University of Reading

Temporal Sensitivity of Rice Seed Quality Development to Environmental Stress

Siti Maslizah Binti Abdul Rahman

Thesis submitted for the Degree of Doctor of Philosophy

School of Agriculture, Policy and Development

University of Reading, United Kingdom

2018

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of University of Reading. It is original and is the result of my own work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

Signature: Date: 28 September 2018

(Mrs SITI MASLIZAH ABDUL RAHMAN)

Dedication

This thesis is especially dedicated to my beloved husband (Farriz) and my kids (Raudhah, Muhaimin & Aleesya) for their unconditional patience, love and support

To unforgettable my beloved father & late mother, thanks for your supports, cares, loves and everything……

Acknowledgement

All praises be to Almighty Allah for giving me guidance, strength and will to complete my study, and peace be upon his final Prophet and Messenger, Muhammad S.A.W.

Deepest in my heart, I wish to express sincere appreciation and thank you to my supervisor, Professor Dr Richard Ellis for his guidance, encouragement, constructive discussion, comment, suggestion and advice during of my study. My sincere gratitude is also to Laurence Hanson, Caroline Hadley and Liam Doherty for his invaluable assistance either in lab and during my field work in PEL. Also thanks to Matthew Richardson, Val Jasper, Tobias Lane and staff in CEL for their assistance while my study was conducted there. I am much indebted and thankful to my friends in UoR as well for their caring, motivation and kind cooperation.

Thanks a lot, to Malaysia government, for supporting and providing scholarship for pursuing my study in U.K. For UiTM, my employer, many thanks for give support during my study leave. I also acknowledged the help and support of all whose names were not mentioned in this thesis.

Abstract

High temperature and drought, together or alone, reduce rice (*Oryza sativa* L.) crop yield. Their effect at different periods during seed development and maturation on rice seed quality, and its development, was investigated with pot-grown plants in controlled-environment growth cabs (28/20°C,11h/13h) or glass-houses (28/20°C, 12h/12h). Ending irrigation early in *japonica* rice cv. Gleva at early- to mid-seed filling (7 or 14 days after anthesis, DAA) resulted in earlier plant senescence, more rapid decline in seed moisture content and initially more rapid seed quality development, but subsequently substantial decline *in planta* in ability to germinate normally. Subsequent seed storage longevity at 40°C with c. 15% moisture content was ultimately greatest in the control (no drought); both drought treatments declined in subsequent longevity *in planta* from 16 or 22 DAA onwards, respectively. A similar investigation but with drought applied later (14 or 28 DAA) also showed poorer seed longevity in drought treatments at harvest maturity (42 DAA). In both investigations, the earlier the drought the greater the damage to subsequent seed quality. Well-irrigated plants exposed to 40/30°C for 3 days provided poorer subsequent seed quality (shorter longevity) the earlier during development they received high temperature (HT) treatment. The effect was greatest with HT at around anthesis and histodifferentiation, with no effect of HT during the seed maturation phase. Damage to seed quality from combining both stresses (drought, HT) was greater than each alone; *indica* rice cv. Aeron 1 was affected less by these stresses than *japonica* cv. Gleva. It is concluded that drought, as well as HT, damages subsequent seed quality in rice, the period around anthesis is the most vulnerable stage of plant development for such damage to seed, and seed quality in *japonica* rices is more vulnerable than in *indica* rices to stress in the production environment.

297 words

Abbreviations and Symbols

&	And
%	Percent
σ	Sigma, standard deviation of seed death in time (days)
°C	Degree Celsius
°N	North (degree)
°S	South (degree)
ABA	Abscisic acid
ANOVA	Analysis of variance
Cab	Cabinet
CEL	Crop and Environment Laboratory
CIAT	International Center for Tropical Agriculture
cv.,	cvs Cultivar, cultivars
cm	Centimetre
d	Day(s)
DAA	Day after anthesis
DAS	Day after sowing
d.f.	Degree of freedom
dw	Dry weight
E	East
e.g.	Exempli gratia; for example
eRH	Equilibrium relative humidity
FAO	Food and Agriculture Organization
Fig., Figs.	Figure, figures
fw	fresh weight
g	Gram
GA	Gibberellic acid

h	Hour
ha	Hectare
Hz	Hertz
i.e.	Id est; that is
Inc.	Incorporation
INGER	International Network for Genetic Evaluation of Rice
IPCC	Intergovernmental Panel on Climate Change
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
kg	Kilogram
L	Litre
LSD	Least significant difference
Ltd	Limited
MARDI	Malaysia Agriculture Research Development Institute
MC	Moisture content
m	Metre
mg	Milligram
mm	Millimetre
n	Number of observation
N	north
NS	Not significant
P	Probability

*p*50	Half viability period
PEL	Plant Environment Laboratory
ppm	Parts per million
RCBD	Randomized complete block design
s.e.	Standard error
sec	Second
s.e.m.	Standard error of means
u	Unified atomic mass unit; equal to 1.660538921(73)×10−27 kg
UoR	University of Reading
µ	Micro, 1x10-6
V	Volt
v/v	Volume by volume
W	Watt
WARDA	West Africa Rice Development Association
wc	water content

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF PLATES

Page

LIST OF APPENDICES

Page

CHAPTER 1

GENERAL INTRODUCTION

This thesis considers the changes of rice (*Oryza sativa* L.) seed quality, including subsequent seed storage longevity, in *japonica* cv. Gleva (and rice cv. Aeron 1) during seed development and maturation under well-watered or limited irrigation conditions or brief elevated high temperature at 40℃. Determination of the potential for subsequent longevity is important in order to know the ability of seed to generate subsequent crops.

Climate change is now tremendously important to the agriculture sector worldwide with consequences for crop yield and also quality. Environmental conditions during rice cultivation affect seed quality (Ellis et al., 1993; Ellis, 2011;Tejakhod and Ellis, 2017) with drought and high temperature important elements to consider in order to reduce crop failure resulting from sowing poor quality seed. In rice, it also well documented that environmental stress has more impact during the reproductive phase compared to other growth phases. Many studies have emphasized the consequence for yield components from environmental stress (Krishnan et al., 2011; Singh et al., 2012), but there has been limited study of the effect of drought during rice cultivation on potential seed longevity.

1.1 *Oryza sativa*

Rice (*Oryza sativa* L.) is a major staple food for the world's population with about two-third of the total rice production grown under irrigation (Maclean et al., 2002). Asia accounts for over 90% of the world's production of rice, with China, India and Indonesia producing the most (FAO, 2017). Rice provides 21% of global human per capita energy and 15% of per capita protein (IRRI, 2013). According to the Human Development Report (HDR) (1997), approximately 70% of the world's poor people live in Asia, where rice is their staple. In countries such as Bangladesh, Vietnam, and Myanmar, the average citizen consumes 150-200 kg rice annually, which accounts for two-thirds or more of caloric intake and approximately 60% of daily protein consumption (IRRI, 2013). However, the growth of rice yield has dropped below 1% per year worldwide with an increase of more than 1.2% per year is required to meet the growing demand for food (Normile, 2008).

Rice is a semiaquatic plant and its production is water intensive (Wassmann et al. 2009; Bouman et al. 2005). The rice plant usually takes 3–6 months from germination to

maturity, depending on the variety and the environment under which it is grown. Following the works of (Kato et al., 1930), the cultivated *Oryza sativa* L. is divided into two major types, *japonica* and *indica.* The classification of *indica* and *japonica* was carried out by pioneer research in the 1920's, based on morphological and physiological differences.

1.1.1 *Japonica* rice

Japonica is found in the cooler zones of the subtropics and in the temperate zones. Mostly planted mainly in the northeast plain and Yangtze River regions of China (Cheng, 1993; Qian, 2007; Wang et al., 2015) and is dominated by inbred varieties (Deng et al., 2006). In terms of plant characteristics, it is a relatively short plant with narrow, dark green leaves and medium-height tillers. *Japonica* rice grains are short and round, do not shatter easily and have low amylose content, making them moist and sticky when cooked (Ricepedia, 2018).

1.1.1.1 *Javanica* rice

Javanica is a primitive type of *japonica*, less thoroughly differentiated, having many intermediate types between typical *indica* and typical *japonica*, both in morphological traits and in compatibility. It has been concluded, therefore, that *javanica* should be classified in an ecological group under *japonica* (Wang et al., 1998). *Javanica* plants have long grains, thick, but fewer culms (stems), and long and broad leaves (Sato, 1996).

1.1.2 *Indica* rice

Indica rice is the major type of rice grown in the tropics and subtropics, including the Philippines, India, Pakistan, Malaysia, Indonesia, central and southern China. In China, *indica* rice is planted mainly in southern China (Cheng, 1993; Qian, 2007; Min et al., 2011, 2012) with mostly hybrid varieties (Deng et al., 2006). *Indica* plants are tall with broad to narrow, light green leaves. The grains are long to short, slender, somewhat flat, tend to shatter more easily and have high amylose content, making them drier and flakier when cooked than *japonica* varieties (Ricepedia, 2018).

1.1.3 *Japonica* rice cv. Gleva

In Spain, rice is sown in May or sometime in June. The crop is usually harvested in October. Gleva is one of popular varieties sown in Spain and most cultivated in Cataluna (delta Del Ebro), which soils are very saline. The temperature during cultivation is 10-12℃ in seed germination stage and increases to 20-25℃ during crop flowering (Kraehmer et al., 2016). For example, mean maximum temperatures in July and August in rice fields in south and

southwest Spain range from 34.0 to 36.5°C and brief peaks above 40°C can occur, whilst night temperatures may on occasion reach 34°C (Duran et al., 2014).

1.1.4 *Indica* rice cv. Aeron 1

IRRI has developed aerobic rice varieties and MARDI (Malaysia Agriculture Research Development Institute) has tested them in local environments. Aeron 1 cultivar was recognized as an aerobic rice, broadly defined as a production system in which input responsive rice varieties with aerobic adaption are grown in non-puddled, non-saturated soils. In Malaysia, Aeron 1 is particularly sown under aerobic conditions. Aerobic rice cultivation is a new concept of growing rice with supplemental irrigation, without the necessity for standing water in the field. This is more efficient in terms of water use than puddle paddy rice. Variety AERON 1/05 showed superior in some yield component characteristics such as longer panicle, higher number of rachis, higher yield and heavier grains (Zainudin et al., 2014). Achieving high yield under aerobic soil conditions requires rice varieties that combine the drought-resistant characteristics of lowland rice. In addition, this aerobic rice, research undertaken by IRRI since 2001 has shown that aerobic rice varieties could produce yields of up to 6 t/ha. However, there had been little progress in the screening and selection of aerobic rice varieties for the tropics and semiarid tropics (Templeton and Bayot, 2011).

1.2 Rice growing phase

Rice varieties can be categorized into three groups based on their growth duration: i) short-duration varieties which mature in 100–120 days, ii) medium duration, 120-140 days and iii) long duration between 140-160 days. They undergo four general growth phases: germination, vegetative, reproductive, and ripening (Yoshida, 1981). This is summarized in Figure 1.1.

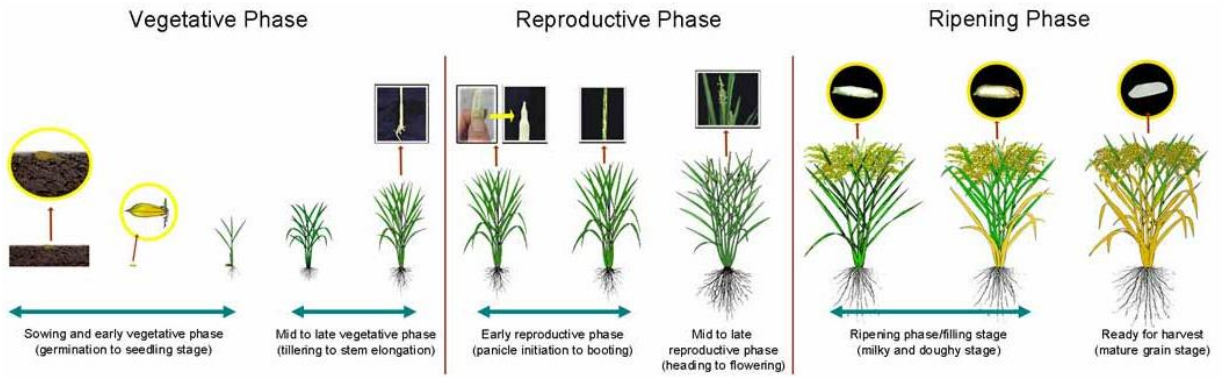


Figure 1.1: Rice growth phase from direct seeding cultivation (From IRRI, 2018)

1.2.1 Seed germination phase

Germination in rice occurs when the first shoots and roots start to emerge from the seed and the rice plant begins to grow. To germinate, rice seeds need to absorb moisture and meet their favourable environment conditions such as a certain amount of water and suitable temperature. Maximum germination of a partially dormant population of seed is achieved at an optimum temperature which is about 27℃ and no germination occurred at 42℃ (Roberts, 1962). Seed dormancy has been defined as the incapacity of a viable seed to germinate under favourable conditions (Bewley,1997; Finch-Savage and Leubner-Metzger, 2006). Delouche (1980) and Kermode (2005) pointed out that dormancy is a means of reducing the adverse effects of environment on seed quality by providing the natural protection against seed deterioration while seed are still on the plant. Induction of seed dormancy usually occurs during the maturation stage (Bewley et al., 2013) parallel with the development of desiccation tolerance (Goldberg et al., 1994) and then develops the capability to maintain their quality under hostile conditions (Delouche and Nguyen,1968). Recently, Shiratsuchi et al. (2017) reported that highly dormant rice seed cv. Hokuriku193 were able to germinate after using steam at 40℃ but this was not necessary for non-dormant rice seed such as *japonica* rice cv. Moeminori.

1.2.2 Vegetative growth phase

The vegetative phase of rice is characterized by the development of tillers and more leaves, and a gradual increase in plant height (Martínez-Eixarch et al., 2013). The number of days the vegetative stage takes varies depends on the rice variety (Hussain et al., 2014; Murshida et al., 2017), with the optimum mean temperature close to 27°C and photoperiods between 11 and 15 hd^{-1} (Summerfield et al., 1992) but is typically between 55 and 85 days after sowing (DAS). The early vegetative phase begins as soon as the seed germinates into

a seedling and ends at tillering. The seedling stage starts right after the first root and shoot emerge and lasts until just

before the first tiller appears. The late vegetative phase starts when tillering begins, which extends from the appearance of the first tiller until the maximum number of tillers is reached. The number of tillers number which later produce panicles depends upon cultivar (Hussain et al., 2014), spacing (Counce et al., 1989; Bhowmik et al., 2013) and year of cultivation (Counce and Wells, 1990). The period of vegetative growth typically takes around 40 DAS in field environments (Counce et al., 1989). The stem begins to lengthen late in the tillering stage and stops growing in height just before panicle initiation about 47-56 days duration to period from culm to panicle emergence, which also signals the end of the vegetative phase (Counce et al., 1996). Tillering in rice is an important agronomic trait for panicle number per unit land area and so grain production (Moldenhauer and Gibbons, 2003). The panicle-bearing tiller rate influences the grain yield of rice (Wang et al., 2007; Badshah et al., 2014). The high tillering capacity is considered desirable trait in rice production, as number of tiller per plant is closely related with number of panicles per plant (Miller et al., 1991), but excessive tillering leads to high tiller abortion, poor grain setting, small panicle size, and can reduce in grain yield (Peng et al., 1994; Ahmad et al., 2005).

1.2.3 Reproductive phase

Reproductive phase consists of four main stages, which are i) booting stage; ii) heading stage; iii) anthesis; and iv) seed grain filling. The first sign that the rice plant is getting ready to enter its reproductive phase is a bulging of the leaf stem that conceals the developing panicle, called the booting stage (Vergara, 1970). At booting stage, senescence of leaves and non-bearing tillers is noticeable at the base of the plant (Rice Knowledge Management Portal, 2018). Then the tip of the developing panicle emerges from the stem and continues to grow, which is called heading. Rice plant is said to be at the heading stage when the panicle is fully visible. As heading progresses, flowering (anthesis) begins. Many studies reports that this period is critical and most sensitive to environmental conditions for example, high temperature (Shi et al., 2016), drought (Kores et al., 2017), and flooding (Tejakhod and Ellis, 2017).

1.2.3.1 Anthesis (flowering) stage

Anthesis (flowering) refers to a series of events between the opening and closing of the spikelet. Anthesis can continue for about 3–9 consecutive days (Cecile-Julia and Dingkuhn, 2013). Throughout my studies, this stage was very important in order to identify when 50% anthesis occurred to detect correct timing to imposed stress during rice plant cultivation. At the beginning of anthesis, tip portions of the lemma and palea begin to open, filaments elongate, and anthers begin to exsert with swelling pollen (Matsui et al., 1999). The filaments elongate further to bring the anthers out resulting in anther dehiscence (Adair, 1934). Consequently, many pollen grains fall onto the stigma, allow pollination to occur. Since the florets of rice are adichogamous (pistils and stamens mature at similar times), most of the florets are self-pollinated at the time of floret opening (Jagoe, 1931). The parts of florets (spikelet) is shown in Figure 1.2.

Pollen grains are viable for only 5 minutes after emerging from the anther, whereas the stigma can be fertilized for 3–7 days and so pollen grains are more sensitive to high temperatures than the stigma (Jagadish et al., 2007). The date of anthesis of individual spikelets varies with the positions of the spikelets within the same panicle. Spikelets on the upper branches anthese earlier than those on the lower branches; within a branch, a spikelet at the tip flowers first (Cao et al., 2015). Time of day for anthesis varies among species and weather conditions. Under optimum temperatures in the tropics, most rice varieties (*O. sativa*) begin anthesis at about 0730 and end at about 1230 hours (Prasad et al., 2006). Jagadish et al. (2007) reported that peak anthesis occurred between 10.30 h and 11.30 h at 29.2℃ for both *indica* and *japonica* rices. When temperatures are low, anthesis may start late in the morning and continue into the late afternoon (Gunawardena et al., 2003; Cruz et al., 2006). Upon double fertilization of the egg and endosperm (polar nuclei) two different structure are formed within the angiosperm seed, the embryo and endosperm, indicate grain seed filling begin to start.

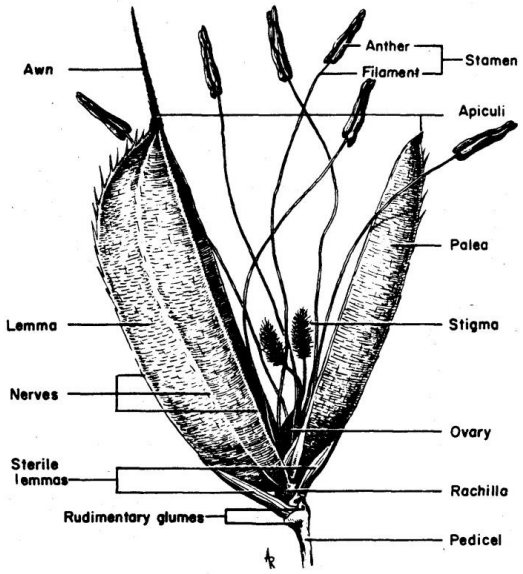


Figure 1.2: Parts of rice spikelet (From Chang and Bernedas, 1965)

1.2.3.2 Grain filling phase (Development of seeds)

Seeds are the single most essential cultivated source of food, with wheat and rice providing the vast majority of food. Besides serving as food, seeds also give rise to the next generation of crops. At grain filling stage, florets on the main stem become immature grains of rice. Formation of grain results mainly from accumulation of carbohydrates in the pistils of the florets. Grain production phase of rice can be subdivided into three distinct sequential stages which are i) histodifferentiation, ii) cell expansion, and iii) seed maturation.

Initially, in phase i) there is the formation of different tissue types within the embryo, endosperm and surrounding seed as a result of extensive cell division which is called histodifferentiation (Borisjuk et al., 1995; Ishimaru et al., 2003). During this stage, the zygote undergoes extensive cell division and differentiation and is accompanied by development of the endosperm, which provide nutrient reserves for the embryo to grow and persist in the mature seed or be reabsorbed during the maturation (Finkelstein, 2004).

In the second phase, major reserves within embryo and storage tissue are laid down which is called cell expansion or seed filling stage (Venkateswarlu, 1976; Shi et al., 2017; Wang et al., 2018). As reserve deposition occurs, water is displaced. The primary source of the carbohydrate is from photosynthesis occurring in the uppermost three to four leaves and the stem (Yoshida, 1981), whilst stem reserves are converted into soluble sugars and

transported into the grains and increases the grain filling rate (Yang et al., 2001b). Hence, the dry weight of the seed is increase and its proportional water content declines. This was supported and well documented by Sinniah and Ellis (1998b) in *Brassica* , Siddique and Wright (2003) in peas and Ellis (2011) in rice. The carbohydrate that accumulates in grain is stored in the form of starch, wherea this starchy portion of the grain is called endosperm (Nakamura et al., 1989). Initially, the starch is white and milky in consistency. When this milky accumulation is first noticeable inside florets on the main stem, the stage defined as milk stage. In this stage, Abayawickrama et al. (2017) suggest it is an early part of seed filling which about 14-15 days after heading (DAH). Prior to pollination, the panicle in most varieties is green, relatively compact and erect. During milk stage, the accumulation of carbohydrate increases spikilet weight. Nagata et al. (2001) reported that in *japonica* rice cv. Tanakari the percentage of ripened spikelets showed the greatest correlation with the total amount of carbohydrate supply per spikelet during 10 to 20 DAH. Since the florets that accumulate carbohydrate first are located near the tip of the panicle, the panicle begins to lean and eventually will turn down. The milky consistency of the starch in the endosperm changes as seed lose their moisture. When the texture of the carbohydrate on the main stem is firm, this stage is called dough stage which occurred at 20-23 DAH which is about at mid early seed filling (Abayawickrama et al., 2017)**.** During the third phase, dry matter accumulation was slow and stops at physiological maturity, where seed begins the maturation phase.

Zhu et al. (1997) and Cao et al. (1992) reported that most *indica* cultivars usually exhibit a faster grain filling rate than *japonica* cultivars. Meanwhile, the hybrid of *japonica/indica* rice usually shows a slower grain filling rate than other hybrid rice varieties (Yuan, 1994, 1998; Peng et al., 1999, 2003).

1.2.4 Ripening (maturation) phase

Agronomically, the duration of filling and ripening is from the date of heading to the time when the maximum grain weight is attained (Krishnan et al., 2011). Grain ripening can be subdivided into milky, dough, yellow, ripe, and maturity stages. These terms are primarily based on the texture and colour and widely used by farmers in observing growing grains.

The length of ripening varies among varieties and depends upon by environmental condition with shorter durations in the tropics than cool temperate regions. Generally, the ripening phase starts when seed filling is completed and moisture content on mother plant

begins to decrease and completed when the grain is mature and ready to be harvested (Egli and TeKrony, 1997).

Physiological maturity (PM), mass maturity (MM) and harvest maturity (HM); those terminology are important which are reflect to seed quality (Siddique and Wight, 2003). Physiological maturity was defined by Shaw and Loomis (1950) as the stage in seed development when the seed reaches its maximum dry seed weight and yield. This same stage was also termed relative maturity by Aldrich (1943), morphological maturity by Anderson (1955), and recently termed as mass maturity by Ellis and Pieta-Filho (1992). Further desiccation on the mother plant need to be continue since the moisture content of the seed is relatively high for mechanical harvesting to apply at PM. Harvest maturity is defined as the first time the seed moisture declines to a harvestable level in those crops harvested as dry seeds and/or fruits (Egli and TeKrony, 1997). Maturity stage affects seed germination and vigour of common vetch (*Vicia sativa* L.). Delaying harvest of common vetch seed may improve seed germination and vigour under wide range of field conditions (Samarah and Mullen, 2004). Early harvest may lower seed germination and late harvest may increase seed losses due to shattering. The time between collection and extraction of seed is very important to maintain high germination and vigour especially seed moisture content at harvest (Siddique and Wright, 2003). In rice seed if they have already dried to moisture content <16%, they are close to the end of maturation drying (Chatelain et al., 2012).

Figure 1.3 shows a general outline of seed development and maturation through the three phases (I-III) histodifferentiation, seed filling (reserve deposition) and seed desiccation (maturation drying). In phase I (histodifferentiation), seed gain in fresh weight (fw) due to cell division and expansion; in phase II , seed gain in dry weight (dw) because of enlargement of storage cells and the deposition of insoluble stored reserves and the final phase is a phase seed loss of their fw as the seeds undergoes maturation drying (Bewley and Black, 1994).

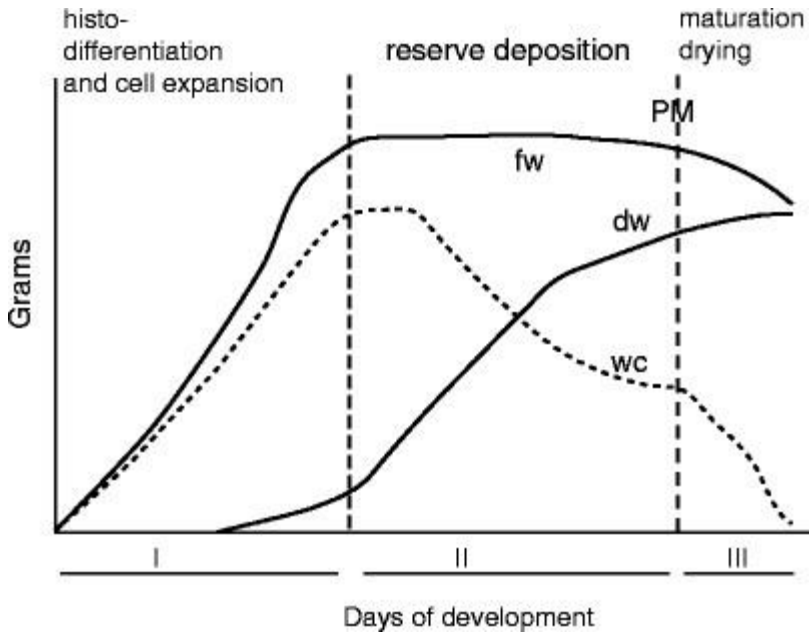


Figure 1.3: Pattern of seed development to show the changes in whole-seed fresh weight (fw), dry weight (dw), and water content (wc) (From Bewley and Black, 1994)

1.3 Seed quality

Seed quality involves the genetic, the physiological quality and the health of seeds. Good seed means that physiological quality, genetic suitability (McDonald, 1998; Nerling et al., 2013) and sufficient healthy seeds. The quality of seed is important to improve as it is relevant not only to yield production but also to secure food sustainability. High seed quality not only ensures a stable source of food, but also provides the propagules for the next, successful growing season

A few studies have shown that seed quality development can continue *in planta* until harvest maturity. Maximum seed viability and seed vigour may be achieved if seeds are harvested at the correct stage of maturity (Ellis et al., 1993; Siddique and Wright, 2003; Ellis, 2011). If harvesting is delayed seed quality may decline due to adverse environmental conditions such as high temperature**,** high humidity, rainfall, over drying, attacks by diseases, pests or damage by birds and animals (Copeland and McDonald, 1999). Therefore, seed should be harvested soon after achieving their maximum seed quality.

Seed physical and physiological qualities develop during seed development. Seed quality traits in terms of seed's ability to germinate and survive air-dry storage are acquired during the progress of seed development and maturation (Hay and Smith, 2003; Probert et al., 2007). Harrington (1972) suggested that developing seeds attain maximum viability and vigour at physiological maturity (end of seed filling) and that they then begin to age,

with viability and vigour declining thereafter. A recent study by Eskandari et al. (2015) concurs with Harrington's hypothesis for sesame seeds. However, several studies contradict the Harrington (1972) hypothesis. Ellis and Pieta-Filho (1992) suggested that the maximum seed dry weight is obtained at the end of seed filling and that this should be defined as mass maturity because seed quality continued to improve beyond this point. In rice, potential longevity in air-dried storage continue to improve for about 2-4 weeks after mass maturity and only then began to deteriorate (Rao and Jackson 1996; Ellis, 2011). With other crops such as pea (*Pisum sativum* L.), harvesting seed crop's at different moisture contents provided evidence that seed vigour improved after physiological maturity and this continued until some time later but then declined as harvesting was delayed (Siddique and Wright, 2003); similarly in pinto bean *Phaseolus vulgaris* cultivars (Ghassemi-Golezani and Mazloomi-Oskooti, 2008).

1.3.1 Seed deterioration

Deterioration of seeds during storage is a great concern and is defined as deleterious change with time (ageing) but it may also be considered as a change that occurs with reduced water content (desiccation damage) or high and low temperatures (denaturation or freezing damage respectively) (Kermode and Bewley, 1985). Studies have shown that as seeds undergo ageing or deterioration, seed germination and seed vigour are progressively lost during storage (Ellis and Roberts, 1981; Kermode and Bewley, 1985). Seeds lose vigour as they age, leading to poor seedling emergence, stand establishment, and low crop yield (McDonald, 1998). Deteriorated seeds have few viable cells that are incapable of organizing growth, or have little or no viability, due to critical cellular constituents being seriously degraded (Jyoti and Malik, 2013). The seed quality and viability during storage depend upon the initial quality of seed and also the manner in which it is stored. Several storage environmental factors contribute to seed deterioration and these conditions may make it difficult to maintain seed viability during storage. Two common practices applied to limit seed deterioration are to reduce seed storage moisture content and/to temperature (Roberts, 1973; Walter et al., 2004).

1.3.2 Desiccation tolerance

Rice produces orthodox seeds which develop desiccation tolerance and can easily be stored for many years in a dry, cool storage environment (Krishnan et al., 2011). During

development, the seeds of many species progressively gain the ability to withstand significant moisture loss (desiccation tolerance) (Bisht and Singh, 2013). At maturity, seed can be characterized by acquisition of its functional traits such as desiccation tolerance, remaining viable after as much as 90–95% of their water has been removed (Dasgupta et al., 1982). In this dehydrated state, the seed can survive for long periods and, unless dormant, will resume full metabolic activity, growth, and development when conditions are suitable for germination (Finch-Savage, 2003). Seed in desiccation tolerance will reduce their metabolic activity to a drastically low level and can be stored at low temperature for long periods in order to prolong viability while retaining their ability to germinate for considerable periods (Buitink and Leprince, 2008).

Immature seeds, with relatively high moisture content (e.g. 60%), are very sensitive to damage from desiccation (Dasgupta et al., 1982; Ellis et al., 1987, 1993; Fischer et al., 1988; Hong and Ellis, 1990, 1992). Similarly, at later stages of seed development such as at the end of the seed-filling phase, seed may still show damage upon desiccation to very low moisture contents (Hong and Ellis, 1992; Ellis et al., 1993; Ellis and Hong, 1994). Immature seeds of several legumes and other dicotyledonous plants have been reported not to germinate when removed from their parent plant in the fully hydrated state, but will only germinate after drying (Dasgupta et al., 1982; Bewley, 1985; Kermode et al., 1986). For example, freshly-harvested seeds of bean *(Phaseolus vulgaris* L.) have been reported to be capable of germinating only after they have first acquired tolerance to rapid desiccation and only after achievement of maximum seed dry weight once net water loss *in situ* has begun (Dasgupta et al., 1982; Kermode et al., 1986).

Rice is an example where an effect of seed production environment on the development of desiccation tolerance to low moisture content has been shown. The achievement of maximum desiccation tolerance to low levels of moisture content (4%) in rice did not occur until some 2-3 weeks after mass maturity, when maturation drying on the mother plant had naturally reduced seed moisture contents to levels below 32% (Ellis and Hong, 1994). Similarly, in Norway maple (*Acer platanoides* L), the developing seeds did not attain desiccation tolerance to very low moisture contents (3%) until 3 to 4 weeks after mass maturity when maturation drying had reduced seed moisture content on the mother plant to about 27-30% (Hong and Ellis 1990, 1992).

1.3.3 Seed longevity

Seed longevity is defined as the capacity to remain viable for long periods during storage in the quiescent dry state. It is one of the characteristics which determines seed quality. Determining seed survival period *ex planta* air-dry, in hermetic storage has shown that seed quality continues to improve during seed development and maturation for some considerable time after seed filling end's for example, in rice (Ellis et al., 1993, 2011), and in barley and wheat (Filho and Ellis, 1991; Ellis and Pieta-Filho, 1992).

Seed loss in viability results in failure to germinate, through ageing, even when there is no dormancy and all environmental requirements such as water and temperature are adequate for reactivation of biochemical processes (Robert, 1972). Ellis and Hong (2007) reported that seed lose their viability in air-dry storage at a rate dependent on seed storage moisture content and temperature, but in addition oxygen is also deleterious particularly with very dry seeds. Therefore, reducing seed moisture content and storage temperature and providing hermetic storage improved the longevity of orthodox seed, and is preferable for long-term seed storage (Pritchard and Dickie, 2003; Ellis and Hong, 2007; Whitehouse et al., 2015). There was a report that safe levels of seed moisture content for storage at -20℃ are about 12-14% in cereals to avoid freezing damage (Zewdie and Ellis, 1991a).

The accumulation of specific soluble carbohydrate has been implicated in the acquisition of desiccation tolerance and improved longevity in orthodox seeds, thus conclude that carbohydrate composition might be used as a diagnostic marker for seed storage category (Steadman et al., 1996). This was associated with Bernal-Lugo and Leopold (1992) and Wang et al. (2018) who reported that soluble sugars and antioxidants have been implicated in protecting seeds from the effects of ageing. Sinniah et al. (1998b) showed that maturing seed accumulated both late-embryogenesis abundant protein and oligosaccharides during maturation drying and these were correlated with subsequent longevity.

The critical levels of moisture content of seeds at which more rapid loss in viability occurs during hermetic storage varies considerably with species, degree of maturity and method of seed handling. There is relationship between seed moisture content and equilibrium relative humidity (eRH) as shown in Figure 1.4 for rice by Whitehouse et al.

(2015). In general, seeds which are extracted at maturity tolerate desiccation to moisture contents in equilibrium with about 10-20% relative humidity (RH). Ellis et al. (1992) reported that in rice, the seed storage moisture content that provides the maximum longevity is in equilibrium with about 10-11 % RH. At 85-90% eRH, rice seed moisture content was about 15% and, seeds are shortest lived because damage accumulates rapidly (Whitehouse et al., 2015).

As well as seed storage environment, the potential storage life of seeds is also affected by the pre and post-harvest environment and seed production practices (Hay et al., 2006; Probert et al., 2007). For example, the longer rice plants are exposed to extreme temperature, particularly during flowering, lower seed viability (Tuay and Saitoh, 2017). This was also shown by Jatoi et al. (2001) in pea, however the effect of temperature on seed longevity was dependent upon the temperature range. Ellis et al. (1993) reported that the longevity of *japonica* rice produced in a warm regime was more damage than *indica/ javanica* rices. Dry conditions at harvest may increase physical injury and reduce quality if seeds are handled at low moisture levels (TeKrony et al.,1987). In addition, Yadav and Ellis (2016) showed that the greatest damage to longevity in wheat (*Triticum aestivum* L.) occurred from rainfall imposed during late in maturation. In contrast, seed longevity of the non-sprouted rice seed fraction in air-dry hermetic storage was not affected greatly by submergence (Tejakhod and Ellis, 2017).

Seed longevity is compromised when seeds are harvested either prematurely or if harvest is delayed. From the time of fertilization and physiological maturity, environmental stresses can have occurred and influence longevity of mature seed. Rao and Jackson (1997) reported that mass maturity in rice was achieved in rice with moisture content between 20-40% with potential longevity was greatest about 2 weeks after mass maturity. Also, the germination ability of seeds in the early stages of development varied significantly, but as mass maturity approached, germination increased to the maximum. Ellis et al. (1993) reported that the deleterious effect of high temperature on seed quality development was not detected until after mass maturity in rice. Moreover, K_i (the initial probit viability) was always greater in the cooler regime than in the warmer regime in later harvest.

It is a major challenge for the conservation of plant biodiversity to improve seed longevity for crop success (Smith et al., 2003). Plant genetic resources are in particular danger and need to be conserved for the future by establishing modern gene banks around the world (Maxted et al., 1997). For many plant species, the most economical methods of

conserving germplasm are to store their seeds in a seed gene bank. In some cases, under this conservation, the longevity of desiccation tolerance of orthodox seeds might extend to hundreds of years (Linington and Pritchard, 2001).

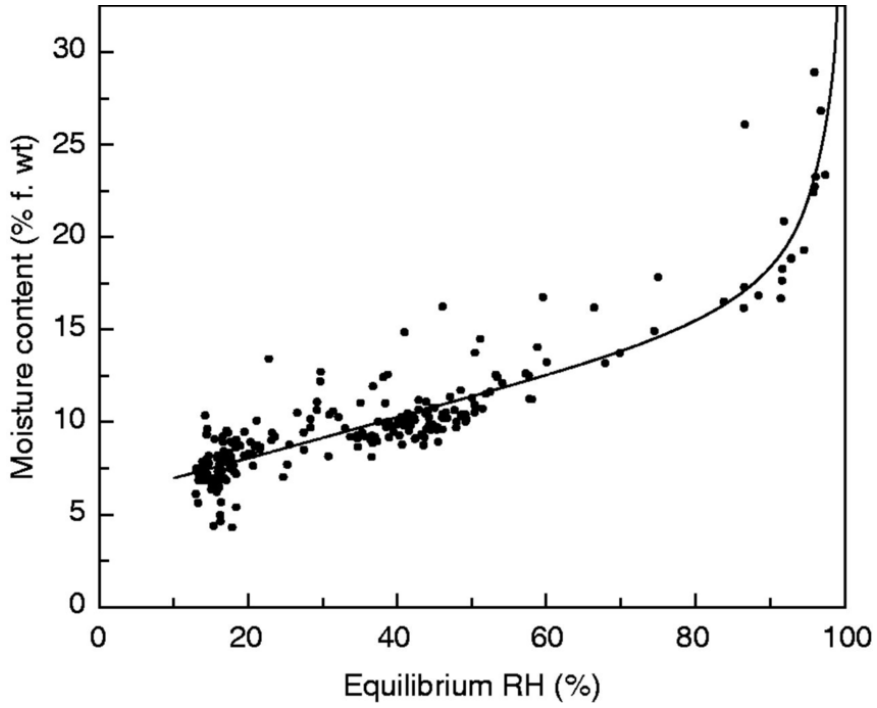


Figure 1.4: Relationship between rice seed moisture content and equilibrium relative humidity (eRH) during seed drying (From Whitehouse et al., 2015).

1.3.4 Seed viability equation

The longevity of orthodox seeds in storage can be predicted using viability equations after the determination of viability constants by controlled ageing experiments (Pritchard and Dickie, 2003). Seed longevity can be quantified by the equation introduced by Ellis and Roberts (1980). Not only in rice seed, this equation been used widely by many studies to predict potential of seed longevity for many other crop species; in peas (Siddique and Wright, 2003) and in wheat (Yadav and Ellis, 2016).

Transforming percent viability to probit viability resulted a straight-line relationship between viability and storage period. The slope of this line is the value of 1/σ and the intercept is the initial viability of the seeds, *K*i. Estimates of percentage germination after different storage periods provided observations for seed survival curves which were fitted by probit analysis according to the modified equation proposed by Ellis and Roberts (1980),

$$v = K_i - p/\sigma \quad (1)$$

where, *v* is probit percentage viability after *p* days in storage in a constant environment. K_i is a constant specific to seed lot. The reduction in seed viability overtime during storage depends on the slope (1/σ) of seed survival curve (percentage are transformed to probits) and relates with storage condition, particularly and σ is the standard deviation of the frequency distribution of seed deaths in time (days). The parameters K_i and σ can be estimated for any constant storage environment by probit analysis. As K_i is assumed to depend only on genotype and seed quality, Ellis and Roberts (1980) described the effect of storage moisture content and temperature on longevity, the parameter σ, in a second equation as,

$$\log_{10}\sigma = K_E - C_w \log_{10} m - C_H t - C_Q t^2 \quad (2)$$

where, *m* and t are the seed storage moisture content (% fresh weight) and temperature (°C), respectively meanwhile K_E, C_w, C_H, C_Q are parameters constant to a species. The temperature range limits to use of this seed viability equation (2) is between -13°C to 90°C and would not differ between different seed lots of a particular species. Therefore, it would possible to predict the longevity of any seed lot of that species by estimating these parameters and K_i under any storage environment. For example, the temperature term of the seed viability equation has been shown to apply between those subzero temperatures used in conventional seed genebanks and the very high temperatures used in certain heated air seed driers. However, the application of the seed viability equation at temperatures cooler than about –20°C or so is not advised (Dickie et al. 1990), bcz thy showed the eqtn appld at least over th rnge -13 to plus 90 c. However, there are limits to the negative logarithmic relation between seed moisture content and seed longevity (Roberts and Ellis, 1989). Lower moisture content limit to the seed viability equation also varies substantially among species. For example, the estimates of the low-moisture content limit of seed

longevity in hermetic storage at 65℃ was between 1.1 to 1.9% for sunflower (*Helianthus annuus* L.) (Ellis et al., 1988) and 4.3 to 4.5% for rice (Ellis et al., 1992). Notes that the main difference in these two species (seed oil vs starch contents) is different in moisture content but similar in equilibrium relative humidity.

1.4 Environmental stresses

Future climate scenarios are worrying. Climate projections indicate that global mean temperatures will rise by 0.4 to 1.6 °C by 2046-2065 and 2.6-4.8 °C by 2081-2100, depending on the emission scenario considered, with the potential to reduce crop production without adaptation (IPCC, 2014). This climatic anomaly is manifested in temperature and rainfall variability and will have negative impact on rice paddy production (Zainal et al., 2014). With the human population unlikely to stabilize before 2070, at around 9 billion (Byrd, 2014) the prospect is that climate change will shape future agricultural practices and threaten natural habitats: and seeds and seed production are at the centre stage of food security and conservation of plant genetic resources.

1.4.1 Drought

Rice production, particularly in Asia, is increasingly constrained by water limitation (Arora, 2006). Agriculture consumes 70% of the fresh water resources, but less water is becoming available for irrigation owing to the global climate change and competition from urbanization and industrial development (Pennisi, 2008). Large areas of lowland and upland rainfed rice occupy 31% and 11% of the global rice-growing area, respectively (Murty and Kondo, 2001; Kamoshita et al., 2008). In particularly, increasing physical water scarcity is a major constraint for irrigated rice production (Bernier et al., 2008; Peng et al, 2009; Mishra et al., 2014). It is estimated that more than 50% of the world's rice production area is affected by drought (Bouman et al., 2005). Evenson et al. (1996) reported that the average annual global reduction of rice production due to drought was about 18 million metric ton.

Rice is more susceptible to drought than other cereals because it is unable to regulate its transpirational water loss as effectively as other cereals (Austin, 1989). Generally, the rice plant uses less than 5% of the water absorbed through roots from the soil (Jose et al., 2004). The rest is lost through transpiration which helps to maintain leaf energy balance of the crop. Decreased leaf water potential leads to stomatal closure and ultimately results in low transpiration which in turn increases leaf temperature (Fukai et al.,

1999). The plant's strategy and adaptation to avoid drought according to Levitt (1980) and Bodner et al. (2015) shown as in Figure 1.5. Water deficit during the vegetative stage of growth may have relatively little effect on grain yield, perhaps owing to compensatory growth or changed partitioning of dry matter after the stress is relieved (Fukai and Lilley, 1994). Plants have a variety of successful strategic adaptions to harsh environments however, adjustment of water uptake to soil-water availability through modifications to seed filling is crucial (Gooding et al., 2002).

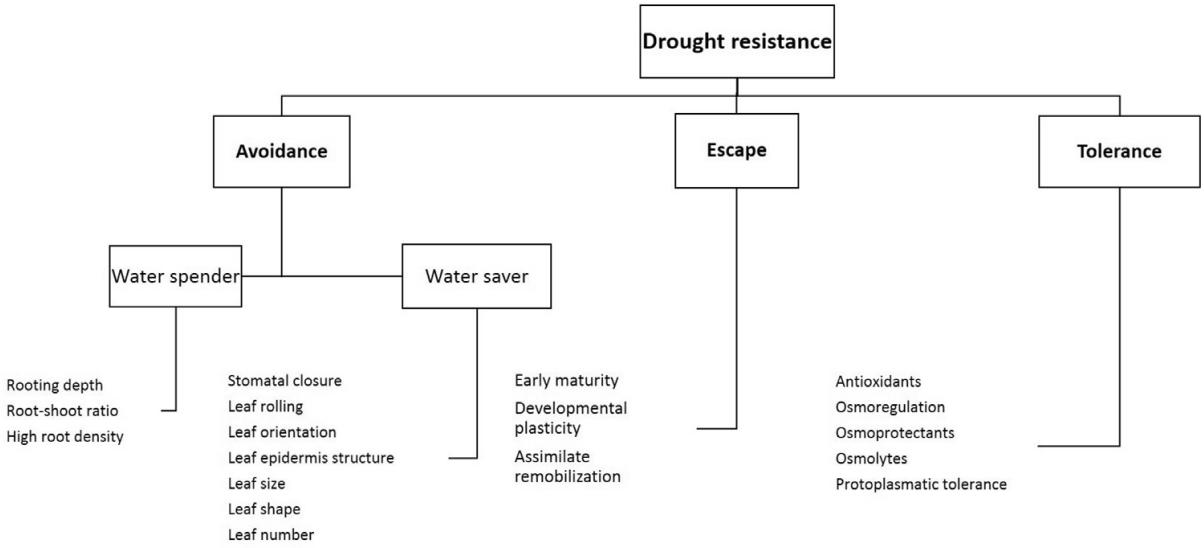


Figure 1.5: Drought resistance strategies and adaptive traits confer it. Avoidance of drought through efficient water uptake is most compatible with high crop yields. Based on Levitt (1980) and Bodner et al. (2015). From Korres et al. (2017).

1.4.1.1 Effect of drought on seed quality

Water deficit during the vegetative stage reduces tiller number, panicle length and grain percentage which prolongs days to maturity (Sikuku et al., 2010); sensitivity to drought is high during mid tillering (Sabetfar et al., 2013). Reduce 1000 seed weight from water limitation imposed on rice cultivars during vegetative growth (Mostajeran and Rahemi-Eichi, 2009), has often been attributed to a limitation in carbohydrate supply reducing grain filling rate and so grain weight (Yang and Zhang, 2006).

In cereals, extensive studies have demonstrated that post anthesis water deficit result in early senescence and increase mobilization of pre-stored assimilates to grains (Kobata et al., 1992; Yang et al., 2001, 2003). Drought during the grain-filling process induces early senescence and shortens the grain-filling period but increases remobilization of assimilates from the straw to the grains (Yang et al., 2001; Plaut et al., 2004). Senescence is a genetically programmed process that involves remobilization of nutrients from

vegetative tissues to grains (Buchanan-Wollaston, 1997 and Ori et al., 1999). Rice grain filling rates were more important than duration of filling for seed weight which is an essential determinant of grain yield in cereal crops (Yang et al., 2008). In wheat, water deficit enhances senescence by accelerating loss of leaf chlorophyll and soluble proteins and was more severe when water limitation was imposed from 14 DAA to maturity than earlier (Moradi, 2011). Anthesis and fertilization are particularly sensitive to drought in rice. Water stress during flowering may reduce the harvest index by as much as 60%, largely as a result of a reduction in grain set (Garrity and O'Toole, 1994). The reduction in grain weight in response to drought during the early periods of grain filling can mainly be attributed to the lower number of endosperm cells (Nicolas et al.,1985) while during the later stages results in the impairment of starch synthesis either because of the limited supply of assimilates for the grain (Blum, 1998) or the direct effects on the synthetic processes in the grain (Yang et al., 2004). In millet, both biomass production and grain yield were severely reduced by drought prior to and at the beginning of flowering but had no affect at the end of flowering (Winkle et al., 1997).

Vieira et al. (1991) reported that seed germination in soya bean was not affected when drought stress was imposed during seed filling in a greenhouse experiment, but yield was reduced. Nichols et al. (1978), working with potted plants of peas observed no effect of drought stress on seed conductivity or germination. Samarah and Alqudah (2011), in barley, that late-drought stress had no effect on standard germination but reduced the germination after the accelerated ageing test. However, for example, in soya bean and pea, water stress during the seed filling period induced a reduction in seed quality assessed by germination and conductivity results, however, this reduction was not seen with earlier water stress (Smiciklas et al., 1989; Fougereux et al., 1997).

It is well-documented that seed sensitivity to drought is particularly acute during the reproductive phase, particularly during meiosis and seed filling (Pirdashti et al., 2004; Fukai and Lilley, 1994; Zeigler, 1995). In rice for example, soil water deficit during the grain filling period induces earlier senescence, shortening the grain-filling period and causing yield losses (Yeo at al., 1996; Bouman and Tong 2001; Sikuku et al., 2010) by reducing the supply of assimilate (Baruah et al., 2006) due to large reduction in total root length (Kato and Okami ,2011). However, McKersie and Ya'acov (1994) reported that the most sensitive phases to drought in rice reproductive development are during booting and anthesis meanwhile O'Toole and Namuco (1983) suggested during panicle exsertion and anther dehiscence. Drought stress later during the reproductive development decreases seed

size rather than seed number and it has been suggested that faster grain filling and enhanced mobilization of stored carbohydrates can minimize the effects of drought rice yield (Zhang et al., 1998). It was reported that water stress has no significant effect on pinto bean (*Phaseolus vulgaris*) seed quality (Ghassemi-Golezani et al., 2010). In contrast, Zehtab-Salmasi et al. (2006) in dill (*Anethum graveolens* L.) and Ghassemi-Golezani et al. (2012) in soyabean (*Glycine max*) showed that water deficit during grain filling led to significant reduction in seed quality, assessed by electrical conductivity and standard germination.

Water stress during early stage of grain filling, results in a reduction of grain weight in rice (Yang et al., 2001), wheat (Zhang et al., 1998), maize (Ober and Setter, 1990). During seed maturation the cell ceases to expand, water content decrease, storage products are synthezised, and free ABA accumulates (Hilhorst and Toorop, 1997). Sinniah et al. (1998) reported that ending irrigation early during seed development in *Brassica* resulted in seed moisture content declining rapidly from 48-50% to about 6%. As plants dry, ABA accumulation in grain filling leads to enhanced water uptake and postpones water shortage in shoots (Brenner and Cheikh, 1995; Yang et al., 2001a).

1.4.1.2 Effect of drought on seed longevity

Extensive reports suggest that drought stress during seed development reduces seed quality (Barnabas et al., 2008; Stagnari et al., 2016), however, there have been limited studies on the effect on seed longevity. The potential storage life of seeds is affected by pre-and post-harvest environments and practices (Probert et al., 2007; Whitehouse et al., 2015). Therefore, water limitation during seed development may also influence seed longevity. Reducing irrigation to rapid-cycling *Brassica* improved maximum seed longevity when irrigation ended at 16 or 24 day after pollination (DAP) (Sinniah et al., 1998). This might happen as seeds possess a wide range of systems (protection, detoxification, and repair) allowing them to survive in the dry state and to preserve high germination ability (Barnabas et al., 2008). In contrast, Samarah and Alqudah (2011) reported that late drought imposed to barley plants reduced seed longevity after a seed ageing test.

1.4.2 High temperature

Rice is very sensitive to high temperature close to the time of anthesis, and most sensitive at about 9 days before anthesis (Yoshida, 1981). The increasing frequency and intensity of short-duration high temperature events (>33℃) pose a serious threat to agricultural production, especially in cereals such as wheat (Modarresi et al., 2010) and rice (Wassmann et al., 2009). The critical temperature (℃) (low, high and optimum) during the development of rice plants shown in Table 1.1.

1.4.2.1 Effect of brief high temperature on seed quality

High temperature stress during the grain filling stage reduces grain quality of rice and this is a serious problem in Asia, e.g. Japan (Tanamachi et al., 2016). An effect of high temperature during seed development on plant under stress and control is shown as in Figure 1.6 (Sreenivasulu et al., 2015). Morita (2008) reported that when rice plants were exposed to high temperature during ripening period, the sink-source balance of the carbohydrate was disrupted. High temperature stress effects during pre-anthesis, particularly during meiosis and growth of the ovaries which may impose an upper limit for potential grain weight (Calderini et al., 1999; Krishnan, 2011). Effect of a short period (8 days) of high temperature (35℃) exposed during grain filling stage (8-15 d from heading) showed that rice plants stimulated the opening stomata yet preserved the diurnal pattern of stomatal variation (Yang and Heilman, 1991) resulting from oxidative damage in photosynthesis and respiration pathway (Fitter and Hay, 1987; Bernabas et al., 2008). However, these incidents often coincide with low water supply. Elevated temperatures reduce the duration between anthesis and physiological maturity which is associated with a reduction in grain weight (Madan et al., 2012; Tanamachi et al., 2016). Reduced grain weight (~1.5 mg per day) can occur for every 1℃ above 15–20℃ (Streck, 2005).

High temperature treatment during grain filling process may cause seeds attributed to a restricted carbohydrate supply and poor performance of vital enzymes (Yang et al., 2001). Also, HT impose at anthesis may reduce anther dehiscence, pollen viability and depressed fertilization, which all lead to reduce spikelet fertility (Matsui et al., 2001; Prasad et al., 2006; Jagadish et al., 2010a, 2010b), thus, yield losses (Baker et al., 2004). Cessation of development of the nucellar epidermis occurred when HT exposed to rice plant (cv. Hinohikari) at 14 day after flowering (DAF), whereas it did not affect grain development (Tanaka et al., 2009) or 1000 grain weight (Tanamachi et al., 2016). In terms of a night

temperature study, Coast et al. (2014) mentioned that high day temperature was less damaging in terms of grain weight compared to night temperature. However, sterility induced by heat stress is more likely to be occurred in warm-humid than hot-arid environments due to humidity effects on transpiration cooling (Cécile-Julia and Dingkuhn, 2013).

High temperature (36/29℃) imposed beginning 10 DAA to maturity had no effect in wheat seed germination of cv. Oum-rabia, whereas it decreased seed germination of cv. Marzak (Grass and Burris, 1995). In contrast with recent studies by Nasehzadeh and Ellis (2017), reported that development of ability to germinate in wheat occurs sooner with higher temperature during seed filling and maturation. The high temperature treatments (34/26℃) showed no effect on the subsequent development of desiccation tolerance whereas, imposed this stress during seed development and seed maturation may affect seed quality. Also *japonica* rice is more sensitive to high temperature than *indica* rices (Ellis et al., 1993; Ellis, 2011).

The severity of seed quality damage by brief period of HT depend also on the duration imposed. In rice for example spikelet fertility was reduced from 90% to 20% by only 2h exposure to 38℃ and 10% by <1h exposure to 41℃ and therefore seed quality in rice may reduce (Jagadish et al., 2007). Similar with wheat crop, plants exposed to 30℃ for 3 consecutive days, during seed grain filling stage showed substantially reduced grain set compared to 30℃ for 1 day (Saini and Aspinall,1982). Seed viability was significantly reduced in rice by 7-d exposure to 38 and 34℃ at 1 to 7 d and 1 to 14 d after anthesis, respectively (Martinez-Eixarch and Ellis, 2015).

Table 1.1: Critical temperatures (°C) for the development of rice plants at different growth stages (From Yoshida, 1978).

Growth stage	Critical temperature (°C)		
	Low	High	Optimum
Germination	16-19	45	18-40
Seedling emergence	12	35	25-30
Rooting	16	35	25-28
Leaf elongation	7-12	45	31
Tillering	9-16	33	25-31
Initiation of panicle primordial	15	-	-
Panicle differentiation	15-20	30	-
Anthesis	22	35-36	30-33
Ripening	12-18	>30	20-29

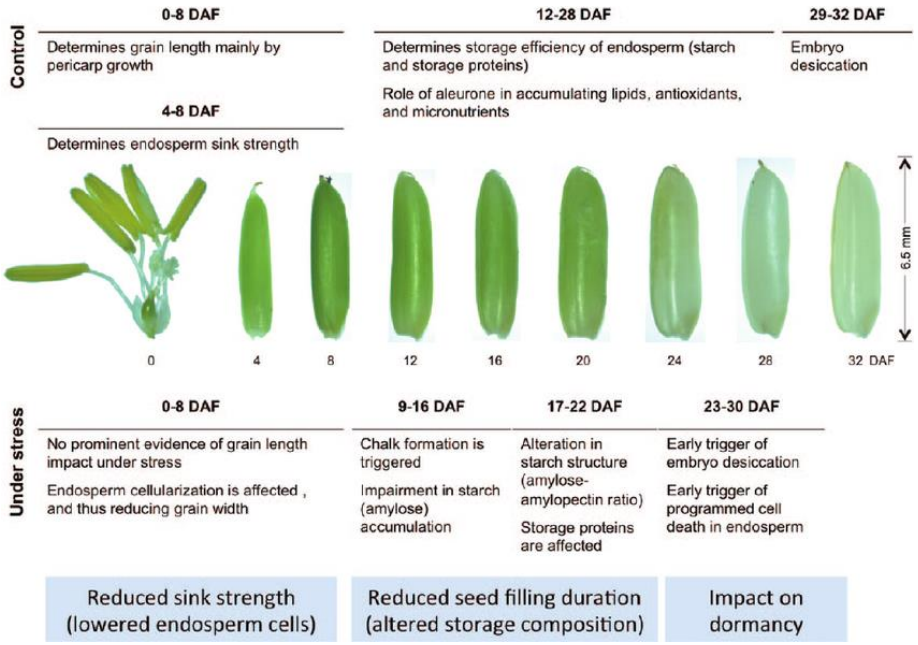


Figure 1.6: The ontology of seed development covering import phase transitions. High temperature stress-induced perturbations occurring during seed development affect the grain quality, cooking quality, and eating quality of rice (From Sreenivasulu et al., 2015)

1.4.2.2 Effect of high temperature on seed longevity

The impact of extreme temperature on rice seed quality, particularly on seed longevity has received less attention than that on yield. Longevity in storage in the same environment is affected by seed production environment and the timing of harvest (Ellis et al., 1993). However, rice varieties are known to vary in longevity, with cultivars from temperate regions normally having shorter life-spans than tropical cultivars (IRRI, 1988). Ellis et al. (1993) and Ellis and Hong (1994) showed that the potential longevity of the *japonica* cultivars which evolved in temperate environments was significantly less when produced under a warm seed production regime (32/24°C) than in a cooler regime (28/20°C). Sanhewe and Ellis (1996) reported for other crops e.g. *Phaseolus vulgaris* L. resulted slightly poor in terms of maximum potential longevity in the warm regimes which tallies with Siddique and Goodwin (1980) in bean. Sanhewe et al. (1996) presented a model of the effect of temperature before harvest on the potential longevity of wheat, indicated that there was a significant positive relation between the rate of increase in potential longevity and temperature.

1.4.2.3 Combined effect of drought and high temperature

The combination of drought and high temperature represent an excellent example of two different abiotic stress conditions that occur in the field simultaneously (Moffat, 2002; Shah and Paulsen, 2003). During HT for example, plants open their stomata to cool their leaves by transpiration. However, if HT is combined with water limitation the plants are unable to open their stomata, so the leaf temperature is higher (Rizhsky et al., 2002). At present, however, information on the combined effect of HT and water limitation on the reproductive development of cereals is rather limited (Barnabas et al., 2008). The combination of HT and drought reduced the duration of grain filling more than each one in wheat (Altenbach et al., 2003; Shah and Paulsen, 2003). Also, it was greater affect than additive effects of HT or drought alone for leaf chlorophyll content, grain numbers and harvest index in spring wheat (Prasad et al., 2011). In sorghum (Machado and Paulsen, 2001) and in lentil crops (*Lens culinaris* M.) (Sehgal et al., 2017), both authors reported that HT appeared to interact with drought by altering soil water content and later inducing damaged cell membranes and photosynthetic traits.

1.5 Research purpose

Drought and high temperature stress are considered to be the two major environmental factors that influence various cellular and whole-plant processes that affect crop yield and quality. Drought coincident with high temperature often occurs in Asia, and this is increasing with global climate change. For example, Malaysia has experienced more frequent drought and severe extended late drought stress, resulting not only reducing seed production but also damaging seed quality. Drought is severe during May to October, particularly in north peninsular Malaysia, with average temperature ranging from 26℃ to 28℃ and annual rainfall during the main season of about 1682 mm (Radin-Firdaus, et al., 2012). For this reason, I used *indica* rice cv. Aeron 1 to see how these stresses may affect Malaysian rice seed quality and *japonica* rice cv. Gleva was chosen because it was known to be vulnerable to high temperature but nothing is known about its response to drought. In addition, the brief high temperature regime 40/30℃ was chosen according to temperature stress events currently occurring in some rice production in Spain and also in Malaysia. Moreover, study on effect of terminal drought and brief high temperature stress during seed development and maturation on seed quality particularly on potential seed longevity was limited. Thus, stress sensitivity during seed development and maturation deserves more attention, especially in the case of short exposures during fertilization and early grain filling. The development of seed needs to be considered, later to adopt appropriate strategies that may target several developmental stages in order to improve the subsequent seed longevity. As a consequence, my studies focused on the impact of ending irrigation early and brief elevated stress during seed development or maturation on rice seed development quality particularly on subsequent seed longevity.

1.6 Objectives

In sum, the objectives of this study are to:

1.6.1 To determine the effect of ending irrigation early and/or brief elevated temperature during seed development and maturation in seed quality of *japonica* cv. Gleva.

1.6.2 To identify the most sensitive stages of seed quality development to ending irrigation early and/or brief elevated temperature during seed development and maturation in seed quality of *japonica* cv. Gleva.

1.6.3 To investigate the effect of the combination of ending irrigation early and/or brief elevated temperature during seed development and maturation in seed quality of *japonica* cv. Gleva and/or *indica* cv. Aeron 1.

1.7 General hypotheses

General hypotheses in this thesis are as follows.

1.7.1 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduces the duration of seed filling phase, final seed dry weight, seed moisture content, and ability of seed to germinate normally,

1.7.2 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduces subsequent seed longevity in air-dry storage,

1.7.3 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduces maximum seed longevity,

1.7.4 Combining ending irrigation early and brief high temperature in cv. Gleva and cv. Aeron 1 during seed development and maturation reduces seed quality compared to each stress alone.

Aspects of hypotheses **1.7.1**, **1.7.2**, and **1.7.3** are tested in Chapters 2 to 5: drought in Chapters 2, 4 and 5; and high temperature in Chapters 3 and 5. Hypothesis **1.7.4** is tested in Chapter 5. Further hypotheses are introduced in each of experimental chapters (2 to 5).

1.8 Thesis outline

In this thesis, there are four main experimental chapters; **Chapter 2**, focuses on the impact of ending irrigation early during early and end of the seed filling phase on subsequent quality of rice cv. Gleva; **Chapter 3,** investigates the effect of brief period of elevated temperature during seed development on seed quality of cv. Gleva; **Chapter 4**, studies the effect of ending irrigation early during the end of the seed filling phase and maturation drying on subsequent rice seed quality (cv. Gleva) ; and **Chapter 5**, Effect of early ending irrigation and/ or elevated temperature combined during seed development on seed quality of *indica* rice cv. Aeron 1 and *japonica* cv. Gleva. The general discussion and conclusion are provided in **Chapter 6**.

CHAPTER 2

Effect of drought on seed development and potential longevity of rice cv. Gleva

2.1 Introduction

One of the main constraints of rice (*Oryza sativa* L.) cultivation and production is water shortage during periods of low rainfall, which affects the vegetative growth rate and grain yield (Tao et al., 2006). Water shortage is often unpredictable and does not occur in all years in a target environment and thus affects the stability of rice grain production and quality, and hence resilience. Drought stress is a prolonged period of inadequate amount of available water in the soil for normal growth and development of the plant leading to permanent wilting and ultimately death (O'Toole, 1982). In rice the effect of drought varies with the variety, degree and duration of stress and when it occurs during development (Kato et al., 2006, Zeigler and Puckridge, 1995)

Seeds are the most basic input for agriculture, and their quality is very crucial to successful crop production. Numerous studies have reported on rice physiology, morphology and also molecular aspects as affected by water deficiency during cultivation. However, there is only limited information of its effect on subsequent seed lifespan (seed longevity) in rice.

Seed longevity is an important trait for both ecology and agronomy values. This study therefore investigated the effect of drought applied to the rice crop on subsequent seed quality. A series of harvests during seed development were made also to identify the timing of changes in seed quality under the different treatments to identify the ideal harvest time in order to obtain seeds of high quality with maximum potential longevity.

2.2 Objectives

This study investigated changes in seed quality, including subsequent seed storage longevity, in rice cv. Gleva during seed development and maturation under well-watered conditions or with terminal drought during the seed-filling stage in order to determine when maximum seed quality was attained and when seed deterioration begins on the mother plant, and the influence of drought on the above.

2.3 Hypotheses

This study investigated changes of seed quality, including subsequent seed storage longevity, in rice cv. Gleva during seed development and maturation under well-watered or limited irrigation conditions. The following hypotheses were tested;

2.3.1 Duration of seed filling and final seed dry weight are reduced, and seed desiccates sooner by ending irrigation early,

2.3.2 Ending irrigation early leads to earlier development of ability to germinate and of ability to tolerate desiccation,

2.3.3 Subsequent seed longevity improves earlier, maximum longevity occurs sooner and reduces by ending irrigation early during seed development and maturation.

Hypotheses 2.3.1-2.3.3 relate to my general hypotheses 1.7.1-1.7.3 in relation to drought, but also consider effects on the temporal pattern of seed development and seed quality development and possible subsequent decline.

2.4 Materials and Methods

This first experiment was sown on 25th November 2014 and carried out in controlled environment growth cabs at the Plant Environment Laboratory (PEL), Shinfield, University of Reading (UoR) (51^0 27′ N latitude and 00^0 56′ W longitude).

2.4.1 Seed selection

Gleva, a high-yielding early-maturing, *japonica* cultivar from Spain was selected for study. Seeds had been stored at 2-4℃ at UoR in a sealed laminated aluminum foil bag since receipt in 2012 from the Institute of Agrifood Research and Technology, Barcelona, Spain. Seeds were tested for viability before sowing. One hundred seeds were selected randomly, by pie sampling, and placed in moist rolled towel papers (Plate 2.1a). There were two replicates of 100 seeds each. They were kept in a loosely-folded clear plastic bag and incubated at 34/11 ^{0}C (16h/8h) (Plate 2.1b) (Ellis et al., 1983). After five days in test, the sample showed 89% germination. This germination test (Plate 2.1c) indicated that this seed sample was suitable for this experiment.

(a) (b) (c)

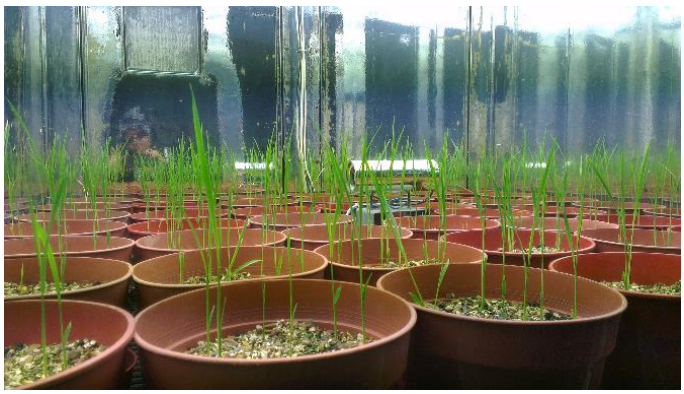
Plate 2.1: (a) Rice cv. Gleva seed on moist paper towels; (b) Moist rolled paper towels in clear plastic bag; (c) Seeds germinated after 5 days at 34/11 ^{0}C.

2.4.2 Planting Medium

Three litre plastic pots were filled with the growing medium. This comprised vermiculite, sand, gravel and peat in the ratio of 4:2:4:1, respectively, mixed with 3kg/m^3 of slow-release fertilizer (Osmocote Pro 3-4M, Everris International BV, The Netherland). The growing medium was wetted with tap water until water drained out and then left to drain for 24 hours.

2.4.3 Plant Husbandry

Seven seeds were sown approximately 15mm deep in each of 48 pots and placed in one growth cab (modified Saxcil cab with internal dimensions 1.4 x 1.4 x 1.5 m). Cool white fluorescent tubes provided 724 µmol m^{-2} s^{-1} (mean of four observations) photosynthetic photon flux density at pot level. The temperature regime was 28/20 ^{0}C day/night (11h/13h thermoperiod synchronized with 11h photoperiod) at 75% relative humidity. At 3 days after sowing (DAS), manual watering began and was continued every day. About 95% seeds had germinated by 7 DAS with first leaves emerged. At 14 DAS, weaker seedlings were thinned to leave 4 seedlings in each pot (Plate 2.2a). Pots were irrigated (water without nutrient adjusted to pH 4.8-5.0) using an automatic drip system after three leaves had emerged at 16 DAS (Plate 2.2b) seven times per day for three minutes. After 42 DAS, irrigation was reduced to three times a day. To minimize the effect of any possible variation in environment, each pot was moved from centre to the sides of cab and vice versa three times a week until 50% of panicles had exserted. At panicle exsertion, treatment labels were shuffled to randomized and then inserted in the pots.



(a)

(b)

Plate 2.2: (a) Rice seedlings at 14 DAS; (b) Start of drip irrigation at 16 DAS

2.4.4 Irrigation Treatments

Three irrigation treatments were applied: Treatment 1 (T1), plants were irrigated throughout the experiment, designated as control; T2 and T3 irrigation was stopped at 7 days after 50% anthesis (DAA) or 14 DAA, respectively. Determination of 50% anthesis was based on the frequency of anthesis observed (Appendix 2.1). Only panicles that anthesed during the period 101-108 DAS were used in the study to limit the anthesis variation. Earlier and later anthesing panicles were cut and removed. Anthesis (0 DAA)

was 105 DAS (i.e. 50% anthesis). All treatments were completely randomized in terms of their location within the single cabinet.

2.4.5 Data Collection

Serial harvests of plants began at 7 DAA and continued at 4-6 days intervals on eight dates, until 40 DAA. Eight plants (2 pots) were harvested from each treatment on each date and the following data recorded on each occasion.

2.4.5.1 Seed moisture content

The two-stage method was necessary to determine moisture content of fresh seeds harvested well before harvest maturity. Panicles were gently threshed by hand to obtain filled seed, with any empty seeds removed. A weighed sample of 100 fresh seeds with 2 replications was pre-dried in an open Petri dish for 1-2 days on top of the constant temperature oven at 130^0 C. Moisture content on the fresh weight basis was determined as:-

$$\text{m.c.}(\%) = \frac{\text{Original wt. of seed - pre-dried wt. of seed}}{\text{Original wt. of seed}} \times 100$$

The pre-dried seeds were ground using a mill grinder, then weighed and place in the oven at 103^0 C for 17 h to determine the moisture content loss in the second stage of drying. The final moisture content of the sample was then calculated by the formula;

$$\text{m.c. } (\%) = (S1 + S2) - \frac{100(S1 \times S2)}{100}$$

where *S1* and *S2* were the moisture loss in the first and second stages, respectively (ISTA, 2010).

2.4.5.2 Seed dry weight

Eight samples of 100 fresh seeds were counted at random by hand sampling method to provide a working sample and weighed to estimate mean seed fresh weight. Seed dry weight was calculated from estimates of seed fresh weight and known seed moisture content (m.c.).

$$\text{Seed dry weight (mg)} = \text{Seed fresh weight (mg)} - \left(\text{Seed fresh weight (mg)} \times \text{Seed moisture content (\%)} \right)$$

2.4.5.3 Germination of fresh seeds

Samples of seeds (100; two replicates of 50) were tested for ability to germinate. Seeds were tested in an incubator at 34/11°C (16h/8h) for 21 days (Ellis et al., 1983) and considered as germinated when radicle protrusion reached more than one-half of the seed's length. Numbers of normal and abnormal seedlings were also counted (ISTA, 2010) and percentage normal germination calculated. Seeds remaining firm after 21 days of test, were pricked in order to promote germination and the test extended to 28 days total test period where necessary.

2.4.5.4 Germination of air-dried seeds

Samples of weighed seeds inside a cotton bag were dried at 15-17^0 C with 12-15% relative humidity. After variable periods, seeds were removed when moisture content was estimated to have reduced to 10-14 %. This was managed by reweighing samples regularly. In early harvest, seeds took 1-2 weeks to dry but in later harvests only 1-2 days. Germination was re-assessed on a sample of dried seeds as outlined above. The remainder of the seed was stored hermetically in laminated aluminum foil bags at 2-4 ˚C initially, then at -20˚C (when all samples were available) until the subsequent determination of longevity.

2.4.5.5 Seed storage longevity

Samples were withdrawn from storage and held sealed overnight at ~20^0 C before opening the packets. The moisture content either was adjusted to 15% moisture content above water at 20˚ C (seed lot in muslin bag placed on a gauze tray above water in a closed container) for 2-40 h or drying over silica gel at 20˚C (depending on initial value), the seed weight being checked regularly. Seed samples were left together in separate muslin bags in a sealed container at 2-4˚C for 3 d to allow moisture to equilibrate within samples and amongst samples. Samples were assumed to be at 14.5-15.5% moisture content based in the original moisture content determination and the change in weight. Moisture content was re-determined at 103^0 C for 17h and the seeds were prepared for longevity determination. About 10-14 samples (depending upon seed available) of 100 seeds were sealed in separate laminated aluminum foil bags for each combination of harvest date and drought treatment. Samples were stored in an incubator maintained at 40 ± 0.5˚ C and withdrawn at regular intervals for up to 40 days for germination testing

The method for the determination of seed storage longevity followed that of Ellis *et al*. (1993). Estimates of percentage germination after different storage periods provided observations for seed survival curves which were fitted by probit analysis according to the following equation (Ellis and Robert, 1980):

$$v = K_i - p/\sigma$$

In this equation, potential longevity, K_i , is the value of seed lot constant (y-axis, where p=0) and v is probit percentage viability after p days in storage in a constant environment.

2.4.6.6 Statistical Analysis

GenStat 17th edition (2015, VSN International Ltd.) was used for probit analysis and to analyze the data in order to evaluate the treatment effects. Analysis of variance (ANOVA) was used and means comparison using Tukey's Multiple range test at 5% error (P= 0.05).

2.5 Results

2.5.1 Seed desiccation, dry weight and ability to germinate normally (fresh and dried-seeds)

The timing of anthesis varied amongst plants, with 50% anthesis was at 105 DAS. Seed moisture content declined with seed development and occurred sooner the earlier irrigation ended (Fig.2.1a). Moisture content was affected significantly by treatment and by period of seed development and by their interaction ($P < 0.001$, Appendix 2.2). When plants were irrigated throughout, seed moisture content declined gradually, reaching 27.4% (s.e. 0.31) at 40 DAA. Where irrigation ended at 7 or 14 DAA, seed moisture content declined sooner and stabilized at 14-15% from about 26 DAA. From 11 DAA onwards, moisture content varied significantly within each harvest amongst treatments (Appendix 2.3). Tukey's test multiple range (Appendix 2.4) showed, seed moisture content in the control was significantly greater for drought treatments from 8 (14 DAA) or 9 (7 DAA) days after irrigation ended onwards.

Seed dry weight was significantly affected by irrigation treatment, period of development, and their interaction (Appendix 2.5). From 11 DAA onwards, seed dry weight varied significantly within each harvest amongst treatments (Appendices 2.5 & 2.6). Seed dry weight was affected by irrigation treatment consistently from 16 DAA onwards (Fig. 2.1b). Where irrigation ended at 7 DAA, seed filling ended at about 11 DAA with subsequent seed weight averaging 8.6mg (s.e. 0.4). Where irrigation ended at 14 DAA, seed filling ended at just after 16 DAA with subsequent seed weight averaging 16.0mg (s.e. 0.44). In contrast in the control, seed filling continues until 40 DAA with final seed weight of 28.4mg (s.e. 0.69).

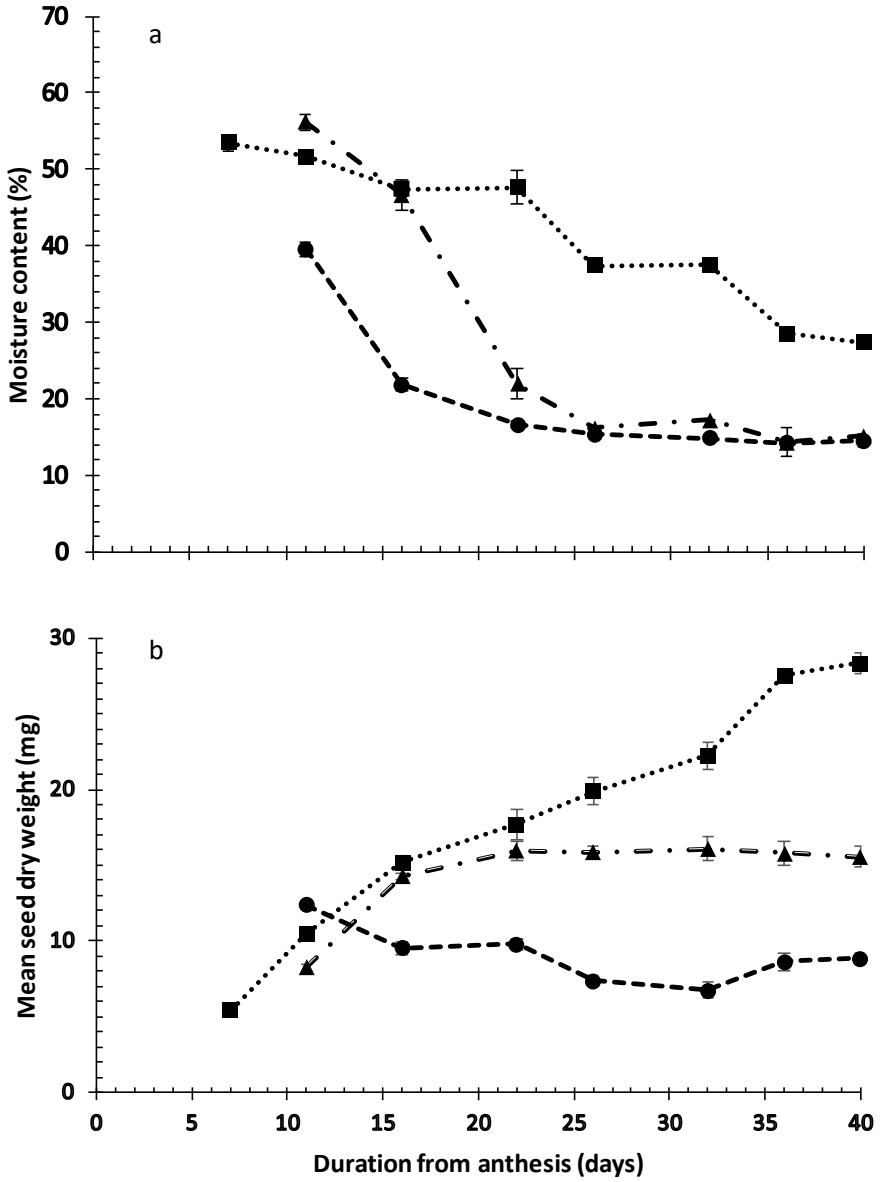


Figure 2.1: Moisture content (a) and dry weight (b) for seeds of *japonica* rice cv. Gleva harvested serially during their development from plants grown in controlled environments at $28/20^0$C (12h/12h), 12 h d^{-1} photoperiod with irrigation throughout (■), irrigation ended at 7 (●), or at 14 DAA (▲) with ±standard errors of mean shown where larger than symbols.

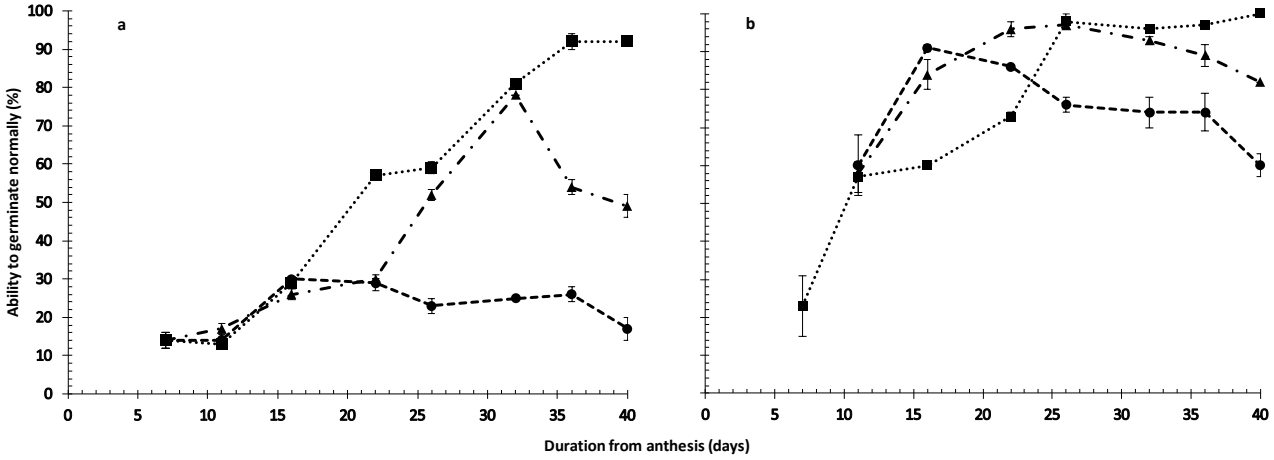


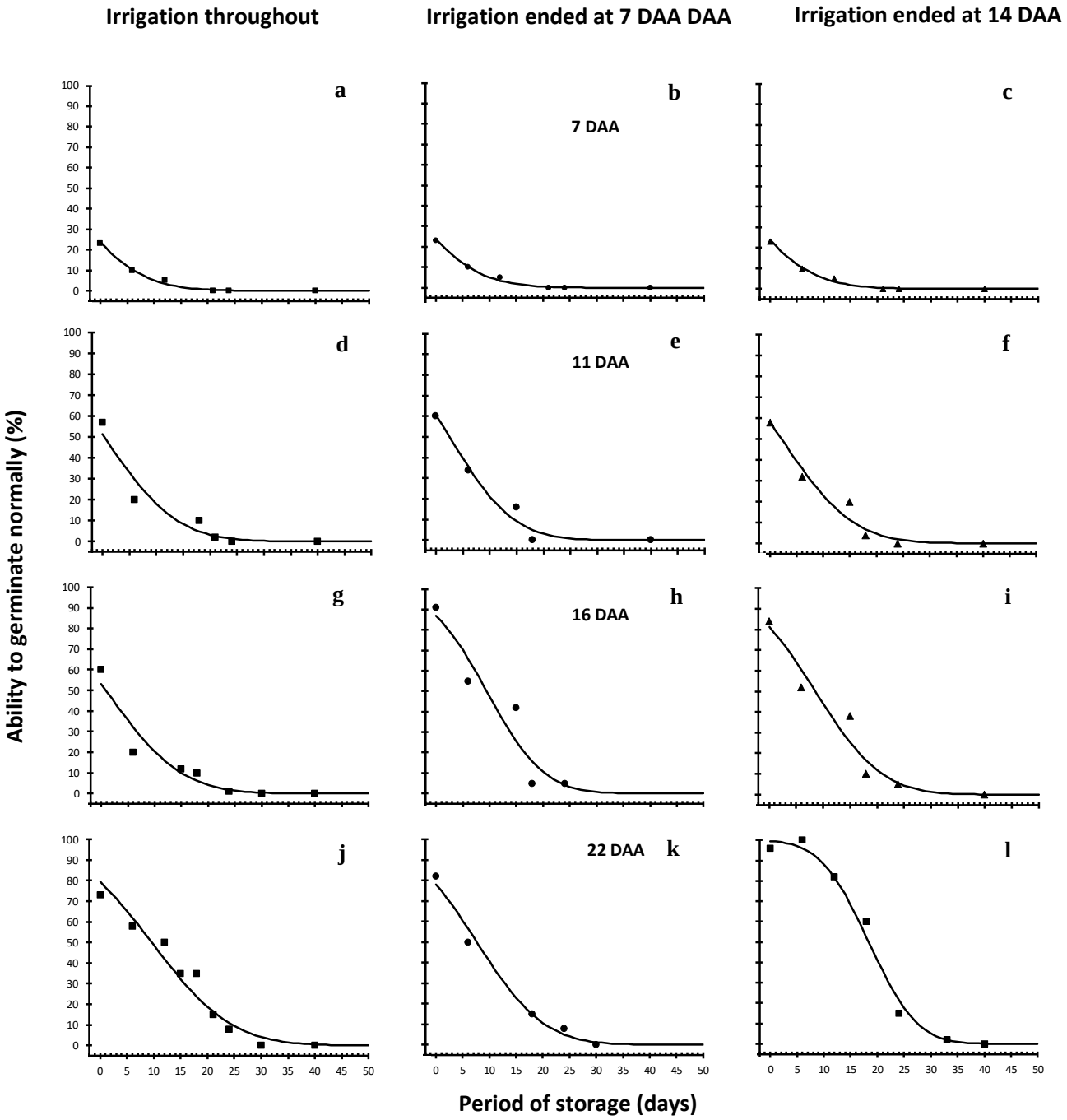
Figure 2.2: Changes in viability of fresh (a) or dried (b) seed, assessed by ability to germinate normally in standard tests at 34/11°C, for seeds of *japonica* rice cv. Gleva harvested serially during their development from plants grown in controlled environments at $28/20^0$C (11h/13h), 11 h d^{-1} photoperiod with irrigation throughout (■), irrigation ended at 7 (●), or at 14 DAA (▲) with ±standard errors of mean shown where larger than symbols.

The viability of fresh and dried seeds (assessed by ability of seed to germinate normally *ex planta*) improved during seed development (Fig. 2.2a & b). The main effect of irrigation treatment was significant for fresh and dried seeds ($P<0.05$), with harvest date and their interaction also significantly (Appendices 2.7 & 2.8).

For fresh seeds, ability to germinate normally for drought at 7 DAA was tremendously lower. Initially, the germination percentage showed increasing pattern only until 16 DAA (4 days after imposed to drought) with a maximum of only 30% germination. This contrasted with 76% normal germination for the dried seeds. From 36 to 40 DAA, only the control provided 100% normal germination for fresh and dried seed samples. In the control, ability to germinate normally for dried seeds was improved greatly towards the final harvest. In drought treatments, however, dried seeds only improved greatly at early harvests and then declined towards the end. The decline began from 16 DAA (irrigation ended 7 DAA), or from 27 DAA (irrigation ended 14 DAA) (Fig. 2.2b). Maximum germination (90%) was recorded in the 7 DAA irrigation treatment at 16 DAA, in the 14 DAA treatment at 22-26 DAA (97%), and in the control at 36-40 DAA (100%). As a consequence, normal germination at 40 DAA differed greatly amongst treatments for dried seeds, with the drought treatments providing poor germination.

2.5.2 Seed storage survival

Seed survival in hermetic storage of the different irrigation treatments harvested at different periods throughout seed development generally conformed well to negative cumulative normal distributions (Fig. 2.3). Comparison of the survival curves within each harvest from 11 to 40 DAA showed that the slopes did not differ significantly in slope amongst treatments except at 22 DAA (Appendix 2.10). In most cases, constraining the curves to a common value of Ki as well as a common value of σ was significant, however. Seed survival curves were fitted separately to each treatment combination and are shown in Figure 2.3a-x. These curves are quantified in Table 2.1. Good fit was provided by the fitted curves in most cases (e.g. Fig. 2.3 a,r,w) with acceptable but slightly poorer fit in a few cases (e.g. Fig. 2.3 h, m,n). The survival curves differed considerably amongst harvest, with brief longevity in the early harvest (Fig. 2.3a-f) to considerable longevity in the control in the last four harvests (Fig. 2.3m,p,s,v).


Irrigation throughout
Irrigation ended at 7 DAA DAA
Irrigation ended at 14 DAA
a
b
c
7 DAA
d
e
f
11 DAA
g
h
i
16 DAA
j
k
l
22 DAA
Ability to germinate normally (%)
Period of storage (days)

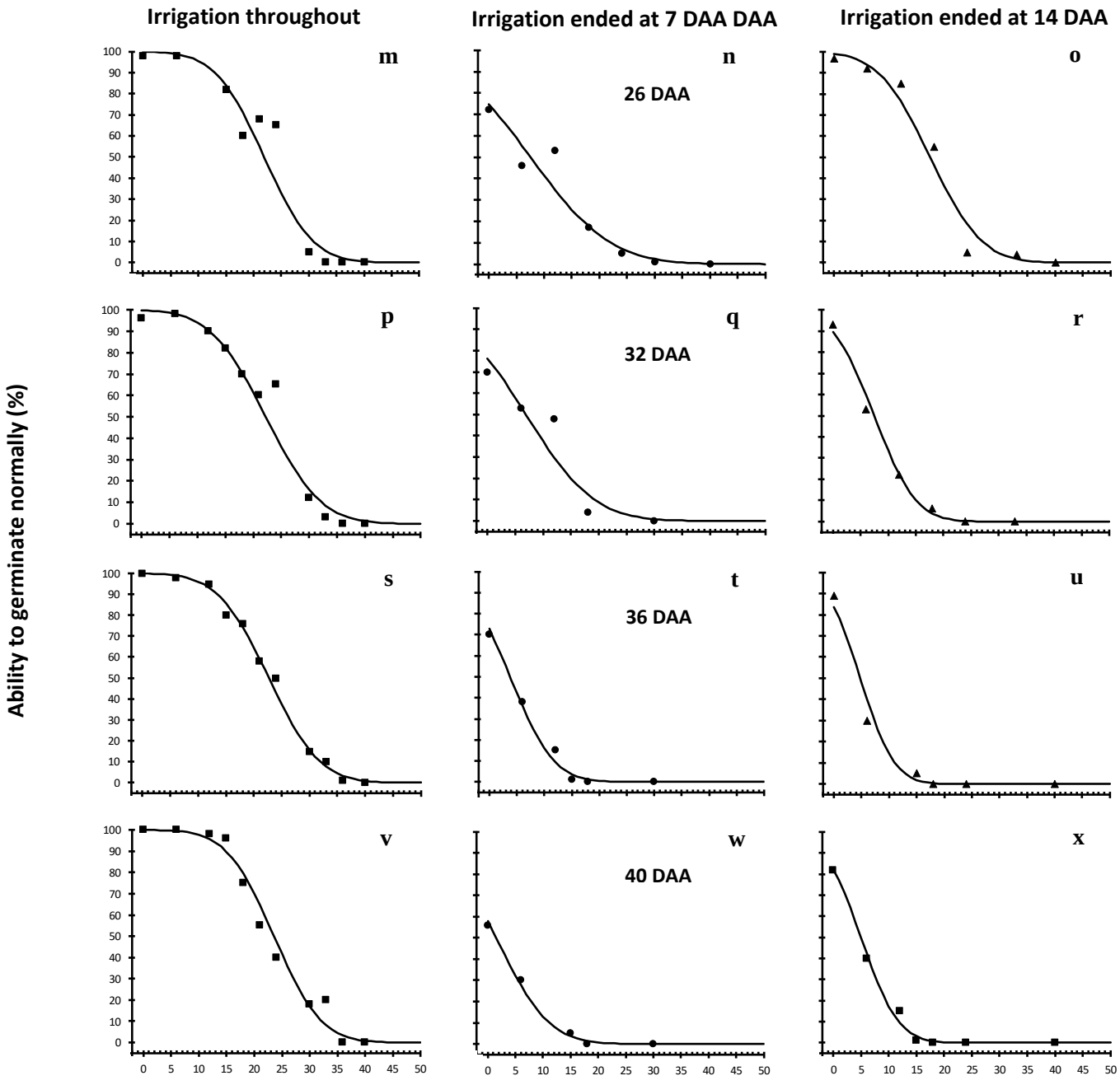


Figure 2.3. Loss in viability (estimated by change in ability to germinate normally) of seeds of *japonica* rice cv. Gleva harvested serially from 7-40 DAA during seed development in a growth cab (28/20 ^{0}C, 11h/13h, 11 h d^{-1} photoperiod) with irrigation throughout (■), irrigation ended at 7 (●), or at 14 DAA (▲) and stored hermetically at 40°C with storage moisture content at 15%±0.5. Best fit survival curves for each treatment combination fitted by probit analysis are shown. These are quantified in Table 2.1. Note that only one sample of seeds was harvested at 7 DAA (before drought treatment were imposed) and these results are repeated in b and c to enable comparison with later harvests.

2.5.3 Longevity (p_{50})

Seed storage longevity (p_{50} estimated by probit analysis) varied considerably during seed development and with treatment with quite different trends apparent over time due to irrigation treatment (Fig. 2.4). Comparison of treatments are made here for p_{50} . This period is the product of *K*i and (1/σ) (Ellis et al., 1980). The period p_{50} was used because the slopes (1/σ) differ amongst the seed survival curves (see above). Hence, it not possible to compare the survival curves using *K*i alone. The period p_{50} is also the most accurate for determination period of longevity. The estimated parameters of the estimates of p_{50} the seed viability equation fitted by probit analysis shown as in Table 2.1.

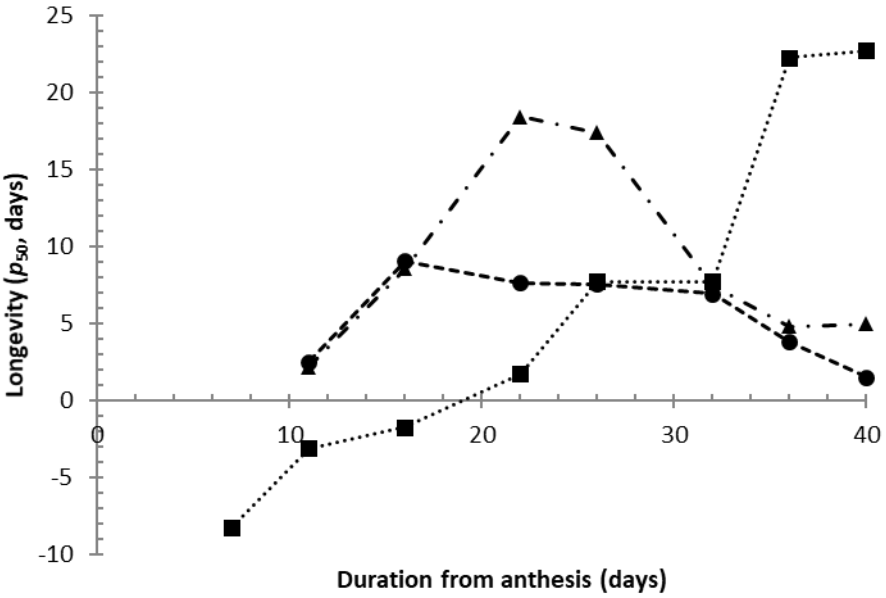


Figure 2.4: Longevity (p_{50} provided by parameters of seed viability equation fitted by probit analysis) of seeds of *japonica* rice cv. Gleva harvested serially during their development from plants grown in a controlled environment and stored hermetically at 40℃ with seed storage moisture content at 15%±0.5. Plants were irrigated throughout (■), or until 7 (●), or 14 DAA (▲). The estimate of p_{50} is provided by the best fit curve for each treatment combination (Table 2.1).

In the control treatment, subsequent longevity after harvest provided a consistent pattern of increase throughout the harvest period reaching the greatest value (23.6d) in the last harvest (40 DAA). The greatest increase occurred between 32 and 36 DAA (Fig. 2.4) when seed moisture content declined from 37.5-28.5% (Fig. 2.1a), the most rapid loss in moisture in the control. Where irrigation ended at 7 DAA, the greatest subsequent longevity (9.5d) was obtained only 9 days later (16 DAA) when seed moisture content had declined

to 21.8% (Fig.2.1a). Longevity declined thereafter, gradually until 32 DAA and then more rapidly. Ending irrigation at 14 DAA provided results intermediate between the two extreme treatments; longevity increased until 22 DAA with maximum value of 18.4d (Fig.2.4) at 21.9% seed moisture content (Fig. 2.1a), then declined only slightly at 26 DAA, and then substantially thereafter reaching values as low as the 7 DAA treatment from 32 DAA onwards.

Table 2.1: Longevity (parameters of seed viability equation fitted by probit analysis) of japonica rice cv. Gleva freshly harvested serially during their development from plants grown in controlled environments; irrigation throughout, irrigation ended at 7 DAA or at 14 DAA in hermetic storage at 40°C with seed storage moisture content at 15%±0.5. The 95% confidence intervals are shown for p_{50}

Treatments	Harvest (DAA)	*K*i		Slope(1/σ)		p_{50} (days)			
		estimate	s.e	estimate	s.e	estimate	s.e.	lower 95%	upper 95%
Irrigation throughout	7	-0.71	0.14	0.085	0.018	-7.8	2.80	-15.0	-4.1
	11	0.03	0.11	0.094	0.009	0.3	1.14	-2.2	2.2
	16	0.08	0.10	0.090	0.009	0.9	1.13	1.1	-1.6
	22	0.82	0.10	0.085	0.006	9.6	0.69	8.2	10.9
	26	3.13	0.19	0.143	0.008	21.9	0.40	21.1	22.7
	32	2.84	0.15	0.128	0.007	22.2	0.41	21.4	23.0
	36	3.13	0.17	0.138	0.007	22.7	0.39	21.9	23.5
	40	3.49	0.18	0.148	0.008	23.6	0.38	22.8	24.3
Ending irrigation at 7 DAA	7	-0.71	0.14	0.085	0.018	-7.8	2.80	-15.0	-4.1
	11	0.27	0.11	0.107	0.011	2.5	0.89	0.6	4.0
	16	1.12	0.12	0.118	0.009	9.5	0.60	0.6	8.3
	22	0.77	0.11	0.101	0.008	7.7	0.77	6.1	9.1
	26	0.66	0.10	0.088	0.007	7.5	0.78	5.9	9.0
	32	0.72	0.11	0.104	0.009	6.9	0.70	5.5	8.2
	36	0.60	0.12	0.160	0.013	3.8	0.55	2.7	4.6
	40	0.20	0.11	0.133	0.014	1.5	0.78	-0.2	2.8
Ending irrigation at 14 DAA	7	-0.71	0.14	0.085	0.018	-7.8	2.80	-15.0	-4.1
	11	0.21	0.11	0.095	0.009	2.2	0.10	0.0	3.9
	16	0.89	0.11	0.104	0.008	8.6	0.68	0.6	7.2
	22	2.60	0.18	0.141	0.010	18.4	0.52	17.4	19.4
	26	2.36	0.17	0.136	0.009	17.4	0.52	16.4	18.4
	32	1.25	0.13	0.168	0.013	7.4	0.47	6.5	8.3
	36	0.98	0.13	0.204	0.019	4.8	0.46	3.9	5.7
	40	0.93	0.13	0.187	0.014	5.0	0.45	4.0	5.8

2.6 Discussion

Comparison amongst treatments showed considerably and consistent effects of the two drought treatments on the progress of seed development and maturation, seed desiccation and filling (Fig. 2.1a & b) and on fresh and dry seed quality development, ability to germinate (Fig. 2.2a & b) and subsequent air-dry seed storage longevity (Fig. 2.3). The drought treatments resulted in more rapid seed development but considerable poorer seed quality later on.

At the end of this study, all chapter 2 hypotheses (2.3.1-2.3.3) in relation with general hypotheses (1.7.1-1.7.3) were accepted as they relate to the effects of drought. The hypotheses for the effects of ending irrigation at 7 and 14 DAA during seed development and maturation can now be answered.

1. Ending irrigation early reduced the duration of seed filling and final seed dry weight and resulted earlier seed desiccation; it was reduced/earlier by the14 DAA treatment and reduced further/earliest by the 7 DAA treatment.

2. Ending irrigation early resulted in ability to germinate (dry seed only) than the control and to tolerate desiccation earlier than control. Both drought treatments were similar in each other and earlier than the control. However, ability for fresh seed to germinate did not develop earlier than the control.

3. Improvement in subsequent seed longevity during seed development and maturation occurred sooner by ending irrigation early; the improvement developed earlier than the control in both the 7 and 14 DAA treatments. The maximum longevity was detected earlier by ending irrigation early, as this was achieved earlier than the control in both the 7 and 14 DAA treatments. This value was greatest for the control; the control provided greater longevity than the 14 DAA treatment, which in turn was greater than the 7 DAA treatment.

In the present study, loss of seed moisture content during seed development and maturation drying occurred earlier under water limitation. This reflects the plant's strategy to respond to water shortage to ensure their survival by producing seed quickly. Water stress during seed development reduced the duration of seed filling (Fig. 2.1) and final seed dry weight (Fig. 2.1b). Seed dried earlier in the seed-filling phase when irrigation ended after the

end of the histodiffentiation phase (7 DAA treatment) and well before seed filling ended (14 DAA treatment). This was supported by Yang and Zhang (2006) who showed that water stress during grain filling increased the remobilization of assimilate from the rice straw to grains and resulted in early senescence. Furthermore, water stress during grain filling induces the conversion of stem reserves into soluble sugars and the transport of these sugars into the grains (Blum, 2005). Nicolas et al. (1985) reported seed dry weight was reduced in response to early drought due to the development of a smaller number of endosperm cells. That is expected to have occurred here because all plants were irrigated until 7 DAA when the histodiffentiation phase was assumed to have been completed. I suggest that the reduction in final seed dry weight from ending irrigation early resulted from earlier plant senescence and reduced period for assimilate to be provided to the developing seeds compared to the control.

It was surprising the seed dry weight in the control continued to increase throughout the investigation (Fig. 2.1a) whilst moisture content remained quite high (about 30% moisture content) (Fig. 2.1a). Ellis et al. (1993) have shown that seed filling ends at about 22 DAA with seed moisture content at about 40%, in similar environments. There was considerable variation in the timing of anthesis amongst plants (Appendix 2.9). It might be possible therefore that the seed filling in later harvests in the control represented those plants that had flowered much later. However, this was prevented in my study because panicles that anthesed from 101-113 DAS were selected for the experiment. Irrigation in the control continued until the final harvest at 40 DAA. This long period of irrigation might explain the long period of seed filling.

From the result for dry seed germination (Fig. 2.2b), it can be concluded that germination capacity (dried seeds) for ending irrigation at 7 DAA increased for about 5 days after attaining maximum seed dry mass (Fig. 2.1b). For ending irrigation at 14 DAA, maximum germination capacity coincided with mass maturity at 22 DAA, meanwhile in the control, it was achieved before mass maturity. The present results for the 7 DAA treatment was consistent with results of Filho and Ellis (1991) who found that in barley, maximum germination was observed between 7-13 days after maximum seed mass. Those results in the control and ending irrigation at 14 DAA were slightly similar to Pereira et al. (2014) with *Syngonanthus elegans*, where maximum germination capacity was reached prior to seed mass maturity.

The stage of seed development and maturation affects seed quality (eg. Martínez-Eixarch and Ellis, 2015; Ellis, 2011; and Sanhewe et al.,1996). Since the timing of development was significantly affected by the irrigation treatments (Appendices 2.0, 2.3, 2.6) it is possible that the different results at any one harvest time for seed quality might result from the more rapid development when irrigation ended early.

During seed development and maturation, fresh and dry seeds for both drought treatments declined in ability to germinate normally in later harvests (Fig. 2.2a & b). Moreover, fresh seed from the earlier drought during seed development showed an extremely low ability to germinate compared to other treatments (Fig. 2.2a). This circumstance might be due to loss in viability or increase in dormancy during seed development and maturation: since dried seeds showed much greater germination (Fig. 2.2b), the latter must be the explanation. In wheat, Yadav and Ellis (2016), showed similar development of ability for seed to germinate in both fresh and dry seed. However, for the control treatment, the present results are similar to Tejakhod and Ellis (2017) which presented that dry seed of rice cv. Gleva able to germinate normally throughout seed development and maturation.

My hypothesis that improvement in subsequent seed longevity during seed development and maturation would occur sooner and maximum longevity occur earlier by ending irrigation early were accepted: improvement of subsequent longevity was developed earlier, and maximum longevity achieved earlier than the control in both the 7 and 14 DAA treatments (Fig. 2.4) This is similar to the conclusion Sinniah et. al. (1998a) with rapid-cycling brasicca. The earlier improvement in longevity also matched the earlier desiccation (Fig. 2.1a). Similarly, onset of ability to germinate and of desiccation tolerance for wheat develops early in development from the first harvest (14 DAA) at which time seeds were only about 10% filled (Ellis & Yadav, 2016). Consistent differences were apparent amongst all three treatments for longevity and compared with seed moisture content: with desiccation to 22% moisture content, longevity improved in all treatments, but beyond 32 DAA, longevity of the control > 14 DAA > 7 DAA irrigation treatments. The declining in longevity for 7 DAA and 14 DAA, respectively with below 22% or 18% seed moisture content was only a small decline however much greater than control towards the end of harvest. These results are compatible with Sinniah et al. (1998a) who reported that when reducing irrigation to rapid-cycling brassica plants, the attainment of maximum potential longevity coincided with the rapid reduction in seed moisture content due to plant drought.

Control provided greater maximum longevity (23.6 d) than the 14 DAA (18.4d) treatment which was greater than the 7 DAA (7.7d) treatment (Table 2.1). Thus, the hypothesis that maximum value of longevity is reduced by ending irrigation early was accepted. This result was unexpected (in terms of the published literature in other species) and is the subject of a future investigation. The result may have been due to the shorter duration of improvement in seed quality in the 7 DAA and to a lesser extent the 14 DAA treatments and the later decline

in longevity occurred sooner because seeds had reached lower moisture contents than in the control.

In relation with seed moisture content, ability of dry seeds to germinate showed similar trends with desiccation to about 30% moisture content, plateaus with further loss in moisture content, but then declines below about 18% moisture content (Fig. 2.5a). The 7 and 14 DAA treatments were similar thereafter, with progressively reduced ability to germinate apparent as moisture content declined from about 22% to 16% (Fig. 2.5a). This decline was not detected in the control, perhaps because seed moisture content *in planta* did not fall below 30% in this study. Longevity increases until close to 22% moisture content (Fig. 2.6b), but the pattern is very different amongst irrigation treatments, and then declines at about 16% moisture content. The value of 16% moisture content is also the point at which Whitehouse et al. (2015) identified that rice seed longevity no longer benefitted from heated-air drying treatments. That is, longevity could not be improved further beyond that occurring *in planta*. The control samples, plants irrigated throughout did not dry below 26% moisture content during the investigation, and so no decline in ability to germinate or longevity was detected.

From this present finding, my next study (Experiment 2) was continuing with similar stress (drought) with some modification, which include treatments ending irrigation during end seed filling and late maturation drying.

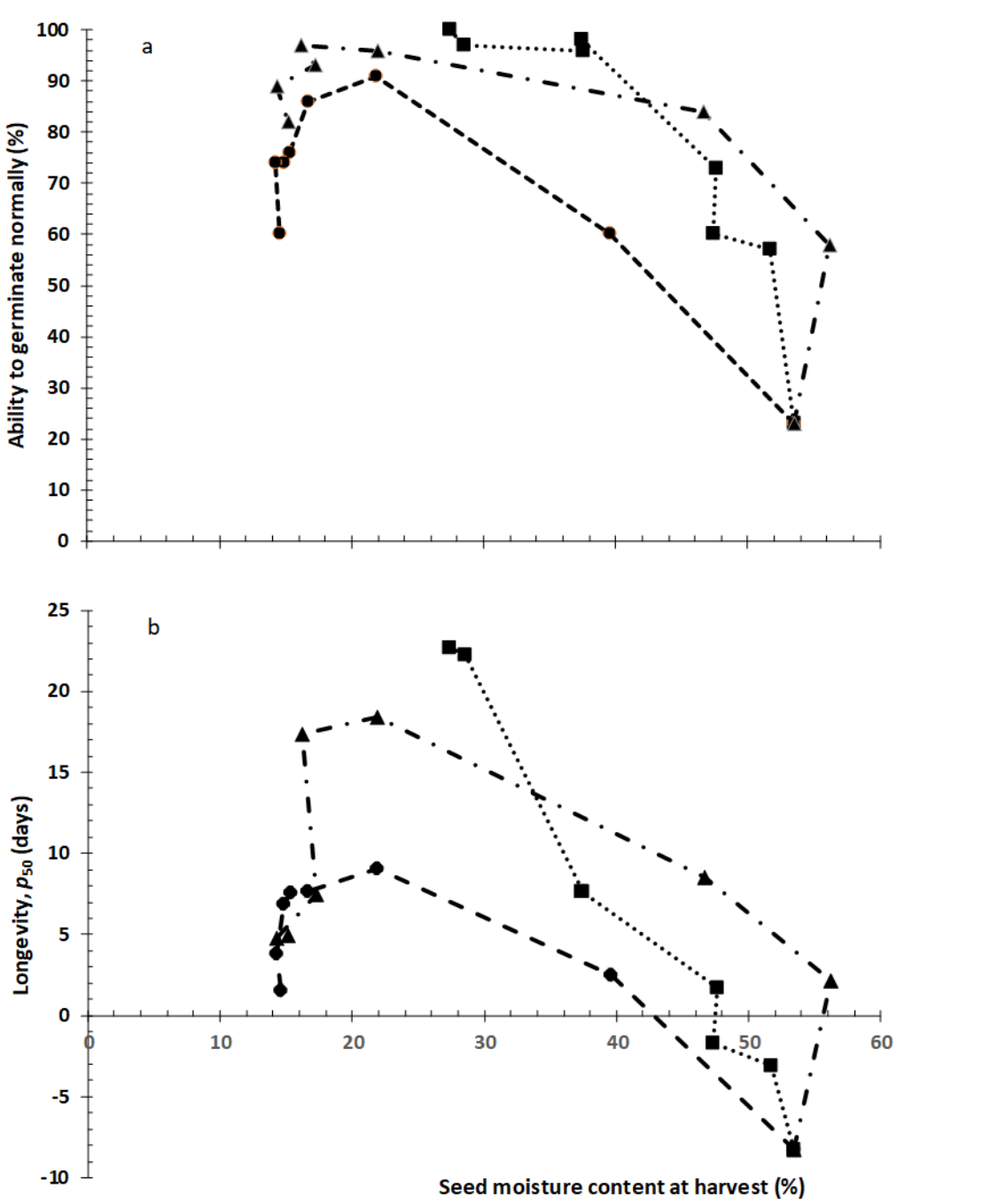


Figure 2.5: Seed moisture content in relation with ability to germinate (dried-seed) (a) and longevity p_{50} (b) for seeds of *japonica* rice cv. Gleva harvested serially during their development from plants grown in controlled environments at $28/20^0$C (11h/13h), 11 h d^{-1} photoperiod with irrigation throughout (■), irrigation ended at 7 (●), or at 14 DAA (▲). Data point linked in chronological order from first harvest (high moisture content) to the final harvest (low moisture content).

2.7 Conclusion

The evidence from the present study indicates that drought treatments had a substantial effect on the pattern of increase and then decline in both seed viability and seed longevity during seed development and maturation of cv. Gleva. The results to date suggest that early plant senescence, from drought, accelerates the development of seed desiccation tolerance and longevity, but longer periods of development from continuous irrigation result in higher seed quality ultimately, with seed deterioration in the mother plant beginning to accumulate once seed moisture content declines to about 16% and so well within the air-dry range.

CHAPTER 3

Effect of a brief period of elevated temperature during seed development on rice seed quality

3.1 Introduction

Abiotic stress such as extreme temperature frequently limits the growth and productivity of major crops species including rice. Short-term heat stress events have occurred frequently across the globe during the past 20-30 years and are likely to increase with global warming (Lobell et al., 2012). Only a limited number of reports have been published on the effect of elevated temperature during seed development on rice seed quality, particularly on seed longevity.

In rice, the effect of high temperature depends on the developmental stage of the rice plant, particularly during reproductive stage. This was proved by many studies in rice, for example in terms of spikelet fertility Martinez-Eixarch and Ellis (2015) and Tuay and Saitoh (2017). Seed quality and potential longevity of *japonica* rice is particularly sensitive to high temperature (Ellis et al., 1993). Similar studies by Zakaria et al. (2002) discovered that *japonica* rice was less resistant to elevated temperature during the seed maturity phase than *indica* or *javanica* rices. Moreover, Ellis (2011) suggested that seed quality development may be less sensitive to high temperatures during late development and maturation than during early seed development.

It is well established that high temperatures during seed development reduce grain yield and seed quality (Morita, 2008; Tanamachi et al., 2016). However, continuous extreme temperatures provided throughout an experiment are unrealistic in terms of the problem in the field. For this reason, I provided more realistic 3-days exposure to high temperature stress in this investigation. Moreover, the issue of which stage during seed development, high temperature most affects seed quality was explored. Thus, this study was focused on brief elevated high temperature at different stages during seed development and their effect on seed quality, particularly on seed longevity. The regime 40/30℃ was chosen according to temperature stress events currently occurring in some rice production in Spain.

In this study, I added an additional seed vigour test which is electrical conductivity (EC) test of seed steep water. The conductivity test is a measurement of electrolytes leaking from seeds based on the principle that the deterioration process is the leaching of the cells of seeds soaked in water due to loss of integrity of cellular systems (Powell and Matthews, 1978).

Changes in the organization of cell membranes occur during the development of seeds prior to physiological maturity, seed desiccation before harvest, and during imbibition prior to germination (ISTA, 1995). However, there are factors which influence the conductivity values as the genotype, size, the initial water content, temperature and time of soaking and the number of seeds per sample (Ramos et al., 2012).

3.2 Objectives

This study investigated changes in seed quality, including seed viability and subsequent seed storage longevity, in the *japonica* rice cv. Gleva in response to 3-days treatment at 40/30℃ at different stages of seed development and maturation.

3.3 Hypotheses

The hypotheses for the investigations were as follows:

3.3.1 Seed moisture content, seed dry weight and seed viability (ability to germinate normally of fresh and air-dried seed) at harvest maturity (42 DAA) are lowest by 3-days treatment at a high temperature of 40/30℃;

3.3.2 Subsequent seed longevity in air-dry storage of seeds harvested at 42 DAA is lowest by 3-days treatment at a high temperature of 40/30℃.

Hypotheses 3.3.1-3.3.2 relate to my general hypotheses 1.7.1 and 1.7.2 in relation to brief high temperature.

Each of the above was tested for different 3-days periods during seed development and maturation, to test the hypothesis that each independent variable above varies in sensitivity to extreme high temperature during seed development.

3.4 Methodology

This study was conducted in a controlled environment glass house and growth cab at the Plant Environmental Laboratory (PEL), Shinfield, University of Reading (UoR) (51º 27′ N latitude and 00º 56′ W longitude). It was carried out using RCBD experimental design with eleven treatments (including a control with no high temperature) provided to five pots of plants in each of four blocks.

3.4.1 Seed Sowing

Seeds of *japonica* rice cv. Gleva were sown in pots in blocks on 11 April 2015 in a temperature and photoperiod controlled heated and vented glasshouse (6.4 x 8.6 m). Pots were arranged on four mobile trolleys (each 3.5 x 2.1m) then kept inside the dark compartments in the close 'garage' until they reached 50% germination (Plate 3.1). Each trolley held 55 experimental pots comprising one block. Four trolleys (blocks) in total held 220 experimental pots. A further 25 pots on each trolley were also used to multiply seeds for later growing seasons and provided a dense stand of plants similar to a small plot field experiment. Seedlings were exposed to sunlight (Plate 3.2) after 11 DAS (22 April 2015).

Plate 3.1: 50% rice (cv. Gleva) seed germinated (11 DAS)

Plate 3.2: Rice (cv. Gleva) seedlings on mobile trolley exposed to daylight at 11 DAS

3.4.2 Vegetative growth

Plants on the mobile trolleys were drawn into the glasshouse from the 'garage' manually in order to expose plants to a 12-hour photoperiod in a temperature of $28/20^0$ C at about 75% relative humidity at 7 am and back in at 7 pm each day. After 13 DAS, plants were provided with drip irrigation five times per day where water pH was controlled in the range between 5.5-6.5 (Plate 3.3a & b). Plants were thinned in early seedling stage (14 DAS) to give four strong plants to a uniform stand.

a

b

Plates 3.3a & b: Rice (cv. Gleva) seedling provided with drip irrigation from 13 DAS. The night (dark) compartment ('garage') are shown closed in b.

3.4.3 Anthesis

The development of anthesis was monitored to obtain the pattern of variation in anthesis date prior to starting the treatments. This pattern is shown in Appendix 3.11. In order to limit the anthesis variation, only panicles that anthesed during the period 81-87 DAS were used in the study. Earlier and later anthesing panicles were cut and removed. Anthesis (0 DAA) was 85 DAS (i.e. 50% anthesis)

3.4.4 High temperature treatment

Treatments began 82 DAS (-3 DAA) and ended up to 27 DAA. Pots in a treatment were transferred to the controlled growth cab (Saxcil) for 3-days at $40/30^0$C with 11h/13h thermoperiod synchronized with 11h photoperiod (Plate 3.4). Each transfer consisted of 20 treatment pots. Treatments T2 to T11 followed each other at 3 days intervals. The treated plants were moved back to the glass house at the end of the 3 days where they remained until maturity. The different periods of high temperature treatment were as below:

1. T1 = control (no high temperature treatment, in glasshouse throughout)
2. T2 = -3-0 DAA
3. T3 = 0 - 3 DAA
4. T4 = 3 - 6 DAA
5. T5 = 6 - 9 DAA
6. T6 = 9 - 12 DAA
7. T7 = 12 - 15 DAA
8. T8 = 15 - 18 DAA
9. T9 = 18 - 21 DAA
10. T10 = 21-24 DAA
11. T11= 24 - 27 DAA

Plate 3.4: General view of (cv. Gleva) plants in cabs during 3-d period at 40/30℃ (11h/13h thermoperiod)

3.4.5 Harvesting

Irrigation of all treatments continued until the end of the experiment when panicles were harvested. The panicles were harvested on 11 August 2015 (42 DAA) at harvest maturity (Plate 3.5). Seeds were threshed gently by hand and empty seeds were removed.

Plate 3.5: Rice (cv. Gleva) plants at harvest maturity (42 DAA)

3.4.6 Data Collection

The methods for the determination of germination and seed storage longevity were similar to Experiment 1.

3.4.6.1 Seed dry weight

The mean of eight samples of 100 seeds was recorded during the seed moisture content determination. Seed dry weight was calculated from estimates of seed fresh weight and seed moisture content.

3.4.6.2 Seed Moisture Content

As seeds were harvested at maturity, the two-stage method was not used. Samples of 10 g (210-250 seeds) were divided into two replicates and placed in a constant temperature oven at 103°C for 17 h and moisture content was calculated as,

$$\text{m.c.}(\%) = \frac{\text{Original wt. of seed - dry wt. of seed}}{\text{Original wt. of seed}} \times 100$$

3.4.6.3 Bulk Conductivity Test of seed steep water

In some species, this test provides a quick, cheap and easy way to distinguish seed lots that differ in quality (Matthews and Bradnock, 1968). This test was included in this experiment to investigate by myself if it could be used for seed development study in rice. Sets of 100 seeds from each sample were soaked in 50 ml for distilled water at 25^0 C for 16h. A control with distilled water without seeds was maintained (control conductivity). Seed leachate was measured with a portable Electrical Conductivity meter (EcoTestr EC high, Eutech Instruments Pte. Ltd.) as electrical conductivity (E.C.) (µS cm^{-1} g^{-1}) (Krishnan et al., 2004). The blank treatment sample was subtracted to provide the result of the solution conductivity. The weight (g) was taken from the seed dry weight determination.

The reading is expressed as µS cm^{-1} g^{-1}. It is calculated as follows,

$$\text{Conductivity (µS cm}^{-1}\text{ g}^{-1}\text{)} = \frac{\text{Solution conductivity – control conductivity}}{\text{Weight (g) of replicate}}$$

(Sharma et al., 2011)

3.4.6.4 Statistical analysis

Similar analyses were carried out as in the earlier study in Experiment 1.

3.5 Results

Elevated high temperature (HT) for 3-days at different periods of seed development considerably affected seed quality (final seed moisture content, dry weight, ability to germinate (fresh seeds)) and subsequent longevity (Figs. 3.1-3.4). All the independent variables above were affected significantly by the HT treatments, however not for electrical conductivity (E.C.) and ability of dried seed to germinate normally (Appendices 3.0-3.8). Comparisons amongst treatments were made using the Tukey's multiple range test (Appendices 3.5-3.8). In each case, final seed moisture content, dry weight, ability to germinate (fresh seeds) of *japonica* rice cv. Gleva was more influenced by HT during the first three period HT treatments (at -3-0, at 3-6 and at 6-9 DAA). Seed longevity was most affected by the first two periods of HT treatment.

3.5.1 Seed moisture content, dry weight and ability to germinate (fresh and dry)

The range of seed moisture contents at 42 DAA for all treatments was in the range 24.7-37.2%. The extreme seed moisture content in these samples was provided by the earliest (-3-0 DAA) timing of exposure to HT with 37.2% (Fig. 3.1a). The last treatment (24-27 DAA) and control provided similar estimates of about 25% seed moisture content. Moisture content was significantly affected by HT treatment (Appendix 3.0).

The control provided the highest value (31.4mg, s.e. 0.7), whilst HT at -3-0 DAA provided significantly lower seed dry weight (20.2mg, s.e. 5.0) with all the remaining in between treatments (Fig. 3.1b, Appendix 3.6). The HT at 3-6 until 24-27 DAA (T4-T11) respectively did not differ significantly and ranged only from 27.4 to 31.4mg (Appendix 3.6). The first three HT treatments (HT at -3-0 until 3-6 DAA) showed a progressive rise in seed dry weight. Hence, HT imposed around anthesis reduced final seed dry weight.

Solute leakage (measured as electrical conductivity) on seed steep water showed no significant difference amongst HT treatments (Appendix 3.2). The range of E.C. was in between 58.0 to 78.3 $\mu S\ cm^{-1}\ g^{-1}$. Hence, this study indicates that E.C. of *japonica* rice cv. Gleva was not influenced by elevated HT during seed development and maturation.

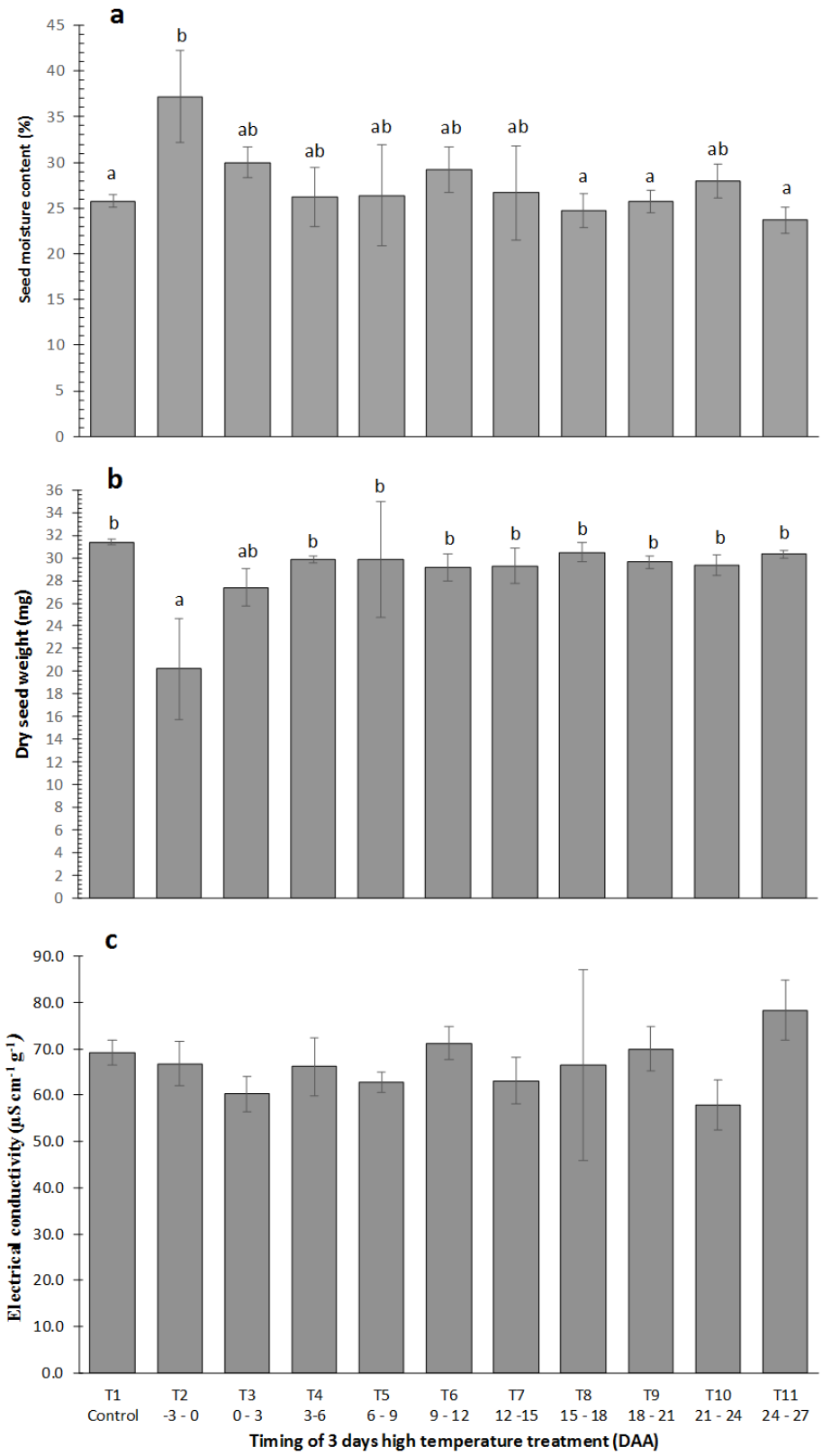


Figure 3.1: Seed moisture content (a), dry weight (b), and electrical conductivity of seed steep water (c) for japonica rice cv. Gleva cultivated in a glasshouse (28/200 C:12h/12h) treated with elevated high temperature during seed development at 40/30°C with 11h/day photoperiod in the growth cab for 3 days at harvest maturity (42 DAA). Different letters indicate significance difference at $P< 0.05$ amongst treatments using the Tukey's multiple range test.

All treatments showed approaching 100% normal germination for seed harvested at 42 DAA, except for the first two high temperature treatments of fresh seeds (-3-0 and 0-3 DAA) where it was depressed (Fig. 3.2). This reduction was much greater for freshly-harvested than dried seeds. Significant difference was detected amongst HT treatment in fresh seeds (Appendix 3.3) but not in dried seeds (Appendix 3.4). The lowest percentage of seed to germinate was in fresh seed treated with HT at -3-0 DAA which achieved about 70% when harvested at 37.2% moisture content (Fig. 3.1a). Drying these seeds below 30% at harvest resulted in a much greater in ability to germinate.

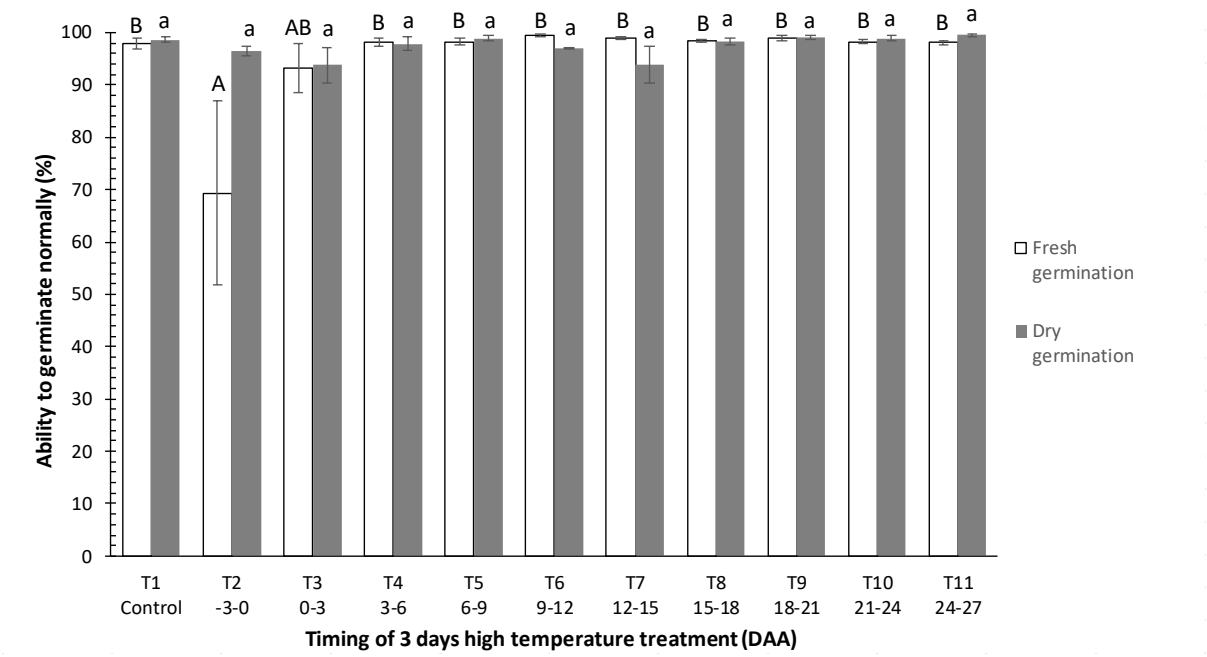


Figure 3.2: Ability to germinate normally of freshly-harvested and dried seed of *japonica* rice cv. Gleva cultivated in glass house ($28/20^0$ C,12h/12h) treated with high temperature during seed development at $40/30^0$ C at the periods shown with 11h/day photoperiod for 3 days in the growth cab and harvested at 42 DAA. Mean values within treatments for freshly-harvested seed with the different capital letter; and within dried seed germination with different small letter indicate significance difference at $P \leq 0.05$ amongst treatments using the Tukey's multiple range test.

3.5.2 Seed storage survival and Longevity (p_{50})

The seed survival curves at 40/30^{0}C with 14.5-15.5% moisture content were fitted by probit analysis. The estimates for *K*i, 1/σ and p_{50} for these survival curves are shown in Table 3.1. The seed survival curves of the 11 treatments (control and HT during different 3-days period in seed development) conformed well to negative cumulative normal distributions (Fig. 3.3). In some cases, the survival curves fit very well e.g. (Fig.3.3b,c,k), and some cases were less well (e.g. Fig. 3.3e,f). Overall, the first HT treatment (-3-0 DAA) provided the shortest survival period (p_{50}=10.6 d) and the second (0-3 DAA) the next (p_{50}=12 d). The longest period was in the control (p_{50}=25.4 d) with the final HT treatments (21-24 DAA, 24-27 DAA) almost as good as the control. Individual results in between were not quite consistent. For example, treatment at 9-12 and 18-21 DAA were particularly low, whereas 3-6 DAA, 6-9 DAA, 12-15 DAA and 15-18 DAA were high (Fig. 3.4).

In Appendix 3.9, the survival curves were compared amongst treatments with different models to describe the seed survival curves fitted by probit analysis. There was no significant difference detected in 1/σ, whereas *K*i did differ significantly. The estimates of *K*i with a common seed survival curve slope are shown in Appendix 3.10. The survival curves for -3-0 DAA provided the lowest estimates of *K*i (1.6), and so the lowest initial viability was detected before it loss in viability towards the end, meanwhile 21-24 DAA was provided the greatest of *K*i value (4.1) (Table 3.1).

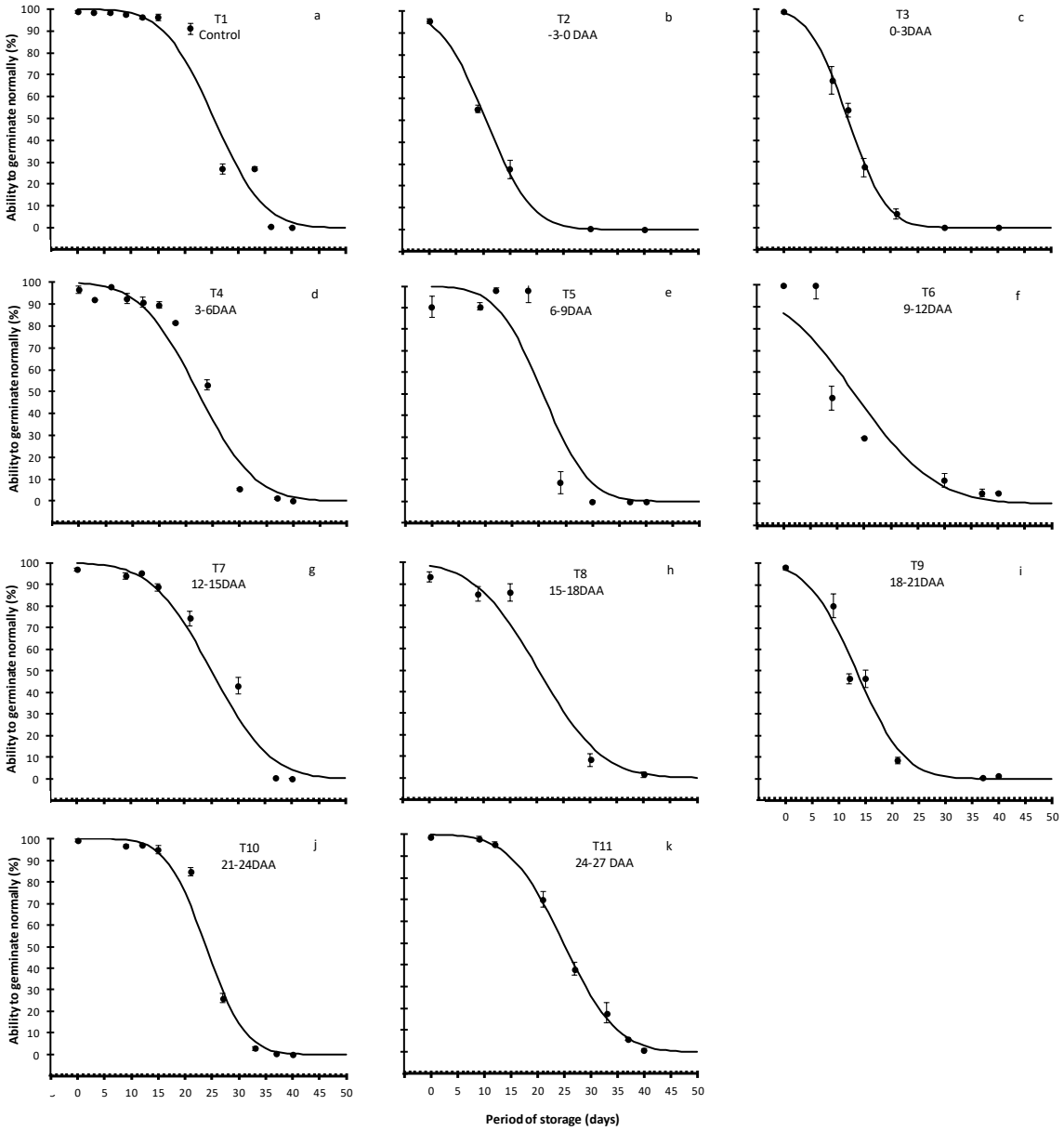


Figure 3.3: Seed survival curves (viability, estimated by ability to germinate) of seeds of *japonica* rice cv. Gleva produced in a glasshouse ($28/20^0$ C,12h/12h) treated with elevated temperature during seed development at $40/30^0$ C with 11h/day photoperiod in the growth cab for 3-days period shown and harvested at 42 DAA. Seeds were stored hermetically at 40°C with the moisture contents shown in Table 3.1. Best fit survival curves for each treatment combination fitted by probit analysis are shown. These are quantified in Table 3.1.

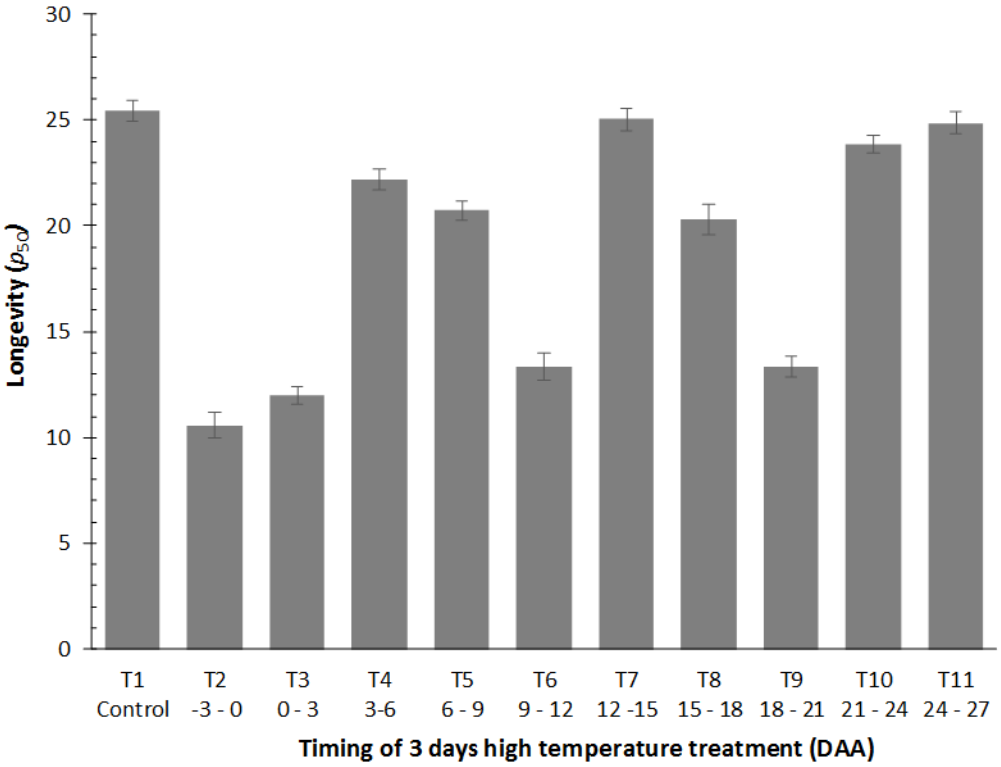


Figure 3.4: Longevity (p_{50} provided by parameters of seed viability equation fitted by probit analysis) of seeds of *japonica* rice cv. Gleva produced in a glasshouse ($28/20^0$ C,12h/12h) treated with high temperature during seed development at $40/30^0$ C with 11h/day photoperiod in the growth cab for 3 days period shown and harvested at 42 DAA, and stored hermetically at 40℃ with seed storage moisture content as shown in Table 3.1.

Table 3.1. Longevity (parameters of seed viability equation fitted by probit analysis) of *japonica* (cv Gleva) rice harvest at maturity (42 DAA) in a glasshouse (28/20^0 C,12h/12h) treated with high temperature during seed development at 40/30^0 C with 11h/day photoperiod in the growth cabinet for 3 days periods shown and harvested at 42 DAA. Seeds were stored hermetically at 40°C with the seed storage moisture contents shown in Table 3.1. The 95% confidence intervals are shown for $p_{50.}$

Treatment	Storage moisture content (%)	K_i		Slope(1/σ)		p_{50} (days)			
		estimate	s.e	estimate	s.e	estimate	s.e.	lower 95%	upper 95%
T1 (Control)	14.8	3.45	0.18	0.1355	0.007	25.4	0.46	24.6	26.34
T2 (3DBA)	14.6	1.57	0.17	0.149	0.015	10.6	0.59	9.5	11.71
T3 (0-3DAA)	14.9	2.10	0.19	0.176	0.014	12.0	0.40	11.2	12.73
T4 (3-6DAA)	15.0	2.63	0.13	0.119	0.0059	22.2	0.48	21.3	23.14
T5 (6-9DAA)	14.5	3.06	0.18	0.148	0.009	20.7	0.44	19.9	21.59
T6 (9-12DAA)	14.8	1.14	0.09	0.085	0.006	13.3	0.65	12.1	14.61
T7 (12-15DAA)	14.5	2.89	0.16	0.115	0.006	25.0	0.56	24.0	26.13
T8 (15-18DAA)	14.7	2.16	0.14	0.106	0.007	20.3	0.71	19.0	21.74
T9 (18-21DAA)	15.1	1.87	0.17	0.140	0.012	13.3	0.48	12.4	14.27
T10 (21-24DAA)	15.2	4.13	0.25	0.172	0.010	23.9	0.42	23.1	24.71
T11 (24-27DAA)	15.5	3.13	0.20	0.126	0.007	24.9	0.52	23.8	25.85

3.6 Discussion

Rice is often cultivated currently in regions where temperatures are already above the optimal for growth (28/22°C), therefore, any further increase in mean temperature or incidents of HT during sensitive stages (e.g. flowering) may adversely affect the performance of the crop (Krishnan et al., 2011). Elevated HT for 3-days in *japonica* rice cv. Gleva did affect seed quality later at harvest maturity (42 DAA). Thus, the null hypothesis set up initially, for all study parameter was rejected for the first two, or the first three HT treatments (-3-0, 0-3, and 3-6 DAA) depending upon the aspect of seed quality studied. The first HT treatment (-3-0 DAA) generally provided the extreme results: the highest seed moisture content and the lowest seed weight; lowest ability to germinate (fresh seeds); and poorest longevity. The combination of highest seed moisture content at 42 DAA and lowest seed weight might reflect a delay to development of some seeds as a result of disruption around the time of anthesis.

Seed desiccation was less sensitive to extreme HT during seed development and maturation as shown in Figure 3.1a. However, it was most pronounced in early histodifferentiation (-3-0 DAA) with highest seed moisture content at harvest (37.2%). This highest seed moisture content might indicate that seed had not yet achieved mass maturity at 42 DAA, perhaps delayed in anthesis as consequence from HT imposed. This is supported by Dunand and Saichuk (2014) who in their review mentioned that most rice seed achieved physiological maturity (mass maturity) while moisture content was between 25-30%.

Present study showed that seed dry weight was not affected by HT in later harvest from 3-6 until 24-27 DAA. This circumstance might due because seed have completed their seed filling as early as 3-6 DAA. Dry seed weight was similar for each 3-days treatment during this seed development period, averaging 30.0mg (Fig. 3.1b). This agrees with Kobata et al. (2018) in their Figure 8, illustrated that rice cv. Koshikari in seed dry weight (g m^{-2}) was not affected from about 6 to 40 DAA when plants were treated at 40°C (HT).

The low seed weight with HT at -3-0 DAA agrees with Wu et al. (2016), where HT at heading caused a lower 1000 grain weight due to reduced photosynthesis and reduced sink size (Makino et al., 1994). Kim et al. (2011), who reported that early seed development was the most critical phase of damage by HT, as at this stage cells were actively multiplying, thus HT limited grain growth by inhibiting subsequent dry matter translocation (Shi et al., 2016).

Electrical conductivity (E.C.) has been used as a possible method for measuring viability and vigour of seedlings in rice and other crops. In my present study, *japonica* rice cv. Gleva was not influenced by elevated HT imposed during seed development

and maturation, by using electrical the conductivity test (Fig. 3.1c) showed no significantly difference between treatment (Appendix 3.1). In contrast, Zhoa et al. (2009) showed in their study that E.C. was suitable to evaluate sweet corn viability as E.C. was can indirectly determine seed membrane integrity of different quality seed lots. It also been listed in the International Rules for Seed Testing (ISTA, 1995) as a testing method for pea. High value of E.C. indicates more leaching of sugars with later increase in seed age and increased injury to endosperm (Abdul-Baki & Anderson 1970). Once the membrane stability is compromised, deterioration is enhanced. However, it can be suggested here that, E.C test was not suitable to evaluate seed viability in rice, even though there are some previous studies where this test was a success for example in some cotyledon spp. such as mungbean (Onwimol et al., 2016). As E.C. test did not give any difference among treatments, this test was not used in later experiments.

Seed viability also depends on harvest time, particularly seed harvest moisture content (Olasoji et al., 2012; Demir et al., 2008) with high seed viability associated with seed desiccation (Ellis, 2011). Ability to germinate *ex planta* in this study after HT treatment was significant was affected was in terms of the maximum germination capacity of fresh seed (Appendix 3.3) but not in dried seed (Appendix 3.4). Almost all treatments for both seeds provided close to 100% ability to germinate normally except for fresh seeds at (-3-0 DAA) with only 69%. The significant effect occurred in fresh seed perhaps because of seed moisture content was considerably high during harvest at that period (-3-0 DAA). This support Ellis (2011) conclusion that low moisture content at harvest will produce high seed viability.

Any effects of HT on seed quality development also depends on species and cultivar, level and duration of stress imposed. To support this, Jagadish et al. (2007) had reported that upland *japonica* rice cv. Azucena showed that high temperature had a significant interaction with temperature duration. For instance, temperature >35℃ at anthesis for five days resulted in sterile spikelets and complete failure seed production. Moreover, Madan et al. (2012) reported that grain yield in hybrid *indica* rice was affected by loss of their superior performance at a temperature of 38℃ for five days during flowering. The present study revealed during the period of 3-6 DAA onwards, seed were less sensitive to short duration 3-days HT than the earlier. This was supported by Martinez-Eixarch and Ellis (2015) who reported that, seed viability in Gleva was significantly reduced by 7-d exposure to $38/34^0$C at 1 to 7 and 1 to 14 DAA.

Seed longevity in air-dry storage is a good, sensitive indicator of differences in seed quality among high viability seed lots (Ellis and Roberts, 1981). In this study, the seed lot which produced a survival curve with a lowest estimate of p_{50} was that for -3-0 DAA HT treatment (Table 3.1) with final moisture content at harvest (42 DAA) was 37.2% (Fig.3.1a). Meanwhile, seed obtained from plants stressed at 21-24 and 24-27 DAA showed almost similar estimates of longevity with the control. The present result thus supports the conclusion of Ellis (2011), where little or no deleterious effect to subsequent seed storage longevity when plants were exposed to more than 28^0C from $2/3^{rd}$ through the seed filling phase (late in seed development).

It is noticeable also, potential longevity considerably coincides with reduction in seed moisture content at harvest (42 DAA) from -3-0 until 3-6 DAA (Fig.3.1a). Present study showed that once seed desiccate from mother plant from 37.2 to 26. 2% seed longevity improve from 10.6 to 22.2 d. whilst, this might indicate that there is relationship between seed longevity improvement and harvest moisture content. Indeed, this results strongly support by Whitehouse et al., (2015) which reported that rice seeds harvested at a moisture content where they are still metabolically active (≥16.5%) are considered to continue their desiccation phase and therefore able to improve longevity. In *Brasicca* for example, the attainment of maximum potential longevity coincided with the reduction in seed moisture content by maturation drying *in planta* to 6-7% (Sinniah et al., 1998a).

In terms of $p_{50,}$ values, the estimates were variable between the third HT treatment at 3-6 DAA and eight at 18-21 DAA (Fig. 3.4). Nevertheless, there is a tendency for them to be between the extremely poor values at -3-0 DAA and 0-3 DAA and the best values in the control and for HT from 21-24 or 24-27 DAA. This is broadly consistent with earlier conclusive that *japonica* rice seed quality was most vulnerable to damage from high temperature dose to anthesis and least vulnerable in late seed filling and thereafter (Ellis, 2011; Martinez-Eixarch and Ellis ,2015). In addition, it was suggested by Cromarty et al. (1982) that seed with high moisture content at harvest are easily seed easily damaged by high temperature.

3.7 Conclusion

In conclusion, well-irrigated plants of *japonica* rice cv. Gleva were able to tolerate 3-days HT treatment during late seed development period, whereas HT imposed around anthesis reduced seed quality. In many field environments, however, plants are subjected to HT and drought stress simultaneously. This is explored in a later experiment.

CHAPTER 4

The effect of drought during seed development and maturation on subsequent rice seed quality (cv. Gleva)

4.1 Introduction

In Experiment 1 (Chapter 2), I showed that terminal drought before the end of the seed filling phase had a substantial effect on the subsequent pattern of increase and then decline in both seed viability and seed longevity during seed development and maturation. Those results suggested that early plant senescence, from drought, accelerates the development of seed desiccation tolerance and longevity, but longer periods of development with irrigation throughout resulted in higher seed quality ultimately. In addition, seed deterioration on the mother plant began to accumulate in the drought treatments once seed moisture content declined to about 16%.

Drought escape by plants usually involves earlier maturity to avoid the most severe period of water scarcity whereas, generally, this adjustment to severe environmental stress limits crop yield especially when it occurs during anthesis and seed filling (Seiler et al., 2011). In addition to its effects on the physiological processes in plants, drought stress during seed development may reduce subsequent seed germination and vigour. As introduced in Chapter 2, the effects of drought on seed quality reported vary amongst different, and perhaps also within, crops (e.g. Yaklich, 1984; Sinniah et al., 1998a)

4.2 Objectives

This study investigated changes in seed quality, including subsequent seed storage longevity, in rice cv. Gleva during seed development and maturation under well-watered conditions or with terminal drought during late seed filling and late maturation drying (i.e. later than in Experiment 1) in order to determine when maximum seed quality was attained and when seed deterioration begins on the mother plant, and the influence of drought on the above.

4.3 Hypotheses

The hypotheses for this study were as follows:

4.3.1. Ending irrigation towards the end of seed filling or late in maturation drying reduce the progress of seed filling and desiccation and maximum seed weight but accelerates the development of ability to germinate in the former but not the latter case;

4.3.2 Ending irrigation towards the end of seed filling or late in maturation drying accelerate the pattern of changes in subsequent longevity, and in the initial improvement and then subsequent decline;

4.3.3 Seed deterioration will be detected *in planta* in all treatments once seed moisture content declines below 16% but will be avoided if maintained above 16-20%.

Hypotheses 4.3.1-4.3.3 relate to my general hypotheses 1.7.1-1.7.3 in relation to drought, but also consider effects on the temporal pattern of seed development and of seed quality development and possible subsequent decline. Also, in relation with Chapter 2 however, in this chapter the hypotheses are tested by drought later in seed development (but with a common treatment for comparison).

4.4. Materials and Methods

Experiment 3 began on 21st May 2016 and plant growth and seed production continued over the summer until the last harvest on 28th September 2016 in a controlled environment photoperiod glass-house at the Crop Environment Laboratory (CEL), Whiteknight Campus, University of Reading (51º 26' N latitude, 0º 57' W longitude).

4.4.1 Seed selection

Seeds of cv. Gleva were multiplied from additional plants grown in the control environment of Experiment 2 for this investigation. Seed were stored dry in a cold room (2-4 ºC) in laminated aluminium foil bags, before use, as in previous experiments. They provided more than 90% germination in tests before sowing (Plate 4.1).

Plate 4.1: Germination test for seed viability; incubated seed of rice cv. Gleva, produced in 2015, after 6 days in test at 34/11 ^{0}C (16h/8h)

4.4.2 Growing medium

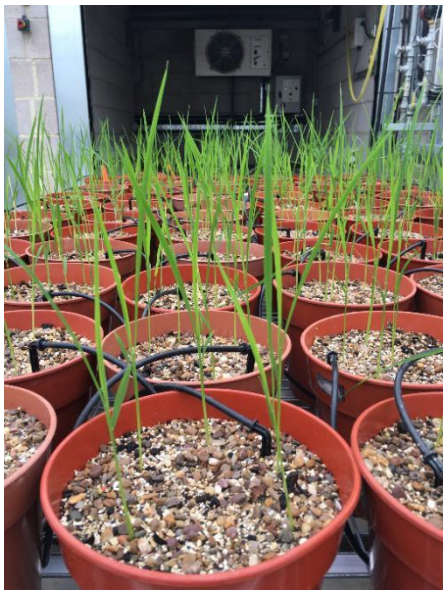
Three litre re-useable plastic pots were filled with the same growing medium as previous experiments and 0.495 kg of the slow-release fertilizer (Osmocote Pro 3-4M, ICL, UK) added at about 3kg/m^3. Pots were three-quarter filled, wetted with tap water, and then left to drain for 24 h.

4.4.3 Plant growth

Seven seeds were sown in each pot and the pots placed on one of four trolleys in the glasshouse dark compartment at 20 °C for germination to occur. Each trolley was 2.2 x 1.4 m and held 63 pots comprising one block. Four trolleys (blocks) in total held 252 pots. Watering by hand started at 3 DAS and was carried out daily.

About 90% of seed had germinated at 13 DAS and the plants were then exposed to full sunlight (Plate 4.2(a)). The temperature in the glass house was controlled at 28/20 ^{0}C day/night (12h/12h) while relative humidity was maintained at about 70-80% by watering the floor of the glasshouse regularly (depending on the forecast). The trolleys automatically began to move out from dark compartment into the glasshouse at 7 a.m. and moved back in to the dark compartment at 7 p.m. to provide a photoperiod of just over 12 h d^{-1} synchronous with thermoperiod.

(a)



(b)

Plate 4.2: (a) Seedlings exposed to sunlight from 13 DAS showing trolleys and dark compartments; (b) Start of drip irrigation at 17 DAS showing irrigation looms.

Plants were provided with drip irrigation (Plate 4.2(b)) from 17 DAS with water (without nutrient) at a pH of 4.5 to 5.5. They were irrigated 7 times per day for three minutes each time from 8 a.m until 7.10 p.m. Plants were thinned to four strong plants per pot at 17 DAS.

4.4.4 Irrigation treatments

For treatment 1 (T1), plants were irrigated throughout the experiment (control); for T2 and T3 irrigation was stopped at 14 DAA or 28 DAA, respectively (i.e. in late seed filling and mid-maturation drying, respectively). All pots (whichever treatment combination) were completely randomized in terms of their location within each trolley.

4.4.5 Flowering

Flowering commenced 65 DAS (25th July 2016) and panicles were marked at 4 to 6 days intervals using sticky tags on the leaves. At 76 DAS (5th August 2016) plants achieved 50% flowering (0 DAA). Only seeds produced from panicles that anthesed between 70-81 DAS, inclusive, were included in the study in order to minimize this source of variation.

4.4.6 Insecticide

At the beginning of flowering, some plants were infested by red spider mites. Hence, the insecticide spray Matador (Koppert B.V., The Netherlands) with Fenazaquin at 1 ml per litre and Savona (Dow Agrosciences Ltd, U.K) with 10 ml per litre fatty acids was applied at 75 and again at 82 DAS.

4.4.7 Harvesting

Serial harvests of plants began at 13 DAA and continued at 6-8 days intervals on seven dates, until 55 DAA. Twelve plants (3 pots) in each block were harvested from each treatment on each date. Seed harvest procedures and all laboratory tests on seeds were carried out as described in Experiment 1.

4.4.8 Statistical Analysis

GenStat 17th edition (2015, VSN International Ltd.) was used to analyse data, as described in Experiment 1.

4.5 Results

4.5.1 Seed desiccation, filling and ability to germinate

The different irrigation treatments affected seed desiccation *in planta* considerably but had little effect on either seed dry matter accumulation or the development of ability to germinate normally (Fig. 4.1).

Seed moisture content showed significant differences due to either irrigation treatment, or harvest date, or their interaction (Appendix 4.0). Not surprisingly, there were no differences amongst treatments at 13 DAA, i.e. before drought was imposed, but later harvests provided significant differences (Appendix 4.1). Differences in the patterns of decline in moisture content (Fig. 4.1a) corresponded with the different irrigation treatments. When irrigation ended at 14 or at 28 DAA, seed moisture content declined rapidly soon afterwards and by 34 DAA it had stabilized at 12-15% in both these treatments. Where plants were irrigated throughout, seed moisture content declined only slightly, reaching 25% at the final harvest (55 DAA).

According to Tukey's multiple comparison test, seed moisture content in the control was significantly greater than both drought treatments from 4 (14 DAA treatment) or 6 (28 DAA treatment) days after irrigation ended onwards (Appendix 4.2).

The main effect of irrigation treatment, regardless of harvest date, was significant (Appendix 4.3): the highest mean moisture content was 26.4% for the control followed by irrigation ended at 28 DAA (20.3%), and finally 14 DAA (17.6%) (Appendix 4.4).

Seed dry weight presented similar trends for each of the treatments, including the control (Fig. 4.1b). Statistically, irrigation treatments showed no significant difference as a main effect but significant differences occurred amongst harvest dates, and both main factors interacted (Appendix 4.5). Considerable seed filling had already occurred before the first harvest at 13 DAA and seed filling was probably completed in all treatments at around 18 DAA or shortly afterwards (Fig. 4.1b). Mean seed dry weight varied amongst treatments only for harvest at 13 and 55 DAA (Appendix 4.6), and amongst harvests within treatments only in irrigation ended at 14 DAA (Appendix 4.7). Ending irrigation at 14 DAA resulted in lighter seed at (only) the first and last harvests (Appendix 4.8). These differences were negligible, however.

Ability of seeds to germinate normally *ex planta* after drying provided a very similar pattern during seed development and maturation amongst all three treatments (Fig.4.1c). Ability to germinate normally was already high at 13 DAA (>75%) and improved thereafter

(P<0.001, Appendix 4.9) reaching full (99-100%) ability to germinate normally in all treatments in later harvests (42 – 55 DAA).

Angular transformation of the data was used for ANOVA and Tukey's multiple comparison for this particular parameter. There was a significant main effect of irrigation treatment and harvest date (Appendix 4.9). In the latter case, the significant effect was detected for seed harvested at 27, 34 and 42 DAA (Appendix 4.10.1-3). In addition, the interaction between irrigation treatments and harvest date was significant (P<0.05, Appendix 5.9), with the 14 DAA drought treatment providing greater ability to germinate than the control at 27 and 34 DAA. As noted above, the differences were small and none were detected in later harvests.

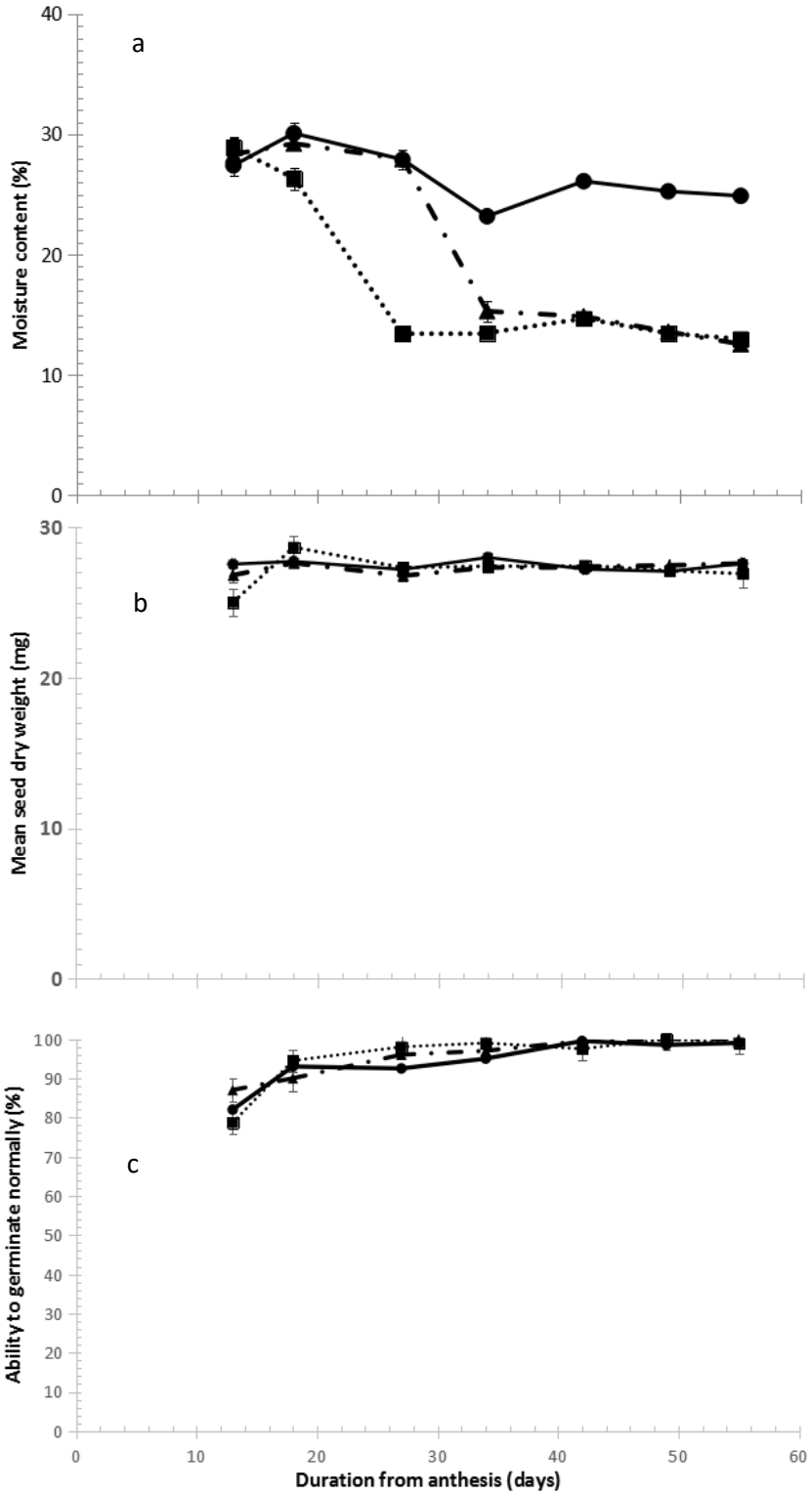
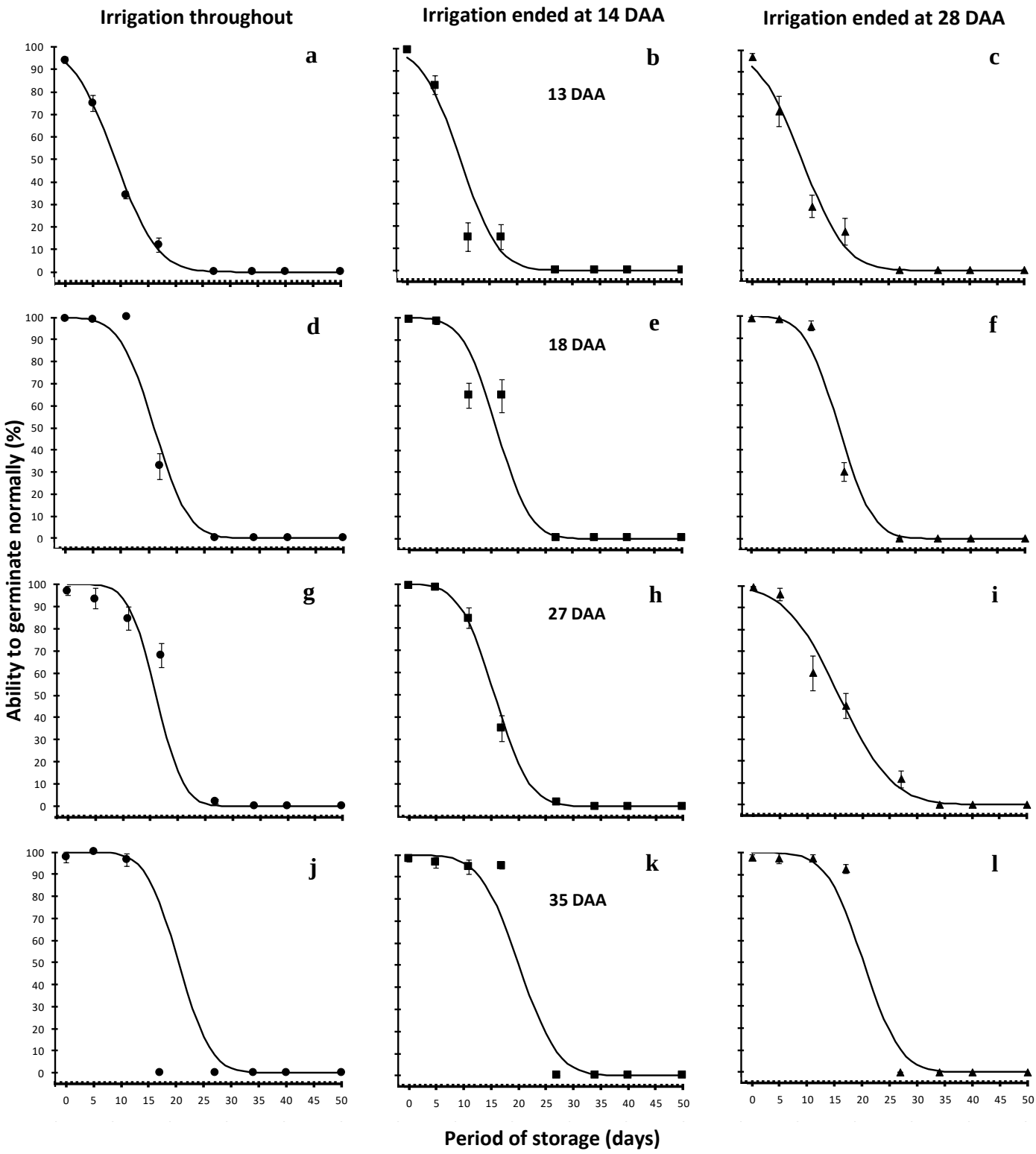


Figure 4.1: Moisture content (a), dry weight (b), and changes in viability of dried (15±0.5% moisture content) seed, assessed by ability to germinate normally in standard tests at 34/11° C (c), for seeds of *japonica* rice cv. Gleva harvested serially during their development from plants grown in a photoperiod glasshouse at 28/20 ^{0}C, (12h/12h), 12 h d^{-1} photoperiod with irrigation throughout (●), irrigation ended at 14 (■), or at 28 DAA (▲).

4.5.2 Seed storage survival

Seed survival in hermetic storage of the different irrigation treatments harvested at different periods throughout seed development generally conformed well to negative cumulative normal distributions (Fig. 4.2): good fit was provided in most cases (e.g Fig.4.2a, h, o) with acceptable but poorer fit in a few cases (e.g. Fig. 4.2e, j, p). Comparison of these curves within each harvest from 13 to 34 DAA showed no significant differences amongst irrigation treatments, but survival periods varied with harvest date (Appendix 4.13). Longevity differed amongst irrigation treatments but only after 34 DAA (Appendices 4.13). Besides that, seed survival curves for each block separately (84 curves) also have been analysed. Little variation occurred amongst the four blocks within a treatment at one harvest date (Appendix 4.14).


Irrigation throughout
Irrigation ended at 14 DAA
Irrigation ended at 28 DAA
a
b
c
13 DAA
d
e
f
18 DAA
g
h
i
27 DAA
j
k
l
35 DAA
Ability to germinate normally (%)
Period of storage (days)

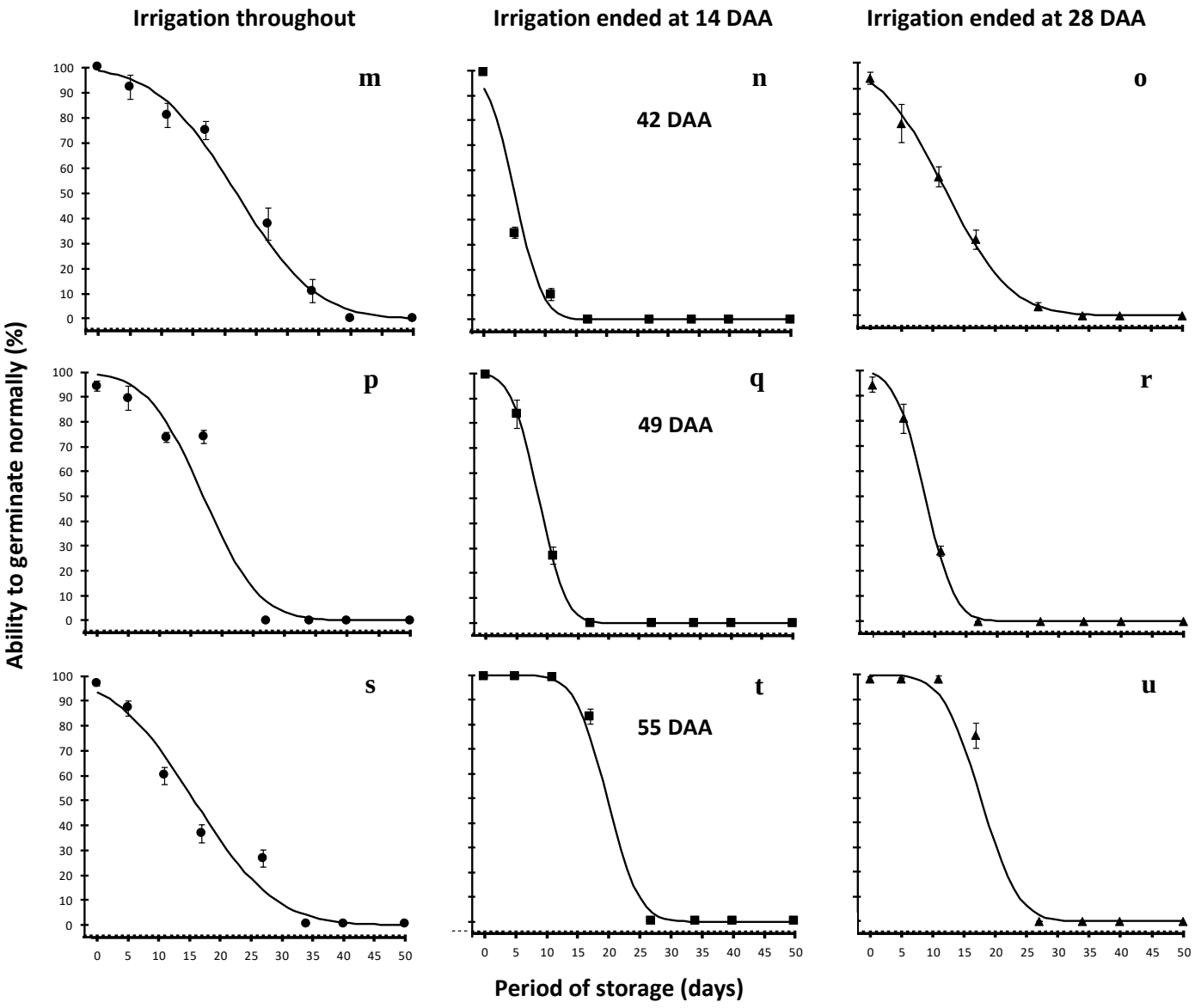


Figure 4.2. Loss in viability (estimated by change in ability to germinate normally) of seeds of *japonica* rice cv. Gleva harvested serially from 13-55 DAA during seed development in a photoperiod glass-house (28/20^{0}C; 12h/12h, 12 h d^{-1} photoperiod) with irrigation throughout (●-column 1), or ended at 14 (■-column 2) or at 28 DAA (▲- column 3) and stored hermetically at 40℃ with 15.0±0.5 moisture content. Results are means of four seed lots, each from separate blocks (for individual seed lot survival curves see Appendix 5.12): vertical bars are ±s.e. where larger than symbols. Best fit survival curves for each treatment combination fitted by probit analysis are shown. These are quantified in Table 5.1.

Table 4.1. Longevity (parameters of seed viability equation fitted by probit analysis) of *japonica* rice cv. Gleva harvested serially during their development from plants grown in glass-houses. Plants were irrigated throughout, or until 14 DAA or 28 DAA. Seed in hermetic storage at 40° C with 15.0 ± 0.5% moisture content. Results for four blocks combined. See Appendix 4.14 for the moisture contents of each seed lot from each block in storage. The 95% confidence intervals are shown for $p_{50.}$

Treatment	Harvest (DAA)	K_i		Slope(1/σ)		p_{50} (days)			
		estimate	s.e	estimate	s.e	estimate	s.e.	lower 95%	upper 95%
Irrigation throughout (T1)	13	1.49	0.14	0.164	0.013	9.1	0.47	8.2	10.0
	18	4.07	0.41	0.254	0.027	16.0	0.43	15.3	16.9
	27	2.50	0.17	0.143	0.010	17.5	0.55	16.5	18.7
	34	4.39	0.35	0.215	0.017	20.4	0.49	19.5	21.4
	42	2.19	0.14	0.100	0.006	21.9	0.65	20.6	23.1
	49	2.39	0.17	0.141	0.009	16.9	0.55	15.9	18.0
	55	1.52	0.11	0.097	0.007	15.7	0.67	14.4	17.0
Irrigation ended at 14 DAA (T2)	13	1.76	0.15	0.182	0.0142	9.6	0.44	8.8	10.5
	18	2.62	0.19	0.160	0.012	16.4	0.52	15.4	17.4
	27	3.10	0.26	0.199	0.017	15.6	0.47	14.8	16.6
	34	3.58	0.26	0.179	0.013	20.1	0.52	19.1	21.1
	42	1.45	0.16	0.285	0.026	5.1	0.34	4.4	5.8
	49	2.49	0.23	0.290	0.025	8.6	0.34	7.9	9.3
	55	4.83	0.41	0.245	0.022	19.7	0.47	18.9	20.7
Irrigation ended at 28 DAA (T2)	13	1.45	0.13	0.157	0.012	9.2	0.48	8.3	10.1
	18	3.85	0.38	0.248	0.025	15.5	0.42	14.7	16.3
	27	2.03	0.14	0.129	0.008	15.7	0.57	14.6	16.8
	34	3.83	0.29	0.189	0.014	20.3	0.51	19.3	21.3
	42	1.44	0.12	0.120	0.008	12.0	0.57	10.9	13.1
	49	1.90	0.17	0.233	0.019	8.2	0.38	7.4	8.9
	55	3.70	0.31	0.210	0.018	17.7	0.48	16.8	18.6

4.5.3 Longevity (p_{50})

The control treatment provided a consistent pattern of increase in subsequent seed longevity (p_{50}) during seed development and maturation from 13 to 42 DAA (Fig. 4.3). Maximum longevity at 42 DAA was 21.9d. Thereafter, longevity declined consistently in the control to only 15.7d at 55 DAA. The two drought treatments (irrigation ended at 14 or 28 DAA) presented an almost identical pattern of increase in subsequent seed longevity to each other and to the control from 13 to 34 DAA (Fig. 4.3, P>0.05, Appendix 4.13). At 34 DAA, the three irrigation treatments provided near identical values (P>0.05, Appendix 4.13) of 20.4 (control), 20.1 (irrigation ended 14 DAA), and 20.3d (irrigation ended 28 DAA) for p_{50}. In contrast to the further improvement in the control, longevity in the two drought treatments declined substantially immediately thereafter to values between 4.1 and 12.0 days at 42 and 49 DAA. Surprisingly, however, longevity in both drought treatments improved substantially and consistently between 49 and 55 DAA with all three treatments providing significant differences in longevity at 55 DAA and the control providing the lowest value (P<0.05, Appendix 4.13).

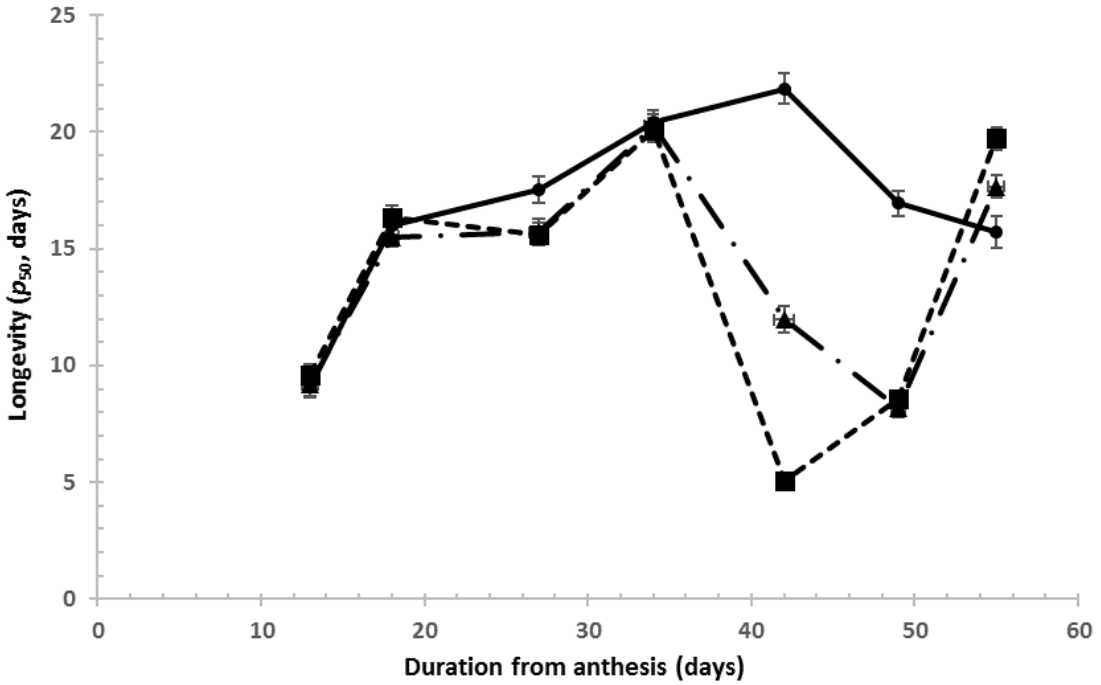


Figure 4.3: Longevity (p_{50} provided by parameters of seed viability equation fitted by probit analysis) of seeds of japonica rice cv. Gleva harvested serially during their development from plants grown in a photoperiod glass-house and stored hermetically at 40℃ with 15.0±0.5 moisture content; vertical bars are ±s.e. where larger than symbols. Plants were irrigated throughout (●), or until 14 (■), or 28 DAA (▲). Each observation derived from results from 4 blocks. The estimate of p_{50} is provided by the best fit curve for each treatment combination (Table 4.1).

During this experiment, the temperature in the glass house was set at 28/20 ^{0}C day/night (12h/12h). The minimum and maximum temperatures varied and were greater and lower an average than the set values (Appendix 4.15).

4.6 Discussion

Drought treatments were imposed later in Experiment 3 than Experiment 1, towards the end of seed filling (T1) and late in maturation drying (T2) and seed harvests were continued well beyond maturity and normal harvest times (to 55 DAA). After seed filling ends, seed enter the ripening phase, where they gradually become hard and turn yellow. Maturation drying phase can represent as little as 19% or as much as 78% of total seed development time (LePrince et al., 2017). Cereal seed are usually at <15% moisture content when dried naturally and then harvested (Bewley, 1986). This was the case for the two drought treatments with rice here, whereas the continuous irrigation treatment provided a greater moisture content. The later value was within the range reported by Whitehouse et al. (2015) for paddy field harvested rice.

At the end of this investigation, the hypotheses set regarding the effects of ending irrigation at the end of seed filling (14 DAA) or in late maturation drying (28 DAA) during seed development and maturation which were related with general hypotheses 1.7.1-1.7.3 can now be answered: -

1. Ending irrigation early resulted in earlier and greater seed desiccation but did not affect the seed filling process and maximum seed dry weight, accelerated the development of ability to germinate of dried seed very slightly but no decline was later detected in any irrigation treatment despite harvest being extended to 55 DAA.
2. Ending irrigation early did not accelerate the pattern of development in subsequent longevity air-dry seed storage longevity, compared with the control, but between 34 and 42 DAA (the normal harvest date) the plant drought treatments resulted in a decline in seed longevity whilst the control continued to improve: and as a result, the control treatment harvested at 42 DAA provided the greatest seed longevity of all treatment combinations; with the two drought treatments providing poorer maximum quality than control.
3. Seed deterioration did occur in both drought treatments when seed moisture content *in planta* was below 16%, but improvement in seed quality was also detected at <16% between 27 and 42 DAA (14 DAA treatment) or 34 and 42 DAA (28 DAA) and again later between 42 and 55 DAA (14 DAA treatment) or 49 and 55 DAA (28 DAA treatment); maintaining seed moisture content *in planta* at between 16-20%, did not enable seed deterioration *in planta* to be avoided: the control was always above this range, but deteriorated after 42 DAA.

4.6.1 Drought effect on seed desiccation and seed filling

The moisture content of seed decreased with the delay in the date of harvesting. The present study showed that seed moisture content was reduced to a minimum of 13.4 % and 15.3% in the drought stress treatments. Also seed desiccation occurred earlier and was greater when plants were subjected to drought. This finding is supported by, Sinniah et al. (1998) for *Brassica* and Siddique and Wright (2003) for pea. Seed desiccation and filling patterns showed that seed had moved from the development to the maturation phase from around 18 DAA and hence reduced seed metabolic activity.

The process of rice grain filling, the accumulation of reserve nutrients in the developing and maturing grain, is sensitive to environmental conditions affecting final yield quantitatively and qualitatively (Yang and Zhang, 2006). Dasgupta et al. (2015) reported that, drought stress imposed for *indica* rice eg cv. IR36 during seed filling had a more pronounced effect than in the flowering stage and affected grain filling. However, in my study drought beginning during late seed filling (14 DAA) had no impact on seed filling.

4.6.2 Drought effect on seed dry weight

Maximum seed dry weight (mass maturity) was obtained 4 days after drought began (14 DAA), only one day earlier than the 28 DAA treatment. This result implies that dry matter accumulation was largely completed before the stress began to be applied (14 DAA) and completed at about around 18 DAA (Fig. 4.1b). Seed filling occurred earlier than in the previous study probably due to higher day temperature in the photoperiod glass-house (Appendix 4.15). Present results, showed that seed dry weight was not affected by both drought treatments imposed, control was 27.6mg meanwhile the other stresses were respectively 26.9mg and 27.6mg. This study is supported by Sikuku et al. (2010), where NERICA 2 rice was not affected by water limitation as it is an early maturing variety. In addition, this circumstance might be due to deposition process of food storage reserves was completed before treatment been imposed. Ellis (2011) reported that non-impact on mean seed weight suggest no effect on yield however, seed quality more sensitive indicator than yield for climate change impacts on crops in real fields.

4.6.3 Drought effect on ability of air-dried seed to germinate normally

Most seeds were already able to germinate normally before drought treatment were imposed (Fig.4.1C). The general temporal pattern of development in the ability of seeds to germinate during seed development and maturation was relatively as expected in the creals; rice (Ellis et al., 2011) and wheat (Yadav and Ellis, 2016). Onset of ability to germinate and of desiccation tolerance was apparent from the first harvest (13 DAA) early in the seed-filling phase (Fig. 4.1C) at which time wheat seeds were only about 10% filled (Yadav and Ellis, 2016). Ability to germinate normally was achieved fully about 75% through the seed-filling phase and so well before mass maturity (Ellis and Pieta, 1992).

4.6.4 Drought effect on subsequent longevity

This study showed that, seed longevity was improved by drought stress during late seed filling and subsequent by during the maturation phase (Fig.4.3). Hence, longevity continued to improve after mass maturity. This supports the conclusions of Ellis and Pieta (1992) in wheat and most environment by Ellis et al. (1993) in which longevity continued improving in rice after maximum seed dry weight (mass maturity) was achieved and until harvest maturity. Drought stress reduced the period over which this improvement occurred, however, such that the control at 42 DAA (close to harvest maturity) provided the greatest longevity (Fig. 4.3). Surprisingly, after the drought treatments, longevity declined greatly (at 42 or 49 DAA) but then their longevity subsequently improved. The late improvement in longevity in both drought treatments is difficult to explain.

In this study, seed survival in hermetic storage of all treatments generally conformed well to negative cumulative normal distribution (Fig.4.2). It well known that viability declines gradually during the early stages of seed storage, later in storage, there is a sharp decline, until the seed only produces a weak seedling that quickly succumbs to hostile environments for example.

4.6.5 Seed deterioration in planta above and below 16% moisture content

There was some evidence to support the suggestion that seeds did not deteriorate *in planta* until their moisture content had declined below 16%. For example, in the drought treatments longevity declined between 35 and 42 DAA when seed moisture content was about 15% whilst in the control longevity improved over this period when moisture content was 26.1% (Fig. 4.3). However, there were contradictory observations where seeds deteriorated above 16% moisture

content and improved below 16% moisture content here. Whitehouse et al. (2015) reported rice at >16.2% moisture content improves considerably in subsequent air-dried longevity when subjected briefly to 45℃. Hence the hypothesis of seed deterioration detected once moisture content fell below 16% are not accepted.

Hypothesis 4.6 can be accepted: the control had the longest period of development and provided greater maximum seed longevity to the drought treatments. The stage of seed filling and maturity at harvest is the vital factor that influences seed longevity and seed establishment. Water deficit during grain filling effect grain affects quality of rice (Yang et al., 2003) and wheat (Saeedipour and Moradi, 2010).

The hypothesis constructed that seed deterioration will be detected *in planta* in all treatments once seed moisture content declines below 16% can be accepted up to a point. Seed deterioration occurred in the two drought treatments after seed moisture content fell below 16% (Fig.4.1a), in terms of 35-42 or 35-49 DAA (Fig. 4.3) but not after only during at 42 and 49 DAA respectively.

However, in the 14 DAA drought treatment longevity improved between 27 and 34 DAA despite moisture content below 16%, whilst improvement also occurred 49- 55 DAA. Meanwhile, in the 28 DAA drought treatment, longevity improved with the same period as 14 DAA with moisture content at 15.3% (below 16%). This study suggested that, particularly after normal harvest date (49 to 55 DAA), irrigation accelerated seed deterioration in contrast to results under drought stress. Delaying harvest increases the risks of seed deterioration in the field (Bewley, 1986). This statement is contradicted by the present study for drought stress but not the control treatment in a glasshouse environment.

4.7 Conclusion

Terminal drought during seed development and maturation accelerated seed desiccation, but the later drought treatments used here had little effect on seed filling or the development of ability to germinate after desiccation. The plant drought treatments did affect the development of subsequent seed longevity, however. Terminal drought treatments resulted in poorer maximum seed longevity, and also subsequent longevity declined *in planta* earlier in the control. Whilst delayed harvest resulted in a decline in longevity in the control, a surprising observation was a second prior of increase in the two drought treatments such as at 55 DAA all three treatments showed similar longevity.

CHAPTER 5

Effect of elevated temperature and drought singly and combined during seed development on seed quality of *indica* rice cv. Aeron 1 and *japonica* cv. Gleva

5.1 Introduction

Indica rice is grown in the tropics and sub-tropics, and these regions provide 80 % of the world's production (Ricepedia, 2018). Rice in these regions is susceptible to damage from periods of high temperature, particularly during anthesis (Jagadish et al., 2007, 2010b). The increase in temperature and drought; resulting from climate change (Korres et al., 2017) may adversely affect both the quantity and quality (seed germination and vigour) of seeds produced worldwide, and these two stresses commonly coincide. However, Krishnan et al. (2011) concluded that any effects of high temperature on seed quality development varied also with species and cultivar, as well as the level and duration of stress imposed.

Although short episodes of high temperature do not greatly affect mean temperature during the whole grain-filling period, they can affect both grain yield and quality in wheat (Stone and Nicolas, 1994), although Sanhewe et al. (1996) showed in wheat, within certain limits, that warmer mean temperature during seed development in the U.K. improved subsequent seed longevity. In contrast, in *Phaseolus vulgaris* L. in warmer conditions increasing temperature resulted in poor subsequent seed quality (Sanhewe and Ellis, 1996). Tolerance and avoidance of high temperature during seed development are potentially useful traits for rice breeding programs for future climates (IRRI, 2017).

The experiments reported in this chapter were planned from the results of the impact of drought (Experiment 1) and elevated temperature (Experiment 2) applied separately during seed development and maturation to the *japonica* rice cv. Gleva. In Experiment 2, only plants treated to high temperature ($40/30^0$C for 3 days) during early seed development (3 DBA (days before anthesis), during anthesis and early histodifferentiation) affected final moisture content and seed dry weight. This treatment also as provided seeds with lower seed longevity than those treated later during the maturation phase. Meanwhile in Experiment 1, terminal drought early in seed development accelerated all aspects of seed development and maturation, but also resulted in (earlier) seed deterioration later on.

In this experiment the effect of terminal drought and brief high temperature singly and in combination were investigated. *Indica* cultivars of rice are often more resilient to high temperature than the *japonica* rices (Ellis et al., 1993; Martinez-Eixarch & Ellis, 2015). Hence, two contrasting cultivars were used, *indica* and a *japonica*. As the cultivars differed in the timing of anthesis, two similar experiments were carried out, one with the *indica* and the other with the *japonica* rice, with similar hypotheses tested.

5.2 Objectives

This study investigated changes in seed quality, including seed viability and subsequent seed storage longevity, in the *indica* rice cv. Aeron 1 and *japonica* rice cv. Gleva in response to terminal drought and brief high temperature exposure, singly and combined, at different stages during seed development and maturation.

5.3 Hypotheses

The hypotheses for the investigations within each cultivar were as follows:

5.3.1 Final seed dry weight, seed moisture content and seed viability (ability to germinate normally of air-dried seed) at harvest (42 DAA) is lowest by either a single period of high temperature (HT) at anthesis or during the seed filling phase, or terminal drought (TD) during the seed filling phase, or both these high temperature and drought stresses combined (HT, TD);

5.3.2 Subsequent seed longevity in air-dry storage of seeds harvested at 42 DAA decline sooner by either a single period of high temperature (HT) at anthesis or during the seed filling phase, or terminal drought (TD) during the seed filling phase, or both these high temperature and drought stresses combined (HT, TD).

Hypothesis 5.3.1 relates to 1.7.1 general hypothesis, whilst 5.3.2. relates to 1.7.2. The whole investigation relates to general hypothesis 1.7.4, where both stresses (high temperature and drought) are applied singly and together.

From previous investigations my expectations were that cv. Gleva was more likely to be affected by these treatments than cv. Aeron 1, that the earlier stress was imposed the greater the effect, and that an interaction between the effects of elevated temperature and drought was likely with seed quality and size reduced further.

5.4 Materials and Methods

This experiment was started on 17th September 2016 and carried out in two controlled-environment growth cabs at the Crop and Environment Laboratory (CEL), Whiteknight Campus, University of Reading (51º 26' N latitude, 0º 57' W longitude).

5.4.1 Cultivars and seed selection

Two contrasting rice cultivars were selected: *indica* cv. Aeron 1 and *japonica* cv. Gleva.

5.4.1.1 cv. Aeron 1

Aeron 1 is an aerobic, high-yielding *indica* rice with early harvest maturity (Zainudin et al., 2014), cultivated in Malaysia. Seeds were provided by Malaysia Agriculture Research Development Institute, Pulau pinang, Malaysia in January 2016. They were then stored at UoR in a sealed laminated aluminum foil bag at 2-4 ℃. The seed was tested for viability similar to Experiment 2. The sample showed 85% germination, after five days in test at 34/11 ^{0}C (16h/8h) to break any dormancy (Ellis et al., 1983).

5.4.1.2 cv. Gleva

Seeds were multiplied from additional plants grown in the control environment of Experiment 2 for this investigation and stored dry in a cold room (2-4 ºC) in laminated aluminium foil bags. Testing at 34/11 ^{0}C (16h/8h) before sowing provided 100% germination.

5.4.2 Planting Medium

Growing medium was prepared in exactly the same way as in Experiment 3.

5.4.3 Plant Husbandry

For each cultivar, seven seeds were sown per pot on 17th September 2016 and placed in two modified Saxil growth cabs (Cabs 1 and 2) with internal dimensions 1.4 x 1.4 x 1.5 m. Each cab contained 24 pots of each variety.

Both cabs were maintained at 28/20 ^{0}C day/night (11h/13h thermoperiod synchronized with 11h photoperiod) at 75% (±10%) relative humidity. As will be discussed the actual relative humidity in both cabs declined greatly late in the investigation. Cool white fluorescent tubes provided about 710-740 µmol m^{-2} s^{-1} photosynthetic photon flux density at pot level.

Manual, daily watering began 3 days after sowing (DAS). After 10 DAS (27th September 2016), cv. Gleva achieved 100% seedling emergence in both cabs, whereas, cv. Aeron 1 achieved 90% and 82%.

At 14 DAS, weaker seedlings were thinned to leave 4 seedlings in each pot (Plate 5.1). Pots were irrigated (water without nutrient adjusted to pH 5.0-5.5) seven times per day for three minutes using an automatic drip system after three leaves had emerged at 16 DAS.

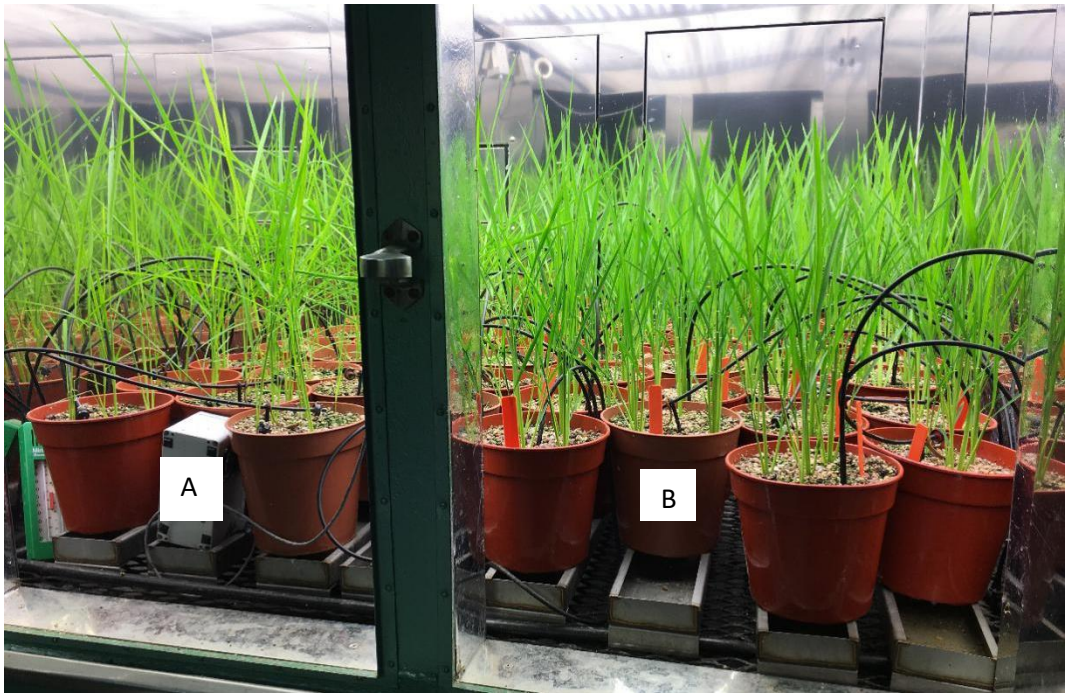


Plate 5.1: Seedlings of cv. Aeron 1 (A) and cv. Gleva (B) in a single controlled environment chamber after thinning at 26 DAS.

5.4.4 Drought and temperature treatments

Six treatments were applied: treatment 1 (T1) irrigated throughout the experiment (designated as control); treatment 2 (T2), plants with high temperature (HT) at 0-3 DAA; treatment 3 (T3) irrigation stopped at 14 DAA; treatment 4 (T4) combination of HT at 0-3 DAA and drought at 14 DAA; treatment 5 (T5), HT at 14-17 DAA with drought at 14 DAA; treatment 6 (T6) HT at 14 DAA. The 3-day high temperature treatment was 40/30℃ day/night (11h/13h thermoperiod synchronized with 11h photoperiod) with relative humidity controlled at 70%.

Cabinets 1 (cab 1) and 2 (cab 2) represented two different blocks. Meanwhile another one cab was used to expose plants to HT treatments 40/30℃ with an 11h/day photoperiod (11h/13h thermoperiod) (Plate 5.2). Plants were returned back to the original cab at the end of the 3-day HT treatment and remained there until maturity. Each variety comprised eight pots per treatment combination (four pots in cab 1 and four pots in cab 2). Treatments within a variety were completely randomized in terms of their location within a cab.

In both cultivars, irrigation ended at 35 DAA (7 days before final harvest, at 42 DAA) for those treatments not subjected to terminal drought at 14 DAA (T1, T2 and T6).

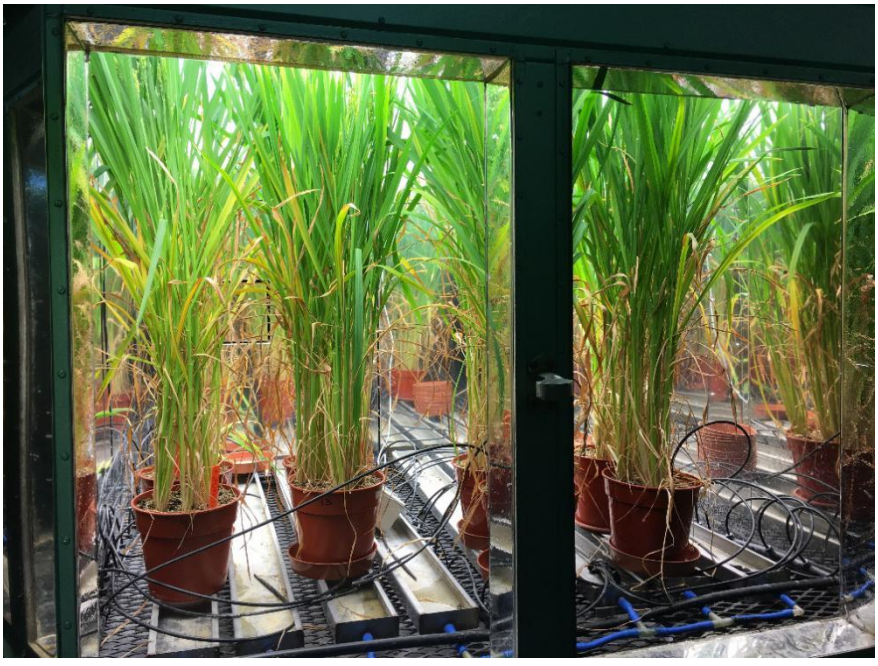


Plate 5.2: Plants (cv Gleva) exposed to HT (40/30℃) for 3-days at 0-3 DAA

5.4.5 Anthesis

The date of anthesis varied amongst cultivars. It also varied amongst cabs for cv. Gleva but not cv. Aeron 1. Cultivar Aeron 1 achieved 50% anthesis on 16th December 2016 (90 DAS). Cultivar Gleva achieved 50% anthesis in cab 1 on 10th January 2017 (114 DAS) but 8-days later (18th January 2017) in cab 2. It found that cv. Gleva was much longer period to anthesis than Aeron 1. Also, cv. Gleva in cab 2 showed longer to anthesis than in cab 1 (Appendix 5.9).

To limit the variation in panicle development amongst plants at treatment times in the experiment, panicles of cv. Aeron 1 that anthesed/exserted before 88 DAS and also those that anthesed after 95 DAS were cut and removed from the investigation (Appendix 5.8). Similarly, in cv. Gleva (cab 1 and 2) only panicles that anthesed 110-118 DAS (cab 1) or 118-126 DAS (cab 2) were included (Appendix 5.9). Thus, due to the different timing of anthesis between cabs, plants of cv. Gleva from cab 1 and 2 were treated at 40/30 ℃ at different times but same time as DAA.

5.4.6 Harvesting

Only one harvest was taken at 42 DAA. For cv. Aeron 1, this was 26th January 2017 for both cabs. For cv. Gleva in cab 1, this was 21st February 2017 and in cab 2 1st March 2017. Seeds were threshed gently by hand and empty seeds removed.

5.4.7 Data Collection

Drought and HT treatment, singly or in combination, in each of Aeron 1 and Gleva affected seed moisture content, final seed dry weight, and the ability to germinate normally at maturity (Figs. 5.1 & 5.2). All analyses were conducted separately as cultivar (Aeron 1 and Gleva) and by cab (Cabs 1 & 2) were treated as four separate experiments. For all three variables (final moisture content, seed dry weight, ability to germinate) in each cab and for each cultivar, significant ($P< 0.05$) effects of the treatments applied were detected (Appendices 5.0-5.3). Germination data was arcsine transformed before statistical analysis (Appendices 5.0C-5.3C). Each independent variable is discussed in turn below. Comparisons amongst treatments were made using the Tukey multiple range test.

5.5 Results

5.5.1 Seed moisture content

Final seed moisture content for Aeron 1 in Cab 1 was between 9.9-10.2% (Fig. 5.1a). Meanwhile, cab 2 was between 10.6-11.2% (Fig. 5.1d). This is a very narrow range. Despite this, significant differences were apparent: in cab 1, the control provided the driest seeds and HT at 0-3 DAA (with and without drought at 14 days) the greatest values; in cab 2, HT at 0-3 DAA (with and without drought at 14 days) and HT at 14-17 DAA provided the greatest values.

Moisture contents at harvest were lower for Gleva and also varied more (6.0-9.4% in cab 1; 7.8-9.2% in cab 2) amongst treatments (Fig.5.2a, d). The control was comparatively low in both cabs, but in Cab 1 (only) the result for HT 14-17 DAA with drought at 14 DAA was particularly low.

5.5.2 Seed dry weight

In both cabs and both cultivars, the control treatment provided the greatest, or equal greatest, seed dry weight (Figs. 5.1b, e; 5.2b, e). In cab 1, combined treatment of HT 0-3 DAA with drought at 14 DAA provided the lowest seed dry weight in each cultivar (Fig. 5.1b; 5.2b). In Aeron 1, all other stress treatments were similar to the control whereas in cv. Gleva all stress treatments were slightly less than control. More variation was apparent amongst treatments in Cab 2: all stress treatments provided reduced seed weight, with HT 14-17 DAA combined with drought at 14 DAA the lowest in both cultivars.

5.5.3 Ability to germinate

The control provided higher ability to germinate than stress treatments. In cv. Aeron 1 in cab 1 most treatments gave close to 100% germination, the exception being HT14-17 DAA combined with drought at 14 DAA where it was reduced (Fig. 5.1c). The remaining results were more variable, but in all three cases ability to germinate was lowest for HT 14-17 DAA combined with drought at 14 DAA (Fig 5.1f, 5.2c & f). Values for cv. Gleva in this regime were considerably lower than those for cv. Aeron 1 and results for cv. Gleva were also more variable across treatments than cv. Aeron 1.

Aeron 1

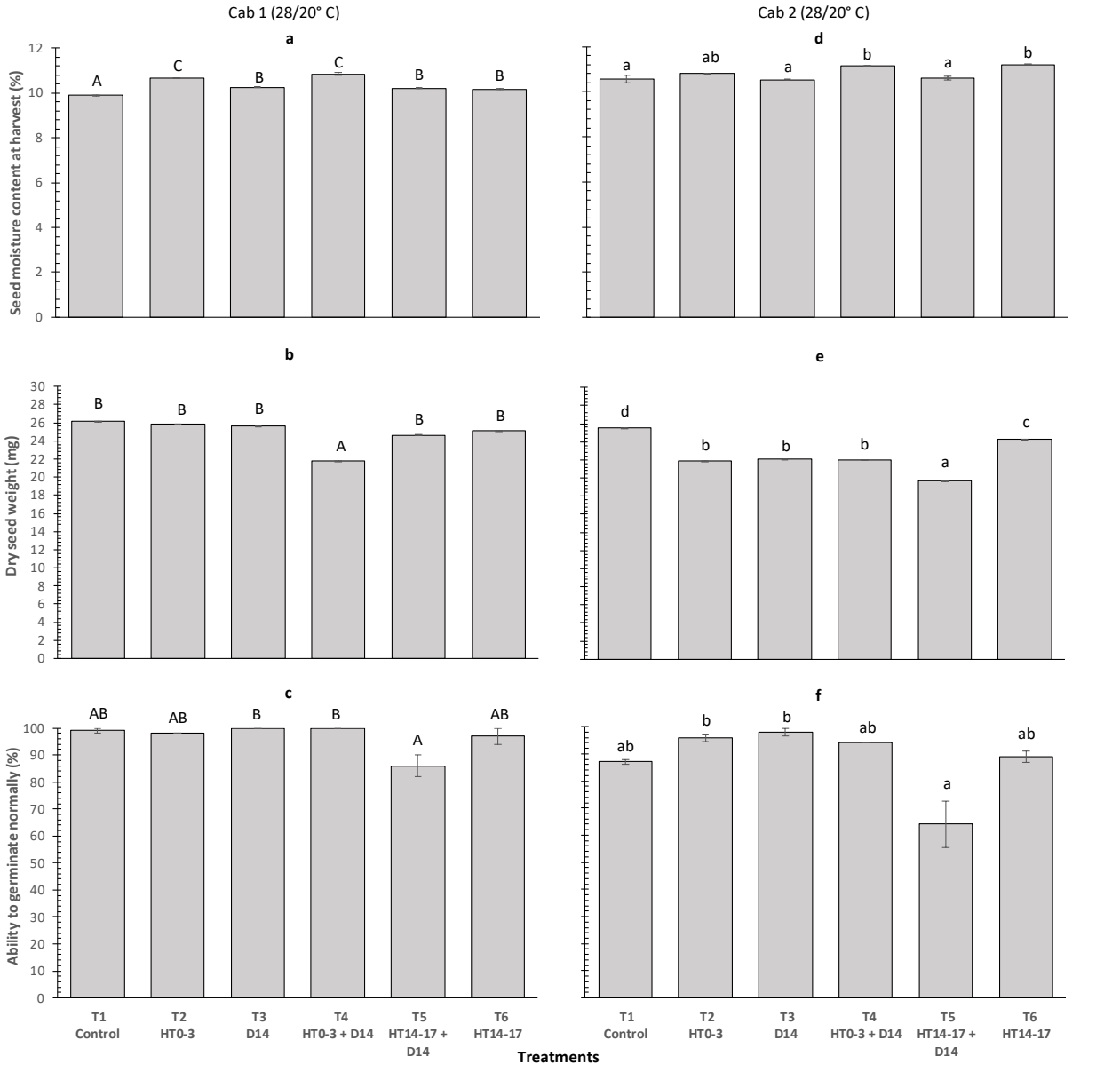


Figure 5.1: Moisture content (**a** & **d**), dry weight (**b** & **e**), and ability to germinate normally in standard tests at 34/11° C (**c** & **f**), for seeds of *indica* rice cv. Aeron 1 harvested at maturity (42 DAA) produced in a growth cabinet (Cabs 1 & 2) at 28/20 ^{0}C (12h/12h, 12 h d^{-1} photoperiod) with irrigation throughout (T1); plants with high temperature (HT) at 0-3 DAA (T2); irrigation stopped at 14 DAA (T3); combination of HT at 0-3 DAA and drought at 14 DAA (T4); HT at 14-17 DAA with drought at 14 DAA (T5); or HT at 14 DAA (T6). Different letters indicate significance difference at $P \leq 0.05$ amongst treatments (Tukey's multiple range test). Mean values within treatments in cab 1 with the different capital letter; and in cab 2 with the different small letter indicate significance difference at $P \leq 0.05$ amongst treatments using the Tukey's multiple range test.

Gleva

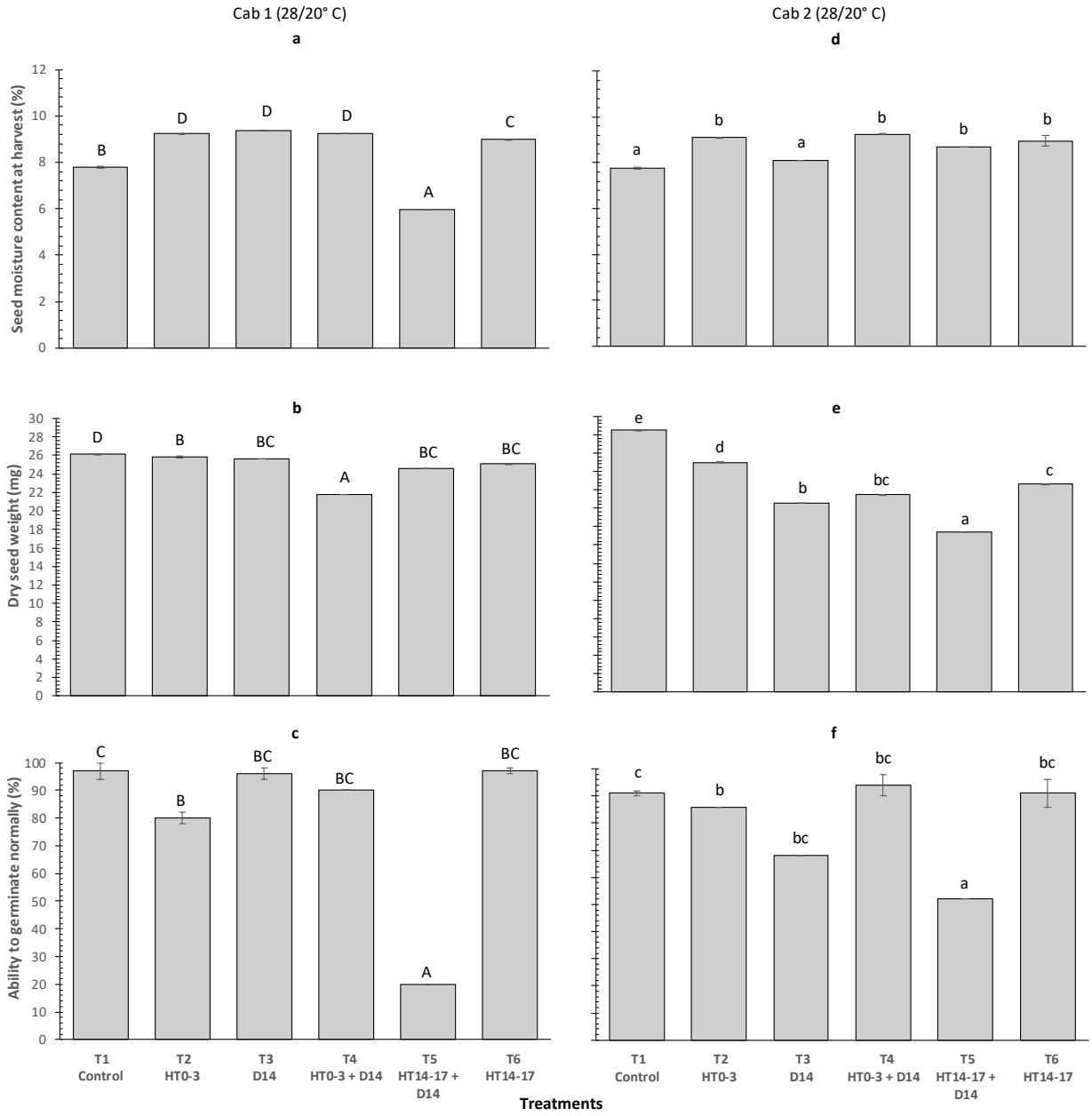


Figure 5.2: Moisture content (**a** & **d**), dry weight (**b** & **e**), and ability to germinate normally in standard tests at 34/11° C (**c** & **f**), for seeds of *japonica* rice cv. Gleva harvested at maturity (42 DAA) produced in a growth cabinet (Cabs 1 & 2) at 28/20 ^{0}C (12h/12h, 12 h d^{-1} photoperiod) with irrigation throughout (T1); plants with high temperature (HT) at 0-3 DAA (T2); irrigation stopped at 14 DAA (T3); combination of HT at 0-3 DAA and drought at 14 DAA (T4); HT at 14-17 DAA with drought at 14 DAA (T5); or HT at 14 DAA (T6). Mean values within treatments in cab 1 with the different capital letter; and in cab 2 with the different small letter indicate significance difference at $P \leq 0.05$ amongst treatments using the Tukey's multiple range test.

5.5.4 Seed storage survival

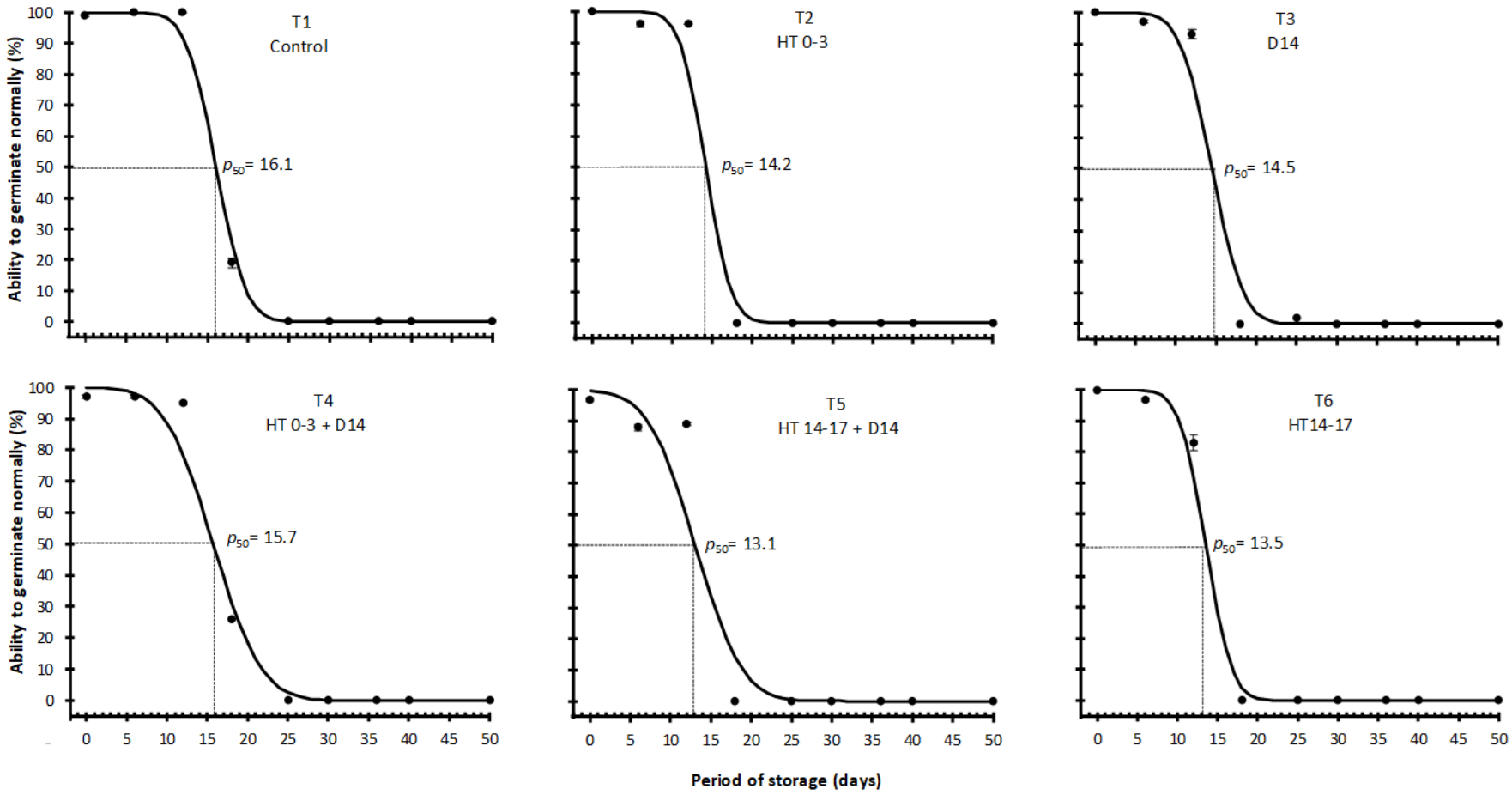
The 24 seed survival curves were described well by negative cumulative normal distributions (Fig. 5.3, 5.4). Comparison of these curves found no significant difference in slope or in slope and intercept combined for cv. Aeron 1 in cab 1 (Appendix 5.4): that is all six curves could be described by a single line. Within cv. Aeron 1 in cab 2 (Appendix 5.5) and in cv. Gleva in cab 1 (Appendix 5.6) and 6 (Appendix 5.7), the curves did differ significantly in slope amongst treatments. Hence, survival curves fitted separately to each treatment combination are shown in Figures 5.3 and 5.4. These are quantified in Table 5.1.

Longevity (p_{50}) varied greatly from -2.5 to 26.5 days (Table 5.1). It was greatest in the control in most cases (cv. Aeron 1, in cab 1; cv. Gleva in both cabs) but not in cv. Aeron 1, cab 2. With the exception of cv. Aeron 1 cab 1 where treatments did not differ, the combined treatment of HT 14-17 DAA with drought at 14 DAA provided the shortest longevity, especially so in cv. Gleva in both cabs. The longevity of seeds of cv. Gleva (Fig.5.4) was more sensitive to the stress treatments than Aeron 1, with all five stress treatments reducing longevity in cv. Gleva in both cabs. Each of HT at 0-3 DAA or 14-17 DAA, or drought at 14 DAA on their own in cv. Gleva were roughly equally damaging to subsequent longevity, but not as damaging as HT at 14-17 DAA combined with drought at 14 DAA.

Estimates of K_i ranged from -0.32 to + 5.58 (Table 5.1). Those for σ were slightly less variable, ranging from 2.54 days ($1/\sigma = 0.394$) to 8.06 days ($1/\sigma = 0.12$).

Aeron 1

A


T1
Control
p_{50}= 16.1
T2
HT 0-3
p_{50}= 14.2
T3
D14
p_{50}= 14.5
T4
HT 0-3 + D14
p_{50}= 15.7
T5
HT 14-17 + D14
p_{50}= 13.1
T6
HT14-17
p_{50}= 13.5
Ability to germinate normally (%)
Period of storage (days)

B

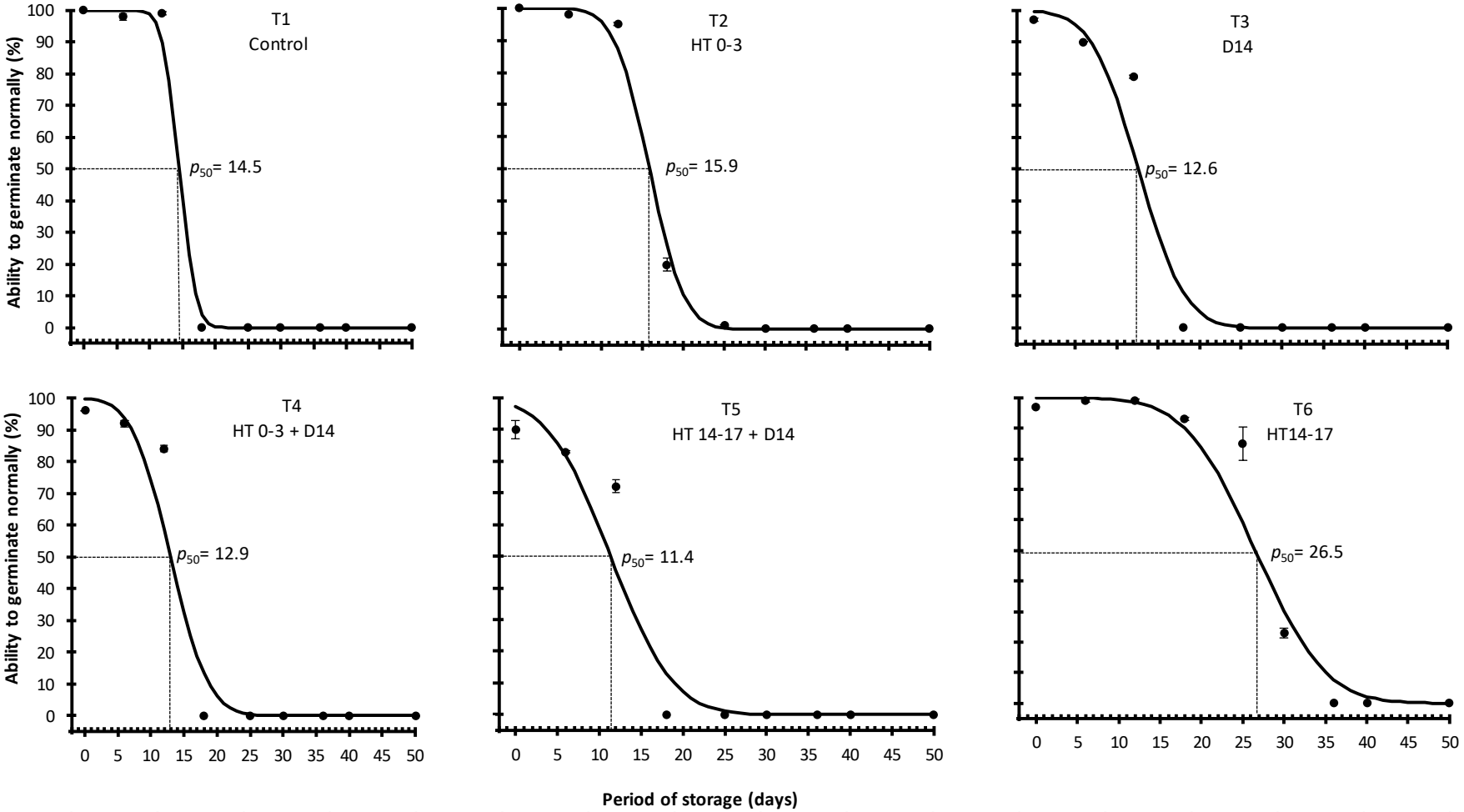
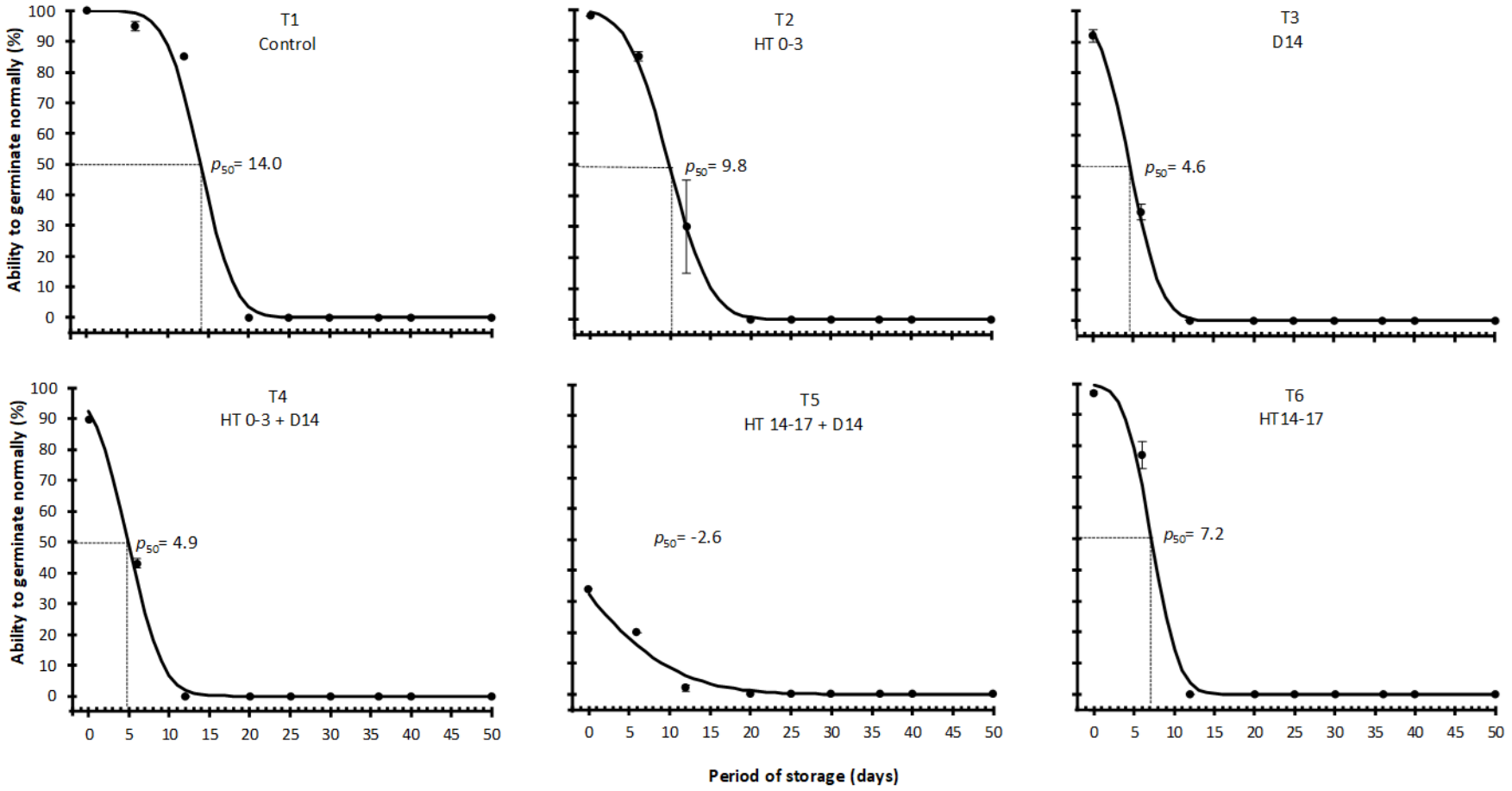


Figure 5.3. Seed survival curves (viability, estimated by ability to germinate normally) of seeds of *indica* rice cv. Aeron 1 produced in a growth cabinet, Cab 1 (A) and Cab 2 (B) at 28/20 ^{0}C (12h/12h, 12 h d^{-1} photoperiod) with irrigation throughout (T1); plants with high temperature (HT) at 0-3 DAA (T2); irrigation stopped at 14 DAA (T3); combination of HT at 0-3 DAA and drought at 14 DAA (T4); HT at 14-17 DAA with drought at 14 DAA (T5); or HT at 14 DAA (T6). Seeds were stored hermetically at 40°C with the moisture contents shown in Table 5.1. Best fit survival curves for each treatment combination fitted by probit analysis are shown. These are quantified in Table 5.1. Estimated seed longevity (p_{50}) is also shown.

Gleva

A


T1
Control
p_{50}= 14.0
T2
HT 0-3
p_{50}= 9.8
T3
D14
p_{50}= 4.6
T4
HT 0-3 + D14
p_{50}= 4.9
T5
HT 14-17 + D14
p_{50}= -2.6
T6
HT14-17
p_{50}= 7.2
Ability to germinate normally (%)
Period of storage (days)

B

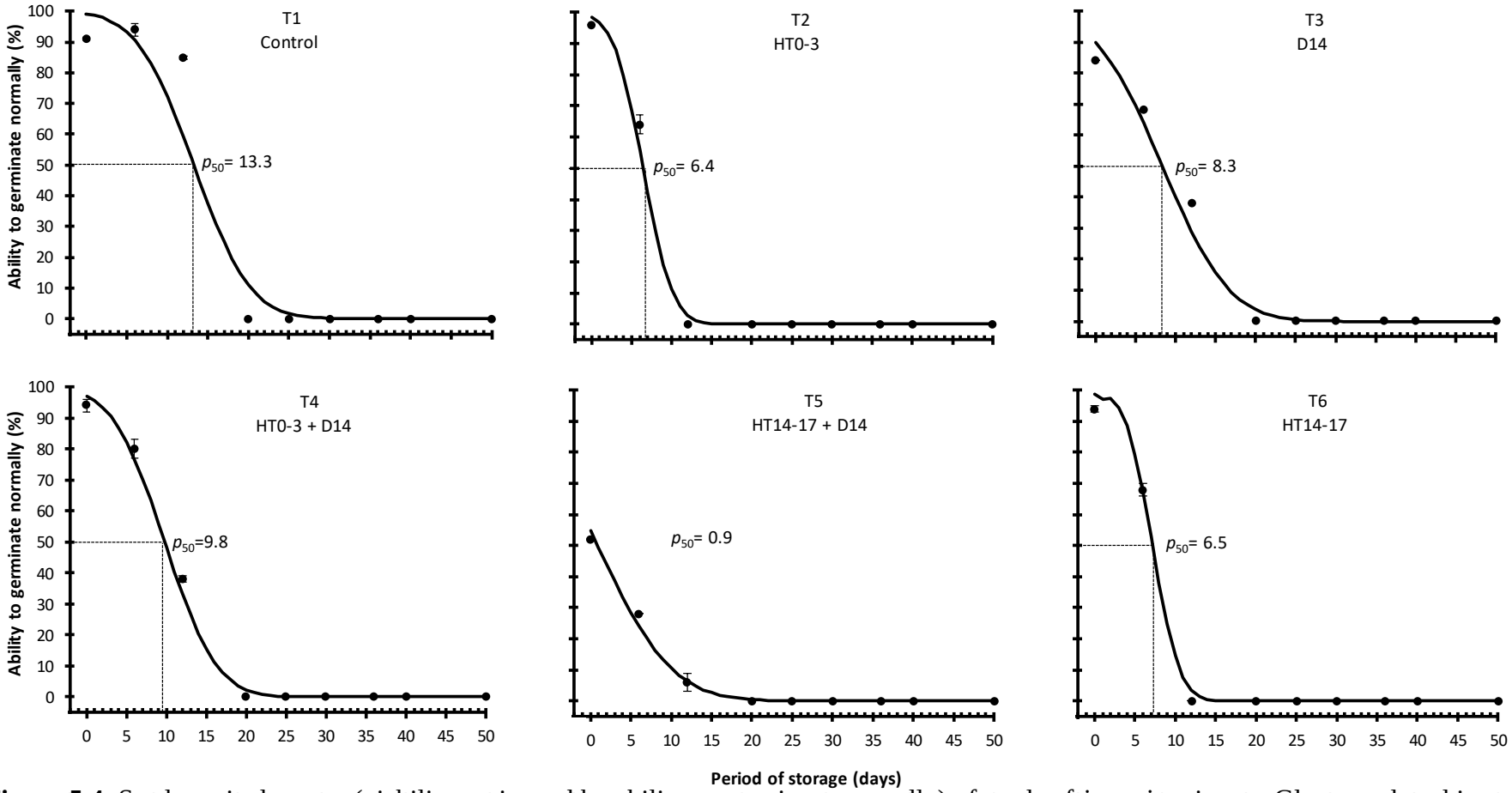


Figure 5.4. Seed survival curves (viability, estimated by ability to germinate normally) of seeds of *japonica* rice cv. Gleva produced in a growth cab 1, Cab 1 (A) and Cab 2 (B) at 28/20 ^{0}C (12h/12h, 12 h d^{-1} photoperiod) with irrigation throughout (T1); plants with high temperature (HT) at 0-3 DAA (T2); irrigation stopped at 14 DAA (T3); combination of HT at 0-3 DAA and drought at 14 DAA (T4); HT at 14-17 DAA with drought at 14 DAA (T5); or HT at 14 DAA (T6). Seeds were stored hermetically at 40℃ with 15.0±0.5 m.c. Best fit survival curves for each treatment combination fitted by probit analysis are shown. These are quantified in Table 5.1. Estimated seed longevity (p_{50}) is also shown.

Table 5.1. Longevity (parameters of seed viability equation fitted by probit analysis) of *indica* (c.v. Aeron 1) and *japonica* (c.v. Gleva) rice harvest at maturity (42 DAA) respectively in growth cab 1 and 2. Plants were irrigated with irrigation throughout (T1), plants with high temperature (HT) at 0-3 DAA (T2), irrigation stopped at 14 DAA (T3); combination of HT at 0-3 DAA and drought at 14 DAA (T4); HT at 14-17 DAA with drought at 14 DAA (T5) or HT at 14 DAA (T6) and stored hermetically at 40℃ with the moisture contents shown in Table 5.1. The 95% confidence intervals are shown for p_{50}. N. B comparisons are valid within individual cabs only. Negative estimates of p_{50} are shown where initial viability was less than 50% and the negative estimates represent extrapolation back to 50% viability.

Cultivar (Cab)	Treatment (Moisture content %)	K_i		Slope(1/σ)		p_{50} (days)			
		estimate	s.e	estimate	s.e	estimate	s.e.	lower 95%	upper 95%
Aeron 1 (Cab 1)	T1 (14.6)	5.52	0.56	0.344	0.035	16.1	0.33	15.4	16.7
	T2 (15.0)	5.58	0.55	0.394	0.038	14.2	0.30	13.6	14.8
	T3 (15.2)	4.68	0.46	0.323	0.031	14.5	0.33	13.8	15.1
	T4 (15.3)	3.32	0.27	0.211	0.016	15.7	0.41	14.9	16.5
	T5 (15.3)	2.83	0.24	0.217	0.017	13.1	0.40	12.3	13.8
	T6 (15.3)	5.25	0.54	0.388	0.040	13.5	0.30	12.9	14.1
Aeron 1 (Cab 2)	T1 (14.8)	7.30	0.68	0.502	0.047	14.5	0.28	14.0	15.1
	T2 (14.9)	4.72	0.45	0.298	0.028	15.9	0.35	15.2	16.5
	T3 (15.0)	2.83	0.24	0.224	0.018	12.6	0.40	11.9	13.4
	T4 (15.5)	2.85	0.24	0.220	0.018	12.9	0.40	12.2	13.7
	T5 (14.8)	1.91	0.16	0.169	0.012	11.4	0.46	10.5	12.2
	T6 (14.8)	3.98	0.27	0.150	0.010	26.5	0.47	25.6	27.5
Gleva (Cab 1)	T1 (15.1)	4.21	0.39	0.300	0.027	14.0	0.39	13.3	14.8
	T2 (14.7)	2.41	0.23	0.246	0.022	9.8	0.40	9.1	10.6
	T3 (15.1)	1.48	0.18	0.324	0.031	4.6	0.33	3.9	5.2
	T4 (15.2)	1.43	0.17	0.292	0.027	4.9	0.40	4.2	5.6
	T5 (15.0)	-0.32	0.12	0.124	0.018	-2.5	1.32	-5.7	-0.6
	T6 (15.5)	2.70	0.30	0.376	0.039	7.2	0.31	6.6	7.8
Gleva (Cab 2)	T1 (15.0)	2.41	0.18	0.182	0.013	13.3	0.46	12.4	14.2
	T2 (14.6)	2.19	0.24	0.341	0.034	6.4	0.32	5.8	7.0
	T3 (14.9)	1.27	0.13	0.152	0.011	8.3	0.51	7.4	9.3
	T4 (15.1)	1.89	0.17	0.194	0.015	9.8	0.44	8.9	10.6
	T5 (14.5)	0.12	0.11	0.137	0.016	0.9	0.80	-0.9	2.2
	T6 (14.8)	2.03	0.22	0.312	0.029	6.5	0.34	5.8	7.1

5.6 Discussion

Drought and high temperature often occur simultaneously, but their effects on crops are usually investigated individually. This was also the case in Experiment 1-3. In rainfed rice ecosystems, plants are often subjected to a combination of abiotic stresses. Simultaneous occurrence of high temperature and drought underlines the potential severity of stress combinations, as well as its physiological, molecular and biochemical aspects (Mittler, 2006) which is expected with raised global temperatures, changes in the distribution of precipitation and intensify drought in arid and semiarid areas (Wrigley and Raper, 2001). In Malaysia for example, paddy fields will be exposed to water scarcity and high temperature as a result of climate change (Zainal et al., 2014), threatening 85% of rice cultivation in this particular region (Radin-Firdaus et al., 2013).

Cereal seed are usually at <15% moisture content when dried naturally (Bewley, 1986). Moisture content in rice at harvest maturity in paddy fields, influence on yield and cooking quality of rice were studied by many authors (Firouzi and Alizadeh, 2011; Ilieva et al., 2014), mainly reporting that seed quality eg. rice milling yield was highest at average highest moisture content at harvest (19.6% moisture content). From the present study, both cultivars showed relatively low in moisture content when harvested at maturity (42 DAA), with Aeron 1 at between 9.9-11.2% (Fig. 5.1a & c), meanwhile Gleva was lower at 6.0-9.4% (Fig.5.2a & c).

When all irrigation to the cabs ended (in the later flowering cv. Gleva at 152 DAS in cab 1 and at 160 DAS in cab 2), relative humidity in each cab declined greatly (Appendix 5.10). The difference in RH between cabs suggest declining RH most probably because irrigation was stopped one week before harvest for control treatment. The seed moisture contents at harvest, cab relative humidity at 42 DAA, and expected eRH from the isotherm of Whitehouse et al. (2015) are compared for each cultivar in each cab in Table 5.2. The lower moisture content of cv. Gleva at harvest compared with cv.Aeron 1 is explained by this reduction in cab relative humidity. However, in both cultivars seed moisture contents were lower at 42 DAA than would be expected from cab relative humidity and seed moisture content isotherm (Table 5.2).

Table 5.2. Seed moisture content (%) range at 42 DAA and relative humidity (%) in cabs compared with expected eRH from isotherm of Whitehouse et al. (2015).

Cultivar	Cab	DAA	DAS	Seed m.c. range (%)	Mean cab RH (%)	Expected eRH (%) Whitehouse et al. (2015) at m.c. range.
Aeron 1	5	42	132	9.9-10.8	72.9	~50%
Aeron 1	6	42	132	10.5-11.2	72.1	~50%
Gleva	5	42	158	6.0-9.4	47.2	~20-40%
Gleva	6	42	166	7.8-9.3	31.0	~20-40%

IRRI (2007) reported that rice seed harvested from paddy fields at optimum grain maturity have an average moisture content about 20−25 %. Higher moisture content results in more losses from poor grain quality while lower moisture content results in more losses from shattering (Ilieva et al., 2014). Since the seed in this study had already dried naturally *in planta* to below 14% moisture content, further desiccation was not required for storage. ISTA (2009) reported that rice seeds, which can withstand drying down to low m.c. of around 5% to 10% and successfully stored at low freezing temperature for long periods. In addition, in terms of seed moisture content at harvest maturity, this study suggests that *japonica* rice (Gleva) was less well adapted to drought and high temperature stresses than *indica* rice (Aeron 1).

Rice in the reproductive stage is more sensitive to high temperature (Prasad et al., 2006; Martínez-Eixarch and Ellis, 2015) and water stress (Sikuku et al., 2010; Sabetfar et al., 2013) than in the vegetative stage. Water deficit during the reproductive stage reduces pollen viability (Rang et al., 2011) and grain yield (Kato et al., 2004). Similarly, this study indicates that high temperature at 14-17 DAA, particularly when combined with drought (Fig. 5.2C & F) caused large reductions in grain weight of *japonica* rice (Gleva). Similar results have been reported studied with other cereal crops by Shah and Paulsen (2003) in wheat; and Savin and Nicolas (1996) in barley. Futhermore, the present study consistently showed the heaviest rice seeds were provided by irrigation throughout (Fig. 5.1C & F; 5.2C & F) compared to those plants subjected to stress. This is supported by Zakaria et al. (2002) in their studies using *japonica*, *indica* and *javanica* rice. Also, Hurkman et al. (2003) reported that high temperature (37/17 °C) from anthesis to maturity caused a significant reduction in the starch accumulation period in developing wheat grains compared with plants grown under control (24/17 °C) conditions.

Cultivar Gleva was more damaged by drought and high temperature stress combined, with only 20% (cab 1) and 52% (cab 2) normal germination (Fig. 5.2B &E) with seed moisture content at harvest of 6.0% and 7.8% (Fig.5.2A & C) respectively. This low viability of *japonica* rice after mother plant stress is supported by Martínez-Eixarch & Ellis (2015). The reported that the viability of Gleva seeds was reduced by extreme-temperature (38/34℃) treatments applied in the 7 or 14 d immediately after anthesis. Also, for other cereals, for example wheat (cv. Marzak) low was germination detected if exposed to heat stress (36/29 ℃) during seed development (Grass and Burris, 1995).

The studies of Chang (1991) and Ellis et al. (1993) revealed that *japonica* rice cultivars often show poorer seed longevity than *indica* rice cultivars. Similarly, in the present study, the maximum potential longevity of *japonica* cultivar (Gleva) was less than *indica* (Aeron 1) (Table 5.1). The combined treatment of HT 14-17 DAA with drought at 14 DAA provided the shortest longevity, especially so in cv. Gleva. Each of HT at 0-3 DAA or 14-17 DAA, or drought at 14 DAA on their own in cv. Gleva were roughly equally damaging to subsequent longevity, but not as damaging as HT at 14-17 DAA combined with drought at 14 DAA. Longevity of a *japonica* rice (cv. Taipei 306) at 32/24°C reached a plateau from mass maturity onwards during the maturation drying phase, whereas longevity continued to improve over this period at 28/20°C and in other (*indica*) cultivars in both temperature regimes resulting in better longevity at harvest maturity (Ellis et al.,1993). In addition, the current study indicates that the treatment of *japonica* (cv. Gleva) plants by high temperatures at 0-3 DAA or 14-17 DAA damages the storage potential of the mature seed (Fig.5.4A & B).

The hypotheses in this Chapter 5 (5.1-5.2), associated with general hypotheses (1.7-1.7.2 and 1.7.4), were accepted. The results lead to the following conclusions. Seed at harvest maturity, were lighter after either a single period of high temperature (HT) at anthesis or during the seed filling phase, or terminal drought (TD) during the seed filling phase, or both these high temperature and drought stresses combined (HT, TD); and yet lighter with combined treatment (HT at 14-17 & drought at 14 DAA). Seed moisture content at harvest (42 DAA) was not greatly affected by treatment, except in cv. Gleva cabs where the combination of HT at 14-17 DAA with drought at 14 DAA provided a very much lower value. Seed viability (ability to germinate normally of air-dried seed) at harvest (42 DAA) was lower after either a single period of high temperature (HT) at anthesis or during the seed filling phase, or terminal drought (TD) during the seed filling phase, or both these high temperature and drought stresses combined (HT, TD), and yet more so with at combined treatment (HT at 14-17 & drought at 14 DAA). Subsequent seed longevity in air-dry storage of seeds, harvested 42 DAA was reduced by either

a single period of high temperature (HT) at anthesis or during the seed filling phase, or terminal drought (TD) during the seed filling phase, or both these high temperature and drought stresses combined (HT, TD), and more damaging by combined treatment (HT at 14-17 & drought at 14 DAA).

5.7 Conclusion

In conclusion, this study suggests that the quality of seed lots of *indica* cultivars (e.g. Aeron 1) produced in Malaysia will be comparatively less affected than *japonica* cultivars (e.g.Gleva) under combined heat and drought stresses. Furthermore, seed quality of either *indica* Aeron 1 and *japonica* Gleva rice is reduced considerably more by the combined stresses than by either stress alone. Combination of both stresses had a significantly greater detrimental effect on rice seed quality particularly on *japonica* cv. Gleva compared with each of the different stresses applied separately. Greater tolerance to heat and drought stress are priority strategies for rice seed quality, to maintain future yield by adapting to climate change. Hence, overcoming the effects of high temperature and water stress on rice production is essential for food security in the future.

CHAPTER 6

GENERAL DISCUSSION

6.1 General summary

The purpose of the study was to investigate whether water limitation and/or brief high temperature during seed development and maturation could affect seed quality especially subsequent longevity, in rice cv. Gleva and/ or cv. Aeron 1. The present findings clearly suggest that both environmental stresses considerably affected seed quality development in rice. Seed quality was more sensitive to water limitation during early grain filling and more sensitive to brief high temperature at histodifferentiation. However, seed seems more tolerant to the imposition of these environmental stresses towards the end of seed filling and during the maturation drying phase. Seed longevity improvement was more damaged by combination of both stresses during seed development, whereas *indica* rice cv. Aeron 1 was more resilient to environmental stress than *japonica* rice cv. Gleva.

6.2 Hypotheses answer

The general hypotheses set in Chapter 1 (1.7.1-1.7.4) are answered below.

6.2.1 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduced the duration of the seed filling phase, final seed dry weight, seed moisture content and the ability of seed to germinate normally. This hypothesis was accepted, but only in terms of the effect of ending irrigation early during early seed filling or well before end of seed filling; not during late seed filling and maturation drying; and similarly, for brief HT treatments only during the period of anthesis and histodifferentiation, less so for later treatments.

6.2.2 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduced subsequent seed longevity improvement in air-dry storage. This hypothesis was accepted by seed produced from water limitation and considerably accepted by imposition of HT around anthesis and soon after; there was some evidence of damage in the period of 3-6 until 18-21 DAA.

6.2.3 Ending irrigation early and/or brief high temperature in cv. Gleva during seed development and maturation reduced maximum seed longevity. This hypothesis was partially accepted; only ending irrigation early during early and towards the end of seed filling) reduced

maximum longevity, with treatment from 7 DAA worse than that from 14 DAA and with no effect towards the end of seed filling and during maturation drying (14 and 28 DAA); maximum value of longevity was lowest when HT was imposed at anthesis and histodifferentiation (-3-0, 0-3 DAA) compared to control.

6.2.4 Combining ending irrigation early with brief high temperature in cv. Gleva and cv. Aeron 1 during seed development and maturation reduced seed quality compared with each single stress. This hypothesis was accepted for simultaneous water limitation at 14 DAA combined with brief HT at 14-17 DAA. The damage was more severe in the *japonica* cv. Gleva than in *indica* cv. Aeron 1.

Generally, the topics 6.2.1 - 6.2.3 were answered in all experimental chapters: Chapters 2, 4, 5 in relation to drought, and Chapters 3 and 5 in relation to high temperature (Chapter 2-5), as well as hypothesis 6.2.3 (except Chapter 5). Paragraph 6.2.4 was covered in Chapter 5.

6.3 Effect of water limitation on seed quality development

In this study, when ending irrigation during early grain filling (7 DAA) or well before the end of the seed filling phase (14 DAA) rice plants were found to senescence earlier and with a shorter duration of seed filling, with the 7 DAA treatment senescence much earlier than 14 DAA. Ending irrigation during late seed filling or maturation phase had much less effect. This is shown by the seed desiccation and final seed dry weight results for water stress during seed development (Figs. 2.1 & 4.1).

Senescence developed earlier in the grain filling phase when irrigation ended at the end of histodifferentiation phase (7 DAA) followed by irrigation ended well before end of seed filling phase (14 DAA). These results are consistent with reports that drought during the grain-filling process induces early senescence and shortens the grain-filling period (Plaut et al., 2004). In the present study, the grain filling period for the control was 40 days, with maximum seed dry weight gained 28.0 mg, meanwhile irrigation ending early for 7 or 14 DAA ended filling at 11 or 22 days with 12.4 mg and 16.0 mg, respectively. There was evidence that seed was far less filled after subjected to water limitation during grain filling process. From this data, grain filling rate was greater (1.13 mg d^{-1}) at irrigation ending early at 7 DAA compared to the control with only 0.73 mg d^{-1}. This was logical to assume that when drought occurred there was more movement of assimilates from straw to grain which agrees with Saini et al. (2000) who reported that drought during seed filling in rice may increase remobilization of assimilates from straw to grains. Early senescence is due to accelerated loss of leaf chlorophyll

and rapid development as a result from accelerating remobilization of soluble sugars in the stem, with both of these having a higher priority than sugar remobilization efficiency as part of the strategy of plants to mitigate stress (Saeedipour and Moradi, 2011).

Dry matter accumulation and ability to germinate were not affected by ending irrigation close to or after seed filling ended (Fig. 4.1b, c). During the period between 14 DAA and 28 DAA, seed reached maximum dry weight. This can be indicated by maximum germination capacity almost at 100% by the time drought begin to impose (Fig.4.1b). This finding of the current study is consistent with those of Samarah and Alqudah (2011) in barley, Vieira et al. (1991) in soya bean and Nichols et al. (1978) in peas, who conclude that late-drought stress during seed filling in a greenhouse (potted plants) had no effect on standard germination but reduced the germination after the accelerated ageing test. In contrast, these results are disagreement with Smiciklas et al. (1989) for soya bean and Fougereux et al. (1997) for peas who reported that water stress during seed filling period induced a reduction in seed quality assessed by germination and conductivity results.

However, what is surprising is that the seed dry weight in the control continued to increase throughout the seed development study period (Fig. 2.1a) with seed moisture content about 30%, which is relatively high for mature seed. Continuous irrigation until the end of harvest (40 DAA) might be the reason of long duration of seed filling. Continue irrigation until harvest is not a normal practice in field conditions: in flooded rice puddle cultivation commonly, water supply will stop about 7-10 days before harvest (Bouman et al., 2007).

In both water limitation studies (Experiment 1 and 3), subsequent seed longevity improvement was advanced by ending irrigation early throughout seed development and maturation phase (Fig. 2.4 & 4.3). Seed longevity improvement was detected earlier if subjected to water limitation during early (7 DAA) and well before end seed filling phase (14 DAA) (Fig.2.4) compared with the control (irrigation throughout). The earlier improvement occurred was matched with the earlier seed desiccation (Fig.2.1a), which consistent to the conclusion Sinniah et al. (1998) with rapid-cycle *brasicca*. However, this did not happen when irrigation ended during the late seed filling phase (14 DAA) or maturation drying (28 DAA) (Fig. 4.3).

In Table 2.1, maximum potential seed longevity was reduced by early water limitation during early and well before end of seed filling (7 and 14 DAA). Control was greatest (23.6 d, K_i = 3.5) followed by ended water at 14 DAA (18.4 d, Ki=2.6), and the least was at 7 DAA (9.5d, K_i=1.1). This finding may due to the shorter duration of improvement in seed quality

and the later decline in longevity occurring sooner because seeds desiccate and reached lower moisture contents than in the control (refer Fig. 2.1 for moisture content). In contrast, maximum potential longevity showed water ended during late and maturation drying had no significant effect (14 and 28 DAA) (Table 4.1). Hence the present study indicates that seed produced by water limitation before mass maturity may have poorer subsequent seed longevity.

From the present study, all drought treatments (Exp. 1 and Exp. 3) showed that maximum longevity was achieved some time after mass maturity. For example ending irrigation early at 14 or 28 DAA, respectively achieved maximum potential longevity (28.7 d or 27.6 d) at 16 days (34 DAA) after mass maturity (18 DAA). This finding was consistent with Ellis et al. (1993) reported that maximum potential longevity in rice was not achieved until 12-19 d after mass maturity. Also, current results support the conclusions of Ellis and Pieta-Filho (1992) and Yadav and Ellis (2016) in wheat which longevity increased progressively reaching maximum values well after mass maturity. However, those findings contradicted the general hypothesis by Harrington (1972) who concluded that seed longevity did not improve after mass maturity.

In relation to seed longevity and seed moisture content during development, the results in this study show that longevity in water limited conditions was reduced between 35 and 42 DAA when seed moisture content was about 15%. Meanwhile longevity with irrigation throughout (control) improved during this period at a moisture content of 26.1% (Fig.4.3). The finding of the current study are consistent with those of Whitehouse et al. (2015) who found that rice seed longevity no longer benefitted from heated-air drying treatments when seed moisture content at harvest was below than 16%.

6.4 Effect of brief HT on seed quality

High temperature during histodifferentiation (-3-0 DAA) and anthesis (3-0 DAA) was more detrimental to seed quality than later HT treatment of (21-24 to 24-27 DAA) in cv. Gleva. This can be seen in figure 3.1-3.4. This finding is in agreement with Martinez-Eixarch and Ellis (2015) who revealed that rice seed quality development most sensitive to high temperature before the end of seed-filling phase and possibly as early as the histodifferentiation phase soon after pollination. Typically, in rice growing regions, days at 33℃ and above are considered critical for rice production (Prasad et al., 2006; Jagadish et al., 2007, 2008). There is also much evidence in other aspects of reproductive biology of greater sensitivity to high temperature at specific developmental stages. In particular, pollination, and so seed set, are particularly

sensitive to brief exposure to high temperature, e.g. in rice (Yang et al., 2001; Jagadish et al., 2010b; Coast et al., 2015; Martinez-Eixarch and Ellis, 2015).

High temperature imposed in early histodifferentiation (-3-0 DAA) provided the highest seed moisture content (37.2%) at harvest compared to other HT periods (Fig. 3.1). This high value for seed moisture content might happen because seed was not yet achieved mass maturity by the time of harvest (42 DAA). Perhaps seed development was disrupted in some panicles due to HT imposed during early cell division stage (-3-0 DAA). Dunand and Saichuk (2014) reported that most rice seed will achieved physiological maturity (mass maturity) while moisture content was between 25-30%. Other reports support this: my results, HT during anthesis caused spikelet fertility (Baker, 2004; Jagadish et al., 2007); and increase in temperature can increase the duration of grain filling (Wheeler et al., 1996; Zahedi & Jenner, 2003). Extended durations of seed filling perhaps prolong the time course for seed to complete their development.

Seed dry weight and ability to germinate were similar with the control (Fig.3.1b,c) if HT was imposed from 3-6 DAA onwards (Fig. 3.1b). In rice, HT at 14 DAF did not affect grain development of cv. Hinohikari (Tanaka et al., 2009) or 1000 grain weight (Tanamachi et al., 2016). The seed dry weight was not affected maybe because by the time HT was imposed during this period, seed had already completed seed filling. This finding further supports the conclusion of Ellis (2011) that increasing temperature from 28/20°C to 34/26°C from two-thirds through the seed-filling phase has no effect on seed dry weight. This was also shown by Zakaria et al. (2001) where some *indica* rice were resistant to elevated temperature during the maturation phase. Hence the quality of seed at mass maturity (Ellis et al., 1993) is less affected by HT. In contrast, the limited assimilate supply to the grain was suggested to be the main factor limiting grain weight under HT stress in rice during early grain filling (Kobata and Uemuki, 2004)

As expected, the control provided the longest survival period, with the HT (21-24 DAA, 24-27 DAA) almost good as control (Fig.3.4), also in Table 5.1 (except Aeron 1 in cab 2). In addition, the current study showed some evidence of damage to subsequent longevity from the third HT treatment (3-6 DAA) until 18-21 DAA, although this was somewhat variable. This was strongly consistent with Ellis (2011) who concluded that high temperature treatment during late seed filling had no effect on potential seed longevity in rice (Ellis, 2011) and,

Martinez-Eixarch and Ellis (2015) where seed exposed to HT during anthesis was more vulnerable.

The observation where damage occurred at 29% and 37% seed moisture content *in planta* contradict with the conclusion of Whitehouse et al. (2015) who reported that longevity improved *in planta* and *ex planta* in room regime until moisture content declined to 16%. Cromarty et al. (1982) reported that if seed moisture *ex planta* is relatively high, seed may be easily damaged by high temperature.

In addition, in experiment 2 (Chapter 3), I used electrical conductivity of seed steep water to test on seed viability. However, the result showed that there was no different in this value as brief HT treatment was imposed during seed development and maturation. As no effect was detected, this test was not used for the later experiments 3 and 4. Perhaps it is not suitable to use in monocotyledonous seed such as rice, but maybe successful for dicotyledonous crops.

6.5 Effect of combining both stresses (drought and high temperature)

The results in Figure 5.2 were consistent with my second experiment (Chapter 3) where HT during late seed filling (14-17 DAA) was less damaging to seed quality (Fig. 3.1). Even HT at this period tolerate to seed quality, however, by combining with water limitation stress, the effect was pronounced. Combining water limitation and HT was more damaging than each single stress, and cv. Aeron 1 was less sensitive than cv. Gleva (Figures 5.1 and 5.2).

It was expected that combining the two stresses would be more damaging and that *japonica* rice cv. Gleva would be more vulnerable. The present study confirmed the earlier findings of Chang (1991) and Ellis et al. (1993) on varietal differences in seed longevity of rice: potential longevity of *japonica* rice was much more susceptible to high temperature compare to *indica* and *javanica* rice (Ellis et al., 1993). Ellis and Hong (1994) studies also showed that even in the cooler regime, the maximum potential longevity of the seeds of *japonica* cultivars was less than that of the *indica* cultivars. Also, investigations in the field confirmed that *japonica* seeds produce longer-lived seeds in cooler than warmer regimes (Rao and Jackson, 1996; 1997). This result may be explained by adaptation of Aeron 1 to both stresses compared to cv. Gleva, due to their different origins and genotypes. Aeron 1 and Gleva were bred and cultivated in Malaysia and Spain, respectively. The average air temperature in Malaysia is between 26-28°C (Radin Firdaus et al., 2012) which is above the average of 22°C in Catalonia (Duran et al., 2017). Also, Aeron 1 is a hybrid rice developed to be cultivated under aerobic conditions where low soil moisture content is common (Zainudin et al., 2014).

6.6 Comparison of previous predictions of σ with current estimates

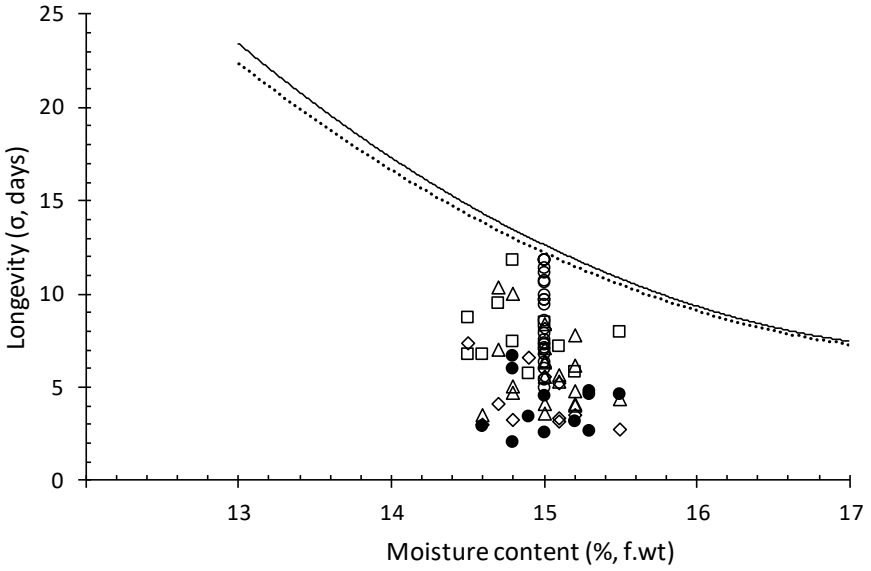


Figure 6.1 Previously-reported negative logarithmic relation between the standard deviation of the frequency distribution of seed deaths in time (σ, days) and seed storage moisture content for rice (*Oryza sativa*) compared with current estimates for all 80 rice seed lots produced in 2015-2017 and stored hermetically at 40°C with the moisture contents shown. The continuous and broken curves are derived from the seed viability equation (Ellis and Roberts, 1980) and the estimates of the viability constants K_E , C_w, C_H and C_Q for rice provided by Ellis and Hong (2007), where the broken curve is derived from constants constrained to a common temperature term for all 12 crops they investigated and the continuous curve only for rice. The symbols are the observations for σ, estimated by probit analysis, reported in the current studies (Chapters 2-5). Open symbols are for rice cv Gleva (○ (April '15), □ (August '15), Δ (2016), ◊ (2017) and solid symbol for cv. Aeron 1 (•).

The constants K_E , C_w, C_H and C_Q of the seed viability are described as species constant, with the value of σ for any one storage environment the same for different seed lots within that species (Ellis and Roberts, 1980; 1981). Ellis and Hong (2007) derived the seed viability constants for twelve crops including rice at different temperatures and moisture content in hermetic storage. The values of constants they published provide predictions of σ for rice in various storage environments. Figure 6.1 compares the predictions of the above authors of σ for rice with my current estimates provided by probit analysis of the survival curves for all experiments with 80 separate rice seed lots. As mentioned by Ellis and Roberts (1980), seed longevity varies exponentially with storage environment from minutes to possible hundreds of years in storage. The independent predictions of σ for rice overestimated every one of the 80 estimates in the present studies (Fig. 6.1). The greatest observation for σ in my research was close to the prediction, but the majority of current observations were very much lower than

values predicted by Ellis and Hong (2007). The majority of seed lots stored at 40℃ with 15% moisture content provided estimates of 4-7 days compared with predictions of around 13 days (Fig.6.1).

Between the two cultivars, the *japonica* rice cv. Gleva was less distant from the predictions than was the case with *indica* rice cv. Aeron 1 (Fig. 6.1). Also, *japonica* cv. Gleva had greater longevity (σ) than *indica* cv. Aeron 1 in similar storage environments (Fig. 6.1). This is suprising. It contradicts Chang (1991) and Tejakhod and Ellis (2017), who both reported that *indica* rice cultivars showed greater longevity in a given storage environment than *japonica* cultivars. Similarly, it contradicts Ellis et al. (1993) who reported estimates of σ in one environment were ranked *indica* > *javanica* > *japonica* rice cultivars. Given the consistent reports in the literature that seed longevity is less in *japonica* and *indica* cultivars of rice, it is difficult to explain why the opposite was found in my research. The only obvious difference is that cultivar Aeron 1 was bred for aerobic production environments, whereas most if not all of the research referred to above was with *indica* cultivars bred for common paddy rice production. Aeron 1 was identified and selected from aerobic rice breeding lines under International Network for Genetic Evaluation of Rice (INGER) programme from IRRI, where the designation of Aeron 1 was a cross from IR76569-259-1-2-1 which includes the Aerob gene (IRRI, 2007). This variety underwent the preliminary selection in local aerobic environments in Malaysia and was identified as having the best plant vigour characterestics and good phenotype acceptability in aerobic conditions (Zainudin et al., 2014).

One of the greatest causes of error in comparisons like Figure 6.1 is small difference in seed moisture content during storage, since estimates of moisture content are subject to error and a small difference in seed moisture content has a comparatively large effect on seed storage life. Moreover, the narrow moisture range might contribute to the error, thus, to make a complete comparison perhaps it would have been better to use a wider range of moisture content contents for example by using much lower or higher seed moisture levels. The number of observations in Figure 6.1 is large and the differences consistent, and so that possible explanation is most unlikely to explain either the shorter longevity of the *indica* cultivar, all the shorter longevity of all seed lots compared to earlier predictions.

The intra-specific differences in the relation between σ and seed storage moisture content here showed that the *japonica* cultivar had greater values than the *indica* cultivar. However, in terms of p_{50} (the product of K_i and σ) my current finding was the opposite (Table

5.1): *japonica* cv. Gleva had shorter p_{50} values than *indica* cv. Aeron 1. Thus, the conclusions of Chang (1991), Ellis et al. (1993) and Tejakhod and Ellis (2017) were consistent with studies in terms of p_{50} (Chapter 5) but not for σ.

6.7 Progress towards fulfilling the original objectives

The high-level objectives of this research study (see section 1.6) were to:

1. determine the effect of ending irrigation early and/or brief elevated temperature during seed development and maturation on seed quality of *japonica* cv. Gleva;
2. identify the most sensitive stages of seed quality development to ending irrigation early and/or brief elevated temperature in *japonica* cv. Gleva;
3. investigate the effect of combining ending irrigation early and/or brief elevated temperature during seed development and maturation on seed quality in two contrasting rices-*japonica* cv. Gleva from Spain and *indica* cv. Aeron 1 from Malaysia.

The above objectives were largely met. Earlier research with *japonica* cv. Gleva had indicated that the ability of seed harvested at maturity to subsequently germinate was damaged by exposure of the plants for 7 days to 34-38°C at 1-7 or 1-14 DAA (Martinez-Eixarch and Ellis, 2015). My study showed that a shorter (3 days) period at high temperature imposed around anthesis reduced subsequent ability to germinate (of seed harvested at harvest maturity) slightly (fresh seed only) but considerably reduced the longevity of seed in air-dry storage. Moreover, I identified a previously unrecognized phenomenon whereby both the ability of mature seeds to germinate and their air-dry seed storage longevity was damaged considerably by drought of the parent plants imposed from 7- 14 DAA onwards. Exposure to high temperature was more damaging to rice seed quality when it occurred around anthesis with little or no damage the more developed the seed when exposed to high temperature; and the earlier that drought and high temperature stress occurred in seed development the more damaging they were to subsequent seed quality, and more so combined than each stress singly, and in all these cases damage was more severe in the *japonica* sv. Gleva than in *indica* cv. Aeron 1.

However, my objectives were not completely met. While it is clear from my research that high temperature is more damaging shortly after anthesis and drought most damaging in early seed development, the precision of timing of greatest sensitivity has not been identified as precisely as it might, neither is clear if temperature or drought immediately before anthesis damage subsequent seed quality, and finally it is not known when the sensitivity to these

stresses ends. Moreover, in my studies the drought stress was terminal and not relieved by subsequent irrigation and hence, the effects of brief drought stress on seed subsequent longevity remains unknown.

6.8 Limitations of study and recommendations

Controlled and field environments differ, as does their respective applications in experimentation. Controlled environments are not normally used to replicate field environments precisely. Controlled environment facilities are expensive to operate and so, where they can be used, field studies are usually considerably cheaper. The differences between controlled and field environments include:

(a) solar radiation in full sun at midsummer is considerably greater than that which an be provided within growth cabs (with glasshouses intermediate);

(b) controlled environments (typically) provide a 'rectangular' diurnal temperature profile (e.g.12 h a day at temperature A; 12h a day at temperature B) whereas in the field the diurnal temperature pattern tends to be sinusoidal;

(c) plant water status is more easily controlled (through irrigation and also relative humidity control but less so in glasshouse than a growth cab for the latter) in a controlled environment;

(d) it is easier to grow plants as a genuine crop in the field than in a controlled environment (although plant population density in the field can often be replicated in controlled environments);

(e) plants in controlled environments are typically grown in pots in an artificial (often sterilized) growing medium, compared to soil in the field;

(f) and weeds, pests and diseases in field and controlled environments often differ.

My studies were better suited to experiments in controlled rather than field environments but were subject to certain limitations.

- Controlled environment investigations do enable particular hypotheses to be tested in well-defined consistent (e.g. from sowing to maturity) conditions. Moreover, they enable the study of a particular crop in a region well beyond its normal cultivation area, as was as the case here for rice, and at any time of the year (for growth cabs but not glass houses here). They also enable investigations to be repeated in the same conditions and so avoiding confounding of different observations (new and old) with other variables that are not constant in ambient conditions (although the unexplained

differences between cabs in my final experiment at the CEL shows that this may sometimes affect controlled environments also).

- Drought is unlikely where rice is cultivated in paddy fields because the crops excess water until the paddy is drained. Generally, in Malaysia for example, farmers growing rice in paddy fields can produce 2 to 3 crops per year, but this depends upon cultivar choices. Sakke et al. (2016) reported that in Malaysia in 1989 the longest cumulative period of drought events (of varying severity) was about 250 days. Often, drought lasts for 2-3 weeks which greatly affect those farmers, especially from east Malaysia (e.g Borneo) that grow rice in rainfed systems (non-puddled soils, and so dependent on rainfall) (Herman et al., 2015). Furthermore, lowland rice producers in peninsular Malaysia during the early 1980s were also badly affected by drought (Chan, 2004). Hence, the drought treatments I imposed during seed development in my studies are not only relevant to the production of rice in aerobic and rainfed systems but also in current extreme seasons to paddy field systems in Malaysia (and beyond). The effect of climate change on future rainfall patterns is uncertain, but increased variability is likely (IPCC, 2014), and so the effect of my drought treatments and the conclusions drawn are relevant to decisions on future rice production systems in many regions.
- Extreme temperatures in Malaysia can reach 41℃ (Suparta and Yatim, 2017) and in Spain they can soar well above 40℃ (Duran et al., 2017). Indeed at the time of writing (August 2018), the media reports that the current maximum day temperatures in Spain and Portugal have been close to the record European value of 48℃ for several days (although as rice is not cropped year round in Spain, cf. Malaysia etc, at this time of year the crop is well past anthesis). Hence, the brief high temperature treatment I applied of 3-days periods at 40/30℃ (11 h photoperiod per day) in experiment 2 and 4 (to study elevated temperature effect on subsequent seed quality) is not unrealistic in terms of current and future field environments for rice seed production.
- It can be argued that the rectangular diurnal temperature profile may be more stressful than the ambient profile, but in ambient conditions high temperature periods are often prolonged and, in those circumstances, higher minimum temperatures are also common. Since my objective was to test hypotheses concerning the temporal sensitivity of seed quality development to high temperature, these small differences in temperature profile between laboratory and field are acceptable. In future climates, IPCC (2014) reported that global mean temperature will rise by 0.4 to 1.6℃ by 2046-2065 and 2.6-4.8℃ by

2081-2100. Nonetheless, to determine the precise impact of periods of high temperature on seed quality development (at the stage where this is known from my research to be most severe) in a given location would require an investigation at that location. Temperatures could be raised temporarily, at the appropriate developmental stage, in the field in selected plots (to provide a contrast with a control) using the approach of Ferris et al. (1998).

- In experiment 4, my initial plan was to make this one whole experiment in order to investigate and compare the effect of the stresses between contrasting rice cultivars (Gleva and Aeron 1) with two cabs. However, it was not possible to do this. First of all, the growth habit of the two cultivars differed with Aeron 1 being much taller. Given the need to replicate as closely as possible crop seed production of each cultivar in the investigation, the two cultivars were therefore kept in separate halves of each cab, providing two different canopies, and so their location was not randomized. It was also expected that anthesis dates for the two cultivars would differ and hence treatment dates would differ as was indeed the case (Appendices 5.8 and 5.9). Hence the decision was made at the outset to carry out two separate but closely similar identical investigations one for each cultivar.
- During the investigation it became apparent that the rice plants were developing differently between cabs (Appendices 5.8 and 5.9). Given that seed lot quality is sensitive to environment and that a seed lot by definition comprises seeds produced, dried and stored under identical conditions, I decided to treat the overall investigation as four separate experiments (each experiment comprising a single cultivar in a single cab) for the purposes of statistical analyses and of reporting. This then enabled the effects detected, or not, to be compared qualitatively amongst the four very similar investigations' conclusions. A simple half-way house approach to the statistical analyses would have been possible, where within each cultivar the cabs were designated as blocks within ANOVA. Another idea would have been to combine all the results and analyses as a split-plot design within ANOVA in which cabs were blocks and cultivars the sub-plots.
- During my studies, I learnt that small difference in seed moisture content affect seed storage longevity considerably and also that adjusting the moisture content of many different seed lots of rice to near identical values and then determining those values accurately is most challenging. It was also a challenge with large numbers of

simultaneous germination test to provide optimum conditions to promote full germination of the viable seeds. Nonetheless, comparison of seed survival curves amongst blocks in Appendices 4.14 A-G in my third experiment showed good agreement. A single vigour test would be less work than determining a seed survival curve, however. Determining longevity was very useful in confirming that the results were not confounded with dormancy. In order to avoid confounding seed dormancy with viability, un-germinated seed were pricked at 21 days and retained in the incubator for up to 40 days. At the end, all the un-germinated seeds were darkened or mouldy, confirming they were not viable.

- My studies were also limited to only two cultivars: contrasting genotypes of aerobic *indica* rice are certainly worthy of similar investigations in order to determine the scope to select cultivars able to continue to produce high quality seeds in future warmer and possibly drier climates. Rice is one of the major crops of Malaysia, and such studies are needed now to improve the quality of rice seeds: Izham et al. (2003) reported that, Malaysia requires about 60,000 tonnes of rice seed per annum, but only produces 53,000 tonnes leaving a deficit of 7,000 tonnes (currently met by imports). Finally, I did not investigate the effect of drought and high temperature stress on rice's food value, particularly cooking quality.

From the above considerations, I recommend that future studies approach the research as outlined below.

- in future research between experimental environments and realistic local conditions, wherever that might be and whether current or future predicted conditions, the real historic data and the forecasted conditions need to be obtained from the relevant meteorological/climatology authority or agency, prior to conducting studies in those environments in controlled environment facilities. The use of daily temperature, relative humidity, light intensity and duration (including a sinusoidal diurnal temperature pattern and, although very expensive, in very high light intensity cabs) would be possible to give a more accurate estimate of the effect of a particular elevated temperature - event if that were the objective of the investigators. This information would enable future studies to be better defined. For example, 7 days of elevated temperature for only 5-6 hours per day above the simulated weather at

a given location would inform local rice producer of the real impact of one climate change phenomenon.

- Also, I would suggest that, it would be better if conducted in a photoperiod-controlled glass house rather than in growth cabs if *indica* rice is to studied as many of these cultivars are typically taller than *japonica* rices. In my study with *indica* rice Aeron 1, maximum plant height resulted in the grain on the tops of panicles nearly touching the fluorescent lights casing in the growth cab. This might cause grain to dry more quickly. This might have happened in my experiment four, where the final seed moisture contents for all treatments (include control) were relatively low (Fig. 5.1 & 2). The fluorescent lights casing in these growth cabs are cooled by ventilation and so the late drop in relative humidity because irrigation ended is a more likely explanation, but close proximity to lights might be a problem in less well-designed growth cabs.
- Serial destructive sampling of plants to harvest seeds at different stages of seed development provides more insight as to the possible mechanisms underlying the effects detected than only one final harvest at maturity. The latter approach was used in Experiment 2 and 4, because the numbers of treatments were too many to allow serial harvests. Serial harvesting throughout seed development was used in Experiments 1 and 3. This approach provided considerable insight into the effects of plant drought at different seed developmental stages on the contrasting trends of seed quality development an understanding which would not have been possible with a single harvest in the same date in all treatments.
- My research has shown that both drought and high temperature stress affect seed quality and that the timing of the stress affects the extent of any damage. Whilst my studies have shown that these stresses during anthesis and histodifferentiation, and early seed filling thereafter are especially damaging to seed quality development, future studies might investigate the possibility of damage in the period immediately preceding anthesis (when pollen and egg cells are formed and develop).
- Seed longevity development studies in rice in response to mother plant environmental stress should be continued because, based on a search on Web of Science, this type of study has seldom been reported.

My general recommendations are as follows.

- farmers should avoid water deficit in their rice crops from anthesis through early and perhaps late seed filling, whereas drought during maturation drying may be helpful, in order to maximize seed quality. This has obvious relevance to rice production in Malaysia using aerobic systems and other regions that use this cropping system. Perhaps, the system of rice intensification (SRI) – a system that was developed in Madagascar that involves a labour-intensive operation by selecting young seedlings and singly spacing in low water condition (Stoop et al., 2002 & Uphoof et al., 2006) can be adopted by farmers with some modification to suit adaptation under aerobic condition. This may reduce water usage in order to mitigate water scarcity resulted from global warming.
- In the face of the warming climate trend, cv. Aeron 1 might be able to become a parent for plant breeding programmes in other rice cultivating countries. The tolerance and escape traits identified in this study for that cultivar may assist plant breeders targeting resilience in warmer climates.
- In reality, Malaysian farmers generally obtain rice seed from government agencies such as MARDI. Hence, most rice farmers do not produce their own seed. Therefore, my study is most relevant to specialist seed producers which supply seed to these agencies. Hopefully, MARDI etc will find this smaller group of farmers easier to communicate with to provide advice. Hybrid rice seed producers should be particularly interested in the above recommendations. Hybrid seed is valuable and so low quality is not tolerated.

6.9 Conclusions

The main conclusions of my research can be summarised as follows:

i. ending irrigation early or brief elevated temperature did affect seed quality of *japonica* rice cv. Gleva and *indica* rice v. Aeron 1.

ii. Ending irrigation early well before seed filling ended accelerated seed desiccation, shortened seed filling duration and reduced maximum seed quality, whereas later treatments (during late seed filling and maturation drying) had little effect on subsequent seed quality.

iii. Water limitation treatments affected the development of subsequent seed longevity, where seed longevity declined *in planta* late in development and maximum longevity was poorer.

iv. Brief exposure to extreme high temperature at anthesis and during the histodifferentiation phase damaged subsequent rice seed longevity.

v. The negative effects on final seed dry weight were not associated with any effects on seed viability, with no evidence of any direct effect of 3-d exposure to elevated temperature during the seed-filling phase (period from 3-6 until 18-21 DAA).

vi. The viability of seeds was only reduced by high temperature treatments applied during the -3-0 or 0-3 DAA period, which coincides with anthesis and histodifferentiation.

vii. Effects on seed quality from combining drought and high temperature stress were more pronounced in cv. Gleva, whilst cv. Aeron 1 was considerably more resilient to these environmental stresses.

In general, the above conclusions show that my study's initial objectives were accomplished.

References

ABAYAWICKRAMA, A. S. M. T., REINKE, R. F., FITZGERALD, M. A., HARPER, J. D.I. & BURROWS, G. E. 2017. Influence of high daytime temperature during the grain filling stage on fissure formation in rice. *Journal of Cereal Science,* 74**,** 256-262

ABDUL-BAKI, A. A. & ANDERSON, J. D. 1970. Viability and Leaching of Sugars from Germinating Barley1. *Crop Science,* 10, 31-34.

ADAIR, CR. 1934. Studies on blooming in rice. *Journal of the American Society of Agronomy*, 26, 965-973.

AHMAD, S.A., HUSAIN, H., ALI, H. & AHMAD, A. 2005. Transplanted fine rice (*Oryza sativa* L.) productivity as affected by plant density and irrigation regimes. *International Journal of Agriculture and Biology*, 7, 445-447.

ALDRICH, S.R., 1943. Maturity measurements in corn and an indication that grain development continues after premature cuttings. *Journal of the American Society of Agronomy*., 35, 667-680.

ALTENBACH, S.B., DUPONT, F.M., KOTHARI, K.M., CHAN, R., JOHNSON, E.L. & LIEU, D. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. *Journal of Cereal Science*, 37, 9–20.

ANDERSON, S.R., 1955. Development of pods and seeds of birdsfoot trefoil, *Lotus corniculatus* L., as related to maturity and seed yields. *Agronomy Journal,* 47, 483-487.

ARORA, V. K. 2006. Application of a rice growth and water model in an irrigated semi-arid subtropical environment. *Agricultural Water Management*, 83, 51-57.

AUSTIN R.B. 1989. Prospects for improving crop production in stressful environments. *In*: Jones, H.G., Flowers, T.J. & Jones, M. B (Eds.). *Plant under stress*. Cambridge University Press, Cambridge, 235- 248.

BADSHAH, M., NAIMEI, T., ZOU, Y., IBRAHIM, M. & WANG, K. 2014. Yield and tillering response of super hybrid rice Liangyoupeijiu to tillage and establishment methods.*The Crop Journal*, 2, 79-86.

BAKER, J.T. 2004. Yield responses of southern US rice cultivars to CO_2 and temperature. *Agricultural and Forest Meteorology*,122, 129–137.

BARNABAS, B., JAGER, K. & FEHER, A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environment,* 31**,** 11-38.

BARUAH, K.K, RAJKHOWA, S.C & DAS, K. 2006. Physiological analysis of growth, yield development and grain quality of some deep water rice cultivars. *Agronomy and Crop Science,* 192, 228-232.

BERNAL-LUGO, I. & LEOPOLD, A. C. 1992. Changes in Soluble Carbohydrates during Seed Storage. *Plant Physiology,* 98, 1207.

BERNIER, J., ATLIN, G.N., SERRAJ, R., KUMAR, A., SPANER, D., 2008. Review: breeding upland rice for drought resistance. *Journal of the Science of Food and Agriculture*, 88, 927–939.

BEWLEY, J.D. 1986. Membrane changes in seeds as related to germination and the perturbations resulting from deterioration in storage. *In:* McDonald, J. R & Nelson (Eds.). *Physiology of seed deterioration*. Madison: Crop Science Society of America, 27-46.

BEWLEY, J.D. 1997. Seed germination and dormancy. *Plant Cell,* 9, 1055-1066.

BEWLEY, J.D. & BLACK, M. 1994. Seeds Physiology of Development and Germination. 3rd Edition, Plenum Press, New York, 445.

BEWLEY J.D., BRADFORD K.J., HILHORST H.W.M., NONOGAKI H. 2013. Longevity, Storage, and Deterioration. *In:* Seeds. Springer, New York.

BHOWMIK, S. K., SARKAR, M. A. R. & ZAMAN, F. 2013. Effect of spacing and number of seedlings per hill on the performance of aus rice cv. NERICA 1 under dry direct seeded rice (DDSR) system of cultivation. *Journal of the Bangladesh Agricultural University,*10.

BISHT, I. S. & SINGH, M. 2013. Genetic and genomic resources of grain legume improvement. Oxford, Elsevier, 322.

BLUM, A. 2005. Drought resistance, water-use efficiency, and yield potentialare they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research,* 56, 1159-1168.

BODNER, G., NAKHFOROOSH, A. & KAUL, H.-P. 2015. Management of crop water under drought: a review. *Agronomy for Sustainable Development,* 35**,** 401-442.

BOUMAN, B. & TOUNG, T.P. 2001. Field water management to save water and increase its productivity in irrigated low land rice. *Agriculture water management,* 49, 11-30.

BOUMAN, B.A.M., PENG, S., CASTAÑEDA, A. R. & VISPERAS, R. M. 2005. Yield and water use of irrigated tropical aerobic rice systems. *Agricultural Water Management,* 74, 87-105.

BOUMAN, B., LAMPAYAN, R. & TUONG, T. P. 2007. *Water management in irrigated rice. Coping with water scarcity*. Los Baños (Philippines): International Rice Research Institute, 54.

BORISJUK, L., WEBER, H., PANITZ, R., MANTEUFFEL, R. & WOBUS, U. 1995. Embryogenesis of Vicia faba L.: Histodifferentiation in Relation to Starch and Storage Protein Synthesis. *Journal of Plant Physiology,* 147**,** 203-218.

BRENNER, M.L. & CHEIKH, N. 1995. Hormones in photosynthate partitioning and seed filling. *In:* Davies, P.J. (ed.). *Plant Hormones*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 649–670.

BUCHANAN-WOLLASTON, V. & AINSWORTH, C. 1997. Leaf senescence in Brassica napus: cloning of senescence related genes by subtractive hybridisation. *Plant Molecular Bioliogy,* 33**,** 821-34.

BUITINK, J. & LEPRINCE, O. 2008. Intracellular glasses and seed survival in the dry state. *Comptes Rendus Biologies***,** 331**,** 788-95.

BYRD, D. 2014. World population unlikely to stabilize in this century. http://earthsky.org/earth/world-population-unlikely-to-stabilize-this-century. Accessed 3 January 2018.

CAO, Z. Z., ZHAO, Q., HUANG, F. D., WEI, K. S., ZAIDI, H., ZHOU, W. & CHENG, F. M. 2015. Effects of high temperature at anthesis on spikelet fertility and grain weight in relation to floral positions within a panicle of rice *(Oryza sativa* L.*). Crop & Pasture Science,* 66, 922–929.

CALDERINI, D. F., REYNOLDS, M. P. & SLAFER, G. A. 1999. Genetic gains in wheat yield and main physiological changes associated with them during the 20th century. *In*: Wheat: Ecology and Physiology of Yield Determination. Satorre, E.H. & Slafer, G.A. (Eds.). Food Product Press, New York, 351–377.

CÉCILE-JULIA, A. & DINGKUHN. M**.** 2013. Predicting temperature induced sterility of rice spikelets requires simulation of crop-generated microclimate. *European Journal Agronomy,* 49, 50– 60.

CHAN, N. W. 2004. Managing Water Resources in the 21st Century: Involving All Stakeholders Towards Sustainable Water Resources Management in Malaysia. Bangi: Environmental Management Programme, Centre for Graduate Studies, Universiti Kebangsaan Malaysia.

CHANG. T.T. 1991. Findings from a 28-yr seed viability experiment. *International Rice Research Newsletter,* 16, 5–6.

CHANG, T. T. & BARDENAS, E. A. 1965. The morphology and varietal characteristics of the rice plant. *The International Rice Research Institute Technical Bulletin,* 4, 40.

CHENG, H. 1993. Total dietary fiber content of polished, brown and bran types of japonica and indica rice in taiwan resulting physiological effects of consumption. *Nutrition Research,* 13, 93-101.

CHENG, K. S. 1993. The Identification of *O. sativa* L. subsp. Ting and *O. sativa* L. subsp. Keng Ting.Kunming. *Yunnan Science and Technology Press*.

COAST, O., ELLIS, R. H., MURDOCH, A. J., QUIÑONES, C. & JAGADISH, K. S. V. 2015. High night temperature induces contrasting responses for spikelet fertility, spikelet tissue temperature, flowering characteristics and grain quality in rice. *Functional Plant Biology*, 42, 149-161.

COUNCE, P.A. & KEISLING. T.C. 1995. Emergence data mediation of rice yield responses to equidistant spacing. *Field Crops Research,* 41,167-171.

COUNCE, P. A. & WELLS, B. 1990. Rice plant-population density effect on early-season nitrogen requirement. *Journal of Production Agriculture,* 3, 390-393.

COUNCE, P. A, MOLDENHAUER, K. & MARX, D. 1989. Rice yield and plant-yield variability responses to equidistant spacing. *Crop Science,* 29, 175-179.

COUNCE, P. A., SIEBENMORGEN, T. J., POAG, M. A., HOLLOWAY, G. E., KOCHER, M. F. & LU, R. 1996. Panicle emergence of tiller types and grain yield of tiller order for direct-seeded rice cultivars. *Field Crops Research,* 47, 235-242.

COPELAND, L.O. & MCDONALD, M. B. 1995. Principle of seed science and technology. New York: Chapmen & Hall, 409.

COPELAND, L. O. & MCDONALD, M. B. 1999. Seed Vigour and Vigour Tests. *In:* COPELAND, L. O. & MCDONALD, M. B. (Eds.). *Principles of Seed Science and Technology*. Boston, MA, Springer US.

CHANG, T.T., & BARDENAS, E. A. 1965. The Morphology and varietal characteristics of the rice plant. Los Baños, The International Rice Research Institute.

CHATELAIN, E., HUNDERTMARK, M. & LEPRINCE, O. 2012. Temporal profiling of the heat-stable proteome during late maturation of Medicago truncatula seeds identifies a restricted subset of late embryogenesis abundant proteins associated with longevity. *Plant, Cell & Environment,* 35, 1440–1455.

CROMARTY, A., ELLIS, R.H. & ROBERTS, E.H. 1982. The Design of Seed Storage Facilities for Genetic Conservation. International Board for Plant Genetic Resources, Rome.

CRUZ, R.T. & O'TOOLE, J.C**.** 1984. Dryland rice response to an irrigation gradient at flowering stage. *Agronomy Journal*, 76, 178-183.

CRUZ, R. P. D., MILACH, S. C. K. & FEDERIZZI, L. C. 2006. Rice cold tolerance at the reproductive stage in a controlled environment. *Scientia Agricola,* 63**,** 255-261.

DASGUPTA, 1., BEWLEY, J.D. & YEUNG, E.C., 1982. Desiccation-tolerant and desiccation-intolerant stages during the development and germination of Phaseolus vulgaris seeds. *Journal of Experimental Botany*, 33,59-68.

DASGUPTA, P., DAS, B. S. & K-SEN, S. 2015. Soil water potential and recoverable water stress in drought tolerant and susceptible rice varieties. *Agricultural Water Management,* 152, 110-118.

DELOUCHE, J.C. 1980. Environmental-effects on seed development and seed quality. *Hortscience,* 15, 775-780.

DELOUCHE, J.C. & NGUYEN, N.T. 1964. Methods for overcoming seed dormancy in rice. *Proceeding Association of Official Seed Analysts*, 54,41–49.

DEMIR, I., ERMIS, S., MAVI, K. & MATTHEWS, S. 2008. Mean germination time of pepper seed lots (*Capsicum annuum* L.) predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Science & Technology,* 36, 21-30.

DENG H. F., HE Q. & SHU F. 2006. Research status and countermeasures of japonica hybrid rice in China. *Hybrid Rice,* 21, 1–6.

DICKIE, J.B., ELLIS, R., KRAAK, H.L., RYDER, K., & TOMPSETT, P.B. 1990. Temperature and seed storage longevity. *Annals of Botany,* 65,197–204.

DUNAND, R. & SAICHUK, J. 2014. Rice growth and development. *In:* Saichuk, J. Louisiana Rice Production Handbook. Crowley (US), LSU Agricultural Center, 34.

DURAN, X., JOSEP, P. M. & REALES, L. 2017. Government Of Catalonia; Institute Of Catalan Studies. *In:* Queralt. A. (Ed). Climate change in Catalonia: Executive summary of the Third Report on Climate Change in Catalonia.

EGLI, D. B. & TEKRONY, D. M. 1997. Species differences in seed water status during seed maturation and germination. *Seed Science Research,* 7**,** 3-12.

ELLIS, R. 2011. Rice seed quality development and temperature during late development and maturation. *Seed Science Research,* 21, 95-101.

ELLIS, R. & PIETA-FILHO, C. 1992. The development of seed quality in spring and winter cultivars of barley and wheat. *Seed Science Research,* 2**,** 9-15.

ELLIS, R.& HONG, T.D. 1994. Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.), *Annals of Botany,* 73,501-506.

ELLIS, R. & HONG, T. 2007. Seed longevity - moisture content relationships in hermetic and open storage. *Seed Science and Technology,* 35**,** 423-431.

ELLIS, R. & ROBERTS, E. 1980. Improved Equations For The Prediction Of Seed Longevity. *Annals of Botany,* 45, 13-30.

ELLIS, R. & ROBERTS, E. 1981. The quantification of aging and survival in orthodox seeds. *Seed Science and Technology,* 9, 373-409.

ELLIS, R., HONG, T. & JACKSON, M. 1993. Seed production environment, time of harvest, and the potential longevity of seeds of 3 cultivars of rice (*Oryza sativa* L.). *Annals of Botany,* 72, 583-590.

ELLIS, R., HONG, T. & ROBERTS, E. 1983. A note on the development of a practical procedure for promoting the germination of dormant seed of grape (Vitis Spp). *Vitis,* 22, 211-219.

ELLIS, R., HONG, T. & ROBERTS, E. 1987. The development of desiccation-tolerance and maximum seed quality during seed maturation in 6 grain legumes. *Annals of Botany,* 59**,** 23-29.

ELLIS, R., HONG, T. & ROBERTS, E. 1988. A low-moisture-content limit to logarithmic relations between seed moisture-content and longevity. *Annals of Botany,* 61**,** 405-408.

ELLIS, R. H., HONG, T. D. & ROBERTS, E. H. 1992. The Low-moisture-content Limit to the Negative Logarithmic Relation Between Seed Longevity and Moisture Content in Three Subspecies of Rice. *Annals of Botany,* 69**,** 53-58.

ELLIS, R. & YADAV, G. 2016. Effect of simulated rainfall during wheat seed development and maturation on subsequent seed longevity is reversible. *Seed Science Research,* 26, 67-76.

ESKANDARI, H., HAMID, A. & ALIZADEH-AMRAIE, A. 2015. Development and maturation of sesame (*Sesamum indicum*) seeds under different water regimes. *Seed Science and Technology* ,43.

EVENSON, R.E., HERDT, R.W. & HOSSAIN, M. 1996. Rice Research in Asia: Progress and Priorities. CAB International in association with the International Rice Research

Institute, Philippines and Wallingford, UK, Wallingford (UK), 418.

FAO. 2017. The future of food and agriculture. *Trends and challenges*. Rome.

FERRIS, R., ELLIS, R. H., WHEELER, T. R. & HADLEY, P. 1998. Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat, *Annals of Botany*, 82, 631–639.

FINKELSTEIN, R. R. 2004. The role of hormone during seed development and germination, in Davies, P.j. (Ed.). *Plant hormones: Biosynthesis, Signal transduction, action.* Kluwer Academic Publicher, Dordrecht, The Netherland, 513-537.

FINCH-SAVAGE, W. E. 2003. Seed development. Onset of desiccation tolerance. Brian. T (Ed.). *Encyclopedia of Applied Plant Sciences.* Oxford, Elsevier.

FINCH-SAVAGE, W. & LEUBNER-METZGER, G. 2006. Seed dormancy and the control of germination. *New Phytologist*, 171, 501-523.

FILHO, C. P. & ELLIS, R. H. 1991. The development of seed quality in spring barley in four environments. I. Germination and longevity. *Seed Science Research,* 1, 163-177.

FIROUZI, S. & ALIZADEH, M. R. 2011. Effect of Whitener Type and Paddy Moisture Content on Rice Grain Damage During Milling Process. *American-Eurasian Journal of Agricultural & Environmental Sciences,* 10, 470-474.

FISCHER, W., BERGFELD, R., PLACHY, C., SCHAFER, R. & SCHOPFER, P. 1988. Accumulation of storage materials, precocious germination and development of desiccation tolerance during seed maturation in mustard (*Sinapis alba* L.). *Botany Acta,* 101, 344-354.

FOUGEREUX, J.A., T. DORE, F. LADONNE & A. FLEURY. 1997. Water stress during reproductive stages affects seed quality and yield of pea. *Crop Science,* 37,1247-1252.

FUKAI, S. & LILLEY, J.M. 1994. Effects of timing and severity of water deficit on four diverse rice cultivars. Phenological dev, Crop growth and grain yield. *Field Crop Research*, 37, 225-234.

FUKAI, S., PANTUWAN, G., JONGDEE, B., & COOPER M. 1999. Screening for drought resistance in rainfed lowland rice. *Field Crop Research*, 64: 61-74.

GARRITY, D.P., & O'TOOLE, J.C. 1994. Screening rice for drought resistance at the reproductive phase. *Field Crop Research,* 39, 99–110.

GHASSEMI-GOLEZANI, K. & MAZLOOMI-OSKOOYI, R. 2008. Effect of water supply on seed quality development in common bean (*Phaseolus vulgaris* var.). *International Journal of Plant Production,* 2**,** 117-124.

GHASSEMI-GOLEZANI, K., CHADORDOOZ-JEDDI, A., NASROLLAHZADEH, S. & MOGHADDAM, M. 2010. Effects of hydro-priming duration on seedling vigour and grain yield of pinto bean (*Phaseolus vulgaris* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 38, 109-113.

GHASSEMI-GOLEZANI, K., LOTFI, R. & NOROUZI, M. 2012. Seed quality of soybean cultivars affected by pod position and water stress at reproductive stages. *International Journal of Plant, Animal and Environmental Sciences*,2, 119-125.

GOLDBERG, R., DEPAIVA, G. & YADEGARI, R. 1994. Plant embryogenesis - zygote to seed. *Science,* 266, 605-614.

GOODING, M. J., PINYOSINWAT, A. & ELLIS, R. 2002. Responses of wheat grain yield and quality to seed rate. *Journal of Agricultural Science*, 138, 317–331.

GRASS, L. & BURRIS, J. S. 1995. Effect of heat stress during seed development and maturation on wheat (Triticum durum) seed quality. Seed germination and seedling vigour. *Canadian Journal of Plant Science,* 75**,** 821-829.

GUNAWARDENA, T. A., FUKAI, S. & BLAMEY, F. P. C. 2003. Low temperature induced spikelet sterility in rice. I. Nitrogen fertilisation and sensitive reproductive period. *Australian Journal of Agricultural Research,* 54**,** 937-946.

HARRINGTON, J.F**.** 1972. Seed storage and longevity. *In*: Kozlowski, T.T. (Ed.). *Seed Biology,* New York, Academic Press, 3, 145-245.

HAY, F.R., SMITH, R.D. 2003. Seed maturity: when to collect seeds from wild plants. *In*: Smith, R.D., Dickie, J.B., Linington, S., Pritchard, H.W. & Probert, R.J. (eds.). *Seed Conservation – Turning Science into Practice.* Royal Botanic Gardens Kew, 97–133.

HAY, F.R., KLIN, J., PROBERT, R.J. 2006. Can a post-harvest ripening treatment extend the longevity of *Rhododendron* L. seeds? *Scientia Horticulturae,* 111, 80–83.

HDR. 1997. Human Development Report. New York. Oxford University Press.

HERMAN, T., MURCHIE, E. H. & WARSI, A.A. 2015. Rice Production and Climate Change: A Case Study of Malaysian Rice. *Pertanika Journal Tropical Agricultural Science*, 38, 321-328.

HILHORST, H.W.M. & TOOROP, P.E. 1997. Review on dormancy, germinability, and germination in crop and weed seeds. *Advances in Agronomy*, 61, 111-165.

HONG, T.D. & ELLIS, R. 1990. A comparision of maturation drying, germination, and desiccation tolerance between developing seeds of *Acer pseudoplatanus* L. and *Acer platanoides* L. *New Phytologist,* 116,589-596.

HONG, T.D. & ELLIS, R. 1992. Development of desiccation tolerance in Norway maple (*Acer platanoides* L.) seeds during maturation drying, *Seed Science Research,* 2, 169-172.

HUANG, M., ZOU, Y., FENG, Y., CHENG, Z., MO, Y., XIA, B. & JIANG, P. 2011. No-tillage and direct seeding for super hybrid rice production in rice–oilseed rape cropping system. *European Journal of Agronomy*, 34, 278-286.

HURKMAN, W.J., MCCUE, K.F., ALTENBACH, S.B., KORN, A., TANAKA, C.K., KOTHARI, K.M., JOHNSON, E.L., BECHTEL, D.B., WILSON, J.D., ANDERSON, O.D. & DUPONT, F.M., 2003. Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Science*, 164, 873–881.

HUSSAIN, S., FUJII, T., MCGOEY, S., YAMADA, M., RAMZAN, M. & AKMAL, M. 2014. Evaluation of different rice varieties for growth and yield characteristics. *Journal of Animal and Plant Sciences*, 24, 1504-1510.

ILIEVA, V., KAROV, I., MIHAJLOV, L., MARKOVA RUZDIK, N. & ILIEVSKI, M. 2014. Effect of rice moisture at harvest and rough rice storage time on milling yield and grain breakage. Conference: International Scientific Conference " *Breeding and Techologies Field Crops*".

IPCC. 2014. Climate Change 2014: Synthesis report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernamental Panel on Climate Change. IPCC, Geneva, Switzerland.

IRRI. 2007. Summary report of the first international aerobic rice observational nursery (1st AERON) 2005, International Network for Genetic Evaluation of Rice (INGER), IRRI, Manila, Philippines.

IRRI. 2013. Rice knowledge bank. International Rice Research Institute, Los Baños, Philippines. http:// knowledgebank.irri.org/step-by-step-production/growth/pests-anddiseases/diseases. Accessed 20 Feb. 2018.

IRRI. 2017. Rice Today. Future rice climate in Africa. April-June 2017,16, 23.

IRRI. 2018. How to develop a crop calender. http://www.knowledgebank.irri.org/step-by-step-production/pre-planting/crop-calendar. Accessed 15 March 2018.

ISHIMARU, T., MATSUDA, T., OHSUGI, R. & YAMAGISHI, T. 2003. Morphological development of rice caryopsis located at the different positions in a panicle from early to middle stage of grain filling. *Functional Plant Bioliogy*,30,1139–1149.

ISTA. 1995. Handbook of seed vigour test methods. Zurich, Switzerland, International Seed Testing Association.

ISTA. 2009. lnternational Rules for Seed Testing. Switzerland. The Intenationa1 Seed Testing Association,

ISTA. 2010. International Rules for Seed Testing. Switzerland, International Seed Testing Association.

IZHAM, A., ZAINOL, A. A., LEONG, A.C. & SHAHADAN, M. 2003. Production of quality seeds and planting materials by MARDI. *Proceeding 3rd National Seed Symposium*, 8-

9 April 2003,Putrajaya Kuala Lumpur, Malaysian Association of Seed Technologies. 32-34.

JAGADISH, S. V. K., CRAUFURD, P. Q. & WHEELER, T. R. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany,* 58**,** 1627-1635.

JAGADISH, S.V.K., CRAUFURD, P.Q. & WHEELER, T.R. 2008. Phenotyping parents of mapping populations of rice (O*ryza sativa* L.) for heat tolerance during anthesis. *Crop Science,* 48,1140–1146.

JAGADISH, S. V. K., CAIRNS, J. LAFITTE, R. WHEELER, T. R. PRICE, A. H. & CRAUFURD, P. Q. 2010a. Genetic Analysis of Heat Tolerance at Anthesis in Rice. *Crop Science*. 50, 1633-1641.

JAGADISH, S. V. K., MUTHURAJAN, R., OANE, R., WHEELER, T. R., HEUER, S., BENNETT, J. & CRAUFURD, P. Q. 2010b. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.) *Journal of Experimental Botany*, 61**,** 143-156.

JAGOE, RB. 1931. Hybridization of selected strain of padi. T*he Malayan Agricultural Journal*, 19, 530-534.

JATOI, S., MUHAMMAD, A., SHAHZAD, N. & RASHID, A. 2001. Seed Deterioration Study in Pea, Using Accelerated Ageing Techniques. *Pakistan Journal of Biological Sciences*, 4, 1490-1494.

JOSE, M., MOHANASARIDA, K. & RESMI, O.W. 2004. Water scarcity in dry seeded lowland rice. Proceedings of the 4th international crop science congress, Brisbane, Australia.

JYOTI & MALIK, C.P. 2013. Seed Deterioration: A Review, *International Journal of Life Sciences Biotechnology and Pharma Research*, 2, 374-385..

KAMOSHITA, A., BABU, R.C., MANIKANDA BOOPATHI, N., FUKAI, S., 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Research*. 109, 1–23.

KATO, Y. & OKAMI, M. 2011. Root morphology, hydraulic conductivity and plant water relations of high–yielding rice grown under aerobic conditions. *Annals of Botany,*108, 575-583.

KATO, S., KOSAKA, H., MARUYAMA, Y. & TAKIGUCHI, Y. 1930. On the affinity of cultivated varieties of rice plants, *Oryza sativa* L. *Journal of Department of Agriculture, Kyushu University*, 2, 241-76.

KATO, Y., SATOSHI, H., AKINIKO, K., ABE, J., URASAKI, K. & YAMAGISHI, J. 2004. Enhancing grain yield of rice (*Oryza sativa* L.) under upland conditions in Japan. 4*th International Crop Science Congress*, Brisbane, Australia.

KATO, Y., ABE, J., KAMOSHITA, A. & YAMAGISHI, J. 2006. Genotypic Variation in Root Growth Angle in Rice (*Oryza sativa* L.) and its Association with Deep Root

Development in Upland Fields with Different Water Regimes. *Plant and Soil*, 287, 117-129.

KERMODE, A. & BEWLEY, J. 1985. The role of maturation drying in the transition from seed development to germination. *Journal of Experimental Botany,* 36, 1906-1915.

KERMODE, A.R, BEWLEY, J.D., DASGUPTA, J. & MISRA S. 1986. The transition from seed development to germination: a key role for desiccation? *HortScience*. 21,1113-18.

KIM, J., SHON, J., LEE, C.K., YANG, W., YOON, Y., YANG, W.H., KIM, Y.G., & LEE, B.W., 2011. Relationship between grain filling duration and leaf senescence of temperate rice under high temperature. *Field Crops Research*, 122, 207–213.

KOBATA, T. & UEMUKI, N. 2004. High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. *Agronomy Journal*, 96,406-414.

KOBATA, T., PALTA, J.A. & TURNER, N.C. 1992. Rate of development of post-anthesis water deficits and grain filling of spring wheat. *Crop Science*, 32, 1238–1242.

KOBATA, T., PALTA, J. A., TANAKA, T., OHNISHI, M., MAEDA, M., KOÇ, M. & BARUTÇULAR, C. 2018. Responses of grain filling in spring wheat and temperate-zone rice to temperature: Similarities and differences. *Field Crops Research*, 215**,** 187-199.

KOBATA, T., PALTA, J. A., TANAKA, T., OHNISHI, M., MAEDA, M., KOÇ, M. & BARUTÇULAR, C. 2018. Responses of grain filling in spring wheat and temperate-zone rice to temperature: Similarities and differences. *Field Crops Research*, 215, 187-199.

KORRES, N. E., NORSWORTHY, J. K., BURGOS, N. R. & OOSTERHUIS, D. M. 2017. Temperature and drought impacts on rice production: An agronomic perspective regarding short- and long-term adaptation measures. *Water Resources and Rural Development,* 9**,** 12-27.

KRAEHMER, H., JABRAN, K., MENNAN, H. & CHAUHAN, B. 2016. Global distribution of rice weeds A review. *Crop Protection*, 80, 73-86.

KRAEHMER, H., THOMAS, C. & VIDOTTO, F. 2017. Rice Production in Europe. *In:* CHAUHAN, B. S., JABRAN, K. & MAHAJAN, G. (Eds.) *Rice Production Worldwide*. Cham: Springer International Publishing.

KRISHNAN, P.,NAGARAJAN,S. & MOHARIR, A.V. 2004. Thermodynamic characterization of seed deterioration during storage under accelerated aging conditions. *Biosystem Engineering*, 89, 425-433.

KRISHNAN, R., RAMAKRISHNAN, B., REDDY, K., REDDY, V. & SPARKS, D. 2011. High-temperature effects on rice growth, yield, and grain quality. *Advances in Agronomy,*111, 87-206.

LEPRINCE, O., PELLIZZARO, A., BERRIRI, S. & BUITINK, J. 2017. Late seed maturation: drying without dying. *Journal of Experimental Botany,* 68**,** 827-841.

LEVITT, J.J. 1980. Responses of plants to environmental stresses, Radiation, salt and other stresses. 2nd Edition. Academic press Inc. London, United Kingdom, 365- 488.

LININGTON, S.H. & PRITCHARD, H.W. 2001. Genebanks. *In*: Levin, S.A. (Ed.). *Encyclopedia of Biodiversity*, Academic Press, San Francisco, 165– 181.

LOBELL, D. B. & GOURDJI, S. M. 2012. The influence of climate change on global crop productivity. *Plant Physiology*, 160, 1686.

MACHADO, S. & PAULSEN, G. M. 2001. Combined effects of drought and high temperature on water relations of wheat and sorghum. *Plant and Soil*, 233**,** 179-187.

MACLEAN, J.L., DAWE, D.C., HARDY, B. & HETTEL, G.P. 2002. Rice almanac (3rd Edition). Philippines, IRRI, WARDA, CIAT & FAO.

MADAN, P., JAGADISH, S. V.K., CRAUFURD, P. Q., FITZGERALD, M., LAFARGE, T., & WHEELER, T. R. 2012. Effect of elevated CO_2 and high temperature on seed-set and grain quality of rice. *Journal of Experimental Botany*, 1-10.

MAKINO, A., NAKANO, H. & MAE, T. 1994. Responses of ribulose-1,5-bisphosphate carboxylase, cytochrome f, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. *Plant Physiology*, 105, 173.

MARTÍNEZ-EIXARCH, M. & ELLIS, R. 2015. Temporal sensitivities of rice seed development from spikelet fertility to viable mature seed to extreme-temperature. *Crop Science*, 55, 354-364.

MARTÍNEZ-EIXARCH, M., ZHU, D.-F., CATALÁ-FORNER, M. D. M., PLA-MAYOR, E. & TOMÁS-NAVARRO, N. 2013. Water, nitrogen and plant density affect the response of leaf appearance of direct seeded rice to thermal time. *Rice Science*, 20**,** 52-60.

MATSUI, T., OMASA, K. & HORIE, T. 1999. Rapid swelling of pollen grains in response to floret opening unfolds anther locules in rice *(Oryza sativa* L.*). Plant Production Science*, 2, 196-199.

MATSUI, T., OMASA, K. & HORIE, T. 2001. The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Production Science*, 4, 90–93.

MATTHEWS, S. & BRADNOCK, W.T. 1968. Relationship between seed exudation and field emergence in peas and French beans. *Horticultural Research*,8, 89-93.

MAXTED, N., FORD-LLOYD, B.V., HAWKES, J.G. 1997. *Plant genetic conservation: the in situ approach*. New York: Chapman & Hall.

MCDONALD, M. B. 1998. Seed quality assessment. *Seed Science Research*, 8, 265-276.

MCKERSIE, B.D. & YA'ACOV, Y.L. 1994. Stress and stress coping in cultivated plants. Kluwer Academic press. USA. pp. 148-177.

MILLER, B. C., HILL, J. E. & ROBERTS, S. R. 1991. Plant Population Effects on Growth and Yield in Water-Seeded Rice. *Agronomy Journal*, 83, 291-297.

MIN, J., ZHU, Z.W., CHEN, N. 2012. Study on the quality and the percentages meeting fine quality standards in Chinese indica conventional rice. *China Rice,* 18, 4–7.

MIN, J., ZHU, Z. W., JIN, L. D., XU, L., ZHANG, L. P., TANG, S. X. 2011. Analysis on grain quality of indica hybrid rice combinations bred during twenty-five years in China. *China Journal Rice Science.,* 25, 201–205.

MITTLER, R. 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Science,*11, 15-19.

MISHRA, A.K., MOTTALEB, K.A., KHANAL, A.R., MOHANTY, S., 2014. Abiotic stress and its impact on production efficiency: the case of rice farming in Bangladesh. *Agriculture, Ecosystems & Environment,* 199, 146–153.

MOFFAT, A.S. 2002. Plant genetics – finding new ways to protect drought-stricken plants. *Science,* 296, 1226–1229.

MOLDENHAUER, K. A. K. & GIBBONS, J. H. 2003. Rice morhpology and development, in: Rice: Origin, History, Technology, and Production. *New Jersey,* 103-128.

MORADI, F. 2011. Effect of drought at the post-anthesis stage on remobilization of carbon reserves and some physiological changes in the flag leaf of two wheat cultivars differing in drought resistance. *Journal of Agricultural Science,* 3, 81-92.

MORITA , S. 2008. Prospect for developing measures to prevent high temperature damage to rice grain ripening. *Japan Journal Crop Science,* 77,1-12. (in Japanese with English summary).

MOSTAJERAN, A. & RAHIMI-EICHI, V. 2009. Effects of Drought Stress on Growth and Yield of Rice (*Oryza sativa* L.) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 5, 264-272.

MURSHIDA, S., UDDIN, M. R., ANWAR, M., SARKER, U., ISLAM, M. M. & HAQUE, M. M. I. 2017. Effect of variety and water management on the growth and yield of Boro rice. *Progressive agriculture,* 28, 26-35.

MURTY, M.V.R. & KONDO, M., 2001. Upland: a simulation model for water balance in upland soils. IRRI Technical Bulletin No. 7. International Rice Research Institute, Los Banos, Philippines, 42.

NAGATA, K., YOSHINAGA, S., TAKANASHI, J. & TERAO, T. 2001. Effects of dry matter production, translocation of nonstructural carbohydrates and nitrogen application on grain fi lling in rice cultivar Takanari, a cultivar bearing a large number of spikelets. *Plant Production Science*, 4, 173-183.

NAKAMURA, Y., YUKI, K., PARK, S.-Y. & OHYA, T. 1989. Carbohydrate Metabolism in the Developing Endosperm of Rice Grains. *Plant and Cell Physiology,* 30**,** 833-839.

NASEHZADEH, M. & ELLIS, R. 2017. Wheat seed weight and quality differ temporally in sensitivity to warm or cool conditions during seed development and maturation. *Annals of Botany,* 120**,** 479-493.

NERLING, D., COELHO, C. M. M. & NODARI, R. O. 2013. Genetic diversity for physiological quality of seeds from corn (*Zea mays* L.) intervarietal crossbreeds. *Journal of Seed Science*, 35, 449-456.

NICHOLS, M. A., SCOTT, D. J., WARRINGTON, I. J. & GREEN, L. M. Pre-harvest treatment effects on some quality criteria of pea seeds. 1978. International Society for Horticultural Science (ISHS), Leuven, Belgium, 113-124.

NICOLAS, M. E., GLEADOW, R. M. & DALLING, M. J. 1985. Effect of Post-anthesis Drought on Cell Division and Starch Accumulation in Developing Wheat Grains. *Annals of Botany*, 55, 433-444.

NORMILE, D., 2008. Reinventing rice to feed the world. *Science*, 321, 330–333.

OBER, E.S. & SETTER, T.L. 2010. Timing of kernel development in water stressed maize: water potentials and abscisic acid concentrations. *Annals of Botany*, 66, 665–672.

OLASOJI, J. O., OLUBUNMI, A., OLUTAYO, A., OLANIPEKUN, S. O., OLOSUNDE, A. & OKOH, J. O. 2012. Effect of time of harvest on physiological maturity and kenaf (*Hibiscus canabinus*) seed quality. *African Journal of Plant Science*, 6, 282-289.

ONWIMOL, D., CHANMPRASERT, W., CHANGSEE, P. & RONGSANGCHAICHAREON, T. 2016. Seed vigour classification using analysis of mean radicle emergence time and single counts of radicle emergence in rice (*Oryza sativa* L.) and mung bean (*Vigna radiata* L. Wilczek). *Agriculture and Natural Resources,* 50, 345-350.

ORI, N., JUAREZ, M. T., JACKSON, D., YAMAGUCHI, J., BANOWETZ, G. M. & HAKE, S. 1999. Leaf senescence is delayed in tobacco plants expressing the maize homeobox gene knotted1 under the control of a senescence-activated promoter. *Plant Cell,* 11**,** 1073-80.

O'TOOLE, J. C. 1982. Adaptation of rice to drought-prone environment. *Drought Resistance in Crops with Emphasis on Rice*, 195-213.

O'TOOLE, J. C., & O. S. NAMUCO. 1983. Role of Panicle Exsertion in Water Stress Induced Sterility. *Crop Sci.* 23, 1093-1097.

PENG, S., KHUSH, G.S., CASSMAN, K.G. 1994. Evolution of the new plant ideotype for increased yield potential, Cassman, K.G. (Ed.), Breaking the Yield Barrier, International Rice Research Institute, Philippines ,5-20.

PENG, S. B., TANG, Q. & ZOU, Y. 2009. Current status and challenges of rice production in China. *Plant Production Science*, 12, 3–8.

PENG, S., CASSMAN, K.G., VIRMANI, S.S., SHEEHY, J., KHUSH, G.S. 1999. Yield potential trends of tropical rice since release of IR8 and the challenge of increasing rice yield potential. *Crop Science* ,39, 1552–1559.

PENG, S., YANG, J., LAZA, R.C., SANICO, A.L, VISPERAS, R.M., SON, T.T. 2003. Physiological bases for heterosis and crop management strategies for hybrid rice in the tropics. *In*: Virmani, S.S., Mao, C.X., Harby, B., (eds.). Hybrid rice for food security,

poverty alleviation, and environmental protection. Los Baños, the Philippines: International Rice Research Institute, 153–172.

PENG, S.B., HUANG, J.L., SHEEHY, J.E., LAZA, R.C., VISPERAS, R.M., ZHONG, X.H., CENTENO, G.S., KHUSH, G.S., CASSMAN, K.G., 2004. Rice yields decline with higher night temperature from global warming. *PNAS*, 101, 9971–9975.

PENNISI, E. 2008. The blue revolution, drop by drop, gene by gene. *Science*. 320, 171-173.

PEREIRA, L. A., DE CARVALHO, M. L. M., NERY, M. C. & TOOROP, P. E. 2014. Embryo morphology indicates physiological maturity better than seed mass in *Syngonanthus elegans* (Eriocaulaceae). *Seed Science and Technology*, 42, 161-170.

PIRDASHTI, H., Z. T. SARVESTANI, G. NEMATZADCH & A. ISMAIL. 2004. Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. 4th Int. *Crop Science Congress*, 1-7.

PLAUT, Z., BUTOW, B. J., BLUMENTHAL, C. S. & WRIGLEY, C. W. 2004. Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. *Field Crops Research*, 86**,** 185-198.

POWELL, A.A. & MATTHEWS, S. 1978. The damaging effect of water on dry pea embryos during imbibition. *Journal of Experimental Botany*, 29, 1215-1229.

PRASAD, P. V. V., BOOTE, K. J., ALLEN, L. H., SHEEHY, J. E. & THOMAS, J. M. G. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, 95**,** 398-411.

PRASAD, P. V. V., PISIPATI, S. R., MOMČILOVIĆ, I. & RISTIC, Z. 2011. Independent and Combined Effects of High Temperature and Drought Stress During Grain Filling on Plant Yield and Chloroplast EF-Tu Expression in Spring Wheat. *Journal of Agronomy and Crop Science*, 197**,** 430-441.

PRITCHARD, H.W. & DICKIE, J.B. 2003. Predicting seed longevity: the use and abuse of seed viability equations. *In*: Smith, R. D., Dickie, J. B., Linington, S. H., Pritchard, H. W. and Probert, R. J. (Eds.). *Seed conservation: turning science into practice*. Royal Botanic Kew, UK. 654-721.

PRITCHARD, H. W. & NADARAJAN, J. 2008. Cryopreservation of Orthodox (Desiccation Tolerant) Seeds. *In:* REED, B. M. (Ed.) *Plant Cryopreservation: A Practical Guide.* New York, NY: Springer New York.

PROBERT, R., ADAMS, J., CONEYBEER, J., CRAWFORD, A. & HAY, F. 2007. Seed quality for conservation is critically affected by pre-storage factors. *Australian Journal of Botany,* 55, 326–335

QIAN, Q. 2007. Breeding by Gene Design in Rice Beijing. *Science Press*.

RADIN-FIRDAUS, R. B., ABDUL LATIFF, I., & BORKOTOKY, P. 2012. The impact of climate change towards Malaysian paddy farmers. *Journal of Development and Agriculture Economics*, 5 , 57-66.

RAMOS, K.M.O., MATOS, J.M.M., MARTINS, R.C.C., MARTINS, I.S. 2012. Electrical conductivity testing as applied to the assessment of freshly collected *Kielmeyera coriacea* Mart. Seeds. *ISRN Agronomy*, 2012, 5.

RANG, Z. W., JAGADISH, S. V. K., ZHOU, Q. M., CRAUFURD, P. Q. & HEUER, S. 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environmental and Experimental Botany,* 70**,** 58-65.

RAO, N. & JACKSON, M. T. 1996. Seed Longevity of Rice Cultivars and Strategies for their Conservation in Genebanks. *Annals of Botany*, 77**,** 251-260.

RAO, N. & JACKSON, M.T. 1997. Effect of sowing date and harvest time on longevity of rice seeds. *Seed Science Research,* 7, 13-20.

RICE KNOWLEDGE MANAGEMENT PORTAL. 2018. http://digitalknowledgecentre.in/listings/rice-knowledge-management-portal-rkmp/. Accessed 5 April 2018.

RICEPEDIA. 2018. *Cultivated rice species*. http://ricepedia.org/rice-as-a-plant/rice-species/cultivated-rice-species. Accessed 1 March 2018.

RIZHSKY, L., LIANG, H.J. & MITTLER, R. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiology*, 130, 1143–1151.

ROBERTS, E.H. 1962. Dormancy in Rice Seed: III. The influence of temperature, moisture, and gaseous environment. *Journal of Experimental Botany*, 13, 75–94.

ROBERTS, E.H. 1973. Predicting the Storage Life of Seeds. *Seed Science and Technology*, 1, 499-514.

ROBERTS, E. & ELLIS, R. 1989. Water and seed survival. *Annals of Botany,* 63**,** 39-52.

SABETFAR, S., ASHOURI, M., AMIRI, E. & BABAZADEH, S. 2013. Effect of drought stress at different growth stages on yield and yield component of rice plant. *Persian Gulf Crop Protection*, 2,14–18.

SAEEDIPOUR, S. & MORADI, F. 2011. Effect of Drought at the Post-anthesis Stage on Remobilization of Carbon Reserves and Some Physiological Changes in the Flag Leaf of Two Wheat Cultivars Differing in Drought Resistance. *Journal of Agricultural Science,* 3,81-92.

SAKKE, N., ITHNIN, H., IBRAHIM, M. H. & SYED HUSSAIN, T. P. R. 2016. Kemarau hidrologi dan kelestarian sumber air di Malaysia: Kajian analisis sifat Lembangan Langat, Selangor (Hydrological drought and the sustainability of water resources in Malaysia: An analysis of the properties of the Langat Basin, Selangor). *Malaysian Journal of Society and Space*, 12, 133-146.

SAINI, H.S. & ASPINALL, D. 1982. Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. *Annals of Botany*, 49, 835-846.

SAINI, H., WESTGATE, M. & SPARKS, D. 2000. Reproductive development in grain crops during drought. *Advances in Agronomy, Vol 68*, 68**,** 59-96.

SAMARAH, N.H., & MULLEN, R.E. 2004. Effect of maturity stage on seed germination and vigour of common vetch (*Vicia sativa* L.). *Seed Technology,* 26, 27-37.

SAMARAH, N. & ALQUDAH, A. 2011. Effects of late-terminal drought stress on seed germination and vigour of barley (*Hordeum vulgare* L.). *Archives of Agronomy and Soil Science,* 57, 27-32.

SANHEWE, A. & ELLIS, R. 1996. Seed development and maturation in Phaseolus vulgaris .1. Ability to germinate and to tolerate desiccation. *Journal of Experimental Botany,* 47, 949-958.

SANHEWE, A., ELLIS, R., HONG, T., WHEELER, T., BATTS, G., HADLEY, P. & MORISON, J. 1996. The effect of temperature and CO2 on seed quality development in wheat (*Triticum aestivum* L). *Journal of Experimental Botany,* 47**,** 631-637.

SATO, Y. I.1996. Origin and dissemination of cultivated rice in the eastern Asia. In: Interdisciplinary perspectives on the origins of the Japanese. International Research Center for Japanese Studies, Tokyo, 143–153.

SAVIN, R. & NICOLAS, M. E. 1996. Effects of Short Periods of Drought and High Temperature on Grain Growth and Starch Accumulation of Two Malting Barley Cultivars. *Functional Plant Biology,* 23**,** 201-210.

SEHGAL, A., SITA, K., KUMAR, J., KUMAR, S., SINGH, S., SIDDIQUE, K. H. M. & NAYYAR, H. 2017. Effects of Drought, Heat and Their Interaction on the Growth, Yield and Photosynthetic Function of Lentil (Lens culinaris Medikus) Genotypes Varying in Heat and Drought Sensitivity. *Frontiers in Plant Science,* 8**,** 1776.

SEILER, C., HARSHAVARDHAN, V. T., RAJESH, K., REDDY, P. S., STRICKERT, M., ROLLETSCHEK, H., SCHOLZ, U., WOBUS, U. & SREENIVASULU, N. 2011. ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *Journal of Experimental Botany,* 62**,** 2615-2632.

SHAH, N. H. & PAULSEN, G. M. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and Soil,* 257**,** 219-226.

SHARMA, P., MISHRA, M., INDRAKUMAR SINGH, N. & S CHAUHAN, J. 2011. Determination of Integrity of Seed Membrane Systems and Mineral Ions Leakage in Horse Gram. *World Journal of Agricultural Sciences,* 7, 476-479.

SHAW, R. H. & LOOMIS, W. E. 1950. Bases for the prediction of corn yields. *Plant Physiology,* 25**,** 225-244.

SHI, W., YIN, X., STRUIK, P. C., XIE, F., SCHMIDT, R. C. & JAGADISH, K. S. V. 2016. Grain yield and quality responses of tropical hybrid rice to high night-time temperature. *Field Crops Research,* 190, 18-25.

SHI, W., XIAO, G., STRUIK, P. C., JAGADISH, K. S. V. & YIN, X. 2017. Quantifying source-sink relationships of rice under high night-time temperature combined with two nitrogen levels. *Field Crops Research,* 202, 36-46.

SHIRATSUCHI, H., OHDAIRA, Y., YAMAGUCHI, H. & FUKUDA, A. 2017. Breaking the dormancy of rice seeds with various dormancy levels using steam and high temperature treatments in a steam nursery cab. *Plant Production Science,* 20, 183-192.

SIDDIQUE, M.A. & GOODWIN, P.B. 1980. Seed vigour in bean (*Phaseolus vulgaris* L. cv. Apollo) as influenced by temperature and water regime during development and maturation. *Journal of Experimental Botany*, 31, 313-323

SIDDIQUE, A.B., & WRIGHT D.2003. Effects of time of harvest at different moisture contents on seed fresh weight, dry weight, quality (viability and vigour) and food reserves of peas (*Pisum sativum* L.). *Asian Journal of Plant Sciences,* 2,983-992.

SIKUKU, P. NETONDO, G. W., ONYANGO J. C. & MUSYIMI, D. M. 2010. Effects of water deficit on physiology and morphology of three varieties of nerica rainfed rice (*Oryza sativa* L.). ARPN *Journal of Agricultural and Biological Science*, 5, 23-28

SINGH, C., KUMAR, B., MEHANDI, S. & CHANDRA, K. 2012. Effect of Drought Stress in Rice: A Review on Morphological and Physiological Characteristics. *Trends in Biosciences*, 5, 261-265.

SINNIAH, U., ELLIS, R. & JOHN, P. 1998. Irrigation and seed quality development in rapid-cycling brassica: Seed germination and longevity. *Annals of Botany,* 82, 309-314.

SINNIAH, U., ELLIS, R. & JOHN, P. 1998. Irrigation and seed quality development in rapid-cycling brassica: Soluble carbohydrates and heat-stable proteins. *Annals of Botany,* 82, 647-655.

SMICIKLAS, K.D., MULLEN, R.E., CARLSON, R.E. & KNAPP. A.D. 1989. Drought induced stress effects on soybean seed calcium and seed quality. *Crop Science*., 29,1519-1523.

SMITH, R.D., DICKIE, J.B., LININGTON, S.H., PRITCHARD, H.W., & PROBERT, R.J. 2003. Seed conservation: turning science into practice. Royal Botanic Gardens, Kew.

STONE, P. J. & NICOLAS, M. E. 1994. Wheat Cultivars Vary Widely in Their Responses of Grain Yield and Quality to Short Periods of Post-Anthesis Heat Stress. *Functional Plant Biology,* 21**,** 887-900.

SUMMERFIELD, R., COLLINSON, S., ELLIS, R., ROBERTS, E. & DEVRIES, F. 1992. Photothermal responses of flowering in rice (oryza-sativa). *Annals of Botany,* 69**,** 101-112.

SREENIVASULU, N., BUTARDO, J. V. M., MISRA, G., CUEVAS, R. P., ANACLETO, R. & KAVI KISHOR, P. B. 2015. Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *Journal of Experimental Botany,* 66, 1737-1748.

STAGNARI, F., GALIENI, A. AND PISANTE, M. 2016. Drought stress effects on crop quality. *In Water Stress and Crop Plants*, P. Ahmad (Ed.), 375-392.

STEADMAN, K. J., PRITCHARD, H. W. & DEY, P. M. 1996. Tissue-specific Soluble Sugars in Seeds as Indicators of Storage Category. *Annals of Botany,* 77**,** 667-674.

STRECK, N. A. 2005. Climate change and agroecosystems: the effect of elevated atmospheric CO2 and temperature on crop growth, development, and yield. *Ciência Rural*, 35**,** 730-740.

SUPARTA, W. & YATIM, A.N.M. 2017. An analysis of heat wave trends using heat index in East Malaysia. *Journal of Physics*: Conference Series, 852.

TANAKA, K., ONISHI, R., MIYAZAKI, M., ISHIBASHI, Y., YUASA, T. & IWAYA-INOUE, M. 2009. Changes in NMR Relaxation of Rice Grains, Kernel Quality and Physicochemical Properties in Response to a High Temperature after Flowering in Heat-Tolerant and Heat-Sensitive Rice Cultivars. *Plant Production Science,* 12**,** 185-192.

TANAMACHI, K., MIYAZAKI, M., MATSUO, K., SURIYASAK, C., TAMADA, A., MATSUYAMA, K., IWAYA-INOUE, M. & ISHIBASHI, Y. 2016. Differential responses to high temperature during maturation in heat-stress-tolerant cultivars of Japonica rice. *Plant Production Science,* 19, 300-308.

TAO, H., BRUECK, H., DITTERT, K., KREYE, C., LIN, S. & SATTELMACHER, B. 2006. Growth and yield formation of rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Research,* 95, 1-12.

TEJAKHOD, S. & ELLIS, R. 2017. Effect of simulated flooding during rice seed development and maturation on subsequent seed quality. *Seed Science Research,* 28, 72-81.

TEKRONY, D.M., EGLI, D.B., WHITE, G.M., 1987. Seed Production and Technology. *In*: Soybeans: Improvement, Production and Uses. Wilcox, J.R. (ed.). 2nd Edition, Agronomy N-16, ASA, CSSA, SSSA, Madison, Wisconsin, USA, 295-353.

TEMPLETON, D. & BAYOT, R. 2011. An impact assessment of the 'Developing a System of Temperate and Tropical Aerobic Rice (STAR) in Asia' project. Impact Assessment Paper 5. Colombo, Sri Lanka: The CGIAR Challenge Program on Water and Food.

THUY, T. L. & SAITOH, K. 2017. Responses of Fourteen Vietnamese Rice (*Oryza sativa* L.) Cultivars to High Temperatures during Grain Filling Period under Field Conditions. *Agronomy*, 7, 57.

VENKATESWARLU, B. 1976. Source-sink interrelationships in lowland rice. *Plant and Soil,* 44, 575-586.

VIEIRA, R.D., D.M. TEKRONY & D.B. EGLI. 1991. Effects of drought stress on soybean germination and vigour. *Journal of Seed Technology,* 15, 12-21.

VIEIRA, R.D., & KRZYZANOWSKI, F.C.1999. "Electrical conductivity test," in Seed Vigour: Concepts and Tests, Krzyzanowski, F.C., Vieira, R. D. & J. B. Franc¸a Neto, J. B.(Eds.), 4.1–4.26, Abrates, London, UK.

VIEIRA, R.D.; CARVALHO, N.M. & SADER, R. 1994. Vigour tests and its possibility of use. p. 31-47. In: Vieira, R.D.; Carvalho, N.M., (Eds.). Seed vigour tests. Funep, Jaboticabal, SP, Brazil (in Portuguese).

VERGARA, B. S. 1970. Plant growth and development. In *Rice Production Manual.* Univ. of the Philippines, Laguna.

WALTERS, C., WHEELER, L. & STANWOOD, P. C. 2004. Longevity of cryogenically stored seeds. *Cryobiology,* 48**,** 229-244.

WANG, X. K., LI, R. H. SUN, C. Q., LI, Z. C., SUN, X. L. & CAI, H. W. 1998. Identification of subspecies Asian cultivated rice and their hybrids. *China Science Bulletin,* 43, 1864-1872.

WANG, F., CHENG, F.-M. & ZHANG, G.P. 2007. Difference in Grain Yield and Quality among Tillers in Rice Genotypes Differing in Tillering Capacity. *Rice Science,* 14, 135-140.

WANG, W., HE, A., PENG, S., HUANG, J., CUI, K. & NIE, L. 2018. The Effect of Storage Condition and Duration on the Deterioration of Primed Rice Seeds. *Front Plant Science,* 9, 172.

WANG Y. Z., WANG X. J., LI Y., XU H., WANG J. Y., ZHAO M. H.2015. Analysis of yield and quality traits and their relationship in *japonica* rice in northern China. *Acta Agronomica Sinica*, 41, 84–92.

WASSMANN, R., JAGADISH, S.V.K., HEUER, S., ISMAIL, A., REDONˇA, E., SERRAJ, R., SINGH, R.K., HOWELL, G., PATHAK, H. & SUMFLETH, K. 2009. Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. *Advances in Agronomy* , 101, 59–122.

WHEELER, T.R., HONG, T.D., ELLIS, R.H., BATTS, G.R., MORISON, J.I.L., HADLEY, P. 1996. The duration and rate of grain growth, and harvest index, of wheat (*Triticum aestivum*) in response to temperature and CO_2 . *Journal of Experimental Botany*. 47, 623–630.

WHITEHOUSE, K., HAY, F. & ELLIS, R. 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**,** 247-259.

WHITEHOUSE, K., HAY, F. & ELLIS, R. 2017. High-temperature stress during drying improves subsequent rice (*Oryza sativa* L.) seed longevity. *Seed Science Research,* 27, 281-291.

WRIGLEY, T. M. L. & RAPER, S. C. B. 2001. Interpretation of High Projections for Global-Mean Warming. *Science,* 293**,** 451.

WINKEL, T., RENNO, J. F. & PAYNE, W. A. 1997. Effect of the timing of water deficit on growth, phenology and yield of pearl millet (*Pennisetum glaucum* (L.) R. Br.) grown in Sahelian conditions. *Journal of Experimental Botany,* 48, 1001-1009.

WU, Y.-C., CHANG, S.-J. & LUR, H.-S. 2016. Effects of field high temperature on grain yield and quality of a subtropical type japonica rice—Pon-Lai rice. *Plant Production Science,* 19, 145-153.

YADAV, G. & ELLIS, R. 2016. Development of ability to germinate and of longevity in air-dry storage in wheat seed crops subjected to rain shelter or simulated supplementary rainfall. *Seed Science Research,* 26, 332-341.

YANG, C.M. & HIELMAN, J.L. 1991b. Short-term High Temperature Effects on Stomatal Behaviors of Rice Plants. II. Occurring at the Grain-filling Stage. *Chinese Agricultural Research,* 40**,** 243-247.

YANG, J. & ZHANG, J. 2006. Grain filling of cereals under soil drying. *New Phytology.,* 169, 223-36.

YANG, J., ZHANG, J., WANG, Z., ZHU, Q. & WANG, W. 2001a. Hormonal changes in the grains subjected to water stress during grain filling. *Plant Physiology*, 127, 315-323.

YANG. J., ZHANG. J., WANG. Z., ZHU, Q. & LIU, L. 2001b. Water deficit induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. *Agronomy Journal,* 93, 196–206.

YANG, J.C., ZHANG, J.H., WANG, Z.Q., LIU, L.J. & ZHU, Q.S. 2003. Post anthesis water deficits enhance grain filling in two-line hybrid rice. *Crop Science*, 43, 2099–2108.

YAKLICH, R. W. 1984. Moisture stress and soybean seed quality. *Journal of Seed Technology,* 9**,** 60-67.

YEO, M.E., CUARTERO J., FLOWERS T.J. & YEO, A.R. 1996. Gas Exchange, Water loss and biomass production in rice and wild Oryza Species in well- watered and water imiting growth conditions. *In*: The International Journal of the German Botanical Society, 110, 32-41.

YOSHIDA, S. 1978. Tropical climate and its influence on rice. IRRI Research Paper Series 20. Los Baños, Philippines, IRRI.

YOSHIDA, S. 1981. Fundamentals of Rice Crop Science, International Rice Research Institute, Los Baños. *Laguna*.

YUAN, L.P. 1994. Increasing yield potential in rice by exploitation of heterosis. *In:* Virmani S. S (Ed.) *Hybrid rice technology, new developments and future prospects*. Los Baños, the Philippines: International Rice Research Institute, 1–6.

YUAN, L.P. 1998. Hybrid rice breeding in China. In: Virmani SS, Siddiq EA, Muralidharan K, eds. Advances in hybrid rice technology. Proceedings of the Third International Symposium on Hybrid Rice, Hyderabad, India. Los Baños, the Philippines: International Rice Research Institute, 27–33

ZAHEDI, M. & JENNER, C. F. 2003. Analysis of effects in wheat of high temperature on grain filling attributes estimated from mathematical models of grain filling. *The Journal of Agricultural Science,* 141**,** 203-212.

ZAINAL, Z., SHAMSUDIN, M. N., MOHAMED, Z. A., ADAM, S. U. & KAFFASHI, S. 2014. Assessing the impact of climate change on paddy production in Malaysia. *Research journal of environmental sciences,* 8, 331-341.

ZAINUDIN, H., SARIAM, O., CHAN, C. S., AZMI, M., SAAD, A., ALIAS, I. & MARZUKHI, H. 2014.Performance of selected aerobic rice varieties cultivated under local condition. *Journal Tropical Agriculture and Food Science*, 42, 175-182.

ZAKARIA, S., MATSUDA, S., TAJIMA S. AND NITTA, Y. 2002. Effect of High Temperature at Accumulation in Seed Ripening Stage on the Reserve in Some Rice Cultivars. *Plant Production Science*, 5, 160-168.

ZEHTAB-SALMASI, S., GHASSEMI-GOLEZANI, K. & MOGHBELI, S. 2006. Effect of Sowing Date and Limited Irrigation on the Seed Yield and Quality of Dill (*Anethum graveolens* L.) . *Turky Journal of Agriculture*, 30, 281-286

ZEIGLER, R. S. & PUCKRIDGE, D. W. 1995. Improving sustainable productivity in rice-based rainfed lowland systems of South and Southeast Asia. *GeoJournal*, 35, 307-324.

ZEWDIE, M. & ELLIS, R. 1991. Survival of tef and niger seeds following exposure to subzero temperatures at various moisture contents. *Seed Science and Technology*, 19, 309-317.

ZHANG, C., WANG, B., LIU, D. & CAI, Z. 1998: Research on drought and flood indices in the northwest China (in Chinese). *Plateau Meteorology*, 17, 381–389.

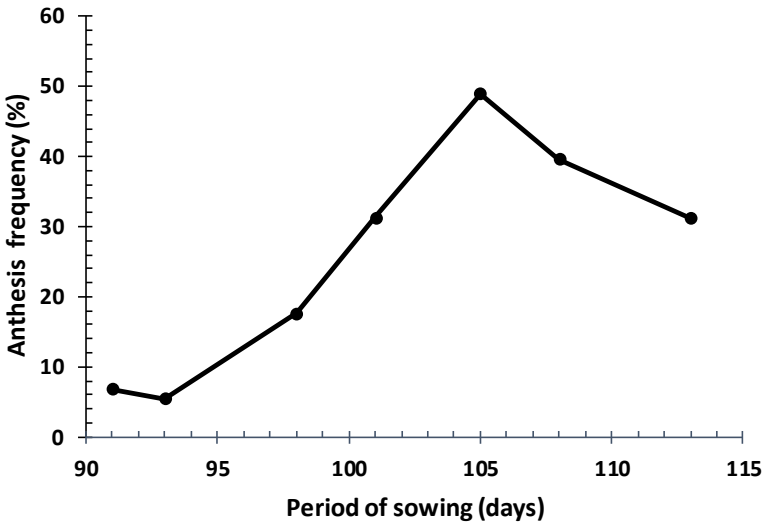
ZHAO, G., YANG, L., WANG, J. & ZHU, Z. 2009. Studies on the rapid methods for evaluating seed vigour of sweet corn. *In:* ZHAO, C. & LI, D., (Eds.). *Computer and Computing Technologies in Agriculture II*, Boston, MA. Springer US, 3, 1729-1738.

ZHU, Q., ZHANG, Z., YANG, J. & WANG, Z. 1997. Source–sink characteristics related with the yield of inter-subspecific hybrid rice, *Scientia Agricultura Sinica*, 30, 52-59.

Appendices

Chapter 2

Appendix 2.1 Flowering frequency (number of panicles anthesis emergence per day) of *japonica* rice cv. Gleva after sowing



Appendix 2.2. Analysis of variance of seed moisture content (%) for different irrigation treatments at different harvests (DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	2526.482	1263.241	630.4	<.001
DAA	7	7818.205	1116.886	557.36	<.001
Treatment.DAA	14	1428.258	102.018	50.91	<.001
Residual	24	48.093	2.004		
Total	47	11821.04			

Appendix 2.3: Analysis of variance of seed moisture content (%) between irrigation treatments at different harvests (DAA).

Appendix 2.3.1 11 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	295.315	147.657	41.29	0.007
Residual	3	10.727	3.576		
Total	5	306.042			

Appendix 2.3.2 16 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	843.591	421.796	129.45	0.001
Residual	3	9.775	3.258		
Total	5	853.366			

Appendix 2.3.3 22 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1095.289	547.644	166.19	<.001
Residual	3	9.886	3.295		
Total	5	1105.175			

Appendix 2.3.4 26 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	623.3354	311.6677	978	<.001
Residual	3	0.956	0.3187		
Total	5	624.2914			

Appendix 2.3.5 32 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	617.246	308.623	105.67	0.002
Residual	3	8.762	2.921		
Total	5	626.008			

Appendix 2.3.6 36 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	269.239	134.62	244.89	<.001
Residual	3	1.6491	0.5497		
Total	5	270.889			

Appendix 2.3.7 40 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	210.724	105.362	1085.16	<.001
Residual	3	0.29128	0.09709		
Total	5	211.015			

Appendix 2.4: Comparison by Tukey's test at 95% confidence intervals of seed moisture content (%) between irrigation treatment within each of eight harvest (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments.

Treatment	Harvest (DAA)							
	7	**11**	**16**	**22**	**26**	**32**	**36**	**40**
Control (T1)	53.4	51.7b	47.4b	47.6b	37.4b	37.5b	28.5b	27.4b
Water ended at 7 DAA (T2)	53.4	39.6a	21.9a	16.7a	15.3a	14.8a	14.3a	14.6a
Water ended at 14 DAA (T3)	53.4	56.2b	46.6b	21.9a	16.2a	17.3a	14.3a	15.1a

Appendix 2.5. Analysis of variance of seed dry weight (mg) for different irrigation treatments at different harvests (DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	38.2321	19.1161	621.03	<.001
DAA	7	33.0528	4.72182	153.4	<.001
Treat.DAA	14	26.2905	1.87789	61.01	<.001
Residual	216	6.64872	0.03078		
Total	239	104.224			

Appendix 2.6: Analysis of variance of seed dry weight (mg) between irrigation treatments at different harvests (DAA).

Appendix 2.6.1 11 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.85267	0.42633	39.7	<.001
Residual	27	0.28996	0.01074		
Total	29	1.14263			

Appendix 2.6.2 16 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1.83495	0.91748	36.81	<.001
Residual	27	0.67289	0.02492		
Total	29	2.50784			

Appendix 2.6.3 22 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	3.44596	1.72298	40.29	<.001
Residual	27	1.15467	0.04277		
Total	29	4.60062			

Appendix 2.6.4 26 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	8.12067	4.06033	69.47	<.001
Residual	27	1.57818	0.05845		
Total	29	9.69885			

Appendix 2.6.5 32 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	18.4709	9.23546	437.79	<.001
Residual	27	0.56959	0.0211		
Total	29	19.0405			

Appendix 2.6.6 36 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	18.4709	9.23546	437.79	<.001
Residual	27	0.56959	0.0211		
Total	29	19.0405			

Appendix 2.6.7 40 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	19.6309	9.81545	403.68	<.001
Residual	27	0.65651	0.02432		
Total	29	20.2874			

Appendix 2.7: Comparison by Tukey's test at 95% confidence intervals of seed dry weight (mg) between irrigation treatment within each of eight harvest (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments.

Treatment	Harvest (DAA)							
	7	**11**	**16**	**22**	**26**	**32**	**36**	**40**
Control (T1)	5.43	10.50b	15.16b	17.68b	19.88c	22.26c	27.49c	28.36c
Water ended at 7 DAA (T2)	5.43	12.37c	9.53a	9.79a	7.39a	6.76a	8.43a	8.83a
Water ended at 14 DAA (T3)	5.43	8.25a	14.29b	15.97b	15.85b	16.08b	15.83b	15.68b

Appendix 2.8. Analysis of variance of fresh seed germination (rad) for different irrigation treatments at different harvests (DAA) using angular transformed data.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1.3422	0.6711	801.53	<.001
DAA	7	2.10811	0.30116	359.69	<.001
Treatment.DAA	14	1.43437	0.10245	122.37	<.001
Residual	24	0.02009	0.00084		
Total	47	4.90477			

Appendix 2.9: Analysis of variance of dried seed germination (rad) for different irrigation treatments at different harvests (DAA) using angular transformed data.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.3928	0.1964	8.23	0.002
DAA	7	4.37582	0.62512	26.2	<.001
Treatment.DAA	14	1.8222	0.13016	5.45	<.001
Residual	24	0.57265	0.02386		
Total	47	7.16348			

In Appendices 2.10. Different models are compared amongst seed survival curves fitted by probit analysis

Test 1= Best fit model; test 2= Common slope model; test 3= Common line model: Not significant shown as (ns), P>0.05; significant (s, P<0.05).

In Appendix 2.10.1- 7, comparison of treatments within a harvest date

Appendix 2.10.1: Comparison of survival curves for seeds harvested at 11 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	41.71	13	3.208
Best model	40.83	11	3.712
Change	0.88	2	0.44
$F_{(2,13)} = 0.11853$			
Slopes different / **not different**	P= **0.889**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	44.74	15	2.983
Best model	40.83	11	3.712
Change	3.91	4	0.9775
$F_{(4,11)} = 0.263335$			
Slope + Ki different / **not different**	P= **0.895**		ns

Appendix 2.10.2: Comparison of survival curves for seeds harvested at 16 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	63.87	15	4.258
Best model	58.88	13	4.53
Change	4.99	2	2.495
$F_{(2,15)} = 0.55077$			
Slopes different / **not different**	P= **0.59**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	138.4	17	8.143
Best model	58.88	13	4.53
Change	79.52	4	19.88
$F_{(4,13)} = 4.388520$			
Slope + Ki different /**different**	P= **0.018**		s

Appendix 2.10.3: Comparison of survival curves for seeds harvested at 22 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	82.43	18	4.58
Best model	53.64	16	3.353
Change	28.79	2	14.395
$F_{(2,18)} = 4.2931$			
Slopes different / not different	P= **0.03**		s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	248	20	12.4
Best model	53.64	16	3.353
Change	194.36	4	48.59
$F_{(4,16)} = 14.491$			
Slope + Ki different /different	P= **0.000**		s

Appendix 2.10.4: Comparison of survival curves for seeds harvested at 26 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	154.9	20	7.746
Best model	124	18	6.886
Change	30.9	2	15.45
$F_{(2,20)} = 2.243$			
Slopes different / **not different**	P= **0.13**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	485.4	22	22.07
Best model	124	18	6.886
Change	361.4	4	90.35
$F_{(4,18)}$ = 13.120			
Slope + Ki different /different	P= **0.000**		s

Appendix 2.10.5: Comparison of survival curves for seeds harvested at 32 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	104.2	18	5.791
Best model	87.95	16	5.497
Change	16.25	2	8.125
$F_{(2,18)}$ = 1.4780			
Slopes different / **not different**	P= **0.13**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	667.8	20	33.39
Best model	87.95	16	5.497
Change	579.85	4	144.96
$F_{(4,16)}$ = 26.371			
Slopes + Ki different / not different	**P= 0.00**		s

Appendix 2.10.6: Comparison of survival curves for seeds harvested at 36 DAA

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	45.07	19	2.372
Best model	30.85	17	1.815
Change	14.22	2	7.11
$F_{(4,17)}$ = 3.917			
Slopes different / **not different**	P= **0.038**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	1001	21	47.66
Best model	30.85	17	1.815
Change			
$F_{(4,18)}$ = 133.629			
Slope + Ki different /different	P= **0.000**		s

Appendix 2.10.7: Comparison of survival curves for seeds harvested at 40 DAA

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	56.62	19	2.98
Best model	48.2	17	2.835
Change	8.42	2	4.21
$F_{(2,19)} = 1.485$			
Slopes different / **not different**	P= **0.2516**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	1117	21	53.21
Best model	48.2	17	2.835
Change	1068.8	4	267.2
$F_{(4,1)} = 94.250$			
Slope + Ki different /different	P= **0.000**		s

CHAPTER 3

Appendix 3.0. Analysis of variance of seed moisture content (%) for high treatments (HT) at different timing of seed development.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HT treatment	10	542.44	54.24	2.59	0.019
Residual	33	690.93	20.94		
Total	43	1233.37			

Appendix 3.1. Analysis of variance of seed dry weight (mg) for HT treatments at different timing of seed development.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HT treatment	10	365.89	36.59	3.51	0.003
Residual	33	344.31	10.43		
Total	43	710.2			

Appendix 3.2. Analysis of variance of electrical conductivity (µS cm^{-1} g^{-1}) of seed steep water for HT treatments at different timing of seed development.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HT treatment	10	1272.9	127.3	0.54	0.849
Residual	33	7783.3	235.9		
Total	43	9056.2			

Appendix 3.3. Analysis of variance of fresh Gleva seed (ability to germinate normally (%)) for HT treatments at different timing of seed development using angular data.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HT treatment	10	3078.3	307.8	2.53	0.022
Residual	33	4011.4	121.6		
Total	43	7089.7			

Appendix 3.4. Analysis of variance of dry Gleva seed (ability to germinate normally (%)) for HT treatments at different timing of seed development using angular data.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
HT treatment	10	0.16065	0.01607	1.56	0.162
Residual	33	0.33969	0.01029		
Total	43	0.50034			

Appendix 3.5: Comparison by Tukey's multiple range test at 95% confidence intervals of seed moisture content at harvest (%) for HT treatments at different timing of seed development.

HT **treatment**	**Seed moisture content (%)**
T1(Control)	25.8a
T2 (-3-0 DAA)	37.2b
T3 (0-3 DAA)	30.0ab
T4 (3-6 DAA)	26.2ab
T5 (6-9 DAA)	26.4ab
T6 (9-12 DAA)	29.2ab
T7 (12-15 DAA)	26.7ab
T8 (15-18 DAA)	24.7a
T9 (18-21 DAA)	25.7a
T10 (21-24 DAA)	28.0ab
T11 (24-27 DAA)	23.7a

Appendix 3.6: Comparison by Tukey's multiple range test at 95% confidence intervals of final seed dry weight (mg) for heat treatments at different timing of seed development.

HT **treatment**	**Seed dry weight (mg)**
T1(Control)	31.41b
T2 (-3-0 DAA)	20.20a
T3 (0-3 DAA)	27.39ab
T4 (3-6 DAA)	29.86b
T5 (6-9 DAA)	29.86b
T6 (9-12 DAA)	29.15b
T7 (12-15 DAA)	29.29b
T8 (15-18 DAA)	30.46b
T9 (18-21 DAA)	29.64b
T10 (21-24 DAA)	29.33b
T11 (24-27 DAA)	30.33b

Appendix 3.7: Comparison by Tukey's multiple range test at 95% confidence intervals of electrical conductivity of seed steep water (µS cm^{-1} g^{-1}) for heat treatments at different timing of seed development.

HT **treatment**	**E.C seed steep water (**µS cm^{-1} g^{-1}**)**
T1(Control)	130.47b
T2 (-3-0 DAA)	49.43a
T3 (0-3 DAA)	54.44a
T4 (3-6 DAA)	65.46ab
T5 (6-9 DAA)	63.59ab
T6 (9-12 DAA)	71.83ab
T7 (12-15 DAA)	60.86ab
T8 (15-18 DAA)	68.56ab
T9 (18-21 DAA)	71.00ab
T10 (21-24 DAA)	44.22a
T11 (24-27 DAA)	75.71ab

Appendix 3.8: Comparison by Tukey's at 95% confidence intervals of fresh & dry Gleva seed (ability to germinate normally (%)) for heat treatments at different timing of seed development using angular data.

HT **treatment**	**Fresh germination**	**Dry germination**
T1(Control)	1.401b	1.444a
T2 (-3-0 DAA)	0.859a	1.362a
T3 (0-3 DAA)	1.277ab	1.277a
T4 (3-6 DAA)	1.409b	1.407a
T5 (6-9 DAA)	1.411b	1.457a
T6 (9-12 DAA)	1.482b	1.452a
T7 (12-15 DAA)	1.434b	1.457a
T8 (15-18 DAA)	1.436b	1.411a
T9 (18-21 DAA)	1.475b	1.460a
T10 (21-24 DAA)	1.414b	1.444a
T11 (24-27 DAA)	1.396b	1.510a

In Appendices 3.9. Different models are compared below to describe the seed survival curves fitted by probit analysis for the 11 seed lots

Test 1= Best fit model; test 2= Common slope model; test 3= Common line model: Not significant shown as (ns, P >0.05); significant (s, P <0.05).

Comparison of survival curves for Gleva seeds harvested at 42 DAA.

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	761.1	77	9.884
Best model	646.1	67	9.643
Change	115	10	11.5

$F_{(10,67)}$=1.1925

Slopes different / **not different** P=0.311862 **NS**

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	1879	87	21.6
Best model	646.1	67	9.643
Change	1232.9	20	61.645

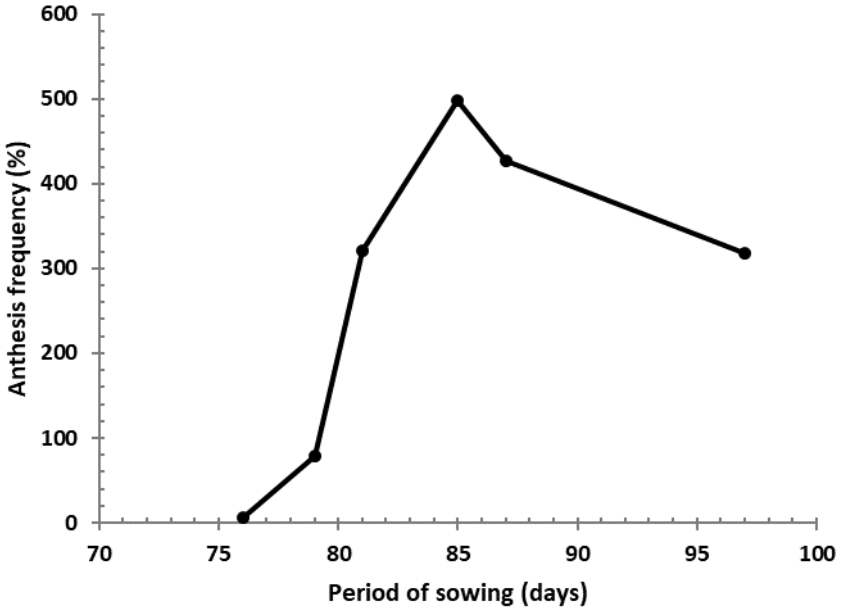
$F_{(20,67)}$=6.3927

Slope + Ki different / not different P=< .00001 **S**

Appendix 3.10. Estimate parameters of seed viability equation fitted by probit analysis of model 2 (common slope model)

Treatment	Storage moisture content (%)	K_i		Slope(1/σ)		p_{50} (days)			
		estimate	s.e	estimate	s.e	estimate	s.e.	lower 95%	upper 95%
T1 (Control)	14.8	3.23	0.083	0.126	0.002	25.5	0.47	24.6	26.42
T2 (3DBA)	14.6	1.34	0.083	0.126	0.002	10.6	0.63	9.4	11.88
T3 (0-3DAA)	14.9	1.49	0.069	0.126	0.002	11.8	0.49	10.8	12.73
T4 (3-6DAA)	15.0	2.78	0.073	0.126	0.002	22.0	0.45	21.1	22.87
T5 (6-9DAA)	14.5	2.67	0.074	0.126	0.002	21.1	0.46	20.2	21.98
T6 (9-12DAA)	14.8	1.67	0.067	0.126	0.002	13.2	0.49	12.2	14.15
T7 (12-15DAA)	14.5	3.14	0.087	0.126	0.002	24.9	0.53	23.8	25.88
T8 (15-18DAA)	14.7	2.48	0.085	0.126	0.002	19.6	0.60	18.5	20.8
T9 (18-21DAA)	15.1	1.69	0.070	0.126	0.002	13.4	0.50	12.4	14.35
T10 (21-24DAA)	15.2	3.05	0.082	0.126	0.002	24.1	0.48	23.2	25.01
T11 (24-27DAA)	15.5	3.14	0.089	0.126	0.002	24.9	0.51	23.9	25.85

Appendix 3.11. Flowering frequency (number of panicles anthesis emergence per day) of *japonica* rice cv. Gleva after sowing



CHAPTER 4

Appendix 4.0. Analysis of variance of seed moisture content (%) for different irrigation treatments at different harvests (DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks	3	10.1	3.37	0.09	0.967
Irrigation treatment	2	1140.57	570.285	459.22	<.001
DAA	6	1946.312	324.385	261.21	<.001
Treat.DAA	12	770.775	64.231	51.72	<.001
Residual	63	78.238	1.242		
Total	83	3935.894			

Appendix 4.1: Analysis of variance of seed moisture content (%) between irrigation treatments at different harvests (DAA).

Appendix 4.1.1 13 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	4.497	2.248	0.62	0.558
Residual	9	32.51	3.612		
Total	11	37.007			

Appendix 4.1.2 18 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	30.861	15.431	9.44	0.006
Residual	9	14.708	1.634		
Total	11	45.57			

Appendix 4.1.3 27 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	560.833	280.416	267.36	<.001
Residual	9	9.439	1.049		
Total	11	570.272			

Appendix 4.1.4 34 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	213.128	106.564	65.39	<.001
Residual	9	14.666	1.63		
Total	11	227.794			

Appendix 4.1.5 42 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	342.602	171.301	663.55	<.001
Residual	9	2.3234	0.2582		
Total	11	344.9254			

Appendix 4.1.6 49 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	342.602	171.301	663.55	<.001
Residual	9	2.3234	0.2582		
Total	11	344.9254			

Appendix 4.1.7 55 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	367.792	183.896	1206.79	<.001
Residual	9	1.3715	0.1524		
Total	11	369.163			

Appendix 4.2: Comparison by Tukey's test at 95% confidence intervals of seed moisture content (%) between irrigation treatment within each of seven harvests (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments.

Treatment	Harvest (DAA)						
	13	**18**	**27**	**34**	**42**	**49**	**55**
Control (T1)	27.4a	30.1b	27.9b	23.2b	26.1b	26.1b	25.3b
Water ended at 14 DAA (T2)	28.9a	26.3a	13.4a	13.5a	14.7a	13.5a	14.7a
Water ended at 28 DAA (T3)	28.9a	29.2b	27.9b	15.3a	14.9a	14.9a	13.6a

Appendix 4.3: Analysis of variance of moisture content (%) between irrigation treatment Regardless of harvest date.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1140.57	570.285	459.22	<.001
Residual	63	78.238	1.242		
Total	83	3935.894			

Appendix 4.4: Comparison by Tukey's at 95% confidence intervals of moisture content (%) between irrigation treatment regardless of harvest date.

Treatment	**Mean Moisture content**
Control (T1)	26.4c
Water ended at 14 DAA (T2)	17.6a
Water ended at 28 DAA (T3)	20.3b

Appendix 4.5: Analysis of variance of seed dry weight (mg) for different irrigation treatments at different harvests (DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks	3	0.8325	0.2775	0.32	0.810
Irrigation treatment	2	1.6913	0.8456	1.55	0.220
DAA	6	16.0761	2.6794	4.92	<.001
treatment.DAA	12	17.9993	1.4999	2.75	0.005
Residual	63	34.3187	0.5447		
Total	83	70.0853			

Appendix 4.6: Analysis of variance of seed dry weight (mg) between irrigation treatment at different harvest date (DAA).

Appendix 4.6.1 Harvesting period at 13 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	13.588	6.794	4.42	0.046
Residual	9	13.834	1.537		
Total	11	27.422			

Appendix 4.6.2 Harvest date at 18 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	2.6603	1.3302	1.56	0.262
Residual	9	7.6659	0.8518		
Total	11	10.3263			

Appendix 4.6.3 Harvest date at 27 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.6400	0.3200	0.67	0.534
Residual	9	4.2728	0.4748		
Total	11	4.9129			

Appendix 4.6.4 Harvest date at 34 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1.0409	0.5205	1.38	0.299
Residual	9	3.3836	0.3760		
Total	11	4.4245			

Appendix 4.6.5 Harvest date at 42 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.1344	0.0672	0.23	0.801
Residual	9	2.6565	0.2952		
Total	11	2.7910			

Appendix 4.6.6 Harvest date at 49 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.3424	0.1712	0.66	0.541
Residual	9	2.3422	0.2602		
Total	11	2.6847			

Appendix 4.6.7 Harvest date at 55 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	1.23107	0.61553	10.26	0.005
Residual	9	0.53990	0.05999		
Total	11	1.77097			

Appendix 4.7: Analysis of variance of seed dry weight (mg) within irrigation treatment

Appendix 4.7.1 Control

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAA	6	2.6728	0.4455	1.58	0.201
Residual	21	5.9053	0.2812		
Total	27	8.5781			

Appendix 4.7.2 water ended at 14 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAA	6	28.4757	4.7460	4.77	0.003
Residual	21	20.8940	0.9950		
Total	27	49.3698			

Appendix 4.7.3 water ended at 28 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
DAA	6	2.9269	0.4878	1.36	0.275
Residual	21	7.5194	0.3581		
Total	27	10.4462			

Appendix 4.8: Comparison by Tukey's test at 95% confidence intervals of seed dry weight (mg) between irrigation treatment within each of seven harvests (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments

Treatment	Harvest (DAA)						
	13	**18**	**27**	**34**	**42**	**49**	**55**
Control (T1)	27.6b	27.7a	27.2a	28.0a	27.2a	27.1a	27.6b
Water ended at 14 DAA (T2)	25.0a	28.7a	27.3a	27.4a	27.5a	27.2a	26.9a
Water ended at 28 DAA (T3)	26.8ab	27.6a	26.8a	27.3a	27.4a	27.5a	27.6b

Appendix 4.9: Analysis of variance of dried seed germination (rad) for different irrigation treatments at different harvests (DAA) using angular transformed data.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks	3	0.01482	0.05	0.1	0.986
Irrigation treatment	2	0.058	0.029	3.27	0.045
DAA	6	2.784	0.464	51.9	<.001
Treatment.DAA	12	240.667	20.056	2.02	0.037
Residual	63	0.563	0.009		
Total	83	3.724			

Appendix 4.10: Analysis of variance of dried seed germination (%) between irrigation treatment at different harvest date (DAA) using angular transformed data.

Appendix 4.10.1 Harvesting at 27 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.086565	0.043282	4.92	0.036
Residual	9	0.079151	0.008795		
Total	11	0.165716			

Appendix 4.10.2 Harvesting at 34 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.092605	0.046303	6.56	0.017
Residual	9	0.06349	0.007054		
Total	11	0.156095			

Appendix 4.10.2 Harvesting at 42 DAA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Irrigation treatment	2	0.078082	0.039041	5.37	0.029
Residual	9	0.065425	0.007269		
Total	11	0.143507			

Appendix 4.11: Comparison by Tukey's test at 95% confidence intervals dried seed germination (rad) between irrigation treatment within each of seven harvest (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments

Treatment	Harvest (DAA)						
	13	**18**	**27**	**34**	**42**	**49**	**55**
Control (T1)	1.0a	1.2a	1.2a	1.3a	1.5b	1.5a	1.5a
Water ended at 14 DAA (T2)	0.9a	1.2a	1.4b	1.5b	1.4a	1.6a	1.5a
Water ended at 28 DAA (T3)	1.0a	1.1a	1.3ab	1.3ab	1.5b	1.6a	1.6a

In Appendices 4.12A and B, different models are compared amongst seed survival curves fitted by probit analysis

Test 1= Best fit model; test 2= Common slope model; test 3= Common line model: Not significant shown as (ns), P>0.05; significant (s, P<0.05).

In Appendix 4.12A.1- 7, comparison of treatments within a harvest date

Appendix 4.12A.1: Comparison of survival curves for seeds harvested at 13 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	44.77	20	2.238
Best model	42.89	18	2.383
Change	1.88	2	0.94
$F_{(2,18)} =$ 0.394461			
Slopes different / **not different**	P=**0.67977**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	45.61	22	2.073
Best model	42.89	18	2.383
Change	2.72	4	0.68
$F_{(4,18)}$ = 0.285355			
Slope + Ki different / **not different**	P=**0.88366**		ns

Appendix 4.12A.2: Comparison of survival curves for seeds harvested at 18 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	114.4	20	5.719
Best model	94.6	18	5.256
Change	19.8	2	9.9
$F_{(2,18)} =$1.88356			
Slopes different / **not different**	P=**0.18083**		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	115.2	22	5.237
Best model	94.6	18	5.256
Change	20.6	4	5.15
$F_{(4,18)} =$ 0.979833			
Slope + Ki different / **not different**	P=**0.44314**		**ns**

Appendix 4.12A.3: Comparison of survival curves for seeds harvested at 27 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	63.25	20	3.162
Best model	46.15	18	2.564
Change	17.1	2	8.55
$F_{(2,18)} =$ 3.334633			
Slopes different / **not different**	P=**0.05862**	0.05862	n.s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	70.54	22	3.206
Best model	46.15	18	2.564
Change	24.39	4	6.0975
$F_{(4,18)} =$ 2.37812			
Slope + Ki different / **not different**	P=**0.09031**		n.s

Appendix 4.12A.4: Comparison of survival curves for seeds harvested at 34 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	236.7	20	11.84
Best model	233.4	18	12.96
Change	3.3	2	1.65
$F_{(2,18)} =$ 0.127315			
Slopes different / **not different**	P=**0.8813**		n.s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	237.6	22	10.8
Best model	233.4	18	12.96
Change	4.2	4	1.05
$F_{(4,18)} =$ 0.081019			
Slope + Ki different / **not different**	P= **0.98719**		**ns**

Appendix 4.12A.5: Comparison of survival curves for seeds harvested at 42 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	126.8	20	6.341
Best model	48.76	18	2.709
Change	78.04	2	39.02
$F_{(2,18)} =$14.40384			

Slopes different / not different P=**0.0002** **s**

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	534.6	22	24.3
Best model	48.76	18	2.709
Change	485.84	4	121.46

$F_{(4,18)} =$ 44.83573

Slope + Ki different / not different P=**0.00001** **s**

Appendix 4.12A.6: Comparison of survival curves for seeds harvested at 49 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	105	20	5.249
Best model	57.3	18	3.183
Change	47.7	2	23.85

$F_{(2,18)} =$ 7.492931

Slopes different / not different P=**0.00429** **s**

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	314.7	22	14.3
Best model	57.3	18	3.183
Change	257.4	4	64.35

$F_{(4,18)} =$20.21678

Slope + Ki different / not different P=**0.00001** **s**

Appendix 4.12A.7: Comparison of survival curves for seeds harvested at 55 DAA

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	182.5	14	13.04
Best model	81.13	12	6.761
Change	101.37	2	50.685

$F_{(2,12)} =$7.49667

Slopes different / not different P=**0 .007719** **s**

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	228.8	16	14.3
Best model	81.13	12	6.761
Change	147.67	4	36.9175

$F_{(4,12)} =$ 5.46036

Slope + Ki different / not different P=**0.00969** **s**

In Appendix 4.12B.1-3, comparison of harvest dates within each irrigation treatment

Appendix 4.12B.1 Comparison of survival curves for irrigation throughout

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	376.3	48	7.839
Best model	230.7	42	5.493
Change	145.6	6	24.26667

$F_{(6,42)} = 4.4177$

Slopes different / not different P=**0.00151** **s**

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	714.7	54	13.23
Best model	230.7	42	5.493
Change	484	12	40.33333

$F_{(12,42)} = 7.34267$

Slope + Ki different / not different P< **.00001** **s**

Appendix 4.12B.2: Comparison of survival curves for irrigation ended 14 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	255.2	48	5.316
Best model	206	42	4.905
Change	49.2	6	8.2

$F_{(6,42)} = 1.671764$

Slopes different / **not different** **P=0.15187** n.s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	1193	54	22.09
Best model	206	42	4.905
Change	987	12	82.25

$F_{(6,42)} = 16.7686$

Slope + Ki different / not different **P< .00001** s

Appendix 4.12B.3: Comparison of survival curves for irrigation ended 28 DAA

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	252.6	48	5.263
Best model	169.3	42	4.031
Change	83.3	6	13.88333

$F_{(6,42)} = 3.444141$

Slopes different / not different **P=0.00741** s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	744.5	54	13.79
Best model	169.3	42	4.031
Change	575.2	12	47.93333

$F_{(12,42)} = 11.89118$

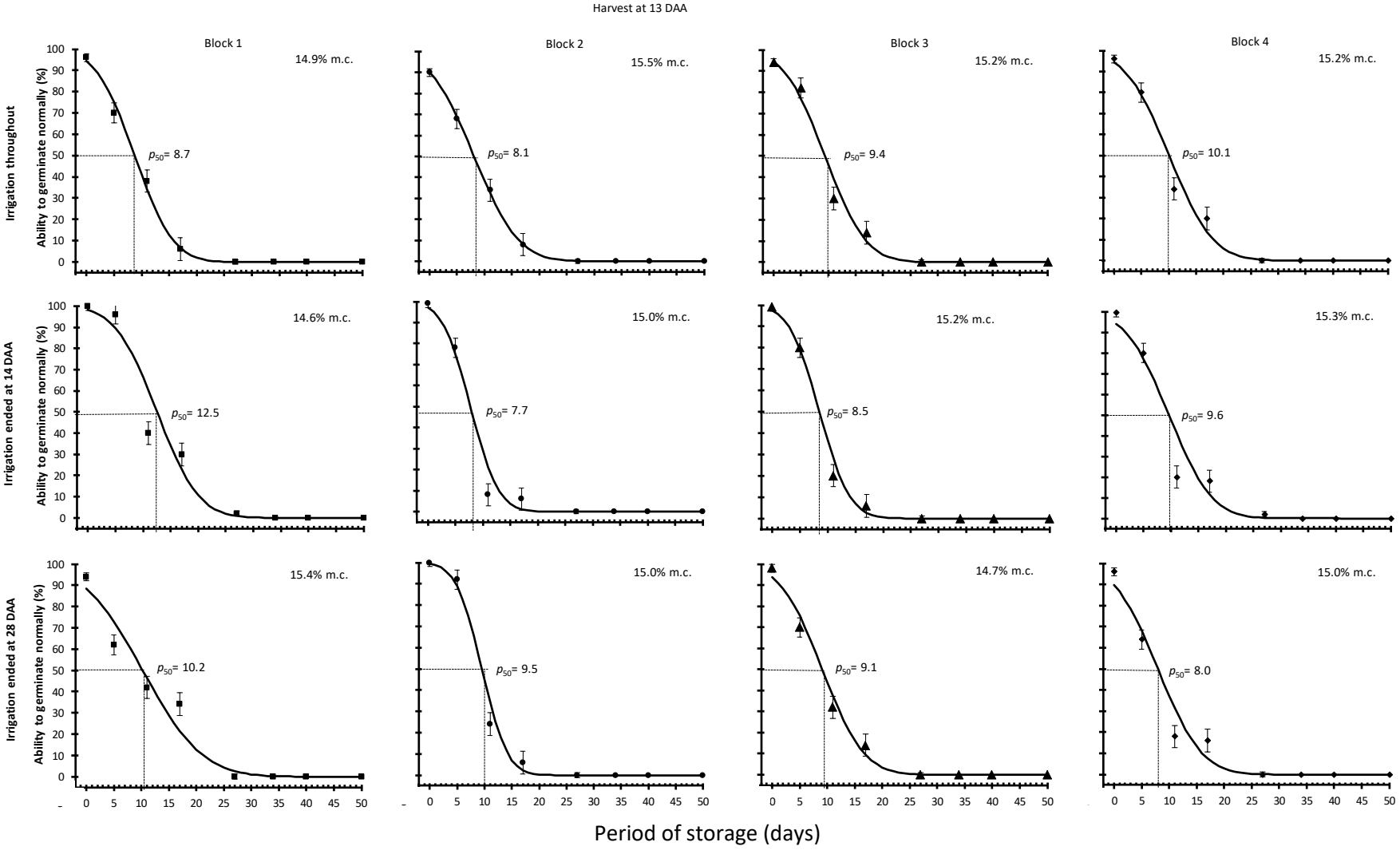
Slope + Ki different / not different **P< .00001** s

Appendix 4.13: Comparison by Tukey's test at 95% confidence intervals of longevity ($p_{50,}$ days) between irrigation treatments within each of seven harvests (DAA). Different letters within a harvest indicate significant differences amongst irrigation treatments.

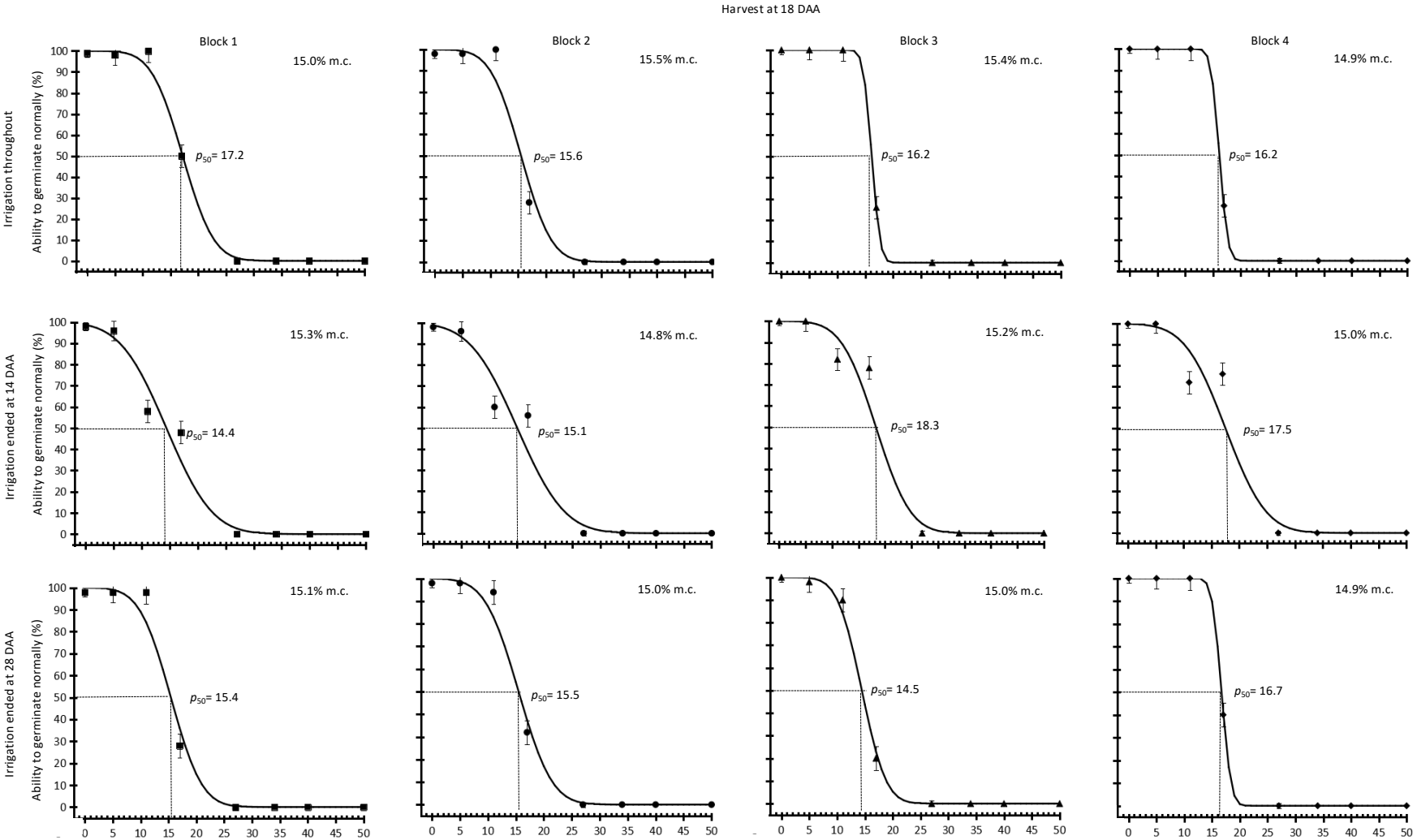
Irrigation treatment	Harvest (DAA)						
	13	**18**	**27**	**34**	**42**	**49**	**55**
Control (T1)	9.1a	16.0a	17.5a	20.4a	21.9c	16.9c	15.7a
ended 14 DAA (T2)	9.7a	16.4a	15.6a	20.1a	5.1a	8.6a	19.7b
ended 28 DAA (T3)	9.2a	15.5a	15.7a	20.3a	12.0b	8.2a	17.7c

Appendix 4.14. Loss in viability (estimated by change in ability to germinate normally) of seeds of rice cv. Gleva harvested serially from 13-55 DAA during seed development in photoperiod glass-house (31.1/18.1°C;12h/12h, 12h/12hd^{-1} photoperiod) with different irrigation treatments of four blocks. Seeds were stored hermetically at 40°C with the moisture content shown (m.c.); estimated seed longevity (p_{50}) is also shown.

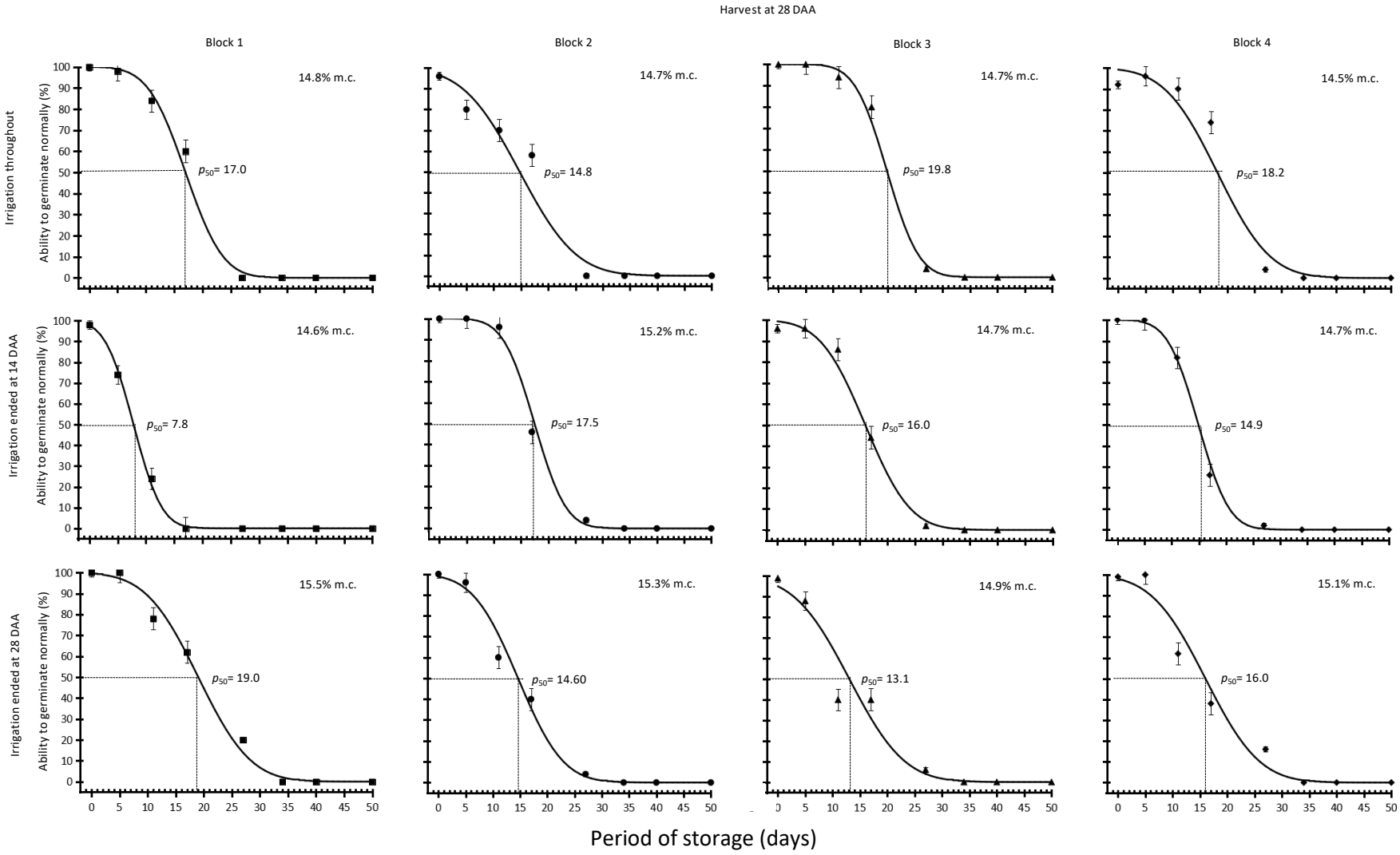
Appendix 4.14A. Comparison of survival curves for seed harvest at 13 DAA



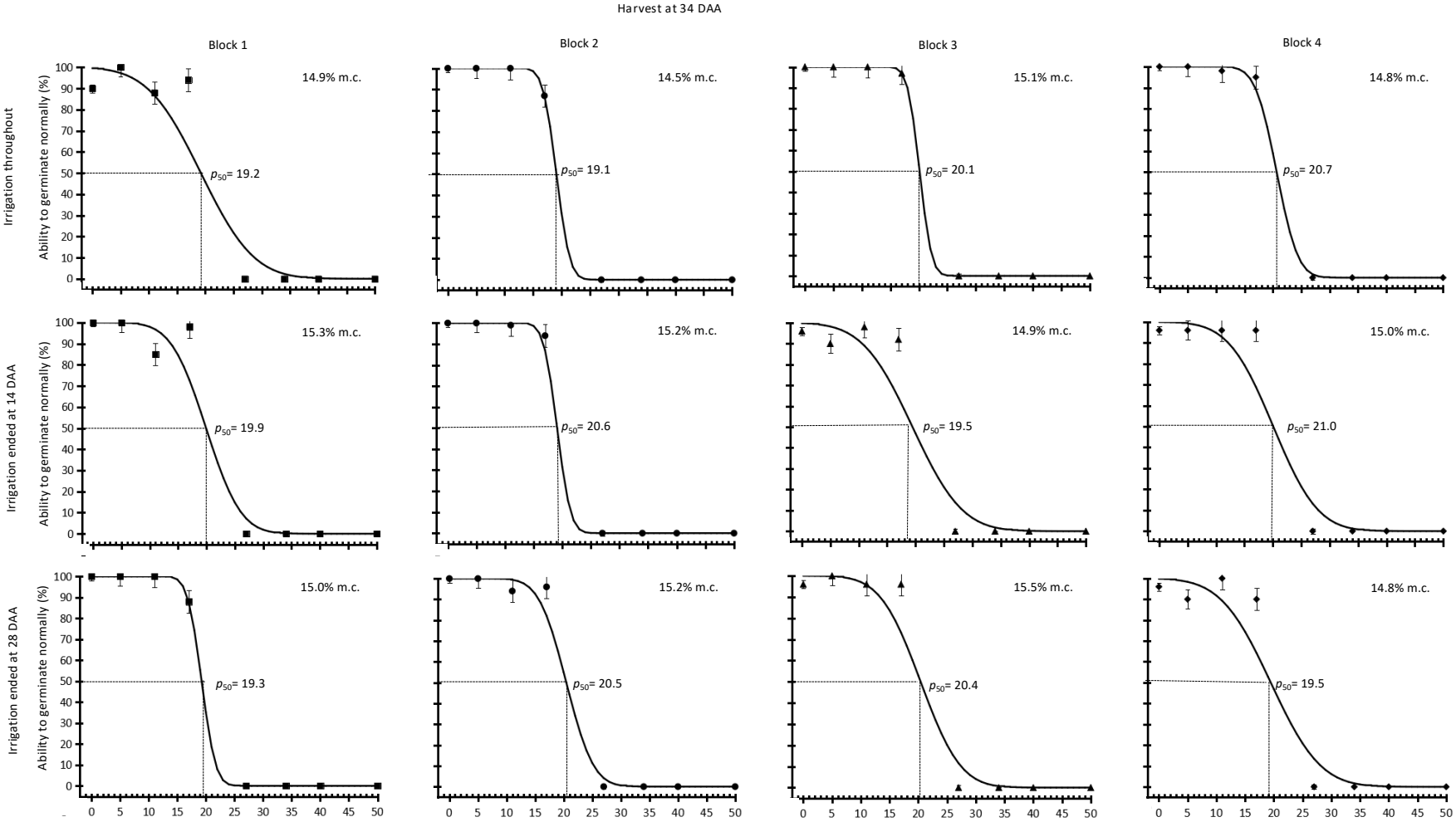
Appendix 4.14B. Comparison of survival curves for seed harvest at 18 DAA



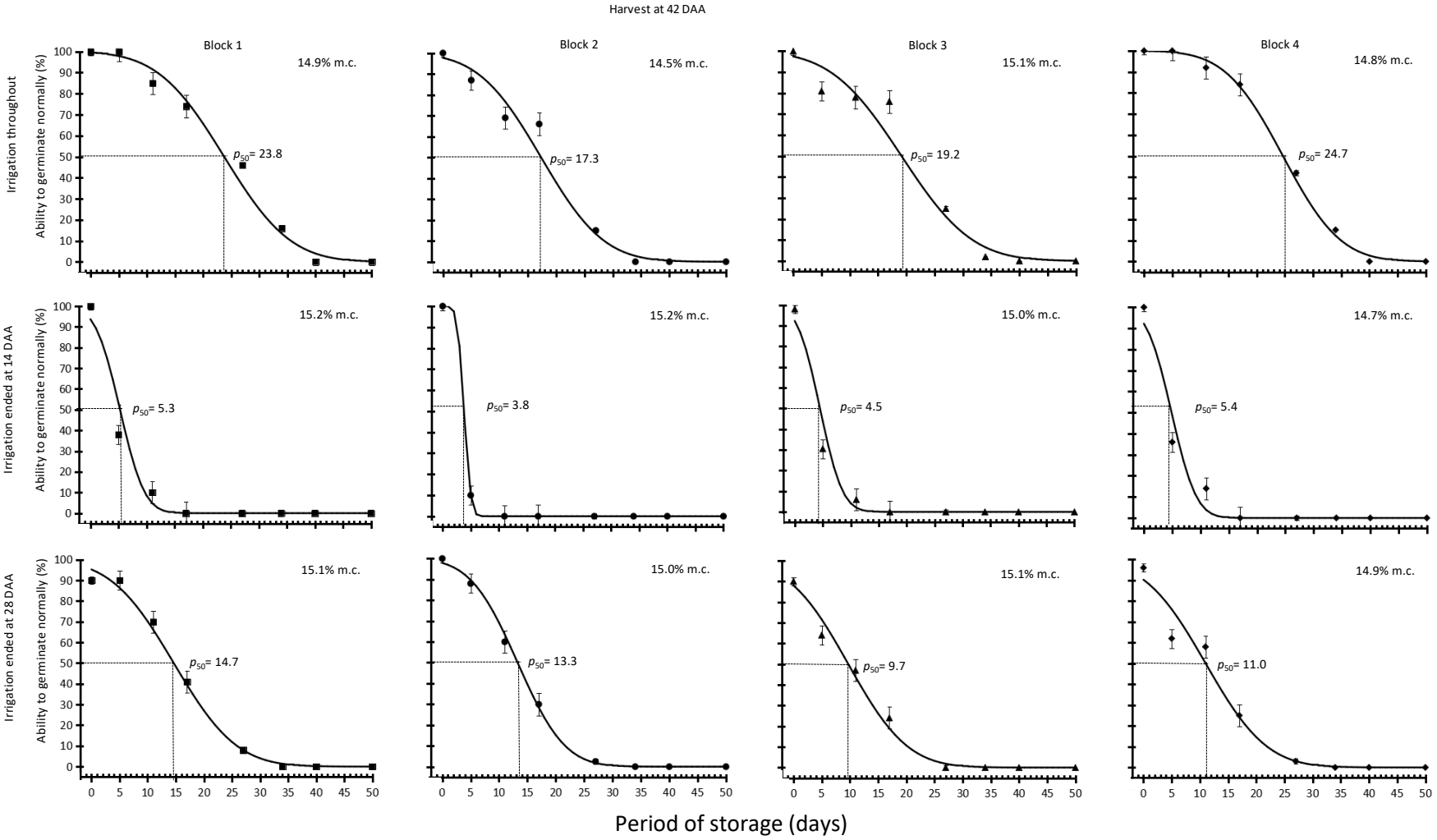
Appendix 4.14C. Comparison of survival curves for seed harvest at 28 DAA



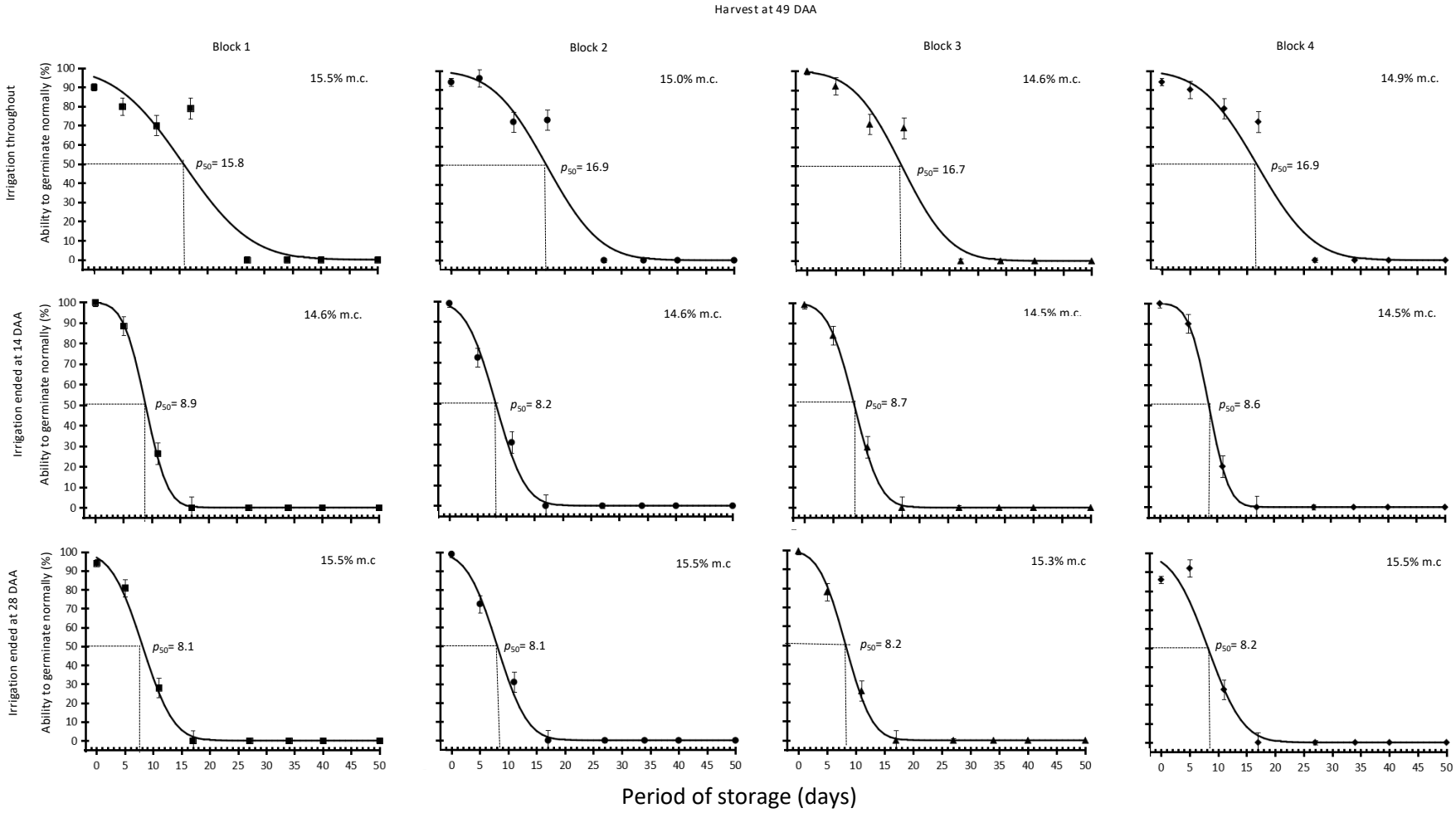
Appendix 4.14D. Comparison of survival curves for seed harvest at 34 DAA



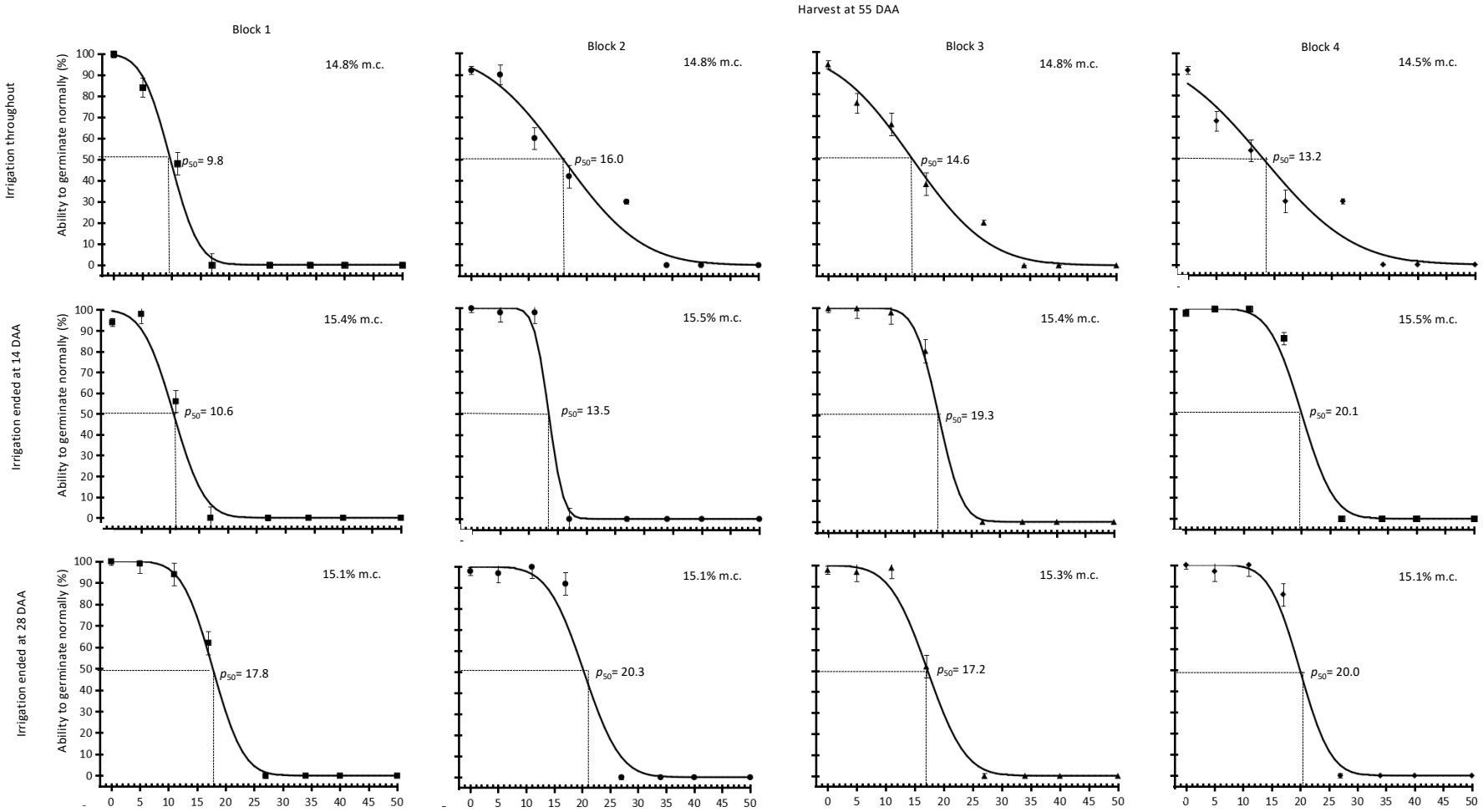
Appendix 4.14E. Comparison of survival curves for seed harvest at 42 DAA



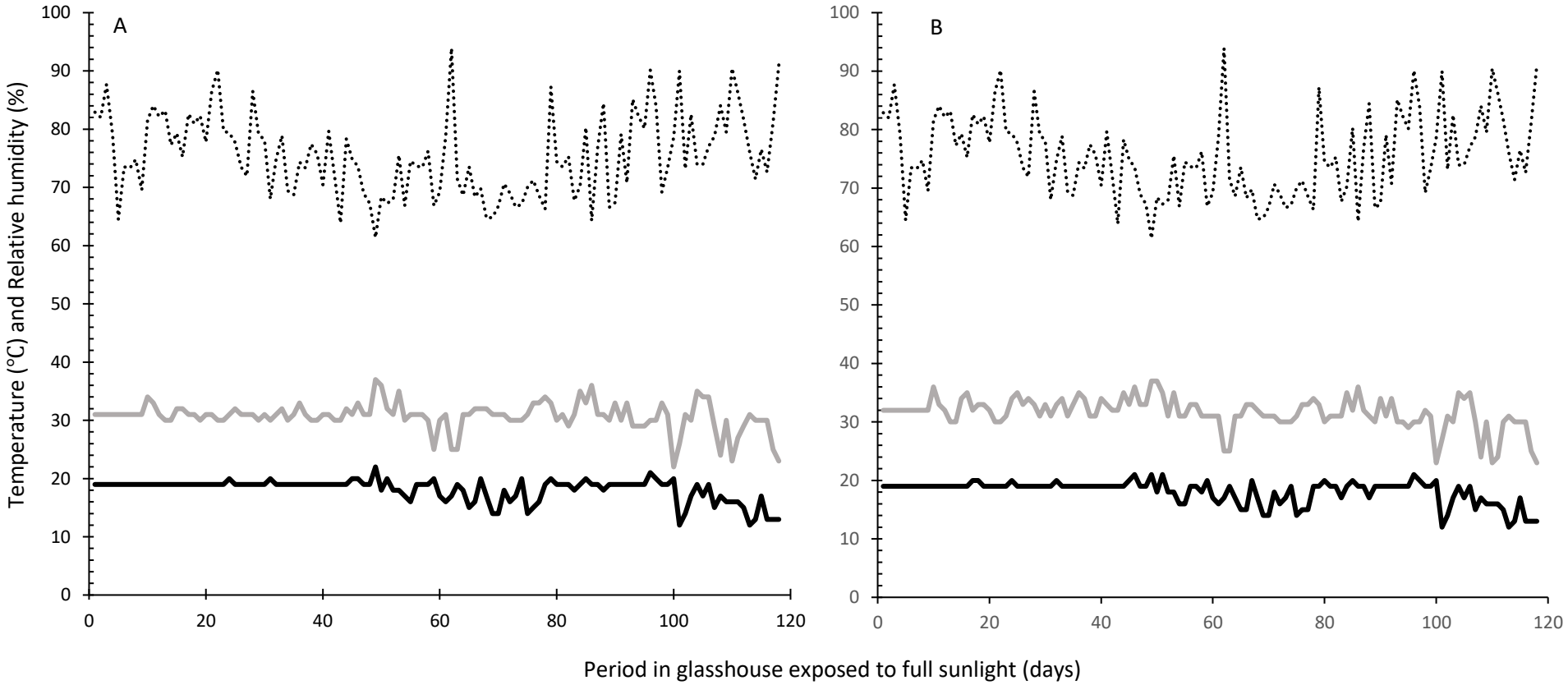
Appendix 4.14F. Comparison of survival curves for seed harvest at 49 DAA



Appendix 4.14G. Comparison of survival curves for seed harvest at 55 DAA



Appendix 4.15. Weather data of minimum (▬) and maximum temperature (▬)(℃) in glass-house and relative humidity (.....) (%) outside the glass-house during June -September 2016. Block 1 and 2 (A); block 3 and 4 (B).



CHAPTER 5

Appendix 5.0 A. Analysis of variance of seed moisture content (%) of Aeron 1 (Cab 1) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.2668	0.25336	84.31	<.001
Residual	6	0.01803	0.00301		
Total	11	1.28483			

Appendix 5.0 B. Analysis of variance of seed dry weight (mg) of Aeron 1 (Cab 1) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.25731	0.05146	28.41	<.001
Residual	6	0.01087	0.00181		
Total	11	0.26818			

Appendix 5.0 C. Analysis of variance of ability of seed to germinate normally (%) of Aeron 1 (Cab 1) for different treatments at harvest maturity (42 DAA) by (angular transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.38665	0.07733	4.98	0.038
Residual	6	0.09322	0.01554		
Total	11	0.47986			

Appendix 5.1 A. Analysis of variance of seed moisture content (%) of Aeron 1 (Cab 2) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.8815	0.1763	13.01	0.004
Residual	6	0.08128	0.01355		
Total	11	0.96278			

Appendix 5.1 B. Analysis of variance of seed dry weight (mg) of Aeron 1 (Cab 2) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.4722806	0.09446	203.78	<.001
Residual	6	0.0027812	0.00046		
Total	11	0.4750618			

Appendix 5.1 C. Analysis of variance of ability of seed to germinate normally (%) of Aeron 1 (Cab 2) for different treatments at harvest maturity (42 DAA) (angular transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.62481	0.12496	6.76	0.019
Residual	6	0.11091	0.01849		
Total	11	0.73572			

Appendix 5.2 A. Analysis of variance of seed moisture content (%) of Gleva (Cab 1) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	17.9743	3.594854	2819.24	<.001
Residual	6	0.00765	0.001275		
Total	11	17.9819			

Appendix 5.2 B. Analysis of variance of seed dry weight (mg) of Gleva (Cab 1) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.64025	0.32805	91.14	<.001
Residual	6	0.0216	0.0036		
Total	11	1.66185			

Appendix 5.2 C. Analysis of variance of ability of seed to germinate normally (%) of Gleva (Cab 1) for different treatments at harvest maturity (42 DAA) (angular transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.99697	0.39939	31.02	<.001
Residual	6	0.07725	0.01287		
Total	11	2.07421			

Appendix 5.3 A. Analysis of variance of seed moisture content (%) of Gleva (Cab 2) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	3.53082	0.70616	36.36	<.001
Residual	6	0.11652	0.01942		
Total	11	3.64734			

Appendix 5.3 B. Analysis of variance of seed dry weight (mg) of Gleva (Cab 2) for different treatments at harvest maturity (42 DAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.46075	0.292151	137.13	<.001
Residual	6	0.01278	0.00213		
Total	11	1.47354			

Appendix 5.3 C. Analysis of variance of ability of seed to germinate normally (%) of Gleva (Cab 2) for different treatments at harvest maturity (42 DAA) (angular transformed data).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.99697	0.39939	31.02	<.001
Residual	6	0.07725	0.01287		
Total	11	2.0742			

In Appendices 5.4-5.7, different models are compared to describe the seed survival curves fitted by probit analysis

Test 1= Best fit model; test 2= Common slope model; test 3= Common line model: Not significant shown as (ns, P >0.05); significant (s, P <0.05).

Appendix 5.4: Comparison of survival curves for Aeron 1 seeds (Cab 1) harvested at 42 DAA.

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	390.8	47	8.314
Best model	332.3	42	7.912
Change	58.5	5	11.7
$F_{(5,42)} = 1.47877$			
Slopes different / not different?	P=0.21717		ns

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	446.7	52	8.591
Best model	332.3	42	7.912
Change	114.4	10	11.44
$F_{(10,42)} = 1.4459$			
Slope + Ki different / not different	P=0.194326		ns

Appendix 5.5: Comparison of survival curves for Aeron 1 seeds (Cab 2) harvested at 42 DAA.

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	495.1	47	10.53
Best model	366.5	42	8.727
	128.6	5	25.72

$F_{(5,42)}$= 2.9472

Slopes different / not different? P=0.022782 s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	1281	52	24.63
Best model	366.5	42	8.727
Change	914.5	10	91.45

$F_{(10,42)}$= 10.479

Slope + Ki different / not different P<0 .00001 s

Appendix 5.6: Comparison of survival curves for T1 (Control) of Gleva seeds (Cab 1) harvested at 42 DAA.

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	118.7	47	2.525
Best model	60.88	42	1.449
Change	57.82	5	11.564

$F_{(5,42)}$= 7.9807

Slopes different / not different? P=0.022782 s

TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	809.8	52	15.57
Best model	60.88	42	1.449
Change	748.92	10	74.892

$F_{(10,42)}$ = 51.6853

Slope + Ki different / not different P<0 .00001 s

Appendix 5.7: Comparison of survival curves for T1 (Control) of Gleva seeds (Cab 2) harvested at 42 DAA.

TEST 1 vs 2

F-test	Res dev	Res d.f.	Res Mean dev
Common slope	222.6	47	4.735
Best model	147.5	42	3.513
Change	75.1	5	15.02

$F_{(5,42)}$= 4.2756

Slopes different / not different?	P=0.003116	s

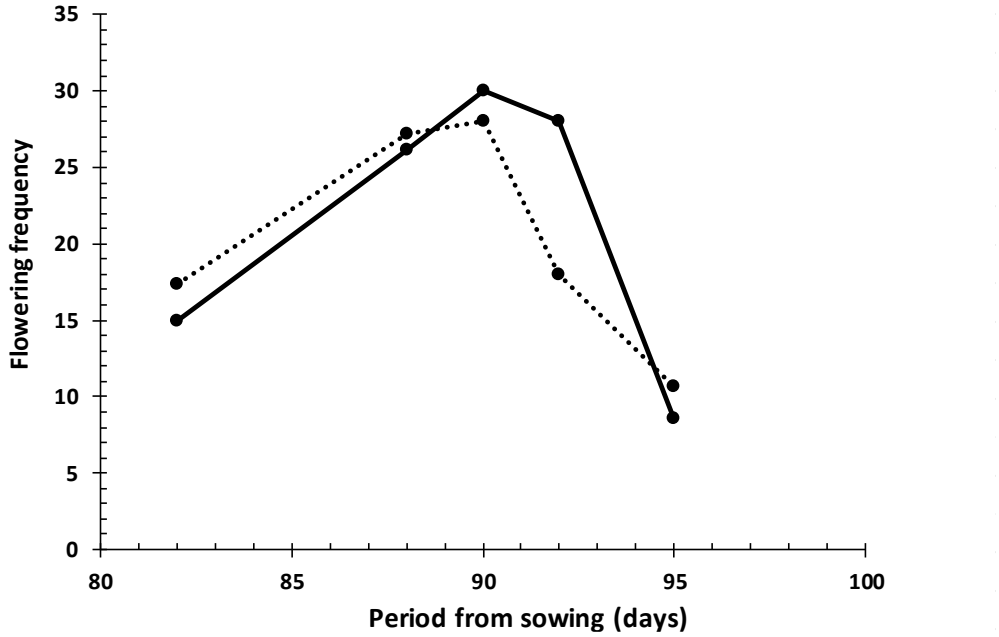
TEST 1 vs 3

F-test	Res dev	Res d.f.	Res Mean dev
Common line	577.1	52	11.1
Best model	147.5	42	3.513
Change	429.6	10	42.96

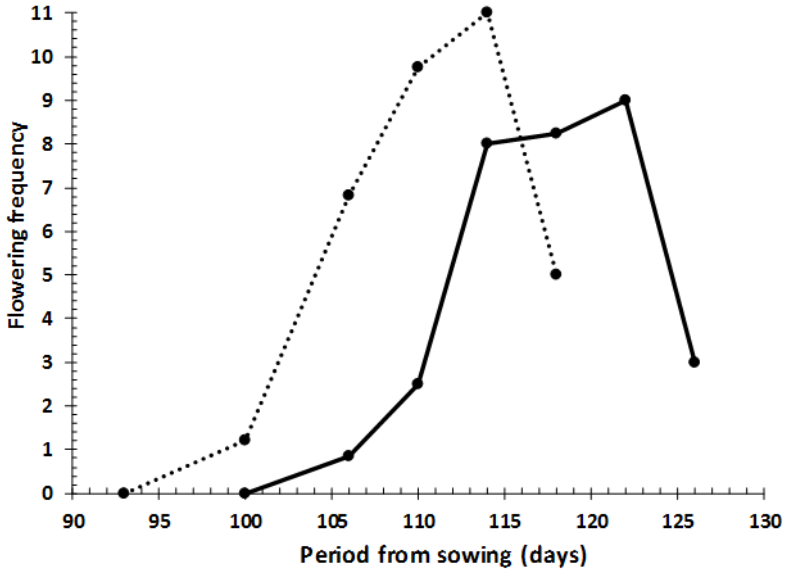
$F_{(10,42)}$=12.2289

Slope + Ki different / not different	P<0 .00001	s

Appendix 5.8. Flowering frequency (number of panicles beginning to anthese each day) of *indica* rice cv. Aeron 1 after sowing; Cab 1 (…) and Cab 2 (—).



Appendix 5.9 Flowering frequency (number of panicles beginning to anthese each day) of *japonica* rice cv. Gleva after sowing; Cab 1 (…) and Cab 2 (—).



Appendix 5.10. Relative humidity of cab 1 (…) and cab 2 (─) at 28/20 ℃. In cv. Aeron 1, the period 0-42 DAA was 90-132 DAS in both cabs. In cv. Gleva, the period 0-42 DAA was 114-156 DAS in cab 1, and 122 to 164 DAS in cab 2.