At a constant temperature there is a negative logarithmic relationship between σ and *m*,

$$\log_{10}\sigma = K - C_{\mathrm{W}} \log_{10} m \quad [4]$$

and *K* is,

$$K = K_{\mathrm{E}} - C_{\mathrm{H}} t - C_{\mathrm{Q}} t^2 \quad [5]$$

where *K* is the intercept, C_{W} is the slope, and *m*, moisture content. The negative logarithmic relationship breaks down at very high (Ibrahim and Roberts, 1983) and very low (Ellis *et al*., 1988) moisture contents. The range of moisture contents over which the relation applies corresponds roughly to region II (Figure 1.1) of isotherms (Roberts and Ellis, 1989). Critical moisture contents for safe storage can be deduced on a species-by-species basis. However it is important to note that high moisture content in seeds is more deleterious at a high than a low temperature therefore the value of critical moisture content is subject to change with a change in temperature (Vertucci and Roos, 1993; Ellis and Hong, 2006). *O. sativa* has a low critical moisture content limit between 4.3 and 4.5%; below this level, at least to 1.5%, has no further effect on longevity (Ellis *et al*., 1992).

Oxygen is beneficial and essential to the prolonged survival of hydrated seeds, i.e. above the upper moisture content limit, but below it is detrimental to seed longevity and especially so at very low moisture contents (Ellis and Hong, 2007). In some species, inert atmospheres (nitrogen, argon or helium) have been shown to help promote longevity. For example, the longevity of lettuce seeds stored at moisture contents below their upper limit (15%) was greater when exposed to nitrogen as opposed to air or pure oxygen (Ibrahim and Roberts, 1983; Ibrahim *et al*., 1983).

1.5. Inter- and intra-specific variations in seed longevity

The previous section has introduced the Ellis and Roberts viability equations which predict the storage longevity of a seed lot in air-dry storage with the assumption being

that under identical, constant storage conditions different seed lots of the same species will follow the same normal distribution of seed deaths over time but their initial viability may differ due to intra-specific variation arising as a result of genetic, environmental influences and/or seed maturity. In contrast, inter-specific variation can result in considerable variation in both initial viability and the distribution of seed deaths over time in seed lots of different species stored under the same conditions which can therefore lead to large differences in viability periods (p_{50}; period for viability to fall to 50%) (Ellis and Roberts, 1980a; Ellis, 1991). The sigmoidal pattern of seed deterioration makes it difficult to summarise ageing kinetics as seeds show loss in vigour before loss in viability. As a result, p_{50} values are most commonly used as a measure of storage longevity because this point is the most accurately estimated (as it is the mean of the frequency distribution) (Walters *et al.*, 2005; Probert *et al*., 2009; Nagel and Börner 2010; Mondoni *et al*., 2011). The half viability period (p_{50}) is a function of both K_i and σ and therefore, unlike σ (in theory), can discriminate between seed lots of the same species which differ in initial germination.

Understanding inter- and intra-species differences in seed longevity is critical to the sustainability of *ex situ* conserved seed collections as it underpins decisions on accession viability re-test intervals and therefore regeneration and/or recollection. Despite there already being species-specific constants (section 1.4) for the improved viability equations for 56 species (Liu *et al*., 2008), due to time constraints and expenditure of seeds it is unlikely that species constants are ever to be experimentally determined for the majority of plant species. Therefore identifying correlates of longevity and understanding the underlying factors which influence longevity will aid the general prediction models.

1.5.1. Inter-specific variation

Providing the initial viability is high, longevity is largely influenced by storage temperature, seed moisture content and oxygen (sections 1.3 and 1.4). However whatever the environment, some species are better at maintaining viability in storage than others. For example, for *Anemone nemorosa*, even when initial viability is high seeds are still only predicted to survive less than 1 year under seedbank storage (Ali *et al.,* 2007). A number of papers have been published which report the inter-species

differences in long-term survival of seeds in genebank storage (Walters *et al*., 2005), under ambient conditions (Nagel and Börner, 2010), or in ultra-dry storage (Pérez-Garćia *et al*., 2008; 2009). When using the Ellis and Roberts (1980a) seed viability equations the predicted time for viability to fall from 97.7% to 84.1% under conventional genebank storage conditions ranged from approximately 30 to 6000 years (Liu *et al*., 2008). Similarly, when the seeds of 18 crop species were stored in open storage for up to 26 years the Ellis's equations predicted viability to remain relatively high for at least the first two years but would decline to 0 within 5-23 years for all crops (Nagel and Börner, 2010). Furthermore, a study by Walters *et al.* (2005) who used the Avrami equation (which describes the kinetics of how a solid transforms from one phase to another at a constant temperature; based on visco-elastic properties Avrami, 1941) to model re-test data from 276 species predicted a difference in p_{50} of 626 years between the shortest (*Bromus sitchensis*; 7 years) and the longest (*Trifolium campestre*; 633 years) lived specimens in a genebank. Other studies which conferred inter-specific variation in air-dry storage longevity (experimental storage at 45°C and 60% RH) reported estimates of p_{50} between the range of 4.7 to 95.5 days in seed lots from 69 related species (Mondoni *et al*., 2011) and between 0.1 and 771 days for seed lots of 195 taxonomically diverse species (Probert *et al*., 2009). Such inter-species studies, by using p_{50}, are in effect considering potential variation in both K_i and sigma. If sigma is considered to be the species-specific constant that would rank species according to their seed longevity, use of p_{50} alone does not take into account the fact that the initial viability and hence longevity can vary between seed lots (independent of species) through K_i. To avoid this potential distortion, Probert *et al*. (2009) did attempt to minimise variation in K_i by only selecting seed lots with germination ≥85%. It could be argued that in doing so, p_{50} is simply a function of sigma and sigma could be used as the measure of relative seed longevity, however, p_{50} is more easily understood.

When using p_{50} values as an estimate of longevity it is possible to group species into various longevity categories (based on a logarithmic scale) which can be of considerable benefit to seedbanks in the effective management of their seed stocks. Such studies amongst different species or genera were able to identify potential correlates of seed storage longevity including; seed mass, composition, relative embryo size, taxonomy and climate (Priestley *et al*., 1985; Pritchard and Dickie, 2003; Walters *et al*., 2005; Probert *et*

al., 2009; Nagel and Börner, 2010; Mondoni *et al*., 2011). Although these correlations are not supported by all studies, there has been a significant advance in understanding the influence of taxonomy and climate on inter-specific differences in longevity. Walters *et al.* (2005) claimed the seeds from some families were inherently short lived (e.g. *Apiaceae*) and others long-lived (e.g. *Malvaceae*). They also reported that species originating from cool, temperate climates tend to produce short-lived seeds and warm and arid climates long-lived seeds (Walters *et al*., 2005). Such climatic correlations were also supported by Probert *et al.* (2009) who showed that species from cool, moist environments, particularly those with small embryos, were relatively short lived in comparison to non-endospermic seeds from hot dry environments. Similarly, Mondoni *et al.* (2011) presented a highly significant relationship between p_{50} and mean annual temperature and rainfall: seeds from cooler wetter climates had shorter life spans.

1.5.2. Intra-specific variation

1.5.2.1. Seed production environment and physiological traits

Studies reporting the long-term survival of seeds also provide information on the variation in longevity within a species. Of the Walters *et al.* (2005) re-test data (see previous section), 84 of the 42,000 accessions analysed were seed lots of *O. sativa* which ranged in p_{50} from 13 to 457 years. Similarly, based on the re-test data for seed lots stored in the active collection at the International Rice Genebank (IRG) for up to 31 years, estimates of p_{50} ranged from 54-997 years (Hay *et al*., 2013). Differences in the longevity between seed samples within the same species could be due to differences in the initial viability when they are first placed into storage (estimated by K_i in the viability equation; see equation [1]) due to differences in the pre or post- harvest environment and/or processing/handling procedures (Ellis *et al*., 1993a, b; Kameswara Rao and Jackson, 1996a, b, c; Ellis, 2011). Further to this, differences could also result from differences in the rate of viability loss (σ^{-1}) during storage which can vary between seed lots within a species due to maturity (Hay *et al*., 1997; 2010) and/or genotype (Ellis *et al*., 1992). These will be further discussed below.

Seeds have evolved to be highly adapted to their natural environment and the effects of a change in the maternal environment during seed development and maturation can affect the acquisition of physiological traits such as desiccation tolerance and longevity. For example in rice, japonica varieties which evolved in temperate environments typically show poorer storage longevity compared with indica varieties of the tropical regions possibly due to their heightened sensitivity to a higher temperature seed production environment (Ellis *et al*., 1993b; Kameswara Rao and Jackson 1996b). If seed quality development is different between varieties then differences in storability may be due to both genotype and genotypic × environmental effects (Hay *et al*., 2013). A recent study on *Wahelenbergia tumidifructa* (Kochanek *et al*., 2010) showed that the effect of temperature on subsequent seed longevity depended on that of the pre-zygotic plant growth environment. Low temperatures during seed development and the ripening phase had detrimental effects on longevity but had either no effect or enhanced seed longevity when low temperatures were provided prior to seed set (Kochanek *et al*., 2010). Similarly in japonica rice, high temperatures have a more damaging effect on seed quality the earlier on in seed development that they occur, and that this effect reduces during late seed filling onwards, suggesting seeds are less sensitive to high temperatures during late development and maturation (Ellis, 2011). Another example of the effect of environment is that the longevity of *Brassica campestris* seeds was greater if maternal plants experienced drought during seed development (Sinniah *et al.*, 1998a).

The effects of intra-species variation have been reported in longevity studies of seeds stored under controlled (Mondoni *et al*., 2011) and uncontrolled conditions (Nagel and Börner, 2010). For example, Mondoni *et al*. (2011) compared the longevity of seed lots of 63 related species from two different climatic regions (alpine and lowland) and found that the seed lots from the same and congeneric species collected at the cooler, wetter alpine region were shorter lived than those collected from lowland. Similarly, Nagel and Börner (2010) reported an increase in the variability in germination between genotypes within a crop species with an increase in storage duration.

1.5.2.2. Seed maturity

Naturally within a seed population, individual seeds vary in the timing of maturation due to variation in the timing of pollination, fertilisation, and environment over the period from flowering to dispersal but nevertheless it is extremely important to harvest seeds as close to peak maturity (or "storage maturity"; Kameswara Rao and Jackson 1996a) as possible as premature harvests can result in seeds which have not yet reached maximum quality in terms of longevity and desiccation tolerance. Immature seed lots generally have a lower initial viability and/or show faster loss in viability (Ellis *et al*., 1993a; Ellis and Hong, 1994; Hay and Probert, 1995). It is important to note that the environmental conditions experienced during maturation and development can affect the relevant timings of developmental stages. For example, a warm seed production environment was capable of bringing forward the time when maximum seed quality was attained in japonica varieties, and reduced the improvement in seed quality that occurs subsequent to mass maturity in indica varieties. It was thought that the hotter temperatures enhanced the progression through development which subsequently resulted in indica seeds which had not fully acquired maximum quality (Ellis *et al*., 1993b). Similarly, Daws *et al*. (2004) found that desiccation tolerance increased in seeds of *Aesculus hippocastanus* which developed in warmer conditions than what they would naturally experience as it enabled development to progress further before seeds were shed.

1.5.2.3. Assessing seed maturity

There are various strategies carried out by collectors to assess the maturity of seeds and so time of collection, however the most reliable is to determine the equilibrium relative humidity (eRH) which can be carried out using portable eRH meters to confirm whether the seeds have equilibrated with ambient conditions. For wild species, the current recommendation for *ex situ* conservation is to collect seeds when they have reached an eRH between 85 and 90% (Hay and Smith, 2003) just prior to dispersal. However seeds of tropical species, or those within fleshy fruits, are unlikely to naturally dry to such low eRHs due to high temperature and humidity conditions. In the case of cultivated species like *O.sativa*, the seeds are also shatter-resistant and therefore fail to show signs of dispersal. Kameswara Rao and Jackson (1996a) measured the changes in seed quality

during ripening in 16 varieties of rice and found that the potential longevity was greatest around 2 weeks after mass maturity, at between 33 and 37 days after 50% anthesis (DAA). The period DAA has since become an acceptable method at IRRI of assessing rice seed maturity status. As well as looking for signs of seed dispersal, other possible indicators are seed coat colour change (Hay *et al*., 2010) and chlorophyll decline (Jalink *et al*., 1999). In the case of rice, "degreening" caused by the breakdown of chlorophyll occurs during the later stages of seed ripening where the hull changes from green in colour to a yellow-brown (Ward *et al*., 1992). The changes in the amount of chlorophyll can be detected by chlorophyll fluorescence analysis (CF) and this has been linked to the maturity status of the seeds (Jalink *et al*., 1999; Costa *et al*., 2014; Hay *et al.,* 2015). However in rice there were highly variable differences between mean CF value and seed storage longevity and therefore it was not identified as a reliable tool to guide harvest time across diverse rice accessions (Hay *et al*., 2015).

1.6. The genetics of longevity

Rice genotypes originating from different ecogeographic regions vary in longevity (Ellis *et al.*, 1992; Kameswara Rao and Jackson, 1996a) due variation in the value of K_E (equation [2]) which subsequently results in differences in σ (Ellis *et al*., 1992). Temperate japonicas are inherently short-lived (Ellis *et al*., 1992) compared with the aus and boro rice varieties which show particularly great longevity (Kameswara Rao and Jackson, 1997). The predicted values of σ (years) are 294 and 729 for temperate japonica and indica varieties, respectively, when stored under typical genebank conditions (-20°C and 15% RH) (estimated using the seed viability constants in the Seed Viability module of the Seed Information Database [Royal Botanic Gardens Kew, 2008]). In recent years, DNA markers and quantitative trait loci (QTL) mapping have aided the identification of genomic regions which could potentially control quantitative traits such as longevity (Tanksley, 1993; Yano and Sasaki, 1997). True breeding lines such as double hybrid (DH), recombinant inbred lines (RILs) and backcross inbred lines (BILs) have been used, due to their genetic consistency over generations and environments, to map QTLs involved in seed storability in an attempt to understand the genetic factors controlling variations in seed longevity. Rice seed longevity QTLs have been identified on multiple chromosomes derived from crosses between Nipponbare (japonica) and Kasalath (indica) (Miura *et al.*, 2002);

between Akihikari (japonica) and Milyang23 (indica) (Sasaki *et al*., 2005); between JX17 (japonica) and ZYQ8 (indica) (Zeng *et al*., 2006); between Asominori (japonica) and IR24 (indica) (Xue *et al*., 2008); and between Nanjing35/USSr5 (japonica) and N22 (indica) (Lin *et al*., 2015). Several of these QTLs were located on chromosome 9 (*qLG-9*, Miura *et al*., 2002; *RC-9-2*, Sasaki *et al*., 2005; *qLS-9*, Zeng *et al*., 2006; *qRGR-9*, Xue *et al*., 2008; *qSSn-9*, Lin *et al*., 2015). The effects of *qLG-9*, *qLS-9* and *qRGR-9* QTLs were confirmed using chromosome substitution lines (CSSLs; (Miura *et al*., 2002; Zeng *et al*., 2006; Xue *et al*., 2008). The reports have shown that the QTL allele from the indica variety increased seed longevity in each population.

A more comprehensive characterisation of QTLs can be achieved by molecular cloning (Yano, 2001), fine-scale mapping and the use of near isogenic lines (NILs) (Lin *et al*., 2000; Monna *et al*., 2002). Sasaki *et al*. (2015) used fine-scale mapping which precisely located the QTL *qLG-9* to a 30kb region in the Nipponbare genome. NILs of *qLG-9* produced by marker assisted selection (MAS) showed greater longevity compared with the control lines. MAS is a helpful tool in identifying/selecting plants with target QTLs and can be used to transfer specific alleles located at target loci to improve the storability of cultivated rice varieties (Miura *et al*., 2002; Zeng *et al*., 2006; Xue *et al*., 2006; Saskai *et al*., 2015).

1.7. Drying seeds for genebank storage

The moisture content of mature cereal seeds at harvest depends on the ambient temperature and relative humidity and will determine the rate of viability loss (section 1.4). At equilibrium relative humidities (eRH) greater than 80% seeds are metabolically active (section 1.2; Figure 1.1) and are at risk of losing viability fast if there is not sufficient oxygen to allow for repair (Roberts and Ellis, 1989). In tropical climates where relative humidity (RH) conditions rarely fall below 80% seeds are harvested at high moisture contents, especially in the wet season. Hence there, but also under temperate conditions in wet periods, seeds may be harvested at moisture contents which are too high (>80% eRH) for safe storage and so require drying to reduce subsequent ageing (to be discussed further in section 1.9) and the probability of insect and fungal damage.

Below 80% there is a negative semi-logarithmic relationship between σ and eRH (Roberts and Ellis, 1989).

1.7.1. Genebank standards for drying orthodox seeds

The conditions and duration of drying depends on the physical characteristics of the seed and the harvest moisture content. For seeds intended for long-term storage, it is recommended that they are dried to a moisture content of between 3 and 7% fresh weight (depending on seed oil content) (FAO/IPGRI, 1994), as at this moisture level the rate of ageing is minimal and viability would therefore be maintained for a long period (section 1.4) (Ellis *et al*., 1989, 1992; Ellis and Hong, 2006). In order to achieve this moisture content it was further recommended that seeds should be dried immediately after harvest in a drying chamber set at 10-25°C and 10-15% RH (FAO/IPGRI, 1994). More recently this was modified to 10-25% RH and 5-20°C (FAO, 2013).

The recommended drying conditions were determined by combining the seed viability equations, developed and quantified from investigations with mature seeds, with equations describing the effect of environment on seed drying rate and seed temperature in constant-temperature heated-air dryers in contrasting species. A relatively low drying temperature was adopted to reduce the rate of ageing during the drying process, particularly when seeds still have high moisture content (Cromarty *et al*., 1982). Similarly the simplicity of the preferred conditions, i.e. a single environment for all orthodox species, provides the advantage that a single drying environment can accommodate many different species (Cromarty *et al*., 1982).

1.7.2. Alternative drying methods

1.7.2.1. Heated-air dryers

The drying rate and equilibrium moisture content is influenced by temperature, the relative humidity of the air, the volume of seed and air flow, as well as the interaction between them (Nellist and Hughes, 1973). Much of the previous research on drying and seed quality has been on cereals with the aim to dry quickly, usually at high temperatures

and/or relative humidities in large seed bulks, to a moisture content of 13-14% which is considered adequate for commercial seed storage and grain milling (Wiset *et al*., 2001).

Heated air drying uses high temperatures to dry seeds rapidly to a desired MC compared with low temperature drying whereby the objective is to control the RH as opposed to temperature so all the layers of seed reach equilibrium. Heated-air dryers, compared with sun drying have the advantage of being able to set suitable drying conditions at any time of the day or night and with an automatic temperature control the rate of seed drying can be maximised whilst avoiding over-heating or over-drying. Understanding heat and mass transfer is important in determining "safe temperatures" at which to dry seeds. The main factors affecting seeds response to high-temperature drying include: species and/or variety, moisture content, exposure time and the dryer design. Types of continuous flow dryers differ in how the seeds flow through the system in relation to the direction of the airflow (Nellist, 1980). The particular design used at IRRI in my research is described in detail in Chapter 2.

A cross-flow dryer is the most common design where the heated air moves across the path of the seeds (Nellist, 1980). Since the seeds are not mixed in these dryers, the seeds nearest the air inlet will dry quicker than the seeds furthest away. Therefore manual mixing is required to reduce the moisture content gradient through the layers of seeds and to allow equilibration. In a concurrent-flow dryer, the air flows in the same direction as the grain but the transfer of heat and moisture from the seeds closest to the inlet causes the air temperature to fall rapidly, stopping seeds from over-heating and ever reaching the same temperature of the air at the inlet. The final type of continuous flow dryer is the counter-flow design where the air flows in the opposite direction to the seeds. This is a very energy-efficient drying system as moisture from the increasingly wet seeds moves into the dry air therefore the temperature of the inlet air and the dry grain at exit is almost the same.

High temperatures are thought to be detrimental especially when seeds are at high moisture contents and during the later stages of drying when evaporative cooling can no longer suppress seed temperature (Cromarty *et al*., 1982). As a result, intermittent drying regimes are usually adopted when drying seeds down to levels safe for storage. The dryer

is usually integrated into a larger system which has a conveyer and tempering units to allow the seeds to continuously pass through the system and allowing moisture gradients to relax between high temperature exposure (active drying phases) which will increase the drying rate during a subsequent drying phase and help maintain seed quality (Mujumdar and Law, 2010).

Despite there being evidence to suggest high temperatures are a major cause of damage, cereals were shown to be particularly tolerant to high temperature drying. Certain other species such as seeds of onion (*Allium cepa*) are highly vulnerable: according to North (1948), air temperature should not exceed 32°C at 12–20% moisture content, or 21°C if moisture content is >20%. Tolerance to high temperature drying was confirmed in rice seed by Crisostomo *et al*. (2011) who showed that initial intermittent high temperature drying (45–50°C), before drying at 15°C/15% RH, resulted in greater subsequent seed quality than drying throughout at 15°C/15% RH. This was not the first time that an alternative drying regime has been reported to be better than the standard genebank drying room conditions (15°C/15% RH) for subsequent seed longevity or quality; Butler *et al*. (2009a) described how the longevity of seeds of foxglove (*Digitalis purpurea*) that were intentionally harvested prematurely, in the post-abscission (i.e. desiccation) phase of seed development, increased when seeds were dried at RH >15 %. Further to this it has been suggested from other studies that, in particular for tropical species, a low drying temperature may curtail late developmental processes in seeds and have a negative impact on subsequent longevity in storage (Hay, 1997). Therefore it may be better to dry harvested seeds of wild species under conditions which would be experienced by the developing seeds *in situ* (Probert *et al*., 2007).

1.7.2.2. Low-input alternatives

In resource-limited countries, particularly those in wet tropical regions, it may be difficult, due to missing apparatus and/or unreliable power sources, and costly to maintain a drying room of a sufficient size to efficiently dry large volumes of seeds (Somado *et al*., 2006). In such cases the use of desiccants (e.g. silica gel, lithium chloride, calcium chloride, molecular sieve and charcoal) are common low input alternatives (Probert, 2003). However, often, they are not able to reduce seed moisture content sufficiently

(Justice and Bass, 1978). A recent study examined the potential of aluminium silicate ceramics (a form of molecular sieve) to dry seed to low moisture contents required for storage (Hay *et al*., 2012; Hay and Timple, 2013). With a greater affinity for water, particularly at low humidity, they are capable of more rapid drying compared with other desiccants. In order to dry freshly harvested seeds which have a moisture content of 22-24% to the recommended 6-7% moisture content prior to storage, a seed to bead ratio of 1 (beads with a capacity of 16%) would be required (Hay *et al*; 2012; Hay and Timple, 2013). Furthermore, if desiccants are not readily available, sun drying which is comparable to silica gel in its drying potential (4-5% moisture content) and subsequent seed viability has proved an effective and affordable method for drying seeds intended for short term storage, i.e. for farm-saved seed (Somado *et al*., 2006).

1.8. Desiccation tolerance and protective mechanisms

Desiccation tolerance is the ability of a seed to survive the removal of almost all cellular water without irreversible damage such that it can germinate fully and rapidly on rehydration. It allows the seed to remain stable for long periods by suspending its metabolic activity and enabling it to survive conditions of environmental stress (Leprince and Buitink, 2010). In seeds which undergo maturation drying, desiccation tolerance is acquired around the time of mass maturity (when the abscission layer forms and the seeds moisture status is independent of the parent plant) but can be influenced by the seed production environment and also genotype (section 1.5). Desiccation tolerance reached its maximum 22 and 14 days after mass maturity (during the maturation drying phase of seed development) in japonica rice grown in cool and warm regimes, respectively (Ellis and Hong, 1994).

Maturation drying corresponds to drought stress in seeds and is thought to prepare the embryo for desiccation and the ability to germinate after desiccation by inducing various cellular and biochemical events including the synthesis of late embryogenesis abundant (LEA) proteins, heat shock proteins (HSPs), accumulation of the raffinose family oligosaccharides and the activation of antioxidant defence-mechanisms (Vertucci and Farrant, 1995; Kermode, 1997; Bailly *et al*., 2004; Buitink and Leprince, 2008; Leprince and Buitink, 2010). Seeds which do not undergo maturation drying, i.e. recalcitrant seeds,

are unable to survive desiccation as the corresponding stress reaction is not induced and therefore the protective mechanisms do not operate (Radwan *et al*., 2014).

1.8.1. Protective proteins

Heat shock proteins (HSPs) are stress related proteins and act as chaperones that protect proteins from unfolding (Hundertmark *et al*., 2011). Late embryogenesis abundant (LEA) proteins are a family of hydrophilic proteins which result from some of the most differentially expressed and highly up-regulated genes expressed in response to water shortage (Hundertmark *et al*., 2011; Leprince and Buitink, 2010; Radwan *et al*., 2014), protecting cellular components from dehydrative stress by stabilizing membranes and protecting proteins from aggregation. The products of these genes are abundant during the post-abscission phase of embryogenesis and they disappear during germination.

There are two main groups of LEA genes which are seed specific and encompass LEA 5 and seed maturation proteins which have also been linked specifically to desiccation tolerance in *Medicago truncatula* (Boudet *et al*., 2006). LEA genes are redundant (the existence of more than one gene performing the same role) making it difficult to isolate their exact role in desiccation tolerance, however studies have reported that their over expression results in enhanced desiccation tolerance, an increase in seedling growth rate and accumulation of other protective molecules such as proline, polyamine, sugars and peroxidase (Figueras *et al*., 2004; Roychoudhury *et al*., 2007; Tunacliffe and Wise, 2007; Liu *et al*., 2009). It is therefore not surprising that desiccation tolerance and seed longevity are thought to be linked. Spatial and temporal expression profiles of LEA polypeptides in *Medicago trunculata* were obtained during maturation (including final maturation drying) where longevity and desiccation tolerance are acquired, and showed that five LEA proteins, representing 6% of the total LEA proteins, accumulated upon the acquisition of desiccation tolerance, after which there was a 30-fold increase in longevity and an accumulation of a further four other LEA proteins which accounted for 35% of the total LEA in mature seeds (Chatelain *et al*., 2012). The differences in the accumulation profiles suggest the LEA proteins have differing roles in seed physiological traits (Chatelain *et al*., 2012).

One class of LEA proteins, dehydrins, are produced in response to any dehydrative force (temperature, drought, salinity) and their abundance is widespread in cells acting upon the nucleus and cytoplasm as an intracellular stabiliser (Campbell and Close, 1997). Dehydrins, together with other protective mechanisms are relevant to desiccation tolerance. In some species such as cotton dehydrin accumulation occurs late in embryogenesis after abscission, whereas in rice dehydrin synthesis can be detected before the seeds have acquired desiccation tolerance and they continue to accumulate thereafter in parallel with an increase in dry weight (Still *et al*., 1994). This late accumulation of dehydrin proteins provides evidence that they are not just required for desiccation tolerance but that they also play a role in seed quality and longevity (Galau *et al*., 1991; Ellis *et al*., 1993a). It is thought therefore that maturation drying, which induces the stress response and therefore the triggering of protective mechanisms, is crucial for desiccation tolerance as well as the storability of seeds. Orthodox seeds retain their viability in storage and synthesise dehydrins in response to maturation drying. Despite recalcitrant plants expressing some dehydrins (Finch-Savage *et al*., 1994; Farrant *et al*., 1996; Han *et al*., 1997; Panza *et al*., 2007; Šunderlíková *et al*., 2009), maturation drying is absent during seed development and their seeds are unable to be stored. Therefore it is likely that certain types of dehydrins are constitutively expressed, acting as some kind of housekeeping genes (Hara *et al*., 2011) or they could be involved in other developmental processes such as germination (Gumilevskaya and Azarkovich, 2010). Seeds of *Arabidopsis* with lower levels of dehydrin expression showed reduced longevity in storage and a reduction in germination when exposed to salt stress compared with wild types, emphasising their role against seed deterioration (Hundertmark *et al*., 2011).

1.8.2. Carbohydrates

The accumulation of carbohydrates and changes in the soluble sugar ratio in dry orthodox seeds have been correlated with the development of desiccation tolerance (Steadman *et al*., 1996; Peters *et al*., 2007; Zhu *et al*., 2007; Moore *et al*., 2009). The oligosaccharide to sucrose ratio was significantly higher in orthodox seeds (>0.143) than recalcitrant (<0.143) seeds and therefore generally is a good indicator of seed storage category (Steadman *et al*., 1996). Metabolic engineering studies which suppress or over-express trehalose in plants affects their desiccation sensitivity and tolerance to drought, salt,

freezing and high temperatures (Leprince and Buitink, 2010). During development and dehydration, glucose, fructose and maltose reduce to undetectable levels while trehalose, sucrose and oligosaccharides – mainly those from the raffinose family (RFO) – accumulate (Zhu *et al*., 2007), aiding the stabilization of intracellular glasses by increasing the viscosity of the cytoplasm and the glass-liquid transition temperature (section 1.8.4) (Buitink and Leprince, 2004; Hoekstra *et al*., 2001). As longevity is related to the molecular mobility of the cytoplasm (Leopold *et al*., 1994; Sun, 1997; Buitink *et al*., 1998a, b), the oligosaccharide to sucrose ratio has also been linked to the storability of seeds (Horbowicz and Obendorf, 1994; Lin and Huang, 1994; Bernal-lugo and Leopold, 1995; Steadman *et al*., 1996). Carbohydrates also act as surfactants, polymers or salts which limit protein aggregation and protect the structure and function of desiccated phospholipids enhancing membrane protection (Caffrey *et al*., 1988; Wang, 2000; 2005) and maintaining membrane integrity (Corbineau *et al*., 2000).

1.8.3. Antioxidants

In plants one of the main forms of damage as a result of environmental stresses is oxidative. Transcriptomics show many of the genes related to antioxidant defence are upregulated in desiccation-tolerant tissues and that their over-expression, e.g. of glutathione S-transferase, enhances seedling growth under numerous stress conditions (Roxas *et al*., 2000). Oxidative stress occurs due to the accumulation of reactive oxygen species (ROS) such as aldehydes which are toxic to cells and are the main contributors to mutagenesis and cellular ageing (Kranner *et al*., 2010). Hydrogen perioxide (H_20_2) is considered the most damaging of ROS due to its stability at biological PH and ability to cross membranes (Bienert *et al*., 2006). Protection from ROS-induced damage by antioxidants (superoxide dismutases, tocopherols, glutathione, catalase and peroxidases) can increase resistance to seed ageing. Aldehyde dehydrogenases play a role in the detoxification of aldehydes and catalase functions to break down hydrogen peroxide which limits germination in low quality seeds (Shin *et al*., 2009; Kibinza *et al*., 2011). Oxidative damage increases in aged seeds, coupled with a reduction in antioxidant defences (Bailly *et al*., 1996; Kibinza *et al*., 2006; Kranner *et al.,* 2006) and it is this imbalance which defines oxidative stress.

1.8.4. Glass transition

Glass transition is the process by which the cytoplasm of a cell enters a highly viscous glassy state during drying or a change in temperature, enabling the seed to survive desiccation for long periods of time (Buitink and Leprince, 2008). The water content at which the transition occurs is dependent upon the temperature, known as the glass transition temperature (T_g); so at a higher cellular water content glass transition occurs at a lower drying temperature than when the cells have a lower water content (Perdon *et al*., 2000; Sun *et al*., 2002). The physical, chemical and biochemical changes which occur during this transition can explain trends in the drying rate and the crystalisation, shrinkage, collapse and fissuring of cells (Cnosson *et al*., 2002). Seeds dry at a faster rate when they are exposed to temperatures above T_g as moisture diffusion is much higher (Perdon *et al*., 2000; Cnosson *et al*., 2002). However drying seeds at high temperature/low RH conditions which result in seeds with a low equilibrium moisture content can cause cellular damage as the high rate of moisture diffusion at the surface causes the outer cells of the seed to transit from a rubbery to glassy state which then reduces the subsequent drying rate and hinders glass transition at the centre. This can be minimised by a tempering period in between drying phases which allows the moisture content gradient of the seeds to relax (section 1.7) (Cnosson *et al*., 2002).

Intracellular glasses were suggested to confer desiccation tolerance as seeds which are desiccation sensitive have a lower T_g compared with desiccation tolerant-seeds (Williams and Leopold, 1995), however they are not void of glasses. It is important to note that the water content at which desiccation–sensitive seeds (recalcitrant) die occurs before glass transition indicating that the formation of glasses is paramount to survival in the dry state but does not confer desiccation tolerance *per se* (Buitink *et al*., 1996; Buitink and Leprince, 2008). A glass is essentially a highly viscous liquid in which molecular diffusion and therefore the probability of a chemical reaction occurring is greatly reduced (Slade and Levine, 1994). The water content of the seed is reduced during drying and so the cytosolic compounds become supersaturated leading to an increase in the cohesive forces between molecules which reduces molecular mobility in the cytoplasm and slows down degradative reactions, i.e. ageing (section 1.9). However, the molecules in a glassy state are not completely restricted in their movement explaining why seeds still age as

deteriorative processes can still occur but just at a slow rate (Buitink and Leprince, 2008). It is this reduced mobility which is responsible for the extreme longevity that orthodox seeds can achieve in dry storage.

The stabilising effect that glasses have on the macromolecular and structural components during storage provides further evidence of their essential role in seed longevity. Proteins showed no sign of aggregation or denaturation after 28 days in dry storage and appeared to remain stable even after several years in open storage (Williams and Leopold, 1995). From the previous section we know that during maturation seeds accumulate non-reducing sugars and LEA proteins which are thought to interact together in the formation of the glassy state. Research has shown that sugars appear to be tightly associated with the protein molecules and that the strength of the hydrogen bonds in a protein-sugar mix is far greater than that of a sucrose glass alone (Walters *et al*., 1997; Wolkers *et al*., 2001).

1.9. Seed ageing and repair

As seeds age they lose vigour making them more sensitive to environmental stresses upon germination, and eventually they will lose the ability to germinate completely i.e. they become non-viable. The rate of ageing is dependent on water content and temperature at which seeds are stored (Roberts, 1973; Ellis and Roberts, 1980a, b) however even when manipulating these factors to a point where longevity is optimised, deterioration can never be inhibited and ageing still occurs, just at a very slow rate (see previous section; Priestley, 1986). Seed longevity and germination are negatively correlated with relative humidity and high storage temperature (section 1.4) as shown in viability studies where seeds are stored under less than ideal conditions (Ellis and Roberts, 1980a, b; Ellis *et al*., 1986; 1988; 1989) or when storage conditions fail to be maintained by the use of inadequate storage containers (Gomez, 2006). Germination tests are the most used method to assess seed viability, however molecular markers (RAPD analysis, quantification of redox activity of non-enzymatic antioxidant compounds and gene expression profiling) have been identified in species which show inherent variability in seed longevity and could therefore be used as tools to show interspecific variation in longevity (Doña *et al*., 2013).

Lipid peroxidation and free radicals are major contributors to seed deterioration due to loss in membrane integrity, reduced energy metabolism, protein carbonylation, impairment of RNA and protein synthesis, and DNA degradation (Corbineau, 2012). This damage occurs in the quiescent state and in cycles of desiccation-rehydration (Waterworth *et al.*, 2010). DNA repair and antioxidant activities (scavenging of reactive oxygen species [ROS]) must occur on imbibition in order for seeds to "recover" from the dry state and optimise their germination performance (Waterworth *et al*., 2010; Dona *et al*., 2013). DNA damage (single strand breaks, double-strand breaks and damage to bases) inhibits effective transcription and replication and arises as a consequence of ROS and breaks which are incurred during DNA replication (Waterworth *et al.*, 2015). Studies have shown that repair occurs early in imbibition with DNA synthesis being observed several hours before cells enter the S-phase (synthesis phase where DNA is replicated) (Elder and Osbourne, 1993). The eukaryotic mechanisms of DNA repair are largely conserved but recent work on *Arabidopsis thaliana* characterised the plant specific DNA ligase (VI) which was shown to be a major determinant in seed quality and longevity; mutants showed an increased sensitivity (delayed germination and reduced seedling vigour) to controlled seed ageing and low temperature germination stress (Waterworth *et al*., 2010).

1.9.1. Priming and rehydration

Slow, asynchronous germination arises as a result of seed ageing (Matthews, 1980). Invigoration treatments by holding seeds at an elevated MC (hydropriming, osmopriming, aerated hydration and humidification) are known as priming and can improve seed quality by increasing the rate and uniformity of germination, attributed by initiation of germination processes, in particular repair (Heydecker *et al*., 1973; Burgass and Powell, 1984; Bailly *et al*., 2000), so that seeds are ready to germinate when sown (Soeda *et al*., 2005). Despite the immediate improvement in seed performance following priming there are contrasting reports of how such a treatment affects subsequent seed longevity following desiccation. For example, lettuce (Tarquis and Bradford, 1992) and pepper (Saracco *et al*., 1995) seeds showed a reduction in longevity post-priming whereas carrot and tomato seeds have both shown an improvement and reduction in longevity depending on the storage, type of hydration treatment and duration (Powell *et al*., 2000 and references therein).

According to the seed viability equations (Ellis and Roberts 1980a; Ellis, 1991) differences in storage longevity post-priming arise as a result of differences in the initial quality of the seed lot (K_i). Improvements are most apparent in deteriorated seed lots due to their requirement for repair prior to germination, whereas high quality seed lots become "over-advanced" after an invigoration treatment meaning they have entered a stage where they have lost desiccation tolerance and therefore become susceptible to drying (Powell *et al*., 2000). Harvesting seeds before they have reached peak maturity will result in seeds which have not reached maximum quality and could therefore compromise the initial quality of the seed lot (section 1.5). Recent research by Butler *et al*. (2009a) showed that priming prematurely-harvested seeds of *Digitalis purpurea* before storage can improve their subsequent longevity by allowing for the continuation of maturation *ex planta*. However, priming did not improve the longevity of the seeds within the population which had already acquired maximum longevity. Another study by Butler *et al*. (2009b) showed that subsequent seed longevity could also be improved by priming at intervals during storage, suggesting that the lower vigour seeds, i.e. those which have a higher level of deterioration and therefore on the cusp of becoming non-viable, are somewhat "rejuvenated" by the rehydration treatment. It was also shown that the storage potential could be increased further by additional cycles of priming during air-dry storage. These two studies suggest that priming and re-drying can act as a maturation or repair treatment of aged or immature seeds (Butler *et al*., 2009a, b).

The effect of priming on subsequent seed storage longevity can be influenced by post-priming treatments as research has shown heat shock (Bruggink *et al*., 1999), mild water stress or slow drying (Gurusinghe and Bradford, 2001) can restore desiccation tolerance in some species by inducing the synthesis of LEA and/or heat shock proteins which confer as protective mechanisms beneficial to storage longevity (section 1.8).

1.10. Thesis aims and objectives

The overall aim of the thesis was to examine the current recommended protocol for drying seed of *Oryza sativa* L. intended for long-term storage and to determine whether an alternative drying method could further improve subsequent seed longevity in storage.

The research focussed on determining the optimal drying conditions which would maximise rice seed storage longevity with the following objectives:

1. Evaluate the effects of high-temperature drying on subsequent rice seed longevity.
2. Determine the optimum combinations of temperature, relative humidity (RH), and duration to dry rice seeds for long-term conservation and whether these optima vary with genotype and/or maturity stage.
3. Investigate the influence of pre-harvest environment on rice seed quality and longevity.
4. Investigate the potential of post-harvest invigoration treatments in improving subsequent rice seed storage longevity.
5. Investigate dehydrin expression during rice seed drying.

CHAPTER 2

THE EFFECT OF HIGH TEMPERATURE DRYING ON SEED LONGEVITY IN RICE (*ORYZA SATIVA* L.)

2.1. Introduction

Rice (*Oryza sativa* L.) shows orthodox seed storage behaviour and the largest and most diverse collection (over 125,000 accessions) is stored in the International Rice Genebank (IRG) at the International Rice Research Institute (IRRI) in the Philippines (Chapter 1). Although seeds remain viable for many decades under genebank storage conditions, over time their viability will decline and regeneration is required to maintain genetic integrity (Cromarty *et al*., 1982; Rao *et al*., 2006). It is therefore important to determine the drying method which could prolong seed storage longevity as longevity underpins the selection of viability re-test intervals and regeneration and recollection strategies (Probert *et al*., 2009). An underestimation of loss in viability and thus longevity, will lead to an accumulation of genetic damage and an overestimation will lead to unnecessarily rapid depletion of genetic stocks (FAO, 2013).

Recommendations for the management of genebank accessions emphasize the importance of initial seed drying to extend the subsequent longevity of seeds during storage (Cromarty *et al*., 1982; FAO/IPGRI, 1994; Rao *et al*., 2006; FAO, 2013), but so far there has been no critical evaluation (impact on subsequent quality or longevity of the seeds) of the conditions that are actually used by genebanks, for any particular species. Rather, the recommendation to dry seeds in genebanks to low (3-7%) moisture contents using cool temperatures combined with very low relative humidity (Cromarty *et al*., 1982) was driven by the requirement for a single, simple, safe procedure for diverse species in all locations worldwide. Despite this, there are various methods for drying seeds and the effect of a specific drying procedure on subsequent storage longevity varies between species and the initial moisture content of the seed. There has been some evidence to suggest that the conventional dryroom held at 15°C/15% RH may not be optimal for the subsequent storage longevity of some species (Chapter 1; section 1.7.1.).

Cultivated Asian rice is the most important food crop of the developing world, being a staple for more than half the global population. It is grown in tropical and semi-tropical regions where the humidity is high and there are often prolonged periods of precipitation which results in the seeds having a high moisture content (>80% RH) at harvest. Based on the evidence that seed quality can be enhanced in seeds harvested prematurely by drying at temperatures close to the natural ambient conditions, a two-stage drying procedure has been recommended for seeds of the dry tropics (Hay, 1997; Probert *et al*., 2007).

2.1.1. Objectives and Hypothesis

The aim of this study was to evaluate the effects of initial high temperature drying for different periods on subsequent rice seed longevity compared with low temperature drying with a specific objective to determine which of the drying regimes provided the greatest storage longevity for the 20 rice accessions used in this study.

H_0: Drying seeds using a two-stage high temperature drying method will have no effect on the subsequent storage longevity of rice seeds compared with the existing genebank drying protocol.

2.2. Materials and Methods

2.2.1. Plant material

Seeds of 20 rice accessions representing five variety groups (aus, aromatic, indica, and temperate and tropical japonica; McNally *et al*., 2009) were sampled from the active collection and held at 50°C for 5 days to break dormancy. They were sown in Block UF on the IRRI Experimental Station (ES) on 23rd November 2012 and transplanted into blocks UB 3-4 (14° 8’ 56.4060”N, 121° 15’ 56.286”W) on 18th December 2012. Normal rice production practices and routine plant protection measures were followed (Reaño *et al*., 2008; Appendix 2.1). Seed lots were harvested between March and April 2013, as close to 35 days after 50% anthesis (DAA) as possible (Table 2.1), as recommended by Kameswara Rao and Jackson (1996a, b, c). The mid-flowering date (50% anthesis) is the midpoint

Table 2.1. Information of the 20 rice (*Oryza sativa*) seed lots used in the study showing date of harvest, the duration from 50% anthesis to harvest date (DAA), seed moisture content (MC) and equilibrium relative humidity (eRH) at harvest.

Accession	Variety name	Variety group [1]	Harvest date 2013	DAA (days)	MC (s.e.) (% f.wt.)	eRH (%)
IRGC 117264	Azucena	tropical japonica	19 Mar	24	22.4 (0.42)	95.9
IRGC 117265	Dom-sufid	aromatic	11 Mar	24	22.7 (0.09)	96.1
IRGC 117266	Dular	aus	19 Mar	37	18.9 (0.11)	92.9
IRGC 117267	FR 13 A	aus	04 Apr	36	16.8 (0.22)	88.4
IRGC 117268	IR64-21	indica	02 Apr	44	14.9 (0.04)	74.4
IRGC 117269	Li-Jiang-Xin-Tuan-Hei-Gu	temperate japonica	11 Mar	38	26.8 (0.36)	96.9
IRGC 117270	M 202	temperate japonica	14 Mar	38	23.4 (0.23)	97.4
IRGC 117271	Minghui 63	indica	15 Apr	33	16.7 (0.06)	91.6
IRGC 117272	Moroberekan	tropical japonica	10 Apr	35	17.7 (0.05)	91.6
IRGC 117273	N 22	aus	05 Mar	29	20.8 (0.12)	91.9

Accession	Variety name	Variety group [1]	Harvest date 2013	DAA (days)	MC (s.e.) (%, f.wt)	eRH (%)
IRGC 117274	Nipponbare	temperate japonica	05 Mar	40	28.9 (0.31)	96.0
IRGC 227275	Pokkali	indica	27 Mar	37	13.7 (0.02)	69.8
IRGC 117276	Sadu-cho	indica	27 Mar	26	13.2 (0.09)	67.8
IRGC 117277	Sanhuangzhan no 2	indica	10 Apr	38	16.2 (0.04)	86.5
IRGC 117278	Swarna	indica	04 Apr	36	18.2 (0.28)	91.7
IRGC 117279	Tainung 67	temperate japonica	15Apr	45	17.3 (0.08)	86.7
IRGC 117280	Zhenshan 97B	indica	14 Mar	38	23.3 (0.24)	96.1
IRGC 117281	Aswina	indica	25 Mar	48	19.3 (0.14)	94.6
IRGC 117282	Cypress	tropical japonica	25 Mar	41	18.8 (0.04)	92.8
IRGC 117283	Rayada	aus	02 Apr	34	16.5 (0.16)	83.8

[1] Variety group taken from McNally *et al*.,(2009).

between the initial date of flowering and completion. This range varies between accessions due to developmental variation, the effect of the environment and the interaction between them, which determines, for example, an accession's photoperiod sensitivity.

2.2.2. Post-harvest treatments

Immediately after harvest, the seeds were threshed and blown to remove debris. A sample taken at random from each accession was placed inside a 3.2 ml sample holder in the measuring chamber of an AW-D10 water activity station used in conjunction with a HygroLab 3 display unit (Rotronic South East Asia Pte. Ltd., Singapore). The temperature and equilibrium relative humidity (eRH) was measured at room temperature once the reading had stabilised, after 20-40 min. Seed moisture content (MC; fresh weight basis) was determined using three 5 g samples from each accession and the high-temperature oven method of ISTA (2013; Appendix 2.2). The samples were ground in a Krups 75 coffee grinder and weighed before being placed at 130°C for 2 h. The samples were removed from the oven and placed over silica gel for 1 h to cool before reweighing.

2.2.3. Seed drying

The flat-bed batch dryer (BD) used at IRRI is a locally fabricated shallow layer dryer approximately 2 m wide and 3-4 m long with a perforated base (Appendix 2.3). Heated air (45°C) is blown into the chamber and forced upwards through the grains which are spread in a thin layer over the perforated base. A simple axial flow fan provides the air and a kerosene burner provides the heat. The decision to dry seeds at 45°C was based on the results of a preliminary small-scale study carried out by Crisostomo *et al*. (2011) which showed the physiological quality of rice seeds could improve after drying at 45-50°C in a batch dryer. This encouraged the further testing of high temperature drying of rice seeds which prior to the release of the genebank standards (1994) was a standard practice at IRRI.

Seeds from each accession were divided into a maximum of seven 300 g samples (depending on quantity available) and placed into 0.2 × 0.33 m (L × W) nylon mesh bags (1

mm-diameter holes). They were stored inside sealed 0.6 × 0.3 × 0.132 m (L × W × H) electrical enclosure boxes (ENSTO Finland Oy) at room temperature (approximately 21.5°C) overnight to reduce drying. The following morning (0800 hrs), one sample was immediately placed in the genebank dryroom (DR; 15°C/15% RH) and the remaining samples (up to six) were placed into the BD at the IRRI ES. The change in weight, and eRH of the DR samples was measured daily at 0800 hrs. The temperature and eRH was measured in the DR using a portable hygroclip SP05 water activity probe used in conjunction with a Hygropalm AWI display unit (Rotronic South East Asia Pte. Ltd., Singapore). Seeds in the BD were exposed to 8 h heated-air drying (0800–1600 hrs) per daily (24 h) cycle. At the end of this 8 h period one sample was removed and re-weighed before a small subsample (approximately 15 g) was taken to determine MC, as described in section 2.2.2. Seed eRH was measured either by using the portable water activity probe or the AW-D10 water activity station. The remainder of the seeds of this sample was transferred within the nylon mesh bag to the DR, where all seed samples completed drying (i.e. equilibrating to 15°C/15% RH; resulting in a MC of 6.1%). The remaining 300 g samples were sealed inside 0.6 × 0.3 × 0.132 m (L × W × H) electrical enclosure boxes at room temperature overnight (1600– 0800 hrs) before they were returned to the BD for the next day's 8 h heated air treatment period. Prior to each BD cycle the eRH of the seed samples was also recorded (Appendix 2.4). Each accession provided different seed samples that had been dried using the BD for up to 6 daily cycles. This protocol resulted in all samples being dried to the same MC but individually differing in the number of daily heated-air drying cycles in the BD (0-6 days). Once equilibrated in the DR (which required up to 14 days), the eRH of the seed samples was first checked using the portable water activity probe. Seeds were then manually sorted, discarding any infected, empty or immature seeds before sealing inside sealed 0.17 × 0.12 m (L × W) laminated aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until experimental seed storage began in June 2013.

2.2.4. Seed storage

Seeds of each treatment combination (accession [20] × drying treatment [7]) were removed from cold storage (2-4°C) and equilibrated to room temperature (21.5°C) before opening. Each sample was split into 5 g subsamples (maximum of 29) and placed into 30

mm-diameter open Petri dishes and held over a non-saturated LiCl solution (60% RH) in a sealed 0.6 × 0.3 × 0.132 m (L × W × H) electrical enclosure box for 7 days at 21.5°C. The RH provided by the solution was checked at weekly intervals, using the water activity-measuring instrument described above, and the bulk solution was adjusted if necessary by adding distilled water, stirring and allowing equilibration before re-checking RH (Hay *et al*., 2008).

Seed MC reached equilibrium with this environment after 7 days. Four 5g subsamples from each treatment combination were taken and seed eRH measured. Three of these subsamples were used to determine MC and the fourth to estimate initial ability to germinate (prior to experimental storage). The remaining 5 g subsamples were each sealed inside 0.12 × 0.09 m (L × W) laminated aluminium foil packets (Moore and Buckle) and then placed in an incubator at 45°C. One packet per treatment combination was removed at 1-to-3 day intervals up to 45 days for germination testing (see below). For some seed lots, where viability was lost before 45 days, sampling was discontinued earlier; for a few seed lots, later samples were at longer intervals due to an unexpectedly slow rate of viability loss. At 21 days (mid-storage) and at the end of the storage experiment, MC was determined using three additional 5 g packets of seeds each time.

2.2.5. Seed germination

Ability to germinate was estimated with four replicates of 30 seeds, sown on two layers of Whatman No. 1 filter paper wetted with 7.5 ml distilled water in 90 mm-diameter Petri dishes. They were incubated at constant 30°C (12 h light and 12 h dark cycle). Germination was scored after 2, 3, 4, 5, 7 and 14 days. Non-germinated seeds were dehulled and tested for an additional 7 days before final scoring. Seeds were scored as germinated when the radicle had emerged by at least 2 mm.

2.2.6. Statistical analyses

Seed drying curves were fitted using a modified version of the Page equation (equation [6]) in GenStat for Windows, Version 15 (VSN International Ltd., Oxford, UK) to show the

relationship between loss in seed moisture content (converted to water content, WC) over time, as follows:

$$\mathrm{WC_t} = (\mathrm{WC_i} - \mathrm{WC_e})\mathrm{e}^{-\mathrm{kt}^{\mathrm{a}}} + \mathrm{WC_e}$$

[6]

where WC_t is water content (g g^{-1} dry weight) at time t, WC_i is the initial water content and WC_e is the equilibrium water content. t is drying time (days) and k, a are equation constants (Raj *et al*., 2010).

Seed survival curves (ability to germinate after different periods of air-dry storage in the experimental regime) were fitted by probit analysis using GenStat thereby fitting the following equation to estimate the period (days) for viability to fall to 50% (p_{50}), K_i and σ:

$$v = K_\mathrm{i} - p/\sigma$$

[1]

where v is the viability (ability to germinate) in normal equivalent deviates (NED) of a seed lot stored for period p (days), K_i is the initial viability (NED) and σ (days) is the standard deviation of the normal distribution of seed deaths in time (Ellis and Roberts, 1980a). The estimate of p_{50} was used as the measure of longevity. For those accessions also showing loss in dormancy during (early) storage, i.e. after-ripening, a probit combined loss in dormancy and loss in viability model was applied:

$$g = (K_\mathrm{d} + \beta_1 p) \times (K_\mathrm{i} - (p/\sigma))$$

[7]

where g = ability to germinate (NED), p, K_i and σ are as in equation [1], K_d is the initial proportion of non-dormant seeds (NED), and β_1 is the probit rate of loss of dormancy (Kebreab and Murdoch, 1999). Equation [7] was fitted using the FITNONLINEAR directive in GenStat. Probit analysis was carried out for all seed lots within an accession simultaneously, fitting the full model (different estimates for all parameters) and reduced models in which one or more parameters were constrained to a common value for all seed lots. An approximate F-test was used to determine the best model.

The difference in longevity (p_{50}) between the highest value from the BD treatments (BD p_{50}) and the DR treatment (DR p_{50}) was calculated as a proportion of the DR p_{50} according to the equation: 100 × ((BD p_{50}-DR p_{50})/DR p_{50}). This use of this calculation continues throughout the entirety of this thesis. Split-line regression analysis was used to explore the relationships between different variables and relative difference in longevity. A modified version of the D'Arcy-Watt equation (D'Arcy and Watt, 1970) was used to describe the relationship between seed MC (converted to water content, WC, as a proportion of dry weight) and eRH, as follows (also fitted using the FITNONLINEAR directive in GenStat):

$$\mathrm{WC} = y + c(\mathrm{eRH}/100) + \frac{k'k(\mathrm{eRH}/100)}{1 + k(\mathrm{eRH}/100)} \quad [8]$$

Where *c*, *k* and *k'* are parameters that relate to the number and strength of weak (*c*) and multi-molecular (*k*, *k*') water-binding sites. Since there was little data at very low water contents, the part of the original equation relating to strong water binding sites was substituted by *y*, i.e.

$$y = \frac{K'K(\mathrm{eRH}/100)}{1 + K(\mathrm{eRH}/100)} \quad [9]$$

The WC values provided by equations [7] and [8] were transformed to fresh weight basis for presentation.

2.3. Results

2.3.1. Seed drying

The pattern of loss in moisture for all seed lots in both drying regimes showed the expected trend of a negative exponential before approaching an asymptote (Figure 2.1). Seeds immediately placed in the DR did not dry as rapidly over the first day as those initially placed in the BD, with the exception of accessions IRGC 117268, -72, -75, -76, -77

Figure 2.1. Drying curves for seed of 20 rice accessions. Initial moisture content (MC; % fresh weight) and equilibrium relative humidity (eRH) was measured before freshly harvested seeds were placed either in the dryroom (DR) or the flat-bed dryer (BD; 8 h day). The eRH of DR seeds were measured in the DR (maintained at 15°C/15%) and MC was estimated based on the initial moisture content and change in sample weight. The eRH of the BD seeds were measured at room temperature (approximately 21°C) and the MC was determined using the high-temperature oven method (ISTA, 2013). The values displayed are the mean eRH or MC ± s.e. (too small to show; Appendix 2.5). The solid red lines are the results of fitting a modified version of the Page equation (equation [6]) to the WC data. The water content (WC; g g^{-1} dry weight) values were transformed in fresh weight basis (%) for presentation.

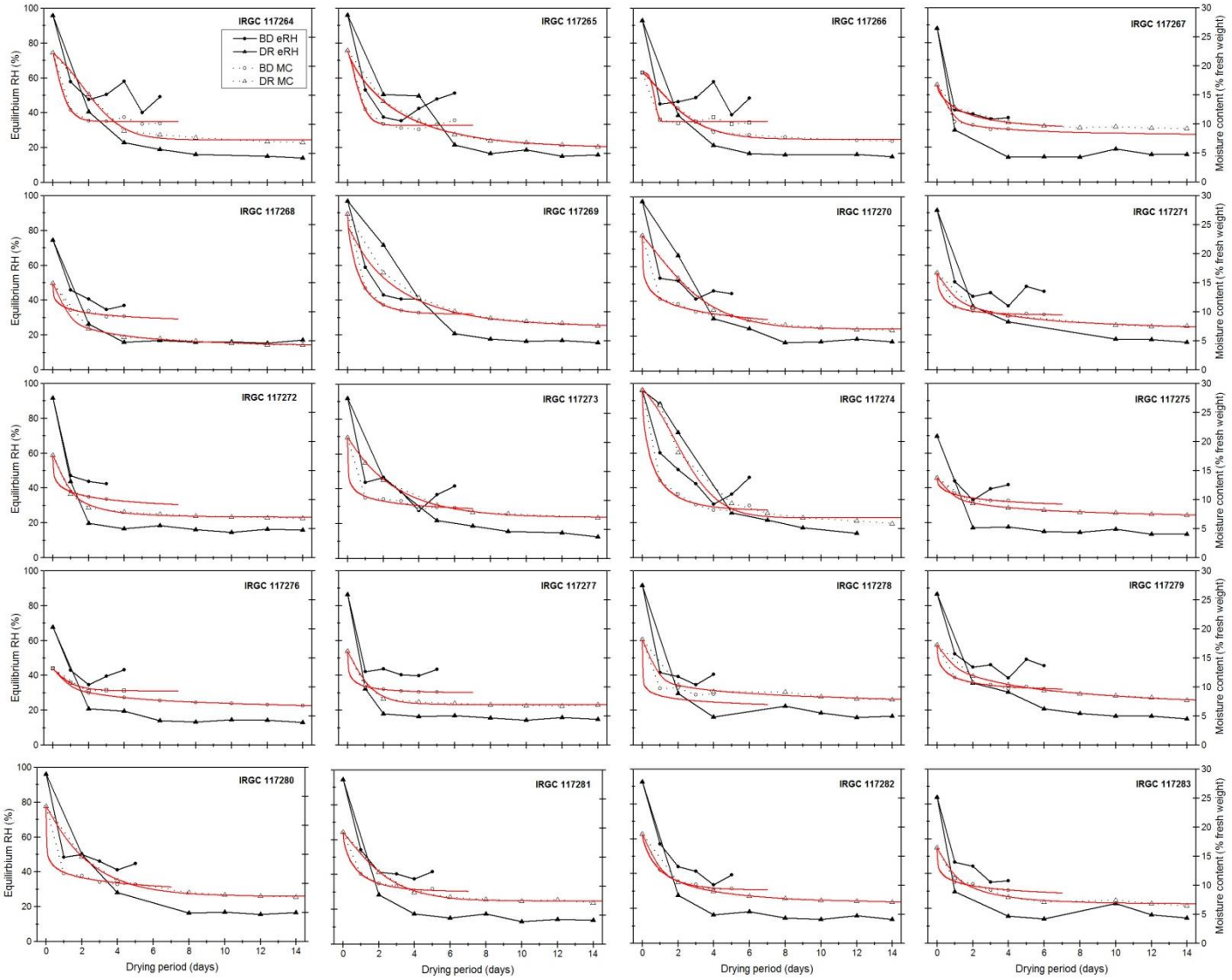

IRGC 117264
BD eRH
DR eRH
BD MC
DR MC
IRGC 117265
IRGC 117266
IRGC 117267
IRGC 117268
IRGC 117269
IRGC 117270
IRGC 117271
IRGC 117272
IRGC 117273
IRGC 117274
IRGC 117275
IRGC 117276
IRGC 117277
IRGC 117278
IRGC 117279
IRGC 117280
IRGC 117281
IRGC 117282
IRGC 117283
Equilibrium RH (%)
Moisture content (% fresh weight)
Drying period (days)

Table 2.2. The results of fitting the modified Page's equation (equation [6]) to show the loss in moisture (% f.wt.) over time (days) when seeds of the 20 rice accessions were dried either in the dryroom (DR) or or the flat-bed dryer (BD; 8 h day). The model was fitted to the water content (WC ; g g^{-1} dry weight) but was transformed to moisture content (MC; % fresh weight) in figure 2.1, therefore both the initial and equilibrium water contents (WC_i and WC_e) and moisture contents (MC_i and MC_e) are shown along with the constants K and a.

Accession	Type of drying	WC_i (s.e.) (g g^{-1} d.wt.)	WC_e (s.e) (g g^{-1} d.wt.)	MCi (% f.wt.)	MCe (% f.wt.)	K (s.e) ($days^{-1}$)	a (s.e)
IRGC 117264	DR	0.288 (0.006)	0.079 (0.004)	22.38	7.33	0.251 (0.055)	1.587 (0.231)
	BD	0.288 (0.007)	0.117 (0.003)		10.49	1.897 (0.279)	1.200 (1.110)
IRGC 117265	DR	0.294 (0.002)	0.063 (0.003)	22.71	5.88	0.468 (0.023)	0.823 (0.050)
	BD	0.294 (0.009)	0.109 (0.004)		9.83	1.674 (0.277)	1.400 (1.430)
IRGC 117266	DR	0.233 (0.005)	0.080 (0.003)	18.88	7.42	0.359 (0.069)	1.272 (0.199)
	BD	0.233 (0.007)	0.117 (0.003)		10.46	3.330 (1.740)	3.927
IRGC 117267	DR	0.202 (0.001)	0.101 (0.001)	16.80	9.18	0.789 (0.028)	0.681 (0.040)
	BD	0.202 (0.003)	0.085 (0.082)		7.81	1.320 (1.860)	0.328 (0.619)
IRGC 117268	DR	0.175 (0.002)	0.037 (0.009)	14.92	3.57	0.954 (0.065)	0.419 (0.110)
	BD	0.175 (0004)	0.084 (0.006)		7.75	1.040 (0.123)	0.349 (0.123)
IRGC 117269	DR	0.367 (0.001)	0.080 (0.001)	26.84	7.42	0.483 (0.010)	0.837 (0.021)
	BD	0.367 (0.001)	0.106 (0.001)		9.55	1.508 (0.018)	0.754 (0.030)

IRGC 117270	DR	0.305 (0.004)	0.078 (0.002)	23.35	7.26	0.310 (0.030)	1.199 (0.087)
	BD	0.305 (0.004)	0.054 (0.005)		5.11	1.030 (0.038)	0.278 (0.032)
IRGC 117271	DR	0.200 (0.002)	0.075 (0.006)	16.68	6.96	0.678 (0.037)	0.572 (0.105)
	BD	0.200 (0.003)	0.104 (0.004)		9.42	1.649 (0.224)	0.560 (0.283)
IRGC 117272	DR	0.215 (0.003)	0.075 (0.002)	17.67	6.96	1.107 (0.067)	0.668 (0.101)
	BD	0.215 (0.001)	0.081 (0.001)		7.52	1.049 (0.087)	0.316 (0.223)
IRGC 117273	DR	0.263 (0.002)	0.076 (0.002)	20.84	7.04	0.437 (0.016)	0.943 (0.037)
	BD	0.263 (0.006)	0.076 (0.007)		7.04	1.453 (0.115)	0.245 (0.067)
IRGC 117274	DR	0.412 (0.015)	0.073 (0.008)	28.88	6.84	0.244 (0.060)	1.562 (0.279)
	BD	0.406 (0.007)	0.089 (0.010)		8.05	1.557 (0.147)	0.615 (0.207)
IRGC 117275	DR	0.159 (0.001)	0.071 (0.004)	13.74	6.61	0.730 (0.030)	0.436 (0.045)
	BD	0.159 (0.006)	0.094 (0.011)		8.55	0.822 (0.197)	0.481 (0.239)
IRGC 117276	DR	0.152 (0.001)	0.062 (0.005)	13.20	5.80	0.655 (0.037)	0.434 (0.050)
	BD	0.152 (0.000)	0.103 (0.000)		9.32	1.074 (0.001)	1.100 (0.003)
IRGC 117277	DR	0.193 (0.001)	0.098 (0.004)	16.17	6.97	1.869 (0.210)	0.391 (0.132)
	BD	0.193 (0.003)	0.075 (0.001)		8.89	1.052 (0.077)	1.011 (0.164)
IRGC 117278	DR	0.223 (0.004)	0.061 (0.010)	18.24	5.71	0.923 (0.080)	0.264 (0.043)
	BD	0.223 (0.005)	0.049 (0.008)		4.63	1.371 (0.132)	0.165 (0.087)

IRGC 117279	DR	0209 (0.001)	0.064 (0.007)	17.28	6.01	0.504 (0.023)	0.512 (0.042)
	BD	0.209 (0.003)	0.102 (0.012)		9.25	1.271 (0.277)	0.465 (0.226)
IRGC 117280	DR	0.303 (0.002)	0.084 (0.002)	23.25	7.76	0.475 (0.031)	0.981 (0.068)
	BD	0.303 (0.004)	0.060 (0.005)		5.64	1.169 (0.246)	0.196 (0.040)
IRGC 117281	DR	0.239 (0.003)	0.080 (0.002)	19.29	7.44	0.436 (0.049)	1.131 (0.115)
	BD	0.239 (0.005)	0.099 (0.011)		9.01	1.277 (0.217)	0.683 (0.327)
IRGC 117282	DR	0.232 (0.004)	0.072 (0.001)	18.81	6.73	0.852 (0.008)	0.545 (0.015)
	BD	0.232 (0.004)	0.100 (0.006)		9.12	1.091 (0.109)	0.803 (0.204)
IRGC 117283	DR	0.198 (0.004)	0.073 (0.004)	16.5	6.76	0.843 (0.091)	0.689 (0.132)
	BD	0.198 (0.004)	0.079 (0.006)		7.33	1.086 (0.092)	0.317 (0.089)

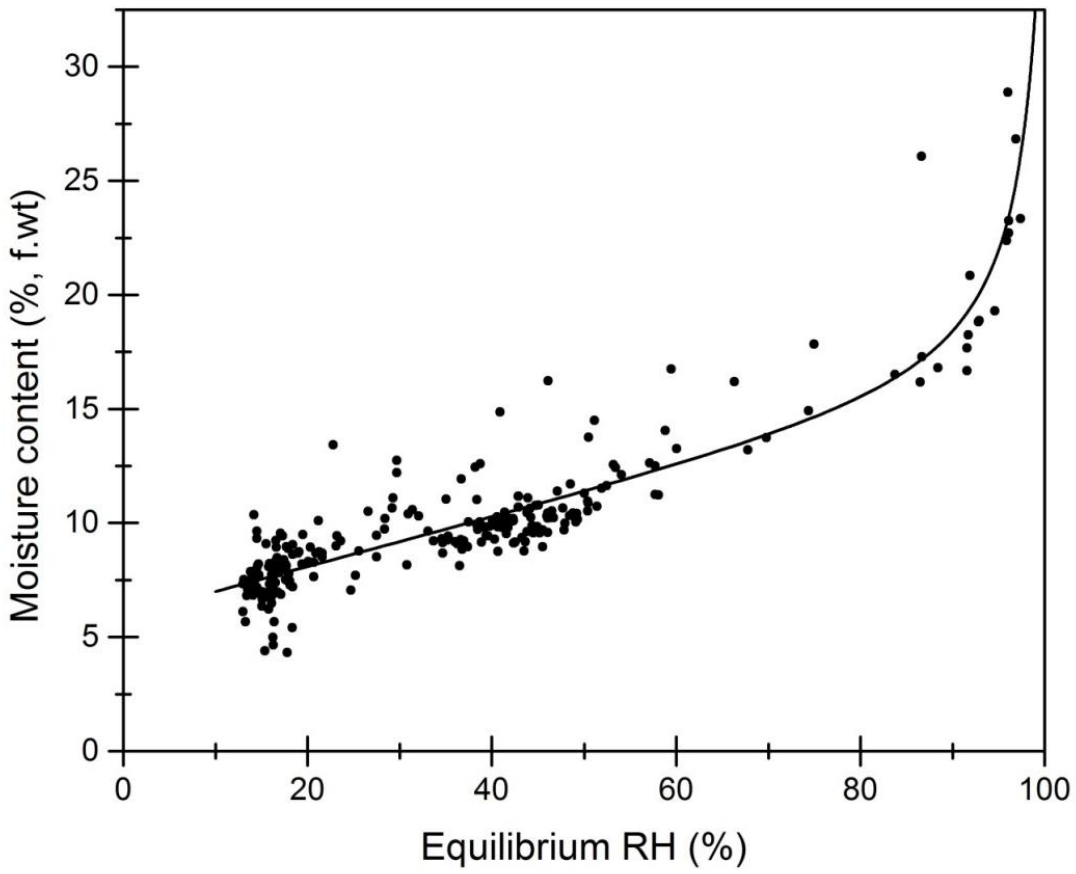


Figure 2.2. Relationship between seed moisture content and equilibrium relative humidity (eRH) during seed drying for 20 rice accessions (data shown in Figure 2.1.). All eRH measurements were made between 20.8 and 24.7°C. Seeds were dried either immediately in the DR or initially in the BD. The solid line is the result of fitting a modified version of the D'Arcy-Watt isotherm equation (equation [9]).

and -83 where drying rates were similar for BD and DR samples (Figure 2.1; Table 2.2). These accessions had the lowest harvest MC. Accession IRGC 117274 showed the highest drying rate in the BD and also had the highest harvest MC. Seeds dried in the BD had a mean eRH of 49.3% (s.e. 1.4) and MC of 11.4% (s.e. 0.3) after the first 8 h while seeds dried in the DR had a mean eRH of 52.1% (s.e. 12.6) and MC of 14.6% (s.e. 2.5) after 1 day. Seeds dried in the DR varied in eRH considerably (17.3 – 7.5%) after the first 2 days. The period for seeds to reach equilibrium inthe DR ranged from 4 to 14 days, whereas BD seeds reached equilibrium between 3 to 5 days with fluctuations in MC thereafter (Figure 2.1).

2.3.2. Seed moisture isotherm

The desorption isotherm for all seed lots shows a shallow slope between 13 and 80-85% eRH (7.3 and 15.5-16.6% MC; Figure 2.2). The MC then increases rapidly with further increase in eRH.

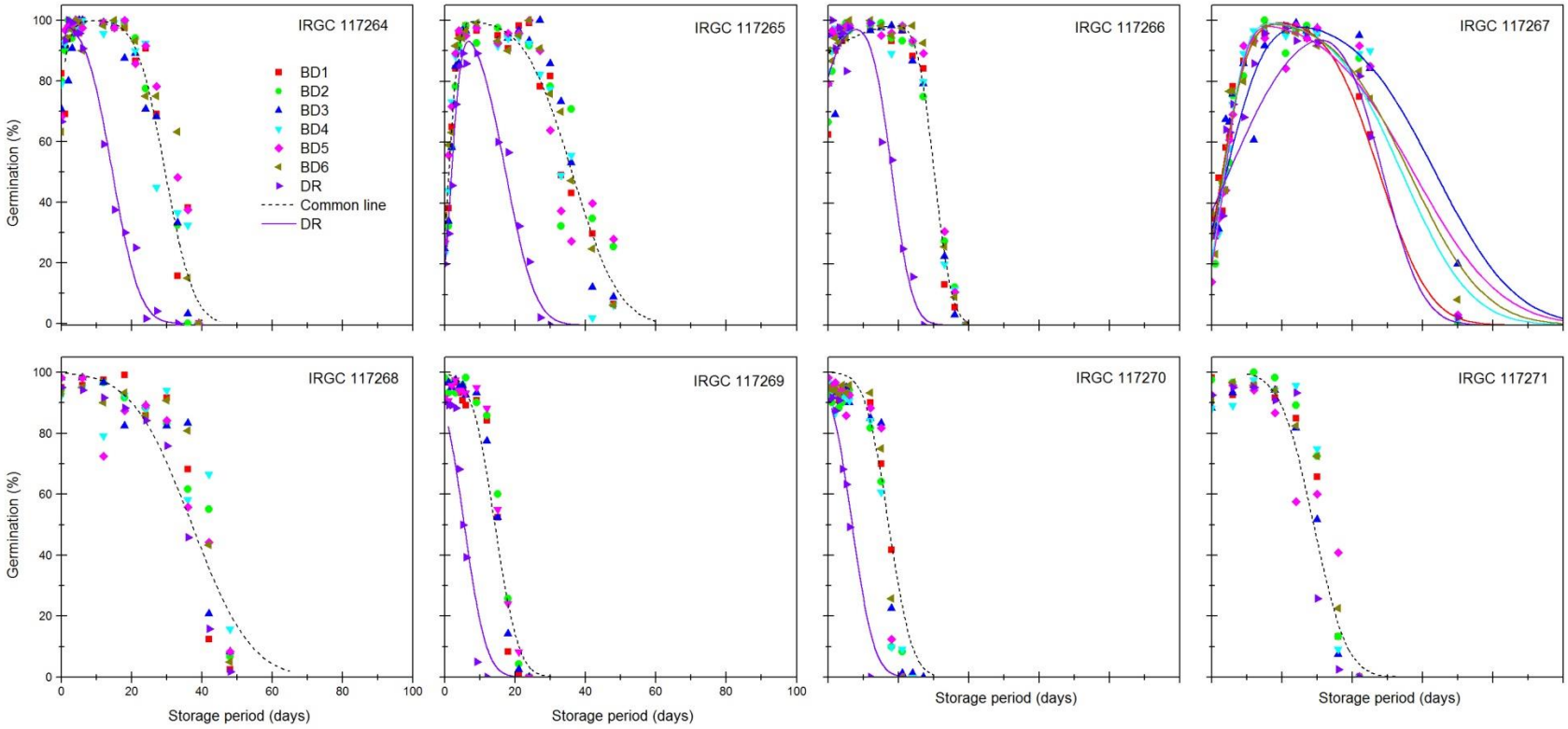
2.3.3. Seed longevity

The mean seed moisture content during experimental storage across all seed lots (accession × drying treatment) was 10.9% (s.e. 0.01). Seeds of some accessions showed dormancy which was lost during early experimental storage (accessions IRGC 117264, -65, -66, -67, -73, -75, -81 and -83); all seed lots ultimately showed a loss in viability (Figure 2.3). These changes in ability to germinate during storage were quantified by either equation [1] or equation [7] (Table 2.3).

Differences in seed longevity were apparent between accessions and, in some cases amongst the different drying treatments within accessions (Figure 2.3; Table 2.3; Appendix 2.5). Three categories of within-accession variation were apparent. For accessions IRGC 117268, -71, -72, -75, -77 and -83, there were no differences in K_i or σ amongst any of the seven different drying treatments. For accessions IRGC 117264, -65, -66, -69, -70, -74, -79 and -82, there were significant differences in K_i and σ (P<0.05) between BD and DR treatments, but not amongst different BD treatments (i.e. initial period of BD drying). For the six remaining accessions (IRGC 117267, -73, -76, -78, -80 and -81), it was not possible to constrain K_i and σ to common values for seeds given different drying treatments (Table 2.3; Appendix 2.5).

Where it was not possible to constrain K_i and σ to common values for BD and DR treatments, at least one of the BD treatments (period of drying in the BD) resulted in an improvement in longevity (p_{50}) compared with drying in the DR (Table 2.3; Appendix 2.5). For example, for accession IRGC 117267, the estimate of p_{50} was 63.7 days for seeds first dried for 3 days in the BD and 48.7 days for seeds dried throughout in the DR; and for

Figure 2.3. Ability to germinate during storage at 45°C and a MC of 10.9% for seeds of 20 rice accessions dried immediately in the dryroom (DR) or initially dried (8 h day^{-1}) in the batch dryer for 1 (BD1), 2 (BD2), 3 (BD3), 4 (BD4), 5 (BD5) or 6 (BD6) days. Equation [1] or [7] were fitted to the data with or without parameter constraints; the results shown are for the model with the fewest parameters that could be fitted without a significant increase in residual deviance compared with the best-fit model (Table 2.6).



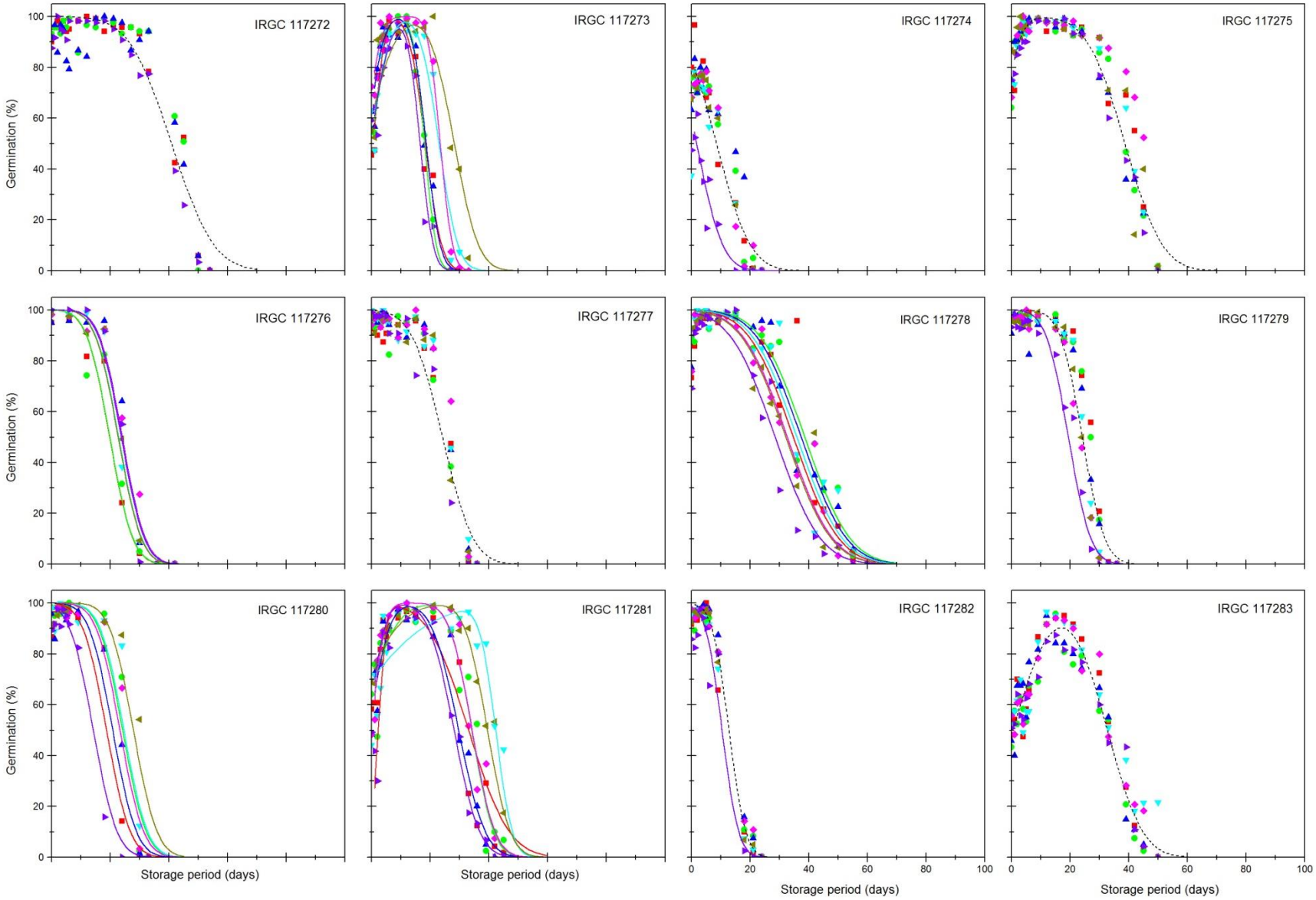

IRGC 117272
IRGC 117273
IRGC 117274
IRGC 117275
IRGC 117276
IRGC 117277
IRGC 117278
IRGC 117279
IRGC 117280
IRGC 117281
IRGC 117282
IRGC 117283
Germination (%)
Storage period (days)
0
20
40
60
80
100

Table 2.3. Results of fitting equation [1] (viability equation; Ellis and Roberts, 1980a) or equation [7] (combined loss in dormancy and loss in viability; Kebreab and Murdoch, 1999) to quantify changes in ability to germinate during hermetic storage at 45°C for 20 *O. sativa* accessions. Samples were immediately dried in the dryroom (DR) or initially dried (8 h day^{-1}) in the batch dryer (BD) for 1 (BD1), 2 (BD2), 3 (BD3), 4 (BD4), 5 (BD5) or 6 (BD6) days. The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant (P=0.05) increase in residual deviance compared with the best-fit model (see Appendix 2.5). The moisture content is the mean and standard error (s.e.) calculated from measurements taken at three stages across the duration of the storage experiment.

			Loss in dormancy		Loss in viability			
Treatment	Model	Seed MC (s.e.) (% f.wt.)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p_{50} relative to DR (%, days)
IRGC 117264								
BD1	Equation [7] K_d, β_1, K_i, and σ^{-1} constrained within BD treatments	10.8 (0.0)	0.81 (0.62)	0.29 (1.37)	4.61 (0.72)	0.16 (0.04)	29.6	102.7
BD2		10.8 (0.0)						
BD3		10.9 (0.0)						
BD4		10.8 (0.0)						
BD5		10.8 (0.0)						
BD6		10.8 (0.0)						
DR		10.9 (0.0)	0.44 (0.33)	1.23 (0.68)	2.39 (0.31)	0.16 (0.02)	14.6	

IRGC 117265								
BD1	Equation [7] K_d, and β_1 constrained within BD treatments	10.5 (0.1)	0.57 (0.50)	0.48 (0.19)	3.40 (0.99)	0.09 (0.05)	36.3	108.6
BD2		10.5 (0.1)						
BD3		10.5 (0.1)						
BD4		10.5 (0.1)						
BD5		10.5 (0.1)						
BD6		10.5 (0.6)						
DR		10.6 (0.0)	0.88 (0.24)	0.45 (0.09)	2.63 (0.48)	0.15 (0.03)	17.4	
IRGC 117266								
BD1	Equation [7] K_d and β_1 constrained within BD treatments	10.9 (0.0)	1.25 (0.50)	0.05 (0.16)	7.71 (1.60)	0.26 (0.08)	30.2	66.9
BD2		10.9 (0.0)						
BD3		10.9 (0.0)						
BD4		10.9 (0.0)						
BD5		10.9 (0.0)						
BD6		10.8 (0.0)						
DR		10.7 (0.0)	0.89 (0.25)	0.15 (0.08)	4.00 (0.72)	0.22 (0.04)	18.1	

IRGC 117267								
BD1		10.9 (0.1)	0.52 (0.44)	0.17 (0.05)	4.23 (2.54)	0.09 (0.07)	47.7	
BD2		10.9 (0.0)	-	-	-	-	-	
BD3	Equation [7]	10.8 (0.0)	0.49 (0.43)	0.12 (0.05)	3.56 (2.56)	0.06 (0.06)	63.7	
BD4	No parameter constrained	10.8 (0.0)	0.80 (0.46)	0.22 (0.07)	3.52 (2.48)	0.07 (0.06)	54.2	30.8
BD5		10.8 (0.0)	0.86 (0.46)	0.22 (0.07)	2.96 (2.45)	0.05 (0.06)	57.5	
BD6		10.7 (0.0)	0.60 (0.45)	0.18 (0.06)	3.37 (2.47)	0.06 (0.06)	56.3	
DR		10.7 (0.0)	0.32 (0.17)	0.07 (0.02)	5.46 (1.18)	0.11 (0.02)	48.7	
IRGC 117268								
BD1		10.7 (0.0)	-	-				
BD2	Equation [1]	10.7 (0.0)	-	-				
BD3		10.7 (0.0)	-	-				
BD4	K_i and σ^{-1} constrained within all treatments (BD and DR)	10.6 (0.0)	-	-	2.78 (0.06)	0.08 (0.00)	37.2	0
BD5		10.7 (0.0)	-	-				
BD6		10.7 (0.0)	-	-				
DR		10.7 (0.0)	-	-				

IRGC 117269								
BD1	Equation [1] K_i and σ^{-1} constrained within BD treatments	11.5 (0.1)	-	-	2.69 (0.06)	0.19 (0.00)	14.3	155.4
BD2		11.5 (0.0)	-	-				
BD3		11.5 (0.1)	-	-				
BD4		11.7 (0.0)	-	-				
DR		11.6 (0.0)	-	-	1.13 (0.05)	0.20 (0.00)	5.6	
IRGC 117270								
BD1	Equation [1] K_i and σ^{-1} constrained within BD treatments	11.0 (0.1)	-	-	3.28 (0.08)	0.19 (0.05)	17.2	149.3
BD2		10.8 (0.1)	-	-				
BD3		10.8 (0.0)	-	-				
BD4		10.8 (0.1)	-	-				
BD5		10.7 (0.1)	-	-				
BD6		10.6 (0.1)	-	-				
DR		10.9 (0.1)	-	-	1.39 (0.06)	0.20 (0.01)	6.9	

IRGC 117271								
BD1	Equation [1] K_i and σ^{-1} constrained within all treatments (BD and DR)	10.6 (0.0)	-	-	3.89 (0.09)	0.13 (0.00)	29.0	0
BD2		10.6 (0.1)	-	-				
BD3		10.6 (0.0)	-	-				
BD4		10.6 (0.0)	-	-				
BD5		10.6 (0.0)	-	-				
BD6		10.6 (0.0)	-	-				
DR		10.6 (0.0)	-	-				
IRGC 117272								
BD1	Equation [1] K_i and σ^{-1} constrained within all treatments (BD and DR)	11.1 (0.1)	-	-	3.60 (0.08)	0.09 (0.00)	41.0	0
BD2		11.1 (0.1)	-	-				
BD3		11.0 (0.1)	-	-				
DR		10.9 (0.1)	-	-				

IRGC 117273								
BD1		10.6 (0.1)	0.18 (0.45)	0.32 (0.14)	4.22 (1.92)	0.23 (0.11)	18.4	
BD2		10.8 (0.0)	0.03 (0.45)	0.20 (0.14)	5.85 (2.25)	0.32 (0.13)	18.2	
BD3	Equation [7]	10.5 (0.2)	0.34 (0.44)	0.24 (0.12)	4.97 (2.11)	0.27 (0.12)	18.7	
BD4	No parameters constrained	10.8 (0.0)	0.12 (0.44)	0.19 (0.13)	4.55 (1.89)	0.20 (0.11)	23.1	70.5
BD5		10.6 (0.0)	0.52 (0.44)	0.25 (0.12)	7.15 (2.22)	0.31 (0.12)	23.5	
BD6		10.3 (0.1)	0.26 (0.44)	0.13 (0.13)	4.37 (1.87)	0.16 (0.10)	28.3	
DR		10.8 (0.0)	0.08 (0.18)	0.19 (0.05)	4.77 (0.86)	0.29 (0.05)	16.6	
IRGC 117274								
BD1		10.7 (0.1)	-	-				
BD2		10.8 (0.0)	-	-				
BD3	Equation [1]	10.8 (0.0)	-	-				
BD4	K_i and σ^{-1} constrained within BD treatments	10.8 (0.0)	-	-	0.94 (0.02)	0.11 (0.00)	8.5	372.2
BD5		10.7 (0.0)	-	-				
BD6		10.8 (0.0)	-	-				
DR		10.8 (0.1)	-	-	0.26 (0.05)	0.14 (0.00)	1.8	

IRGC 117275								
BD1	Equation [7] K_i and σ^{-1} constrained within all treatments (BD and DR)	11.3 (0.1)	0.77 (0.09)	0.19 (0.03)	3.85 (0.18)	0.10 (0.01)	38.7	0
BD2		11.4 (0.2)						
BD3		11.3 (0.0)						
BD4		11.3 (0.0)						
BD5		11.2 (0.0)						
BD6		11.2 (0.1)						
DR		11.5 (0.3)						
IRGC 117276								
BD1	Equation [1] σ^{-1} constrained within all treatments (BD and DR)	10.8 (0.0)	-	-	3.20 (0.10)		19.8	
BD2		10.9 (0.0)	-	-	3.21 (0.10)		19.7	
BD3		10.9 (0.0)	-	-	3.89 (0.11)		24.1	
BD4		10.9 (0.0)	-	-	3.70 (0.11)	0.16 (0.00)	22.9	1.3
BD5		10.9 (0.1)	-	-	3.87 (0.11)		24.0	
BD6		10.9 (0.0)	-	-	3.67 (0.11)		22.7	
DR		10.9 (0.1)	-	-	3.84 (0.11)		23.8	

IRGC 117277								
BD1	Equation [1] K_i and σ^{-1} constrained within all treatments (BD and DR)	10.8 (0.0)	-	-	2.93 (0.05)	0.12 (0.00)	24.2	0
BD2		10.7 (0.0)	-	-				
BD3		10.7 (0.0)	-	-				
BD4		10.7 (0.0)	-	-				
BD5		10.5 (0.0)	-	-				
BD6		10.6 (0.0)	-	-				
DR		10.7 (0.0)	-	-				
IRGC 117278								
BD1	Equation [1] σ^{-1} constrained within all treatments (BD and DR)	10.5 (0.1)	-	-	2.87 (0.06)		34.8	
BD2		10.5 (0.0)	-	-	3.19 (0.06)		38.7	
BD3		10.5 (0.1)	-	-	3.09 (0.06)		37.4	
BD4		10.6 (0.1)	-	-	2.97 (0.06)	0.08 (0.00)	35.9	35.3
BD5		10.6 (0.1)	-	-	2.70 (0.06)		32.7	
BD6		10.5 (0.0)	-	-	2.66 (0.06)		32.2	
DR		10.6 (0.0)	-	-	2.36 (0.05)		28.6	

IRGC 117279								
BD1	Equation [1] K_i and σ^{-1} constrained within BD treatments	11.0 (0.1)	-	-	3.99 (0.08)	0.17 (0.00)	24.1	23.6
BD2		11.0 (0.0)	-	-				
BD3		11.1 (0.0)	-	-				
BD4		11.1 (0.0)	-	-				
BD5		11.1 (0.0)	-	-				
BD6		11.2 (0.1)	-	-				
DR		11.0 (0.1)	-	-	3.23 (0.08)	0.17 (0.00)	19.5	
IRGC 117280								
BD1	Equation [1] σ^{-1} constrained within all treatments (BD and DR)	11.3 (0.2)	-	-	2.96 (0.09)		18.8	
BD2		11.2 (0.0)	-	-	3.82 (0.11)		24.2	
BD3		11.0 (0.0)	-	-	3.29 (0.10)		20.9	
BD4		11.0 (0.0)	-	-	3.89 (0.11)	0.16 (0.00)	24.7	95.1
BD5		11.0 (0.0)	-	-	3.68 (0.11)		23.4	
BD6		11.0 (0.0)	-	-	4.40 (0.12)		27.9	
DR		11.1 (0.0)	-	-	2.25 (0.07)		14.3	

IRGC 117281								
BD1		10.9 (0.0)	1.05 (0.61)	0.45 (0.17)	3.05 (1.38)	0.09 (0.05)	33.1	
BD2		10.9 (0.0)	0.42 (0.54)	0.11 (0.13)	5.80 (1.81)	0.17 (0.06)	34.2	
BD3	Equation [7]	10.9 (0.0)	0.17 (0.56)	0.18 (0.14)	4.19 (1.53)	0.14 (0.05)	29.8	
BD4	No parameters constrained	10.9 (0.1)	0.52 (0.52)	0.05 (0.12)	9.61 (3.06)	0.23 (0.08)	42.4	50.4
BD5		10.6 (0.0)	0.03 (0.57)	0.27 (0.16)	5.65 (1.69)	0.16 (0.05)	34.4	
BD6		10.7 (0.0)	0.61 (0.54)	0.08 (0.13)	6.52 (2.06)	0.17 (0.06)	39.6	
DR		10.9 (0.0)	0.27 (0.23)	0.23 (0.06)	4.13 (0.63)	0.15 (0.02)	28.2	
IRGC 117282								
BD1		10.9 (0.1)	-	-				
BD2		11.0 (0.0)	-	-				
BD3	Equation [1]	10.9 (0.0)	-	-				
BD4	K_i and σ^{-1} constrained within BD treatments	10.8 (0.0)	-	-	2.83 (0.06)	0.22 (0.00)	12.8	
BD5		10.8 (0.1)	-	-				21.9
BD6		10.8 (0.1)	-	-				
DR		11.0 (0.1)	-	-	2.35 (0.00)	0.22 (0.00)	10.5	

IRGC 117283								
BD1	Equation [7] K_d, β_1, K_i, and σ^{-1} constrained within all treatments (BD and DR)	10.9 (0.1)	0.05 (0.05)	0.10 (0.01)	3.47 (0.19)	0.11 (0.01)	32.8	0
BD2		11.0 (0.1)						
BD3		11.0 (0.1)						
BD4		10.8 (0.1)						
BD5		10.7 (0.1)						
DR		10.8 (0.1)						

IRGC 117264, the estimate of p_{50} was 29.6 days for seeds first dried in the BD, and 14.6 days for seeds dried throughout in the DR. For the accessions where K_i and σ could not be constrained across BD treatments, most accessions showed an improvement in p_{50} after the first day in the BD compared with the DR, which was either then maintained or increased (Table 2.3; Appendix 2.5) until the day the seeds reached equilibrium in the BD (Figure 2.1). For example, for accession IRGC 117281 the estimate of p_{50} was 33.1 days after the first 8 h in the BD and by the end of the fourth daily cycle it had increased to 42.4 days when seeds had reached the minimum eRH of 37.4%. Relative improvement in longevity of seeds initially placed in the BD relative to those dried in the DR throughout ranged from 0% (accessions IRGC 117268, -71, -72, -75, -77 and -83) to 372% (accession IRGC 117274) (Table 2.3). The improvement was more than 100% (i.e. longevity was more than doubled) for 5 of the 20 accessions. These highly variable differences in subsequent seed longevity depending on drying treatment amongst the 20 accessions were further examined by investigating the possibility that they might be dependent upon known differences in their seed production history. Split-line regressions accounted for 66.3, 85 and 65.8% of the variance in the case of the relationship between improvement in longevity and harvest date, harvest moisture content and DR p_{50}, respectively (Figure 2.4A-C). The respective breakpoints occurred on 30th March 2013, at a harvest MC of 16.2%, or a DR p_{50} of 24.2 days. There was no relationship apparent between the improvement in longevity and DAA (Figure 2.4D).

2.4. Discussion

The current study used alternate temperature cycling (8 h 45°C /16 h ambient) for the high temperature treatment and it is clear that in the case of rice, subsequent longevity might be improved more than 3-fold if seeds are not initially dried in the conditions used by the genebank at IRRI (Table 2.3; Figure 2.3; Figure 2.4) which reflected genebank standards at the time of installation. This is in agreement with the preliminary study by Crisostomo *et al*. (2011). Such improvements in rice seed longevity could potentially greatly reduce the number of genebank accessions that have to be regenerated each year due to declining viability. In general, seeds dried in the BD initially dried more quickly, but did not reach MC as low as those obtained when seeds were dried in the DR, with increases in seed moisture observed after 3-4 days (Figure 2.1). Therefore with drying in

the BD alone, seeds cannot reach low moisture contents required for genebank storage. However the drying curves of the DR in the present study were not complete replicas of those shown by Crisostomo *et al.* (2011), where the drying rates were similar between the two regimes, reaching a lower eRH of 20% within 4 days (Crisostomo *et al*., 2011). It is probable that the difference between experiments could be a result of the efficiency of the DR, with all harvests occurring before the peak period between late April and early May, the DR efficiency was not likely to have been compromised during the current experiment. The BD lacks a dehumidification system and is operated in an open environment. Since the ambient conditions in the dry season at IRRI are warm (25-30°C) and humid (80-90%), even when the air is heated there is a limit to the extent to which the RH can be reduced, and hence how effective it will be for drying seeds to low moisture contents. It is therefore perhaps surprising that for 14 of the 20 accessions, there was such a benefit of drying with the BD compared with the DR and furthermore, that the benefit was maintained over several drying cycles (Table 2.3; Figure 2.3), even after there were increases in seed MC in the equilibrium phase (Figure 2.1).

Seeds of the different accessions did not respond in the same way (Table 2.3; Figure 2.3). This was not obviously related to variety group, although the improvement in longevity when seeds were dried in the BD compared with the DR was greatest for three of the four temperate japonica varieties (Figure 2.4). The seeds of these accessions had the lowest p_{50} when they were dried throughout in the DR (Figure 2.4C). Seeds of temperate japonica varieties are known to be short-lived in storage (Ellis *et al*., 1992, 1993b; Kameswara Rao and Jackson, 1997; Xue *et al*., 2008; Hay *et al*., 2013) but it seems that it might be possible to improve the longevity of seeds of such accessions more than those of non-temperate japonica varieties by changing the drying regime. That is, one reason why they are so short-lived, especially when regenerated in a tropical environment, may be because genebank drying conditions are not optimal for drying seeds of these varieties in particular. In terms of current practice, we suggest that genebanks using low temperature low humidity environments to dry rice seeds delay harvesting accessions until after MC has declined naturally to below about 16%, if ambient conditions allow, since at this value and below, the contrasting drying temperatures provided similar longevity (Figure 2.4B). If seeds are unlikely to dry to this MC due to high ambient humidity, high temperature drying might be superior with respect to seed longevity.

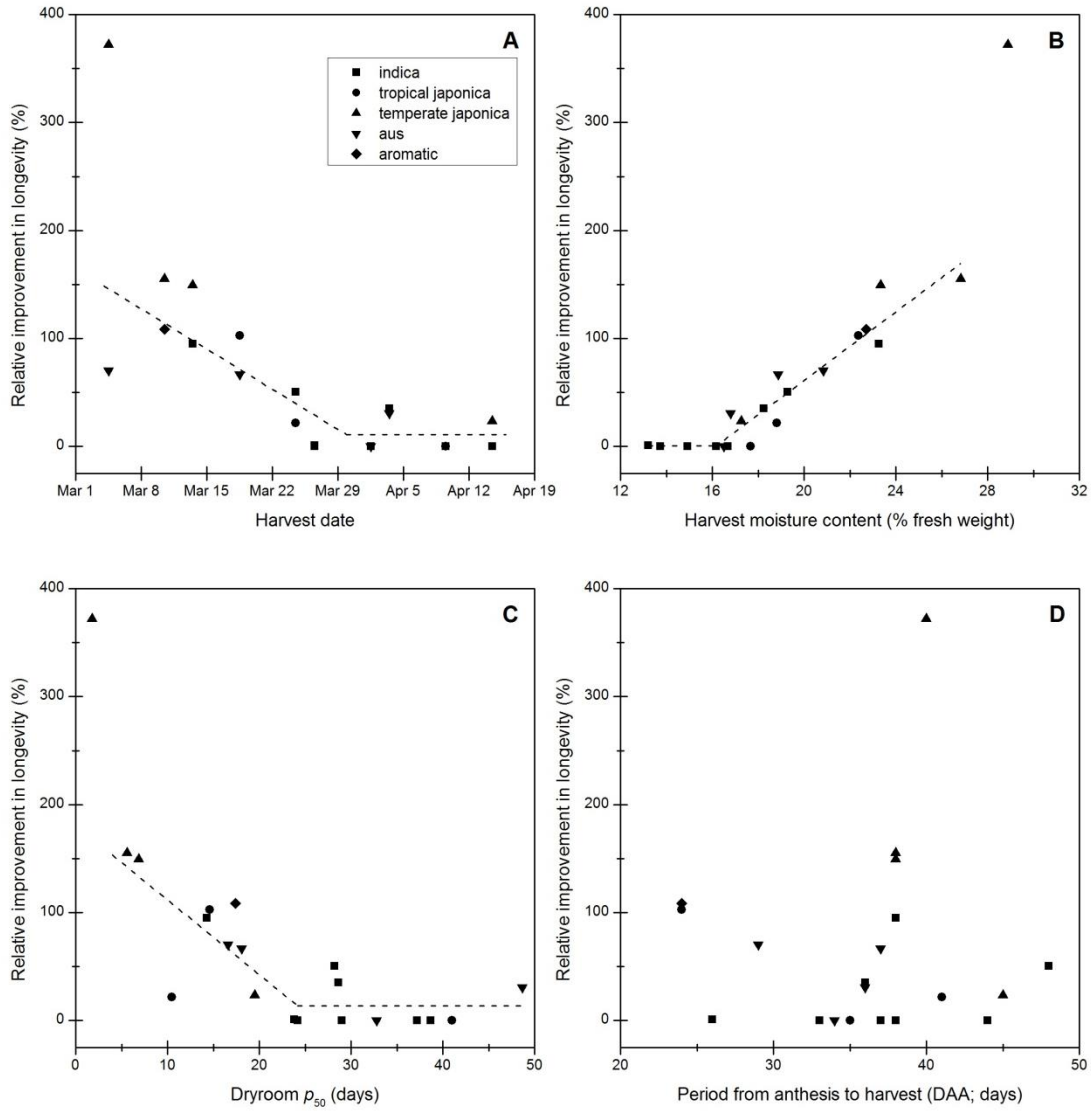


Figure 2.4. Split-line relationships between the relative improvement in longevity (%) between the two drying treatments (BD p_{50}/DR p_{50}) for 20 rice accessions and **A.** harvest date, **B.** seed moisture content and **C.** DR p_{50}. The outlying data point (372% relative improvement in longevity; accession IRGC 117274) was not included in the analyses. No significant regression line could be fitted between improvement in longevity and period from anthesis to harvest **D** (Appendix 2.6). A relative improvement in longevity of 100% is equivalent to a doubling in longevity of BD compared with DR treatments.

This is not the first time that an alternative drying regime has been reported to be better than standard genebank drying room conditions (15°C/15% RH) for subsequent seed longevity or quality. As well as the previous research on rice by Crisostomo *et al*. (2011), Butler *et al*. (2009a) described how the longevity of seeds of foxglove (*Digitalis purpurea* L.) that were intentionally harvested prematurely, in the post-abscission (i.e. desiccation) phase of seed development, increased when seeds were dried at RH >15%. It has been well-reported how seed quality including longevity increases during the desiccation phase of seed development and results from gene expression and metabolite studies have shown that seeds are metabolically active during this phase (Angelovici *et al*., 2010 and references therein). Chatelain *et al*. (2012) further suggested, based on proteomic studies, that the desiccation phase from the end of seed-filling (mass maturity) onwards should be divided into two, the first when there is increase in seed longevity and then a final, maturation drying stage. Based on data from Kameswara Rao and Jackson (1996a, b, c), we can assume that all seed lots had reached mass maturity and were therefore in the desiccation phase of seed development when harvested. Such studies have shown that harvesting seeds later in this phase increases seed longevity however, across the 20 seed lots produced for this study, there was not a significant relationship between DR p_{50} and DAA (graph not shown), nor between relative improvement in longevity with the BD treatment and DAA (Figure 2.4D). Although a negative association has not been highlighted, the results do suggest that DAA alone does not determine seed longevity. Plotting the p_{50} data against harvest date, it is clear that it was only those seeds that were harvested earlier in the season (i.e. in March) that responded positively to the BD treatment (Figure 2.4A). These seeds were also the seeds that happened to have the lowest p_{50} for DR-dried seeds and, perhaps most importantly, a MC greater than 16.2% (Figures 2.4B and 2.5). This coincides with the part of the moisture desorption isotherm where, at higher MCs, seeds become metabolically active i.e. at eRH >80-85% (Figure 2.2; Vertucci and Leopold, 1984; Walters *et al.*, 2002). Therefore, it appears that seeds harvested later in the season and irrespective of DAA, had already acquired greater longevity due to on-plant drying i.e. they had already entered the first, 'increasing longevity' part of the desiccation phase (Chatelain *et al*., 2012), triggered by decline in ambient RH and a long dry phase (without rain) after the end of March (coinciding with the breakpoint shown in Figure 2.4A). If the seeds had already dried on the plant to a MC where sequence of development with respect to time (DAA) once they have reached

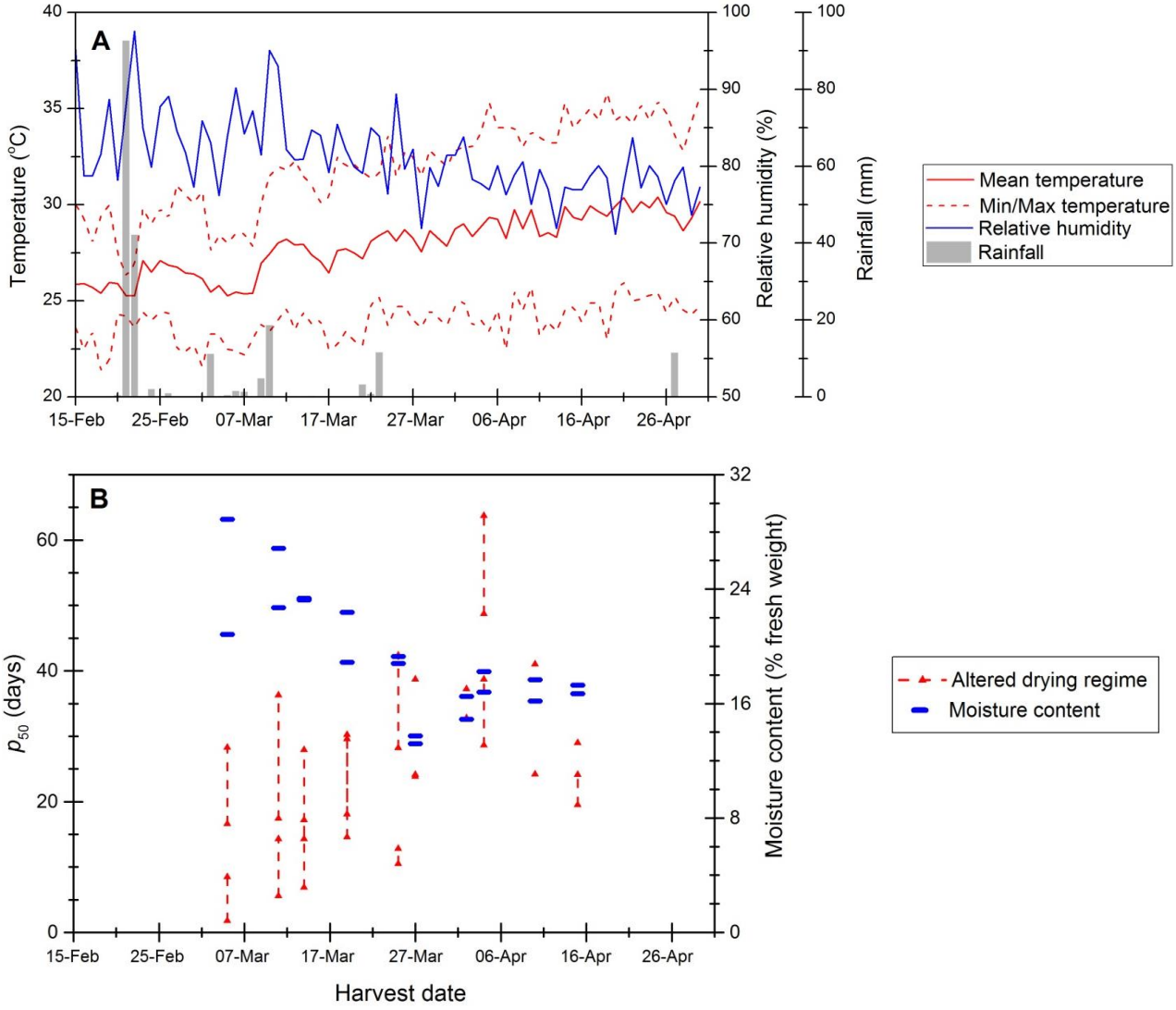


Figure 2.5A. Weather data for the IRRI experimental station over the harvesting period (provided by the IRRI Climate Unit). **B.** Changes in the period of viability to fall to 50% (p_{50}) for seeds harvested on different dates and dried in the DR throughout (lower red triangle) or in the BD (upper red triangle; result shown for the optimum number of drying days). If there is no dashed line, there was not a significant improvement in longevity with the BD treatment. The harvest moisture contents of seeds of the 20 accessions are also shown (short blue horizontal lines).

mass maturity; rather, due to the high humidity of the growing environment at IRRI, the rice seeds stay in a pre-desiccation state where increases in longevity are limited. It is only when they experience some desiccation that substantial 'accumulation of seed longevity' is activated. If rice seeds regenerated at IRRI for long-term storage in the genebank are harvested in the dry season before ambient RH has decreased and hence with high MC (>16.2 %), they should be dried at a high temperature to allow continued metabolism such as the accumulation of proteins that may be involved in stabilizing tissues during seed storage. If they have already dried to MC <16.2%, they are perhaps in that second, final maturation drying phase (Chatelain *et al*., 2012) and will not respond to high-temperature drying. These results also, not for the first time, raise important questions about the value of using single seed lots to make comparisons of seed longevity between genotypes (for example, for genetic association studies), even if they are harvested at the same 'stage' (DAA) of seed development.

Very moist seeds are expected to be more sensitive to damage in heated-air dryers than seeds with low MC (Nellist, 1980; McDonald and Copeland, 1997). This represents an apparent contradiction to the results presented here. However, evaporative cooling by the moist seeds may have maintained seed temperatures very much cooler than the 45°C air temperature and closer to the ambient temperature (approximately 30°C; Figure 2.5) when seeds would normally dry *in situ*. In contrast, the genebank drying room they were no longer metabolically active, drying at a higher temperature (i.e. in the BD) did not improve the longevity *cf*. seeds directly dried directly in the drying room, but nor did it have a negative effect. It is suggested that rice seeds do not strictly follow a temperature of 15°C is very much cooler than the seeds would experience *in situ* and again, the actual temperature experienced by the seeds might be reduced further due to evaporative cooling. Moreover, traditional heated-air drying investigations used mature seeds with moist samples created by "wetting up" dry seeds or perhaps, from harvests delayed by heavy rain well beyond harvest maturity. Such differences could well explain the apparent contradictions with the conventions of heated-air commercial seed drying.

The results also appear to contradict the damaging effect of high seed production temperatures on developing and maturing seeds of japonica rice (Ellis *et al*., 1993b; Kameswara Rao and Jackson, 1996a). However, Kameswara Rao and Jackson (1996a) did

suggest that planting should be timed so that seeds ripen when weather conditions are both cool and dry, based on the rainfall data for that year (1994 dry season harvest) and indeed, there was significant rainfall by the time the last harvest was made, in May, in that study (although it should be noted that ambient RH that year, not presented in that publication but obtained from the IRRI Climate Unit, did not reach the same low values as in March-April 2013). Furthermore, recent investigations point to the phase of rice seed quality development most sensitive to high temperature being before the end of the seed-filling phase (Ellis, 2011) and possibly as early as the histodifferentiation phase soon after pollination (Martínez-Eixarch and Ellis, 2015). It should also be noted that, in addition to the higher temperature, seeds in the BD would have been exposed to higher RH than the 15% of the drying room. It has been reported elsewhere, most notably for pre-dispersal seeds of foxglove, that drying at 15% RH is not optimum for subsequent seed storage longevity (Hay and Probert, 1995; Butler *et al*., 2009a); thus, both RH and temperature during drying may be important for the accumulation of seed longevity. Determining an optimum drying regime and/or potentially customizing drying regimes depending on production history (e.g. harvest DAA, harvest MC) might also be difficult since these variables are not independent: the rate of drying will depend on both the temperature and RH of the air (and indeed the flow of air around the seeds), and changing the temperature of the air will also change the RH. It should not be forgotten however, that even if a different initial drying regime is identified as being better that the current regime, it may still be necessary to equilibrate the seeds after the initial drying, to ensure the seeds are at an appropriate MC for long-term storage.

The results presented in this chapter show clear evidence that, for rice, initial drying with hot air, for example by using a flat-bed BD, can result in seed lots with significantly greater subsequent longevity in storage than for those dried immediately under low temperature, low humidity conditions, particularly if the seeds are harvested when their MC is relatively high. This theme will be considered further in subsequent chapters.

CHAPTER 3

THE EFFECT OF REHYDRATION AND RE-DRYING ON RICE SEED (*ORYZA SATIVA* L.) LONGEVITY

3.1. Introduction

The ability of seeds to tolerate desiccation to low moisture contents required for long-term storage is acquired during the maturation phase of seed development (Ellis and Hong, 1994). Seed longevity, i.e. the duration of survival in air-dry storage, increases after the acquisition of desiccation tolerance (Ellis and Hong, 1994; Hay and Smith, 2003; Ellis, 2011), or perhaps more specifically, during the first part of the desiccation phase ("the late maturation phase") before final maturation drying (Chatelain *et al*., 2012). The previous chapter indicated that seeds must experience some desiccation before they can "accumulate longevity" which is why the longevity of seeds, which are still in a pre-desiccation state at harvest due to the high humidity of the growing environment, can increase in response to high temperature drying (Chapter 2). It is thought that the loss in moisture is a critical factor controlling maturation processes, by inducing the stress response and other protective mechanisms (Radawan *et al*., 2014) which significantly increase seed longevity. Once seeds have equilibrated to low moisture contents (<20% RH) all of the multimolecular and most of the weakly bound water has been removed and seeds are developmentally "fixed".

The moisture content of the seeds at harvest depends on the temperature and relative humidity (RH) of the air, and determines their pre-harvest metabolic activity and subsequent rate of viability loss. In the wet tropics, ambient humidity rarely falls below 80% meaning seeds are harvested at high moisture contents that coincide with the part of the moisture sorption isotherm where there is bulk water and metabolic activity can occur at a rate that increases as water content increases to full imbibition levels (Vertucci and Leopold, 1986). With a sufficient availability of oxygen, seeds at high RH are capable of repairing damage which may have accumulated, through normal metabolic processes, during seed development. Seeds usually activate such repair mechanisms during the

imbibition stage of germination which is crucial to maintain viability and germination vigour (Powell and Matthews, 2012). Although seeds do not need to be fully hydrated to initiate repair processes it is unlikely that they are fully functional below 98% eRH (Ibrahim and Roberts, 1983; Vertucci and Farrant, 1995; Pammenter and Berjak, 1999). Invigoration treatments such as priming can induce repair mechanisms in seeds. Lower quality seeds, i.e. seeds which have already accumulated a considerable amount of damage, show an improvement in longevity in response to priming (Butler *et al*., 2009b; Powell *et al.*, 2000) whilst higher quality seeds are likely to become "over-advanced" and lose desiccation tolerance as a result (Śilwińska and Jendrzejczak, 2002; Powell *et al*., 2000). However in such cases post-priming treatments such as heat shock (Bruggink *et al*., 1999) and slow drying (Gurusinghe and Bradford, 2001) have been reported to restore desiccation tolerance in some species by inducing the synthesis of antioxidants and/or protective proteins which can stabilise the seed during storage (Close, 1996; Hoekstra *et al*., 2001; Rajjou and Debeaujon, 2008).

3.1.1. Objectives and Hypotheses

The aim of the research described in this chapter was to investigate the potential benefit of rehydrating and re-drying seeds on subsequent seed longevity. The results from the BD_DR drying treatment reported in the previous chapter (Chapter 2) for the three accessions evaluated in the present chapter (IRGC 117265, -76 and -80) were re-evaluated in the context of the current investigation.

Hypothesis 1: High temperature drying of seeds which have already been partially dried in the dryroom will not significantly improve their storage longevity compared with solely drying in the dryroom or compared with immediate post-harvest high temperature exposure.

Hypothesis 2: Rehydrating seeds during post-harvest drying treatments followed by high or low temperature drying will significantly improve the longevity of seeds in storage compared with dryroom drying.

3.2. Materials and methods

The materials and methods for the BD_DR drying treatment (2013 dry season) are described in Chapter 2. However, the information (sowing date, transplanting date and harvest date, and the initial seed moisture content (MC) and equilibrium relative humidity (eRH) at harvest) for accessions IRGC 117265, -76 and -80 are also included in Table 3.1.

3.2.1. Plant material

Three accessions, IRGC 117265, -76 and -80, were planted for the 2014 dry season (DS) harvest, representing two varietal groups (aromatic and indica; McNally *et al*., 2009). Seeds were sampled from the GRC active collection and held at 50°C for 5 days to break dormancy before they were sown and later transplanted at the International Rice Research Institute (IRRI) experimental Station (ES) (14° 9’ 3.5742”N, 121° 15’ 54.504”W). Normal rice production practices and routine plant protection measures were taken (Reaño *et al*., 2008; Appendix 2.1). Seeds for each accession were sown on two separate dates, producing two (A and B) individual harvests (Table 3.1). Seeds for each accession and both plantings were harvested at 35 days after 50% anthesis (DAA).

3.2.2. Post-harvest treatments

Immediately after harvest, the seeds were subjected to the same post-harvest protocol as stated in Chapter 2. After being threshed and blown, the temperature and equilibrium relative humidity (eRH) was measured and moisture content (MC) determined (Chapter 2; section 2.2.2).

3.2.3. Seed drying

Seeds from each harvest from each accession were divided into five 200 g samples and placed into 0.2 × 0.33 m (L × W) nylon mesh bags (1 mm-diameter holes) and stored sealed in 0.6 × 0.3 × 0.132 m (L × W× H) electrical enclosure boxes (ENSTO Finland, Oy) at room temperature (21.5°C) until 1600 hrs when they were transferred to the genebank

Table 3.1. Information of the three rice (*Oryza sativa*) seed lots harvested for the experiments (DR_BD; BD_DR) described in this chapter showing sowing, transplanting and harvest dates, and the initial seed moisture content (MC) and equilibrium relative humidity (eRH) at harvest.

Accession	Variety name	Variety group	Season (harvest)	Sowing date	Transplanting date	Harvest date	MC (s.e.) (% f.wt.)	eRH (%)
IRGC 117265	Dom-sufid	aromatic	2014 (A)	30 Nov 2013	21 Dec 2013	26 Mar 2014	22.2 (3.10)	94.5
			2014 (B)	01 Jan 2014	22 Jan 2014	01 May 2014	20.2 (0.17)	97.5
			2013	23 Nov 2012	18 Dec 2012	11 Mar 2013	22.7 (0.09)	96.1
IRGC 117276	Sadu-cho	indica	2014 (A)	30 Nov 2013	21 Dec 2013	26 Mar 2014	25.7 (0.15)	97.6
			2014 (B)	01 Jan 2014	22 Jan 2014	01 May 2014	23.3 (0.10)	99.7
			2013	23 Nov 2012	18 Dec 2012	27 Mar 2013	13.2 (0.09)	67.8
IRGC 117280	Zhenshan 97B	indica	2014 (A)	30 Nov 2013	21 Dec 2013	26 Mar 2014	25.6 (0.11)	97.1
			2014 (B)	06 Jan 2014	27 Jan 2014	30 Apr 2014	18.9 (0.17)	84.4
			2013	23 Nov 2012	18 Dec 2012	14 Mar 2013	23.3 (0.24)	96.1

dryroom (DR) maintained at 15°C/15% RH. The following morning (0800 hrs) after 16 h in the DR, four of the five samples were removed and subjected to different treatments (Box 3.1). Two of the samples removed from the DR were placed in the batch dryer (BD) for 8 h high temperature (approximately 45°C) drying. The remaining two samples were transferred to sealed electrical enclosure boxes where they lay suspended above water for 7 days at room temperature (21.5°C) to rehydrate (R). At the end of the 8 h drying cycle in the BD (0800 – 1600 hrs), one of the two samples was transferred back to the DR where the seeds remained until equilibrium**,** and the other sample was rehydrated for 7 days prior to equilibrium drying in the DR. The samples which were rehydrated for 7 days immediately after the initial DR period (16 h) were both then transferred to the BD for a cycle of drying before one sample underwent an additional 7-day rehydration cycle prior to final drying in the DR and the other sample was transferred directly from the BD to the DR.

Box 3.1. Drying treatments: sequence of drying room (DR; 15°C/15% RH), batch dryer (BD; 45°C) and/or rehydration (R; 100% RH) treatments. Initial DR-drying occurred for 16 h, BD-drying for 8 h, and rehydration for 7 days. All seeds experienced final drying in the DR for a further 14 days.

Treatments:
a. Dryroom
b. Dryroom_Batch dryer_Dryroom
c. Dryroom_Batch dryer_Rehydration_Dryroom
d. Dryroom_Rehydration_Batch dryer_Dryroom
e. Dryroom_Rehydration_Batch dryer_Rehydration_Dryroom

Between each drying or rehydration stage, seed samples were weighed and the eRH measured at room temperature. All samples completed their last stage of drying in the genebank DR irrespective of whether they had received a high temperature drying and/or rehydration cycle (Box 3.1). Once seeds had equilibrated in the DR, the eRH was checked (in the DR; 15°C/15% RH) using a portable hygroclip SP05 water activity probe used in

conjunction with a Hygropalm AWI display unit (Rotronic South East Asia Pte. Ltd., Singapore), before they were manually sorted, discarding any infected, empty or immature seeds (as in Chapter 2). The clean seeds were sealed inside 0.24 × 0.16 m (L × W) laminated aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until they were required for storage experiments which commenced in June 2014.

3.2.4. Seed storage

Seeds of each treatment combination (accession [3] × drying treatment [5]) were removed from cold storage (2-4°C) and equilibrated to room temperature (21.5°C) before opening. Each sample was split into 29 × 5 g subsamples which were placed into 30 mm-diameter open Petri dishes. The dishes were then placed in a VC^3 0034-M climate chamber (Vötsch Industrietechnik, Germany) set at 60% RH and 21.5°C where they remained for 4-5 days to approach equilibrium, resulting in a moisture content of approximately 10.9%. The RH and temperature conditions were monitored using a QRDL datalogger (Centor Thai, Bangkok, Thailand) and the weight of three 5 g subsamples positioned approximately in the middle of the chamber were monitored daily. Moisture uptake ceased after between 4 and 5 days with seeds showing no further increase in weight.

Once equilibrium had been reached, four of the 5 g subsamples from each treatment combination were removed to measure seed eRH after which three of these were used to determine MC and the fourth to estimate initial germination (prior to experimental storage). The remaining subsamples (25) were each sealed inside individual laminated aluminium foil packets (0.11 × 0.08 m [L × W]) (Moore and Buckle) before being placed in an incubator at 45°C. One packet per treatment combination was removed at 3-day intervals up to 54 days for germination testing. For some seed lots, where viability was lost before day-54, sampling was discontinued earlier; for other seed lots, later samples were at longer intervals due to a slow rate of viability loss, therefore germination tests were made after storage day-54 in some samples. At mid-storage (day-27) and end of storage (day-54), moisture content determinations were conducted using three additional 5 g packets of seeds each time.

3.2.5. Seed germination

The protocol for seed germination testing was as described in Chapter 2.

3.2.6. Statistical analysis

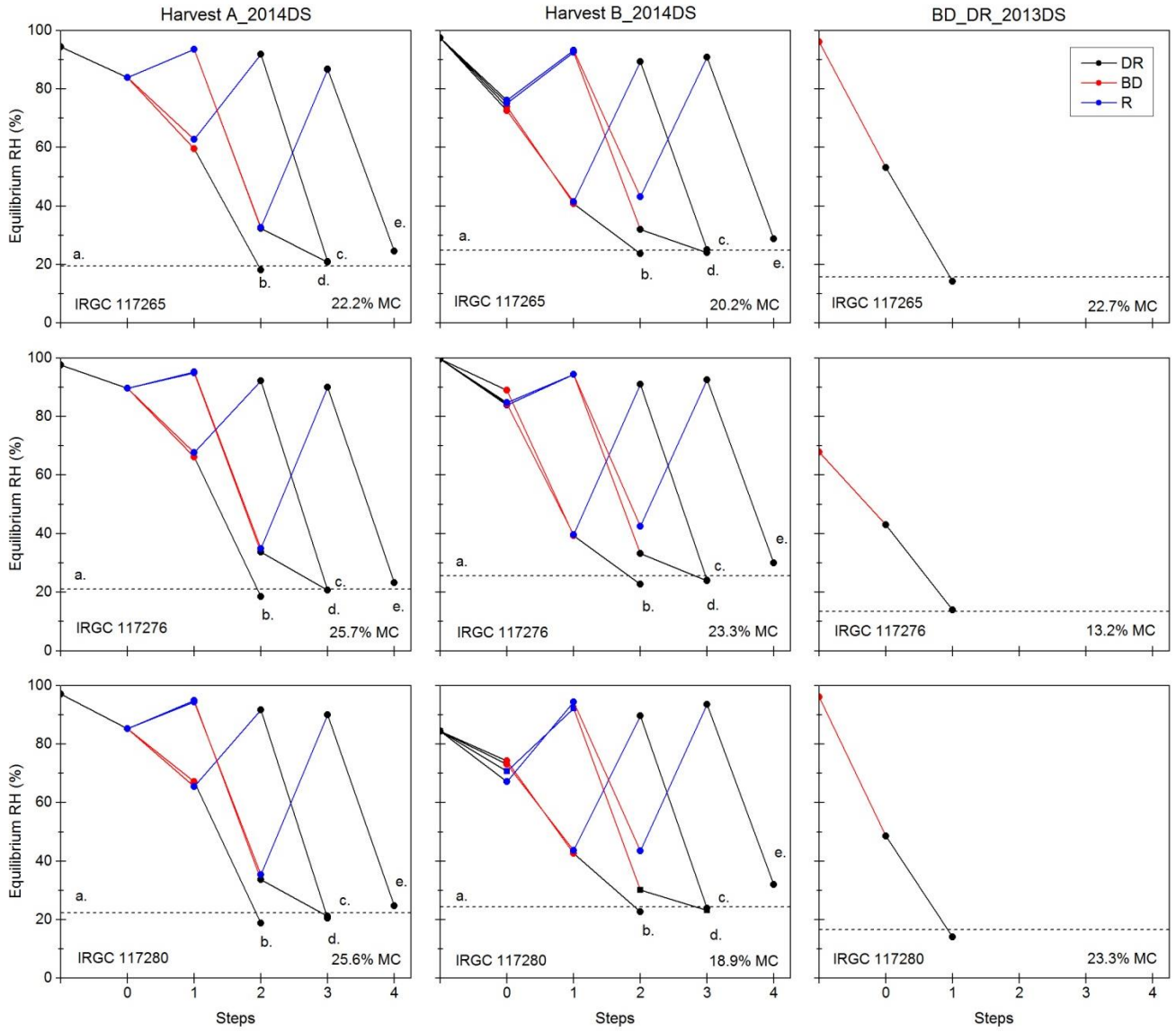
Probit analysis, fitting the Ellis and Roberts (1980a) viability equation (equation [1]) as in Chapter 2, was carried out using GenStat for Windows, Version 15 (VSN International Ltd., Oxford, UK) to estimate the period (days) for viability to fall to 50% (p_{50}), K_i and σ. For those seed lots which showed a reduced initial viability, asymmetry in the survival data (not symmetrical about 50%) and a systematic pattern of residuals when fitting equation [1] (accessions IRGC 117276 and -80), the "control mortality" parameter ("immunity" in GenStat) was included in the probit analysis. The control mortality parameter is the estimate of the proportion of "non-responding" seeds within the population (Mead and Gray, 1999). Furthermore, as in Chapter 2, data for seeds of accession IRGC 117265 which showed a loss in dormancy during storage was fitted using a probit combined loss in dormancy and loss in viability model (equation [7]) using the FITNONLINEAR directive in GenStat.

3.3. Results

3.3.1. Change in eRH during the various post-harvest treatments

The change in the eRH of seeds subjected to different post-harvest treatments was highly consistent between accessions in each harvest (Figure 3.1). Likewise, across different drying/rehydration regimes, the same treatment step had a similar effect on eRH for a given accession × harvest. For example, the eRH of seed lots from accession IRGC 117276 from harvest A after being dried in the BD following initial drying in the DR (DR_BD_DR; DR_BD_R_DR) were 66.2% (eMC 14.4%) and 67.6% (eMC 14.4%), respectively. However when the timings of such treatments differed between seed lots, there were substantial differences in eRH (Figure 3.1). Seeds which experienced high temperature drying after a 7-day rehydration period (DR_R_BD_DR; DR_R_BD_R_DR) dried to a much lower level in comparison with seeds that had been dried in the BD after initial drying in the DR without

Figure 3.1. Changes in eRH of rice seeds of three accessions harvested on two separate dates during the 2014 dry season (DS) and subjected to five different drying/rehydration regimes (a-e; columns 1 and 2), and of the same three accessions harvested during the 2013 DS (column 3) subjected to immediate high temperature drying (BD; 8 h) prior to final drying in the dryroom (DR) (BD_DR; Chapter 2). The data point at step 0 represents the eRH after drying either in the DR for 16 h (first two columns from the left) or after drying in the BD for 8 h (third column). The dashed line in each graph represents the eRH of the DR control sample after 14 days. The eRH for all seed lots was measured at room temperature, except when the BD_DR seed lots were transferred to the DR, the seeds were then measured under the DR conditions (15°C/15% RH) using a portable water activity reader. In all graphs the initial data point on the y-axis represents the eRH of the seeds at harvest. The values displayed are the mean eRH ± s.e. (too small to show; Appendix 3.1) Harvest moisture content (MC; % fresh weight) is indicated in the lower right-hand corner of each graph.


Harvest A_2014DS
Harvest B_2014DS
BD_DR_2013DS
DR
BD
R
Equilibrium RH (%)
Steps
IRGC 117265
22.2% MC
IRGC 117265
20.2% MC
IRGC 117265
22.7% MC
IRGC 117276
25.7% MC
IRGC 117276
23.3% MC
IRGC 117276
13.2% MC
IRGC 117280
25.6% MC
IRGC 117280
18.9% MC
IRGC 117280
23.3% MC
a.
b.
c.
d.
e.

a rehydration step (DR_BD_DR; DR_BD_R_DR), despite the duration of drying being the same for both sets of treatments. Further to this, the difference in eRH between these seed lots (DR_BD_DR and DR_R_BD_DR) was greater from harvest A compared with harvest B (Figure 3.1) with eRH values from accession IRGC 117265 differing by 27.2% (4.6% difference in eMC) and 8.8% (1% difference in eMC), respectively. This effect was less prominent between the different rehydrated seeds lots from each accession × harvest. After a 7-day rehydration period the eRH of the different seed lots, although similar, were slightly higher in seeds which experienced rehydration immediately after initial drying in the DR. However, once seeds were transferred to the DR for final drying, they all equilibrated to the same level after 14 days, which was similar to the DR control, despite the MC of the seeds differing substantially prior to equilibrium drying.

Seed lots with the same post-harvest treatment schedule, but from different harvests responded differently to the various treatments. Generally seeds from harvest B showed a greater percentage loss in moisture during drying and a greater percentage gain in eRH during rehydration compared with seeds from harvest A.

Compared with the BD_DR seed lots from each accession, immediate drying in the DR for 16 h after harvest did not dry seeds to such low levels in comparison to when seeds were dried immediately in the BD for 8 h. The eRH of DR_BD_DR seeds after step 1 (DR-drying) were 73.9, 88.9 and 74.3% for accessions IRGC 117265, -76 and -80, respectively, compared with 53.2, 67.8 and 48.5% for BD_DR seeds. Further to this, the rate of water loss was also reduced for seeds in the DR_BD_DR treatment when they were placed in the BD compared with seeds that were immediately dried at the high temperature after harvest, with the exception of seeds from accession IRGC 117276 from harvest B.

3.3.2. Sorption isotherms

The MC-eRH relationship for seeds after different steps in the various drying/rehydration regimes generally appeared to be consistent with the moisture desorption isotherm determined for the seed lots described in Chapter 2, with the exception of seeds which had only experienced drying, most notably when seed MC/eRH was higher (i.e. >60% eRH;

Figure 3.2. The relationship between moisture content (MC) and equilibrium relative humidity (eRH) for seeds from three accessions × two harvests (2014 dry season). Seed MC was estimated (based on initial MC and change in sample weight) and eRH determined after each step (*) of the various drying/rehydration regimes: Initial (■), desorption (● ; DR*_DR*_BD*_DR*), desorption-adsorption (▲ ; DR_R*_BD_R_DR), desorption-adsorption-desorption (▼ ; DR_R_BD*_R_DR), desorption-adsorption-desorption-adsorption (◆ ; DR_R_BD_R*_DR) and desorption-adsorption-desorption-adsorption-desorption (◀ ; DR_R_BD_R_DR*). Hence, the data represent both de- and adsorption isotherms. For reference the desorption isotherm (dashed line) was determined for seeds lots described in Chapter 2 (BD_DR).

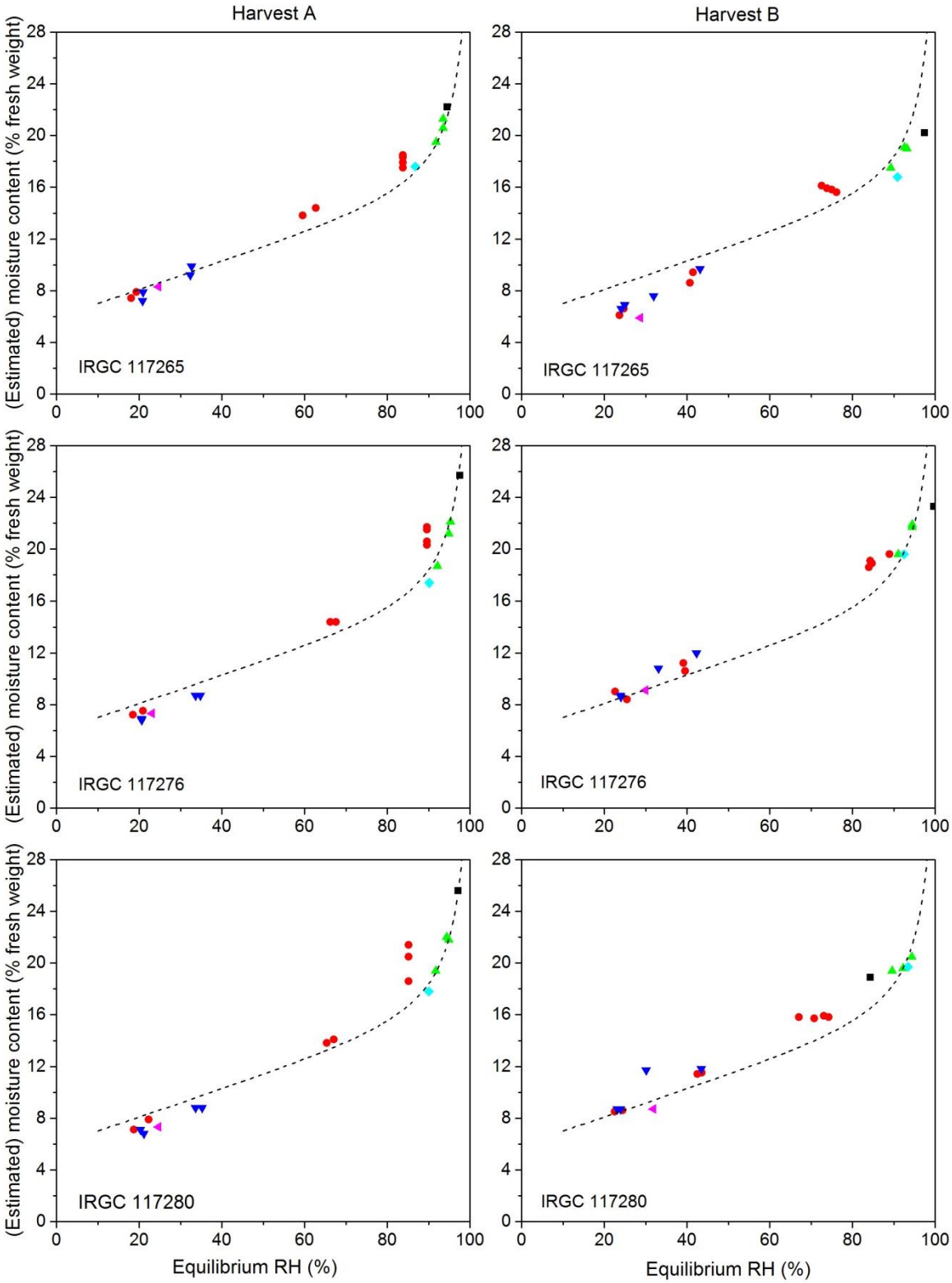

Harvest A
Harvest B
(Estimated) moisture content (% fresh weight)
IRGC 117265
IRGC 117276
IRGC 117280
Equilibrium RH (%)

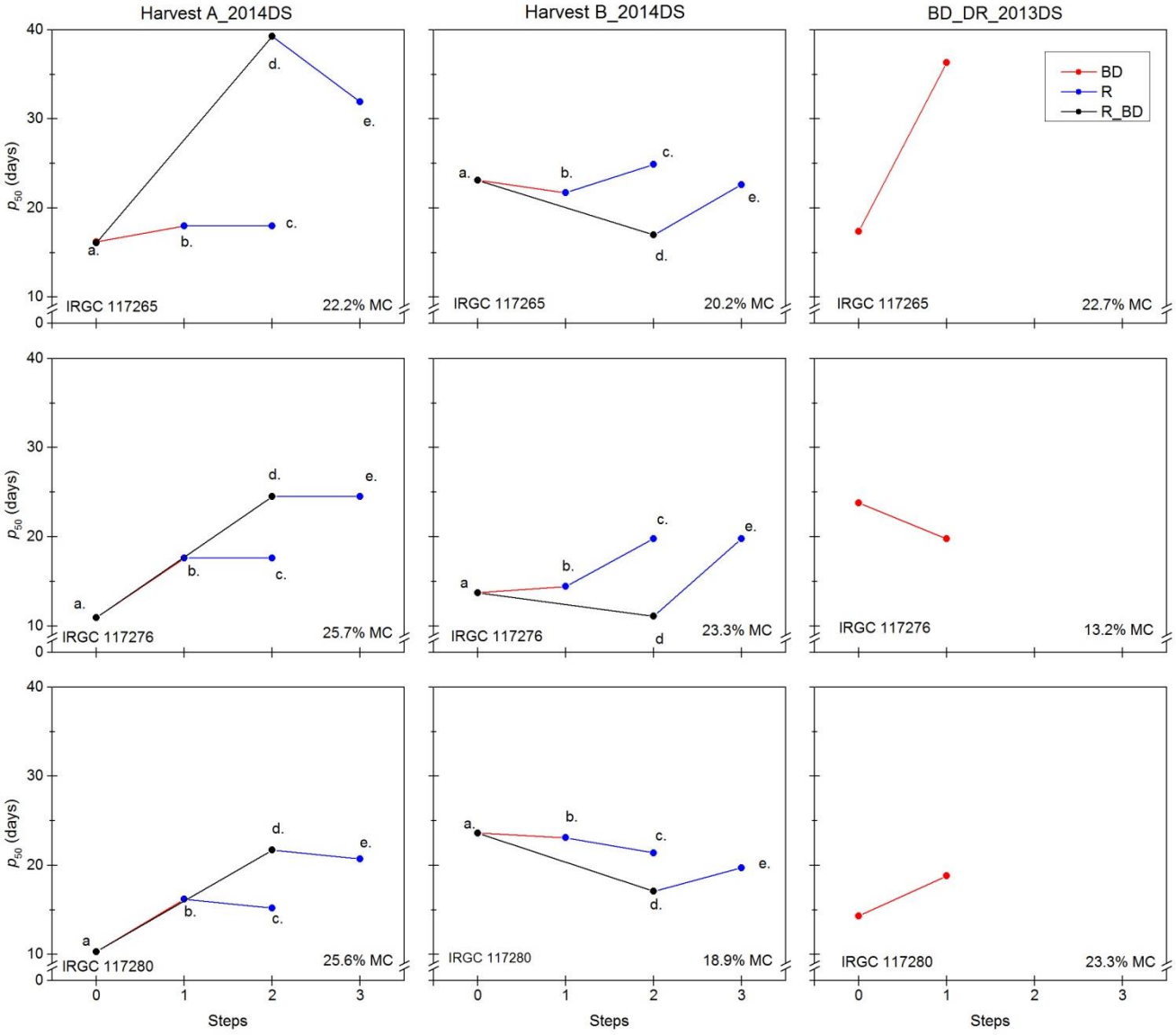
Figure 3.2). With respect to desorbing seeds, seeds which were rehydrated once (DR_R, DR_BD_R; desorption-adsorption) or twice (DR_R_BD_R; desorption-adsorption-desorption-adsorption) in between the post-harvest drying stages (DR_BD_DR) are lower indicating the effect of hysteresis (section 1.2.1).

3.3.3. The effect of rehydrating partially dried seeds (DR) prior to high temperature exposure (DR_R_BD_DR) on subsequent storage longevity

The mean seed moisture content during experimental storage across all seed lots from Harvests A and B was 11.0% (s.e. 0.02) and 11.1% (s.e. 0.03), respectively. During storage all seed lots showed a sigmoidal pattern of loss in viability with no viable seeds remaining after 60 days (Appendix 3.2).

Seed longevity varied between accessions and amongst the drying treatments within accessions (Appendix 3.3). However for all accessions harvested at the higher MC (harvest A), rehydrating seeds prior to re-drying in the BD improved their subsequent storage longevity compared with the other post-harvest treatments; including the DR control (Figure 3.3; Appendix 3.3). The estimated p_{50} values for those seed lots from harvest A were 39.3, 24.5 and 21.7 days for accessions IRGC 117265, -76 and 80, respectively, compared with 17, 11.1 and 17.1 days from harvest B (Appendix 3.3). The rehydration treatment increased K_i in all accessions, prolonging the shoulder of the survival period before viability began to decline. The rate of viability loss (σ^{-1}) was lower in accessions IRGC 117265 and -80 when seeds had experienced rehydration compared with the DR seeds, but higher in accession IRGC 117276 (Appendix 3.3). For accessions IRGC 117276 and -80 where the "control mortality" parameter was included in the models, rehydration reduced the proportion of responding seeds within each seed lot compared with when rehydration was not applied. However the higher K_i value of these rehydrated seed lots (DR_R_BD_DR) from harvest A indicates that a high proportion of the total responding seeds remained viable for longer during storage, shown by the long lag period (compared with the control; DR) (Appendices 3.2 and 3.3). Rehydration prior to re-drying in the BD reduced the storage potential of seed lots from harvest B, which were harvested at lower MCs, compared with non-rehydrated seed lots (DR_BD; DR) (Figure 3.3). Estimated p_{50}

Figure 3.3. The longevity (p_{50}) of rice seeds of three accessions × two harvests (2014 dry season) subjected to five different drying/rehydration regimes (a. DR; b. DR_BD_DR; c. DR_BD_R_DR d. DR_R_BD_DR; e. DR_R_BD_R_DR), and of the same three accessions (2013 dry season) subjected to immediate high temperature drying (BD; 8 h) prior to final drying in the dryroom (DR) (BD_DR; Chapter 2). The data point at step 0 represents the DR control and the other stages of the post-harvest treatments follow thereafter (steps, but later DR steps not shown). Seeds from harvest A were sown on the 30th November 2013 and harvested the 26th March 2014, and seeds from harvest B were sown on the 1st January (IRGC 117265, -76) or the 6th January 2014(IRGC 117280) and harvested 1st May 2014. Seeds from the 2013DS were sown on 23rd November 2012 and harvested on 11th, 27th or 14th March 2013 for accessions IRGC 117265, -76 and -80, respectively. The harvest moisture content (MC; % fresh weight) of the seeds is positioned in the lower right-hand corner.


Harvest A_2014DS
Harvest B_2014DS
BD_DR_2013DS
BD
R
R_BD
p_{50} (days)
Steps
IRGC 117265
22.2% MC
IRGC 117265
20.2% MC
IRGC 117265
22.7% MC
IRGC 117276
25.7% MC
IRGC 117276
23.3% MC
IRGC 117276
13.2% MC
IRGC 117280
25.6% MC
IRGC 117280
18.9% MC
IRGC 117280
23.3% MC

values were 6.1, 2.6 and 6.5 days lower for accessions IRGC 117265, -76 and -80, respectively compared with the DR control. Rehydrated seed lots had higher K_i values compared with the control (and a higher proportion of non-responding seeds) and lost viability faster (Appendix 3.3).

3.3.4. The effect of rehydration after high temperature exposure (DR_BD_R_DR) on subsequent storage longevity

For all seed lots in all accessions harvested at the higher MC (harvest A), rehydration increased the storage longevity compared with solely drying in the DR. Estimates of p_{50} were 1.8, 6.7 and 4.9 days higher in accessions IRGC 117265, -76 and -80, respectively (Appendix 3.3). Despite the higher proportion of non-responders in accessions IRGC 117276 and -80 compared with the control, the higher K_i values suggests that a high proportion of the responding seeds maintained viability after rehydration (Appendix 3.3). Furthermore, in accessions IRGC 117265 and -76, rehydration had no effect on the longevity compared with non-rehydrated seeds (DR_BD_DR) as both these survival curves could be constrained to a common line without a significant increase in residual deviance (P>0.05) (Appendices 3.2 and 3.3). In accession IRGC 117280 however, longevity of the rehydrated seed lot was slightly lower compared to non-rehydrated seeds, with estimates of p_{50} differing by 1 day (Figure 3.3).

Rehydrated seed lots (DR_BD_R_DR) from harvest B showed less of an improvement in longevity compared with the DR control than seed lots from harvest A. However, for all accessions the rehydrated seed lots showed a higher K_i value and for those accessions where the "control mortality" parameter was applied, a higher proportion of non-responders compared with the DR control. The values of σ^{-1} were 0.08, 0.11 and 0.04 $days^{-1}$ greater in the rehydrated seed lots compared with the control, for accessions IRGC 117265, -76 and -80, respectively.

3.3.5. The effect of rehydration before and after high temperature exposure (DR_R_BD_R_DR) on subsequent storage longevity

Two rehydration cycles (7 days each) between the post-harvest drying stages (i.e. inserted within DR_BD_DR) improved subsequent seed longevity compared with the control when seeds were harvested at higher MC (harvest A; Figure 3.3). Values of p_{50} were estimated at 15.7, 13.6 and 10.4 days greater for accessions IRGC 117265 -76 and -80, respectively (Appendix 3.3). The additional cycle of rehydration followed by drying increased the longevity compared with seeds that were rehydrated after drying in the BD (DR_BD_R_DR) in all accessions but reduced the longevity compared with seed lots rehydrated prior to high temperature drying (DR_R_BD_DR) in accessions IRGC 117265 and -80 (Appendix 3.2). In accession IRGC 117276 however, the survival curves for the two treatments (DR_R_BD_DR and DR_R_BD_R_DR) could be constrained to a common line, indicating that an additional rehydration cycle had no effect on subsequent longevity compared with only rehydrating seeds prior to high temperature drying (Appendix 3.2). In seed lots harvested at lower MCs two cycles of rehydration increased the rate of viability loss for all accessions compared with the DR controls. The higher K_i values and reduction in σ prolonged the shoulder of the survival curves and steepened the subsequent slope which led to the crossing of the two survival curves (DR_R_BD_R; DR) in accessions IRGC 117265 and -76, (Appendix 3.2). Longevity was reduced in IRGC 117265 and -80 compared with the control with the differences in p_{50} relative to the DR being -2.2 and -16.5%, respectively (Appendix 3.3).

3.4. Discussion

The results from Chapter 2 demonstrated the beneficial effects of drying seeds in the BD immediately after harvest. It was hypothesised that high temperature drying would only be beneficial to seeds which are dried immediately after harvest, or when they are at a pre-drying MC >16.2% (Chapter 2). However would rehydrating seeds, to ensure a MC greater than 16.2% prior to high temperature drying increase their longevity in storage?

3.4.1. The effect of rehydration during post-harvest drying (DR_BD_DR) on seed longevity

The results of this chapter demonstrate clear differences in how seeds respond to various post-harvest treatments, when harvested at different MCs. The MC of the seeds at harvest gives an indication of whether or not seeds are mature (and hence at risk of ageing and so potentially may also benefit from repair) or immature (and likely to benefit from conditions which might allow them to complete their development), and therefore of their initial quality. High quality seeds are seeds which have attained maximum ability to germinate, desiccation tolerance and longevity, and accumulated very little net damage to date. Powell *et al*. (2000) demonstrated the importance of initial quality which was shown to affect seeds response to post-harvest priming treatments. It was suggested that low vigour seeds would benefit more from priming than high vigour seeds due to their requirement for continued maturation if immature (Butler *et al*., 2009a, b) and/or repair processes (Powell *et al*., 2000). This was supported by the difference in longevity (p_{50}) between the seed lots harvested on different dates (differing in harvest MC) within each accession (Figure 3.3). For seeds harvested at the higher MC, although all post-harvest drying treatments increased subsequent seed storage longevity compared with the DR control, the greatest improvement was provided by the DR_R_BD_DR treatment (Figure 3.3) in which, longevity more than doubled. Improvement in p_{50} relative to the DR was 143, 125 and 111% in IRGC 117265, -76 and -80 respectively, however the DR-dried seeds for these high MC seed lots show the lowest values of $p_{50,}$ as reported in Chapter 2 (Figure 3.3; Appendix 3.2). The increase in K_i and extending of the survival curves was exaggerated by the increase in non-responders (Appendices 3.2 and 3.3). Therefore rehydration prior to high temperature drying was responsible for extending the longevity of a high proportion of the total responding seeds (short- and long-lived seeds) in storage. What is interesting to note is that those seeds which were dried in the BD following rehydration (DR_R_BD_DR; DR_R_BD_R_DR), dried to a much lower moisture content compared with seeds dried immediately in the BD after DR-drying (DR_BD_DR; DR_BD_R_DR) (Figure 3.1). This suggests that initially drying seeds in the DR altered their water binding properties which therefore resulted in water being less tightly bound upon rehydration. The eRH and moisture content of these de-adsorption seeds (Figure 3.2) aligned with the desorption isotherm (fitted using the BD_DR data from Chapter 2; Figure

3.2), but were slightly lower than the desorbing seeds from this experiment. However only 'partial' de- and adsorption isotherm data were available which limits the accuracy of observations.

Metabolic activity increases with increase in availability of water at eRH greater than 80-85% (Vertucci and Leopold, 1984, 1986). After partial drying in the DR, seed lots harvested at the higher MCs still had an eRH above 80% (Figure 3.1) and were therefore likely to still be metabolically active. Although it is thought that certain metabolic processes such as cellular repair are not fully functional until approximately 98% eRH, it is likely that the rehydration cycle after DR-drying brought seeds at least closer to reaching this fully functional capacity enabling them to initiate repair processes and to allow the continuation of maturation processes such as the accumulation of protectants which are involved in increasing the storability of seeds, both of which contribute to an increase in seed quality. In accordance with Butler *et al*. (2009), in an aged population, rehydration "rejuvenates" short-life seeds which are on the cusp of losing viability by allowing the seeds time to repair and to subsequently maintain viability during storage. Differences were observed in the longevity of seed lots which were rehydrated at different times (Figure 3.3). After a 7-day rehydration period each seed lot reached a similar high eRH (>80%), despite there being a considerable difference in the MC prior to rehydration (Figure 3.1). This indicates that the MC of the seeds after rehydration cannot explain the observed differences in longevity which instead are more likely to be attributed to the seeds response to prior experiences and their biochemical status. The beneficial effect of post-harvest rehydration/drying cycles on seeds harvested close to maturity depends on the level of damage the seeds have accumulated previously as a result of ageing. Seeds which have suffered a substantial amount of damage are not able to reach the same potential longevity that was previously attained. It has been suggested that the last aspects of seed quality which are attained are the first lost during ageing (Butler *et al*., 2009b). Contrastingly, post-harvest priming treatments can allow the continuation of seed development *ex planta* when seeds are harvested before they have attained maximum quality (Demir and Ellis, 1992a).

Improvements in seed quality are not infinite and presumably there is a "maximum longevity" that any developing cohort of seeds can attain which can explain why two

cycles of rehydration did not further improve seed storage longevity compared with some of the other post-harvest treatments (Figure 3.3; Appendix 3.2). An additional rehydration cycle during post-harvest drying improved subsequent seed longevity compared with the control when seeds were harvested at a higher MC but reduced the longevity of seeds harvested at a lower MC in the case of accessions IRGC 117265 and -80. The increase in K_i and σ^{-1} indicated that two cycles of rehydration was more beneficial to the short-lived seeds but reduced the longevity of the longer-lived seeds. As a consequence, the population becomes more uniform i.e. the seed lot is more homogenous, hence the steeper survival curve compared with the DR.

An increase in water content can weaken the glassy state which serves as a physical stabiliser and protector against deteriorative reactions in dry seeds (Bernal-Lugo and Leopold, 1998). This can cause seeds to transit from a state of relative stability to dynamic seed ageing. Therefore the overall response of a seed lot to post-harvest rehydration is dependent on the proportion of short- and long-lived individuals within the cohort as they will make the transition from a stable to an ageing state at different times during the rehydration period. This can explain the differences in longevity observed between the seed lots harvested on separate occasions (Figure 3.3). It is not known whether the effects of multiple cycles of rehydration and desiccation are additive in the seeds but past research on sugar-beet (Śliwińska and Jendrzejczak, 2002) and foxglove (Butler *et al*., 2009b) has suggested a cumulative effect. Parera and Cantlifee (1994) thought that upon rehydration, seeds resume the initial pre-emergence seedling processes from the point prior to the last dehydration period. Therefore it is possible that the longer-lived seeds within the seed lot were “over-advanced” by the second cycle of rehydration causing seeds to enter the germination phase where they lose desiccation tolerance. Bodsworth and Bewley (1981) found that some of the germination advancement gained by osmoprimed seeds could be lost upon re-drying.

3.4.2. The effect of re-drying on seed longevity

The method of re-drying after rehydration could be influencing the beneficial effects of rehydration on subsequent storage longevity. Results from priming studies have shown improvements in the longevity of seeds which are re-dried at a slower rate or held under

mild water stress post-priming (Bruggink *et al*., 1999; Gurusinghe and Bradford, 2001; Butler *et al*., 2009b). Such re-drying treatments are suggested to improve the tolerance of seeds to dehydration as it allows seeds to initiate protection mechanisms whilst slowly re-equilibrating with the environment (Kermode and Finch-Savage, 2002; Soeda *et al*., 2005; Butler *et al*., 2009b). Similarly, other research has shown that priming associated with a stress treatment, e.g. heat shock (Bruggink *et al*., 1999), can restore desiccation tolerance in some species by inducing the synthesis of LEA and/or heat shock proteins which confer protective mechanisms beneficial to storage longevity, or can lead to an increase in antioxidant production which could improve seeds' quality after re-drying (Lira *et al*., 2015).

In support of this, the results of this experiment show an improvement in the longevity of seeds harvested at a higher MC, but not at the lower MC, when re-dried post rehydration in the BD (DR_R_BD_DR) as opposed to in the DR (DR_BD_R_DR) (Figure 3.3; Appendix 3.2). However it is unclear whether the differences in longevity are due to the method of re-drying or timing of high temperature exposure (before or after rehydration). But if drying after rehydration is beneficial, it can be assumed to have a similar effect on the seeds as maturation drying *in situ*, as long as seeds have not reached a MC where they no longer show benefit from high temperature drying (16.2% MC; Whitehouse *et al*., 2015) or where they become developmentally fixed prior to rehydration. Continuous wetting and drying of barley seeds during maturation, due to frequent periods of rainfall, increased their subsequent storage longevity (Ellis and Pieta Filho, 1992).

Priming of already mature seeds (high vigour) has shown to have little or even a negative effect on subsequent storage longevity (Butler *et al*., 2009a). For example, pre-germination of barley seeds induced by rain close to harvest, increases the seeds initial quality (rapid and uniform germination) but reduces their potential longevity (K_i) affecting seed storability (Gualano *et al*., 2014). In relation to what has been discussed, based on the harvest moisture content of the seeds, seed lots from harvest B (lower MC category) are assumed to be more mature in terms of development of seed quality/longevity (although seeds were harvested at the same DAA), in comparison with seeds from harvest A due to the different environment experienced. This can explain the greater longevity of the DR control seed lots from harvest B and possibly why rehydration treatments

generally did not improve storage longevity further (Figure 3.4; Table 3.4). Once the abscission layer is formed, the MC of the seeds fluctuates with the rise and fall in RH. It is thought that these natural cycles of dehydration and rehydration could be having a priming effect on the seeds, with seeds being able to accrue longevity during moisture loss, and initiate repair upon rehydration when normal metabolism resumes. However, once seeds dry to an eRH of less than 80%, metabolic processes are no longer fully functional and hence seeds are unable to continue to increase in longevity. Therefore it is assumed that seeds which are at a lower MC at harvest are likely to have already acquired high initial seed quality (Whitehouse *et al*., 2015) and so are recommended to be dried immediately to equilibrium with 15% RH in the DR.

3.4.3. Initial drying in the DR vs. BD

The results from Chapter 2 demonstrated the beneficial effects of high temperature drying on subsequent seed longevity when rice seeds were harvested at a MC greater than 16.2% (Figure 3.4). Similarly, there were clear differences in the longevity of seeds dried in the BD after initial drying in the DR between the two harvests, within each accession. Although DR_BD_DR seeds harvested at the higher MC (harvest A) showed a lower longevity (p_{50}) in storage compared with seeds from harvest B (Figure 3.3; Appendix 3.2), they showed a greater improvement compared with the DR control. As each of the seed lots differed in their harvest MC it was possible to investigate whether this was a determining factor in how seeds respond to the high temperature drying after initial drying in the DR. The relative improvement in longevity (i.e. the difference in p_{50} between the seed lots which experienced a delay in high temperature drying [DR_BD_DR] and the DR, calculated as a proportion of DR p_{50}) was plotted against harvest moisture content, as in Chapter 2 (Figure 3.4). High temperature drying after initial drying in the DR can improve the subsequent storage longevity compared with solely drying in the DR when seeds are harvested at MCs above 21.8%. This breakpoint is much higher compared with when seeds are immediately placed in the BD (Chapter 2). The split line regression analysis from Chapter 2 showed an improvement in the longevity of BD_DR seeds when harvested at a MC greater than 16.2%. At moisture contents below these respective breakpoints seed lots, do not appear to benefit from high temperature drying. Previously,

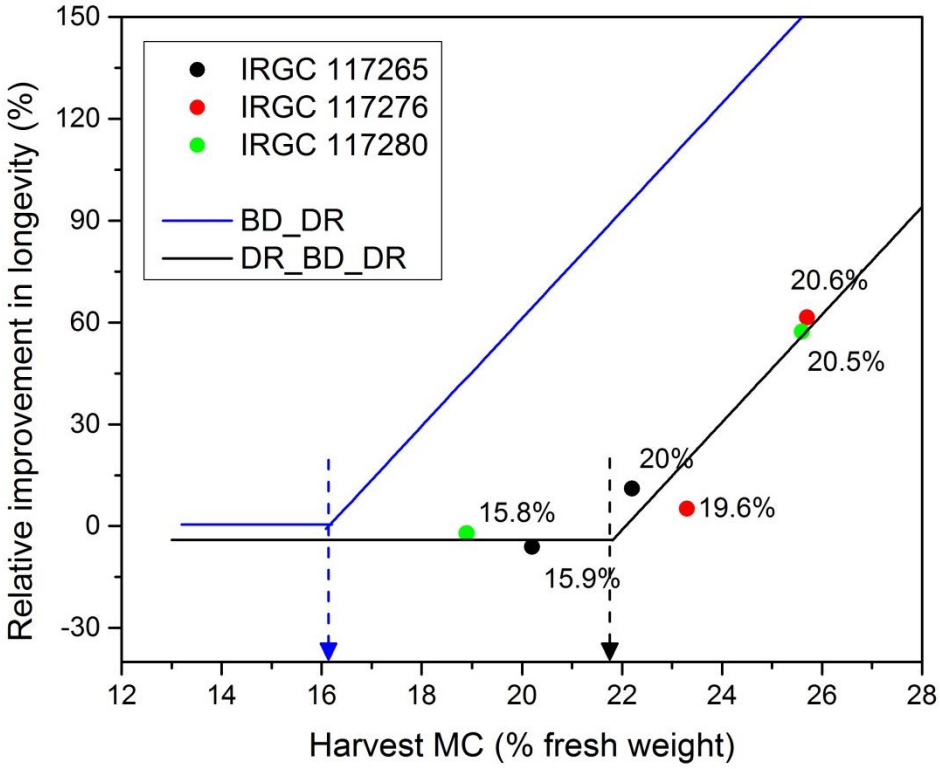


Figure 3.4. Relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between the two drying treatments calculated as a proportion of the DR p_{50} [[DR_BD_DR - DR p_{50}]/ DR p_{50}]; as in Chapter 2) for the three rice accessions and harvest moisture content (MC; % fresh weight). The blue line represents the split-line relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between the highest value from the BD treatments (BD p_{50}) and the DR treatment (DR p_{50}) for 20 rice accessions and initial moisture content from Chapter 2 (Figure 2.4). Constrained linear regression (slope the same as BD_DR seeds) was applied to the four data points which showed an improvement in longevity. The percentage values next to each data point represent the estimated moisture content (eMC; % fresh weight) of the seeds prior to drying in the BD. The harvest moisture content was determined using the high-temperature oven method (ISTA, 2013). The eMC was calculated from the moisture content at harvest and subsequent change in sample weight. The dashed lines indicate the respective breakpoints when drying under each regime. A relative improvement in longevity of 100% is equivalent to a doubling in longevity compared with DR treatments.

the benefits of high temperature drying were only apparent in seed lots which had a harvest MC (or "predrying MC") greater than 16.2% (see Chapter 2) but when exposing seeds, which had been intially dried in the DR, to high temperature drying this breakpoint shifted (Figure 3.4). Seeds with a harvest MC below 21.8% reached MCs which were too low (<16.2%) after 16 h of drying in the DR to benefit from high temperature drying. In comparison, seeds with a harvest MC greater than 21.8% were still at a MC which coincided with the part of the moisture sorption isotherm (Figure 3.2) where seeds are still metabolically active and therefore the longevity of these seeds increased upon high temperature exposure. The graph highlights the importance of harvest MC rather than the MC prior to high temperature exposure, as at any harvest MC greater than 16.2% seeds will benefit more from immediate drying in the BD compared with delayed high temperature drying (Figure 3.4).

To conclude, the estimates of p_{50} were greater when seeds were immediately dried in the BD for one day compared with seeds that experienced a delay in high temperature drying, with the exception of the DR_BD_DR seed lot from accession 117280 from harvest B (Figure 3.3). Further to this, the longevity of the BD_DR seeds from accession IRGC 117276 was slightly lower in comparison to the DR control. The estimates of p_{50} were 19.8 and 23.8 days for the BD_DR and DR seeds respectively (Figure 3.3). However despite what seems to be negative results, the estimates of p_{50} of BD_DR seeds from accessions IRGC 117276 and IRGC 117280 increased with the duration of drying. The highest values being recorded after three days (24.1 days; IRGC 117276) and six days (27.9 days; IRGC 117280) (Chapter 2; Table 2.6) which were not only higher than the DR control (Table 2.6) for each accession but also were higher compared to the DR_BD_DR seeds from both harvests. Therefore, when comparing the improvement in longevity (based on the highest p_{50} values) of seed lots dried according to either regime, immedately drying in the BD (for up to 6 days) yields a much greater improvement in longevity at any given harvest MC i.e. there is no added benefit to initially drying seed in the DR (Figure 3.3).

CHAPTER 4

THE EFFECT OF RE-HYDRATING RICE SEEDS (*ORYZA SATIVA* L.) AFTER DIFFERING DURATIONS OF DRYING IN THE DRYROOM OR BATCH DRYER

4.1. Introduction

The previous chapter reported the differing response of seeds to rehydration during the post-harvest drying phase. The within accession variability observed was attributed to differences in harvest moisture content which indicated seeds may be at different stages of maturity and therefore respond differently to post-harvest treatments.

During the post-abscission phase of seed development, seed quality continues to increase (Ellis *et al*., 1993a, b), providing seeds are metabolically active, until the "maximum" is reached for that cohort of seeds. The maximum quality attained *in situ* is dependent upon the genotype, the pre-harvest environment and the interaction between them. The continuation of such developmental events can continue *ex planta* if seeds are harvested prematurely and held at conditions similar to what they would naturally encounter *in situ* (Hay, 1997; Hay and Probert, 2005; Probert *et al.*, 2007; Butler *et al*., 2009a), or upon rehydration if seeds were dried too quickly for maximum quality to be attained (Butler *et al*., 2009a). However, if seeds have already attained maximum quality (mature seeds) such moist post-harvest treatments may lead to seed ageing or, in the case of rehydration, an over-advancement of seeds whereby they lose desiccation tolerance and storage potential (Hong and Ellis, 1992b).

4.1.1. Objectives and Hypotheses

The aim of the experiment described in this chapter was to investigate the effects of rehydration after differing durations of post-harvest drying in the batch dryer (BD) or the dryroom (DR) on subsequent seed quality and longevity, and to determine whether or not

seeds (harvested at different moisture contents) can resume maturation events and/or repair processes after differing durations of drying and re-drying.

Hypothesis 1: Rehydrating seeds after differing durations of drying in the batch dryer or dryroom will improve their storage longevity storage compared with non-rehydrated seeds.

4.2. Materials and methods

Seeds of accessions IRGC 117265, -76 and -80 were planted on two separate dates to achieve two 2014 dry season (DS) harvests (A and B) for each accession, both made at 35 days after 50% anthesis (DAA), differing in harvest moisture content (Table 4.1).

Immediately after harvest, the seeds were given the same post-harvest protocol as stated in Chapter 2 (Appendix 2.1). After being threshed and blown, equilibrium relative humidity (eRH) was measured (as described in Chapter 2; section 2.2.2) and moisture content (MC; % fresh weight) determined (Appendix 2.2).

4.2.1. Seed drying and rehydration

Seeds of each accession × harvest were divided into ten 200 g samples and placed into nylon mesh bags (as described in the previous chapters). All seed samples were stored in electrical enclosure boxes (as before) at room temperature (21.5°C) until the following morning when the drying treatments began. At 0800 hrs, four of the samples from each accession × harvest were transferred to the DR maintained at 15°C/15% RH and the remaining samples were placed in the BD (45°C). Of the samples transferred to the DR, one for each accession acted as the control and so remained in the DR until equilibrium was reached. After 8 h of drying in the DR (1600 hrs) one sample from each accession was removed and the eRH measured (as previously described) and weight recorded before they were transferred to a hermetically-sealed box (0.6 × 0.3 × 0.132 m [L × W × H]) where they were held over water at room temperature for 7 days to rehydrate (as Chapter 3). This same protocol was followed after 3 and 6 days of drying in the DR; at

Table 4.1. Information of the three rice (*Oryza sativa*) seed lots used in this experiment showing dates (December 2013-May 2014) of sowing, transplanting, and harvest. The initial seed moisture content (MC; % fresh weight) and equilibrium relative humidity (eRH) was measured at harvest.

Accession	Variety name	Variety group	Harvest	Sowing date	Transplanting date	Harvest date	MC (s.e.) (% f.wt.)	eRH (%)
IRGC 117265	Dom-sufid	aromatic	A	11 Dec	01 Jan	01 Apr	20.5 (0.34)	96.1
			B	11 Jan	01 Feb	13 May	17.2 (0.15)	85.8
IRGC 117276	Sadu-cho	indica	A	11 Dec	01 Jan	03 Apr	20.7 (0.06)	95.1
			B	18 Jan	08 Feb	13 May	17.4 (0.10)	82.6
IRGC 117280	Zhenshan 97B	indica	A	16 Dec	06 Jan	03 Apr	23.6 (0.19)	96.6
			B	25 Jan	22 Feb	13 May	15.4 (0.06)	73.8

1600 hrs on each of the specified days, a sample from each accession was removed from the DR and allowed to rehydrate. After each of the three samples per accession had undergone a 7-day rehydration cycle, the eRH was measured and weight re-recorded before they were returned to the DR to dry to equilibrium.

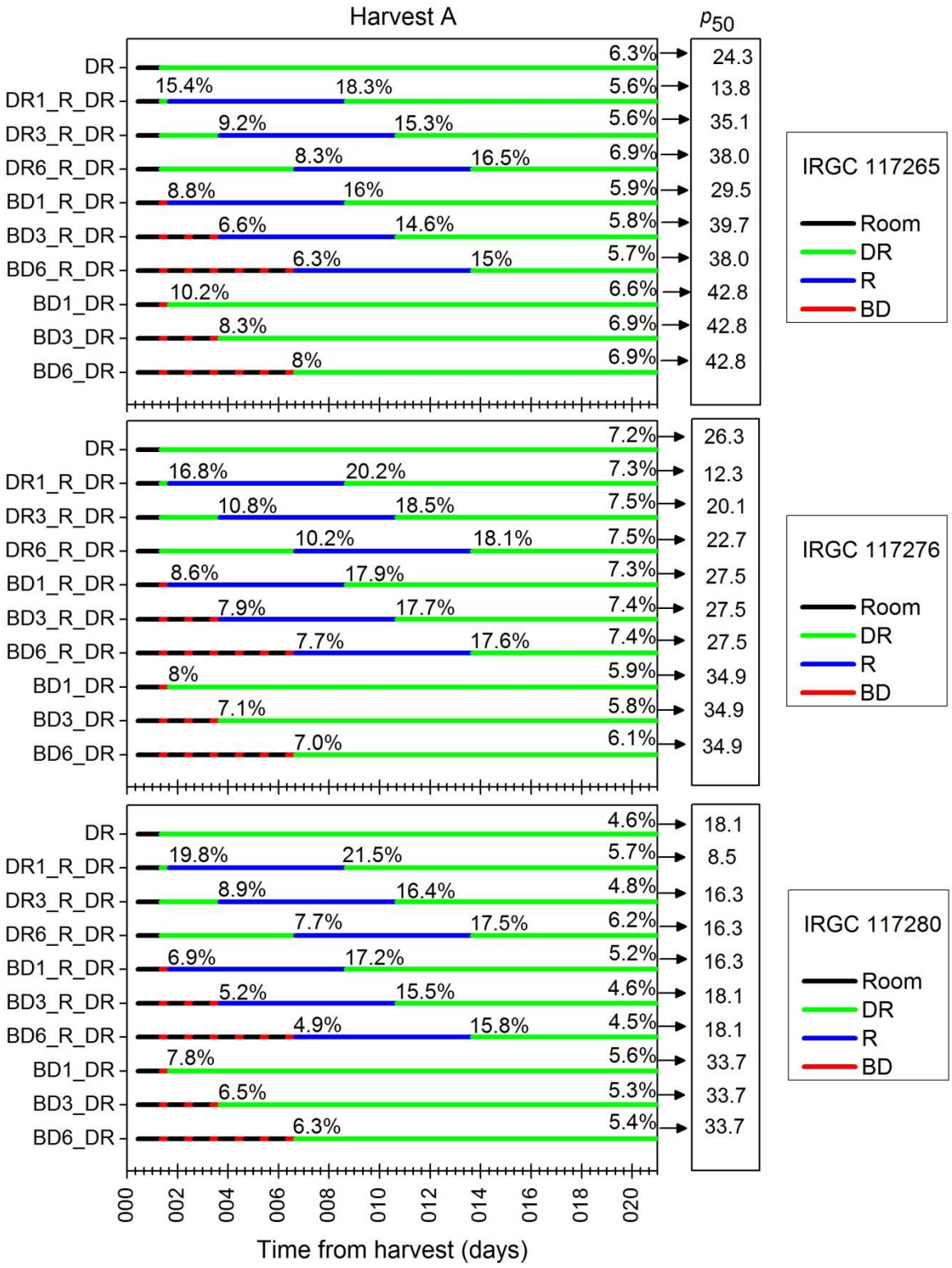
Of the samples which were dried in the BD (see Chapters 2 and 3) following harvest, as opposed to the DR, two from each accession × harvest were removed after 8 h high temperature drying and the eRH and weight was measured. One of the samples from each accession × harvest was transferred to a hermetically sealed box to rehydrate for 7 days and the other was transferred immediately to the DR where it remained until equilibrium. The remaining samples from the BD were sealed inside empty electrical enclosure boxes at room temperature overnight (1600–0800 hrs) before they were returned to the BD for the next 8 h high temperature drying cycle. After the third and sixth cycles in the BD, the same protocol for the seed samples were followed as described above. As with the DR-dried samples, the eRH and sample weight was determined after rehydration, before seeds were placed for final drying in the DR.

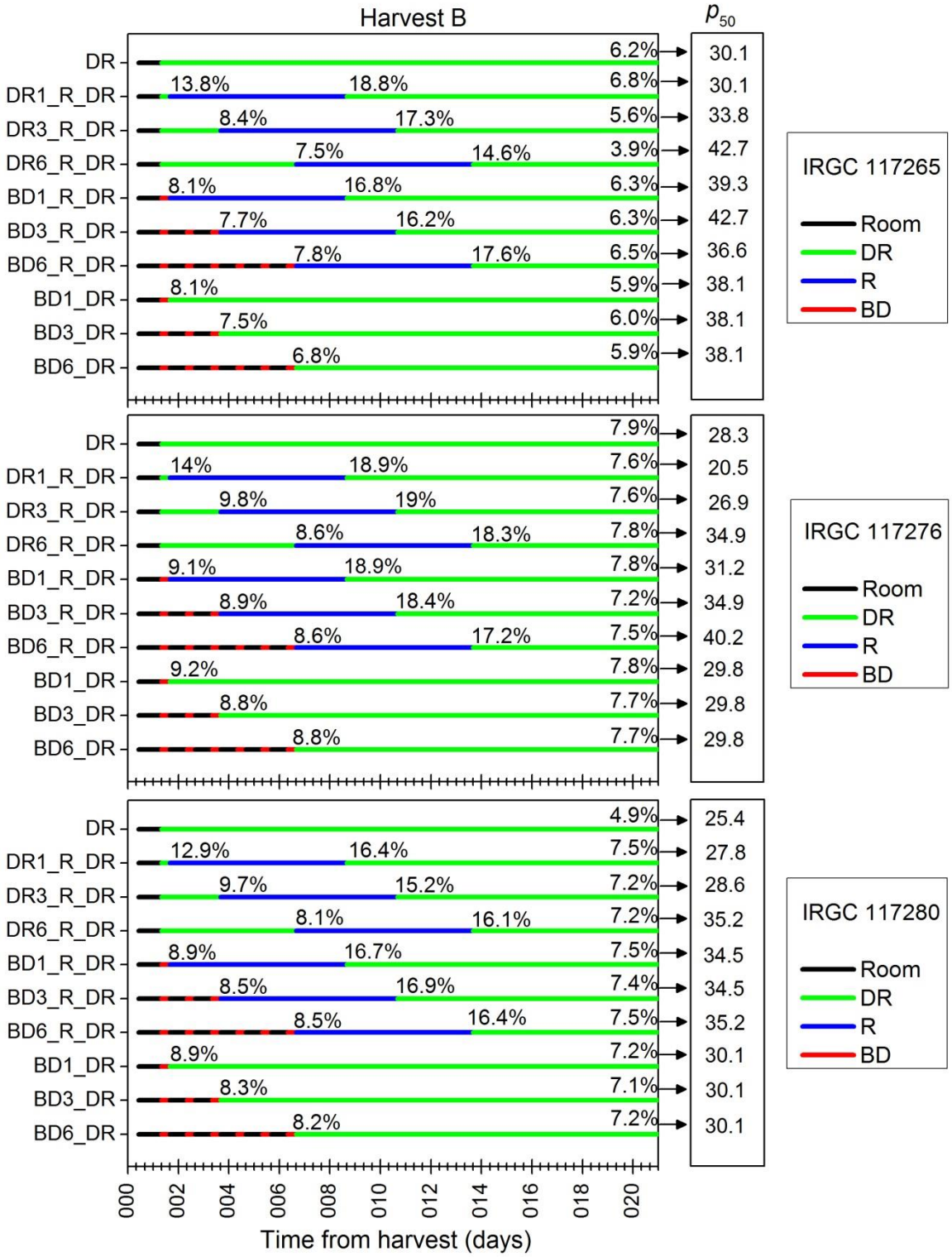
To summarise, each accession from each harvest consisted of six samples that had been dried using the BD and three samples that had been dried in the DR for 1, 3 or 6, 8-hour cycles. The DR sample and one of the BD samples for each drying period were rehydrated for 7 days following drying in either regime before final drying in the DR (Figure 4.1). Once all samples had equilibrated in the DR, they were sealed inside laminated aluminium foil packets and stored at 2-4°C until experimental seed storage.

4.2.2. Seed storage

The same seed storage protocol was followed as described in Chapter 3. The seeds from each treatment combination (accession [3] × drying-rehydration treatment [10]) were equilibrated in the VC3 0034-M climate chamber (Vötsch Industrietechnik, Germany) to 60% RH and at 21.5°C resulting in a MC of approximately 10.9% before subsamples were sealed inside individual aluminium foil packets and placed in an incubator at 45°C. A sample was removed for germination testing (as described in Chapter 3) at 3-day intervals until viability was lost. The interval period was lengthened in seed lots showing a slow

Figure 4.1. The order and duration of the post-harvest drying treatments for accessions from harvests A and B. Harvest moisture content (MC; % fresh weight) of each seed lot from harvest A was 20.5, 20.7 and 23.6%, and from harvest B was 17.2, 17.4 and 15.4% for accessions IRGC 117265, -76 and -80, respectively. The MCs were estimated from initial MC and the change in sample weight of the seed samples after drying/rehydration is positioned above the line. The longevity (p_{50}) of each seed lot is shown in the boxes on the right. The fitted curves (Appendix 4.1) are quantified in Appendix 4.2.


Harvest A
p_{50}
DR
DR1_R_DR
DR3_R_DR
DR6_R_DR
BD1_R_DR
BD3_R_DR
BD6_R_DR
BD1_DR
BD3_DR
BD6_DR
6.3%
24.3
15.4%
18.3%
5.6%
13.8
9.2%
15.3%
5.6%
35.1
8.3%
16.5%
6.9%
38.0
8.8%
16%
5.9%
29.5
6.6%
14.6%
5.8%
39.7
6.3%
15%
5.7%
38.0
10.2%
6.6%
42.8
8.3%
6.9%
42.8
8%
6.9%
42.8
IRGC 117265
Room
DR
R
BD
7.2%
26.3
16.8%
20.2%
7.3%
12.3
10.8%
18.5%
7.5%
20.1
10.2%
18.1%
7.5%
22.7
8.6%
17.9%
7.3%
27.5
7.9%
17.7%
7.4%
27.5
7.7%
17.6%
7.4%
27.5
8%
5.9%
34.9
7.1%
5.8%
34.9
7.0%
6.1%
34.9
IRGC 117276
Room
DR
R
BD
4.6%
18.1
19.8%
21.5%
5.7%
8.5
8.9%
16.4%
4.8%
16.3
7.7%
17.5%
6.2%
16.3
6.9%
17.2%
5.2%
16.3
5.2%
15.5%
4.6%
18.1
4.9%
15.8%
4.5%
18.1
7.8%
5.6%
33.7
6.5%
5.3%
33.7
6.3%
5.4%
33.7
IRGC 117280
Room
DR
R
BD
000
002
004
006
008
010
012
014
016
018
020
Time from harvest (days)


Harvest B
p_{50}
DR
DR1_R_DR
DR3_R_DR
DR6_R_DR
BD1_R_DR
BD3_R_DR
BD6_R_DR
BD1_DR
BD3_DR
BD6_DR
6.2%
13.8%
18.8%
6.8%
8.4%
17.3%
5.6%
7.5%
14.6%
3.9%
8.1%
16.8%
6.3%
7.7%
16.2%
6.3%
7.8%
17.6%
6.5%
8.1%
5.9%
7.5%
6.0%
6.8%
5.9%
30.1
30.1
33.8
42.7
39.3
42.7
36.6
38.1
38.1
38.1
IRGC 117265
Room
DR
R
BD
7.9%
14%
18.9%
7.6%
9.8%
19%
7.6%
8.6%
18.3%
7.8%
9.1%
18.9%
7.8%
8.9%
18.4%
7.2%
8.6%
17.2%
7.5%
9.2%
7.8%
8.8%
7.7%
8.8%
7.7%
28.3
20.5
26.9
34.9
31.2
34.9
40.2
29.8
29.8
29.8
IRGC 117276
Room
DR
R
BD
4.9%
12.9%
16.4%
7.5%
9.7%
15.2%
7.2%
8.1%
16.1%
7.2%
8.9%
16.7%
7.5%
8.5%
16.9%
7.4%
8.5%
16.4%
7.5%
8.9%
7.2%
8.3%
7.1%
8.2%
7.2%
25.4
27.8
28.6
35.2
34.5
34.5
35.2
30.1
30.1
30.1
IRGC 117280
Room
DR
R
BD
000
002
004
006
008
010
012
014
016
018
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Time from harvest (days)

rate of viability loss. Germination (criterion normal seed development) was scored after 3, 5, 7 and 14 before non-germinated seeds were dehulled and tested for an additional 7 days before final scoring. MC determinations (as described in previous chapters) were conducted using three 5 g replicates prior to storage and at the mid- and end storage points.

4.3. Results

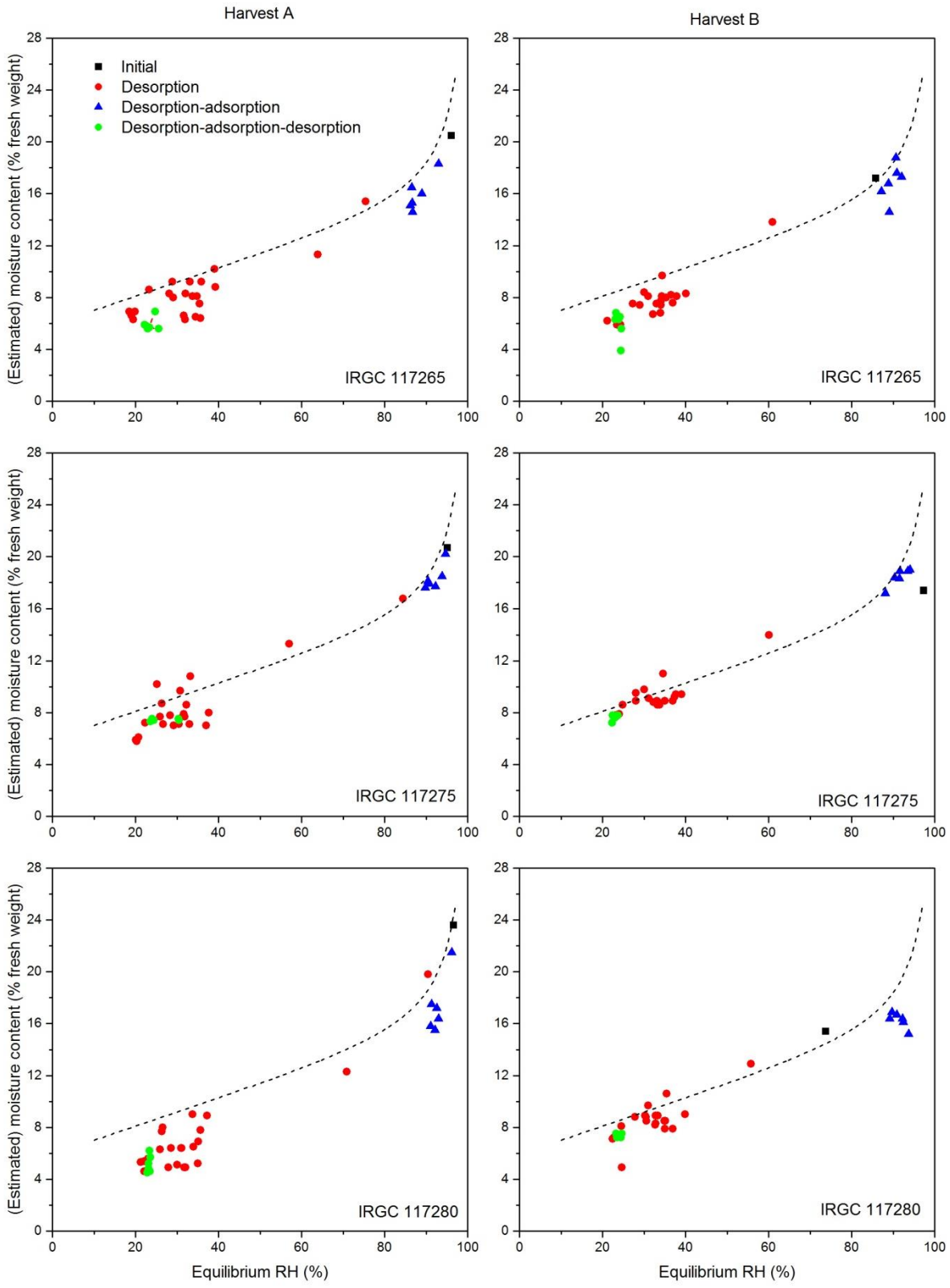
4.3.1. Loss of moisture during drying

Seeds reached a lower estimated moisture content (eMC; % fresh weight) after 1, 3 and 6 days of drying in the BD than those dried for these periods in the DR (Figure 4.1). All seeds lost the most moisture during the first day, irrespective of the drying regime, followed by a gradual decline. Seeds which had been dried for 1 day, in either the BD or the DR, reached a higher eMC after rehydration than seeds which had been dried for 3 or 6 days. However, all seeds, with exception to seeds of accession IRGC 117265 from harvest B which had been dried for 6 days in the DR prior to rehydration (DR6_R_DR), reached an eMC >15% after rehydration (Figure 4.1). All seed lots within each accession which were harvested at the lower MC (harvest B) reached a lower eMC after drying/rehydration compared with seeds which were harvested at a higher MC (harvest A). However once seeds were transferred to the DR for final drying, they all reached equilibrium within 14 days and were at a similar eMC as the DR control despite the eMC of the different seed lots differing substantially prior to equilibrium drying.

4.3.2. Sorption isotherms

The MC-eRH relationship of seeds after drying appeared to be consistent with the moisture desorption isotherm determined for the seed lots described in Chapter 2, with the exception of some of the seeds at low MC/eRH which were notably lower (Figure 4.2). Seeds which were rehydrated for 7 days (desorption-adsorption) were positioned on, or just below the moisture desorption isotherm. They were also slightly lower than the desorbing seeds in this study at high MC.

Figure 4.2. The relationship between moisture content (MC; % fresh weight) and equilibrium relative humidity (eRH) for seeds from the three accessions × two harvests. Seed MC was estimated (based on initial MC and the change in sample weight) and eRH was determined after each drying/rehydration treatment. The red symbols indicate seeds that had only experienced drying and were hence following a desorption isotherm; the blue symbols indicate the MC/eRH relationship for seeds that were adsorbing water following desorption; green symbols represent desorbing seeds following one cycle of desorption and adsorption. The black symbol is the MC/eRH of seeds at harvest.


Harvest A
Harvest B
Initial
Desorption
Desorption-adsorption
Desorption-adsorption-desorption
(Estimated) moisture content (% fresh weight)
Equilibrium RH (%)
IRGC 117265
IRGC 117275
IRGC 117280

4.3.3. Seed survival curves

Accession 117265 showed considerable dormancy prior to experimental storage, particularly for Harvest A, which was lost during early storage (Appendix 4.1). Seed survival curves, with or without early loss in dormancy, were described well by the approaches referred to in previous chapters. Substantial and significant differences in the fitted seed survival curves were detected amongst many treatments either in terms of K_i, σ^{-1}, or both ($P<0.05$) (Appendices 4.1 and 4.2). Where differences were not significant, this is highlighted in Appendix 4.2 by common estimates of K_i, σ^{-1}, or both.

4.3.3.1. Dryroom vs batch dryer (no rehydration)

Longevity was greater in DR-dried seeds which had been harvested at the lower MC (harvest B) and in BD seeds harvested at the higher MC (harvest A) (Figure 4.1). Of the seeds from harvest A, drying in the BD for 1, 3 or 6 days resulted in a greater longevity (p_{50}) compared with the DR in all accessions (Figure 4.1; Appendix 4.1). The survival curves for these seed lots could be constrained to a common line (Appendix 4.1) without a significant increase in the residual deviance (Appendix 4.2). Hence, drying seeds for more than one day provided no additional benefit to subsequent seed longevity. The increase in K_i and reduction in the rate of viability loss (σ^{-1}) compared with DR seeds resulted in an improvement in longevity of 76.1, 32.7 and 86.1% for accessions IRGC 117265, -76 and -80, respectively (Figure 4.3). As seed lots from harvest B showed a reduction in longevity when dried in the BD but an increase in longevity when dried in the DR (Figure 4.1), the relative improvement in longevity of BD seeds (BD_DR) was lower than that of seeds harvested at the higher MC (Figure 4.3). Despite this, estimates of p_{50} still exceeded that of the DR control resulting in improvement in longevity of 26.5, 5.3 and 18.5%, for accessions IRGC 117265, -76 and -80 respectively (Figure 4.3).

4.3.3.2. Batch dryer_rehydration_dryroom vs batch dryer (no rehydration)

Despite rehydration reducing the longevity of BD seeds from harvest A, the values of p_{50} still exceeded that of the DR control in accessions IRGC 117265 and -76 (Figures 4.1 and 4.3). There was no significant difference in the longevity of the seed lots which were

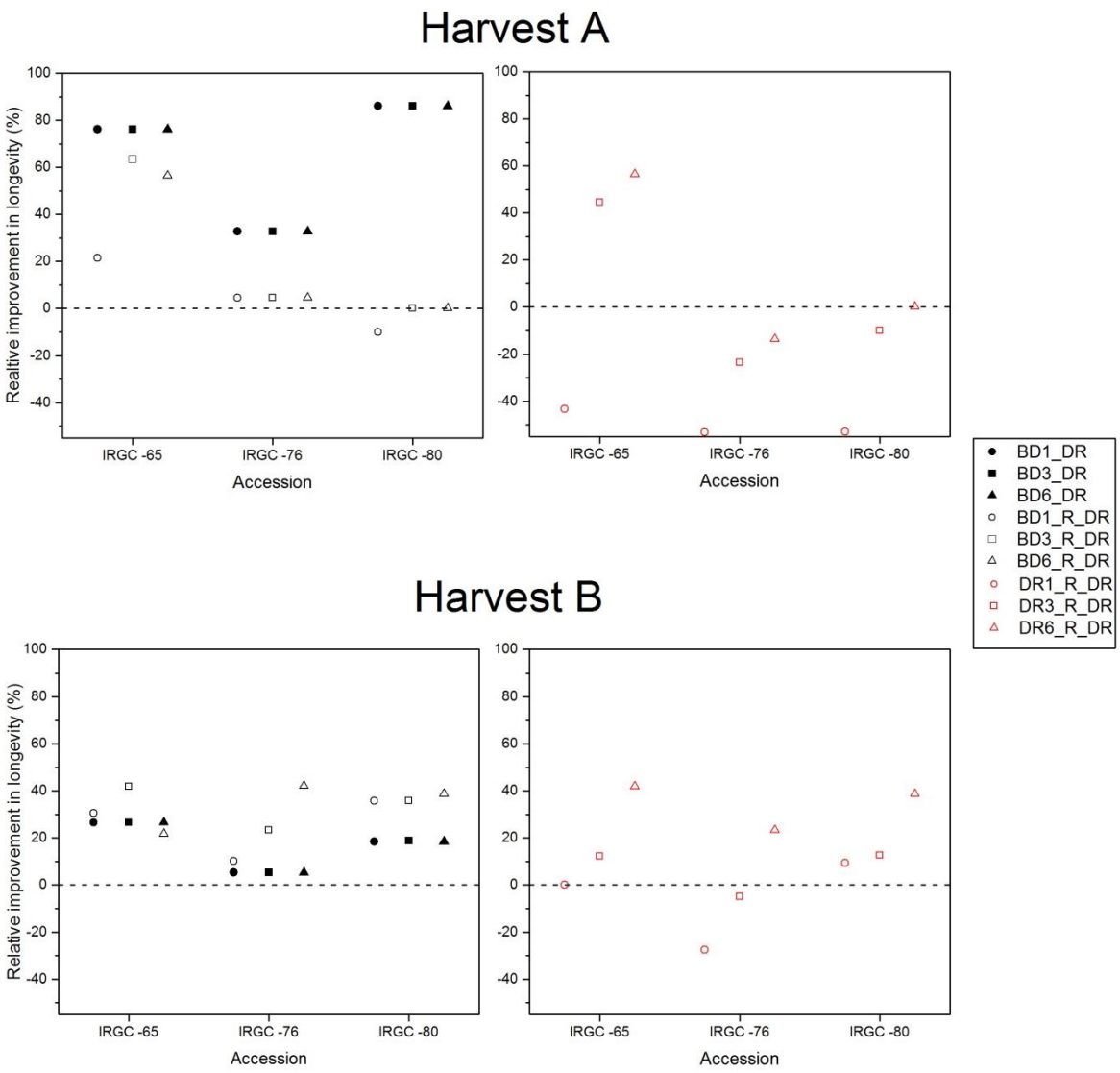


Figure 4.3. The relative improvement in longevity (%; [BDp_{50}-DR p_{50}]/DRp_{50}; [BD_R_DRp_{50}-DRp_{50}]/DRp_{50} and [DR_R_DRp_{50}-DRp_{50}]/DRp_{50}) of seeds immediately dried in the batch dryer (BD; black symbols) or dryroom (DR; red symbols) for 1 (circle), 3 (square) or 6 (triangle) days (closed symbols), after which some samples underwent a 7-day rehydration treatment (open symbols). All seed lots from each accession (IRGC 117265, -76 and -80) were harvested at 35 DAA. The symbols positioned above the dashed line represent an improvement in longevity compared with the DR control (the survival curves for the respective treatment(s) and the dryroom treatment could not be fitted by a common line without a significant increase in the residual deviance (Appendix 4.2B).

rehydrated after 1, 3 and 6 days of drying in the BD (BD_R_DR) in accession IRGC 117276 and after 3 and 6 days of drying in accession IRGC 117280, suggesting that the timing of rehydration is not influencing the storage potential of the seeds. Unlike in harvest A, BD drying alone (BD_DR) did not produce the greatest longevity in storage in seeds from harvest B. In fact, rehydrating seeds after initial drying in the BD (BD_R_DR *cf.* BD_DR) increased their subsequent storage longevity for each accession. Seed longevity was highest when rehydration occurred after 3 days of drying in accession IRGC 117265 and after 6 days in 117276 and –80. These seed lots showed the highest values of p_{50} compared with the other treatments (Figure 4.1) but also the greatest improvement in longevity compared with seeds that had only been dried in the BD (Figure 4.3). As the survival curves for all three of the rehydrated BD seed lots within each accession could not be constrained to a common line, this suggests that the timing of rehydration during post-harvest drying in the BD influences how the seeds from harvest B respond to water uptake. Overall, the estimates of p_{50} for seeds from each accession which were dried for different durations in the BD prior to a rehydration period (BD_R_DR) were greater in seeds harvested later in the dry season and at lower MC (harvest B), the opposite to what was observed when seeds were dried only in the BD for 1, 3 or 6 days before drying to equilibrium in the DR (BD_DR).

4.3.3.3. Dryroom_rehydration_dryroom vs dryroom (no rehydration)

Although estimates of p_{50} for seeds from each accession which were dried in the DR were greater when harvested at a lower MC (harvest B), longevity could be further improved upon rehydration. In general rehydrating seeds after initial drying in the DR improved their longevity compared with DR-drying when seeds were harvested at the lower (harvest B) but not at the higher (harvest A) MC (Figure 4.3). Rehydrating seeds after 1-day of drying in the DR produced the lowest estimates of p_{50} in all accessions from both harvests (Figure 4.1). These seed lots generally showed the highest rates of loss in viability compared with any other treatment (Appendix 4.2) and showed a reduction in longevity compared with the DR control, with the exception of seeds of accessions IRGC 117265 and -80 from harvest B.

4.4. Discussion

Seeds with a lower harvest MC (harvest B) tended to show greater longevity when only dried in the DR compared with seeds harvested with a higher MC (Figure 4.1). Furthermore, irrespective of whether seeds were dried initially in the DR or BD prior to rehydration, improvement in longevity as a consequence of the rehydration treatment was greatest in those seed lots harvested at the lower MC in all accessions (Figures 4.1 and 4.3). In terms of seed quality development, it is assumed that seeds harvested at a lower MC have progressed further through the first stage of the desiccation phase of seed development where there is an increase in longevity (Chatelain *et al*., 2012) with declining ambient RH, and so may not be metabolically active at harvest (Chapter 2). Metabolism can be reinstated in seeds upon rehydration allowing the continuation of maturation and/or initiation of repair processes (Powell *et al*., 2000; Butler *et al*., 2009b). However, if seeds have already acquired maximum longevity and moved into the second part of the desiccation phase where damage can be accrued at a rate that will increase as moisture content increases with fluctuating environmental conditions, as may be the case for seeds harvested at a lower MC (harvest B), seed quality can be compromised.

The improvement in longevity of seeds from harvest B, which were rehydrated after initial drying in the DR, increased with the duration of drying in all accessions, indicating that some drying is required for seeds to benefit from rehydration (Pieta Filho and Ellis, 1991; Butler *et al*., 2009a). Desiccation has been shown to promote the ability of immature seeds to germinate (Kermode and Bewley, 1985; Kermode *et al*., 1986). Hence, it maybe that DR1_R seeds, particularly for harvest A (high harvest MC), did not show an improvement in longevity because the seeds had not dried sufficiently after just 1 day in the DR and were still at a relatively high MC (>15%; Figure 4.1) . As the longevity of seeds from both harvests were able to increase when rehydrated after 3 and 6 days of drying, it is suggested that seed quality development can be reinstated and/or repair processes initiated by rehydration only when seeds have dried to very low MCs. Similar results were reported for seeds of *Digitalis purpurea*, although no critical moisture content was found below which developmental events are terminated for this temperate woodland species (Butler *et al*., 2009a).

Rehydration reduced the subsequent longevity of BD-dried seeds (i.e. BD1_R_DR, BD3_R_DR and BD6_R_DR seeds *cf*. BD1_DR, BD3_DR and BD6_DR seeds, respectively) for harvest A, but not from harvest B (Figure 4.3). Drying in the BD immediately after harvest increased the longevity of seeds compared with drying in the DR, shown by the increase in K_i and σ (Appendix 4.2). However, a proportion of seeds lost desiccation tolerance after rehydration, shown by the reduction in K_i, which reduced the subsequent longevity of the seed lot. Therefore it is thought that rehydration after differing durations of drying in the BD counteracted the beneficial effects of high temperature drying on the storage longevity of the seeds harvested at a high MC. Contrastingly, seeds which were harvested at a lower MC not only showed a reduced benefit to high temperature drying compared with seeds from harvest A, but rehydration after differing durations of drying in the BD was able to improve the subsequent longevity of BD seeds. Although the beneficial effects of drying in the BD did not increase after the first day (i.e. seed lots dried for 1, 3 and 6 days could be constrained to a common line), rehydration after the differing drying durations did not increase the longevity of the seeds to the same level. It was observed that rehydration after 3 (IRGC 117265) or 6 (IRGC 117276 and -80) days in the BD improved longevity the most in all accessions. Seed lots showed an increase in σ following rehydration compared with seeds which has only been dried in the BD, indicating that rehydration helped to maintain the viability of the longer-lived seeds within the population for longer periods in storage.

The benefits of initial high temperature drying on seed longevity have been documented in Chapters 2 and 3. Seeds which are harvested when still in the phase when seed longevity is still increasing on the mother plant benefit from an initial high temperature drying period as they are still able to accrue longevity (Whitehouse *et al*., 2015). Hence, BD seeds from all accessions from harvest A showed, not only the greatest longevity in storage compared with the other treatments, but also the greatest improvement in longevity compared with the DR (Figure 4.3). High temperatures may induce the stress response within seeds allowing the continued metabolism of protectants and other metabolic pathways involved in aiding the stabilisation of the seed during desiccation and survival in air-dry storage (Chapter 2; Whitehouse *et al*., 2015). However, high temperatures are thought to promote ageing, especially when seeds are at high MCs. Therefore rehydration after drying in the BD may allow the seeds time to repair damage

incurred during drying. In all accessions × harvest treatment combinations, rehydration post BD-drying reduced the rate of viability loss compared with seeds solely dried in the BD indicating its beneficial effect on the maintenance of seed viability during storage.

Within a seed lot, individuals differ in their ability to limit ageing reactions (e.g. antioxidants and protective proteins) (Kibinza *et al*., 2011). In accordance with Butler *et al.* (2009b), it is suggested that repair can occur during rehydration, as in priming, by allowing seeds to recover the capacity to germinate under standard conditions. However, the effectiveness of the rehydration treatment depends on the physiological age (i.e. the amount of damage already accumulated) as well as water status of the seeds prior to rehydration. High vigour seeds (lower levels of deterioration) are at a more advanced physiological stage after a priming treatment and therefore are more prone to deterioration, whereas low vigour seeds benefit from priming as it gives them time to repair metabolic lesions before they advance into the germination stage (Varier *et al*., 2010). As seeds age they accumulate oxidative damage, mainly in the form of reactive oxygen species (ROS) which can be scavenged by antioxidants such as superoxide dismutases (SOD) and catalases (CAT), which increase during priming (Bailly *et al*., 2000; Kibinza *et al*., 2011). The inhibition of catalase synthesis during priming of sunflower seeds reduced the ability of the seeds to repair indicating that this enzyme is a key determinant of seed recovery (Kibinza *et al*., 2000). It is therefore suggested that rehydrated seed lots may show an increase in levels of antioxidants which help seeds to resist oxidative damage. This could explain why all rehydrated seed lots, with the exception of the DR1_R_DR seeds, showed greater subsequent longevity in storage compared with the DR control.

To conclude, if seeds are harvested at a high MC, as is typical in wet tropical climates, the benefits of immediate drying in the BD far outweigh those from rehydration. However, the longevity of seeds which have already dried to low MC *in planta* by the time of harvest, and which would therefore show a limited response to high temperature drying, could benefit from a period of rehydration after drying in the BD.

CHAPTER 5

THE EFFECT OF INTERMITTENT VS. CONTINUOUS DRYING AT DIFFERENT TEMPERATURES ON THE LONGEVITY OF RICE SEEDS (*ORYZA SATIVA* L.) HARVESTED AT DIFFERENT MATURITIES

5.1. Introduction

Much of the research described in previous chapters has focused on high temperature drying of rice seeds prior to genebank storage. According to Nellist (1980), there is an upper temperature limit for safe drying which varies between species and depends on the moisture content (MC) of the seeds – as seeds dry, the maximum safe drying temperature increases; it also varies with the design of the drier and differs depending upon whether the maximum air or seed temperature is stated. Consequently, the values of safe temperatures for seed drying reported in the literature are not consistent. North (1948) claimed air temperature when drying onion seeds should not exceed 32°C at 12-20% MC or 21°C if the MC is above 20%, and Harrington (1972) suggested a temperature limit of 45°C when drying cereal seeds and 35°C for vegetable seeds.

5.1.1. Objectives and hypotheses

The previous chapters have shown that rice seed is tolerant to a high drying (air) temperature of 45°C. The aim of the experiment described in this chapter was to investigate the "limits" of drying temperature by initially drying rice seeds, harvested at 25, 35, and 45 days after 50% anthesis (DAA), at temperatures between 15-60°C, at a constant RH of 30%, prior to equilibrium drying in the genebank dryroom (DR). The effects of temperature, DAA, and the duration of drying (intermittent vs. continuous) at these temperatures, on subsequent seed longevity will be reported.

Hypothesis 1: Seed longevity will increase with the increase in temperature up to 45°c.

Hypothesis 2: Drying seeds at 60°C will reduce their longevity compared with drying in the dryroom.

Hypothesis 3: Seed longevity will not be influenced by the duration of drying (intermittent or continuous) or harvest maturity (DAA).

5.2. Materials and Methods

Staggered sowing of seeds of accession IRGC 117265 was conducted to enable simultaneous harvesting of seeds at 25, 35 and 45 DAA. Seedlings were raised in a seed bed before being transplanted to plots on the International Rice Research Institute (IRRI) upland site (14° 9' 3.5742"N, 121° 15' 54.504"W). In total, 2 kg of seeds at each DAA were harvested on 3rd April 2015. Immediately after harvest, the temperature and equilibrium relative humidity (eRH; %) was measured and moisture content (MC; % fresh weight) determined as described previously (section 2.2.2) (Figure 5.1).

Table 5.1. Dates (December 2014-April 2015) of sowing, transplanting, heading and harvest for seeds of accession IRGC 117265 harvested at 25, 35 and 45 days after 50% anthesis (DAA). The moisture content (MC; % fresh weight) and the equilibrium relative humidity (eRH) is recorded.

Maturity stage (DAA)	Sowing date	Transplanting date	Heading date	Harvest date	Harvest MC (s.e) (% f.wt)	eRH (%)
25	23rd Dec	12th Jan	10th Mar	3rd Apr	23.3 (0.1)	96.6
35	11th Dec	31st Dec	28th Feb	3rd Apr	18.9 (0.1)	86.4
45	01st Dec	21st Dec	18th Feb	3rd Apr	18.1 (0.0)	86.0

5.5.1. Seed drying

Immediately after harvest seeds at each maturity stage were divided into ten 200 g samples and placed into 0.2 × 0.33 m (L × W) nylon mesh bags (1 mm-diameter holes) in which they were stored inside sealed 0.6 × 0.3 × 0.132 m (L × W × H) electrical enclosure

boxes (ENSTO Finland, Oy) at room temperature (21.5°C) overnight to limit drying until the treatments began the following morning (0800 hrs).

A sample from each maturity stage was transferred directly to the DR and the remaining samples were placed over a saturated solution of $MgCl_2$ in sealed electrical enclosure boxes (where there was no movement of air), and transferred to either the genebank DR, maintained at 15°C, or to incubators at 30, 45 and 60°C. $MgCl_2$ was chosen due to its stability at temperatures between 15°C (33% RH) and 60°C (29.3% RH) (Rockland, 1960; Winston and Bates, 1960; Young, 1967). Saturated solutions were prepared by adding excess $MgCl_2$ to 700 ml of water. This resulted in a solid mass upon cooling. Solutions were left overnight to equilibrate at each of the four temperatures producing a relative humidity (RH) of approximately 30% RH (Rockland, 1960; Winston and Bates, 1960; Young, 1967). To ensure the solution remained saturated the RH was checked daily, before and after drying, at room temperature (21.5°C) using the AW-D10 water activity-measuring instrument (Chapter 2; section 2.2.2) and the bulk solution was adjusted if necessary by adding excess $MgCl_2$, stirring and allowing equilibration before re-checking RH. Seed samples were exposed to both intermittent (In) and continuous (Con) drying for 3 days at each temperature regime before being transferred for final drying for 11 days in the DR. Intermittent drying cycles took place between 0800 and 1600 hrs and during the non-active drying phase (1600–0800 hrs) seeds were sealed in electrical enclosure boxes (without $MgCl_{2)}$ at 21.5°C. The change in weight and eRH of samples was monitored daily, at 1600 hrs for intermittent dried seeds and 0800 hrs for continuous dried seeds, until samples were transferred to the DR where this was extended to a 3-day interval. Dry seeds were sealed inside 0.16 × 0.24 m (L × W) laminated aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until experimental storage began in May 2015.

5.2.2 Seed storage

Seeds from each maturity stage [3] × drying treatment [9] were split into 29 × 5 g subsamples which were placed into 30 mm-diameter open Petri dishes in a VC^3 0034-M climate chamber (Vötsch Industrietechnik, Germany) set at 60% RH and 21.5°C to equilibrate for 4-5 days to a MC of 10.9%.

As in Chapters 3 and 4, once equilibrium had been reached, four of the 5 g subsamples from each treatment combination were removed to measure seed eRH. Three of these subsamples were used to determine MC and the fourth to estimate initial germination. The remaining subsamples (25) were each sealed inside individual aluminium foil packets (0.12 × 0.08 m [L × W]) before being placed in an incubator at 45°C. One packet per treatment combination was removed at 3-day intervals up to 54 days for germination testing (following the same protocol in Chapter 3; section 3.2.4). Moisture content determinations were made at mid- and end of storage. The protocol for seed germination testing was as described in the previous chapters but scoring occurred only after days 3, 5, 7 and 14 after which any non-germinated seeds were dehulled and tested for an additional 7 days before final scoring.

5.2.3. Statistical analysis

Probit analysis was carried out using GenStat for Windows, Version 15 (VSN International Ltd., Oxford, UK) fitting either the Ellis and Roberts (1980a) viability equation (equation [1]) or the combined loss in dormancy and loss in viability model (equation [7]; Kebreab and Murdoch, 1999); with or without an additional parameter e.g. “mortality” parameter (“immunity” in GenStat), to estimate the proportion of responding seeds (Mead and Gray, 1999).

5.2.4. Sorption isotherms

Adsorption isotherms were determined at the four different temperatures, 15, 30, 45 and 60°C using seeds from accession IRGC 117265 from a 35 DAA treatment which had been dried to equilibrium in the DR (15°C/15% RH). Three 5 g samples of seeds were placed into 30 mm-diameter open Petri dishes and held over LiCl solutions maintained at 15, 30, 45, 60, 75 and 90% RH at each temperature. The eRH of the seed samples was determined, at room temperature, after 5, 7, 10 and 14 days. When seeds had reached equilibrium the three samples were removed and their MC (% fresh weight) determined.

5.3. Results

5.3.1. Seed drying

As expected, the harvest MC of the seeds reduced with increasing maturity; i.e. seeds harvested at 25 DAA had a higher MC compared with seeds at 45 DAA (Figure 5.1). Seeds harvested at 25 DAA showed a greater moisture loss, at least over the first day (0-1), in all drying regimes (including the DR control) compared with seeds harvested later in maturity and so at lower initial MCs (Figure 5.2; Table 5.2). Most seed lots lost the most moisture over the first day of drying however some seeds did show an increase in the amount of moisture lost over the second day (1-2) when dried at the lower temperatures (15 and 30°C) (Table 5.2). Similarly, in the warmer temperature regimes seeds which were dried continuously under all drying regimes generally showed a faster rate of drying than equivalent intermittent treatments, and reached a lower estimated MC (eMC) after each

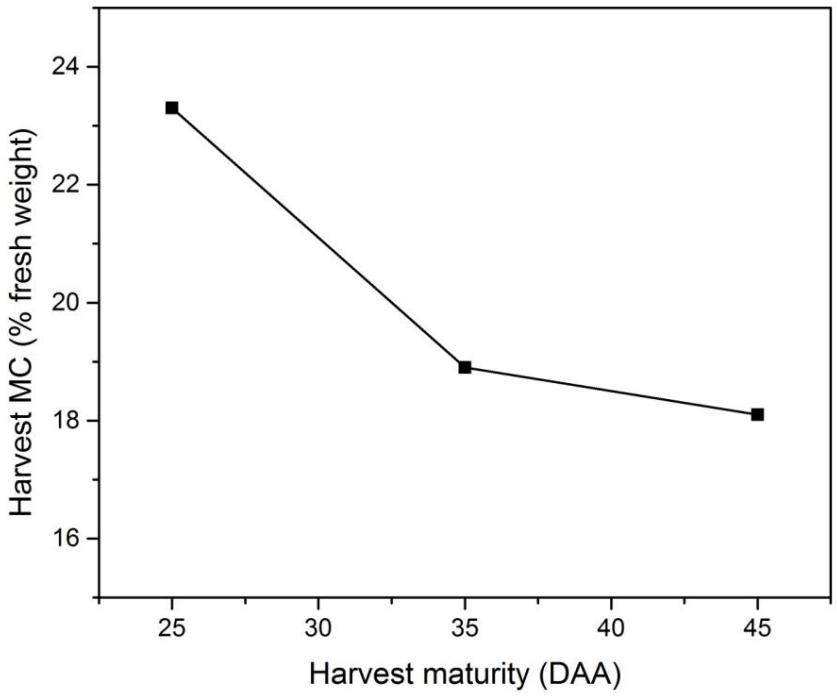


Figure 5.1. The moisture content (MC) of the rice seeds from accession IRGC 117265 at each stage of maturity (DAA) determined from three 5 g subsamples using the high-temperature oven method (ISTA, 2013).

Figure 5.2. Drying curves for accession IRGC 117265 harvested on 3rd April 2015. The harvest moisture content (MC; % fresh weight) and equilibrium relative humidity (eRH) was measured before seeds from each maturity stage (25, 35 and 45 DAA) were placed immediately in the dryroom (DR; 15°C/15%) or over saturated magnesium chloride solutions ($MgCl_2$; 30% RH) at 15, 30, 45 or 60°C for 3 days of initial continuous (Con; 24 h day^{-1}) (solid symbols) or intermittent (In; 8 h day^{-1}) (open symbols) drying. During the 14-day drying period, seed MC (circles) was estimated (eMC) based on the harvest MC (determined using the high-temperature oven method [ISTA, 2013]) and the subsequent change in sample weight, and eRH was measured (squares) daily for the first 3 days and at 3-day intervals thereafter. The standard error of the mean was calculated for the changes in eRH but not for eMC (because the moisture content was estimated based on the change in weight of one sample). The eRH values shown are the mean values from four replicates. Standard errors bars are too small to show in the figure and are reported in Appendix 5.1. The arrows indicate when the samples were moved to the DR for final drying.

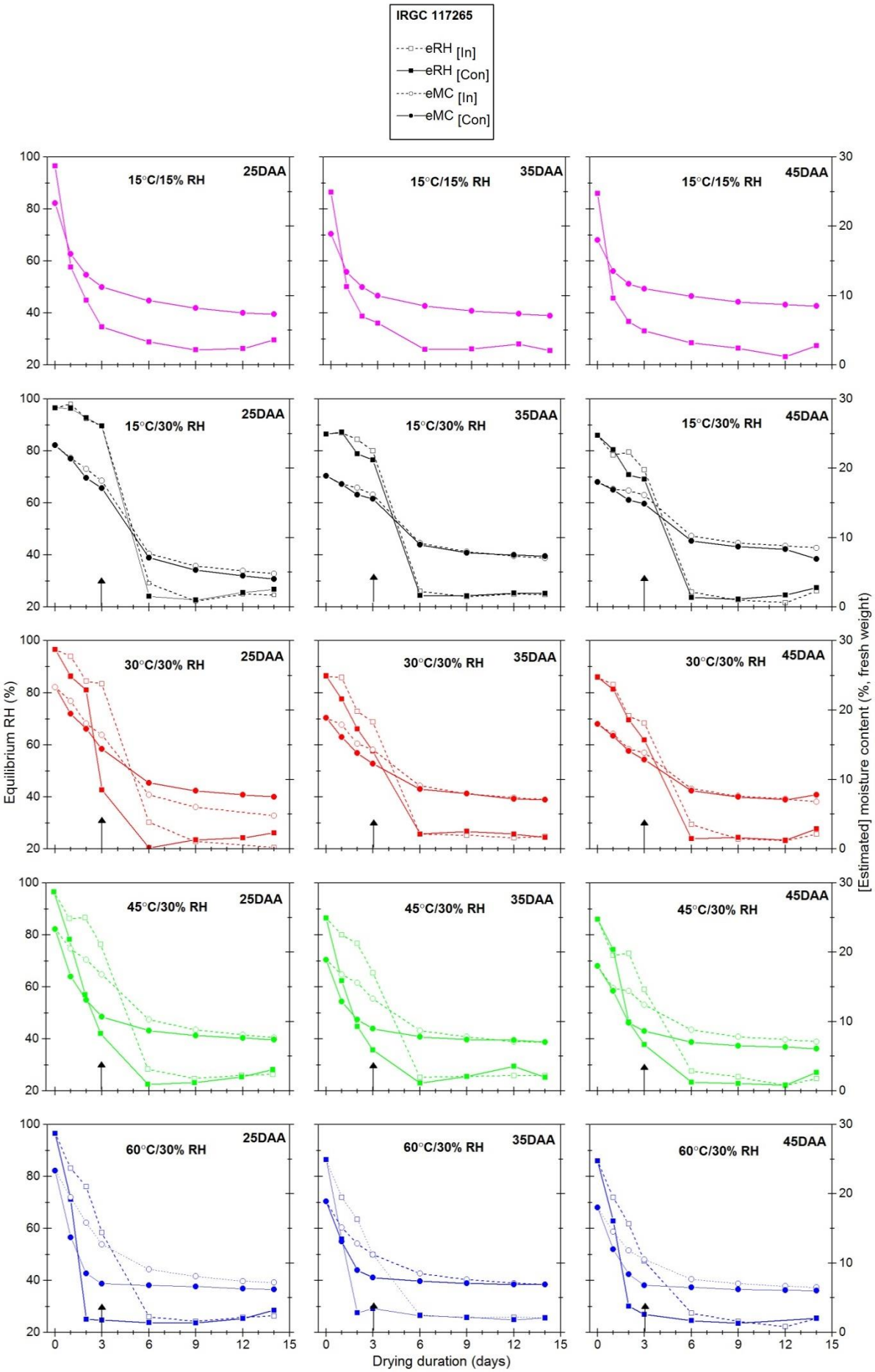

IRGC 117265
eRH [In]
eRH [Con]
eMC [In]
eMC [Con]
15°C/15% RH
25DAA
35DAA
45DAA
15°C/30% RH
30°C/30% RH
45°C/30% RH
60°C/30% RH
Equilibrium RH (%)
[Estimated] moisture content (%, fresh weight)
Drying duration (days)

Table 5.2. The moisture content (MC; % fresh weight) of seed lots from accession IRGC 117265 harvested at 25, 35 and 45 days after 50% anthesis (DAA) was determined using the high-temperature oven method (ISTA, 2013). Changes in seed weight were monitored after 1, 2 and 3 days of intermittent (In) and continuous (Con) drying to estimate reduction in seed moisture content (%).

		Reduction in seed MC (% f.wt)		
Harvest maturity (DAA)	Treatment	0-1d	1-2d	2-3d
25 DAA	15°C/30% $RH_{[In]}$	1.8	1.6	1.7
	15°C/30% $RH_{[Con]}$	1.9	2.8	1.5
	30°C/30% $RH_{[In]}$	2.0	3.2	1.7
	30°C/30% $RH_{[Con]}$	3.8	2.2	2.9
	45°C/30% $RH_{[In]}$	2.8	1.6	2.1
	45°C/30% $RH_{[Con]}$	6.8	3.4	2.4
	60°C/30% $RH_{[In]}$	3.8	3.7	3.1
	60°C/30% $RH_{[Con]}$	9.6	5.2	1.5
	DR	7.3	3.0	1.8
35 DAA	15°C/30% $RH_{[In]}$	1.2	0.5	1.0
	15°C/30% $RH_{[Con]}$	1.2	1.5	0.6
	30°C/30% $RH_{[In]}$	1.0	2.7	0.9
	30°C/30% $RH_{[Con]}$	2.8	2.3	1.5
	45°C/30% $RH_{[In]}$	2.1	1.2	2.3
	45°C/30% $RH_{[Con]}$	6.0	2.6	1.3
	60°C/30% $RH_{[In]}$	3.8	2.3	1.6
	60°C/30% $RH_{[Con]}$	5.8	4.1	1.1
	DR	5.5	2.2	1.2

Harvest maturity (DAA)	Treatment	Reduction in seed MC (% f.wt) 0-1d	1-2d	2-3d
45 DAA	15°C/30% $RH_{[In]}$	1.1	0.2	0.7
	15°C/30% $RH_{[Con]}$	1.2	1.5	0.5
	30°C/30% $RH_{[In]}$	1.5	2.2	0.5
	30°C/30% $RH_{[Con]}$	1.8	2.2	1.2
	45°C/30% $RH_{[In]}$	3.3	0.4	2.0
	45°C/30% $RH_{[Con]}$	3.7	4.6	1.2
	60°C/30% $RH_{[In]}$	3.6	2.7	1.3
	60°C/30% $RH_{[Con]}$	6.1	3.6	1.6
	DR	4.6	1.8	0.7

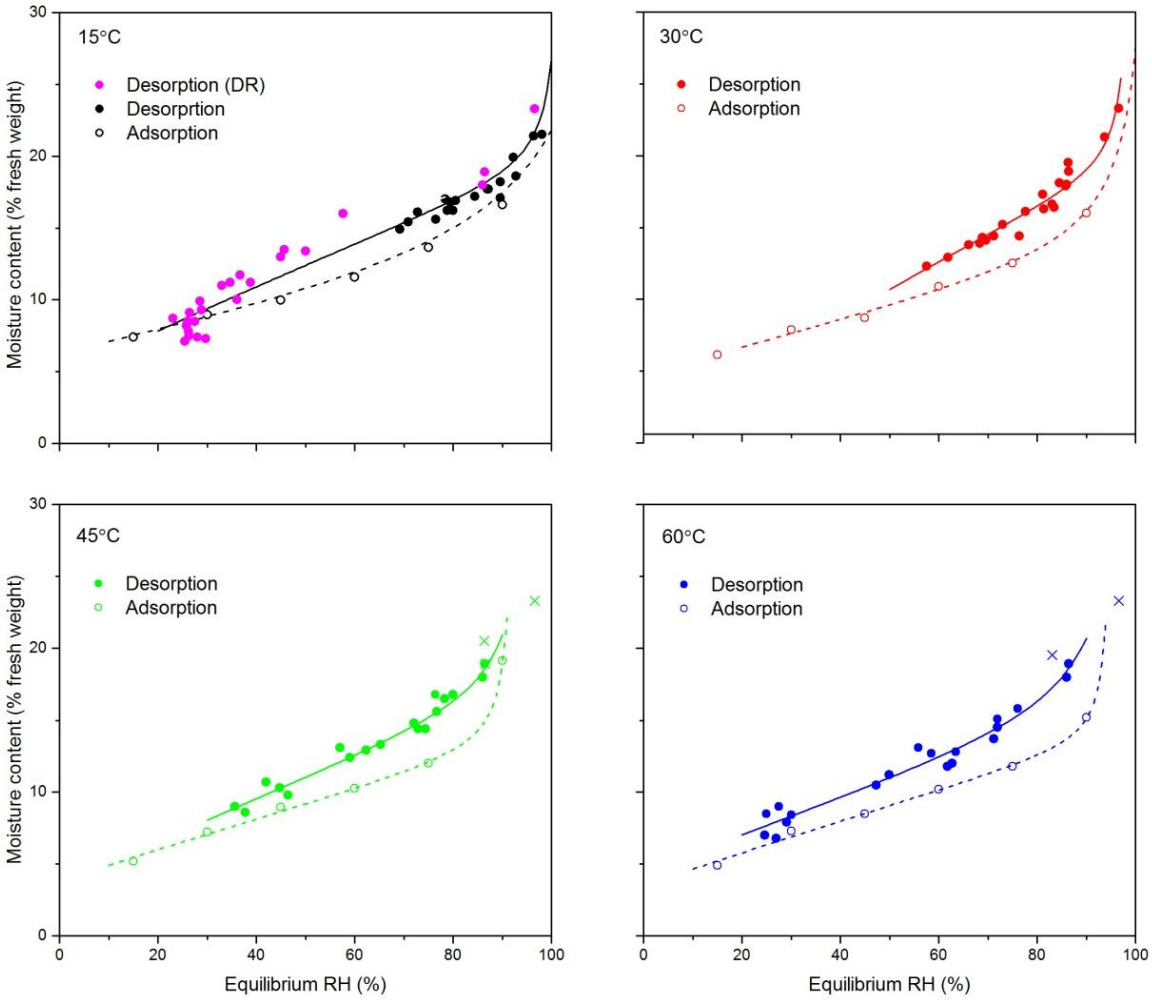
day of drying, compared with intermittently dried seeds, with the exception of seeds dried at the higher temperatures (45 and 60°C) which showed a reduction in the drying rate, and therefore moisture loss, the closer the seeds were to equilibrium (day-2 or -3), which as expected occurred earlier in continuous compared with intermittently dried seeds (Figure 5.2; Table 5.2).

Seeds dried either intermittently or continuously showed an increase in moisture loss over the first day the higher the drying temperature. For example, seeds showed a mean moisture loss of 1.4% (s.e. 0.22) and 1.4% (s.e. 0.23) when dried intermittently and continuously at 15°C/30% RH compared with 3.7 (s.e. 0.07) and 7.2% (s.e. 1.22) when seeds were dried at 60°C/30% RH. This trend was not consistent for all days or between maturity stages (Figure 5.2). Compared with the DR-dried seeds, seeds at all maturity stages did not dry as fast or reach such low eMCs during the first 3 days of drying when dried at 15°C/30% RH and 30°C/30% RH (Figure 5.2; Table 5.2), only when seeds were dried continuously at 45 or 60°C did the drying rate and total percentage moisture loss surpass the DR (Figure 5.2; Table 5.2), resulting in seeds which were near equilibrium after 3 days of drying. Irrespective of the difference in harvest MC, all seed lots which were dried continuously at 60°C/30% RH reached the lowest MC after 3 days compared with seeds dried at the other alternate temperature regimes. Despite the final MC of seeds differing between the four temperature regimes, once seeds were transferred to the DR they reached equilibrium after 3 further days.

5.3.2. Isotherms

The adsorption isotherms for seeds from accession IRGC 117265 held at 15, 30, 45 and 60°C (Figure 5.3) showed the effect of temperature on the eRH/MC relations in seeds. The MC of the seeds at equilibrium with 30% RH when dried at the four different temperature regimes (15, 30, 45 and 60°C at 30%) ranged between 6.8% (60°/30% RH) and 8.9% (15°C/30% RH). At all temperatures, the seeds showed a shallow slope until 80-90% eRH when the MC increased more rapidly with further increase in eRH. The increase in the steepness of the slope occurred earlier and was greater in seeds held at the higher temperatures (45 and 60°C). Desorbing seeds were at higher MCs than the fitted adsorption isotherm at all temperatures, more notably when seeds were at an

Figure 5.3. The relationship between moisture content (MC) and equilibrium relative humidity (eRH) for seeds from accession IRGC 117265. Seeds were dried at 15, 30, 45 or 60°C with 30% RH (desorption; solid symbols) or in the genebank drying room (DR) maintained at 15°C/15% RH (desorption; pink symbols). Seed MC was estimated (based on initial MC and change in sample weight) and eRH determined after days 1-3 of drying at each temperature (with the exception of the DR control), prior to final drying in the DR. Adsorption isotherms were also determined at the same temperatures using seed from accession IRGC 117265 which had been dried immediately in the DR after harvest (open symbols). Seeds were equilibrated (up to 14 days) over LiCl solutions maintained at 15, 30, 45, 60, 75 and 90% RH. As the RH of LiCl solutions vary little with temperature (in comparison with eRH of the seeds) the MC of the seeds was plotted against the expected eRH of the solutions. Moisture content (MC) was determined, from three 5g samples, using the high-temperature oven method (ISTA, 2013). The lines are a result of fitting a modified version of the D'Arcy-Watt isotherm equation (equation [9]; the outlying data points [×] were not included in the model fitting).


15°C
Desorption (DR)
Desorprtion
Adsorption
30°C
Desorption
Adsorption
45°C
Desorption
Adsorption
60°C
Desorption
Adsorption
Moisture content (% fresh weight)
Moisture content (% fresh weight)
Equilibrium RH (%)
Equilibrium RH (%)
0
10
20
30
0
20
40
60
80
100

intermediate MC/eRH (between 60 and 80% RH), showing the effect of hysteresis (Figure 5.3).

5.3.3. The effect of temperature on seed longevity

The mean seed moisture content during experimental storage across all seed lots (maturity × drying treatment) was 10.8% (s.e. 0.03). Some seed lots, depending on the drying regime they were exposed to, showed initial dormancy that was lost during early experimental storage and all seed lots showed a loss in viability (Appendix 5.2). Significant differences in seed longevity (P<0.05) were apparent between seed lots harvested at different maturity stages and amongst the drying treatments at each stage of maturity (Appendix 5.2). The fitted curves are quantified in Appendix 5.3. Dryroom drying resulted in seeds with the lowest storage longevity, and drying continuously for 3 days at 45°C/30% RH resulted in the greatest longevity for seeds at all maturity stage (Figure 5.4; Appendix 5.2). The longevity of seeds dried in the DR increased with the increase in stage of maturity at harvest and therefore the benefits of drying under any of the alternative regimes showed a reduced improvement in comparison, but never a negative effect. For example, the relative improvement in longevity when seeds were dried continuously at 45°C/30% RH dropped from 179.8% at 25 DAA to 59.5% at 45 DAA (Appendix 5.2). The level of dormancy reduced as the drying temperature increased. DR-dried seeds showed the highest level of dormancy amongst all drying regimes and seeds dried at 60°C showed the lowest.

The longevity (values of p_{50}) of seeds harvested at different DAA increased with drying temperature up to 45°C (Figure 5.4). The observed increase in p_{50} was not always a result of an increase in K_i but all seed lots, other than those dried at 60°C, showed a reduction in the rate of viability loss as harvest maturity increased (Appendix 5.3). Seeds dried at 45°C/30% RH consistently showed the slowest rate of viability loss (σ^{-1}) compared with seeds dried at the lower temperatures, including the DR. Seeds harvested at a higher MC benefitted the most from drying at 45°C/30% RH showing the highest estimates of p_{50} (Figure 5.4) and improvement in longevity (Figure 5.5C) compared with the DR control, which was largely due to increases in K_i. The survival curves for seeds dried intermittently or continuously at 45°C/30% RH could be constrained to a common line when harvested

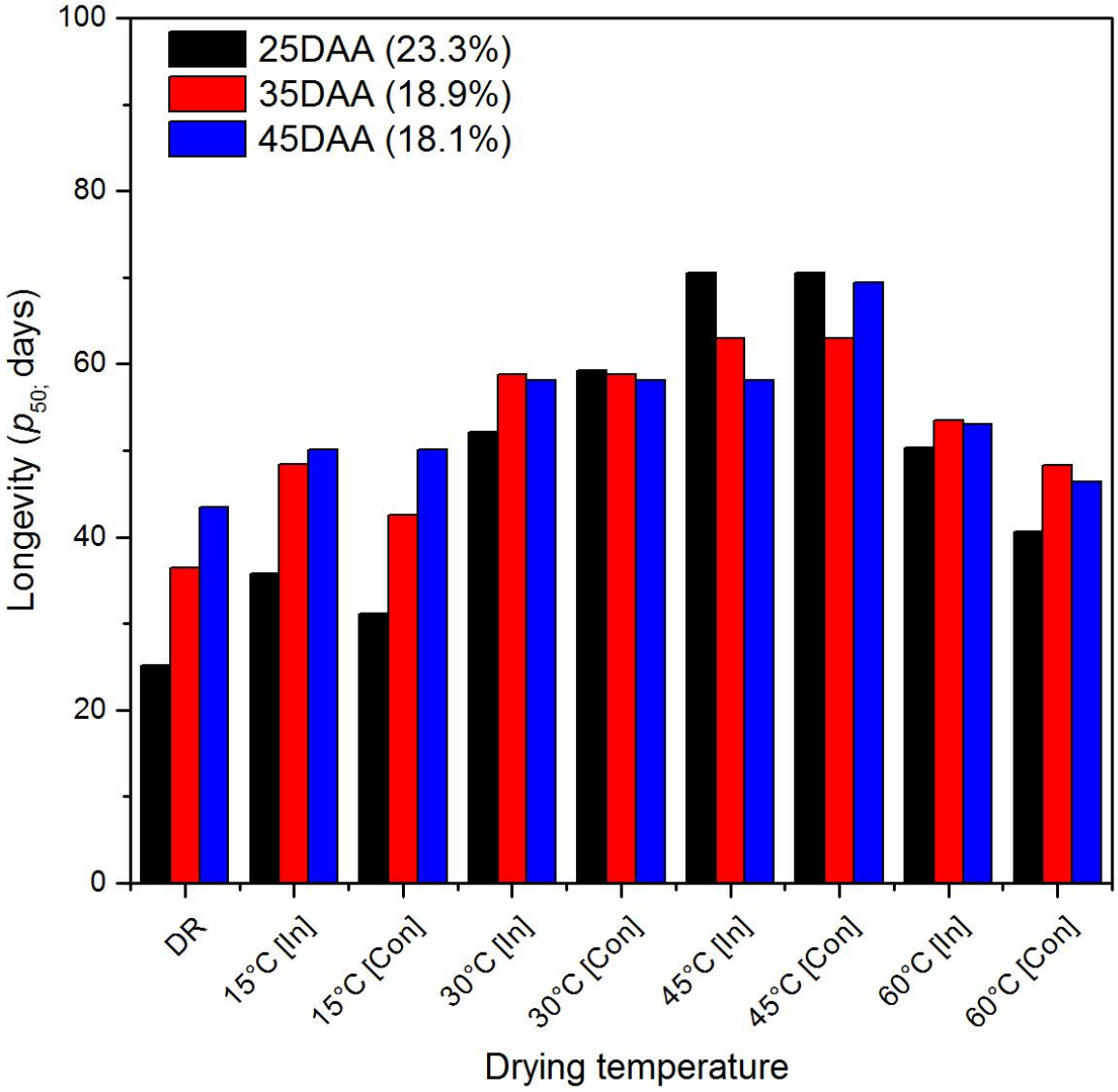


Figure 5.4. The longevity (p_{50}) of seeds from accession IRGC 117265 harvested at 25, 35 and 45 DAA when dried either intermittently or continuously at the five different regimes. Estimates of p_{50} resulted from fitting either the Ellis and Roberts viability equation (equation [1]) or the combined loss in dormancy and loss in viability model (equation [7]; Kebreab and Murdoch, 1999); with or without an additional parameter e.g. "mortality" parameter ("immunity" in GenStat), to estimate the proportion of responding seeds (Mead and Gray, 1999). The fitted curves are quantified in Appendix 5.3).

at 23.3 (25 DAA) and 18.9% (35 DAA) MC, but continuous drying provided a greater longevity in storage compared with intermittent drying when seeds were harvested at the lowest MC (18.1%; 45 DAA) (Figure 5.4). At each temperature regime, other than at 60°C, survival curves for seeds dried intermittently and continuously from at least one maturity stage could be constrained to a common line (Figure 5.4; Appendix 5.2). The maturity stage at which this was observed in each regime does not appear to be random. As the drying temperature increased there was a gradual shift from high maturity seeds (45 DAA), which were at the lowest MC at harvest, which showed the greatest improvement in longevity (compared with the DR) when dried at 15°C/30% RH, to less mature seeds (25 DAA) which showed the greatest improvement when dried at 45°C/30% RH, with a bridging effect at 35 DAA in between (30°C/30% RH) (Figure 5.4). Of the seed lots whose survival curves (intermittent/continuous) within each temperature regime could not be constrained, intermittent drying produced higher estimates of p_{50} and resulted in the greatest improvement in longevity compared with DR-dried seeds when seeds were dried at 15 and 60°C, whereas continuous drying produced greater longevity at 30 and 45°C (Figures 5.4 and 5.5).

It was consistently observed at all maturity stages that amongst the four 30% RH drying regimes, seed lots dried intermittently or continuously at 15°C/30% RH showed the lowest longevity in storage (Figure 5.4) and the lowest improvement in longevity compared with when seeds were dried in the DR (Figure 5.5A), despite estimates of p_{50} increasing with the increase in DAA and/or reduced harvest MC. This same trend was also observed in DR-dried seeds but which subsequently resulted from an increase in *K*i, unlike in seeds dried at 15°C/30%. Seed lots showed a reduction in K_i, but an increase in σ, with an increase in DAA, the values of which (K_i) were even lower in seeds harvested at 25 and 35 DAA when dried continuously (Appendix 5.2). There was no significant difference in the rate of viability loss when seeds harvested at 25 DAA were dried at 15°C/30% RH or in the DR at 15°C/15% RH. However values of K_i were greater in seeds dried at 15°/30% RH compared with the DR and therefore they showed an improvement in longevity of 42.1 and 23.4% when dried intermittently and continuously, respectively (Figure 5.5A; Appendix 5.3).

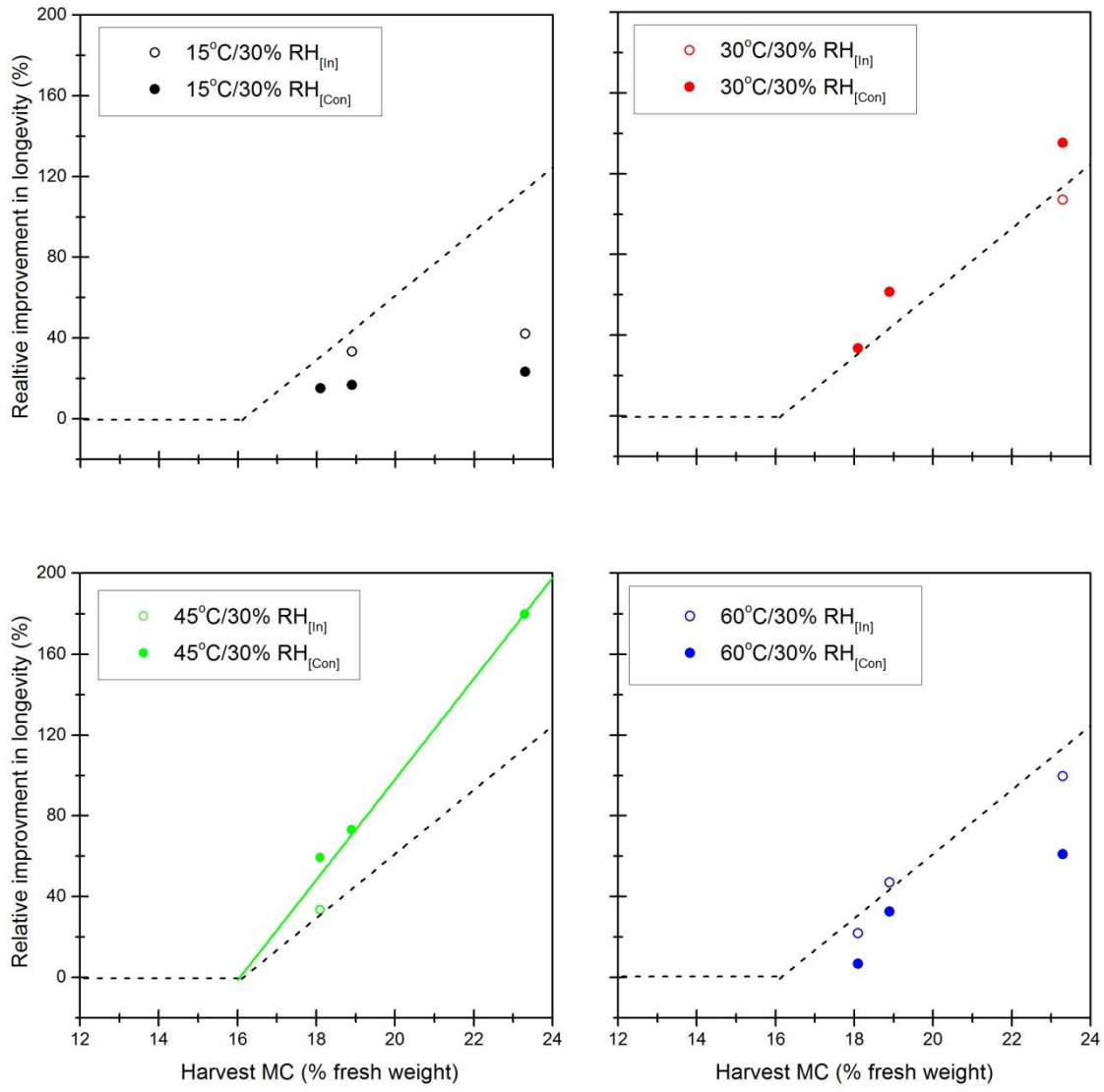


Figure 5.5. The relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) for each of the four 30% RH drying regimes (**A.** 15°C/30%; **B.** 30°C/30%; **C.** 45°C/30%; **D.** 60°C/30%) calculated as a proportion of the DR p_{50}; as in Chapter 2) for accession IRGC 117265 and harvest moisture content (MC; % fresh weight). A relative improvement in longevity of 100 % is equivalent to a doubling in longevity compared with DR treatments. Linear regression (solid line) is shown for seeds dried at 45°C/30% RH and accounted for 98.4% of the variance. The dashed line represents the split-line relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between the highest value from the BD treatments (BD p_{50}) and the DR treatment (DR p_{50}) for 20 rice accessions and initial moisture content from Chapter 2 (Figure 2.4)

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As previously mentioned it was not possible to constrain any parameters when seeds from all stages of maturity were dried intermittently or continuously at 60°C/30% RH (Appendices 5.2 and 5.3), with estimates of p_{50} being greater in seeds dried intermittently at 60°C/30% RH compared with continuously (Figure 5.4). Seed lots dried at 60°C showed a reduction in longevity compared with those dried at 45°C/30% RH and 30°C/30% RH, at all maturity stages (Figure 5.4), which meant they also showed a reduced improvement in longevity (relative to the DR) in comparison (Figure 5.5D). Despite the longevity of seeds dried either intermittently or continuously at 60°C/30% RH being significantly lower when harvested at the lowest MC (Figure 5.4), their improvement in longevity was still higher compared with when seeds were dried at 15°C (15°C/15% RH and 15°C/30% RH) (Figure 5.5A and D).

5.4. Discussion

Seeds require drying immediately after harvest to minimise the subsequent rate of ageing. But the tolerance of orthodox seeds to desiccation depends on the stage of maturity, and the drying conditions, especially the rate of drying (Hay and Probert, 1995). The drying rate is influenced by temperature, RH and airflow (Nellist, 1980). In this experiment seeds were dried at four different temperatures at 30% RH in a sealed environment. As the drying rate increased with increase in temperature at any given RH (Table 5.2), the moisture content reached after 3 days of drying differed between the drying regimes (Figure 5.2).

5.4.1. Drying at 15°C

Low temperature drying, at 15°C, resulted in seeds with a lower storage longevity compared with high temperature drying (Figure 5.4). Nonetheless, drying seeds at 15°C/30% RH still led to an improvement in their subsequent storage longevity compared with drying at the recommended lower RH (15°C/15% RH), consistent with the genebank drying room (DR), even though the drying rate was lower (Figure 5.5A; Table 5.2). Similar results have been reported in seeds of *Digitalis purpurea* which showed an increased ability to tolerate rapid desiccation (15°C/15% RH) after pre-drying at 32% RH (Hay and Probert, 1995). It is thought that the observed increases in desiccation tolerance and

therefore subsequent storage longevity are not a result of slow drying *per se* but rather that holding seeds at an elevated RH after harvest allows for the continuation of maturation and ripening events which can lead to an increased resistance to ageing before seed moisture drops below the level where metabolism ceases (Welbaum and Bradford, 1989; Leprince *et al*., 1993). The rate of drying did not differ between seeds which were dried intermittently or continuously at 15°C/30% RH and therefore cannot account for the observed differences in longevity following these two drying treatments (Figure 5.4; Table 5.2). Since intermittently-dried seeds harvested at 25 and 35 DAA were still at an RH greater than 80% (Figure 5.2) after the first and second days of drying, it is possible they continued to increase in quality during the inactive drying period.

5.4.2. Drying at 30°C and 45°C

Initial drying of seeds at temperatures greater than 15°C resulted in significantly greater longevity compared with the DR in all seed lots. Despite the differences in drying rate between the seed lots dried intermittently and continuously at 30°C/30% RH and 45°C/30% RH, there were generally no, or only small, differences in longevity (Figures 5.4). This indicates that the observed differences in longevity were due to the effects of drying at a higher temperature. The metabolic activity of the seeds is affected by their water content and the availability of oxygen (Vertucci *et al*., 1985). If conditions are favourable, the rate at which enzymatic and metabolic reactions occur will generally increase with an increase in temperature. Seeds undergo a coordinated series of events in response to desiccation which prepares the seeds to survive air-dry storage (Hoekstra *et al*., 2001). It is thought that high temperatures are not only likely to promote the metabolic processes and protective mechanisms which are associated with desiccation (since both represent stresses), but will also increase the rate at which they occur. It is the increased accumulation of the products from these reactions e.g. antioxidants and protective proteins, which increase the capacity of the seeds to tolerate desiccation. This may explain the increased improvement in longevity of seeds dried at 45°C/30% RH compared with drying at 30°C (Figure 5.5B and C).

As seen in seeds dried at a similar temperature (45°C) in the batch dryer (BD) (Chapters 2 and 4), the longevity of seeds dried at 45°C/30% RH increased with the increase in harvest

MC, with seeds showing an improvement in longevity (compared with the DR) of 180% when harvested at the highest MC (23.3%) (Figure 5.5C). Despite the similarity in the effects of drying at 45°C, seeds appeared to benefit more from drying either intermittently or continuously at 45°C/30% RH compared with intermittent drying in the BD, shown by an increase in the slope of the fitted regression line (Figure 5.5C). The harvest moisture content above which seeds dried at 45°C/30% show an improvement in longevity is consistent with when seeds are dried in the BD as the regression line passes through the same breakpoint at 16.2% MC. Below this MC, there was no benefit to drying in the BD. As the temperature of these drying regimes were the same, the observed differences in the improvement in longevity must be a consequence of drying at different RHs. The BD lacks a dehumidification system and is operated in an open environment. Based on the ambient conditions at IRRI (30°C/85% RH) it can be estimated that heating the air to 45°C will reduce the RH to approximately 40% RH (based on air moisture relations as calculated using Cactus2000). These conditions (45°C/30% RH) were expected to dry seeds at a slower rate but due to a lack of airflow surrounding the seeds in the $MgCl_2$ set up, seeds dried slower at 45°C/30% RH compared with in the BD. As a result seeds were still at a high MC (>16.2%), at least when harvested at the highest MC (25 DAA), after drying intermittently or continuously at 45°C/30%, allowing seeds to continue to increase in longevity (Figure 5.5C).

5.4.3. Drying at 60°C

Drying seeds at 60°C reduced the longevity compared with drying at 30 and 45°C (Figure 5.4), but improved the longevity compared with drying in the DR (Figure 5.5D). It has been reported in the literature that the upper temperature limit for safe drying of onion seeds should not exceed 21°C if seeds are at a MC over 20% (North, 1948) or in the case of cereals, should not exceed 35°C (Harrington, 1972). The temperature limit varies between species and the values reported in the literature are a more general recommendation e.g. for a collective of genera. The actual temperature limit for the safe drying of rice seeds prior to long-term storage (*cf.* medium-term storage) has not been determined. When the seeds are subjected to high temperatures, progressive removal of water occurs which can result in physical damage. Although the rate of drying was greater when seeds were dried at 60°C compared with at lower temperatures, their improvement in longevity was

significantly greater compared with seeds dried at 15°C/30% RH (Figures 5.5A and D; Table 5.2). Similarly, seeds dried at a faster rate in the BD, compared with at 60°C/30% RH, and showed a greater improvement in longevity. These observations indicate that the damage caused by large moisture gradients is unlikely to account for the reduced improvement in longevity (Figure 5.5). Instead it is more probable that the metabolic pathways and processes involved in the accumulation of longevity in seeds were likely to have been slowed and/or impaired by the high temperature. High temperatures result in a reduced energy metabolism which reduces RNA and protein biosynthesis (McDonald, 1999; Corbineau *et al*., 2002), contributing to cellular deterioration. In addition, the rates of metabolic processes were also likely to have reduced due to a decline in the activity of enzymes which occurs at temperatures past a critical limit. However, it is possible that normal energy metabolism and the activity of enzymes may have been reinstated during the non-drying period when temperatures are at a level which permits normal cellular activity. Normal energy metabolism was reported to recover in sunflower seeds following a 48 h treatment at 45°C when they were transferred to 25°C (Corbineau *et al*., 2002). This may explain the greater longevity observed in seeds dried intermittently at 60°C compared with seeds dried continuously. Although it is probable that the seeds would have accumulated damage during this non-active phase due to them being at an intermediate RH (50-75%) whereby ageing occurs more rapidly (Roberts and Ellis, 1989) and repair processes are limited, the benefits of allowing seeds to resume normal cellular activity must outweigh the detrimental effects of ageing which may have occurred.

To conclude, the results of this experiment provide further support that drying high moisture content rice seeds at the recommended low temperature, low humidity conditions consistent with a genebank drying room are not optimum for subsequent seed storage longevity. A schematic diagram (Figure 5.6), based on the current results, represents how the different drying regimes could be influencing the longevity of rice seeds and therefore depicting possible strategies, by altering the drying conditions, of how to optimise seed storage longevity. It has been observed that rice seeds show an increased improvement in longevity in response to drying at temperatures greater than 15°C, up to 45°C at least (the potential benefits of drying between 45 and 60°C are unknown). In addition, the results suggest that the longevity of seeds could be further improved by drying at a slower rate at any given temperature. Therefore optimum

longevity could be achieved if slower drying at 45°C (or at a temperature between 45-60°C), which currently shows the greatest improvement in longevity compared with the DR, led to further increases in the storage potential of rice seeds.

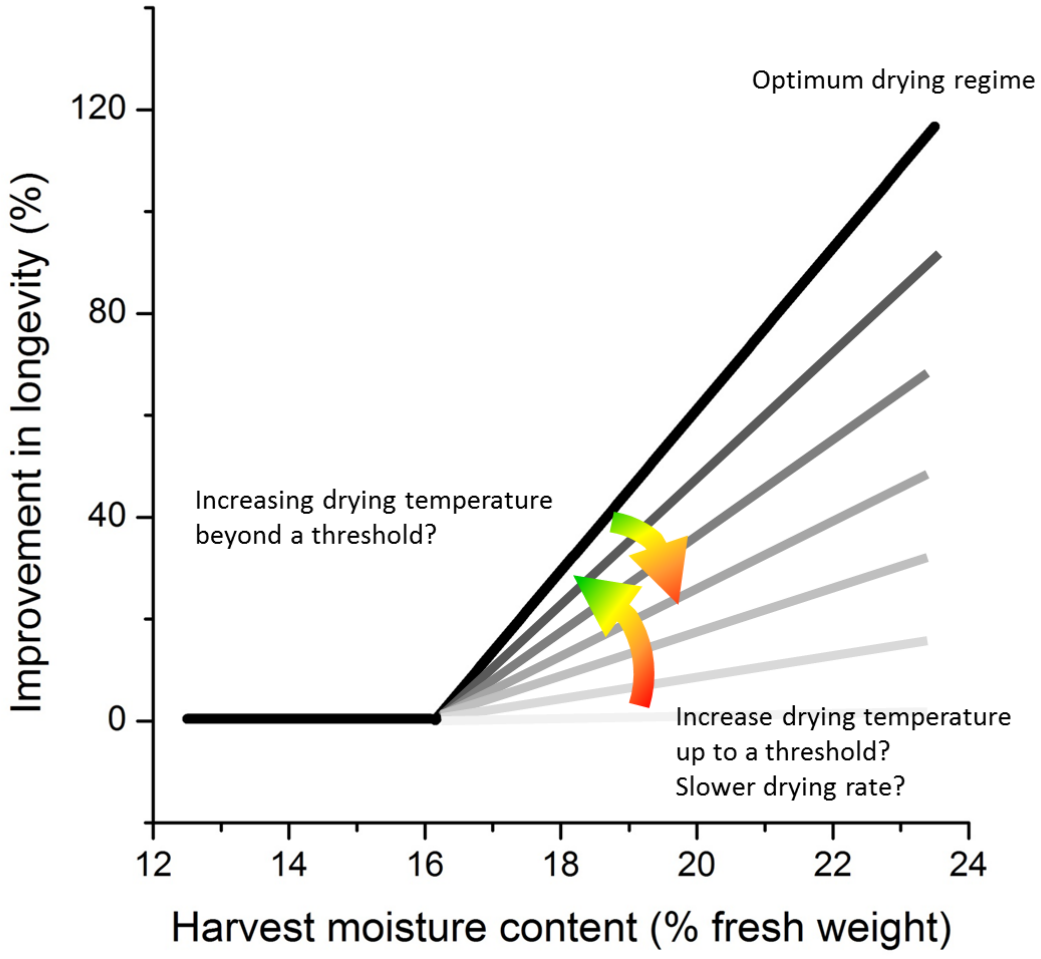


Figure 5.6. A schematic representation of how the longevity of rice seeds might be influenced by drying under different regimes.

CHAPTER 6

THE EFFECT OF DRYING UNDER A RAPID OR STEPPED HIGH TEMPERATURE DRYING REGIME ON RICE SEED (*ORYZA SATIVA* L.) LONGEVITY

6.1. Introduction

From the previous chapter we saw the storage longevity of seeds increase with the increase in drying temperature up to 45°C; storage longevity was reduced for seeds exposed to drying at 60°C. The results also revealed that the longevity of seeds could potentially be further increased by altering the drying rate at 45°C. Much of the research presented so far in this thesis has focussed on the initial drying of seeds at different temperatures prior to final drying in the DR. This chapter presents the results from experiments where rice seeds were subjected to a rapid high temperature drying regime or a stepped drying regime, both capable of drying seeds to an equilibrium moisture content (MC) of 6.1% (estimated using Cromarty's equation executed in the Seed Information Database [Royal Botanic Gardens Kew, 2008]), and hence safe for long-term storage. The effect of these different drying treatments on subsequent seed storage longevity (compared with the conventional low temperature, low humidity drying conditions of a dryroom; 15°C/15% RH) will be shown.

Hypothesis 1: The storage longevity of seeds dried under either drying regime (stepped or rapid) will be greater compared with seeds dried solely in the dryroom.

Hypothesis 2: The stepped drying regime will improve subsequent seed storage longevity compared with rapid drying.

6.2. Materials and Methods

6.2.1. Plant material

Three accessions (IRGC 117265, -76 and -80) from two variety groups (aromatic and indica) were planted in the 2014 dry (DS) and wet season (WS). Following the standard rice growing protocol, all seeds were sampled from the genebank active collection and given an after-ripening treatment at 50°C for 5 days prior to sowing in upland plots (14° 9' 3.5742"N, 121° 15' 54.504"W) on the International Rice Research Institute (IRRI) experimental Station (ES).

Table 6.1. Dates (November 2013-April 2015) of sowing and harvest for seeds of accession IRGC 117265 harvested at 35 days after 50% anthesis (DAA) during the 2014 dry season (DS) and wet season (WS). The moisture content (MC; % fresh weight) and the equilibrium relative humidity (eRH) is recorded.

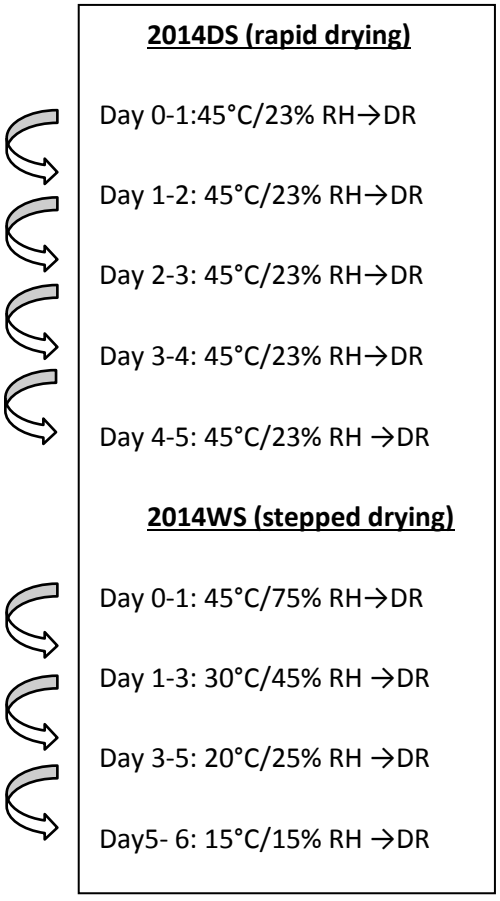
Season	Accession	Harvest	Sowing date	Harvest date	MC (% f.wt) (s.e.)	eRH (%) (s.e.)
2014DS	IRGC 117265	A	30 Nov	26 Mar	20.2 (0.2)	97.5 (1.2)
		B	6 Jan	6 May	13.0 (0.1)	56.8 (0.9)
	IRGC 117276	A	30 Nov	26 Mar	23.3 (0.1)	99.7 (0.4)
		B	11 Jan	6 May	17.1 (0.0)	83.9 (0.3)
	IRGC 117280	A	30 Nov	26 Mar	18.2 (0.4)	85.6 (0.7)
		B	16 Jan	6 May	17.8 (0.1)	87.2 (0.2)
2014WS	IRGC 117265	A	13 Jun	15 Oct	31.0 (0.2)	99.3 (0.4)
		B	11 Jul	8 Nov	18.8 (0.1)	90.0 (0.5)

Seeds from each accession were sown on two separate dates in the 2014DS and the 2014WS to achieve a total of two harvests (A and B) for each accession per season, all made at 35 DAA and which differed in harvest moisture content (MC) (Table 6.1).

However, in the 2014WS, only the data gathered from harvests of IRGC 117265 were analysed as accessions IRGC 117276 and 117280 were affected by Tungro disease and therefore were unable to be utilised fully for the purpose of this experiment. All seed lots followed the same post-harvest handling procedures as in previous experiments and the initial moisture content (MC; % fresh weight) and equilibrium RH (eRH) measured as already described (section 2.2.2). Seed lots were subjected to a rapid or a stepped high temperature drying treatment immediately after harvest in the 2014DS and 2014WS, respectively.

6.2.2. Seed drying

After harvest seeds from each accession were divided into five (2014WS experiment) or six (2014DS experiment) 200 g samples and placed into 0.2 × 0.33 m (L × W) nylon mesh bags and a sample from each was immediately placed into the DR until it approached equilibrium. The remaining samples were transferred to the VC^3 0034-M climate chamber (Vötsch Industrietechnik, Germany) set at the following drying treatments:



For all seed lots in each experiment, once samples had equilibrated in the DR, they were sealed inside 0.24 × 0.17 m (L × W) laminated aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until experimental seed storage.

6.2.3. Seed storage

The same seed storage protocol was followed as described in Chapter 3. The seeds from each treatment combination (accession [3] or [1] × drying treatment [6] or [5]) per harvest were equilibrated to 60% RH in the VC3 0034-M climate chamber (Vötsch Industrietechnik, Germany) before subsamples were sealed inside individual aluminium foil packets and placed in an incubator at 45°C. A sample was removed for germination testing (as described in Chapter 3) at 3-day intervals until viability was lost. The interval period was lengthened in seed lots showing a slow rate of viability loss. Germination was scored after 3, 5, 7 and 14 days before non-germinated seeds were dehulled and tested for an additional 7 days before final scoring. MC determinations (as described in previous chapters) were conducted using three 5 g replicates prior to storage and at the mid- and end storage points.

6.3. Results

Seeds from harvests A, which occurred earlier in each of the seasons, were at a higher MC at harvest compared with seeds from harvest B (Table 6.1). In the case of accession 117625, these values were much greater in the 2014WS than the 2014DS. When comparing the drying curves of seeds dried under the different regimes (including the DR), the drying rates appeared to be dependent on MC of seeds at harvest (Figure 6.1). All seed lots reached a lower MC when dried at 45°C/23% RH compared with when dried at 45°C/75% RH however, both regimes dried seeds at a faster rate compared with drying in the DR (Figures 6.1 and 6.2).

All seed lots dried at 45°C/23% RH reached equilibrium during the 5-day drying period, but exact timings differed between accessions and were influenced by the harvest MC (Figure 6.1). Once seeds were transferred to the DR there was very little change in the MC of the seeds. Seeds harvested at the higher harvest MC (harvest A) reached equilibrium

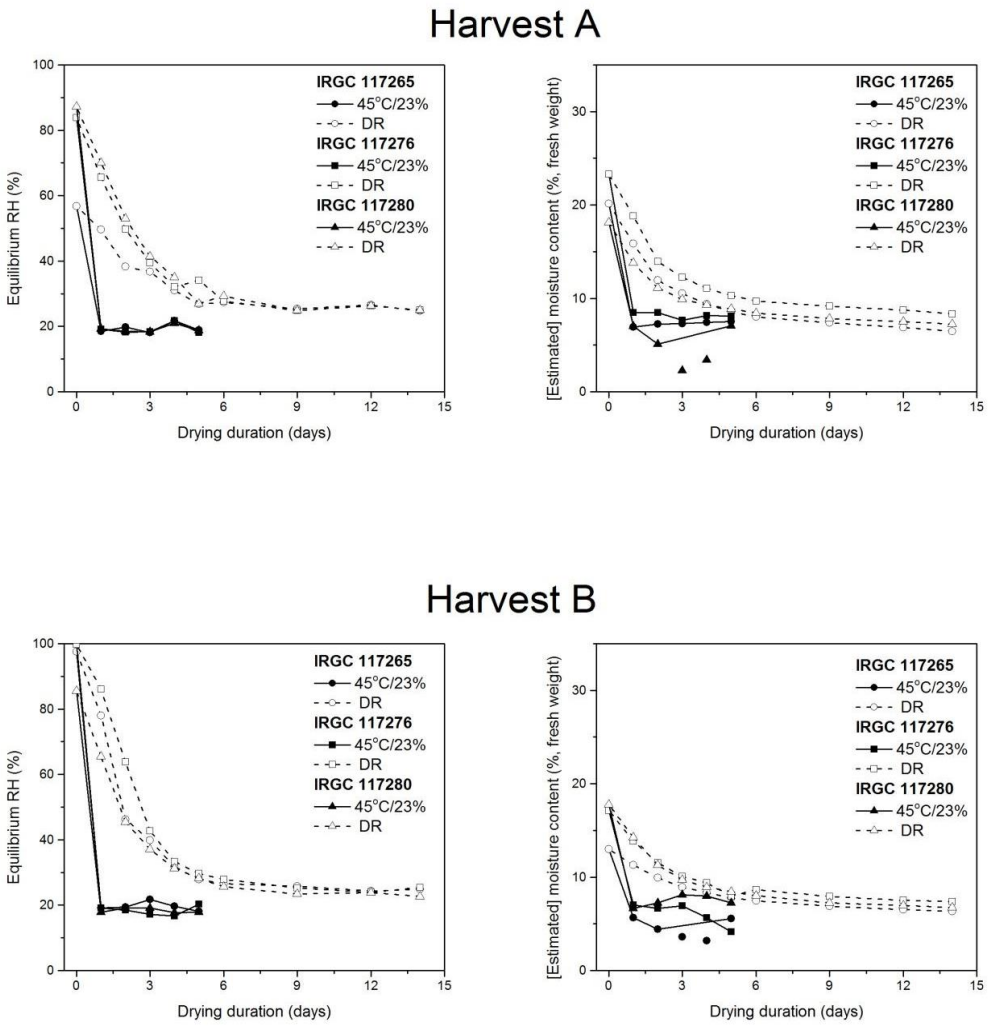


Figure 6.1. Drying curves for accessions IRGC 117265, -76 and -80 harvested in the 2014 dry season (DS) at 35 days after 50% anthesis (DAA). Moisture content (MC) and equilibrium relative humidity (eRH) at harvest was measured before seeds were dried either in the dryroom (DR; 15°C/15% RH) (open symbols) until equilibrium or initially in the climate chamber (45°C/23% RH) for up to 5 days before final drying in the DR (closed symbols). Initial MC was determined using the high-temperature oven method (ISTA, 2013) and the MC during drying was estimated based on the initial MC and the change in sample weight. The eRH values shown are the mean of four replicates (standard errors of the means are too small to show; Appendix 6.1). The standard errors were unable to be generated for the change in MC as this was estimated based on the change in weight from only sample.

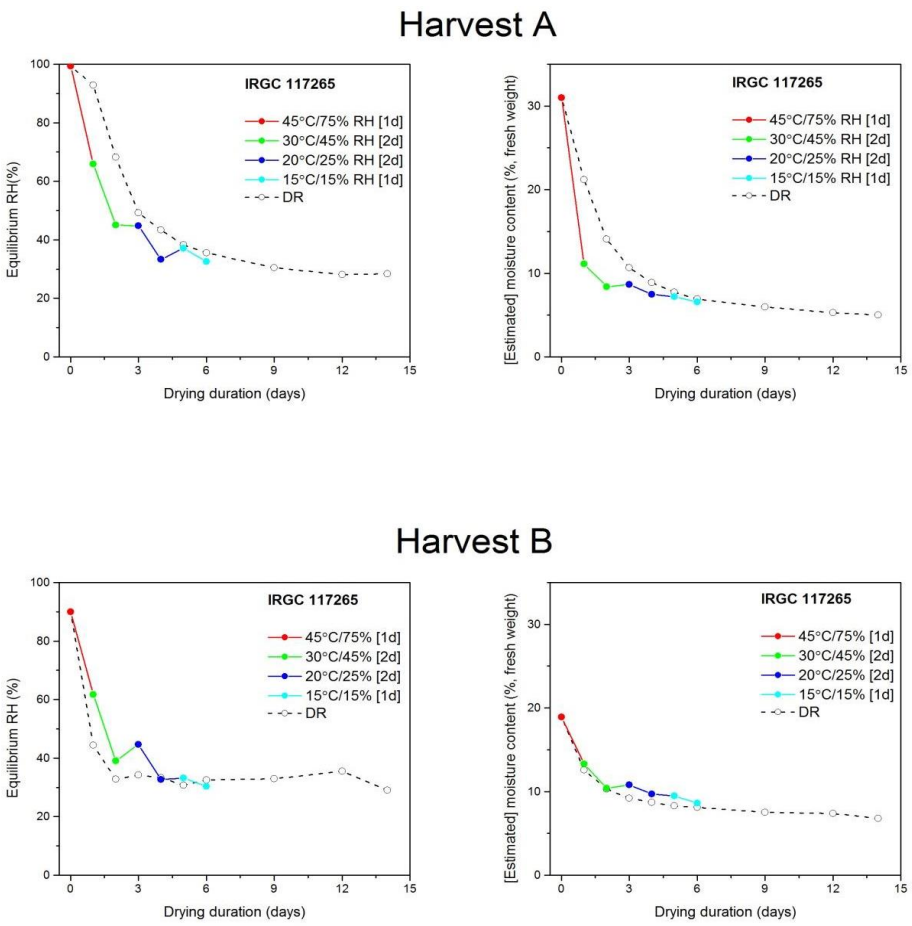


Figure 6.2. Drying curves for accession IRGC 117265 harvested in the 2014 wet season (WS) at 35 days after 50% anthesis (DAA). Moisture content (MC) and equilibrium relative humidity (eRH) at harvest was measured before seed lot were either dried either in the dryroom (DR; 15°C/15% RH) (open symbols) until equilibrium or in the climate chamber under the gradual high-temperature drying conditions (45°C/75% $RH_{[1d]}$; 30°C/45% $RH_{[2d]}$; 20°C/25% $RH_{[2d]}$; 15°C/15% $RH_{[1d]}$) (closed symbols) before being transferred to the DR for final equilibrium drying. Initial MC was determined using the high-temperature oven method (ISTA, 2013) and the MC during drying was estimated based on the initial MC and the change in sample weight. The eRH values shown are the mean from four replicates (standard errors of the means are too small to show; Appendix 6.1). The standard errors were unable to be generated for the change in MC as this was estimated based on the change in weight from only sample.

faster compared with seeds from harvest B. Regardless of harvest MC, all seeds lost the most moisture during the first day of drying and those seed lots which had already reached equilibrium after the first day showed an uptake of moisture during the second day (Figure 6.1). In contrast, when drying seeds at the conventional low temperature, low humidity conditions (15°C/15% RH) in the DR; moisture loss was more gradual with seeds typically reaching an equilibrium moisture content of between 8.4 and 6.4% after 14 days of drying (Figure 6.1). Seeds also lost the most moisture during the first day of drying, but overall dried at a slower rate compared with seeds dried at 45°C/23% RH.

Seeds harvested in the 2014WS and subjected to the stepped drying regime also lost the most moisture during the first day, more notably in seeds from harvest A than harvest B (Figure 6.2). In general the drying rate was similar between seed lots dried in the DR and under the gradual high temperature regime with exception of the first day of drying in seeds from harvest A where seeds dried at a rate which was 10-times faster at 45°C/75% RH compared with the DR (Figure 6.2). The drying rate slowed after the second day as seeds approached equilibrium (day 6), and after 6 days of drying, the MC of the seeds were equal to that of the DR and changed very little thereafter. In contrast, seeds harvested at the lower MC (harvest B) dried at a much slower rate compared with seeds from harvest A, almost identical to seeds dried in the DR (Figure 6.2). After day 1 of drying, the temperature and RH was changed from 45°C/75% RH to 30°C/45% RH, at which point seed samples from harvest B dried at a slightly slower rate and were at a higher MC compared with seeds dried in the DR. Equilibrium was reached after approximately 6 days of drying under either regime.

6.3.1. Seed longevity

The mean seed MC during experimental storage across all seed lots (accession × drying treatments) was 11.0% (s.e. 0.02) in 2014DS and 11.1% (s.e. 0.02) in 2014WS. Accession IRGC 117265 showed a loss in dormancy during early experimental storage and all seed lots within each accession showed loss in viability with increasing storage duration (Appendices 6.2 and 6.3). Differences in seed longevity were significant (P<0.05) between accessions and amongst the drying treatments within accessions when seeds were dried under either regime (Appendices 6.3 and 6.5). Despite the differences, all seeds from

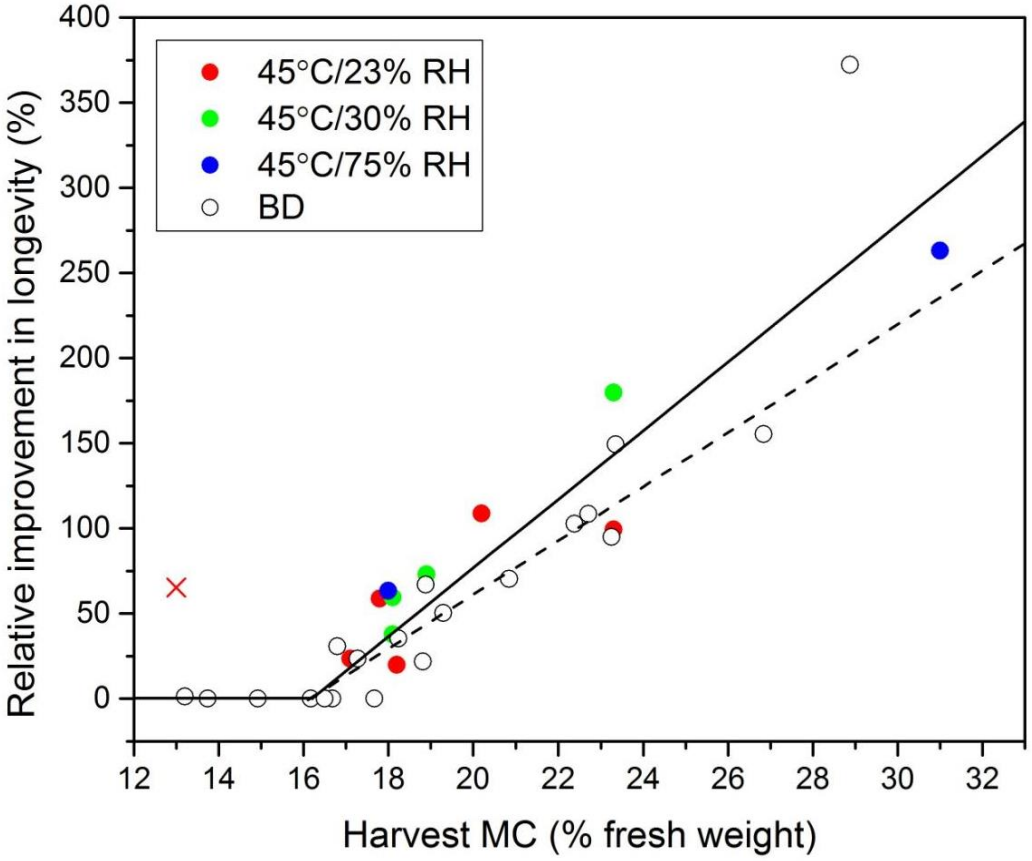


Figure 6.3. Relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between seeds dried at the rapid high temperature drying regime (45°C/23% RH; solid red symbols) and the stepped drying regime (45°C/75% [0-1d]; 30°C/45% RH [1-3d]; 20°C/25% RH [3-5d]; 15°C/15% RH [5-6d]; solid blue symbols) calculated as a proportion of the DR; as in previous chapters) for the three rice accessions IRGC 117265, -76 and 80 and harvest moisture content (MC; % fresh weight). The relative improvement in longevity of seeds dried under the two regimes outlined in this chapter was analysed against the batch dryer (BD; 45°C/40%RH) data (Chapter 2; open symbols) and the $MgCl_2$ (45°C/30% RH; closed green symbols) data (Chapter 5; closed green symbols). The solid black line is a result of split-line regression analysis for all drying treatments and accounted for 83.3% of the variance. The outlying data point (×) at 45°C/23% RH was not included in the analysis. A relative improvement in longevity of 100% is equivalent to a doubling in longevity compared with DR treatments. The dashed split-line regression represents the relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between the highest value from the BD treatments (BD p_{50}) and the DR treatment (DR p_{50}) for 20 rice accessions and initial moisture content from Chapter 2 (Figure 2.4).

each accession which were dried at either of the high temperature regimes showed an improvement in longevity compared with when seeds were dried in the DR (Figure 6.3). It was also observed that within each accession the same seed lot from both harvests showed the greatest improvement in longevity when dried at either one of the regimes despite the difference in harvest MC. In accession IRGC 117265, seeds from both harvests which were dried for 3 days at 45°C/23% RH resulted in the greatest improvement, whereas in accessions IRGC 117276 and -80, the greatest improvement was achieved after the first and fifth day of drying, respectively (Appendices 6.2 and 6.3). The observed improvements in longevity when dried at the rapid high temperature regime was attributed to an increase in K_i in accession IRGC 117265, an increase in K_i and σ in accession IRGC 117276 and an increase in σ in accession IRGC 117280. Seeds of accession IRGC 117265 showed a greater proportion of dormant seeds during early storage when harvested at a higher MC; dormancy was reduced with the increase in duration of drying (Appendix 6.3).

Seeds from accession IRGC 117265 which were harvested in the WS and subjected to the stepped drying regime showed an improvement of 263% (harvest A) and 63.3% (harvest B) compared with the DR control (Figure 6.3; Appendix 6.5). The survival curves of seed lots removed at each stage of the drying phase (after days 1, 3, 5 and 6) could be constrained to common values therefore no further improvements occurred after the first day (Appendices 6.4 and 6.5). The improvement in longevity was greater when seeds were harvested at the higher MC (Figure 6.3; Appendix 6.5).

6.4. Discussion

Within the literature much of the research on drying rates focuses on recalcitrant seeds due to their susceptibility to desiccation. However orthodox seeds are desiccation tolerant and can be dried to moisture contents in the water sorption regions I and II (typically 15-20% RH; section 1.2.2) without damage (Roberts and Ellis, 1989). The response of orthodox seeds to drying depends not only on the conditions of drying but on the maturity of the seeds. Seeds are only able to tolerate rapid drying once they have entered the post-abscission phase of seed development, coinciding with their ability to survive desiccation to very low moisture levels (Ellis and Hong, 1994). All seeds in these

experiments were harvested during the post-abscission phase of seed development which accounts for their ability to tolerate drying at either of the high temperature regimes investigated in this chapter which dried seeds at a faster rate, at least over the first day, compared with drying in the DR (Figures 6.1 and 6.2). The improvement in longevity when drying seeds under either of these faster regimes was notably greater when seeds were harvested at the higher MC (Harvest A) (Figure 6.3) indicating that an increase in the drying rate, which occurs as a result of an increase in MC, is not likely to be compromising the quality of the seeds.

6.4.1. The effect of drying at different RHs

Generally, the results of this chapter are consistent with those from previous chapters which concluded that compared with drying at the currently recommended low temperature conditions (FAO, 2013), rice seeds initially dried at 45°C show a significantly greater storage longevity. However it was observed in Chapter 5 that seeds could show an increased benefit to drying at 45°C if the rate of drying was reduced by drying in a closed system at 45°C/30% RH (Chapter 5). Therefore it was thought that drying seeds at a faster rate at 45°C would reduce the improvement in longevity compared with drying at a slower rate as rapid dying reduces the time that seeds can benefit from high temperatures (before RH drops below 80%) which subsequently lead to an increase in resistance to dehydration (Kermode, 1990; Hay, 1997; Hay and Probert, 1995). At first glance, when only comparing the longevity data of seeds which showed the greatest longevity in storage when dried at 45°C/23% RH (3 days; high rate) and 45°C/75% RH (1 day; low rate), the results appear to support this theory. Not only did drying seeds of accession IRGC 117265 from both harvests for 1 day at the slower regime (45°C/75%) show a greater longevity in storage (Appendix 6.5) compared with when seeds of the same accession were dried for 3 days at 45°C/23% RH (Appendix 6.3), but also seeds showed a greater relative improvement (compared with the DR) (Table 6.2). In order to test for further support for this hypothesis, the relative improvement in longevity of seeds dried under these two regimes (45°C/75% RH and at 45°C/23% RH) were compared against when seeds were dried at the alternate high temperature regimes; 45°C/40% RH (BD; Chapter 2) and 45°C/30% RH ($MgCl_2$; Chapter 5) described in previous chapters (Table 6.2) and harvest MC (Figure 6.3).

Table 6.2. The drying rate and relative improvement in longevity (compared with the dryroom) of seeds from accession IRGC 117265 dried at the different drying regimes.

Drying regime	Method	Chapter	Harvest MC (s.e.) (%)	Moisture lost (% day^{-1})	Relative improvement (%)
45°C/75% RH	Chamber	Chapter 6	31.0 (0.2)	19.9	263.0
			18.8 (0.1)	05.5	63
45°C/40% RH	BD	Chapter 2	22.7 (0.1)	10.2	23
45°C/30% RH	$MgCl_2$	Chapter 5	23.3 (0.1)	02.8	180
			23.3 (0.1)	06.8	180
			18.9 (0.1)	02.1	73
			18.9 (0.1)	06.0	73
			18.1 (0.0)	03.3	38
			18.1 (0.0)	03.7	60
45°C/23% RH	Chamber	Chapter 6	20.2 (0.2)	13.3	109
			13.0 (0.1)	07.4	65

The relative improvement in longevity when seeds were dried at 45°C/23% RH (high rate) and 45°C/75% RH (low rate) was consistent with the split-line relationship between the relative improvement in longevity and harvest MC when seeds were dried in the BD (Figure 2.5; Chapter 2). The re-analysis of the relationship when incorporating all the data from seeds dried at the different rates at 45°C increased the slope of the split-line regression from to 15.9 to 20.2 % MC^{-1} but the break point remained the same (16.2%) showing there is a high level of consistency between the moisture content above which seeds show an improvement in longevity when dried at 45°C (Figure 6.3). Further to this, seeds which were dried in the chamber at the fastest regime of the four did not, as was hypothesised, show in the least improvement in longevity across all MCs. In fact, drying seeds at the second slowest regime 45°C/40% RH (BD; Chapter 2) resulted in the least improvement compared with drying under any of the alternate regimes across all MCs. In light of this evidence it is not possible to attribute the improvement in longevity to the effects of drying at different RHs as all seeds appeared to benefit to a similar degree from high drying temperature (Figure 6.3).

6.4.2. Improved longevity: a result of drying at 45°C

At higher temperatures metabolic activity within the seeds will increase. Not all reactions have the same relative rate of change in response to temperature and many of the temperature coefficients in the literature are quoted as Q_{10} values i.e. the factor by which the metabolic reaction increases with every 10°C increase in temperature. Seeds undergo a coordinated series of events during desiccation which enables them to survive air-dry storage (Hoekstra *et al*., 2001). The ability of seeds to carry out metabolic processes associated with preventing oxidative damage and maintaining cells' structural integrity at an increased rate could help seeds to accrue mechanisms that increase their storability (longevity). Further to this, heat shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins are rapidly synthesised in response to dehydrative and/or temperature stress. They act as chaperones stabilising membranes and protecting proteins from aggregation (Hundertmark *et al*., 2011). Studies have reported that their over expression results in enhanced desiccation tolerance and the accumulation of other protective molecules such as proline, polyamine, sugars and peroxidase (Figueras *et al*., 2004; Roychoudhury *et al*., 2007; Tunacliffe *et al*., 2007; Liu *et al*., 2009) which also contribute

to the survival of seeds in air-dry storage. One class of LEA proteins, dehydrins, is relevant for desiccation tolerance (Galau, Hughes and Dure, 1986; Blackman *et al*., 1992; Bradford and Chandler, 1992; Hundertmark *et al*., 2011). They are also produced in response to any dehydrative force (temperature, drought, salinity) (Hundertmark *et al*., 2011; Leprince and Buitink, 2010; Radwan *et al*., 2014), and are thought to continue to accumulate, along with other protective proteins during the drying process, contributing to the overall longevity of the seeds (Sinniah *et al*., 1992a; Chatelain *et al*., 2012). Sinniah *et al*. (1998a and b) showed that LEA proteins accumulated comparatively late in seed development, during maturation drying, but could be induced to accumulate earlier post anthesis by the imposition of water stress. Therefore conditions, i.e. high temperature drying here, which allow for the continued synthesis of such protective proteins and sugars are likely to account for the increased longevity when seeds were dried at 45° compared with the DR.

To conclude based on an accumulation of results from the previous chapters, high temperature drying of seeds at 45°C still results in the greatest improvement in longevity compared with drying in the DR. It is thought that high temperatures promote the metabolic processes and protective mechanisms which are associated with desiccation, and increase the rate at which they occur. It is the increased accumulation of products from these reactions e.g antioxidants and protective proteins, which promote the ability of seeds to survive air-dry storage.

CHAPTER 7

EFFECT OF HARVEST MOISTURE CONTENT AND THE ROLE OF DEHYDRINS IN THE STORAGE LONGEVITY OF RICE SEEDS (*ORYZA SATIVA* L.)

7.1. Introduction

Based on the results presented so far in this thesis, two main conclusions can be drawn. Firstly, drying prematurely-harvested rice seeds at the conventional low temperature, low humidity conditions consistent of a genebank dryroom (DR) does not yield the greatest longevity in storage compared with drying at higher temperatures under various regimes, and secondly, the moisture content (MC) of seeds at harvest affects how the seeds respond to various post-harvest treatments and therefore their subsequent storage longevity. The experiment described in this chapter considered these two factors with the aim to explore why seeds, which have not entered/completed maturation drying *in situ,* benefit from high temperature drying.

It is thought that high temperature drying induces a similar stress response that the seeds experience during maturation drying, which triggers the induction of protective mechanisms such as the synthesis of dehydrins and other protective proteins which aid cellular stabilisation during storage. Dehydrin proteins are temporally regulated during seed development and generally accumulate during embryo expansion and in response to desiccation (Bewley *et al*., 2006). In rice, dehydrin synthesis can be detected before seeds have acquired desiccation tolerance and continues to increase thereafter (Still *et al*., 1994) indicating that they are not only relevant to desiccation tolerance but that they may also play a role in in seed quality and longevity (Galau *et al*., 1991; Ellis *et al*., 1993a).

The questions specifically addressed in this chapter were:

- Can seeds harvested before maturation drying is complete and subjected to hot-air drying reach the same longevity as those dried *in situ*?

- If seeds cannot dry *in situ*, what is the maximum period they can remain at high moisture contents in the field and still benefit from high temperature drying?
- Does the above vary between intermittent and continuous drying?
- Is hot-air drying stimulating the stress response, and hence further accumulation of dehydrins?

Hypotheses

Hypothesis 1: The longevity of seeds that have dried to low moisture contents *in situ* will not benefit from high temperature drying.

Hypothesis 2: The longevity of seeds maintained at high moisture contents *in situ* will still benefit from high temperature drying, irrespective of period on the mother plant (days after 50% anthesis; DAA).

Hypothesis 3: Intermittent drying is more beneficial to subsequent longevity than continuous drying when seeds are dried at high temperatures.

Hypothesis 4: Seeds which benefit from high temperature drying will show higher levels of dehydrins compared with seeds dried in the dryroom and with seeds that have already dried to low moisture contents *in situ,* irrespective of maturity (DAA).

7.2. Materials and methods

7.2.1. Plant material

Seeds from accession IRGC 117265 were sown on 6th December 2014 for dry season (DS) seed production (November 2014 - May 2015). Seedlings were transplanted in the screen house, CS09, on 26th December 2015. The area was approximately 224m^2 which had originally been divided into three plots: a control plot (59m^2), where the pre-harvest environment was free from manipulation; a restricted drying, or misting plot (105 m^2); and an enhanced drying plot (60 m^2). However due to timing constraints and costing it was not possible to complete the fan-assisted drying system which had been designed for

the enhanced drying plot. As a result this plot became an extension of the control, resulting in a control plot with a new total area of 120m^2 (Figure 7.1). From this point forward, the control and misting plots will be referred to as plots 1 and 2, respectively. The plots were separated by plastic sheeting fixed between the ceiling and the floor of the screen house to limit influence of the misting treatment on the control plot. The temperature conditions within the screen house were monitored using QRDL dataloggers (Centor Thai, Bangkok, Thailand) attached to wooden sticks which were embedded among the rice plants, positioned at two locations within each plot. The roofing and walls were built from fine fiberglass mesh supported by steel pipes, allowing the plants almost full exposure to ambient conditions (e.g. rainfall).

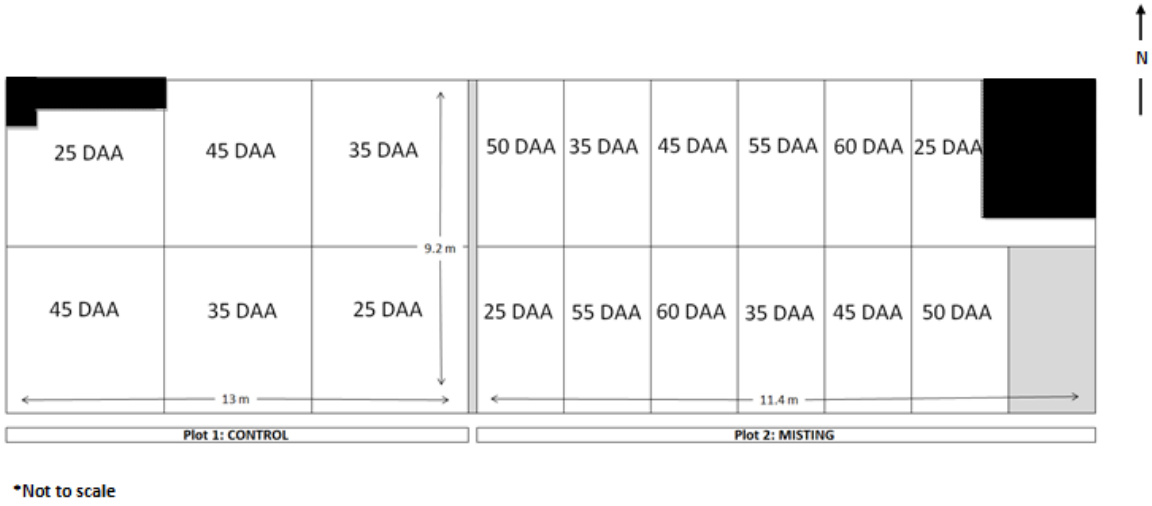


Figure 7.1. Final layout of the CS09 screen house. The total plot size (224 m^2) was sub-divided into two plots which were further segregated for harvests at different maturity stages from 25 -45 DAA (control plot) and from 25-60 DAA (misting plot). The black areas were not available for planting and the grey area was excess land.

All material was sown simultaneously and harvests occurred at 10-day intervals from 25 DAA to 45 DAA in both plots but at 5-day intervals thereafter, up to 60 DAA in the misting plot. The misting plot had twice as many scheduled harvests as the control specifically to test the maximum period that seeds can benefit from high temperature drying when they are maintained at high MC *in situ*. At each harvest, two 1 kg samples of seeds were harvested from two randomly assigned (minimum 12 m^2) sections within each of the plots

(Figure 7.1). These acted as biological replicates (1 and 2) which were later randomly assigned to either storage experiment A or B (Table 7.1).

7.2.2. Seed development and the application of in situ drying treatments

To monitor seed development 20 panicles were removed from each of the plots at 2-day intervals from 10 until 20 DAA. The seeds were removed from the panicle and the equilibrium relative humidity (eRH) was measured, following the same procedure as previously described (Chapter 2; section 2.2). Approximately 300 of the seeds (100 per replicate) were used to determine the seed dry weight using the low-oven temperature method (Appendix 2.2; ISTA, 2013), and three 5 g samples were used to determine the seed fresh weight (moisture content) using the high-temperature oven method (Appendix 2.2; ISTA, 2013). Once the seeds had reached their maximum dry weight, the misting treatment began. A round of manual misting using a knapsack sprayer at 30 PSI, spraying 300 ml per square metre, was originally scheduled every 2 h from 0900 to 1700 hrs from 20 DAA, but was later increased to five rounds every hour from 39 DAA as seed MC (fresh weight) determinations revealed very little difference in the seed MC between the two plots.

In addition to monitoring seed development, fluctuations in seed MC were monitored every 2 days during a 10-day period between 35 and 45 DAA. For this, from the 5th April 2015, 10 random panicles were removed from the plants in each plot at 0400, 1200 and 2000 hrs. As before, the seeds were removed from the panicles, the eRH measured and the MC (fresh weight) determined following the same protocol as described previously (Chapter 2; section 2.2).

7.2.3. Seed drying

Harvesting commenced on 26th March 2015 (25 DAA) and ended 30th April 2015 (60 DAA) (Table 7.1). At each harvest 2 × 1 kg of seeds was collected from each plot and immediately the eRH was measured and the MC (fresh weight) determined (Table 7.1).

Table 7.1. Harvest date, duration from anthesis (DAA), equilibrium relative humidity (eRH) and moisture content (MC; % fresh weight) for each harvest of each biological replicate (Rep.) from plots 1 and 2 at each maturity stage. Each replicate was randomly assigned either storage experiment (Exp.) A or B.

			Plot 1: Control			Plot 2: Misting		
Maturity stage (DAA)	Harvest date	Rep.	Exp.	eRH (%)	MC (s.e.) (% f.wt.)	Exp.	eRH (%)	MC (s.e.) (% f.wt)
25	26th Mar	1	A	96.1	22.8 (0.12)	B	96.9	22.3 (0.07)
		2	B	91.8	21.2 (0.03)	A	96.0	21.8 (0.09)
35	5th Apr	1	A	78.2	15.0 (0.03)	A	86.5	16.8 (0.07)
		2	B	82.9	16.1 (0.09)	B	87.6	17.3 (0.03)
45	15th Apr	1	B	73.3	13.6 (0.21)	B	83.8	16.1 (0.03)
		2	A	71.8	13.4 (0.10)	A	82.2	15.9 (0.09)
50	20th Apr	1				A	89.7	17.5 (0.06)
		2				B	88.2	17.3 (0.03)
55	25th Apr	1				A	86.5	18.2 (0.07)
		2				B	96.3	21.2 (0.15)
60	30th Apr	1				A	97.3	21.2 (0.07)
		2				B	98.9	22.3 (0.07)

Seeds from each replicate [2] × plot [2] were divided into three 200 g samples and placed into nylon mesh bags, as described in previous chapters, and stored inside sealed electrical enclosure boxes at room temperature (21.5°C) overnight. The following morning (0800 hrs), one sample was immediately placed in the genebank dryroom (DR; 15°C/15% RH) where it remained until equilibrium and the remaining samples were transferred to the batch dryer (BD). Seeds were dried in the BD for a total of 3 days either intermittently (In), for 8 h per day (0800 – 1600 hrs), or continuously (Con) for 24 h per day. Intermittently dried seeds were removed after each 8 h cycle and the eRH and sample weight was recorded before seeds were stored, as before, until the following morning when they were returned to the BD at 0800 hrs for the next 8 h cycle. After the 3-day drying period, the eRH and weight of all samples were recorded before they were transferred to the DR for final equilibrium drying. Once in the DR eRH and change in

weight was recorded at 3-day intervals. For those seed samples which were immediately transferred to the DR after harvest, the eRH and change in weight was measured daily for the first 3 days and then at 3-day intervals thereafter. Once seeds had reached equilibrium in the DR they were manually sorted, discarding any infected, empty or immature seeds and sealed inside aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until experimental storage began.

7.2.4. Seed storage

The same seed storage protocol was followed as described in Chapter 3. Seed samples were equilibrated in the VC3 0034-M climate chamber (Vötsch Industrietechnik, Germany) to 60% RH and at 21.5°C before being placed in an incubator at 45°C. Germination testing occurred at 3-day intervals until viability was lost and germination (criterion normal seedling development) was scored after 3, 5, 7, 14 and 21 days. MC determinations (as described in previous chapters) were conducted prior to storage and at the mid- and end storage points.

7.2.5. Statistical analysis

For seed lots which showed a loss in dormancy during storage, probit analysis, fitting the combined loss in dormancy and loss in viability model (equation [7]; Kebreab and Murdoch, 1999) to estimate K_d, θ_1, p_{50}, K_i and σ. The Ellis and Roberts (1980a) viability equation (equation [1]) was fitted for those seed lots with no dormancy at the beginning of experimental storage, combined with the "controlled mortality" parameter ("immunity" in GenStat) to estimate the proportion of "non-responding" seeds within the population (Mead and Gray, 1999) for some seed lots which showed a reduced initial viability.

7.2.6. Dehydrin expression

7.2.6.1. Collection and drying of seeds

Seeds used were of each replicate × maturity stage from each plot. Samples (approximately 30 individual seeds) of seeds were taken at harvest (pre-drying; PD) and after drying intermittently (BD_In) and continuously (BD_Con) for 3-days in the BD, as well as after 3 days of continuous drying in the DR. In addition to these samples, a sample of seeds was collected from the plants at 15 DAA – a mid-way point in seed development, prior to mass maturity. The samples were placed in labelled foil wrap and frozen in liquid nitrogen until they were transported to the Plant Molecular Biology laboratory on the main International Rice Research Institute (IRRI) campus where they were stored at -80°C until required.

7.2.6.2. Protein extraction

The protocol followed was in accordance with the procedure developed for extracting protein from roots and nodules of *Medicago truncatula* (Mathesius *et al*., 2001 and 2003) in: *Medicago truncatula* handbook, Version 2007. All required regents were purchased from Sigma-Aldrich Pte Ltd (Nucleos, Singapore).

Prior to protein extraction, three solutions were prepared. The quantities were sufficient for the extraction of all 70 samples:

- 10% Trichloroacetic acid (TCA) acetone (25 g TCA in 250 ml acetone and 175 mg 0.07% Dithiothreitol [DTT])
- 100% DTT acetone (500 ml acetone and 350 mg 0.07% DTT).
- Solubilisation buffer (20 g of 4% sodium dodecyle sulphate (SDS) dissolved in 250 ml of sterilised water, including 100 ml Tris(hydroxymethyl)aminomethane (Tris) × HCl and 1.54 mg 0.07% DTT).

The seed samples were removed from storage at -80°C and ground to a fine white powder in liquid nitrogen using a pestle and mortar. The ground tissue (0.5 g) was

scraped out of the mortar with a pre-cooled spatula and transferred to a 2 ml Eppendorf tube, including 2 ml of TCA acetone solution. The samples were vortexed thoroughly and placed at -80°C for 1 h. The tubes were centrifuged at 14,000 rpm for 10 min and the supernatant was discarded. The pellet was then suspended in 2 ml 100% DTT acetone and vortexed before transferring back to -80°C for 1 h. The samples were centrifuged again (as above) and the supernatant discarded. This step was repeated one more time. After removing the second volume of DTT acetone, the Eppendorf tubes were left open on the bench for at least 1 h to allow the pellet to dry. Once dried, 500 µl of the solubilisation buffer was added to the tubes which were then vortexed before being centrifuged at 15,500 rpm for 10 min. The liquid containing the extracted protein was pipetted out and collected in a clean new, labelled 1.5 ml Eppendorf tube. The protein sample was kept at -80°C until used for subsequent 1D gel electrophoresis.

7.2.6.3. Protein concentration

The Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Pierce Biotechnology; Rockford, USA) was used to quantify the total protein concentration of each sample. The nine BSA (diluted albumin) standards were prepared by diluting one albumin Standard (BSA) ampule (containing bovine serum albumin at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide) with the same solubilisation buffer that was used for protein extraction, into 1.5 ml Eppendorf tubes labelled A-I. The Working Reagent was prepared by mixing 200 ml of BSA Reagent A (contains sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) with 4 ml of BSA Reagent B (containing 4% cupric sulphate). Before the next step of the procedure could be completed, the protein samples required precipitation as they contained DTT which interferes with the BCA protein assay. The acetone precipitation procedure was performed firstly by pipetting 50 µl of each protein (BSA) standard and sample into 1.5 ml Eppendorf tubes and mixing with 200 µl cold (-20°C) acetone. The tubes were vortexed before being incubated for 30 min at -20°C. Each tube was centrifuged for 10 min at maximum speed and the supernatant discarded. Tubes were then left open at room temperature for 30 min to allow any remaining acetone to evaporate. Next, 50 µl of ultrapure water and 50 µl 5% SDS were added to each of the protein pellets and the tubes were vortexed. Once the pellet had dissolved, 25 µl of each standard or protein sample

was pipetted into a microplate well with 200 µl of the Working Reagent. The plate was then covered and incubated for 30 min at 37°C. After cooling, the absorbance was measured at 562 nm on a plate reader. The absorbance readings from the BSA standards was plotted to produce the standard curve from which the protein concentration of each sample could be determined (Appendix 7.6).

7.2.6.4. Gel electrophoresis

The proteins were separated by electrophoresis on 10% 1D mini gels; two 1.5 ml gels were prepared by mixing 6.25 ml sterilised water with 5 ml 30% acrylamide solution, 3.75 ml 4 × Tris PH 8.8, 75 µl 10% ammonium persulphate (APS), and 20 µl tetramethylethylenediamine (Temed) and pipetting in-between two glass plates. 1 ml butanol was pipetted on the top of the gels to remove any air bubbles before they were left to set for 30 min. Once set, the butanol was poured off and the stacking gel was prepared (3.05 ml sterilised water, 0.65 ml 30% acrylamide, 1.25 ml 4 × Tris PH 6.6, 50 µl 10% APS and 15 µl Temed). This was pipetted on top of the solidified gel and a 10-lane, 1.5 mm comb inserted before being left for 40 min to set. Once set, the comb was removed from the gels and they were transferred to an electrophoresis bath filled with 1 × SDS (diluted from 10 x SDS [30.3 g Tris, 144.1 g glycine, 10 g SDS] with 1000 ml of sterilised water) running buffer.

The protein samples were prepared for loading into the wells by mixing 20 µl of sample in a clean Eppendorf tube with 4 µl 6 × SDS dye (pre-mixed loading buffer [3.7 ml 4X Tris-HCl/SDS buffer (pH 6.8), 3 ml glycerol, 1 g SDS, 3 ml 2-mercaptoethanol, 6 mg Bromophenol blue] diluted to 10 ml with sterilised water and stored at 1 ml aliquots at -20°C]). The tubes were heated in a water bath at 100°C for 5 min before allowing them to cool down over ice. The protein ladder (3 µl) was loaded into the first well in each gel followed by the protein samples. The gels were run for 1 h 30 min at 120 V (or until the protein had run to the end). Each gel was then carefully removed from in-between the two glass plates, the stacking gel removed and discarded, and transferred to individual trays containing sterilised water to stop the gels from drying out until they were ready for blotting.

7.2.6.5. Protein blotting with semi-dry systems

A piece of transfer membrane and 6 sheets of Whatman filter paper were cut to the same size as the gel. The filter paper were placed together in a tray containing Nielsen buffer (5.82 g Tris, 2.93g glycine, 4 ml 10% SDS, and 200 ml methanol in 1000 ml distilled water) and left for 30 min at room temperature on a rocking platform. The membrane was activated in methanol for 30 sec before being washed in sterilise water for 5 min. The membrane was then left to equilibrate in a Nielsen buffer.

The Trans-Blot SD Semi-Dry cell apparatus (Bio-Rad Inc; California, USA) was prepared by applying some of the buffer onto the base of the electrode before mounting 3 sheets of Whatman paper saturated in Nielsen buffer onto the anode. The equilibrated transfer membrane was then placed on top of the filter paper stack and any air bubbles between the membrane and filter paper were removed by rolling a test tube over the surface of the membrane. The gel was placed on top of the membrane, and again any air bubbles removed to ensure intimate contact between the gel and membrane before completing the transfer stack by placing the final 3 sheets of filter paper on top of the gel and screwing the electrode in place. The apparatus was run at a constant 350 mAmp for 1 h.

7.2.6.6. Immunoprobing

Immobilised proteins were probed with specific antibodies to identify the dehydrin antigen present. A polyclonal antibody was designed using two peptides with a conserved region for two target dehydrins (LOC_Os11g26750 and LOC_Os11g26760) on chromosome 11 (see section 7.4.1).

The Trans-Blot SD Semi-Dry cell apparatus was disassembled and the membrane removed. The membrane was first immersed (50 ml) in blocking buffer (5% non-fat dried milk powder dissolved in phosphate-buffered saline (PBS) containing 1% polysorbate-20 (Tween-20) on a rocking platform to fill all protein binding sites with a non-reactive protein. After 1 h, the blocking buffer was decanted and the membrane was washed (50 ml) 3 times in antisera buffer (5% non-fat dried milk powder in PBS containing 0.1% Tween-20), 10 min each time. A final 50 ml of antisera buffer was then added to the

membrane and the primary antibody (20 µl) was pipetted into the solution and left overnight. The following day the blot was exposed to the secondary antibody (goat anti-rabbit (Bio-Rad Inc; California, USA) directed against the primary antibody. The primary antibody solution was decanted and the membrane was washed again, 3 times in 50 ml of the antisera buffer for 10 min. The secondary antibody (8 µl) was pipetted into the solution and left for 1 h at room temperature on a rocking platform. The solution was discarded after the allotted time and the membrane was washed in sterilised water three times for 10 min each.

7.2.6.7. Visualisation protocol

For visualisation of the protein, the Novex ECL Chemiluminescent substrate reagent kit (Thermo Fisher Scientific Pte Ltd; Singapore) was used. Reagents A (luminol) and B (an enhancer [stable peroxide solution]) were mixed together in equal volumes and pipetted onto the membrane and left for 5 min. The damp membrane was then sealed in plastic and placed inside the Western Blot exposure cassette which protects the screen from light during exposure. In the dark-room a sheet of photographic paper was exposed to the membrane for 3-5 sec to capture the chemilumiescent signals. The film was then developed in the dark-room.

7.3. Results

7.3.1. Changes in dry weight and moisture content during seed development

The mean seed dry weight (DW) increased progressively from 10 DAA, coinciding with the steady decline in moisture content (MC) and reached its highest value of 20.4 mg at 20 DAA (Figure 7.2A). This supports the results previously shown in experiment by Kameswara Rao and Jackson, (1996a) where the dry weight of seeds from 16 cultivars of rice, representing both indica and japonica genotypes which were also grown in the dry season at IRRI, reached a maximum at 21 DAA. Mass maturity usually coincides with the end of seed filling which occurred in these 16 cultivars between 18.5 and 21.6 DAA (mean 19.6 s.e. 0.32). Based on these preliminary results it was assumed here that seeds had reached mass maturity by 20 DAA and so signified the beginning of the application of the

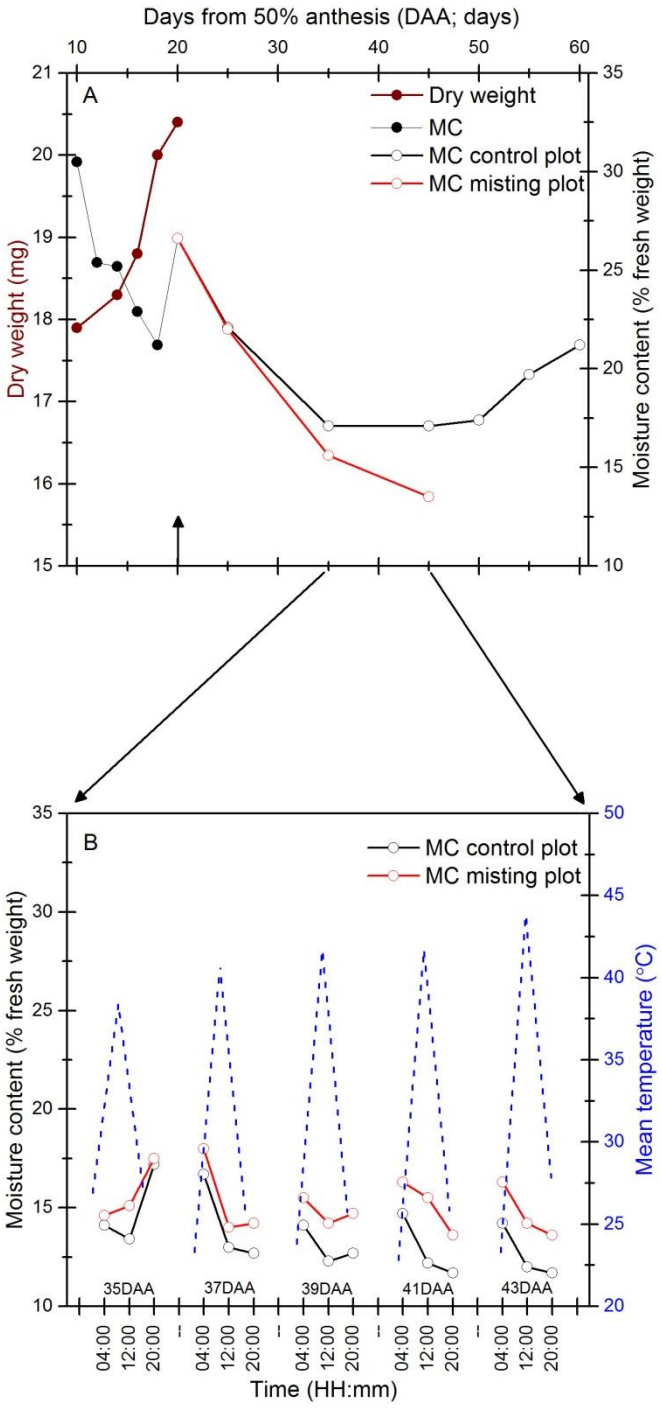


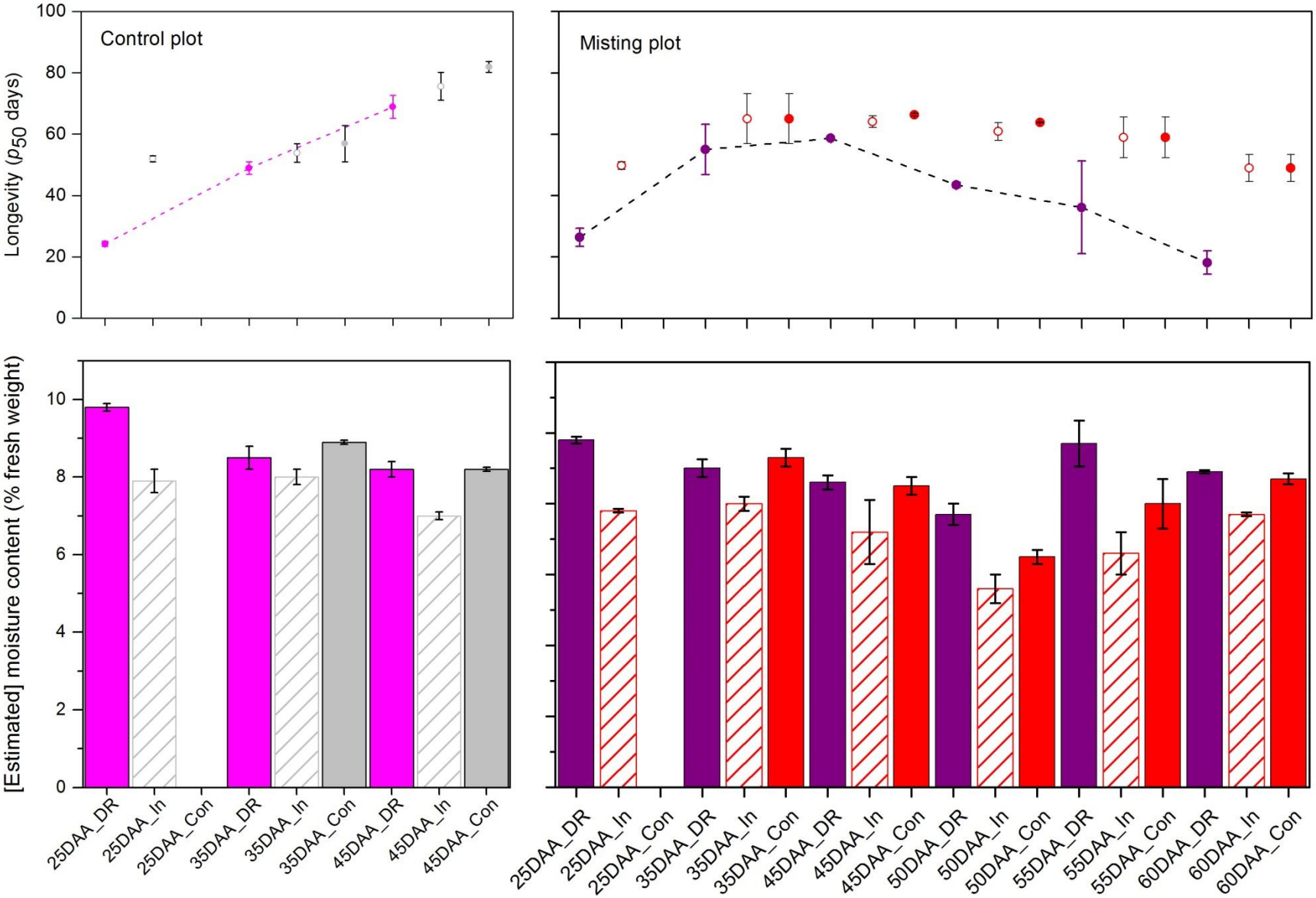
Figure 7.2. A) Changes in *Oryza sativa* L. seed dry weight (DW; solid brown circles) and moisture content on a % fresh weight basis (MC; solid black circles) during seed development from 10 days after 50% anthesis (DAA) to mass maturity (20 DAA). The changes in the mean MC of seeds from both plots (based on two biological replicates) at each harvest (25-60 DAA). B) Changes in the ambient temperature (°C; broken lines) inside the screenhouse and MC (symbols as in A) of seeds from both plots between 0400 – 2000 hrs at 35, 37, 39, 41, and 43 days after 50% anthesis (DAA).

misting treatment (indicated by the arrow on the graph). Following mass maturity the moisture content of the seeds from the control plot, i.e. no *in situ* drying treatment, declined with the increase in time from 50% anthesis to harvest (DAA) (Figure 7.2A), coinciding with the natural rise in ambient temperature (Figure 7.2B). The mean temperature within the screenhouse was much higher (values recorded as high as 45°C) compared with the ambient conditions outside as the fiberglass mesh walls reduce airflow. At 20 DAA the mean (both replicates) seed MC was 26.6% (s.e. 0.35), much higher than that recorded at 18 DAA (21.2% s.e. 0.18) as it was determined just after a period of rainfall, and had declined to 13.5% (s.e. 0.1) by 45 DAA (Figure 7.2A). The increase in temperature, which peaks daily around mid-day (1200 hrs) during the dry season, facilitated the natural drying process of the seeds during the day. In contrast, the mean MC of the seeds from the misting plot was higher than seeds from the control at 35 and 45 DAA, irrespective of the increase in ambient temperature. These differences in MC of the seeds between the two plots were apparent even over a 24 h time scale whereby the MC of the seeds naturally fluctuates with the rise and fall in temperature and humidity (Figure 7.2B). The differences were greater from 39 DAA when the misting treatment was enhanced from one round every 2 h to five rounds every hour between 0900-1700 hrs daily. The MC of seeds from both plots fluctuated between 0400 and 2000 hrs but the MC of the seeds from the misting plot were always higher compared with seeds from the control plot. Generally the MC of seeds increased during the evening/night as temperatures reduced and hence ambient RH increased allowing seeds to take up moisture. As the temperatures rose throughout the day, the MC of the seeds declined (Figure 7.2B) as the atmosphere can hold more water at higher temperatures.

7.3.2. Seed drying

All seeds reached a MC <10% after 3 days of drying in either in the DR or the BD (Figure 7.3). Seeds at all stages of maturity which were dried intermittently in the BD (BD_In) for 3 days reached the lowest MC compared with seeds dried at the other regimes despite the total drying time being only a third of that experienced during continuous drying. Seeds from both plots which were harvested at 35 and 45 DAA and dried in the DR, reached a lower MC after 3 days of drying compared with seeds continuously dried in the BD. Whereas beyond 45 DAA, seeds from the misting plot which were dried continuously

Figure 7.3. The estimated mean ± s.e. (Reps 1 and 2) moisture content (eMC; % fresh weight) (bar chart) and the longevity (p_{50}) (scatter plot) of the seed lots from each plot which were harvested between 25 and up to 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR). The eMC was calculated from the MC at harvest and the change in sample weight. The p_{50} values shown are for the model with the fewest parameters that could be fitted without a significant increase in residual deviance compared with the best-fit model (P>0.05; Appendices 7.2 and 7.4). Data from seeds harvested at 25 DAA and continuously dried in the BD was not able to be obtained due to a fault in the operation of the BD.


Control plot
Misting plot
Longevity (p_{50} days)
[Estimated] moisture content (% fresh weight)
0
20
40
60
80
100
0
2
4
6
8
10
25DAA_DR
25DAA_In
25DAA_Con
35DAA_DR
35DAA_In
35DAA_Con
45DAA_DR
45DAA_In
45DAA_Con
50DAA_DR
50DAA_In
50DAA_Con
55DAA_DR
55DAA_In
55DAA_Con
60DAA_DR
60DAA_In
60DAA_Con

in the DR reached a mean eMC of 8.7% (s.e. 0.4) which was higher than when seeds were dried continuously in the BD (7.8%; s.e. 0.4). As the conditions of drying under each regime did not change over the duration of this experiment it is possible that seeds harvested later in maturity do not respond as well to low temperature drying as seeds harvested at earlier stages.

7.3.3. Seed longevity

There were slight variations in the longevity of seeds harvested from the different plots and in some cases between the replicates within each plot (Figure 7.3), however, despite this, the same trends were observed when drying seeds either in the BD or the DR. First, the longevity of DR-dried seeds increased with the increase in maturity up to 45 DAA before declining, with lowest values recorded at 60 DAA. The proportions of desiccation tolerant seeds increased as seeds approached 45 DAA, shown by the increase in values of K_i (Appendices 7.2 and 7.4), and declined thereafter. As result, seeds dried in the BD showed an improvement in longevity throughout, but which improvement diminished in magnitude up to 45 DAA but increased thereafter (Figure 7.5). In particular, seeds harvested from the misting plot, which remained at a higher MC throughout development, showed the greatest benefit from high temperature drying at 60 DAA. For example, seeds (from replicate 1) harvested at 25 and 60 DAA which were at a MC of 22.3% showed an improvement in longevity of 107 and 212%, respectively (Appendix 7.2). Secondly, the seeds (of both replicates) harvested from both plots and at all stages of maturity showed the greatest longevity in storage when dried in the BD compared with the DR (Figure 7.4). This percentage increase (compared with drying in the DR) was related to harvest MC (Figure 7.4) i.e. seeds at a higher MC responded better to high temperature drying. For example, seeds (from replicate 2) harvested at 50 DAA at a MC of 17.5%, and at 60 DAA at a MC of 21.2% showed an improvement in longevity of 50 and 142.3%, respectively (Appendix 7.4). This increase in longevity did not always coincide with an increase in K_i but BD seed lots consistently showed a slower loss in viability compared with DR seeds (Appendix 7.4). The majority of the seed lots from each plot (replicate 1 or 2) × maturity stage which were dried intermittently or continuously in the BD could be constrained to a common line without an increase in the residual deviance

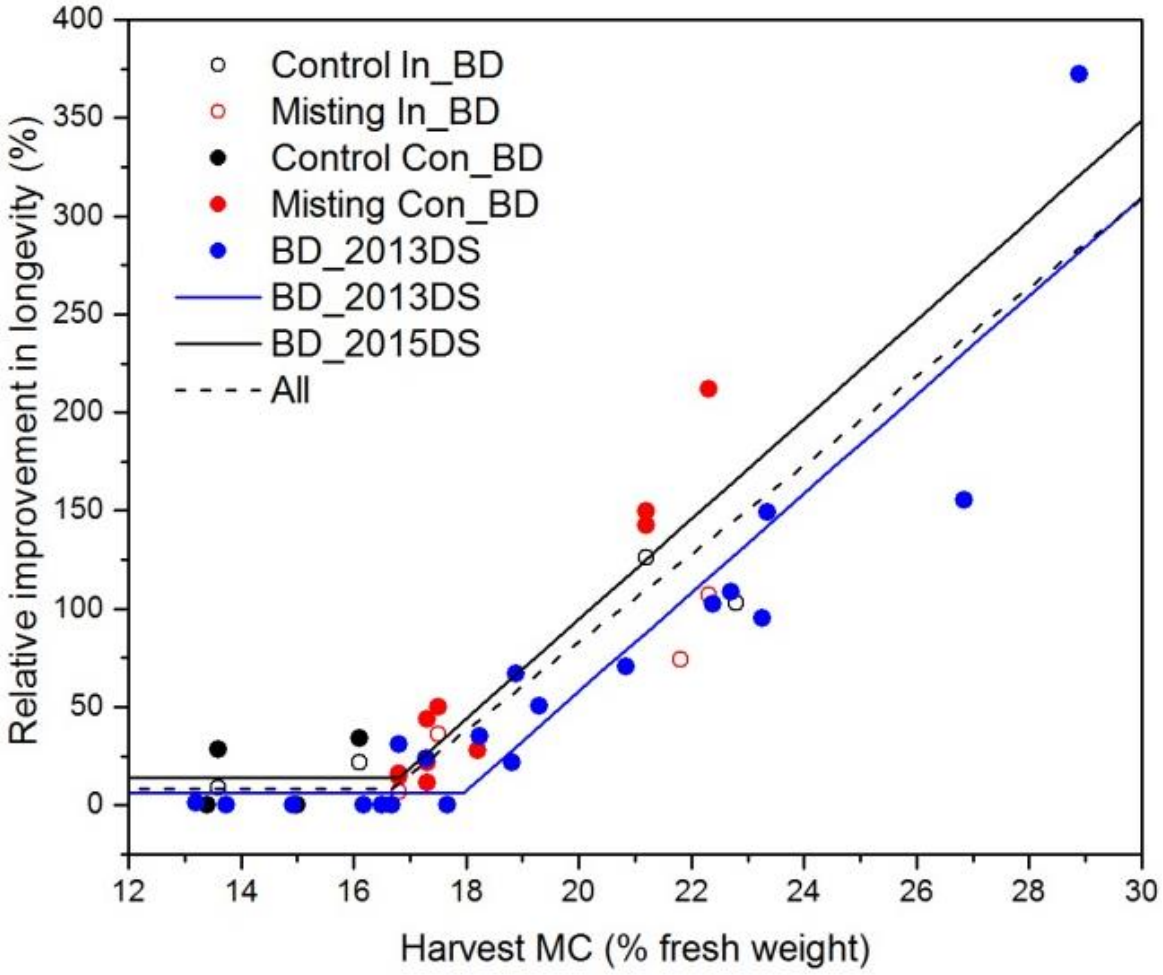


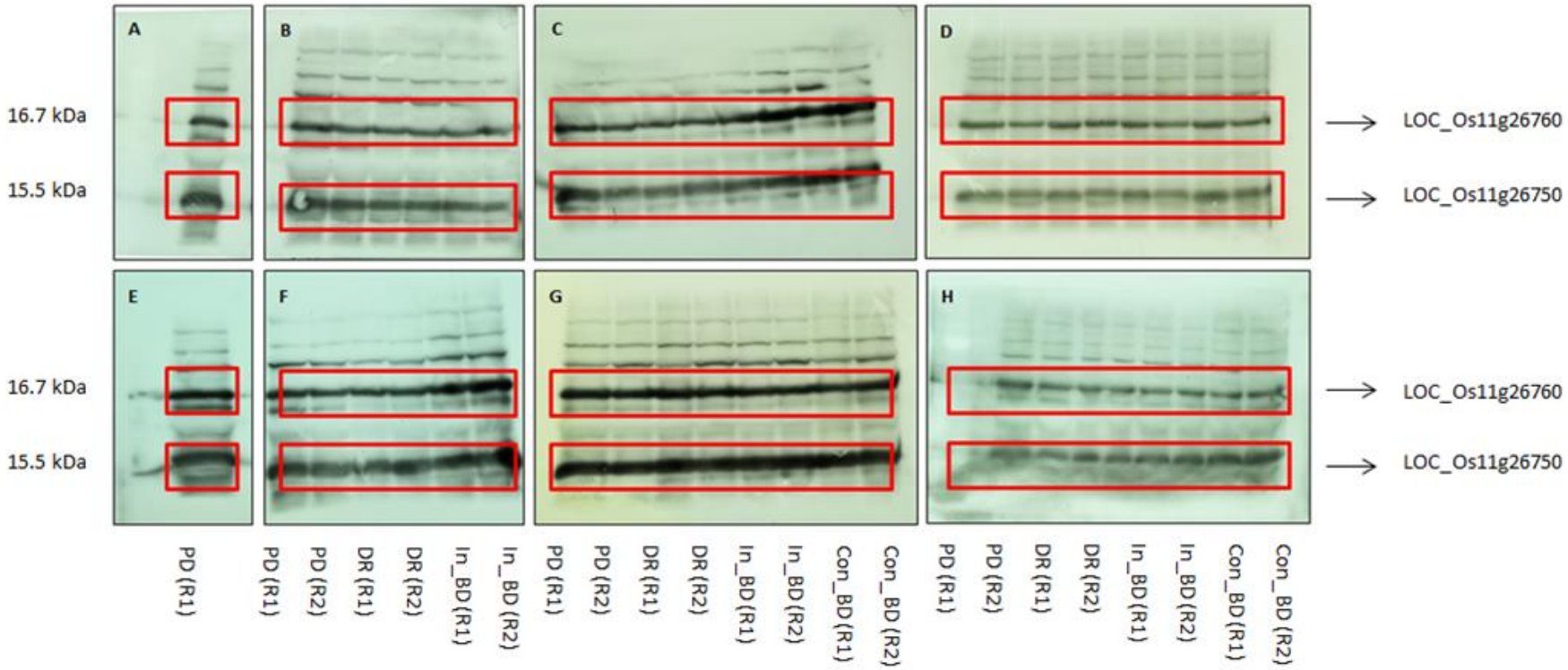
Figure 7.4. The relationship between the relative improvement in longevity (%; as calculated in previous chapters) from BD drying and harvest moisture content (MC) when seed lots (replicates 1 and 2) from each plot (control and misting) which were harvested between 25 and 60 days after 50% anthesis (DAA) were dried either intermittently (8 h day^{-1}) or continuously (24 h day^{-1}) in the batch dryer (BD) for 3 days prior to final drying in the genebank dryroom (DR) (solid, black line). The blue line represents the split line relationship between the relative improvement in longevity for 20 rice accessions and initial moisture content from Chapter 2. The split line regression was re-fitted incorporating the previously excluded "outlier" (Figure 2.4). The dashed line represents the relationship between these two factors for all seeds (2013; Chapter 2 and 2015 DS; present experiment) dried either intermittently or continuously in the BD which could be fitted without a significant (P<0.05) increase in the residual deviance compared with when fitting the regressions for each experiment individually.

(P>0.05) (Appendices 7.2, 7.4), but for those seed lots where the survival curves could not be constrained, continuous drying in the BD resulted in significantly greater longevity, by reducing the rate of probit viability loss (Figure 7.3; Appendices 7.2 and 7.4). Where the longevity of BD seeds varied between plots, seeds harvested from the control plot showed a greater longevity in storage compared with seeds harvested from the misting plot, regardless of harvest MC. In contrast, of the seeds dried in the DR, those which were harvested at a lower MC, irrespective of the plot, showed a greater longevity in storage (Table 7.1; Figure 7.3). Despite the slight variations in the values of p_{50}, the plot type or the duration of drying did not appear to significantly influence the improvement in longevity when seeds were dried in the BD (data not shown). When compiling the longevity data from all seed lots, split line regression accounted for 80.2% of the variance between the relative improvement in longevity and harvest MC (Figure 7.4). Further to this there was no significant difference (P>0.05) in the relationship between the relative improvement in longevity and harvest MC when seeds were harvested in the 2013 (Chapter 2) or the 2015DS and dried either intermittently or continuously in the BD, hence a common split line regression could be fitted to all data and accounted for 80.4% of the variance (Figure 7.4). The break point was re-positioned at 16.7%, below which value seed lots showed a limited response to drying in the BD.

7.3.4. Dehydrin expression

The change in dehydrin expression in seeds pre- and post-drying was monitored during development by Western Blot analysis. In addition to the harvests from 25-60 DAA, the dehydrin expression was measured in fresh seeds at 15 DAA. These seeds acted as somewhat of a control (“baseline”) to which the seeds from later harvests could be compared to as they were harvested approximately mid-way through seed development, before mass maturity, and therefore before application of *in situ* drying treatments. The two rows of thick bands in each of the sample blots (Figure 7.5) represent the two dehydrins, LOC_Os11g26750 and LOC_Os11g26760. The higher molecular weight dehydrin (LOC_Os11g26760), predicted at 16.7 kDa represented by the band closer to the top of the blot and the band nearest the bottom represent the other dehydrin (LOC_Os11g26750) which has a slightly lower molecular weight of 15.5 kDa in comparison. The surrounding “minor” bands are thought to be oligomers of these two

Figure 7.5. Western blots showing the expression of the two target dehydrin proteins (red boxes) in fresh (PD) and dried (BD; DR) seeds of accession IRGC 117265 harvested from the control and the misting plot at different stages of maturity (days after 50% anthesis; DAA). A) Control plot at 15 DAA, B) Control plot at 25 DAA, C) Control plot t 45 DAA, D) Misting plot at 55 DAA, E) Misting plot at 15 DAA, F) Misting plot at 25 DAA, G) Misting plot at 45 DAA and H) Misting plot at 60 DAA. Each lane (left to right) represents seeds from one of the plots (replicate 1 [R1] or 2 [R2]) × treatment: pre-drying (PD), and 3 days of drying either continuously in the DR (DR), intermittently in the batch dryer (In_BD) or continuously in the batch dryer (Con_BD). Although not all Western blots are shown, the results were the same at all maturity stages × drying treatment from each plot.



particular two dehydrins.

The level of dehydrin expression did not appear to differ between seeds harvested at different maturity stages from either plots 1 (control) or 2 (misting) (Figure 7.5). The intensity of the bands was the same for all seed lots harvested between 25 and 60 DAA. There also appeared to be no difference in dehydrin expression between seed lots harvested pre- (15 DAA) and post-mass maturity (25-60 DAA). Furthermore the level of expression of these two dehydrins did not differ between fresh (pre-dried) and dried seeds. There was no change in the intensity of the bands between pre-dried (PD) seeds and seeds which had been dried either in the DR or the BD. Seed lots dried intermittently in the BD did not appear to show any difference in dehydrin expression compared with continuous drying in the BD or the DR in either of the plots.

7.4. Discussion

The results of this chapter provide further support of the benefits of initially drying seeds at a high temperature in the BD. Seeds which are at a MC greater than 16.7% can show a more then 2-fold increase in longevity when dried either intermittently or continuously in the BD compared with drying in the DR at the lower temperature (Figure 7.4). From the results presented throughout this thesis, it is apparent that once seeds have attained mass maturity they do not follow a strict sequence of development with respect to time (DAA); rather their progression through development and increase in quality is determined by the processes which occur during desiccation, when seeds are still metabolically active (Angelovici *et al*., 2010). During the post-abscission phase of seed development the MC of the seeds is independent of the parent plant and naturally starts to decline (Kameswara Rao and Jackson 1996a, b). The aim of the misting treatment was to limit this natural drying process (maturation drying) and ensure seeds remained in the first stage of the post-desiccation phase where they can continue to accrue longevity (Chatelain *et al*., 2012), perhaps in response to hot-air drying. As expected, following mass maturity, the MC of the seeds from both plots, but more notably in the control than the misting plot, declined with the increase in time from 50% anthesis to harvest (Figure 7.2A). As the longevity of the DR seed lots increased during this time, up to 45 DAA (Figure 7.4), seeds dried in the BD subsequently showed a reduced relative improvement

in longevity with DAA as a result (Figure 7.5). However after 45 DAA the longevity of DR-dried seeds from the misted plot began to decline and so, contrastingly, the relative improvement in the longevity of seeds increased after 45 DAA.

At 45 DAA the seeds from both plots had already dried to a MC which coincides with the part of the isotherm (<80% RH) where damage can be accrued at a rate that will increase as moisture content increases with fluctuating environmental conditions (Roberts and Ellis, 1989). Therefore an increase in the MC of seeds thereafter, as a result of the misting treatment, was likely to have compromised their quality. In support of this statement the longevity (p_{50}) of BD-dried seeds tended to be lower when seeds were harvested later in the season (after 45 DAA) compared with earlier despite both seed lots being at the same (or similar) MC at harvest. This was more notable when seeds harvested later in the season were at a higher MC. For example, seeds harvested at 55 DAA and dried in the BD (intermittent or continuous) showed p_{50} values of 52.4 (replicate 1) and 65.6 (replicate 2) when harvested at 21.2 and 18.2% MC, respectively. However seeds which were harvested after 45 DAA still showed an increase in longevity when dried in the BD compared with the DR, suggesting not only that the benefits associated with drying seeds at a high temperature must outweigh the detrimental effects of ageing which may have occurred but also, seeds can continue to increase in quality in response to high temperatures providing they are at a MC where metabolic activities resume, irrespective of whether they had already previously made the transition into the second stage of the desiccation phase. This did not appear possible when seeds are dried at low temperatures post-harvest hence the increased reduction in longevity with time from 45 DAA to harvest. As a result, there did not appear to be a maximum time seeds could remain at a MC indicative of the first stage of the desiccation phase and still benefit from high temperature drying as seeds at 60 DAA still showed an improvement in longevity compared with the DR seed lots. A recent experiment by Ellis and Yadav (2016) provides some evidence that the quality of dry cereal seeds can be further improved *in planta* if the MC of the seeds increases and hence, are re-dried. Wheat seeds which were exposed to simulated rainfall at different stages of development and maturation showed initial, immediate damage but thereafter an increase in subsequent seed longevity providing they were allowed time to re-dry before harvest (Ellis and Yadav, 2016). This indicates

that the seeds are able to initiate repair mechanisms and reverse the previously accrued damage whilst at a high MC.

Generally the longevity of seeds did not differ when dried intermittently or continuously in the BD as most seed lots (from each replicate) at each maturity stage could be constrained to a common line. This indicates, not only that the total exposure time to high temperatures was not impacting on the storage potential of the seeds but also there is no additional benefit of allowing the equilibration of water, at room temperature, within seed tissues. However in the instances where differences were detected, continuous drying resulted in greater longevity in storage compared with intermittent drying, despite seeds being at a higher MC after 3 days of continuous drying (Figure 7.4).

As previously mentioned the BD does not have a dehumidification system and so there is a limit to the extent the RH can be reduced under ambient conditions. However as the ambient temperature drops during the evening the RH can be further reduced compared with when temperatures are higher during the day. Therefore it is probable that MC of the seeds dried continuously in the BD was undergoing constant cycles of desiccation and rehydration as the limit of the drying conditions at 45°C changed throughout a 24 hr period. For example, between 1200 and 0400 hrs the ambient temperature can drop from 44 to 23°C (Figure 7.2A). Based on the amount of water the air can hold at 23°C and 85% RH, which is less compared with when temperatures are higher during the day, heating the air to 45°C will reduce the RH to approximately 25% RH (based on air moisture relations as calculated using Cactus2000) which is able to dry seeds to a lower MC (eMC 6.7%; estimated using Cromarty's equation executed in the Seed Information Database [Royal Botanic Gardens Kew, 2008]) compared with later in the day when heating the air to 45°C will only reduce the RH from 85 to 81% RH resulting in an eMC 14.2%. It is thought that these fluctuations in the MC is having a similar beneficial priming effect (Chapter 3) on the seeds which occurs naturally *in situ* (Figure 7.2), allowing seeds to accrue longevity during moisture loss, and initiate repair upon rehydration when normal metabolism resumes. This goes against current recommendations when drying seeds in a less controlled environment which states seeds should be stored in air-tight containers over night to prevent any uptake in moisture when ambient air humidity rises (Hay and

Probert, 2011). Nevertheless, this may explain the higher longevity of seeds dried continuously as opposed to intermittently (Figure 7.4).

7.4.1. Dehydrin expression and seed longevity

There are clear benefits of initial high temperature drying on the storage potential of seeds which are harvested at a MC where they are still metabolically active, irrespective of whether seeds had previously dried to low MC *in situ* where metabolism ceased. Metabolism can resume upon rehydration allowing seeds to benefit from high temperature exposure. Further there also appears to be no limit in regards to developmental progress as to when seeds stop benefitting from high temperature drying. It was hypothesised that high temperatures were inducing a stress response in the seeds which triggered protective processes and the accumulation of protective proteins, such as dehydrins, which are involved in the stabilisation of tissues during storage. This hypothesis is supported by previous studies which provided evidence that the accumulation of soluble carbohydrates (sugars) and heat stable proteins during development were associated with desiccation tolerance and potential longevity (Sinniah *et al*., 1998b). For example, in seeds of brassica, the total oligosaccharide ratio and the 58 kDa heat stable protein independently showed a significant correlation with the difference in K_i, which arose as a result of the different irrigation treatments on the mother plant and changed the timing of maturation and hence, the attainment of maximum seed quality (Sinniah *et al*., 1998a). From these results it was suggested that both protective sugars and proteins are equally likely to be required for the development of high seed quality (Sinniah *et al*., 1998b), but the accumulation of heat stable proteins are more likely to account for the differences in longevity between seed lots as they accumulate comparatively late in seed development, during maturation drying, coinciding with the increase in potential longevity which continues once seeds have acquired maximum desiccation tolerance. Despite this evidence, the results from protein expression analysis presented in this chapter did not support this theory.

The two dehydrins LOC_Os11g26750 and LOC_Os11g26760 were selected from the possible eight which are expressed in rice (Kawahara *et al*., 2013), based on microarray data showing the individual dehydrin expression during seed development (Kapoor *et al*.,

2007; Appendix 7.5). The expression of the two identical target proteins typically increases late in seed development, from approximately 5-10 days after pollination (DAP) till 29 DAP (Kapoor *et al*., 2007), encompassing embryo morphogenesis (5-10 DAP), embryo maturation (11-20 DAP) and dormancy and desiccation tolerance (21-29 DAP). Desiccation tolerance is acquired before mass maturity in rice, and increases thereafter with seeds not tolerating desiccation to low MC required for storage until later in development, coinciding with an increase in seed longevity (Ellis and Hong, 1994). As seeds require desiccation tolerance to have any longevity in air-dry storage, it is thought that desiccation tolerance to very low moisture contents and the ability to survive air-dry storage may have a common cause (Hong and Ellis 1992a; Ellis and Hong, 1994). Therefore as the expression of the targeted dehydrins were the highest (compared with earlier in development) during the last recorded stages (21-29 DAP), when seeds begin to acquire desiccation tolerance, there was reason to believe that they were involved in conferring/promoting/enhancing seed longevity.

The level of expression of the two dehydrins did not appear to differ between seeds harvested at different stages of maturity in either plot. Although the main harvests began significantly later in comparison to the earlier study (Kapoor *et al*., 2007), the dehydrin expression was expected to differ, at least between seeds harvested pre- (15 DAA) and post-mass maturity (25-60 DAA). However, this was not apparent from the blots (Figure 7.5), suggesting that the accumulation of these dehydrins peaks prior to mass maturity, and do not increase further, irrespective of the seeds' metabolic status (i.e. misting plot seeds remained at an RH greater than 80%). As the desiccation tolerance and the longevity of seeds continues to increase post-mass maturity, during final maturation drying (Chatelain *et al*., 2012), this suggests that the level of dehydrin protein alone does not confer desiccation tolerance (Finch-Savage *et al*., 1994; Still *et al*., 1994), at least to low moisture contents, unless however, the proteins were being "utilised" and re-synthesised at the same rate, then the overall expression level detected within the seed would not change. Dehydrins are labelled as "intrinsically disordered proteins" (IDP) meaning that they have no defined secondary or tertiary structure (Graether and Boddington, 2014) which is why they do denature. They do, however, gain structure when they associate with other macromolecules which can change their oligomeric state. This would be detected by an increase in the intensity of the minor bands, but this was also

not apparent between seeds harvested at different stages of maturity (Figures 7.2 and 7.5) indicating they are not being utilised during development.

In addition, the level of protein expression did not appear to change during drying, either at the low (DR; 15°C/15%) or high temperature (BD; 45°C) despite seeds from both plots showing an increase in longevity when dried either intermittently or continuously in the BD compared with the DR (Figures 7.4 and 7.6). This suggests that these dehydrins are not responsive to desiccation to low MCs, or to high temperatures and so they cannot account for the differences observed in the subsequent storage longevity of seeds. This supports the previous research on brassica seeds which showed desiccation tolerance before they had accumulated a significant amount of group 2 (dehydrin) and group 3 LEA proteins, the timing of which was also not affected by post-harvest drying (Bettey *et al*., 1998). As a result they came to the same conclusion which was that LEA proteins are not absolutely required for desiccation tolerance. In the case of the two dehydrins used in this experiment, it is possible that these dehydrins could play more of a "house-keeping" role (Hara *et al*., 2011), or that they are involved in other developmental processes in which case their expression would be related to a specific developmental stage.

Dehydrin expression can be measured at the protein or mRNA level. Methods such as quantitative polymerase chain reaction (qPCR) which detect changes in the level of RNA are more sensitive compared with Western blot analyses which detect changes in the protein content (AJ Kohl 2016. pers. comm). Therefore it is possible that the expression level between the different seed lots may have differed but was too small to be detected at the protein level, despite the very high protein concentrations (Appendix 7.6). However, during drying RNA degrades very quickly and therefore its expression can only be measured once seeds have become fully imbibed and are metabolically active (AJ Kohl 2016. pers. comm). As a result, RNA expression analysis cannot be used to understand changes that occur in response to seed drying. An alternative representative of a type of stress protein whose expression could be monitored are heat shock proteins (HSPs) which also accumulate in seeds during the late stages of development (Close *et al*., 1993; Wehmeyer *et al*., 1996). Following their expression in brassica during development it was observed that their synthesis was induced by post-harvest drying when harvested during the stage of development when desiccation tolerance increases (Bettey *et al*., 1998). It

was thought that mRNA for HSPs are synthesised but not translated until the seeds experience dehydration stress e.g. rapid drying, reduced irrigation (Bettey *et al*., 1998), heat treatment (DeRocher and Vierling, 1994).

To conclude, the benefits of high temperature drying have been confirmed in rice seeds as long as they are still metabolically active at harvest, irrespective of DAA and whether they had already previously made the transition into the second phase of the post desiccation phase. This has major implications on how seeds, which are regenerated in different climates, are best handled post-harvest.

CHAPTER 8

GENERAL DISCUSSION

8.1. Finding improved conditions to dry rice germplasm

The long-term preservation of the genetic diversity of cultivated Asian rice (*Oryza sativa* L.) can be ensured by storing their orthodox seeds at a low temperature (-20°C) and moisture content (3-7%) in genebanks. Breeders rely on the genetic resources of rice germplasm as they can be used to produce more high yielding varieties and/or improve their resistance to a wider range of biotic/abiotic stresses (Hay *et al*., 2013). Therefore, it is critical that genebanks effectively manage accessions, by monitoring their viability at regular intervals and regenerating them when germination falls (Cromarty *et al*., 1982; Rao *et al*., 2006; FAO, 2013; Hay and Probert, 2013). The regeneration procedure is one of the most crucial components of genebank management (Ellis *et al*., 1985; Rao *et al*., 2006; van Treuren *et al*., 2013), however it is highly expensive and can involve the loss of genetic diversity by genetic drift due to selection pressures, handling errors and outcrossing (Allard, 1970). As a result, the frequency of regeneration should be minimised through the maximisation of seed storage longevity (Kameswara Rao and Jackson, 1996a).

The longevity of seeds in storage is affected by the conditions of storage - longevity increases with decrease in temperature and moisture content (MC) - and the pre- and post-harvest environments and processes (Chapter 1). The main aims of this thesis were to determine the optimum combinations of temperature, relative humidity and duration to dry rice seeds for long-term conservation and to see whether these optima varied with genotype. This discussion will cover how the pre- and post-harvest environment and practices affect the seeds response to drying conditions and the implications of this for *ex situ* conservation.

8.1.1. Overview of drying facilities at International Rice Research Institute (IRRI)

Prior to the 1990s, it was common practice to dry rice seeds intended for the genebank at IRRI at high temperatures (between 45 and 50°C). The dryroom (DR) facility, with external dimensions 2.4 × 8 × 7.6 m (H × L × W) and a refrigeration and dehumidifying system, was built in the early 1990s and, following advice from experts since this was prior to the publication of the FAO genebank standards, was programmed to run at 15 ± 2°C, 15 ± 5%. The genebank standards were published in 1994 (FAO, 1994) and the recommended drying conditions were 10-25°C and 10-15% RH. It was thought that high drying temperatures could potentially damage seeds, especially those at a high MC (Nellist 1980; Cromarty *et al*., 1982; McDonald and Copeland, 1997). Hence, since the installation of the DR, it has been routine for seeds to be dried immediately after harvest in net bags in the DR for 14 days when equilibrium (6.1% MC; estimated using Cromarty's equation executed in the Seed Information Database [Royal Botanic Gardens Kew, 2008]) should be reached.

Technical specifications identify the dryroom as having a capacity of 16 000 kg (14-16% MC) and an accumulation rate of 4000 kg within a 1.5 to a 2 month period. However, when the weight (kg) of all incoming material was recorded during the 2013 dry season (DS) (Feb-May), it revealed that the majority (7000 kg) of seed was received in April which subsequently led to a peak cumulative total of 9000 kg by the end of May (Appendix 8.1). As this exceeds the recommended accumulation rate at any one time, the efficiency of the DR reduced around the time of peak harvest, with seed samples showing a slower rate of moisture loss and a greater time to equilibrate (data not shown), most notably when individual sample sizes were large in volume. Despite this, the DR had not yet reached its absolute capacity. Therefore the stability of the DR conditions is dependent on how close it is to reaching capacity at any one time, but also the volume and rate of material influx. Despite the recommendations for the management of genebank accessions emphasising the importance of initial seed drying to maximise subsequent storage longevity (Cromarty *et al*., 1982; Rao *et al*., 2006; FAO, 2013), the conditions at which IRRI dries seeds or the efficiency of the DR during the harvest season has not been critically evaluated before.

8.1.2. Experimental set-up

As the conditions of the DR (15°C/15% RH) comply with the current genebank standards (FAO, 2013) for all the experiments the DR seed lots acted as a control, considered as the "baseline" against which the effects of other drying treatments were compared. In trying to determine potentially superior conditions to dry rice seeds, a number of different methods/drying equipment were used. Firstly, the locally fabricated batch dryer (BD) (Chapters 2, 3, 4 and 7) dries seeds in an open environment using hot air generated by a kerosene gas burner which on average heated air to approximately 45°C. However, with no in-built dehumidification system the RH cannot be controlled or reduced below perhaps about 35% (estimate based on prevailing ambient conditions and air-water relations). In order to control the temperature and humidity conditions more precisely, saturated salt solutions were considered a practicable alternative (Chapter 5). Although it was possible to compare different treatments (different salts) simultaneously, the RH of different saturated salt solutions varies with temperature to a greater or lesser extent, depending on the salt. Further to this, in order to try to maintain the drying environment (temperature and RH), seeds were placed above the salt solution in a hermetically sealed box. Thus there was no airflow through the seeds; rather the process was passive, relying on the fact that there was only a limited bulk and hence reasonable exposure of all the seeds to the air. The final piece of equipment used in this thesis to dry rice seeds was the climate chamber (Vötsch Industrietechnik, Germany) (Chapter 6), a hermetically sealed system, with an inbuilt de-humidifier, heater and cooler which is able to maintain a highly controlled and stable environment at the temperature and humidity conditions programmed. However, since only one chamber was available, it was not possible to compare different environments using seeds from the same seed lot harvested at the same time. Also, there was no airflow within the chamber.

8.2. The effect of high temperature drying on rice seed longevity

The results from the numerous experiments described and/or discussed in this thesis show that drying seeds, within the recommended low temperature (5-20°C) and low humidity conditions (10-25% RH; FAO, 2013), at 15°C/15% RH to low moisture contents for genebank storage are not optimal for all samples of the rice accessions studied.

Rather, rice seeds can show up to a 3-fold increase in longevity (Chapter 2) when dried intermittently (8 h day^{-1}), for up to 6 days, immediately after harvest at a higher temperature (45°C) in the batch dryer (BD) prior to equilibrium drying in the dryroom (DR). This improvement did not appear to be limited to a specific period in relation to seed maturity (days after 50% anthesis; DAA), or affected by the total exposure time (at least up to 72 h continuous drying) (Chapter 7). Even when high temperature drying was delayed after harvest, until seeds had undergone 16 h of drying in the DR, seeds still showed an improvement in longevity compared with drying solely in the DR; however this improvement was limited and generally lower compared with immediate high temperature drying (Chapter 3). Despite the observed benefits, seed lots (both between and within accessions) did not benefit to the same extent from high temperature drying. The results from drying seeds under more controlled conditions whereby the rate of drying at 45°C could be altered by changing the RH conditions (Chapters 5 and 6) confirmed that the variation in the improvement in longevity when drying seeds at 45°C was not due to drying at different RHs but rather a result of pre-harvest factors.

8.3. The harvest moisture content of the seeds affects their response to high temperature drying

It is clear from the results that not only do rice seeds continue to increase in quality after mass maturity (Chapter 2) therefore contradicting the early hypothesis made by Harrington (1972), but their progression through development and increase in quality is determined by the processes which occur during desiccation (specifically maturation drying; Galau *et al*., 1991) when seeds are still metabolically active (Angelovici *et al*., 2010), as opposed to being dictated by time (DAA) which has been widely reported for other species (e.g. TeKrony *et al*., 1980; Kameswara Rao and Jackson, 1996 a, b, c; TeKrony and Egli, 1997). Chatelain *et al*. (2012) proposed, based on proteomic studies, that the desiccation phase should be divided into two, the first when there is increasing seed longevity and then a final maturation drying stage. This has particular implications for seeds grown in the wet tropics where climate conditions typically limit the drying process and also, in the case of rice, when paddy fields are not drained in the period approaching harvest i.e. the plants are not maturing into terminal drought unlike other cereals (Appendix 2.1).

When compiling the longevity data from all seed lots dried at 45°C and at different relative humidities throughout this thesis, split-line regression accounted for 81.5% of the variance between the relative improvement in longevity (%; relative to the DR) and harvest MC which could be fitted without significant (P<0.05) increase in the residual deviance compared with fitting the regressions for each experiment individually ($F_{(5,75)}$=0.96; P=0.45). This indicates that all seeds benefitted to a similar degree to drying at 45°C, irrespective of the RH conditions, when harvested at a MC ≥16.5% (Figure 8.1). Therefore this implies that seeds which are unable to dry down to low MC (≤16.5%) *in situ* are still in the first part of the desiccation phase and therefore can continue, when exposed to high temperatures, to accrue longevity. However, if seeds have already dried on the plant to a MC at which they are no longer metabolically active (≤16.5%), they are thought to have entered the second part of the desiccation phase of development and therefore show a limited response to high temperature drying (Figure 8.1). The consistency in the relationship between the relative improvement in longevity and harvest MC when seeds from different harvest seasons and at different DAA were dried at different durations under different regimes at 45°C confirms that the temperature of drying is the most important factor which enables seeds to continue to accrue longevity *ex planta*.

Recent evidence has emerged which provides some support of how the pre-harvest environment, with respect to drying, can influence the longevity of cereal seeds (Ellis and Yadav, 2016). The subsequent longevity of wheat seeds could be improved *in planta* if the MC of the seeds increased, in response to simulated rainfall, at different stages of development and maturation providing they were allowed time to re-dry before harvest – albeit that the improvement was the reversal of damage to longevity from simulated rainfall (Ellis and Yadav, 2016; *in press*). Previously, the beneficial effects of wet-drying cycles on seed longevity have been demonstrated for mature seeds *ex planta* (Villiers and Edgecumbe, 1975; Butler *et al*., 2009b) and are supported by the results outlined in Chapters 3 and 4. It was concluded that seeds which are at a high MC (>80%), as result of past environmental conditions (pre- or post-harvest), can continue to increase in longevity *ex planta* in response to high temperature drying. Despite this, the positive effects of an invigoration treatment, when combined with subsequent desiccation, are not consistently shown in the literature with reports of some seeds showing a reduction

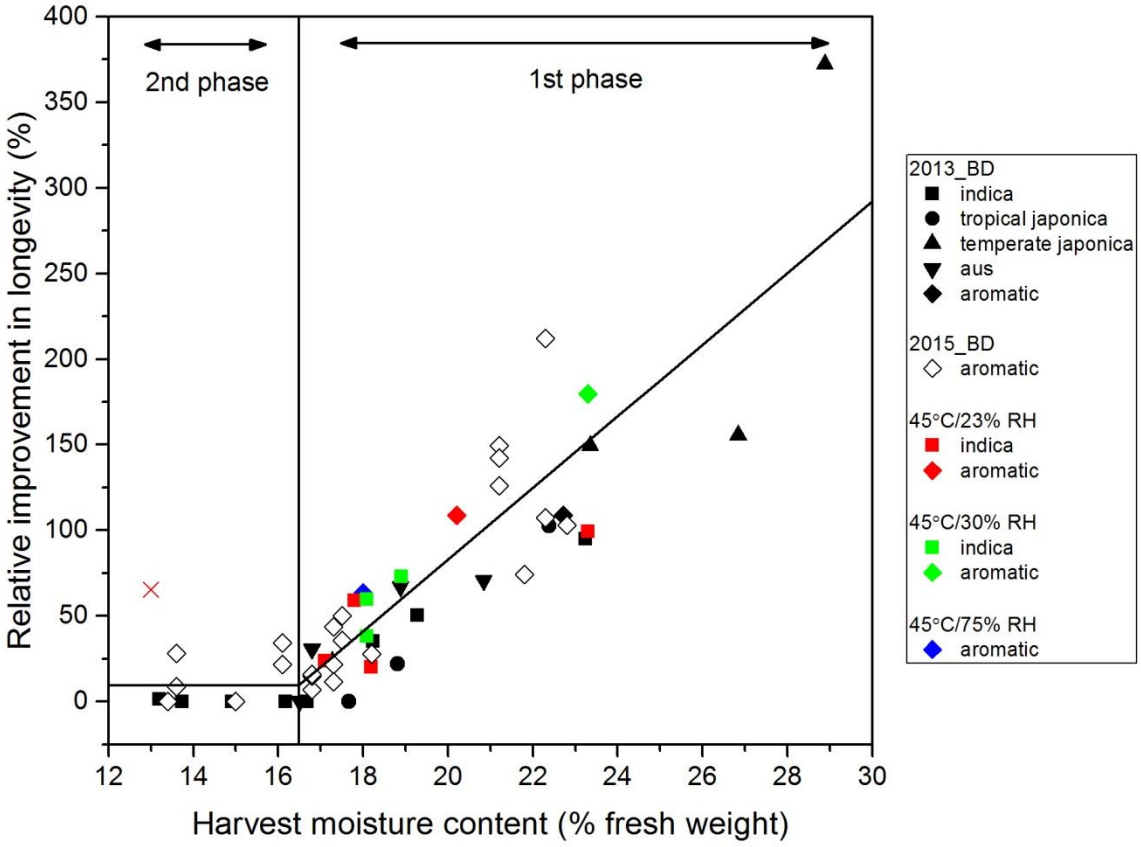


Figure 8.1. Relationship between the relative improvement in longevity (%; difference in longevity (p_{50}) between seeds dried at 45°C and at different relative humidities calculated as a proportion of the DR p_{50}; as in previous chapters) and harvest moisture content (MC; fresh weight) for the 20 rice accessions harvested during the 2013, 2014 and/or 2015 dry and wet seasons. All seed lots were harvested between 24 and 60 days after 50% anthesis (DAA). The solid line is a result of split-line regression analysis for all drying treatments which could be fitted without significant (P<0.05) increase in the residual deviance compared with fitting the regressions for each experiment individually, and accounted for 81.5% of the variance. The outlying data point (×) at c. 13% moisture content was not included in the analysis. A relative improvement in longevity of 100% is equivalent to a doubling in longevity compared with DR treatments. The solid line passes through the 16.5% moisture content breakpoint above which seeds show an increase in longevity in response to high temperature drying. This breakpoint is suggested to delineate the two phases of the drying process proposed by Chatelain *et al*. (2012).

in longevity following priming (Heydecker and Gibbins, 1978; Argerich *et al*., 1989; Tarquis and Bradford, 1992).

How past environmental events (pre- or post-harvest) can influence the seeds response to different post-harvest drying treatments is a novel concept and has not been researched until now. Rather, previous research has focused on how pre-harvest conditions can alter the progression through development including phases of seed development which are most sensitive to specific conditions. For example it is known that very heavy and/or prolonged periods of rainfall late in seed development can reduce grain yield and seed quality (Tu *et al*., 1988; Olivares *et al*., 2009), causing damage and viviparous germination (Hirano, 1979). Similarly, high temperatures during certain stages of development can cause sterility (flowering stage) and/or reduce grain filling (ripening stage). In rice, seeds are most sensitive to high temperatures between the histodifferentiation phase, soon after pollination (Martinez-Eixarch and Ellis, 2014) and the end of seed filling (Ellis, 2011).

8.4. The effect of high temperature drying on K_i and σ

In the Ellis and Roberts (1980a) viability equations, which predict the longevity of a seed lot in air-dry storage, it is assumed that under the same storage conditions, different seed lots of the same species will deteriorate at the same rate (σ^{-1}). Any apparent seed lot differences in longevity are therefore due to differences in the initial viability (K_i) which can arise as a result of genetic or environmental influences or seed maturity (Ellis and Roberts, 1980a, 1989; Ellis, 1991).

Seeds which are harvested when still in the phase when longevity is still increasing on the mother plant benefit from high temperature drying as they are still able to accrue longevity (Whitehouse *et al*., 2015). High temperatures may induce a stress response within seeds, similar to that experienced during maturation drying, allowing the continued metabolism of protectants and other metabolic pathways involved in aiding the stabilisation of the seed during desiccation and survival in air-dry storage. This hypothesis is supported by previous studies which provided evidence that the accumulation of soluble carbohydrates (sugars) and heat stable proteins during

development were associated with desiccation tolerance and potential longevity (Sinniah *et al*., 1998b). For example, in seeds of brassica, the total oligosaccharide ratio and a 58 kDa heat stable protein independently showed a significant correlation with differences in K_i, which arose as a result of the different irrigation treatments on the mother plant and changed the timing of maturation and hence, the attainment of maximum seed quality (Sinniah *et al*., 1998a). From these results it was suggested that both protective sugars and proteins are equally likely to be required for the development of high seed quality (Sinniah *et al*., 1998b), but the accumulation of heat stable proteins are more likely to account for the differences in longevity between seed lots as they accumulate comparatively late in seed development, during maturation drying, coinciding with the increase in potential longevity which continues once seeds have acquired maximum desiccation tolerance. Despite this evidence, the results presented in Chapter 7 do not support this theory, as the expression of the two heat stable proteins studied here (16.7 and 15.5 kDa dehydrins), targeted due to their accumulation typically late during rice seed development, did not change after mass maturity or as a result of high temperature exposure and so were concluded not to account for the differences observed in the potential longevity of rice seeds (Chapter 7).

The observed differences in the longevity between and within accessions appear to be due to differences in the estimates of not only K_i, but also σ. As stated above, according to the Ellis and Roberts (1980a) viability equation, the standard distribution of seed deaths in time (σ) is assumed to be constant for a given species. In the seed viability equation the relationship between longevity (σ, d) under set storage conditions (temperature and MC) is described by equation [2] (section 1.4.1). K_E is equivalent to log σ at 1% MC and 0°C and can hence be considered to be a measure of inherent longevity. The value of K_E has been found to vary with seed maturity (Hay *et al*., 1997; Zanakis *et al*., 1993) and between ecotypes (Hay *et al.,* 2003), mutants (Lyall *et al*., 2003) and subspecies within a species (Ellis *et al*., 1992), which subsequently results in differences in σ (Demir *et al*., 2009). These results contradict the view that σ is a measure of longevity which is constant for all seed lots within a species (Ellis and Roberts 1980a), highlighting K_E as being a potentially useful parameter when evaluating the environmental and post-harvest effects on longevity within a species.

The value of K_E has been shown to vary among the three subspecies of rice (indica, temperate and tropical japonica) and hence account for the observed differences in longevity (Ellis *et al*., 1992). The values of σ for indica and temperate and tropical japonica varieties were estimated using the seed viability constants in the Seed Viability module of the Seed Information Database (Royal Botanic Gardens Kew, 2008), under the conditions of experimental storage used throughout this thesis (45°C and 10.9% MC) and showed that seeds of temperate japonica varieties (σ =8) are predicted to lose viability twice as fast as those of indica varieties (σ=19) (dashed lines; Figure 8.2). However, the observed values, most notably when seeds were dried in the DR, were not consistent with that of the estimates for each variety, with observed values being considerably lower (Figure 8.2). Further, the variation between seed lots of the same variety dried under the different regimes (DR vs. non-DR-drying) and the variation between seed lots of the same variety subjected to any one of the experiments e.g. (2015_BD) indicates the pre-storage environment i.e. pre- and post-harvest environment is influencing the longevity of the seeds in storage. Clearly, in the context of the results presented in this thesis, it would be desirable to determine the species constants, in particular K_E (and hence C_W) for seeds that have been dried either in the DR or at 45°C to see whether hot-air drying is indeed altering the inherent longevity of the seeds.

8.5. Modelling the improvement in longevity

The extent to which high temperature drying can improve the longevity of rice seeds is dependent upon the MC of the seeds at harvest (all Chapters; Whitehouse *et al*., 2015). As mentioned above, seeds which are harvested at a MC below 16.5% show a limited or no improvement compared with seeds dried at 15°C/15% RH (Figures 8.1 and 8.3B). This is because the longevity of seeds dried at a lower temperature increases with the decrease in MC (Figure 8.3A), usually coinciding with the increase in DAA and/or increase in ambient temperature which occurs as the season progresses. When compiling the longevity data from all seed lots dried in the DR, linear regression accounted for 55% of the variance between the estimates of p_{50} and harvest MC which could be fitted without

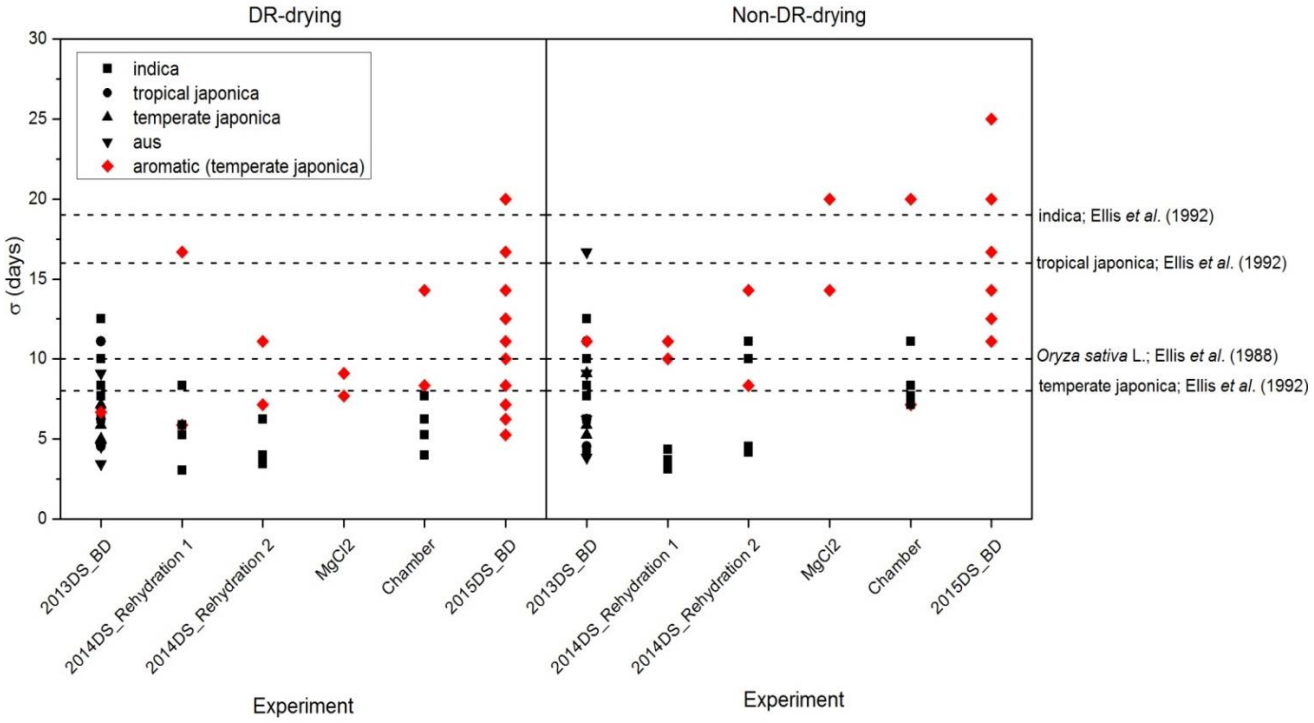


Figure 8.2. The standard deviation of the frequency distribution of seed deaths in time (σ) for seed lots of each of the 5 variety groups (indica, tropical japonica, temperate japonica, aus and aromatic; McNally *et al*., 2009) in each experiment which were dried either to equilibrium to 15% RH in the dryroom (DR) or initially dried under an alternative regime (Non-DR-drying) and stored at 45°C and 10.9% MC. The values shown in the non-DR-drying graph represent the seed lots which showed the greatest longevity in storage (p_{50}) out of all treatments within each experiment. The red symbols in each graph represent the aromatic variety, accession IRGC 117265, which was included in all experiments.

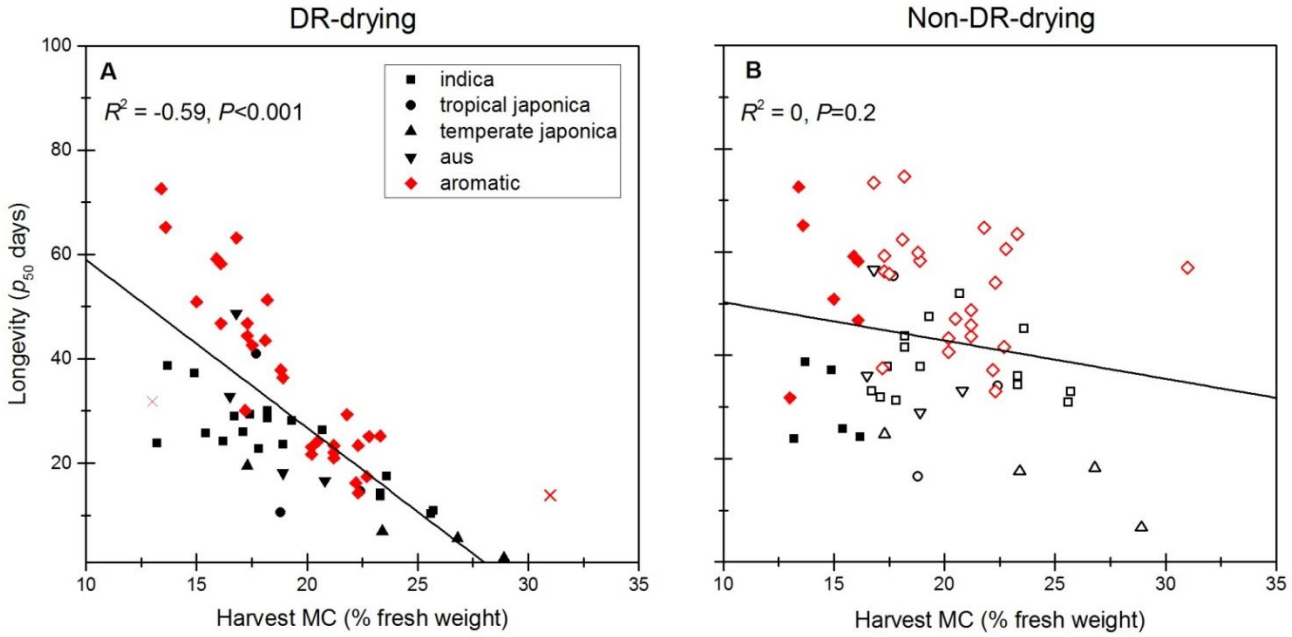


Figure 8.3A. The relationship between the longevity (p_{50}) and harvest moisture content (MC; % fresh weight) of seed lots, of all varieties, from all experiments dried to equilibrium in the dryroom (DR; 15°C/15% RH). The solid black line is a result of linear regression (the outlying data point (×) was not included) and accounted for 55% of the variance. **B.** This relationship assuming an improvement in longevity for the DR seed lots (which had a MC greater than 16.5%) had they been dried at 45°C (open symbols). This was modelled using the equation of the split-line regression showing the relationship between the relative improvement in longevity and harvest MC in Figure 8.1. The seed lots with a MC below 16.5% (solid symbols) were not corrected as they would not be expected show any improvement in longevity. The solid line is a result of regression analysis, confirming there is no relationship between longevity (p_{50}) and harvest MC when seeds are dried at 45°C.

significant (P<0.05) increase in the residual deviance (Figure 8.3A). Seeds which are harvested at lower MCs have already acquired greater longevity due to on-plant drying and are no longer metabolically active. Therefore they are unable continue to increase in quality and hence benefit from immediate drying to equilibrium with 15% RH.

Contrastingly, seeds which are harvested at a MC above 16.5% are still able to accumulate longevity in response to high temperature drying (Figure 8.1). Post-harvest drying at 45°C enables seed maturation to resume, promoting the metabolic processes and protective mechanisms associated with the stress response and increase the rate at which they occur. It is the increased accumulation of products from these reactions e.g antioxidants and protective proteins, which prepares the seeds to withstand desiccation to low MCs required for storage and promotes their ability to survive air-dry storage (Chapter 6). Based on the relationship between the improvement in longevity when seeds are dried at 45°C (Figure 8.1) and harvest MC, it was possible to model the improvement in longevity for the DR seed lots had they been dried at 45°C (Non-DR-drying; Figure 8.3B). Unlike DR-dried seeds there is no relationship (P>0.05) between p_{50} and harvest MC when seeds are dried at 45°C. The almost horizontal regression line shows that all seeds, irrespective of harvest MC, are able to reach their maximum potential longevity when dried at 45°C (Figure 8.3B). The response of seeds to high temperature drying does not appear to be influenced by variety group, although the values of p_{50} were substantially lower for three of the four temperate japonica varieties compared with seeds from any of the alternative varieties at the same/similar MC, reiterating the inherent short-lived nature of temperate japonicas (Ellis *et al*., 1992, 1993; Kameswara Rao and Jackson 1997; Xue *et al*., 2008; Hay *et al*., 2013).

The ability of genebanks to predict the longevity improvement when drying seeds at an alternative regime has great value as such predictions allow them to make informed decisions on how they can improve the management and regeneration of their accessions.

8.6. Timing of harvest

There have been many studies where the change in rice seed quality has been monitored over the course of development in order to identify an optimum time to harvest which coincides with maximum seed quality Ellis *et al*., 1993b; Ellis and Hong, 1994; Kameswara Rao and Jackson, 1996a ,b ,c, 1997). Based on the results from these experiments, IRRI aims to harvest all rice seeds at 35 DAA in concordance with the preliminary research conducted by Kameswara Rao and Jackson (1996a, b, c) which suggested 35 -37 DAA to be the optimum window of maturity at which seed longevity is at its greatest. However the results presented in this thesis suggest that due to the high humidity of the growing environment, and the paddy environment, progression through development can be restricted. As a result the quality of seeds cannot be accurately predicted, post mass maturity, with respect to time (DAA), rather it would be more informative to measure the eRH and/or MC of the seeds from which an appropriate post-harvest drying regime can be decided which will maximise the longevity of the seeds in storage. Further to this, changing the time of harvest (on a 24 hr scale) to coincide with the most humid part of the day has the potential to further improve the storage longevity of seeds when dried at a higher temperature. For example, on particularly wet or humid days, when the seeds are unlikely to dry much on the plant, seeds may benefit from being harvested early (before 8am) as they have an even higher MC (evidence from Chapter 7) and hence respond even more to high temperature drying.

8.7. Conflicts with the genebank standards

Recommendations for the management of genebank accessions emphasise the importance of initial seed drying to extend the subsequent storage longevity of seeds (Cromarty *et al*., 1982; FAO/IPGRI, 1994; Rao *et al*., 2006; FAO, 2013). However until now there has not been a critical evaluation (impact on subsequent quality or longevity) of the recommended conditions to dry mature seeds prior to genebank storage, rather the conditions were derived based on the low MC limit, i.e. below which there is no further improvement in seed longevity (Ellis and Hong, 2006 and references therein), and the drying conditions necessary to achieve this MC (without jeopardising seed quality). Mature seeds at high MC would be more sensitive to damage in heated-air dryers (Nellist,

1980; McDonald and Copeland, 1997) and so a low temperature combined with a low RH was adopted.

The 1994 genebank standards recommended to dry seeds of orthodox species in a drying chamber at 10-25°C and 10-15% RH (FAO/IPGRI, 1994) but were recently modified to a lower temperature (5-20°C) and broader humidity (10-25% RH) range (FAO, 2013). These conditions, in particular the lower drying temperature of the modified standards, represent an apparent contradiction to the results presented in this thesis which consistently show that high temperature drying of rice seeds harvested at a high MC can significantly improve their storage longevity. However, I only considered one set of conditions (15°C/15% RH) within the recommended temperature and humidity ranges which equates to an equilibrium MC of 6.1% in rice (estimated using Cromarty's equation executed in the Seed Information Database [Royal Botanic Gardens Kew, 2008]) therefore it cannot be concluded that seeds will show the same level of response to drying at 45°C compared with drying at any of the other alternative set of temperature and humidity conditions which dry seeds to an estimated equilibrium MC of between 4.8 (20°C/10% RH) and 8.7% (5°C/25% RH).

It is important to remember that these standards were developed based on their suitability to dry mature seeds of a very diverse range of species (all crops and wild relatives with orthodox seeds) from all locations worldwide to a low MC (which depends on the oil content) for storage and that they are not necessarily the optimum drying conditions for all species and/or for seeds of the same species grown in different environments. However they should not be dismissed as they are still a useful standard for genebanks to follow when drying orthodox seeds from multiple species which have already attained maximum on plant longevity. For non-genebank purposes, it is encouraged for example, that seed companies, producers, and farmers, especially those handling a limited number of species, research alternative post-harvest drying methods/conditions in order to maximise the quality of their seeds.

8.8. Implications for *ex situ* conservation

Long-term conservation of germplasm comes at a cost and with genebanks mostly relying on public funding, resources are often limited. The main costs incurred by genebanks are for viability monitoring and accession regeneration, the frequency of which can be reduced by ensuring seeds are at their maximum possible longevity when placed into storage (Probert *et al*, 2009). The cost-efficiency of regeneration is maximised only when seed quantity is sufficient to provide enough for use before viability drops below 85% (Sackville Hamilton and Chorlton, 1997), however this is difficult to predict and an under- and overestimation of loss in viability or utilisation will incur more frequent regeneration (FAO, 2013). Therefore in this situation, increasing the potential storage longevity of the seeds will only increase the regeneration interval if stocks are large enough to meet the likely demand (Sackville Hamilton and Chorlton, 1997; Sackville Hamilton *et al*., 2002) i.e. a higher quality seed lot calls for a larger sample size to be stored.

8.8.1. Use of p_{50} as a measure of longevity

Throughout this thesis, p_{50} has been used to compare the longevity of different seed lots, for example as a consequence of different drying treatments. It has been used in many publications on seed longevity (Priestley *et al*., 1985; Walters *et al.*, 2005; Probert *et al*., 2009; Nagel and Börner 2010; Mondoni *et al*., 2011; Hay et al., 2013), not least since it is the most meaningful index (compared with e.g. σ) and accurately estimated (as it is the mean of the frequency distribution). Although genebanks usually have a viability standard of 85%, p_{85} values have smaller standard errors and therefore it can be difficult to identify differences in longevity between seed lots. The consequence of using p_{50} instead of p_{85} when comparing seed lots is that, if σ is varying, it is possible that e.g. seed lot A has a higher p_{85} than seed lot B, but a lower p_{50}. Although σ did appear to vary considerably (Figure 8.3A), there were only a few cases where an improvement in p_{50} of an alternative drying treatment cf. dryroom did not result in an improvement in p_{85}. The p_{50} is also easier to use if viability is low to start with and/or if there is a high proportion of non-responders in the population of ageing seeds. But, when estimating the p_{50} using the viability model in combination with the control mortality parameter, it is important to note that the estimated p_{50} value is the point when there is 50% germination of the

responding seeds which can obscure the fact that a seed lot had a proportion of seeds that were not part of the ageing (responding) population when placed in storage. In relation to the results presented in this thesis, seed lots which showed a substantial proportion of non-responders (approximately 15%) at the beginning of storage were generally those which experienced a rehydration treatment during the drying treatment (DR and/or BD). Although the post-harvest invigoration treatments (Chapters 3 and 4) helped to develop an understanding of how the physiological status of the seeds can affect their response to certain post-harvest treatments I do not recommend them as a post-harvest treatment (Objective 4; section 1.10).

8.9. Future research and concluding remarks

To conclude, there is clear evidence that high temperature drying can significantly improve the storage longevity of rice seeds when harvested at a MC >16.5% (Objectives 1 and 3; section 1.10). Therefore I suggest that genebanks which are using low temperature, low humidity environments to dry rice seeds should delay harvests until after MC has declined naturally to below 16.5%, if ambient conditions allow. If seeds are unlikely to dry to this MC due to high ambient humidity, they should modify their drying protocol and initially dry seeds at a higher temperature. The precise temperature to use would need to be investigated in each crop species of concern and in the case of rice, perhaps for different variety groups. Although 45°C was identified as an optimum temperature for post-harvest drying, the response appeared to be independent of RH. As temperature appears to be the determining factor influencing longevity, at least when drying at 45°C, it is possible that there may not be an optimum temperature and humidity combination at which to dry rice seeds (Objective 2; section 1.10). Research involving the use of genetic studies, for example, GWAS to identify regions of the genome which influence longevity and the response of seeds to different drying treatments could help strengthen and build upon the conclusions drawn in this thesis. Although the results reported in this thesis did not support the role of dehydrins in increasing subsequent seed longevity in response to high temperature drying (Objective 5; section 1.10), they may be present in minor QTLs that do influence longevity and they should not be ruled out. Alternative genetic approaches have the potential to validate their role in seed longevity (see section 1.6) and should be a key research focus for the future.

In light of the research presented in this thesis, the International Rice Research Institute plans to modify their post-harvest drying procedure. An additional drying room, set to run at 40°C/30% RH, has been installed to initially dry all freshly harvested rice seeds for 3 days prior to drying in the genebank drying room (15°C/15% RH). Ultimately, FAO should revise the genebank standards for drying to reflect the results of the research described in this thesis as this will ensure rice seeds are at their maximum longevity when placed into storage and will reduce the genebanks' annual financial expenditure.

In the future it would be worth testing in independent studies the beneficial limits of high temperature on rice seeds produced in other climatic regions and/or whether tailoring the planting schedule to coincide with the driest conditions within specific climatic zones could enhance the potential longevity of seeds produced in these regions. This could have huge implications on how rice seeds are managed in genebanks situated in different climatic regions to ensure seeds have the maximum longevity when they are first placed into storage. From an evolutionary perspective, it is not surprising that rice seeds are tolerant to such high temperatures as farmers have been sun drying for thousands of years where temperatures can reach up 40°C (Somado *et al*., 2006) and temperatures of up 70°C have been suggested as suitable (J Van Asbrouck 2016. pers. comm.) Therefore it is possible that rice seeds have been selected to withstand high-temperature drying. However future experiments should not be restricted to rice and explore the benefits of high temperature drying on other economically important cereal crops, specifically those grown in a similar climate.

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APPENDICES

Appendix 2.1A. Rice production practices and plant protection (Reaño *et al.*, 2008).

Sowing

- Seeds were sown evenly in rows in modified wet beds (5-10 × 0.8 × 0.1 m [L × W × H]) formed from paddied soil and covered with top soil
- Seedbeds were managed by applying 10 kg of Nitrogen (N) per hectare and applying granular insecticide at a rate of 3 g/ha to control ants, crickets and nematodes. The seedbeds were intermittently irrigated.

Transplanting and plant production

- Seedlings were transplanted after 21 days into field plots under the lowland ecosystem. They were transplanted by hand in straight lines 25 cm apart and filling 21 hills per row. Two rows were left vacant between plots.
- Plots were given numbers from left to right and right to left in alternating rows and entries were clearly labelled on bamboo stakes next to each plot.
- Pre-emergent herbicides ("Sofit") were applied at a rate of 1 L/ha immediately after transplanting followed by 5-days of irrigation (submerged in 2-3 cm of standing water).
- Hand weeding occurred before fertiliser application, 30-days after transplanting.
- Fields were managed using alternate wetting and drying to avoid fast wilting of plants, with wetting occurring after each sampling. Although sufficient water was made available at flowering, plants did not mature into terminal drought.

Fertiliser application

- The recommended fertiliser rate for the dry season at IRRI is 90-30-30 kg Nitrogen (N) -Phosphorus (PO_4) - Potassium (K_20) per hectare. Basal application of fertiliser 30-30-30kg N-P-K occurred at 0 days after transplanting (DAT), followed by a top dressing with 30-0-0 from UREA at 30 DAT, after hand weeding, and secondary dressing (only in the dry season) of fertiliser 30-0-0 kg at the panicle stage.

Pest management

- Preventive application of systemic insecticide “Carbofuran” at a rate of 3 g/ha and molluscicide, “Bayluscide”, at a rate of 1 L/ha occurred after transplanting and pesticide application against stemborers and bugs at 50 DAT.

Harvesting

- Plants were harvested at the specified days after 50% anthesis (DAA) specified in each experiment.
- Panicles were cut and placed into labelled 0.7 × 0.4 m (L × W) cloth bags and taken to be hand threshed. The seeds were then cleaned using blowers to remove inert matter, weed seed and half-filled grains before being transferred in into 0.2 × 0.33 m (L × W) nylon mesh bags (1 mm-diameter holes) labelled with the plot number, accession and date of harvest and transported to the Genetic Resources Laboratory on the main IRRI campus where the initial moisture content and equilibrium relative humidity (eRH) was measured.

Appendix 2.1B. Table summarising routine field operations

Field Operation	Location	Schedule	Remarks
Seedbed preparation	Experimental station seedbed area	21 Days before transplanting to allow distribution of labels in the seedbed	ES in charge of land preparation for the seedbed area
Seed preparation	Seed processing lab	At least a week before target sowing	To accommodate breaking dormancy
Land preparation	Field	At least 1 month before target transplanting	Included ploughing; puddling (2x), to break soil particles; Harrowing and final levelling at least a day before transplanting
Seed sowing	Seedbed	20 days before transplanting	Sowing date is day 1
Irrigation	Seedbed	As needed and during pulling	To facilitate pulling level of water must cover the bed
Weeding seedbed	Seedbed area	3 days before pulling	If weed population is high if not no weeding is done
Labelling (for Field)	Seedbed area	A day before target pulling	Labels for field plantings
Final levelling/fertilizer incorporation	Field	2 days before transplanting	To allow field lay-out and seedling distribution
Pulling	Seedbed	A day or at transplanting date	If the number of entries is minimal to complete pulling, distribution and transplanting In a day
Pesticide application	Seedbed	As may be required	Pest monitoring is needed
	Field	After transplanting pre-emergence herbicide application is a must for better weed control while insecticides as needed.	Snail control is done after transplanting as seedlings are susceptible to snail attack at this early stage
Fertilizer application	Field	Basal/ at final	Using Complete

		leveling or a day after transplanting	fertilizer (14-14-14) NPK at the rate of 30-30-30kg/ha each.
Irrigation	Field	As needed during land preparation; and crop growth duration	Critical at first 5 days after transplanting and at maximum tillering to flowering stage and grain filling stage.
Weeding	Field	At 3 weeks to 1 month after transplanting to allow tillering and for more efficient fertilizer utilization	First top dressing is done after weeding
		Second weeding if necessary at around 50 days after transplanting	In preparation for the second top dressing
Fertilizer application	Field	At tillering stage after first weeding	Use N fertilizer at rate of 30-0-0 from either UREA or Ammonium Sulphate
		Second top-dressing at panicle initiation	Use N fertilizer at the rate of 30-0-0 from UREA or Ammonium sulphate (recommendation is based on our soil fertility status)
Replanting	Field	5-10 days after transplanting	To fill missing hills
Rouging	Seedbed	7-10 days after sowing	Remove seedling off the row
	Field	Two weeks after transplanting	Remove off the row plants
		Flowering	Remove off-types
		Before Harvest	Remove off-types; authenticate using remnants
Harvesting	Field	At 30 to 35 days after 50% anthesis	Depending on the requirement of the expt. Shattering may be harvested earlier
Threshing	Head house	Same day as in harvesting	
Seed Blowing	Head house	Right after threshing	

Appendix 2.2. Seed moisture content determination (ISTA, 2013).

High-temperature oven method

- The weight of three small aluminium dishes (including lids) was measured on a zero-ed balance and recorded (M1).
- Three 5 g samples of seeds were separately ground in a Krups 75 coffee grinder and placed into each of the aluminium dishes. The inside of the grinder was brushed to remove any remnants of ground seed in-between each grinding. Each aluminium dish + seed sample was reweighed and recorded (M2).
- The three dishes were transferred to the oven (lids removed) at 130°C for 2 h before being removed (lid immediately replaced) and placed over silica gel to cool at room temperature for 1 h.
- The dishes were then reweighed again (M3) and the moisture content (MC; % fresh weight) was calculated using the following equation:

$$\mathrm{MC}\ (\%\ \mathrm{f.wt.}) = \frac{(\mathrm{M2} - \mathrm{M3})}{(\mathrm{M2} - \mathrm{M1})} \times 100$$

Low-temperature oven method

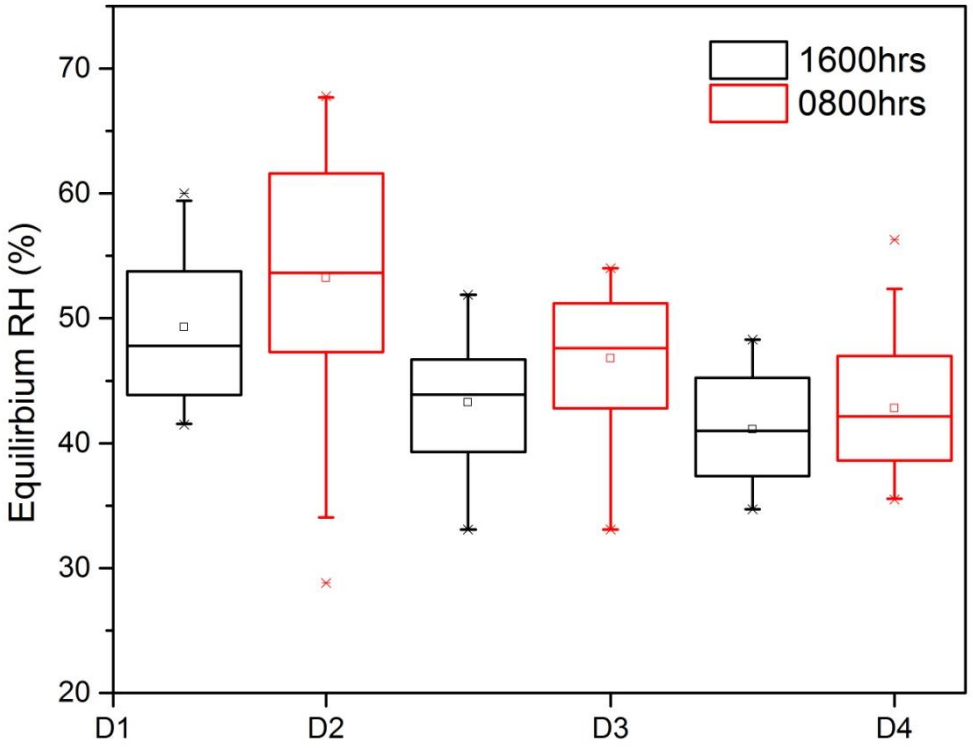
- The weight of three small aluminium dishes (including lids) was measured on a zero-ed balance and recorded (M1).
- 100 individual seeds were counted and placed into each of the three aluminium dishes which were then weighed (including lid).
- The three dishes each containing 100 seeds were transferred to the oven (lids removed) at 103°C for 17 h before being removed (lid immediately replaced) and placed over silica gel at room temperature for 1 h.
- The dishes were then reweighed (M3) and the dry weight (DW; mg) of each individual seed was determined using the following equation:

$$\mathrm{DW}\ (\mathrm{mg/seed}) = \frac{(\mathrm{M3} - \mathrm{M1})}{\mathrm{N}} \times 1000$$

where N represents the number of seeds

Appendix 2.3. The locally fabricated heated-air, flat-bed batch dryer (BD) located at International Rice Research Institute's (IRRI) experimental station (ES).

Appendix 2.4. Box plot dipicting the range in equilibrium relative humidity (eRH) measurements taken at 1600 hrs after days 1, 2 and 3 of drying in the batch dryer (BD) and at 0800 hrs the following day prior to the next cycle of drying for the 20 accessions of rice (*Oryza sativa* L.) from the 2013 dry season (DS) BD experiment (Chapter 2). The box is determined by the 25th and 75th percentile, the medium is represented by the line within the box and the open square dipicts the mean. The horizontal line ("whiskers") signify the 5th and 95th percentile and the crosses (x) mark the minimum and maximum values.



Appendix 2.5. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model within each of the 20 *Oryza sativa* accessions dried either immediately in the dryroom (DR) or initially in the batch dryer (BD) for up to 6 days (BD1, BD2., BD3, BD4, BD5 & BD6) (Figure 2.3; Table 2.3). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Accession	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
	BD1-6					
	Common line	743	99	7.505		
	Best model	651.7	79	8.250		
IRGC 117264	Change	91.3	20	4.565	0.533	0.94^{NS}
	All					
	Common line	2361	103	22.920		
	Best model	651.7	79	8.250		
	Change	1709.3	24	71.221	8.633	<0.001***
	BD1-6					
	Common line	781.4	114	6.854		
	Best model	673	94	7.160		
IRGC 117265	Change	108.4	20	5.420	0.757	0.76^{NS}
	All					
	Common line	1979	70	28.27		
	Best model	673	94	7.160		
	Change	1306	-24	-7.600	-7.600	<0.001***
	BD1-6					
	Common line	632.6	104	6.083		
	Best model	493.1	84	5.871		
IRGC 117266	Change	139.5	20	6.975	1.188	0.28^{NS}
	All					
	Common line	632.6	104	6.083		
	Best model	1987	108	18.4		
	Change	-1354.4	-4	338.600	18.402	<0.001***
	All					
IRGC 117267	Common slope	1411	106	13.310		
	Best model	1100	96	11.460		
	Change	311	10	31.100	2.714	<0.005**
	All					
IRGC 117268	Common line	654.0	54	12.11		
	Best model	457.6	10.90	42		
	Change	196.4	12	16.367	1.502	0.16^{NS}

	BD1-4					
	Common line	320.1	43	7.444		
	Best model	289.0	37	7.810		
IRGC 117269	Change	31.1	6	5.183	0.664	0.68^{NS}
	All					
	Common line	1527	56	27.270		
	Best model	316.2	48	6.587		
	Change	1210.8	8	151.350	22.977	<0.001***
	BD1-6					
	Common line	806.4	45	17.92		
	Best model	533.9	35	15.25		
IRGC 117270	Change	272.5	10	27.250	1.787	0.10^{NS}
	All					
	Common line	1805	52	34.72		
	Best model	561.9	40	14.05		
	Change	1243.1	12	103.592	7.373	<0.001***
	All					
IRGC 117271	Common line	708.9	45	15.75		
	Best model	529.6	33	16.05		
	Change	179.3	12	14.942	0.931	0.53^{NS}
	All					
IRGC 117272	Common line	468.8	46	10.19		
	Best model	375.8	40	9.395		
	Change	93	6	15.500	1.650	0.16^{NS}
	All					
IRGC 117273	Common slope	595.6	96	6.204		
	Best model	455.9	84	5.427		
	Change	139.7	12	11.642	2.450	0.007*
	BD1-6					
	Common line	600.8	65	9.244		
	Best model	528.2	55	9.603		
IRGC 117274	Change	72.6	10	7.260	0.756	0.68^{NS}
	All					
	Common line	1089	76	14.33		
	Best model	573.2	64	8.957		
	Change	515.8	12	42.983	4.799	<0.001***
	All					
IRGC 117275	Common line	857.6	129	6.648		
	Best model	633.4	105	6.032		
	Change	224.2	24	9.342	1.549	0.07^{NS}

IRGC 117276	All					
	Common slope	361.4	46	7.857		
	Best model	278.1	40	6.953		
	Change	83.3	6	13.883	1.997	0.09^{NS}
IRGC 117277	All					
	Common line	930.5	76	12.24		
	Best model	860.7	64	13.45		
	Change	69.8	12	5.817	0.432	0.94^{NS}
IRGC 117278	All					
	Common slope	559.7	93	6.018		
	Best model	523.7	87	6.020		
	Change	36	6	6.000	0.997	0.43^{NS}
IRGC 117279	BD1-6					
	Common line	708.4	62	11.43		
	Best model	517.1	52	9.945		
	Change	191.3	10	19.130	1.924	0.06^{NS}
	All					
	Common line	940.4	71	13.25		
	Best model	564	59	9.560		
	Change	376.4	12	31.367	3.281	0.001***
IRGC 117280	All					
	Common slope	565.8	45	12.57		
	Best model	522.1	39	13.39		
	Change	43.7	6	7.283	0.544	0.77^{NS}
IRGC 117281	All					
	Common slope	1502	124	12.11		
	Best model	1175	112	10.49		
	Change	327	12	27.250	2.598	0.004**
IRGC 117282	BD1-6					
	Common line	97.59	39	2.502		
	Best model	58.28	29	2.010		
	Change	39.31	10	3.031	1.956	0.08^{NS}
	All					
	Common line	194.5	47	4.138		
	Best model	100.8	35	2.880		
	Change	93.7	12	7.808	2.711	0.01**
IRGC 117283	All					
	Common line	489.6	109	4.492		
	Best model	354.3	89	3.981		
	Change	135.3	20	6.765	1.699	0.05^{NS}

Appendix 2.6. The coefficients (s.e.) of the fitted split-regressions between the relative improvement in longevity (%) between the two drying treatments (BD p_{50}/DR p_{50}) for the 20 rice accessions and harvest date (Figure 2.4A), seed moisture content (Figure 2.4B) and DR p_{50} (Figure 2.4C). No significant regression line could be fitted between improvement in longevity and period from anthesis to harvest (Figure 2.4D).

Parameters	Figure 2.4A	Figure 2.4B	Figure 2.4C	Figure 2.4D
Breakpoint x (s.e.)	42.87 (3.92)	16.17 (0.56)	24.20 (0.74)	40.00 (0.819)
Breakpoint y (s.e.)	11.20 (10.7)	0.43 (7.70)	13.46 (9.15)	71.4 (28.6)
Slope (s.e.)	-5.31 (1.28)	15.86 (1.28)	-6.93 (1.19)	1.47 (4.04)
% variance accounted for	66.3	85	65.8	*

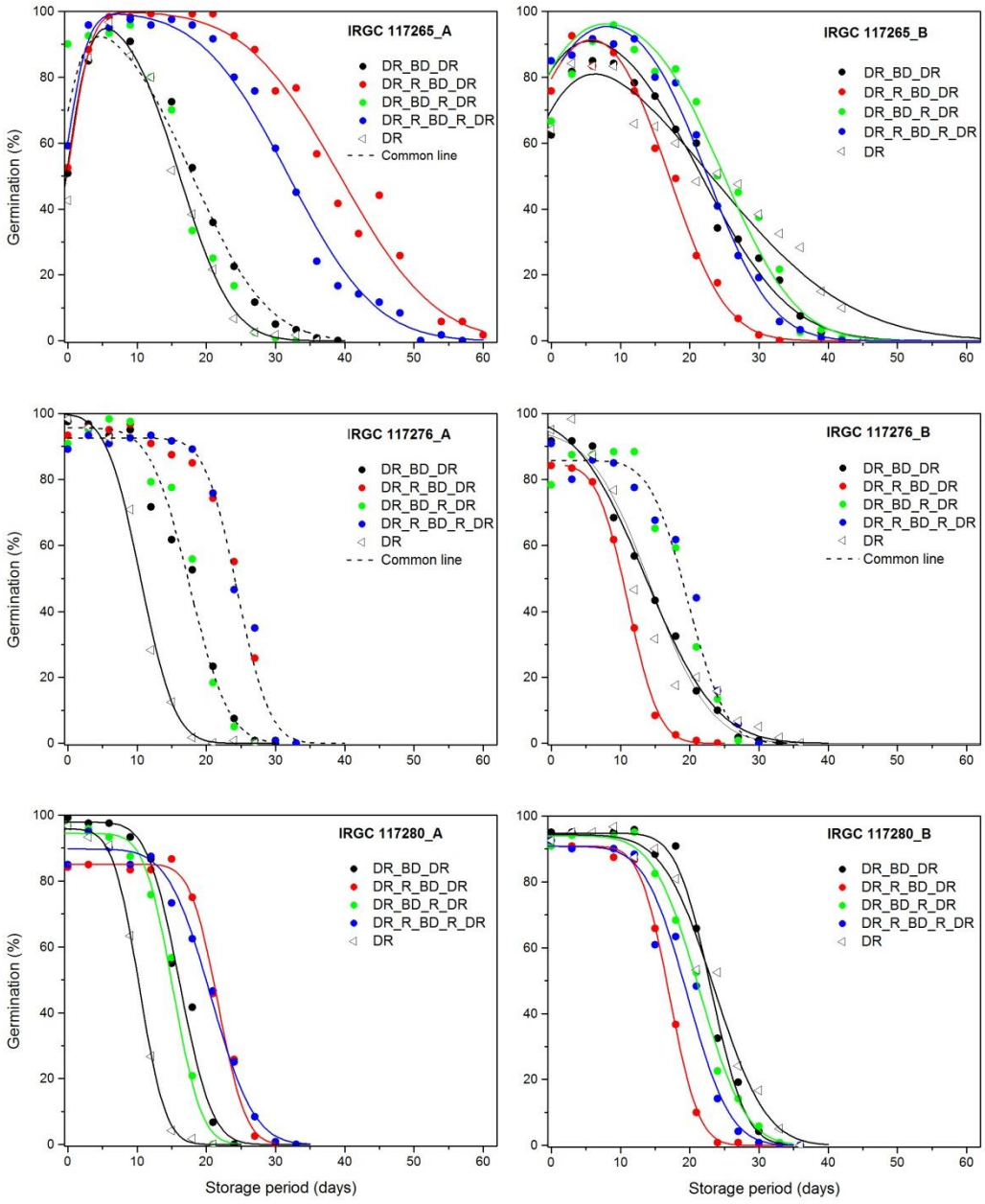
*the residual variance exceeded the variance of the response variate.

Appendix 3.1. Mean eRH values ± (s.e.) of rice seeds of three accessions harvested on two separate dates (A and B) in the 2014 dry season (DS) and harvested on one occasion during 2013DS after each stage (step) of drying, in either the dryroom (DR) or batch dryer (BD), or rehydration (R). Seeds from the 2014DS were subjected to five different drying/rehydration regimes (a. DR; b. DR_BD_DR; c. DR_BD_R_DR; d. DR_R_BD_DR; e. DR_R_BD_R_DR) and seeds from the 2013DS were subjected to immediate high temperature drying (BD; 8 h) prior to final drying in the dryroom (DR) (BD_DR; Chapter 2). Asterisks (*) indicate where s.e. could not be generated. Values are depicted in Figure 3.1.

	2014DS: Harvest A					**2014DS: Harvest B**					**2013DS**
Steps	a	b	c	d	e	a	b	c	d	e	BD_DR
					IRGC 117265						
-1	19.4 (0.2)	94.5 (0.5)	94.5 (0.5)	94.5 (0.5)	94.5 (0.5)	24.8 (0.3)	97.5 (1.2)	97.5 (1.2)	97.5 (1.2)	97.5 (1.2)	96.1*
0		83.9 (0.6)	83.9 (0.6)	83.9 (0.6)	83.9 (0.6)		73.9 (1.0)	72.6 (0.5)	75.1 (0.1)	76.2 (0.6)	53.2*
1		59.6 (0.5)	62.7 (0.8)	93.5*	93.5*		40.8 (0.1)	41.5 (0.1)	92.6 (0.3)	93.3 (0.4)	14.2*
2		18.1 (0.2)	91.9*	32.4 (0.2)	32.7 (0.1)		23.7 (0.2)	89.3 (0.3)	32.0 (0.3)	43.2 (1.0)	-
3		-	21.0 (0.9)	20.8 (0.1)	86.8 (0.4)		-	25.0 (0.1)	24.1 (0.2)	90.9 (0.1)	-
4		-	-	-	24.6 (0.4)		-	-	-	28.7 (0.8)	-
					IRGC 117276						
-1	21.0 (0.3)	97.6 (0.7)	97.6 (0.7)	97.6 (0.7)	97.6 (0.7)	25.5 (0.4)	99.7 (0.4)	99.7 (0.4)	99.7 (0.4)	99.7 (0.4)	67.8*
0		89.7 (0.9)	89.7 (0.9)	89.7 (0.9)	89.7 (0.9)		89.0 (0.7)	84.3 (0.8)	84.0 (0.9)	84.8 (0.9)	42.9*
1		66.2 (0.4)	67.6 (0.4)	94.9*	95.3*		39.2 (0.0)	39.6 (0.3)	94.5 (0.5)	94.5 (0.4)	13.7*
2		18.5 (0.1)	92.2*	33.7 (0.0)	34.9 (0.0)		22.6 (0.2)	91.1 (0.2)	33.1 (0.3)	42.4 (0.3)	-
3		-	20.6 (0.1)	20.6 (0.2)	90.1 (0.3)		-	24.0 (0.0)	23.9 (0.6)	92.5 (0.2)	-
4		-	-	-	23.1 (0.4)		-	-	-	30.0 (0.2)	-

					IRGC 117280					
-1	22.3 (0.2)	97.1 (0.4)	97.1 (0.4)	97.1 (0.4)	97.1 (0.4)	84.4 (0.2)	84.4 (0.2)	84.4 (0.2)	84.4 (0.2)	96.1*
0		85.2 (0.5)	85.2 (0.5)	85.2 (0.5)	85.2 (0.5)	74.3 (0.7)	73.1 (0.8)	70.8 (1.3)	67.1 (2.0)	48.5*
1		67.1 (0.2)	65.4 (0.4)	94.9*	94.4*	42.6 (0.4)	43.6 (0.5)	92.3 (0.3)	94.4 (0.4)	13.0*
2		18.7 (0.1)	91.7*	33.7 (0.1)	35.3 (0.1)	22.6 (0.1)	89.7 (0.3)	30.2 (0.0)	43.5 (0.4)	-
3		-	20.4 (0.4)	21.2 (0.2)	90.0 (0.4)	-	23.9 (0.2)	23.2 (0.5)	93.5 (0.1)	-
4		-	-	-	24.7 (0.3)	-	-	-	31.9 (0.4)	-

Appendix 3.2. Survival curves resulting from fitting models to quantify changes in ability to germinate during hermetic storage (45°C, 60% RH) for three rice accessions (IRGC 117265, -76 and -80) dried immediately in the dryroom (DR) before undergoing a cycle (45°C) of high temperature drying in the BD. Some seed lots experienced either one or two rehydration periods (R; 7 days). The control curve (DR; open symbols) is the response of seed lots dried solely in the genebank DR (15°C, 15% RH). The dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). The fitted curves are quantified in Appendix 3.3.



Appendix 3.3A. The results of fitting the viability model (Ellis and Roberts, 1980a), including the mortality parameter (Mead and Grey, 1999) or the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for samples initially dried in the dryroom (DR) before being transferred to the batch dryer (BD), with or without a 7-day rehydration (R) cycle. The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant (P<0.05) increase in residual deviance compared with the best-fit model. The moisture content (MC; % fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment.

			Loss in dormancy		Loss in viability			
Treatment	Model	Seed MC (s.e.) (% f.wt.)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117265_A								
DR_BD_DR		11.1 (0.1)	0.56 (0.84)	0.33 (0.49)	2.11 (1.01)	0.12 (0.05)	18.0	11.1
DR_R_BD_DR	K_d , β_1, K_i, and σ^{-1} constrained within DR_BD_DR and DR_BD_r_DR treatments & β_1 constrained within DR_r_BD_DR; DR_r_BD_r_DR and DR treatments	11.0 (0.0)	0.05 (0.34)	0.37 (0.05)	3.62 (0.57)	0.09 (0.03)	39.3	142.6
DR_BD_R_DR		11.0 (0.1)	0.56 (0.84)	0.33 (0.49)	2.11 (1.01)	0.12 (0.05)	18.0	11.1
DR_R_BD_R_DR		11.0 (0.0)	0.27 (0.35)	0.37 (0.05)	3.20 (0.54)	0.10 (0.03)	31.9	96.9
DR		10.9 (0.0)	0.10 (0.15)	0.37 (0.05)	2.72 (0.23)	0.17 (0.01)	16.2	-

				Loss in dormancy		Loss in viability			
Treatment	Model	Model	Seed MC (s.e.)	K_d (s.e.)	β_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}	Difference in p_{50} relative to DR
			(% f.wt.)	(NED)	(days)	(NED)	($days^{-1}$)	(days)	(%)
IRGC117265_B									
DR_BD_DR			11.1 (0.1)	0.97 (0.41)		2.14 (0.36)	0.10 (0.01)	21.7	-6.1
DR_R_BD_DR			11.0 (0.0)	0.84 (0.42)		2.60 (0.41)	0.15 (0.02)	17.0	-26
DR_BD_R_DR		β_1 constrained within all treatments	11.0 (0.1)	0.94 (0.41)	0.15 (0.03)	2.98 (0.39)	0.12 (0.02)	24.9	7.8
DR_R_BD_R_DR			11.0 (0.0)	0.82 (0.41)		3.06 (0.41)	0.14 (0.02)	22.6	-2.2
DR			11.2 (0.1)	0.70 (0.19)		1.45 (0.14)	0.06 (0.01)	23.1	-

Treatment	Model	Seed MC (s.e.) (% f.wt.)	Proportion of non-responders (s.e.)	Loss in viability: K_i (s.e.) (NED)	Loss in viability: σ^{-1} (s.e.) (days^{-1})	Loss in viability: p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117276_A							
DR_BD_DR	K_i and σ^{-1} constrained within DR_BD_DR and DR_BD_R_DR treatments & within DR_R_BD_DR and DR_R_BD_R_DR treatments	11.1 (0.1)	0.044 (0.008)	3.92 (0.21)	0.22 (0.01)	17.6	61.5
DR_R_BD_DR		11.1 (0.1)	0.075 (0.007)	6.62 (0.39)	0.27 (0.01)	24.5	124.8
DR_BD_R_DR		11.1 (0.1)	0.044 (0.008)	3.92 (0.21)	0.23 (0.01)	17.6	61.5
DR_R_BD_R_DR		11.1 (0.1)	0.075 (0.007)	6.62 (0.39)	0.27 (0.01)	24.5	124.8
DR		10.8 (0.0)	0	2.11 (0.12)	0.19 (0.01)	10.9	-
IRGC 117276_B							
DR_BD_DR		11.1 (0.1)	0.051 (0.025)	2.10 (0.20)	0.15 (0.01)	14.4	5.1
DR_R_BD_DR	K_i and σ^{-1} constrained within DR_BD_R_DR and DR_R_BD_R_DR treatments	11.1 (0.1)	0.154 (0.024)	3.14 (0.34)	0.29 (0.03)	11.1	-19.0
DR_BD_R_DR		11.1 (0.1)	0.143 (0.012)	4.56 (0.31)	0.23 (0.01)	19.8	44.5
DR_R_BD_R_DR		11.1 (0.1)					
DR		11.4 (0.1)	0	1.69 (0.09)	0.12 (0.01)	13.7	-

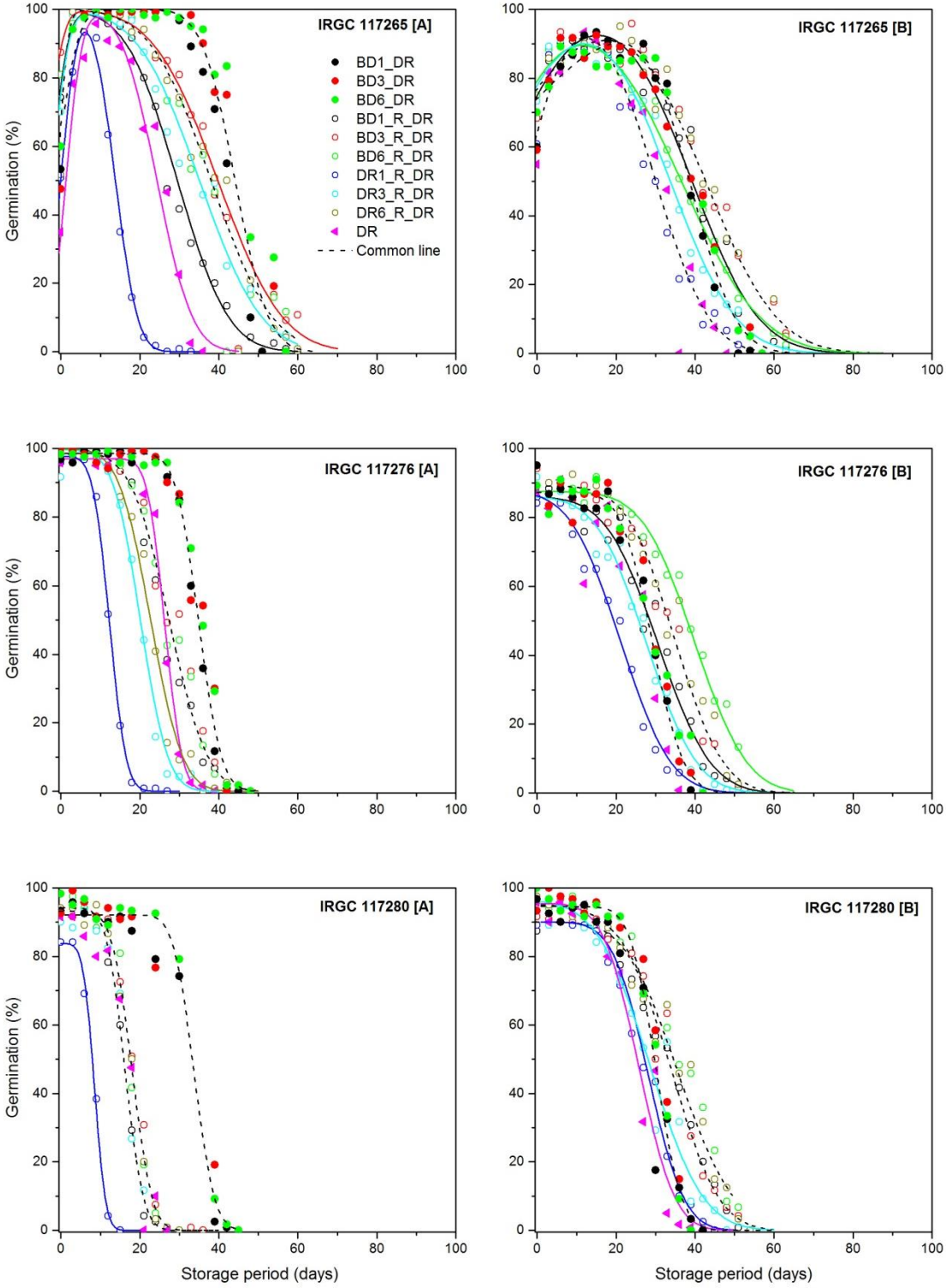
			Loss in viability				
Treatment	Model	Seed MC (s.e.) (% f.wt.)	Proportion of non-responders (s.e.)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC117280_A							
DR_BD_DR_DR	No parameters constrained	11.0 (0.1)	0.021 (0.008)	4.39 (0.34)	0.27 (0.02)	16.2	57.3
DR_R_BD_DR_DR		11.0 (0.1)	0.149 (0.015)	6.86 (0.71)	0.32 (0.03)	21.7	110.7
DR_BD_R_DR_DR		11.1 (0.1)	0.056 (0.012)	4.65 (0.44)	0.31 (0.03)	15.2	47.6
DR_R_BD_R_DR		11.0 (0.1)	0.103 (0.016)	4.21 (0.35)	0.20 (0.02)	20.7	101.0
DR		10.9 (0.1)	0.041 (0.014)	3.42 (0.31)	0.33 (0.03)	10.3	-
IRGC117280_B							
DR_BD_DR	No parameters constrained	11.0 (0.1)	0.052 (0.010)	5.86 (0.45)	0.25 (0.02)	23.1	-2.1
DR_R_BD_DR		11.0 (0.1)	0.091 (0.016)	5.11 (0.45)	0.30 (0.02)	17.1	-27.5
DR_BD_R_DR		11.1 (0.1)	0.060 (0.013)	4.11 (0.29)	0.19 (0.01)	21.4	-9.3
DR_R_BD_R_DR		11.0 (0.1)	0.092 (0.016)	4.10 (0.34)	0.21 (0.02)	19.7	-16.5
DR		11.4 (0.0)	0.057 (0.011)	4.02 (0.29)	0.17 (0.01)	23.6	-

Appendix 3.3B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seed lots from accessions IRGC 117265, -76 and -80 which were dried immediately in the dryroom (DR) before undergoing a cycle (45°C) of high temperature drying in the BD. Some of these seed lots experienced either one or two rehydration periods (R; 7 days) (Appendices 3.2 and 3.3A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Accession	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
IRGC 117265_A	All					
	Common slope	283.3	63	4.497		
	Best model	105.8	55	1.924		
	Change	177.5	8	22.188	11.532	<0.001***
	DR_BD_DR & DR_BD_R_DR					
	Common line	337.9	33	10.240		
	Best model	254.8	29	8.786		
	Change	83.1	4	20.775	2.365	0.07^{NS}
	DR_R_BD_DR; DR_R_BD_R_DR & DR					
	Common AR slope	87.11	37	2.354		
	Best model	83.05	35	2.373		
	Change	4.06	2	2.03	0.855	0.43^{NS}
	Common slopes	173.7	39	4.454		
	Best model	83.05	35	2.373		
	Change	90.65	4	22.663	9.550	<0.001***
IRGC 117265_B	All					
	Common AR slope	116.9	57	2.051		
	Best model	102.5	53	1.934		
	Change	14.4	4	3.6	1.861	0.13^{NS}
	Common slope	234.5	61	3.844		
	Best model	102.5	53	1.934		
	Change	132	8	16.5	8.532	<0.001***

	DR_BD_DR & DR_BD_R_DR					
	Common line	58.83	17	3.461		
	Best model	46.53	14	3.324		
	Change	12.3	3	4.1	1.233	0.33^{NS}
	DR_R_BD_DR & DR_R_BD_R_DR					
	Common line	38.16	21	1.817		
	Best model	37.24	18	2.069		
	Change	0.92	3	0.307	0.148	0.93^{NS}
	All (excl. DR)					
IRGC 117276_A	Common line	595	41	14.51		
	Best model	83.77	32	2.618		
	Change	511.23	9	56.803	21.697	<0.001***
	DR_BD_DR & DR_BD_R_DR & DR					
	Common line	447	27	16.56		
	Best model	135.1	21	6.432		
	Change	311.9	6	51.983	8.082	<0.001***
	DR_R_BD_DR & DR_R_BD_R_DR & DR					
	Common line	1053	31	33.97		
	Best model	125.8	25	5.032		
	Change	927.2	6	154.533	30.710	<0.001***
	DR_BD_R_DR & DR_R_BD_R_DR					
	Common line	40.29	18	2.238		
	Best model	32.86	15	2.191		
IRGC 117276_B	Change	7.43	3	2.477	1.130	0.37^{NS}
	DR_BD_DR; DR_R_BD_DR & DR					
	Common slope	121.4	27	4.495		
	Best fit	61.27	25	2.451		
	Change	60.13	2	30.065	12.266	<0.001***
	All treats					
IRGC 117280_A	Common slope	81.13	35	2.318		
	Best model	57.76	31	1.863		
	Change	23.37	4	5.843	3.136	0.03*
	All treats					
IRGC 117280_B	Common slope	112	46	2.434		
	Best model	73.75	42	1.756		
	Change	38.25	4	9.563	5.446	0.01**

Appendix 4.1. Seed survival curves fitted by probit analysis for three rice accessions (IRGC 117265, -76 and -80) either dried immediately in the dryroom (DR) or batch dryer (BD) for up to 6 days. Some seed lots were rehydrated (R) over water for 7 days after 1, 3 and 6 days of drying at either regime before final equilibrium drying in the DR (open symbols). The results shown are for the model with the fewest parameters that could be fitted without a significant increase in residual deviance compared with the best-fit model. The dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). The fitted curves are quantified in Appendix 4.2. All seed lots were harvested at 35 days after 50% anthesis (DAA) at two different times from two sowings during the 2014 dry season (DS); [A] and [B] respectively.


IRGC 117265 [A]
IRGC 117265 [B]
IRGC 117276 [A]
IRGC 117276 [B]
IRGC 117280 [A]
IRGC 117280 [B]
BD1_DR
BD3_DR
BD6_DR
BD1_R_DR
BD3_R_DR
BD6_R_DR
DR1_R_DR
DR3_R_DR
DR6_R_DR
DR
Common line
Germination (%)
Storage period (days)

Appendix 4.2A. Results of fitting models; viability equation (Ellis and Roberts, 1980a) with/without the mortality parameter (Mead and Grey, 1999) or the combined loss in dormancy and loss in viability (Kebreab and Murdoch, 1999) to quantify changes in ability to germinate during hermetic storage at 45°C and MC shown for the three rice accessions (IRGC 117265, -76 and -80). Samples were immediately dried in the batch dryer (BD) or dryroom (DR) after harvest for up to 6 days. Some seed lots were rehydrated (R) over water for 7-days after 1, 3 and 6 days of drying in either regime before final equilibrium drying in the DR. The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant (P<0.05) increase in residual deviance compared with the best-fit model. The moisture content (MC; % fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment.

Treatment	Model	Seed MC (s.e.)	Loss in dormancy		Loss in viability			Difference in p_{50} relative to DR
			K_d (s.e.)	θ_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}	
		(% f.wt.)	(NED)	(days)	(NED)	(days^{-1})	(days)	(%)
IRGC 117265 [A]								
BD1_DR	K_d, θ_1, K_i, and σ^{-1} constrained within BD1_DR, BD3_DR and BD6_DR & within BD6_R_DR and DR6_R_DR treatments & θ_1 constrained within BD1_R_DR, BD3_R_DR, DR1_R_DR, DR3_R_DR and DR treatments	10.8 (0.1)						
BD3_DR		10.7 (0.1)	0.31 (0.59)	0.24 (0.15)	6.62 (1.23)	0.15 (0.05)	42.8	76.1
BD6_DR		10.7 (0.1)						
BD1_R_DR		11.0 (0.1)	0.76 (0.39)	0.31 (0.04)	2.90 (0.64)	0.10 (0.03)	29.5	21.4
BD3_R_DR		10.9 (0.0)	1.21 (0.42)		3.08 (0.63)	0.08 (0.02)	39.7	63.4
BD6_R_DR		11.0 (0.1)	0.42 (0.45)	0.52 (0.19)	3.12 (0.76)	0.08 (0.03)	38.0	56.4
DR1_R_DR		10.8 (0.1)	0.01 (0.35)	0.31 (0.04)	3.15 (0.73)	0.23 (0.04)	13.8	-43.2
DR3_R_DR		10.9 (0.0)	0.70 (0.39)		2.83 (0.64)	0.08 (0.03)	35.1	44.4
DR6_R_DR		10.8 (0.1)	0.42 (0.45)	0.52 (0.19)	3.12 (0.76)	0.08 (0.03)	38.0	56.4
DR		10.7 (0.1)	0.40 (0.16)	0.31 (0.04)	3.44 (0.29)	0.14 (0.01)	24.3	-

Treatment	Model	Seed MC (s.e.) (% f.wt.)	Loss in dormancy K_d (s.e.) (NED)	Loss in dormancy θ_1 (s.e.) (days)	Loss in viability K_i (s.e.) (NED)	Loss in viability σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117265 [B]								
BD1_DR	K_d, θ_1, K_i, and σ^{-1} constrained within	10.9 (0.1)						
BD3_DR	BD1_DR, BD3_DR and BD6_DR	10.9 (0.1)	0.64 (0.34)	0.04 (0.06)	4.33 (0.67)	0.11 (0.02)	38.1	26.5
BD6_DR	& within	10.9 (0.1)						
BD1_R_DR	BD3_R_DR and DR6_R_DR	10.9 (0.1)	0.68 (0.22)	0.07 (0.01)	2.96 (0.57)	0.08 (0.02)	39.3	30.6
BD3_R_DR	& within	10.9 (0.1)	0.74 (0.29)	0.05 (0.05)	2.99 (0.53)	0.07 (0.02)	42.7	41.9
BD6_R_DR	DR1_R_DR and DR treatments	10.9 (0.0)	0.84 (0.24)	0.07 (0.01)	2.31 (0.55)	0.06 (0.02)	36.6	21.6
DR1_R_DR	& θ_1 constrained within	10.9 (0.1)	0.37 (0.33)	0.12 (0.05)	2.82 (0.65)	0.09 (0.02)	30.1	0
DR3_R_DR	BD1_R_DR, BD6_R_DR, and	10.8 (0.1)	0.78 (0.23)	0.07 (0.01)	2.57 (0.57)	0.08 (0.02)	33.8	12.3
DR6_R_DR	DR3_R_DR treatments	10.9 (0.1)	0.74 (0.29)	0.05 (0.05)	2.99 (0.53)	0.07 (0.02)	42.7	41.9
DR		10.9 (0.1)	0.37 (0.33)	0.12 (0.05)	2.82 (0.65)	0.09 (0.02)	30.1	-

Treatment	Model	Seed MC (s.e.) (% f.wt.)	Loss in viability: Proportion of non-responders (s.e.)	Loss in viability: K_i (s.e.) (NED)	Loss in viability: σ^{-1} (s.e.) ($days^{-1}$)	Loss in viability: p_{50} (days)	Loss in viability: Difference in p_{50} relative to DR (%)
IRGC 117276 [A]							
BD1_DR		10.7 (0.2)					
BD3_DR		10.8 (0.1)	0.014 (0.003)	7.61 (0.28)	0.22 (0.01)	34.9	32.7
BD6_DR	K_i and σ^{-1} constrained within BD1_DR, BD3_DR and BD6_DR & within BD1_R_DR, BD3_R_DR and BD6_R_DR treatments	10.7 (0.1)					
BD1_R_DR		11.0 (0.1)					
BD3_R_DR		10.9 (0.1)	0.009 (0.003)	3.59 (0.11)	0.13 (0.00)	27.5	4.6
BD6_R_DR		11.0 (0.1)					
DR1_R_DR		11.0 (0.1)	0.011 (0.010)	3.73 (0.28)	0.30 (0.02)	12.3	-53.2
DR3_R_DR		10.9 (0.1)	0.004 (0.006)	3.69 (0.20)	0.18 (0.01)	20.1	-23.6
DR6_R_DR		10.9 (0.1)	0.004 (0.004)	3.57 (0.19)	0.16 (0.01)	22.7	-13.7
DR		10.9 (0.2)	0.030 (0.007)	7.51 (0.51)	0.29 (0.02)	26.3	-

			Loss in viability				
Treatment	Model	Seed MC (s.e.) (% f.wt.)	Proportion of non-responders (s.e.)	K_i (s.e.) (NED)	σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117276 [B]			-				
BD1_DR	K_i and σ^{-1} constrained within BD1_DR, BD3_DR & BD6_DR & within BD3_R and DR6 _R treatments & σ^{-1} constrained within BD1_R, BD6_R, DR1_R & DR3_R	11.1 (0.0)					
BD3_DR		11.2 (0.0)	0.128 (0.009)	4.68 (0.25)	0.16 (0.01)	29.8	5.3
BD6_DR		11.2 (0.0)					
BD1_R_DR		11.0 (0.1)	0.150 (0.015)	3.22 (0.14)	0.10 (0.01)	31.2	10.2
BD3_R_DR		10.9 (0.0)	0.109 (0.009)	3.64 (0.18)	0.10 (0.00)	34.9	23.3
BD6_R_DR		10.9 (0.0)	0.133 (0.013)	4.14 (0.16)	0.10 (0.01)	40.2	42.0
DR1_R_DR		11.0 (0.1)	0.123 (0.022)	2.12 (0.12)	0.10 (0.01)	20.5	-27.6
DR3_R_DR		11.0 (0.1)	0.139 (0.018)	2.78 (0.13)	0.10 (0.01)	26.9	-4.9
DR6_R_DR		11.0 (0.0)	0.109 (0.009)	3.64 (0.18)	0.10 (0.00)	34.9	23.3
DR		11.1 (0.0)	0.178 (0.019)	6.73 (0.78)	0.24 (0.03)	28.3	-

			Loss in viability				
Treatment	Model	Seed MC (s.e.) (% f.wt.)	Proportion of non-responders (s.e.)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117280 [A]							
BD1_DR		11.5 (0.0)					
BD3_DR	K_i and σ^{-1} constrained within BD1_DR, BD3_DR and BD6_DR & within BD1_R_DR, DR3_R_DR & within BD3_R_DR; BD6_R_DR; DR6_R_DR & DR treatments	11.4 (0.1)	0.078 (0.006)	8.16 (0.44)	0.24 (0.01)	33.7	86.1
BD6_DR		11.3 (0.0)					
BD1_R_DR		11.2 (0.0)	0.058 (0.014)	4.38 (0.28)	0.27 (0.02)	16.3	-9.9
BD3_R_DR		11.0 (0.1)	0.068 (0.07)	4.51 (0.19)	0.25 (0.01)	18.1	0
BD6_R_DR		11.2 (0.0)					
DR1_R_DR		11.2 (0.1)	0.162 (0.025)	3.77 (0.51)	0.44 (0.05)	8.5	-53.0
DR3_R_DR		11.2 (0.1)	0.058 (0.014)	4.38 (0.28)	0.27 (0.02)	16.3	-9.9
DR6_R_DR		11.2 (0.1)	0.068 (0.07)	4.51 (0.19)	0.25 (0.01)	18.1	0
DR		11.5 (0.2)					-

Treatment	Model	Seed MC (s.e.) (% f.wt.)	Loss in viability: Proportion of non-responders (s.e.)	Loss in viability: K_i (s.e.) (NED)	Loss in viability: σ^{-1} (s.e.) ($days^{-1}$)	Loss in viability: p_{50} (days)	Loss in viability: Difference in p_{50} relative to DR (%)
IRGC 117280 [B]							
BD1_DR		11.0 (0.0)					
BD3_DR	K_i, and σ^{-1} constrained within BD1_DR,	11.0 (0.1)	0.054 (0.006)	5.84 (0.29)	0.19 (0.01)	30.1	18.5
BD6_DR	BD3_DR and BD6_DR &	11.0 (0.0)					
BD1_R_DR	within	10.9 (0.1)					
	BD1_R_DR and		0.100 (0.008)	3.73 (0.17)	0.11 (0.00)	34.5	35.8
BD3_R_DR	BD3_R_DR &	10.9 (0.0)					
BD6_R_DR	within	11.0 (0.1)	0.048 (0.008)	3.07 (0.14)	0.09 (0.00)	35.2	38.6
	BD6_R_DR, and						
DR1_R_DR	DR6_R_DR &	11.0 (0.0)	0.099 (0.012)	3.95 (0.20)	0.14 (0.01)	27.8	9.4
DR3_R_DR	σ^{-1} constrained within DR1_R_DR &	10.9 (0.0)	0.036 (0.014)	2.69 (0.18)	0.09 (0.01)	28.6	12.6
DR6_R_DR	DR treatments	11.0 (0.0)	0.048 (0.008)	3.07 (0.14)	0.09 (0.00)	35.2	38.6
DR		10.9 (0.0)	0.045 (0.011)	3.61 (0.18)	0.14 (0.01)	25.4	-

Appendix 4.2 B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seed lots from accessions IRGC 117265, -76 and 80 which were immediately dried in the batch dryer (BD) or dryroom (DR) after harvest for up to 6 days. Some seed lots were rehydrated (R) over water for 7-days after 1, 3 and 6 days of drying in either regime before final equilibrium drying in the DR (Appendices 4.2 and 4.3A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Accession	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
IRGC 117265_A	BD1-6					
	Common line	374.6	54	6.938		
	Best model	357.3	46	7.768		
	Change	17.3	8	2.163	0.278	1.00^{NS}
	BD6_R_DR & DR6_R_DR					
	Common line	145.3	41	3.543		
	Best model	118.2	37	3.195		
	Change	27.1	4	6.775	2.121	0.09^{NS}
	BD1_R_DR; BD3_R_DR; DR1_R_DR; DR3_R_DR & DR					
	Common AR slope	168.6	62	2.72		
	Best model	151	58	2.604		
	Change	17.6	4	4.4	1.690	0.16^{NS}
	BD1_R_DR; BD3_R_DR; DR1_R_DR; DR3_R_DR & DR					
	Common slope	423.4	66	6.414		
	Best model	151	58	2.604		
	Change	272.4	8	34.05	13.076	<0.001***

IRGC 117265_B	BD1-6					
	Common line	159.6	59	2.704		
	Best model	122	51	2.393		
	Change	37.6	8	4.7	1.964	0.07^{NS}
	BD3_R_DR & DR6_R_DR					
	Common line	86.65	46	1.884		
	Best model	83.96	42	1.999		
	Change	2.69	4	0.673	0.336	0.85^{NS}
	DR1_R_DR & DR					
	Common line	87.79	43	2.042		
	Best model	72.23	39	1.852		
	Change	15.56	4	3.89	2.100	0.10^{NS}
	BD1_R_DR; BD6_R_DR & DR3_R_DR					
	Common AR slope	108	61	1.771		
	Best model	97	58	1.672		
	Change	11	3	3.667	2.193	0.10^{NS}
	BD1_R_DR; BD6_R_DR & DR3_R_DR					
	Common slope	167.6	64	2.619		
	Best model	97	58	1.672		
	Change	70.6	6	11.767	7.037	<0.001***
IRGC 117276_A	BD1-6					
	Common line	91.36	32	2.855		
	Best model	65.43	26	2.516		
	Change	25.93	6	4.322	1.718	0.16^{NS}
	BD1_R_DR; BD3_R_DR & BD6_R_DR					
	Common line	83.44	39	2.139		
	Best model	59.19	33	1.794		
	Change	24.25	6	4.042	2.253	0.06^{NS}
	BD1_R_DR; BD3_R_DR & BD6_R_DR & DR					
	Common line	239.3	52	4.601		
	Best model	80.02	43	1.861		
	Change	159.28	9	17.698	9.510	<0.001***
	DR1_R_DR; DR3_R_DR; DR6_R_DR & DR					
	Common slope	165.3	39	4.239		
	Best model	75.89	36	2.108		
	Change	89.41	3	29.803	14.138	<0.001***

IRGC 117276_B	BD1-6					
	Common line	75.71	31	2.442		
	Best model	60.73	25	2.429		
	Change	14.98	6	2.497	1.028	0.4^{NS}
	BD1-6 & DR					
	Common line	200.1	41	4.881		
	Best model	107.3	32	3.352		
	Change	92.8	9	10.3111	3.076	0.009**
	BD3_R_DR & DR6_R_DR					
	Common line	62.87	33	1.905		
	Best model	57.76	30	1.925		
	Change	5.11	3	1.703	0.885	0.46^{NS}
	BD3_R_DR; DR6_R_DR & DR					
	Common line	363.6	42	8.658		
	Best model	83.62	36	2.323		
	Change	279.38	6	46.563	20.044	<0.001***
	BD1_R_DR; BD6_R_DR; DR1_R_DR; & DR3_R_DR					
	Common slope	97.68	55	1.776		
	Best model	93.34	52	1.795		
	Change	4.34	3	1.447	0.806	0.49^{NS}
	BD1_R_DR; BD6_R_DR; DR1_R_DR; & DR3_R_DR					
	Common line	887.8	61	14.55		
	Best model	93.34	52	1.795		
	Change	794.46	9	88.273	49.177	<0.001***
	BD1_R_DR; BD6_R_DR; DR1_R_DR; DR3_R_DR & DR					
	Common slope	156.3	62	2.521		
	Best model	119.2	58	2.055		
	Change	37.1	4	9.275	4.513	0.003**

IRGC 117280_A	BD1-6					
	Common line	102.2	28	3.650		
	Best model	68.94	22	3.134		
	Change	33.26	6	5.543	1.769	0.15^{NS}
	BD1_R_DR; DR3_R_DR;					
	Common line	20.26	13	1.558		
	Best model	13.92	10	1.392		
	Change	6.34	3	2.11	1.518	0.27^{NS}
	BD1_R_DR; DR3_R_DR & DR					
	Common line	58.62	21	2.791		
	Best model	21.91	15	1.461		
	Change	36.71	6	6.118	4.188	0.01**
	BD3_R_DR; BD6_R_DR; DR6_R_DR					
	Common line	33.90	27	1.256		
	Best model	24.39	21.1	1.161		
	Change	9.51	6	1.585	1.365	0.3^{NS}
	BD3_R_DR; BD6_R_DR; DR6_R_DR & DR					
	Common line	55.87	35	1.596		
	Best model	32.28	26	1.245		
	Change	23.49	9	2.61	2.096	0.07^{NS}
	BD1_R_DR; BD3_R_DR; BD6_R_DR; DR3_R_DR; DR6_R_DR					
	Common line	118.1	43	2.747		
	Best model	38.3	31	1.236		
	Change	79.8	12	6.65	5.380	0.01**
	BD1_R_DR; BD3_R_DR; BD6_R_DR; DR3_R_DR; DR6_R_DR & DR					
	Common line	395.2	53	7.456		
	Best model	240.2	43	5.584		
	Change	155	10	15.5	2.776	0.009**

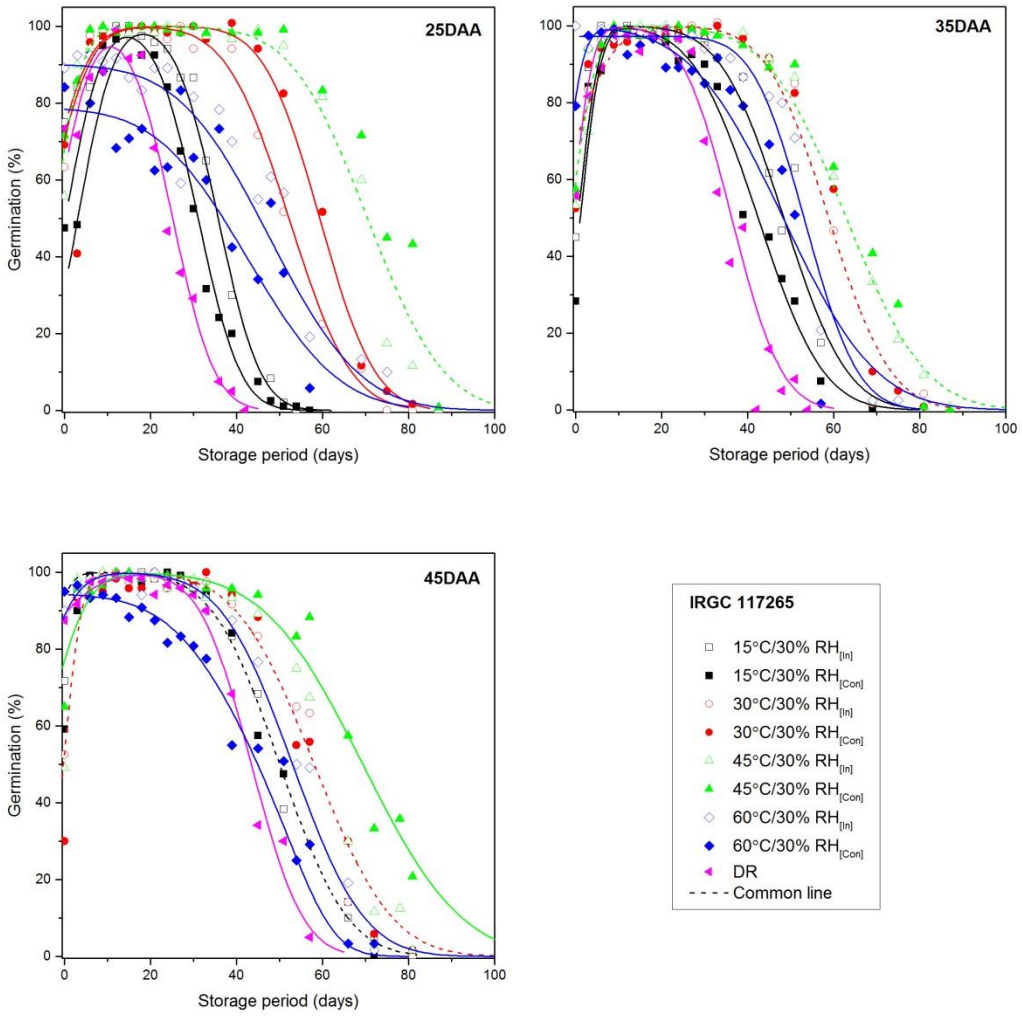
IRGC 117280_B	BD1-6					
	Common line	155.2	31	5.007		
	Best model	110.6	25	4.423		
	Change	44.6	6	7.433	1.681	0.17^{NS}
	BD1-6 & DR					
	Common line	310.1	44	7.047		
	Best model	119.3	35	34.07		
	Change	190.8	9	21.2	6.222	<0.001***
	BD1_R_DR & BD3_R_DR					
	Common line	48.68	35	1.391		
	Best model	43.79	32	1.369		
	Change	4.89	3	1.63	1.191	0.33^{NS}
	BD1_R_DR; BD3_R_DR &DR					
	Common line	406.9	47	8.658		
	Best model	92.54	41	2.257		
	Change	314.36	6	52.393	23.214	<0.001***
	BD6_R_DR & DR6_R_DR					
	Common line	90.33	33	2.737		
	Best model	88.40	30	2.947		
	Change	1.93	3	0.643	0.218	0.88NS
	BD6_R_DR; DR6_R_DR & DR					
	Common line	534.1	45	11.87		
	Best model	137.1	39	3.517		
	Change	397	6	66.167	18.813	<0.001
	DR1_R_DR & DR3_R_DR					
	Common slope	156	37	4.223		
	Best model	117.7	35	3.363		
	Change	38.3	2	19.15	5.694	0.007**
	DR3_R_DR & DR					
	Common slope	143.1	23	6.222		
	Best model	105.2	22	4.784		
	Change	37.9	1	37.9	7.922	0.01**
	DR1_R_DR & DR					
	Common slope	68.34	23	2.971		
	Best model	61.21	22	2.782		
	Change	7.13	1	7.13	2.563	0.13^{NS}
	DR1_R_DR & DR					
	Common line	88.12	25	3.525		
	Best model	61.21	22	2.782		
	Change	26.91	3	8.97	3.224	0.04*

Appendix 5.1. Mean equilibrium relative humidity (eRH) values ± (s.e.) during drying intermittently (In) and continuously (Con) at 15, 30, 45 and 60°C and 30% RH and in the genebank drying room (DR). After 3 days, all samples were moved to the DR.

Drying duration (days)	DR	15°C/30% $RH_{[In]}$	15°C/30% $RH_{[Con]}$	30°C/30% $RH_{[In]}$	30°C/30% $RH_{[Con]}$	45°C/30% $RH_{[In]}$	45°C/30% $RH_{[Con]}$	60°C/30% $RH_{[In]}$	60°C/30% $RH_{[Con]}$
25DAA									
0	96.6 (0.6)	96.6 (0.6)	96.56 (0.6)	96.6 (0.6)	96.6 (0.6)	96.6 (0.6)	96.6 (0.6)	96.6 (0.6)	96.6 (0.6)
1	57.7 (0.2)	98.1 (0.4)	96.35 (0.5)	93.8 (0.4)	86.3 (0.2)	86.3 (0.2)	78.3 (0.7)	83.1 (0.4)	71.2 (0.5)
2	45.0 (0.2)	92.3 (0.3)	92.80 (0.5)	84.5 (0.6)	81.2 (0.3)	86.6 (0.2)	57.0 (0.4)	76.1 (0.2)	25.0 (0.8)
3	34.7 (0.3)	89.6 (0.4)	89.55 (0.3)	83.5 (0.4)	42.7 (0.2)	76.4 (0.5)	42.0 (0.3)	58.5 (0.3)	24.7 (0.9)
6	28.9 (0.2)	29.2 (0.6)	24.05 (0.5)	30.2 (0.4)	20.5 (0.2)	28.3 (0.1)	22.5 (0.3)	26.0 (0.3)	23.7 (0.2)
9	25.8 (0.4)	22.3 (0.5)	22.70 (0.6)	22.9 (0.4)	23.5 (0.3)	24.7 (0.1)	23.2 (0.5)	24.3 (0.2)	23.7 (0.5)
12	26.3 (0.5)	24.9 (0.2)	25.58 (0.6)	-	24.2 (0.4)	26.0 (0.4)	25.4 (0.3)	25.8 (0.4)	25.4 (0.6)
14	29.7 (0.5)	24.6 (0.1)	26.75 (0.1)	20.5 (0.3)	26.1 (0.3)	26.3 (0.4)	28.2 (0.6)	26.4 (0.5)	28.5 (0.4)
35DAA									
0	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)	86.5 (0.1)
1	50.1 (0.3)	87.0 (0.4)	87.2 (0.2)	85.8 (0.3)	77.7 (0.1)	80.0 (0.2)	62.3 (0.4)	72.0 (0.2)	55.9 (1.5)
2	38.8 (0.1)	84.4 (0.3)	78.8 (0.4)	73.0 (0.2)	66.1 (0.1)	76.7 (0.2)	44.8 (0.7)	63.5 (0.9)	27.5 (0.6)
3	36.1 (0.7)	80.0 (0.4)	76.5 (0.3)	68.9 (0.3)	57.6 (0.4)	65.3 (0.0)	35.7 (0.5)	49.9 (0.8)	29.1 (1.3)
6	26.0 (0.3)	26.0 (0.2)	24.4 (0.2)	25.7 (0.2)	25.6 (0.7)	25.1 (0.2)	23.1 (0.1)	26.6 (0.3)	26.5 (0.4)
9	26.2 (0.7)	24.0 (0.2)	24.3 (0.4)	25.2 (0.5)	26.7 (0.4)	25.6 (0.2)	25.6 (0.5)	25.6 (0.2)	25.8 (0.2)
12	28.0 (0.5)	24.9 (0.4)	25.4 (0.4)	24.2 (0.3)	25.7 (0.4)	25.8 (0.5)	29.5 (0.3)	25.8 (0.3)	24.9 (0.2)
14	25.5 (0.5)	24.7 (0.2)	25.3 (0.4)	24.8 (0.4)	24.5 (0.4)	25.8 (0.5)	25.3 (0.4)	25.6 (0.1)	25.6 (0.5)

					45DAA				
0	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)	86.0 (1.0)
1	45.7 (0.2)	78.4 (0.6)	80.5 (0.4)	83.1 (0.2)	81.4 (0.3)	72.1 (0.4)	74.4 (0.1)	72.0 (0.2)	62.8 (0.3)
2	36.7 (0.2)	79.5 (0.1)	70.9 (0.5)	71.2 (0.2)	69.6 (0.1)	72.9 (0.1)	46.5 (0.4)	61.8 (0.3)	30.1 (0.5)
3	33.0 (0.7)	72.8 (0.3)	69.2 (0.1)	68.4 (0.1)	61.9 (0.3)	59.1 (0.3)	37.8 (0.2)	47.3 (0.4)	27.0 (1.0)
6	28.6 (0.4)	25.7 (0.7)	23.6 (0.4)	29.4 (0.3)	23.9 (0.3)	27.6 (0.7)	23.3 (0.2)	27.4 (0.6)	24.6 (0.4)
9	26.5 (1.1)	22.6 (0.3)	23.1 (0.4)	23.8 (0.4)	24.4 (0.3)	25.4 (0.4)	22.8 (0.3)	24.3 (0.2)	23.4 (0.6)
12	23.1 (0.2)	21.6 (0.4)	24.5 (1.1)	23.1 (0.1)	23.3 (0.2)	22.1 (0.2)	22.2 (1.3)	22.2 (0.2)	-
14	27.5 (0.4)	26.2 (0.3)	27.5 (0.2)	25.7 (0.4)	27.7 (0.5)	24.8 (0.6)	27.1 (0.3)	25.4 (0.2)	25.5 (0.3)

Appendix 5.2. Ability to germinate when tested during experimental storage at 45°C and 60% RH for seeds of accession IRGC 117265 harvested at 25, 35 and 45 days after 50% anthesis (DAA) on the 3rd April 2015. Seed lots were either immediately dried after harvest in the dryroom (DR; 15°C/15%) or were subjected to 3 days of continuous (Con) or intermittent (In) drying at 15°C, 30°C, 45°C or 60°C and 30% RH (maintained by a saturated $MgCl_2$ solution) prior to DR drying. The combined loss in dormancy and loss in viability model was applied to the data (equation [7]; Kebreab and Murdoch, 1999). For those seed lots which showed a complete loss in dormancy, survival curves were fitted using the Ellis and Roberts (1980a) viability model. Seed lots which showed initial viability <100% an additional parameter was applied to probit analysis to determine the proportion of responding seeds within the population (Mead and Gray, 1999). The coloured dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). The fitted curves are quantified in Appendix 5.3.



Appendix 5.3A. The results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for samples harvested on 3rd April 2015 which were either immediately dried after harvest in the dryroom (DR; 15°C/15%) or were subjected to 3 days of continuous (Con) or intermittent (In) drying at 15°C, 30°C, 45°C or 60°C and 30% RH (maintained by a saturated $MgCl_2$ solution) prior to DR drying. For those seedlots which showed complete loss in dormancy the viability model was applied, and for seeds which showed a reduced initial viability an additional parameter was applied to either model to determine the proportion of responding seeds within the population (Mead and Gray, 1999). The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant ($P < 0.05$) increase in residual deviance compared with the best-fit model. Asterisks (*) indicate when s.e. could not be generated. The moisture content (MC; % fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment.

Maturity	Treatment	Model	Seed MC (s.e.)	Loss in dormancy		Loss in viability			
				K_d (s.e.)	β_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}	Difference in p_{50} relative to DR
(DAA)			(%, f.wt)	(NED)	(days)	NED	(days^{-1})	(days)	(%)
25DAA	15°C/30% $RH_{[In]}$	K_d, β_1, K_i and σ^{-1} constrained within 45°C/30% $RH_{[In\ \&\ Con]}$ & β_1 and σ^{-1} constrained within 15°C/30% $RH_{[In\ \&\ Con]}$ and DR & β_1 constrained within 30°C/30% $RH_{[In\ \&\ Con]}$ & within 60°C/30% $RH_{[In\ \&\ Con]}$	10.8 (0.1)	0.49 (0.32)	0.16 (0.02)	4.66 (0.31)	0.13 (0.01)	35.8	42.1
	15°C/30% $RH_{[Con]}$		10.7 (0.1)	0.04 (0.30)		4.05 (0.29)		31.1	23.4
	30°C/30% $RH_{[In]}$		10.6 (0.0)	0.54 (0.32)	0.13 (0.02)	4.60 (0.89)	0.09 (0.03)	52.2	107.1
	30°C/30% $RH_{[Con]}$		10.7 (0.1)	0.62 (0.31)		5.85 (1.00)		59.3	135.3
	45°C/30% $RH_{[In]}$		10.8 (0.1)	0.47 (0.55)	0.19 (0.11)	5.07 (1.20)	0.07 (0.04)	70.5	179.8
	45°C/30% $RH_{[Con]}$		10.8 (0.1)						
	60°C/30% $RH_{[In]}$[¶]		10.4 (0.1)	-	-	2.86 (0.21)	0.06 (0.00)	50.7	101.2
	60°C/30% $RH_{[Con]}$[¶]		10.5 (0.2)	-	-	2.56 (0.19)		45.6	80.9
	DR		10.8 (0.1)	0.30 (0.14)	0.16 (0.02)	3.28 (0.18)	0.13 (0.01)	25.2	-

[¶] Immunity values were 0.091 (0.002) and 0.212 (0.021) for 60°C/30% $RH_{[In]}$ and 60°C/30% $RH_{[Con]}$, respectively.

Maturity	Treatment	Model	Seed MC (s.e.)	Loss in dormancy		Loss in viability			Difference in p_{50} relative to DR (%)
				K_d (s.e.)	β_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}	
(DAA)			(%, f.wt)	(NED)	(days)	NED	(days^{-1})	(days)	
35DAA	15°C/30% $RH_{[In]}$	β_1, constrained within 15°C/30%$_{[In \& Con]}$; 60°C/30%$_{[Con]}$ and DR & K_d, β_1, K_i and σ^{-1} constrained within 30°C/30% $RH_{[In \& Con]}$ & within 45°C/30% $RH_{[In \& Con]}$	10.9 (0.0)	0.16 (0.44)	0.27 (0.04)	4.20 (0.91)	0.09 (0.02)	48.4	33.3
	15°C/30% $RH_{[Con]}$		10.9 (0.1)	0.31 (0.42)	0.27 (0.04)	3.58 (0.86)	0.08 (0.02)	42.5	16.8
	30°C/30% $RH_{[In]}$		10.8 (0.1)	0.50 (0.45	0.11 (0.09)	5.18 (0.89)	0.09 (0.02)	58.8	61.5
	30°C/30% $RH_{[Con]}$		10.6 (0.0)						
	45°C/30% $RH_{[In]}$		10.7 (0.0)	0.24 (0.46)	0.22 (0.10)	4.34 (0.82)	0.07 (0.02)	63.0	73.1
	45°C/30% $RH_{[Con]}$		10.6 (0.0)						
	60°C/30% $RH_{[In]}$[¶]		11.1 (0.2)	-	-	5.58 (0.33)	0.10 (0.01)	53.5	47.0
	60°C/30% $RH_{[Con]}$		10.8 (0.0)	0.89 (0.50)	0.27 (0.04)	2.84 (0.90)	0.06 (0.02)	48.3	32.7
	DR		10.9 (0.2)	0.03 (0.19)	0.27 (0.04)	4.00 (0.37)	0.11 (0.01)	36.4	-

[¶] Immunity values were 0.027 (0.006) for 60°C/30% $RH_{[In]}$

				Loss in dormancy		Loss in viability			
Maturity (DAA)	Treatment	Model	Seed MC (s.e.) (%, f.wt)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) NED	σ^{-1} (s.e.) (days^{-1})	p_{50} (days)	Difference in p_{50} relative to DR (%)
45DAA	15°C/30% $RH_{[In]}$	K_d, β_1, K_i and σ^{-1} constrained within 15°C/30% $RH_{[In\ \&\ Con]}$ & within 30°C/30% $RH_{[In\ \&\ Con]}$; 45°C/30% $RH_{[In]}$ & β_1 constrained within 45°C/30% $RH_{[Con]}$ and 60°C/30% $RH_{[In]}$	10.9 (0.1)	0.50 (0.37)	0.24 (0.07)	4.05 (0.71)	0.08 (0.02)	50.1	15.2
	15°C/30% $RH_{[Con]}$		10.9 (0.1)						
	30°C/30% $RH_{[In]}$		10.9 (0.1)	0.06 (0.56)	0.31 (0.10)	4.08 (1.08)	0.07 (0.03)	58.1	33.6
	30°C/30% $RH_{[Con]}$		10.8 (0.0)						
	45°C/30% $RH_{[In]}$		10.8 (0.0)						
	45°C/30% $RH_{[Con]}$		10.7 (0.1)	0.74 (0.36)	0.12 (0.02)	3.85 (0.79)	0.06 (0.02)	69.4	59.5
	60°C/30% $RH_{[In]}$		10.9 (0.0)	1.21 (0.39)	0.12 (0.02)	4.06 (0.80)	0.08 (0.02)	53.0	21.8
	60°C/30% $RH_{[Con]}$[¶]		10.9 (0.0)	-	-	2.84 (0.20)	0.06 (0.00)	46.4	6.7
	DR		10.8 (0.1)	1.22 (0.17)	0.08 (0.02)	4.61 (0.34)	0.11 (0.01)	43.5	-

[¶] Immunity values were 0.0559 (0.012) for 60°C/30% $RH_{[Con]}$

Appendix 5.4B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seed lots from accession IRGC 117265 which were either immediately dried after harvest in the dryroom (DR; 15°C/15%) or were subjected to 3 days of continuous (Con) or intermittent (In) drying at 15°C, 30°C, 45°C or 60°C and 30% RH (maintained by a saturated $MgCl_2$ solution) prior to DR drying (Appendices 5.2 and 5.3A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Maturity	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
25 DAA	15°C/30% [In & Con]					
	Common AR slope	121.1	39	3.106		
	Best model	105.1	37	2.841		
	Change	16	2	8	2.816	0.07^{NS}
	15°C/30% [In & Con]					
	Common slope	126.6	41	3.088		
	Best model	105.1	37	2.841		
	Change	21.5	4	5.375	1.892	0.13^{NS}
	15°C/30% [In & Con]					
	Common line	194.9	31	6.286		
	Best model	105.1	37	2.841		
	Change	89.8	-6	-14.967	-5.268	<0.001***
	15°C/30% [In & Con] & DR					
	Common slope	226.9	56	4.052		
	Best model	150.1	50	3.003		
	Change	76.8	6	4.26	4.262	0.001***
	30°C/30% [In & Con]					
	Common AR slope	125.3	38	3.298		
	Best model	119.7	36	3.325		
	Change	5.6	2	2.8	0.842	0.44^{NS}
	30°C/30% [In & Con]					
	Common slopes	169.9	40	4.247		
	Best model	119.7	36	3.325		
	Change	50.2	4	12.55	3.774	0.01**

	30°C/30% [In & Con] & DR					
	Common AR slope	154.5	52	2.971		
	Best model	451.4	107	4.219		
	Change	-296.9	107	4.219	1.279	0.006***
	45°C/30% [In & Con]					
	Common line	267.2	45	5.937		
	Best model	221	41	5.389		
	Change	46.2	4	11.55	2.143	0.09^{NS}
	45°C/30% [In & Con] & DR					
	Common line	2495	49	50.92		
	Best model	221	41	5.389		
	Change	2274	8	284.25	52.746	<0.001***
	60°C/30% [In & Con]					
	Common slope	152.8	24	6.368		
	Best model	151.9	23	6.604		
	Change	0.9	1	0.9	0.136	0.71^{NS}
	60°C/30% [Con]					
	Common line	249.2	26	9.586		
	Best model	151.9	23	6.604		
	Change	97.3	3	32.43	4.911	0.008**
	15°C/30% [In & Con]; 60°C/30% [Con] & DR					
	Common AR slope	261	54	4.834		
	Best model	229	51	4.489		
	Change	32	3	10.667	2.376	0.08^{NS}
	15°C/30% [In & Con]; 60°C/30% [Con] & DR					
	Common slopes	345.3	57	6.057		
	Best model	229	51	4.489		
35 DAA	Change	116.3	5	19.383	4.318	0.001***
	30°C/30% [In & Con]					
	Common line	189	45	4.2		
	Best model	168	41	4.098		
	Change	21	4	5.25	1.281	0.29^{NS}
	45°C/30% [In & Con]					
	Common line	203.9	48	4.249		
	Best model	194.7	44	4.425		
	Change	9.2	4	2.3	0.520	0.72^{NS}

	15°C/30% [In & Con]					
	Common line	82.86	41	2.021		
	Best model	77.55	37	2.095		
	Change	5.36	4	1.34	0.640	0.64^{NS}
	15°C/30% [In & Con] & DR					
	Common line	182.7	45	4.061		
	Best model	77.55	37	2.095		
	Change	105.2	8	13.15	6.277	<0.001***
	15°C/30% [In & Con] & 45°C/30% [Con]					
	Common line	556.3	61	9.12		
	Best model	128	53	2.414		
	Change	428.3	8	53.538	22.178	<0.001***
	15°C/30% [In & Con] & 60°C/30% [In]					
	Common line	157.2	58	2.71		
	Best model	110.7	50	2.214		
45 DAA	Change	46.5	8	5.813	2.625	0.02**
	30°C/30% [In & Con]					
	Common line	217.5	39	5.576		
	Best model	179.9	35	5.139		
	Change	37.6	4	9.4	1.829	0.15^{NS}
	30°C/30% [In & Con] & DR					
	Common line	523.6	43	12.18		
	Best model	179.9	35	5.139		
	Change	343.7	8	42.963	8.360	<0.002***
	30°C/30% [In & Con] & 45°C/30% [In]					
	Common line	283.8	58	4.894		
	Best model	232.7	50	4.655		
	Change	51.1	8	6.388	1.372	0.23^{NS}
	30°C/30% [In & Con] & 45°C/30% [In & Con]					
	Common line	516.7	78	6.624		
	Best model	208.3	66	3.156		
	Change	308.4	12	25.7	8.143	<0.001***

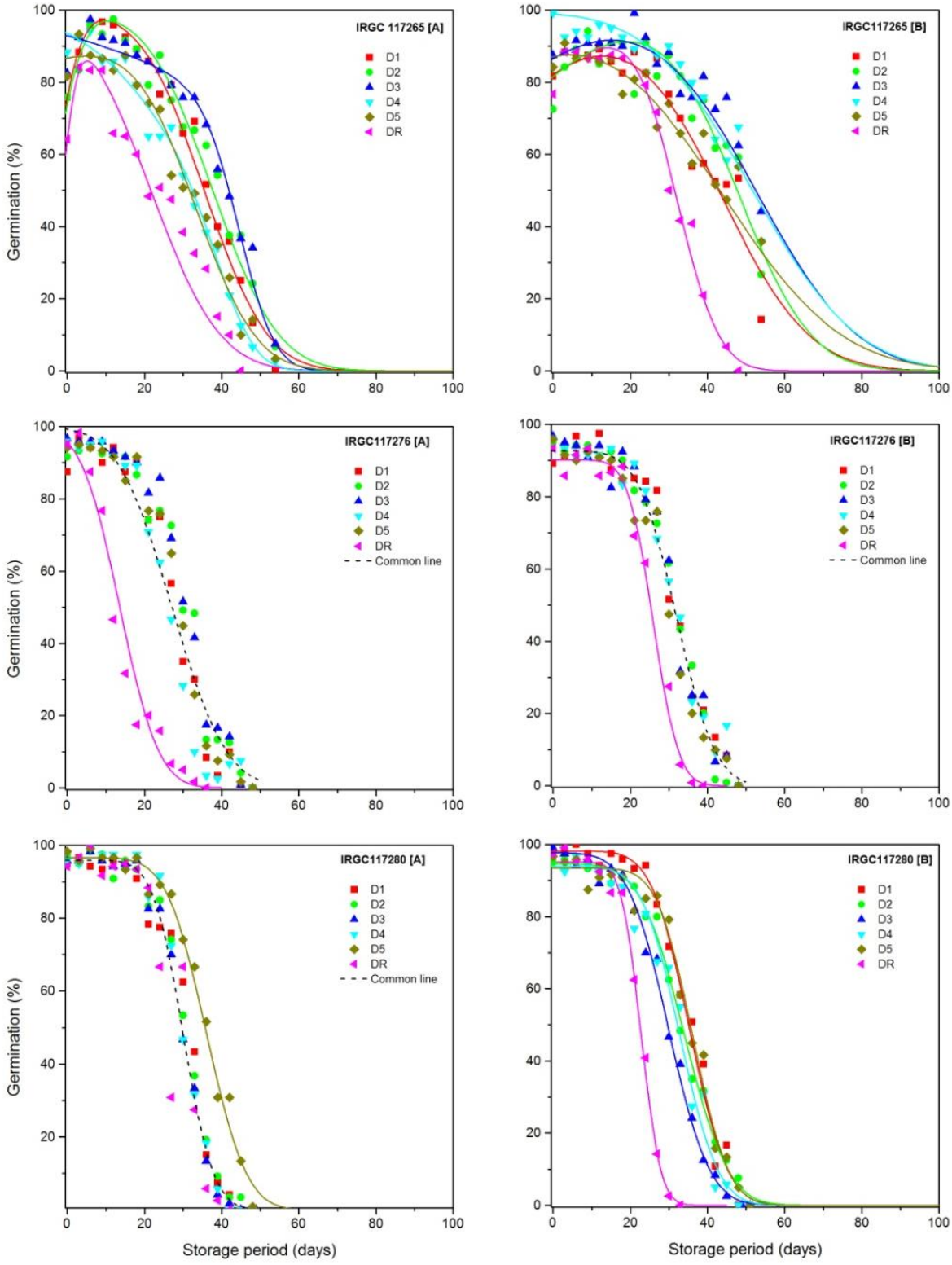
30°C/30% [In & Con] ; 45°C/30% [In] & 60°C/30% [Con]					
Common line	434.3	75	5.791		
Best model	265.9	63	4.221		
Change	168.4	12	14.033	3.325	0.001***
45°C/30% [Con] & 60°C/30% [In]					
Common AR	112.7	42	2.683		
Best model	100.9	40	2.523		
Change	11.8	2	5.9	2.338	0.11^{NS}
45°C/30% [Con] & 60°C/30% [In]					
Common slope	183.4	44	4.168		
Best model	100.9	40	2.523		
Change	82.5	4	20.625	8.175	<0.001**
45°C/30% [Con]; 60°C/30% [In] & DR					
Common AR slope	204	58	3.517		
Best model	153.7	55	2.795		
Change	50.3	3	16.767	5.999	0.001***

Appendix 6.1. Mean eRH values ± (s.e.) of seeds from three accessions (IRGC 117265, -76 and -80) harvested on two separate occasions during the 2014 dry season (DS) and dried either in the dryroom (DR; 15°C/15% RH) until equilibrium or initially in the climate chamber (45°C/23% RH*) for up to 5 days, and of seeds from accession IRGC 117265 harvested, also on two separate occasions, during the 2014 wet season (WS) and dried either in the DR or under a stepped drying regime in the climate chamber (*).

	2014DS: Harvest A		2014DS: Harvest B	
Days	**Chamber***	**DR**	**Chamber***	**DR**
		IRGC 117265		
0	97.5 (1.2)	97.5 (1.2)	54.8 (1.3)	54.8 (1.3)
1 (45°C/23% RH)*	19.1 (1.0)	78.0 (0.3)	18.5 (0.3)	49.6 (0.2)
2 (45°C/23% RH)*	19.4 (0.8)	46.3 (0.3)	19.8 (0.7)	38.2 (0.5)
3 (45°C/23% RH)*	21.7 (0.4)	39.8 (0.3)	18.1 (0.4)	36.7 (0.8)
4 (45°C/23% RH)*	19.6 (0.8)	31.8 (0.5)	21.7 (0.7)	31.0 (0.2)
5 (45°C/23% RH)*	18.1 (0.6)	27.8 (0.2)	18.9 (0.3)	26.9 (0.2)
6	-	26.6 (0.2)	-	27.4 (0.3)
9	-	25.8 (0.3)	-	25.4 (0.3)
12	-	24.3 (0.3)	-	26.6 (0.3)
14	-	24.8 (0.3)	-	24.8 (0.3)
		IRGC 117276		
0	99.7 (0.4)	99.7 (0.4)	83.9 (0.3)	83.9 (0.3)
1 (45°C/23% RH)*	19.2 (0.7)	86.1 (0.3)	19.1 (0.5)	65.7 (0.2)
2 (45°C/23% RH)*	18.5 (0.3)	63.8 (1.1)	18.2 (0.8)	49.7 (0.6)
3 (45°C/23% RH)*	17.2 (0.4)	42.7 (0.7)	18.4 (0.6)	39.5 (0.5)
4 (45°C/23% RH)*	16.7 (0.3)	33.3 (0.4)	21.4 (0.5)	34.6 (0.6)
5 (45°C/23% RH)*	20.3 (0.1)	29.7 (0.2)	18.1 (0.4)	34.2 (0.6)
6	-	27.8 (0.3)	-	27.7 (0.4)
9	-	25.3 (0.3)	-	24.9 (0.3)
12	-	24.0 (0.3)	-	26.3 (0.3)
14	-	25.5 (0.4)	-	25.0 (0.2)
		IRGC 117280		
0	85.6 (0.7)	85.6 (0.7)	87.4 (0.1)	87.4 (0.1)
1 (45°C/23% RH)*	17.8 (0.2)	65.5 (0.4)	19.3 (0.5)	70.0 (0.5)
2 (45°C/23% RH)*	19.1 (0.5)	45.5 (0.2)	18.5 (0.6)	53.0 (0.2)
3 (45°C/23% RH)*	19.1 (0.7)	37.1 (0.5)	18.4 (0.5)	41.5 (0.4)
4 (45°C/23% RH)*	17.7 (0.3)	31.2 (0.4)	21.0 (0.6)	35.1 (0.6)
5 (45°C/23% RH)*	17.8 (0.5)	28.3 (0.3)	18.8 (0.3)	27.0 (0.7)
6	-	25.6 (0.2)	-	29.3 (0.3)
9	-	23.5 (0.1)	-	24.9 (0.3)
12	-	23.8 (0.3)	-	26.4 (0.2)
14	-	22.7 (0.1)	-	25.0 (0.2)

	2014WS: Harvest A		2014WS: Harvest B	
Drying duration	**Chamber***	**DR**	**Chamber***	**DR**
0	99.3 (0.4)	99.3 (0.4)	90.0 (0.5)	90.0 (0.5)
1 (45°C/75% RH)*	66.0 (0.4)	92.9 (1.1)	61.7 (1.0)	40.4 (0.6)
2 (30°C/45% RH)*	45.1 (1.5)	68.2 (0.6)	39.0 (0.9)	32.8 (0.5)
3 (30°C/45% RH)*	44.8 (0.5)	49.3 (0.6)	44.7 (1.0)	34.2 (1.0)
4 (20°C/25% RH)*	34.3 (0.3)	43.4 (0.4)	32.7 (0.6)	33.3 (1.6)
5 (20°C/25% RH)*	37.2 (0.9)	38.3 (0.1)	33.2 (0.8)	30.7 (0.2)
6 (15°C/15% RH)*	32.6 (0.3)	35.6 (0.3)	30.3 (0.6)	32.5 (0.4)
9		30.4 (0.4)		32.9 (0.7)
12		28.2 (0.3)		35.5 (0.9)
14		28.3 (0.9)		29.1 (2.5)

Appendix 6.2. Survival curves fitted by probit analysis for rice seeds, from accessions IRGC 117265, -76 and -80, harvested in the 2014 dry season (DS) and experimentally stored at 45°C and 60% RH. Samples were immediately dried in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at 45°C/23% RH for up to 5 days before being transferred for final equilibrium drying in the DR. The results shown are for the model with the fewest parameters that could be fitted without a significant increase in residual deviance compared with the best-fit model. The dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). All seed lots were harvested at 35 days after 50% anthesis (DAA) on two separate occasions; A and B respectively. Survival curves are quantified in Appendix 6.3.


IRGC 117265 [A]
IRGC117265 [B]
IRGC117276 [A]
IRGC117276 [B]
IRGC117280 [A]
IRGC117280 [B]
D1
D2
D3
D4
D5
DR
Common line
Germination (%)
Storage period (days)

Appendix 6.3A. Results of fitting models; viability equation (Ellis and Roberts, 1980a) with/without the mortality parameter (Mead and Grey, 1999) or the combined loss in dormancy and loss in viability (Kebreab and Murdoch, 1999) to quantify changes in ability to germinate during hermetic storage at 45°C and moisture content (MC) shown for the three accessions (IRGC 117265, -76 and -80). Samples from the 2014 dry season (DS) were immediately dried in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at 45°C/23% RH for up to 5 days before being transferred to the DR for final equilibrium drying. The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant increase (P<0.05) in the residual deviance compared with the best-fit model. The MC (%, fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment. Astericks (*) indicate those seed lot where the mortality parameter could not be applied.

			Loss in dormancy		Loss in viability			
Treatment	Model	Seed MC (s.e.) (%, f.wt)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p_{50} relative to DR (%)
IRGC 117265 [A]								
1d→DR		11.1 (0.2)	0.72 (0.53)	0.18 (0.34)	2.76 (0.44)	0.08 (0.02)	35.5	63.6
2d→DR		11.0 (0.2)	0.65 (0.53)	0.16 (0.34)	2.81 (0.44)	0.07 (0.02)	37.7	73.7
3d→DR	No parameters constrained	10.9 (0.2)	1.47 (0.50)	0.02 (0.32)	6.22 (1.51)	0.14 (0.03)	45.3	108.8
4d→DR		11.0 (0.2)	1.52 (0.50)	0.04 (0.32)	5.56 (2.22)	0.13 (0.05)	42.9	97.7
5d→DR		11.0 (0.2)	1.13 (0.52)	0.01 (0.33)	2.63 (0.87)	0.08 (0.02)	32.8	51.2
DR		11.2 (0.1)	0.49 (0.23)	0.30 (0.16)	1.54 (0.17)	0.07 (0.01)	21.7	-
IRGC 117265 [B}								
1d→DR		11.0 (0.2)	0.95 (0.29)		2.39 (0.78)	0.06 (0.02)	43.1	35.6
2d→DR		10.9 (0.2)	0.90 (0.26)		3.21 (0.84)	0.07 (0.02)	47.8	50.3
3d→DR	β_1 constrained within all treatments	10.8 (0.2)	1.13 (0.29)	0.03 (0.01)	2.57 (0.80)	0.05 (0.02)	52.6	65.4
4d→DR		10.8 (0.2)	2.72 (1.53)		2.43 (0.76)	0.05 (0.02)	51.7	62.6
5d→DR		10.8 (0.2)	1.32 (0.39)		1.77 (0.76)	0.04 (0.02)	43.3	36.2
DR		11.2 (0.1)	0.90 (0.13)		3.67 (0.36)	0.12 (0.01)	31.8	-

				Loss in viability			
Treatment	Model	Seed MC (s.e.)	Proportion of non-responders	K_i (s.e.)	σ^{-1} (s.e.)	p_{50} (s.e.)	Difference in p_{50} relative to DR
		(%, f.wt)	(s.e.)	(NED)	(days^{-1})	(days)	(%)
IRGC 117276 [A]							
1d→DR		11.1 (0.1)					
2d→DR		11.0 (0.1)					
3d→DR	K_i and σ^{-1} constrained within days 1-5	11.0 (0.2)	*	2.44 (0.04)	0.09 (0.00)	27.1 (0.18)	99.3
4d→DR		11.0 (0.1)					
5d→DR		10.9 (0.1)					
DR		11.4 (0.1)	*	1.72 (0.09)	0.13 (0.01)	13.6 (0.34)	-
IRGC 117276 [B]							
1d→DR		11.0 (0.2)					
2d→DR		11.0 (0.1)					
3d→DR	K_i and σ^{-1} constrained within days 1-5	10.9 (0.1)	0.073 (0.004)	4.06 (0.12)	0.13 (0.00)	32.1 (0.20)	23.5
4d→DR		10.9 (0.1)					
5d→DR		10.9 (0.1)					
DR		11.0 (0.0)	0.098 (0.015)	4.97 (0.43)	0.19 (0.02)	26.0 (0.45)	-

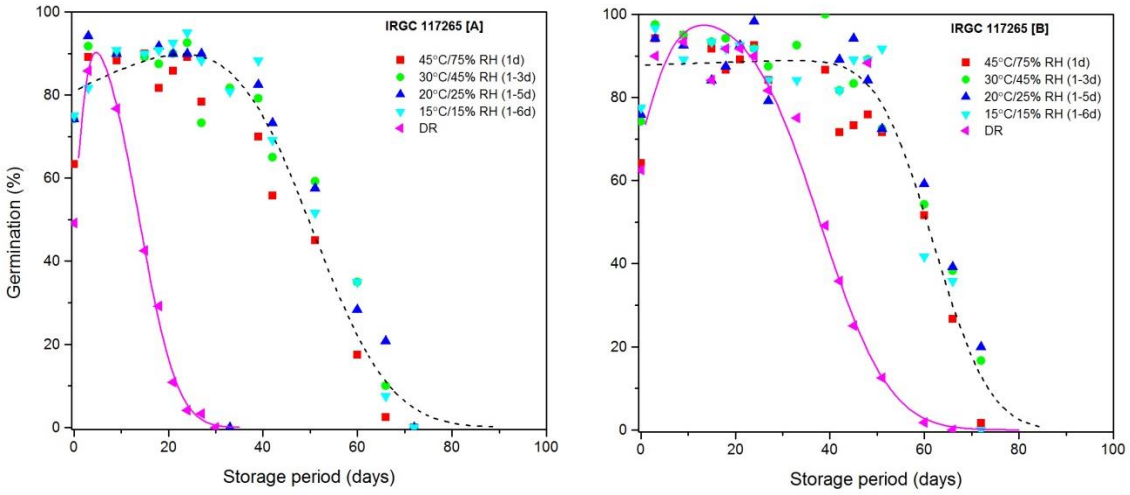
			Loss in viability				
Treatment	Model	Seed MC (s.e.) (%, f.wt)	Proportion of non-responders (s.e.)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (s.e.) (days)	Difference in p_{50} relative to DR (%)
IRGC 117280 [A]							
1d→DR	K_i and σ^{-1} constrained within days 1-4 and DR	11.1 (0.2)	0.041 (0.004)	4.83 (0.13)	0.16 (0.00)	30.1 (0.16)	0
2d→DR		11.0 (0.2)					
3d→DR		11.0 (0.2)					
4d→DR		11.0 (0.1)					
5d→DR		11.0 (0.2)	0.034 (0.007)	4.44 (0.28)	0.12 (0.01)	36.1 (0.41)	19.9
DR		10.9 (0.1)	0.041 (0.004)	4.83 (0.13)	0.16 (0.00)	30.1 (0.16)	-
IRGC 117280 [B]							
1d→DR	No parameters constrained	11.0 (0.1)	0.018 (0.007)	4.59 (0.27)	0.13 (0.01)	35.3 (0.38)	54.8
2d→DR		10.9 (0.1)	0.053 (0.011)	3.80 (0.23)	0.11 (0.01)	34.0 (0.46)	32.9
3d→DR		10.9 (0.1)	0.023 (0.008)	3.60 (0.20)	0.12 (0.01)	29.9 (0.41)	31.1
4d→DR		10.9 (0.1)	0.061 (0.010)	4.24 (0.29)	0.13 (0.01)	32.9 (0.44)	44.3
5d→DR		10.9 (0.1)	0.066 (0.009)	4.99 (0.32)	0.14 (0.01)	36.2 (0.41)	58.8
DR		10.9 (0.1)	0.049 (0.010)	5.59 (0.43)	0.25 (0.02)	22.8 (0.29)	-

Appendix 6.3B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seed lots from accessions IRGC 117265, -76 and 80 which were immediately dried in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at 45°C/23% RH for up to 5 days before being transferred to the DR for final equilibrium drying (Appendices 6.2 and 6.3A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Accession	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
IRGC 117265_A	1d-5d & DR					
	Common AR slope	294.4	87	3.384		
	Best model	239	82	2.915		
	Change	55.4	5	11.08	3.801	0.004**
	1d-5d & DR					
	Common slope	307.2	92	3.339		
	Best model	239	82	2.915		
	Change	68.2	10	6.82	2.340	0.02*
IRGC 117265_B	1d-5d & DR					
	Common AR slope	109.7	65	1.688		
	Best model	99.51	60	1.658		
	Change	10.19	5	2.038	1.229	0.31^{NS}
	1d-5d & DR					
	Common slope	202.9	70	2.899		
	Best model	99.51	60	1.658		
	Change	103.39	10	10.339	6.236	<0.001***
IRGC 117276_A	1d-5d					
	Common line	604.6	83	7.284		
	Best model	504.6	75	6.729		
	Change	100	8	12.5	1.858	0.08^{NS}
	1d-5d & DR					
	Common line	1438	87	16.52		
	Best model	478.9	77	6.22		
	Change	959.1	10	95.91	15.420	<0.001***

	1d-5d					
	Common line	203.6	82	2.483		
	Best model	175.5	74	2.372		
IRGC 117276_B	Change	28.1	8	3.513	1.481	0.18^{NS}
	1d-5d & DR					
	Common line	459.8	92	4.998		
	Best model	193.9	82	2.365		
	Change	265.9	10	26.59	11.243	<0.001***
	1d-4d & DR					
	Common line	246.2	75	3.283		
	Best model	183.1	63	2.906		
IRGC 117280_A	Change	63.1	12	5.258	1.809	0.07^{NS}
	1d-5d & DR					
	Common line	601.6	91	6.611		
	Best model	215.9	76	2.841		
	Change	385.7	15	25.713	9.051	<0.001***
	1d-5d & DR					
IRGC 117280_B	Common slope	208.5	74	2.817		
	Best model	136.6	69	1.979		
	Change	71.9	5	14.38	7.266	<0.001***

Appendix 6.4. Survival curves fitted by probit analysis for accession IRGC 117265 harvested in the 2014 wet season (WS) and experimentally stored at 45°C and 60% RH. Samples were: immediately dried in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at a gradual drying regime of: 45°C/23% $RH_{[1d]}$; 30°C/45% $RH_{[2d]}$; 20°C/25% $RH_{[2d]}$; 15°C/15% $RH_{[1d]}$. At the end of each drying stage (days 1, 3, 5 and 6) a sample of seeds was removed and transferred to the DR for final equilibrium drying. The results shown are for the model with the fewest parameters that could be fitted without a significant increase in residual deviance compared with the best-fit model. The dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). All seed lots were harvested at 35 days after 50% anthesis (DAA) on two separate occasions; A and B respectively. The survival curves are quantified in Appendix 6.5.



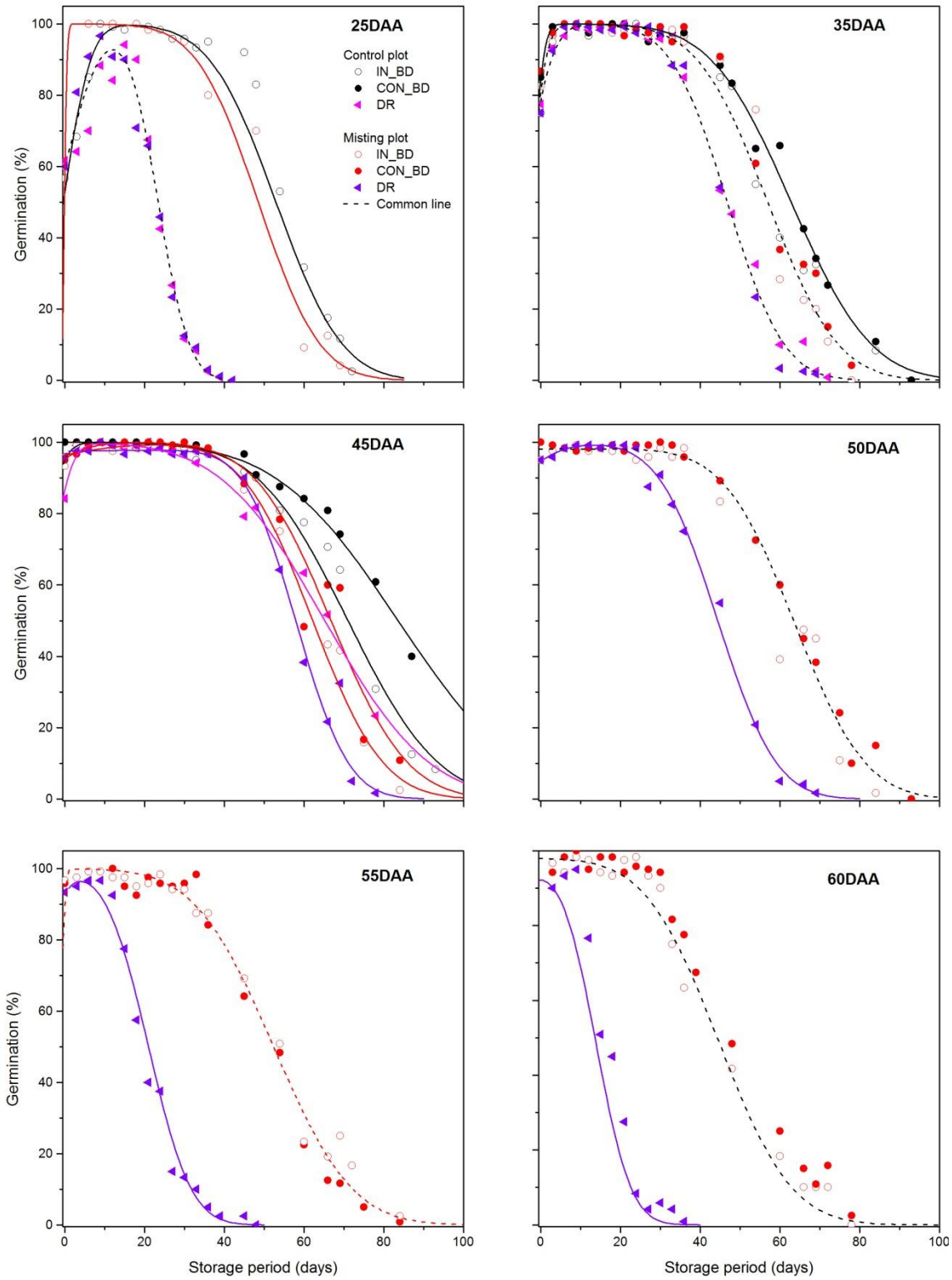
Appendix 6.5A. Results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) to quantify changes in ability to germinate during hermetic storage for seeds dried immediately in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at a gradual drying regime of: 45°C/23% $RH_{[1d]}$; 30°C/45% $RH_{[2d]}$; 20°C/25% $RH_{[2d]}$; 15°C/15% $RH_{[1d]}$. At the end of each drying stage (days 1, 3, 5 and 6) a sample of seeds was removed and transferred to the DR for final equilibrium drying. The parameters shown are for the simplest model that could be fitted without a significant increase (P<0.05) in the residual deviance compared with the best-fit model. The MC (%, fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration.

Treatment	Model	Seed MC (s.e.)	Loss in dormancy		Loss in viability			
			K_d (s.e.)	θ_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}	Difference in p_{50} relative to DR
		(%, f.wt)	(NED)	(days)	(NED)	(days^{-1})	(days)	(%)
IRGC 117265 [A]								
D1: 45°C/75% RH (1d)	K_d, θ_1, K_i and σ^1 constrained within days 1-6	11.1 (0.2)	0.87 (0.49)	0.02 (0.31)	3.83 (0.92)	0.08 (0.05)	50.1	263.0
D3: 30°C/45% RH (2d)		11.1 (0.2)						
D5: 20°C/25% RH (2d)		11.1 (0.1)						
D6: 15°C/15% RH (1d)		11.0 (0.1)						
DR		11.0 (0.2)	0.00 (0.24)	0.42 (0.16)	2.18 (0.39)	0.16 (0.02)	13.8	-
D1: 45°C/75% RH (1d)	K_d, θ_1, K_i and σ^1 constrained within days 1-6	11.1 (0.2)	1.16 (0.52)	0.00 (0.13)	6.52 (1.38)	0.11 (0.03)	61.9	63.3
D3: 30°C/45% RH (2d)		11.1 (0.2)						
D5: 20°C/25% RH (2d)		11.0 (0.2)						
D6: 15°C/15% RH (1d)		11.1 (0.2)						
DR		11.1 (0.2)	0.49 (0.25)	0.14 (0.06)	3.28 (0.37)	0.09 (0.01)	37.9	-

Appendix 6.5B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seed lots from accession IRGC 117265 which were either immediately in the dryroom (DR; 15°C/15% RH) or initially in a climate chamber set at a gradual drying regime of: 45°C/23% $RH_{[1d]}$; 30°C/45% $RH_{[2d]}$; 20°C/25% $RH_{[2d]}$; 15°C/15% $RH_{[1d]}$ (Appendices 6.4 and 6.5A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

Accession	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
IRGC 117265_A	D1-6					
	Common line	229.3	56	4.095		
	Best model	172.8	44	3.927		
	Change	56.5	12	4.708	1.199	0.31^{NS}
	D1-6 & DR					
	Common line	2028	60	33.8		
	Best model	172.8	44	3.927		
	Change	1855.2	16	115.95	29.526	<0.001***
IRGC 117265_B	D1-6					
	Common line	377.8	67	5.638		
	Best model	314.3	55	5.715		
	Change	63.5	12	5.292	0.926	0.53^{NS}
	D1-6 & DR					
	Common line	1162	71	16.36		
	Best model	314.3	55	5.715		
	Change	847.7	16	52.981	9.271	<0.001***

Appendix 7.1. Ability to germination during experimental storage at 45°C and 60% RH for seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (storage experiment A). The combined loss in dormancy and loss in viability model was applied to the data. For those seed lots which showed a complete loss in dormancy, survival curves were fitted using the Ellis and Roberts (1980a) viability model. Seed lots which showed a reduced initial viability an additional parameter was applied to probit analysis to determine the proportion of responding seeds within the population (Mead and Grey, 1999). The coloured dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). Survival curves are quantified in Appendix 7.2.


25DAA
Control plot
IN_BD
CON_BD
DR
Misting plot
IN_BD
CON_BD
DR
Common line
35DAA
45DAA
50DAA
55DAA
60DAA
Germination (%)
Storage period (days)
0
20
40
60
80
100

Appendix 7.2A. The results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (storage experiment A). For those seedlots which showed complete loss in dormancy the viability model (Ellis and Roberts, 1980a) was applied, with (¶) or without the "controlled mortality" parameter ("immunity" in GenStat). The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant (P<0.05) increase in residual deviance compared with the best-fit model. The moisture content (MC; % fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment.

CS09 Batch dryer Storage experiment A									
Plot	Treatment	Seed MC (s.e.) (% f.wt.)	Maturity (DAA)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (days)	Difference in p50 relative to the DR (%)
Control	IN_BD (R1)	10.8 (0.0)	25 DAA	0.06 (0.32)	0.19 (0.09)	4.05 (0.52)	0.08 (0.02)	52.9	126.1
	DR (R1)	10.8 (0.1)		0.24 (1.01)	0.12 (0.25)	3.82 (1.43)	0.16 (0.04)	23.4	-
Misting	IN_BD (R2)	10.7 (0.1)		0.25 (0.33)	2.88 (*.**)	3.99 (0.55)	0.08 (0.02)	48.5	107.3
	DR (R2)	10.8 (0.1)		0.24 (1.01)	0.12 (0.25)	3.82 (1.43)	0.16 (0.04)	23.4	-
Control	IN_BD (R1)	10.7 (0.0)	35 DAA	0.89 (0.40)	0.32 (0.12)	4.11 (0.60)	0.07 (0.01)	56.9	21.6
	CON_BD (R1)	10.7 (0.0)		1.04 (0.19)	0.46 (0.18)	3.99 (0.22)	0.06 (0.00)	62.8	34.2
	DR (R1)	10.9 (0.1)		0.79 (0.40)	0.19 (0.35)	4.19 (0.49)	0.09 (0.01)	46.8	-
Misting	IN_BD (R1)	10.8 (0.1)		0.89 (0.40)	0.32 (0.12)	4.11 (0.60)	0.07 (0.01)	56.9	21.6
	CON_BD (R1)	10.7 (0.1)		0.89 (0.40)	0.32 (0.12)	4.11 (0.60)	0.07 (0.01)	56.9	21.6
	DR (R1)	10.9 (0.1)		0.79 (0.40)	0.19 (0.35)	4.19 (0.49)	0.09 (0.01)	46.8	-

Control	IN_BD (R2)	10.7 (0.1)		1.65 (0.36)	0.23 (0.05)	3.98 (0.40)	0.06 (0.01)	71.0	8.9
	CON_BD (R2)	10.7 (0.1)		9.29 (*.**)	0.23 (0.05)	3.48 (0.40)	0.04 (0.01)	83.6	28.2
	DR (R2)	10.7 (0.1)		1.12 (0.14)	0.23 (0.05)	3.17 (0.17)	0.05 (0.00)	65.2	-
Misting	IN_BD (R2)	10.8 (0.0)	45 DAA	1.79 (0.49)	0.03 (0.03)	4.48 (1.40)	0.07 (0.02)	62.2	6.9
	CON_BD (R2)	10.8 (0.1)		1.68 (0.50)	0.08 (0.05)	4.41 (1.39)	0.07 (0.02)	66.8	14.8
	DR (R2)	10.9 (0.1)		1.97 (0.20)	0.00 (0.01)	5.70 (0.66)	0.10 (0.01)	58.2	-
Misting	IN_BD (R1)[¶]	10.9 (0.0)		-	-	4.56 (0.21)	0.07 (0.00)	63.8	43.7
	CON_BD (R1)[¶]	10.9 (0.1)	50 DAA	-	-	4.56 (0.21)	0.07 (0.00)	63.8	43.7
	DR (R1)	11.0 (0.0)		1.61 (0.17)	0.08 (0.03)	3.84 (0.17)	0.09 (0.00)	44.4	-
Misting	IN_BD (R1)	10.9 (0.1)		1.94 (0.57)	2.35 (0.34)	3.37 (0.46)	0.06 (0.02)	52.4	149.5
	CON_BD (R1)	10.9 (0.0)	55 DAA	1.94 (0.57)	2.35 (0.34)	3.37 (0.46)	0.06 (0.02)	52.4	149.5
	DR (R1)	10.9 (0.1)		1.51 (0.26)	0.17 (0.16)	2.50 (0.18)	0.12 (0.01)	21.0	-

Misting	IN_BD (R1)[¶]	10.9 (0.0)		-	-	3.09 (0.11)	0.07 (0.00)	44.6	211.9
	CON_BD (R1)[¶]	11.0 (0.1)	60 DAA	-	-	3.09 (0.11)	0.07 (0.00)	44.6	211.9
	DR (R1)[¶]	11.1 (0.1)		-	-	2.02 (0.02)	0.14 (0.01)	14.3	-

[¶] Immunity values generated by GenStat were: 0.019 (0.002) for seed lots In_BD and Con_BD at 50 DAA; 0.019 (0.005) for seed lots In_BD and Con_BD at 60 DAA; and 0.020 (0.00) for DR seed lot at 60 DAA.

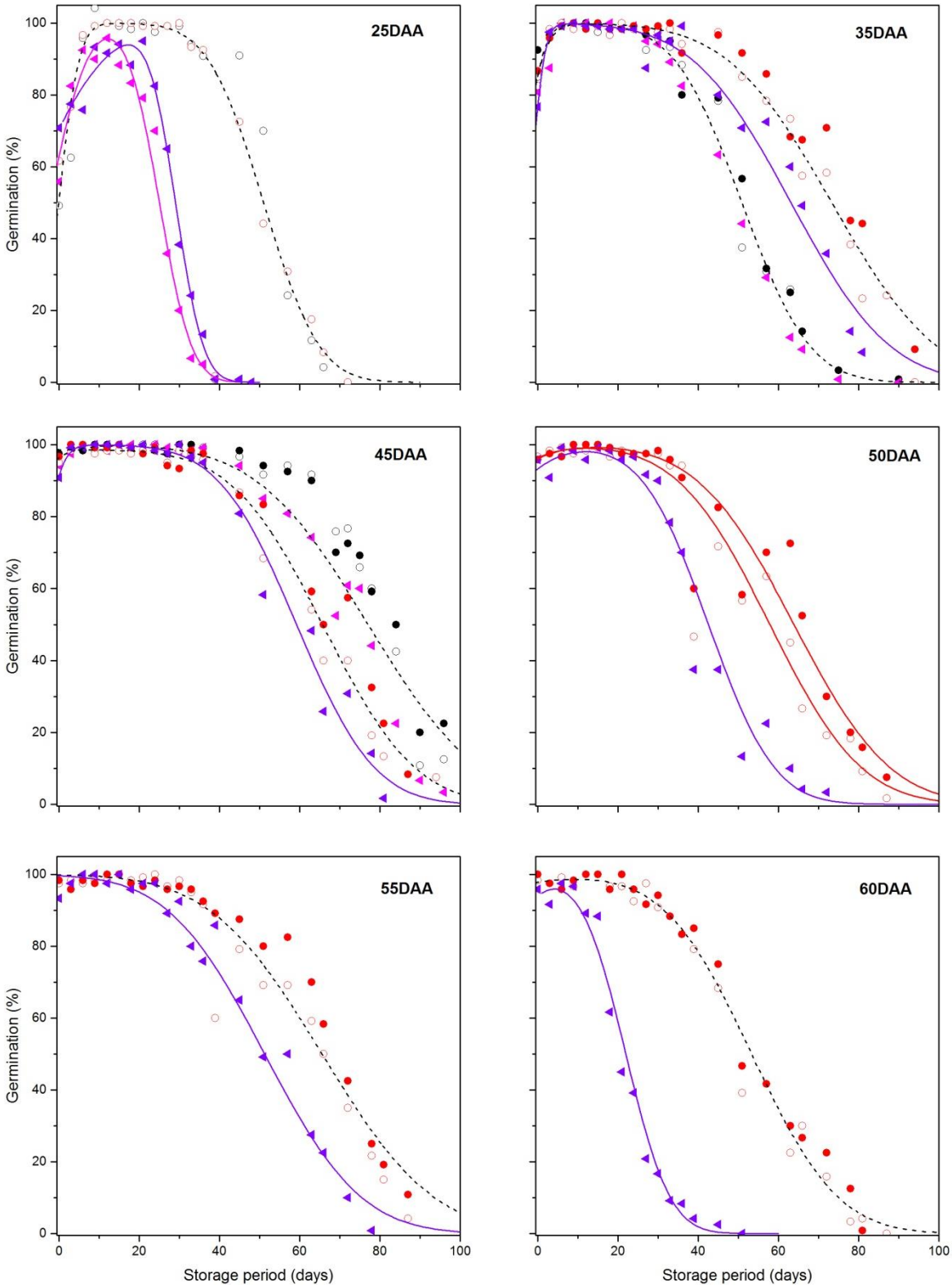
Appendix 7.2B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (storage experiment A; Appendices 7.1 and 7.2A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

CS09 Batch dryer storage experiment A						
Maturity	Treatment	Res dev	Res d.f.	Res Mean dev	F	P
25DAA	IN_BD (Control R1 & Misting R2)					
	Common AR slope	68.05	35	1.944		
	Best model	50.22	31	1.62		
	Change	17.83	4	4.458	2.752	<0.04*
	DR (Control R1 & Misting R2)					
	Common line	872.8	44	19.84		
	Best model	840.9	40	21.02		
	Change	31.9	4	7.975	0.379	0.82^{NS}
	DR (Control R1); DR (Misting R2) & IN_BD (Control R1)					
	Common line	2680	48	55.84		
	Best model	857.6	47	18.25		
	Change	1822.4	1	1822.4	99.858	<0.001***
	DR (Control R1); DR (Misting R2) & IN_BD (Misting R2)					
	Common line	1518	36	42.17		
	Best model	858.4	47	18.26		
	Change	659.6	-11	-59.964	-3.284	<0.001***
35 DAA	IN_BD (Control R1 & Misting R1) & Con_BD (Misting R1)					
	Common line	166.5	65	2.562		
	Best model	136.1	57	2.388		
	Change	30.4	8	3.8	1.591	0.15^{NS}

	IN_BD (Control R1 & Misting R1); & Con_BD (Control R1 & Misting R1)					
	Common line	262.4	85	3.088		
	Best model	159.8	73	2.19		
	Change	102.6	12	8.55	3.904	<0.001***
	DR (Control R1 & Misting R1)					
	Common line	83.54	47	1.778		
	Best model	71.39	43	1.66		
	Change	12.15	4	3.0375	1.830	0.14^{NS}
	DR (Control R1 & Misting R1)& Con_BD (Control R1)					
	Common line	618.6	66	9.372		
	Best model	101	58	1.742		
	Change	517.6	8	64.7	37.141	<0.001***
	All treats					
	Common AR	197.1	96	2.053		
	Best model	148.9	91	1.637		
	Change	48.2	5	9.64	5.889	<0.001***
	IN_BD (Control R2) & Con_BD (Control R2)					
	Common AR	55.69	48	1.16		
	Best model	54.92	46	1.194		
	Change	0.77	2	0.385	0.322	0.73^{NS}
45 DAA	IN_BD (Control R2) & Con_BD (Control R2)					
	Common slopes	173.2	50	3.465		
	Best model	54.92	46	1.194		
	Change	118.28	4	29.57	24.765	<0.001***
	IN_BD (Control R2); Con_BD (Control R2) & DR (Control)					
	Common AR	129	63	2.047		
	Best model	84.34	60	1.406		
	Change	44.66	3	14.887	10.588	<0.001***
	IN_BD (Misting R2) & Con_BD (Misting R2)					
	Common AR slope	108.5	47	2.308		
	Best model	94.07	45	2.09		
	Change	14.43	2	7.215	3.452	0.04*

	IN_BD (Misting R2) & DR (Misting R2)					
	Common AR slope	108.5	47	2.308		
	Best model	94.07	45	2.09		
	Change	14.43	2	7.215	3.452	0.04*
	Con_BD (Misting R2) & DR (Misting R2)					
	Common AR slope	108.5	47	2.308		
	Best model	94.07	45	2.09		
	Change	14.43	2	7.215	3.452	0.04*
50 DAA	IN_BD (Misting R1) & Con_BD (Misting R1) Viability model (incl. immunity parameter)					
	Common line	74.76	36	2.077		
	Best model	63.03	33	1.91		
	Change	11.73	3	3.91	2.047	0.13^{NS}
55 DAA	IN_BD (Misting R1) & Con_BD (Misting R1)					
	Common line	87.33	45	1.941		
	Best model	74.32	41	1.813		
	Change	13.01	4	3.253	1.794	0.15^{NS}
	IN_BD (Misting R1); Con_BD (Misting R1) & DR					
	Common line	1923	49	39.25		
	Best model	74.32	41	1.813		
	Change	1848.68	8	231.085	127.460	<0.001***
60 DAA	IN_BD (Misting R1) & Con_BD (Misting R1)					
	Common line	108.6	35	3.104		
	Best model	95.78	32	2.993		
	Change	12.82	3	4.273	1.428	0.25^{NS}
	IN_BD (Misting R1); Con_BD (Misting R1) & DR					
	Common line	2126	48	44.3		
	Best model	134.5	42	3.203		
	Change	1991.5	6	331.917	103.627	<0.001***

Appendix 7.3. Ability to germination during experimental storage at 45°C and 60% RH for seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (Storage experiment B). The combined loss in dormancy and loss in viability model was applied to the data. For those seed lots which showed a complete loss in dormancy, survival curves were fitted using the Ellis and Roberts (1980) viability model. Seed lots which showed a reduced initial viability an additional parameter was applied to probit analysis to determine the proportion of responding seeds within the population (Mead and Grey, 1999). The coloured dashed lines correspond to treatments which could be constrained to a single curve (P>0.05). Survival curves are quantified in Appendix 7.4


25DAA
35DAA
45DAA
50DAA
55DAA
60DAA
Germination (%)
Storage period (days)
0
20
40
60
80
100

Appendix 7.4A. The results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (storage experiment B). For those seedlots which showed complete loss in dormancy the viability model (Ellis and Roberts 1980a) was applied, with (¶) or without the "controlled mortality" parameter ("immunity" in GenStat). The parameters shown are for the simplest model (fewest parameters) that could be fitted without a significant (P<0.05) increase in residual deviance compared with the best-fit model. The moisture content (MC; % fresh weight) is the mean and s.e. calculated from measurements taken at three stages across the duration of the storage experiment.

CS09 Batch dryer Storage experiment B									
Plot	Treatment	Seed MC (s.e.) (% f.wt.)	Maturity (DAA)	K_d (s.e.) (NED)	θ_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) ($days^{-1}$)	p_{50} (days)	Difference in p50 relative to the DR (%)
Control	IN_ BD (R2)	10.8 (0.1)	25 DAA	0.02 (0.34)	0.25 (0.07)	4.67 (0.71)	0.09 (0.03)	51.0	103.2
	DR (R2)	10.7 (0.1)		0.33 (0.40)	0.14 (0.11)	3.99 (0.79)	0.16 (0.02)	25.1	-
Misting	IN_BD (R1)	10.6 (0.1)		0.02 (0.34)	0.25 (0.07)	4.67 (0.71)	0.09 (0.03)	51.0	74.1
	DR (R1)	10.6 (0.1)		0.54 (0.39)	0.66 (0.10)	5.40 (0.98)	0.19 (0.03)	29.3	-
Control	IN_BD (R2)	10.8 (0.0)	35 DAA	1.06 (0.44)	0.20 (0.26)	3.83 (0.43)	0.08 (0.01)	50.9	0
	CON_BD (R2)	10.6 (0.1)		1.06 (0.44)	0.20 (0.26)	3.83 (0.43)	0.08 (0.01)	50.9	0
	DR (R2)	10.8 (0.0)		1.06 (0.44)	0.20 (0.26)	3.83 (0.43)	0.08 (0.01)	50.9	-
Misting	IN_BD (R2)	10.8 (0.1)		1.06 (0.55)	0.24 (0.33)	3.57 (0.53)	0.05 (0.01)	73.3	16.0
	CON_BD (R2)	10.6 (0.1)		1.06 (0.55)	0.24 (0.33)	3.57 (0.53)	0.05 (0.01)	73.3	16.0
	DR (R2)	10.9 (0.1)		0.75 (0.24)	0.37 (0.15)	3.26 (0.23)	0.05 (0.00)	63.2	-

Control	IN_BD (R1)	10.7 (0.0)							
	CON_BD (R1)	10.6 (0.1)		1.80 (0.40)	0.09 (0.10)	3.52 (0.30)	0.05 (0.00)	77.1	0
	DR (R1)	10.8 (0.0)							-
Misting	IN_BD (R1)¶	10.7 (0.0)	45 DAA	-	-	3.67 (0.14)	0.06 (0.00)	66.0	11.5
	CON_BD (R1)¶	10.6 (0.0)		-	-	3.67 (0.14)	0.06 (0.00)	66.0	11.5
	DR (R1)	10.9 (0.1)		1.37 (0.27)	0.23 (0.12)	3.87 (0.26)	0.07 (0.00)	59.2	-
Misting	IN_BD (R2)	10.7 (0.0)		1.80 (0.55)	0.07 (0.03)	3.22 (0.54)	0.06 (0.01)	57.9	35.9
	CON_BD (R2)	10.6 (0.0)	50 DAA	1.76 (0.53)	0.07 (0.03)	3.39 (0.55)	0.05 (0.01)	63.9	50
	DR (R2)	10.9 (0.1)		1.50 (0.23)	0.07 (0.03)	3.29 (0.23)	0.08 (0.05)	42.6	-
Misting	IN_BD (R2)	10.7 (0.1)		-	-	2.99 (0.07)	0.05 (0.00)	65.6	27.9
	CON_BD (R2)	10.7 (0.1)	55 DAA	-	-	2.99 (0.07)	0.05 (0.00)	65.6	27.9
	DR (R2)	10.9 (0.0)		-	-	2.72 (0.09)	0.05 (0.00)	51.3	-

Misting	IN_BD (R2)	10.8 (0.0)		2.03 (0.73)	0.04 (0.29)	3.15 (0.50)	0.06 (0.02)	53.3	142.3
	CON_BD (R2)	10.8 (0.0)	60 DAA	2.03 (0.73)	0.04 (0.29)	3.15 (0.50)	0.06 (0.02)	53.3	142.3
	DR (R2)	10.9 (0.0)		1.59 (0.31)	0.11 (0.14)	2.54 (0.22)	0.12 (0.01)	22.0	-

[¶] Immunity value generated by GenStat was 0.0136 (0.003) for seed lots Misting In_BD and Con_BD (R2) at 45 DAA

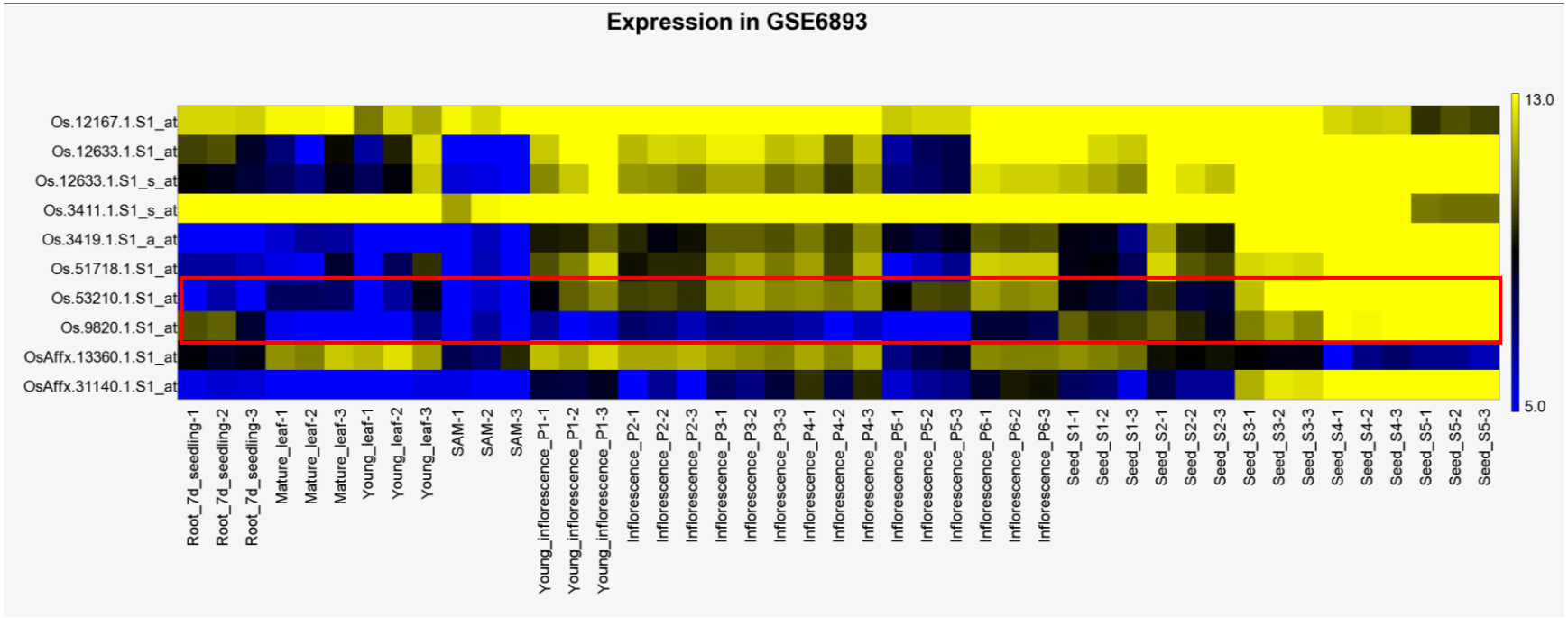
Appendix 7.4B. Results of probit analysis generated in GenStat. The F-test was used to determine the simplest model that could be fitted (where one or more parameters are constrained to a common value for all seed lots) compared with the best-fit model for all seeds from each plot (replicate 1 [R1] or 2 [R2]) which were harvested between 25 and 60 days after 50% anthesis (DAA) and dried for 3 days either intermittently (In; 8 h day^{-1}) or continuously (Con; 24 h day^{-1}) in the batch dryer (BD) or dryroom (DR) prior to final drying in the DR (storage experiment A; Appendices 7.1 and 7.2A). Superscript letters in P column indicate significance at the * 5%, ** 1% and *** 0.01% level and NS is not significant.

CS09 Batch dryer storage experiment B						
25DAA	IN_BD (Control R2 & Misting R1)					
	Common line	95.14	42	2.265		
	Best model	82.28	38	2.244		
	Change	9.86	4	2.465	1.098	0.37^{NS}
	DR (Control R2 & Misting R2)					
	Common slopes	130.8	38	3.442		
	Best model	93.36	36	2.593		
	Change	37.44	2	18.72	7.219	0.002***
	IN_BD (Control R2 & Misting R1) & DR (Control R2)					
	Common line	1857	46	40.36		
	Best model	82.28	38	2.244		
	Change	1771.72	8	221.465	98.692	<0.001***
	IN_BD (Control R2 & Misting R1) & DR (Misting R1)					
	Common line	1523	47	34.2		
	Best model	79.23	39	2.032		
	Change	1443.77	8	180.471	88.815	<0.001***
35 DAA	IN_BD (Control R2); Con_BD (Control R2) & DR (Control R2)					
	Common line	185.4	73	2.539		
	Best model	150.4	65	2.314		
	Change	35	8	4.375	1.891	0.08^{NS}

	IN_BD (Misting R2) & Con_BD (Misting R2)					
	Common line	219.2	58	3.779		
	Best model	198.9	54	3.684		
	Change	20.3	4	5.075	1.378	0.25^{NS}
	IN_BD (Misting R2); Con_BD (Misting R2) & DR (Misting R2)					
	Common line	324.7	62	5.237		
	Best model	198.9	54	3.684		
	Change	125.8	8	15.725	4.268	0.001***
	IN_BD (Control R2); Con_BD (Control R2); & DR (Control R2 & Misting R2)					
	Common line	473.3	77	6.147		
	Best model	150.4	65	2.314		
	Change	322.9	12	26.908	11.628	<0.001***
	IN_BD (Control R1) & Con_BD (Control R1)					
	Common line	827.3	65	12.73		
	Best model	719.9	61	11.8		
	Change	107.4	4	26.85	2.275	0.07^{NS}
	IN_BD (Control R1); Con_BD (Control R1) & DR (Control R1)					
	Common line	903.7	69	13.1		
	Best model	719.9	61	11.8		
	Change	183.8	8	22.975	1.947	0.07^{NS}
45 DAA						
	IN_BD (Control R1); Con_BD (Control R1); DR (Control R1) & DR (Misting R1)					
	Common line	1291	89	14.51		
	Best model	764.7	77	9.931		
	Change	526.3	12	43.858	4.416	<0.001***
	IN_BD (Misting R1) & Con_BD (Misting R1) Viability model (incl. Immunity parameter)					
	Common line	71.58	38	1.884		
	Best model	59.19	35	1.691		
	Change	12.39	3	4.13	2.442	0.08^{NS}

50 DAA	IN_BD (Misting R2) & Con_BD (Misting R2) & DR (Misting R2)					
	Common AR slope	247.9	72	3.444		
	Best model	245.1	69	3.553		
	Change	2.8	3	0.9333	0.263	0.85^{NS}
	IN_BD (Misting R2) & Con_BD (Misting R2) & DR (Misting R2)					
	Common slopes	335.2	75	4.469		
	Best model	245.1	69	3.553		
	Change	90.1	6	15.017	4.226	0.001***
55 DAA	IN_BD (Misting R2) & Con_BD (Misting R2)					
	Common line	162.5	43	3.78		
	Best model	147.9	41	3.608		
	Change	14.6	2	7.3	2.023	0.15^{NS}
	IN_BD (Misting R2); Con_BD (Misting R2) & DR (Misting R2)					
	Common line	459.3	63	7.29		
	Best model	233.2	59	3.953		
	Change	226.1	4	56.525	14.299	<0.001***
60 DAA	IN_BD (Misting R2) & Con_BD (Misting R2)					
	Common line	134.3	53	2.535		
	Best model	128.3	49	2.619		
	Change	6	4	1.5	0.573	0.62^{NS}
	IN_BD (Misting R2); Con_BD (Misting R2) & DR (Misting R2)					
	Common line	2028	57	35.58		
	Best model	128.3	49	2.619		
	Change	1899.7	8	237.463	90.669	<0.001***

Appendix 7.5. Expression data for reproduction development in rice (Kapoor *et al*., 2007). The red box represents the expression pattern of the target two dehydrins, LOC_Os11g26750 (array element Os.9820.1.S1_at) and LOC_Os11g26760 (array element Os.53210.1.S1_at).



Appendix 7.6. Protein concentration (µg/ml) for each seed lot (randomly assigned to storage experiment [A] or [B]) calculated using BCA Protein Assay (Thermo Scientific™ Pierce™). Seeds were collected at the pre-drying stage (PD), and after 3 days of continous drying in the dryroom (DR), 3 days of intermittent drying in the batch dryer (BD_In) and 3 days of continuous drying in the batch dryer (BD_Con). Intermittent drying lasted for 8 h between 0800 and 1600 hrs and continuous drying for 24 h.

Plot 1

	PD		DR		BD_In		BD_Con	
Maturity stage (DAA)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)
15DAA	1.5	1802.2	-	-	-	-	-	-
25DAA_A	3.5	4802.2	3.3	4508.1	2.8	3772.8	-	-
25DAA_B	3.5	4802.2	3.5	4802.2	3.5	4802.2	-	-
35DAA_A	3.5	4802.2	2.5	3331.6	3.5	4802.2	2.9	3919.9
35DAA_B	2.0	2655.1	2.6	3478.7	2.6	3478.7	2.6	3478.7
45DAA_A	2.5	3361.0	3.5	4802.2	2.3	3037.5	1.9	2449.3
45DAA_B	2.9	3846.3	2.5	3331.6	2.6	3478.7	2.0	2596.3

Plot 2

	PD		DR		BD_In		BD_Con	
Maturity stage (DAA)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)	Absorbance (562 nm)	Concentration (µg/ml)
15DAA	3.5	4802.2	-	-	-	-	-	-
25DAA_A	2.8	3758.1	2.1	2743.4	2.2	2890.4	-	-
25DAA_B	2.7	3669.9	3.5	4802.2	2.3	3037.5	-	-
35DAA_A	2.0	2537.5	2.3	3037.5	1.8	2302.2	2.5	3331.6
35DAA_B	3.0	4022.8	3.5	4802.2	1.8	2302.2	2.5	3331.6
45DAA_A	2.0	2625.7	3.0	4066.9	3.5	4802.2	2.5	3331.6
45DAA_B	2.3	2964.0	2.7	3625.7	2.8	3772.8	1.6	2008.1
50DAA_A	3.0	4111.0	3.5	4802.2	2.9	3919.9	3.4	4655.1
50DAA_B	3.1	4169.9	3.5	4802.2	3.5	4802.2	2.9	3919.9
55DAA_A	3.5	4802.2	2.1	2743.4	2.2	2890.4	2.3	3037.5
55DAA_B	3.0	4008.1	2.9	3919.9	2.5	3331.6	2.6	3478.7
60DAA_A	2.3	3066.9	2.5	3331.6	2.2	2890.4	2.4	3184.6
60DAA_B	3.1	4199.3	2.8	3772.8	2.5	3331.6	3.5	4802.2

Appendix 8.1. Weight (kg) of material from the 2013 dry season (DS) that entered the dryroom per week (columns) and the cumulative weight (kg) (line) during the same period.