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High-Temperature Stress During Drying Improves Subsequent Rice (*Oryza sativa* L.) Seed Longevity

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High-Temperature Stress During Drying Improves Subsequent Rice (*Oryza sativa* L.) Seed Longevity

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Abstract

Post-harvest drying prolongs seed survival in air-dry storage; previous research showed benefit from drying moist rice seeds at temperatures greater than recommended for genebanks (5-20°C). The aim of this study was to determine whether there is a temperature limit for safely drying rice seeds, and to explore whether the benefit to longevity is caused by high-temperature stress or continued seed development. Seeds of two rice varieties were harvested at different stages of development and dried initially either over silica gel, or intermittently (8 h d^{-1}) or continuously (24 h d^{-1}) over $MgCl_2$ at temperatures between 15 and 60°C for up to 3 d. Seeds dried more rapidly the warmer the temperature. Subsequent seed longevity in hermetic storage (45°C with 60% equilibrium RH) was substantially improved by increase in drying temperature up to 45°C in both cultivars, and also with further increase from 45 to 60°C in cv. 'Macassane'. The benefit of high-temperature drying to subsequent longevity tended to diminish the later the stage of development at seed harvest. Intermittent or continuous drying at high temperatures provided broadly similar improvements to longevity, but with the greatest improvements detected in a few treatment combinations with continuous drying. Heated-air drying of rice seeds harvested before maturity improved their subsequent storage longevity by more than that which occurred during subsequent development *in planta,* which may have resulted from the triggering of protection mechanisms in response to high-temperature stress.

Introduction

Temperature, particularly extreme values, affects the rate at which crops develop from sowing to maturity, and their yield, which limits their range of cultivation (Evans, 1993). In rice (*Oryza sativa* L.), duration to maturity, and so crop adaptation, is affected by temperature, photoperiod, and possibly diurnal temperature amplitude, with considerable variation amongst genotypes in responsiveness to these environmental variables (Collinson *et al*., 1992; Yin *et al*., 1996). Seed set in rice is vulnerable to low temperatures (12-20°C) during the reproductive phase or to high day (35-38.5°C) or night (30-35°C) temperatures in the early reproductive period up to and including anthesis, with considerable variation amongst genotypes in resilience to such temperature stress (Yoshida, 1981; Jagadish *et al.*, 2008; Coast *et al*., 2015; Martinez-Eixarch and Ellis, 2015). The wide diversity of rice germplasm enables it to be cropped over a wide geographical distribution globally across temperatures ranging from as cool as 15°C to exceeding 40°C (Zhang *et al*., 2005; Wassman *et al*., 2009). In addition to effects on yield, rice seed quality is also affected by high temperature during seed development, particularly during histodifferentiation and early seed-filling (Ellis *et al*., 1993; Ellis and Hong, 1994; Ellis, 2011; Martinez-Eixarch and Ellis, 2015).

Seed quality is important for the long-term conservation of germplasm as it affects seed storage longevity in genebanks (Hay and Smith, 2003). Rice seed quality - such as ability to germinate, desiccation tolerance and survival period under air-dry storage - begins to be acquired early in seed development and, in favourable environments, continues to improve *in planta* until late in maturation drying (Ellis *et al*., 1993; Ellis and Hong, 1994; Ellis, 2011). Moreover, rice seeds, which were metabolically active at harvest (≥ 16.2% moisture content), improved considerably (up to 3-fold in more moist samples) in subsequent air-dry longevity when subjected to initial, intermittent (8 h d^{-1}) high-temperature (45°C) air-drying *ex planta* in comparison with immediate post-harvest drying at 15°C and 15% RH (Whitehouse *et al*., 2015). This latter drying regime is within the multi-species range of 5-20°C with 10-25% RH recommended for genebanks (FAO, 2013).

The importance of seed drying in order to extend subsequent seed storage longevity in genebanks is well recognized (Cromarty *et al*., 1982; FAO, 1994, 2013; Rao *et al*., 2006), but high temperatures are usually avoided to reduce the risk of seed deterioration, especially when seeds are at a high moisture content (Nellist, 1980; Cromarty *et al*., 1982; McDonald and Copeland, 1997). Heated-air seed drying practices recognize safe-drying temperatures (maximum value avoiding damage to ability to germinate) which vary with initial moisture content, design of drier, or whether air or seed temperature is specified (Nellist, 1980). However, much of the research involving heated-air seed drying used rewetted mature, dry seeds (Nellist, 1980). Compared with many other species, safe drying temperatures reported for rice seeds are comparatively high: for example, 48.9°C at 20% moisture content or above, 51.7°C at 15-20% moisture content, and as high as 60°C for initial seed moisture contents of 15% or below (Lewis, 1950). Harrington (1972) suggested a cooler limit of 45°C for drying all cereal seeds, whilst Yamashita (1993) recommended an air temperature of only 40°C for rice seeds with ≥ 24% moisture content. Similarly, Jittanit *et al*. (2010) reported that rice seeds dried at 40°C for 50-250 minutes showed > 85% germination, whereas germination was adversely affected at ≥ 60°C. They also reported less damage from drying in a batch than a fluidized-bed dryer at any one temperature. With traditional sun-drying, on the other hand, temperatures can reach up to 52°C and if carried out carefully rice seed viability can be maintained well thereafter (Regalado and Brena, 2006).

Many investigations of high-temperature drying in rice relate to milling quality. Drying at high temperatures creates intra-kernel moisture content gradients which can lead to fissure formation and reduced milling yield if seeds cool before tempering (Chen *et al*., 1997; Cnossen and Siebenmorgen, 2000, 2002; Schluterman and Siebenmorgen, 2007). Hence for milling rice, Lewis (1950) recommended cooler temperatures than for seed: 40.6°C at 20% or more, 46.1°C at 15-20%, and 51.7°C for initial seed moisture contents of 15% or below. More recently, two-stage drying (initial high-temperature and high velocity followed by ambient conditions with reduced air flow) has been used to dry high-moisture content seeds to reduce damage to milling yield (Jittanit *et al*., 2010; and references therein). The beneficial high-temperature procedure for rice seed longevity reported by Whitehouse *et al*. (2015), as above, was in fact a multi-stage treatment: an

initial diurnal cycle (8/16h, 45°C/15°C for 1-6 d) followed by a final period at 15°C (with 15% RH).

We report here an investigation of the high-temperature limits for seed longevity when drying rice seeds harvested at different moisture contents and days after anthesis (DAA) in order to explore whether maturing rice seeds, which are still metabolically active at harvest, benefit from high-temperature exposure due to continued development or a stress-related response *ex planta*.

Material and Methods

Plant material

Seeds of an aromatic variety of rice from the International Rice Genebank Collection, accession IRGC 117265 (McNally *et al*., 2009), and of a commonly-grown indica variety, 'Macassane', were planted for harvest in the 2015 dry season (DS) and 2016 wet season (WS), respectively (Fig. 1A, C). Seeds were sampled either from the International Rice Genebank (IRG) active collection (4°C) (IRGC 117265) or the storage facility (20°C and 30% RH) at the upland site ('Macassane') and held at 50°C for 5 d to break dormancy. Staggered sowing of seeds from IRGC 117265 was conducted to enable simultaneous harvests at 25, 35 and 45 d after 50 % anthesis (DAA) on two separate occasions, 13 days apart, to achieve two seed lots per stage of maturity differing in harvest moisture content (Table 1 and Fig. 1A). For 'Macassane' however, all seeds were sown on 7 June 2016 and harvested on three separate dates at 34, 36 and 38 DAA (Table 1 and Fig. 1B). All seedlings, from both varieties, were raised in a seed bed before being transplanted to plots on the International Rice Research Institute (IRRI) Ziegler experimental station (ZES) (14°9'3.5742"N, 121°15'54.504"W) and normal rice production practices and plant protection measures were followed. Seeds were harvested on April 2015 and October 2016 for IRGC 117265 and 'Macassane', respectively (Table 1 and Fig. 1A, C).

Immediately after harvest, the seeds of each variety were threshed and blown to remove debris and half-filled grains before they were transported to the laboratory where the seeds underwent manual cleaning - discarding any empty, damaged and/or diseased

seeds - and the initial temperature, equilibrium relative humidity (eRH; %) and the moisture content (MC; % fresh weight) was measured. The eRH was measured at room temperature (21.5°C) using an AW-D10 water activity station in conjunction with a HygroLab 3 display unit (Rotronic South East Asia, Singapore). Seed MC (fresh weight basis) was determined using three 5 g samples from each seed lot (harvest x DAA) using the high-constant-temperature oven method (ISTA, 2013).

Seed drying over saturated $MgCl_2$ (IRGC 117265)

Seeds of IRGC 117265 at each maturity stage and from A and B harvests were divided into 9 x 200 g samples and placed into 0.2 × 0.33 m (L × W) nylon mesh bags (1 mm-diameter holes) in which they were stored inside sealed 0.6 × 0.3 × 0.132 m (L × W × H) electrical enclosure boxes (ENSTO, Porvoo, Finland) at room temperature (21.5°C) overnight to limit drying until the treatments began the following morning (0800 h). A sample from each maturity stage was transferred directly to the genebank drying room maintained at 15°C and 15% RH (Fig. 1B). As the drying room conditions comply with the current genebank standards (FAO, 2013), the treatments dried throughout therein provided a control, i.e. the baseline against which the effects of other drying treatments were compared. To provide the other drying environments, the remaining seed samples were placed over a 700 ml super-saturated solution of $MgCl_2$ in sealed electrical enclosure boxes, producing an RH of approximately 30%, and transferred either to the drying room (15°C), or to incubators at 30, 45 or 60°C (Fig. 1B). Within these drying environments, there was no airflow through the seeds; rather the drying process was passive with the desiccant absorbing moisture from the seeds, without affecting equilibrium RH within the container, and the temperature driving the evaporation of moisture from the seeds to their surroundings. Seed samples were exposed to intermittent (Int; 8 h day^{-1}) and continuous (Cont; 24 h) drying treatments for 3 d at each temperature regime before final drying for 11 d in the drying room (i.e. equilibrating to 15°C/15% RH; expected MC 6-7%). During the non-active drying phase (Int; 1600–0800 h) seeds were sealed in empty (without $MgCl_2$) electrical enclosure boxes at 21.5°C until the following morning (Fig. 1B). The change in weight and eRH of samples was monitored daily, at 1600 h for intermittently-dried seeds and at 0800 h for continuously-dried seeds, until samples were transferred to the drying room where this was subsequently recorded every 3 d. The

dried seeds were then sealed inside 0.16 × 0.24 m (L × W) laminated aluminium foil packets (Moore and Buckle, Saint Helens, UK) and stored at 2-4°C until experimental storage began.

Seed drying over silica gel ('Macassane')

Seeds of 'Macassane' harvested at 34, 36 and 38 DAA were divided into 25 x 200 g samples and placed into nylon mesh bags (as described above) (Fig. 1D). A sample was immediately transferred to the drying room (15°C with 15% RH) and the remaining samples were stored inside individual 0.3 × 0.3 × 0.132 m (L x W x H) electrical enclosure boxes containing 400 g of (previously-dried) silica gel (granular 0.2-1 mm; Sigma-Aldrich, Singapore). A box was transferred to either the genebank drying room (15°C) or to incubators at 30, 45 and 60°C at 10 minute intervals (time taken to measure sample eRH; i.e. ensured all samples were dried for the same period) until there were a total of 6 boxes at each temperature (Fig. 1D). The change in weight and eRH (as described above) was monitored for each sample after 2, 4, 8, 12, 16, 24, 72, 144, 216, 288 and 336 h. A sample was removed and transferred from each high-temperature regime after 2, 4, 8, 12, 16 and 24 h to the drying room for final drying (Fig. 1D). At 14 d after harvest, samples from all drying treatments were removed, final weight and eRH of each recorded, before they were sealed inside laminated aluminium foil packets and stored as described above.

Seed storage

Seed samples were removed from temporary cold storage (2-4°C) and equilibrated to room temperature (21.5°C) before opening. Each sample was split into 29 × 5 g subsamples which were placed into 30 mm-diameter open Petri dishes in a VC^3 0034-M climate chamber (Vötsch Industrietechnik, Germany) set at 60% RH and 21.5°C for 4-5 d. Once equilibrium had been reached (approximately 10.9% MC), four of the 5 g subsamples from each treatment combination were removed to measure seed eRH; three of these were then used to determine MC and the fourth to estimate initial ability to germinate. The remaining subsamples (25) were each sealed inside individual aluminium foil packets (0.12 × 0.08 m [L × W]) before being placed in an incubator at 45°C. One packet per treatment combination was removed from storage at 45°C at 3-day intervals

up to 54 d to test ability to germinate. Moisture content determinations, using three additional 5 g packets of seeds, were made at the mid-point and end of storage. These analyses confirmed seed storage moisture content was stable during experimental storage.

Seed germination

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

Statistical analysis

Seed survival curves were fitted by probit analysis using GenStat for Windows, Version 17 (VSN International Ltd., Hemel Hempsted, UK). As seeds from both varieties typically show dormancy, a probit model combining loss of dormancy (during early storage) with loss of viability [1] was applied using the FITNONLINEAR directive in GenStat.

$$g = (K_{\mathrm{d}} + \beta_1 p) \cdot (K_{\mathrm{i}} - (p/\sigma)) \ , \qquad [1]$$

where g is the ability to germinate in normal equivalent deviates (NED), K_{d} is the initial proportion of non-dormant seeds (NED), β_1 is the probit rate of loss in dormancy and p is the storage period (d); K_{i} and σ are as in the Ellis and Roberts (1980) viability equation:

$$v = K_{\mathrm{i}} - p/\sigma, \qquad [2]$$

where v is the viability (NED) of a seed lot stored for period, p, K_{i} is the initial viability (NED) and σ (days) is the standard deviation of the normal distribution of seed deaths in time. The time for viability to fall to 50% (p_{50}, product of K_{i} and σ) was also estimated and used as a measure of longevity. For those seed lots which showed a reduced initial viability and a systematic pattern of residuals when only fitting equation [1], the “control mortality” parameter (“immunity” in GenStat), which estimates the proportion of “non-

responding" seeds (i.e. dead or empty) within the population (Mead and Gray, 1999), was included in the probit analysis. Probit analysis was carried out for all seed lots simultaneously, fitting the full model (different estimates for all parameters).

Results

Seed drying rate

The harvest eRH of all the seed lots was high (80.8-99.9%; Table 1). For each seed production, the moisture content of seeds harvested at the lowest DAA was highest (21.9-32.0%). Moisture content reduced with increasing maturity, but sometimes the intermediate sample provided the lowest value: 'Macassane' seeds at 34 DAA were the most-moist and IRGC 117265 seeds at 35 DAA (harvest B) the driest (16.3%). All drying treatments, including the control, provided the characteristic negative logarithmic decline, with progressively more rapid moisture loss as temperature increased from 15-60°C (Figs. 2A-D and 3A). Later harvests, with lower initial MC, showed slower drying rates at all temperatures; this was most apparent at 15°C. Most of this effect of later harvest date affected moisture loss over the first day only. The pattern of drying of seeds in the drying room was similar to 45°C/30% RH over the first 3 d of continuous drying at all maturity stages (Fig. 2C, D), whereas seeds dried faster at all temperatures over silica gel compared with the drying room (Fig. 3A).

Subsequent seed storage longevity

Significant differences in survival curves ($P < 0.05$) were apparent between some, but not all, seed lots harvested at different maturity stages and amongst drying treatments at each maturity stage. Therefore, longevity derived from the best-fit survival curves for each treatment combination are shown (Tables S1 and S2).

In both experiments, low-temperature drying (15°C) resulted in seeds with shorter longevity compared with high-temperature drying (Figs. 2E-H and 3B). Nonetheless, drying seeds over $MgCl_2$ at 15°C still led to an improvement in their subsequent storage longevity compared with drying at lower RH (15% RH) in the drying room (Fig. 2E-H). In

'Macassane', however, relative improvement between the drying room and silica gel treatments at 15°C depended on the duration of drying (Fig. 3B). Drying seeds in the drying room throughout (shown by broken lines) resulted in progressively greater longevity from 25 to 45 DAA in harvest A of IRGC 117265 (Fig. 2E, G), a small increase from 34-36 DAA but similar longevity at 36 and 38 DAA in 'Macassane' (Fig. 3B), or an increase from 25-35 DAA but then decline from 35-45 DAA in harvest B of IRGC 117265 (Fig. 2F, H).

In both varieties at all harvests, the higher the temperature of drying from 15 to 45°C the greater the longevity (Figs. 2E-H, 3B). In 'Macassane', this was also the case with further increase in temperature from 45 to 60°C (Fig. 3B), whereas 60°C provided poorer, or sometimes similar, longevity compared with 45°C in IRGC 117265, but was always greater compared with drying at 15 or 30°C (Fig. 2E-H). There was a tendency for the more mature seed at harvest to show a slightly smaller benefit to longevity from high-temperature drying.

Within a temperature regime, the longevity of seeds of IRGC 117265 exposed to high-temperature intermittently was broadly similar to the equivalent continuous treatment (Fig. 2E-H). At 30 and 45°C, continuous high-temperature drying provided slightly greater longevity than intermittent drying, but *vice versa* at 15 and 60°C (Fig. 2E-H).

The longevity of seeds of 'Macassane' dried over silica gel was determined repeatedly on samples drawn after successive periods of drying. This showed that at the warmer temperatures of 45 and 60°C, and to a lesser extent at 30°C, the early periods of drying provided greater enhancement of longevity than the later periods (Fig. 3B). Comparing 60°C with progressively cooler temperatures, the warmer regimes continued to provide a benefit to longevity later in drying than was observed in cooler conditions.

Discussion

The moisture content of mature seeds at harvest depends on the ambient temperature and relative humidity and will affect the subsequent rate of viability loss if seeds are not dried *ex planta*. In tropical climates, RH conditions rarely fall below 80% and so, seeds are

harvested at moisture contents too high for safe storage, especially in the wet season. The genebank standards recommend drying seeds immediately after harvest to between 3 and 7% MC before long-term storage (FAO, 2013), because the rate of ageing is minimized at these values (Ellis *et al*., 1989, 1992; Ellis and Hong, 2006). But the tolerance of orthodox seeds to desiccation and storage depends on the stage of maturity and the drying conditions, especially the rate of drying (Hay and Probert, 1995).

In this experiment, all rice seeds were metabolically active (MC > 16.2%; Whitehouse *et al*., 2015) and assumed to have reached mass maturity before harvest (Kameswara Rao and Jackson, 1996a, b). Hence, they were still within the desiccation phase of seed development where seed quality traits can still be accrued (Galau *et al*., 1991; Angelovici *et al*., 2010; Chatelain *et al*., 2012). In accordance with this, and similar to other studies (Ellis *et al*., 1993; Kameswara Rao and Jackson, 1996a, b; Hay and Smith, 2003; Ellis, 2011), the longevity of seeds dried in the drying room (control; broken lines) increased the later the seeds were harvested i.e. 25-35 DAA (Fig. 2E, G) until, depending on the environmental conditions, longevity plateaued (Fig. 3B) or declined (Fig. 2F, G). However, as previously shown by Whitehouse *et al*. (2015) longevity continued to increase *ex planta* when seeds, from both cultivars and all harvests, were exposed to initial drying at temperatures > 15°C (Figs. 2 and 3). The longevity of seeds dried intermittently or continuously for 3 d over $MgCl_2$ (30% RH) continued to increase with drying at 30 to 45°C (Fig. 2E-H), with a further increase seen from 45 to 60°C in 'Macassane' seeds dried for 1 d over silica (Fig. 3B). The latter corresponds with the report that mature rice seeds can survive drying temperatures as high as 60°C (Lewis, 1950). As reported by Whitehouse *et al*. (2015), these results provide further confirmation that the quality of the seeds cannot be accurately predicted post mass maturity with respect to developmental time (DAA), as the pre-harvest environment (ambient temperature and humidity conditions, as well as the seed production environment) can restrict the seeds progression through development and through maturation drying in particular.

Research has shown that the environmental conditions experienced during development and maturation can affect the relative timings of developmental stages (Hirana, 1979; Tu *et al*., 1988; Olivares *et al*., 2009; Ellis, 2011; Martinez-Eixarch and Ellis, 2014). For example, a warm seed production environment reduced the improvement in seed quality

development that occurs subsequent to mass maturity in indica varieties of rice as the hotter temperatures enhanced the progression through development which subsequently resulted in seeds which had not fully acquired maximum quality (Ellis *et al*., 1993). Continuation of such developmental events can, however, continue *ex planta* if seeds are held at conditions similar to those they would naturally encounter *in situ* (Hay, 1997; Hay and Probert, 2005; Probert *et al*., 2007). Rice seeds typically experience field temperatures between 27 and 30°C and so perhaps it is not surprising that subsequent longevity improved *ex planta* in response to drying at temperatures > 15°C (Figs. 2E-H and 2B.) However, improvements in seed quality are not infinite and presumably there is a "maximum longevity" that any developing cohort of seeds can attain. A typical developmental response would involve a continual increase in longevity before plateauing, and although increases in longevity would be slower at temperatures cooler than 45-60°C, all seed lots would be expected to eventually reach the same level of longevity. This was not observed in this study, rather, drying rate and subsequent storage longevity both increased with the increase in drying temperature, when seed lots were dried over silica gel (Fig. 3). This suggests desiccation shock is more likely to account for the variation in longevity, whilst not ruling out a small contribution from continued development as it is likely that the two are connected, i.e. the desiccation shock accelerates the developmental events associated with increase in longevity during maturation drying. However, as some seed lots dried at a similar rate e.g. 15°C/15% RH and 45°C/30% RH (Fig. 2C, D) but with substantial differences in longevity (Fig. 2G, H), and all seed lots generally showed the greatest benefit to longevity within the first 2 h of drying, which was greater the greater the temperature (Fig. 3), there is clearly an effect of temperature rather than desiccation alone.

High temperatures are thought to induce stress responses within seeds, similar to that which triggers maturation drying, which is likely to promote the metabolic processes and protective mechanisms associated with desiccation (since they both represent stresses), including synthesis of late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs), accumulation of raffinose family oligosaccharides, and activation of antioxidant defence-mechanisms (Vertucci and Farrant, 1995; Kermode, 1997; Bailly *et al*., 2004; Buitink and Leprince, 2008; Leprince and Buitink, 2010); high temperature will also increase the rate at which the above occur. This is supported by previous studies

which provided evidence that the accumulation of soluble carbohydrates and heat stable proteins during development were associated with desiccation tolerance and potential longevity (Sinniah *et al.*, 1998). However, these metabolic pathways and processes involved in the accumulation of longevity can be slowed/impaired when temperatures pass a critical limit (McDonald, 1999; Corbineau *et al.*, 2002). Seed temperature during high-temperature drying would have increased towards the air temperature, albeit with some delay due to evaporative cooling as moisture was lost from the seeds; this may explain why seeds still benefit from drying at higher temperatures, up to 45°C, even after 3 d, but also why longevity was greatest when seeds were dried for 1 d at 60°C (Fig. 3B) but not after 3 d (Fig. 2E-H). Furthermore, it is possible that normal energy metabolism and enzyme activity may have been reinstated in metabolically-active seeds (>16.2% MC) during the non-drying period, as reported by Corbineau *et al*. (2002), which may explain the greater longevity observed in seeds dried intermittently at 60°C compared with seeds dried continuously (Fig. 2E-H).

Interestingly, prior to the 1990s, it was common practice to dry rice seeds intended for storage in the IRGC at high temperature (45-50°C) and it was only after the publication of the FAO genebank standards (FAO, 1994), which recommended cool temperatures combined with relatively low humidity to dry seeds prior to storage, that the drying room facility was installed and operated at 15°C and 15% RH. The standards were derived based on the low MC limit, i.e. below which there is no further improvement in longevity (Ellis and Hong, 2006 and references therein), and the drying conditions necessary to achieve this equilibrium MC (without jeopardising seed quality). The FAO wanted a single, simple, safe procedure for diverse species from all locations worldwide and due to the vulnerability of some species to high temperatures, especially when mature seeds were at high MC (Nellist, 1980; Cromarty *et al*., 1982; McDonald and Copeland, 1997), low temperature and humidity conditions were adopted (FAO, 1994, 2013). The genebank standards have recently been modified from 10-25°C and 10-15% RH (FAO, 1994) to a lower temperature (5-20°C) and broader humidity (10-25% RH) range (FAO, 2013) which further contradicts the results presented in this study which show drying rice seeds, harvested before maturity, at higher temperatures and at a higher humidity (30% RH) can significantly improve their subsequent storage longevity by more than that which occurred during subsequent development *in planta*. This could benefit farmers in

resource-limited countries, particularly those in wet tropical regions, where it is difficult to dry large volumes of seeds to low MC. The quality of freshly harvested seeds could be maintained better (e.g. to the next growing season) by drying seeds to an intermediate MC using heated air.

To conclude, there is clear evidence that drying freshly-harvested, high moisture content rice seeds under low temperature, low humidity conditions is not optimum for subsequent seed storage longevity. Rice seeds showed improvement in longevity in response to drying for a total of 3 d at temperatures greater than 15°C, up to at least 45°C (but drying at 60°C for more than 1 d may damage some seeds). Further studies could involve testing, in independent studies, the beneficial limits of high-temperature drying on rice seeds produced/regenerated in other climatic regions. This could have huge implications on how rice seeds are managed by genebanks globally to ensure maximum longevity when first placed into storage. However, future research should investigate high-temperature drying of fresh moist seeds more widely, addressing other economically-important crops, especially those with poor seed storage longevity and where harvesting seeds at a range of maturities and hence, perhaps moisture content, is inevitable.

Supplementary material

Tables S1 and S2.

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Table 1. Dates of sowing and harvest and the seed moisture content (MC; % fresh weight) and equilibrium relative humidity (eRH; %) at harvest of rice accession IRGC 117265 and cv. 'Macassane' harvested 25-45 or 34-38 days after 50 % anthesis (DAA), respectively.

Accession (harvest) / cultivar and seed maturity (DAA)	Sowing date	Harvest date	Harvest MC (s.e.) (% f.wt.)	eRH (%)
IRGC 117265 (harvest A)				
25	23 Dec 2014		23.3 (0.1)	96.6
35	11 Dec 2014	3 Apr 2015	18.9 (0.1)	86.4
45	1 Dec 2014		18.1 (0.0)	86.0
IRGC 117265 (harvest B)				
25	5 Jan 2015		21.9 (0.1)	91.5
35	24 Dec 2014	16 Apr 2015	16.3 (0.1)	80.8
45	14 Dec 2014		16.8 (0.1)	82.9
'Macassane'				
34		4 Oct 2016	32.0 (1.2)	99.9
36	7 Jun 2016	6 Oct 2016	23.8 (2.0)	96.8
38		8 Oct 2016	20.9 (0.3)	91.5

Figure Legends

Fig.1. Illustration of the experimental design showing the factorial combination of different seed production and seed drying treatments for rice accession IRGC 117265 (A and B) and cv. 'Macassane' (C and D). Seeds were sown (S) for harvest (H) in either the 2015 dry season (DS) or the 2016 wet season (WS). On graphs B and D the dashed lines (B and D) represent the drying room (15°C/15% RH) and the solid black lines represent alternative drying conditions. The solid grey lines show the duration seeds were held in an air-tight box at room temperature (21.5°C).

Fig. 2. Seed drying curves (A, B, C, D) at 15 (●), 30 (▲), 45 (◆), or 60°C (▼) over saturated $MgCl_2$ (30% RH), either intermittently (8 h d^{-1}; A, B) or continuously (24 h d^{-1}; C, D), for rice accession IRGC 117265 harvested on 3 April 2015 (Harvest A) or 16 April 2015 (Harvest B) after 25, 35 or 45 days after 50% anthesis (DAA). After these initial treatments seeds were dried further in the drying room (15°C/15% RH, 11 d). Broken lines show loss in moisture content for seeds dried throughout in the drying room (DR) control (15°C/15% RH; ○). Loss in moisture content during drying was estimated based on the initial determination and subsequent change in sample weight. Seed longevity at 45°C and 60% RH (p_{50}; days ± s.e.) after each drying treatment, provided by the best-fit model for each sample (DAA x temperature) for intermittent (E, F) or continuous drying (G, H), is also shown. For clarity, the control values (open circles joined by broken lines) in E and F are repeated in G and H, respectively.

Fig. 3. Seed drying curves (A) at 15 (●), 30 (▲), 45 (◆), or 60°C (▼) over silica gel for rice cv. 'Macassane' seeds harvested at 34, 36 and 38 days after 50% anthesis (DAA) during 2016 wet season (WS). Broken lines show loss in moisture content for seeds dried throughout in the drying room (DR) control (15°C/15% RH; ○). Loss in moisture content during drying was estimated based from the initial determination and subsequent change in sample weight. Seed longevity (B) during storage at 45°C and 60% RH (p_{50}; days ± s.e.) after each drying treatment (DAA x temperature x drying duration), from both harvests, was estimated from the best-fit model for each sample.

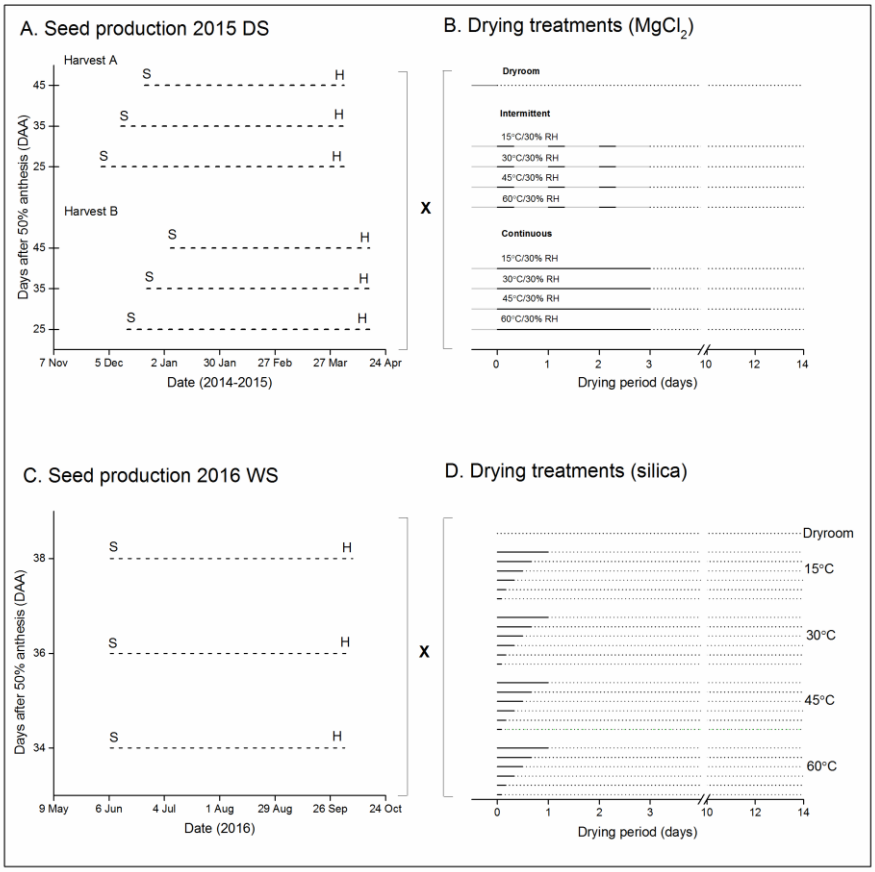
A. Seed production 2015 DS
Harvest A
Harvest B
S
H
Days after 50% anthesis (DAA)
45
35
25
45
35
25
7 Nov
5 Dec
2 Jan
30 Jan
27 Feb
27 Mar
24 Apr
Date (2014-2015)
x
B. Drying treatments (MgCl2)
Dryroom
Intermittent
15°C/30% RH
30°C/30% RH
45°C/30% RH
60°C/30% RH
Continuous
15°C/30% RH
30°C/30% RH
45°C/30% RH
60°C/30% RH
0
1
2
3
10
12
14
Drying period (days)
C. Seed production 2016 WS
S
H
Days after 50% anthesis (DAA)
38
36
34
9 May
6 Jun
4 Jul
1 Aug
29 Aug
26 Sep
24 Oct
Date (2016)
x
D. Drying treatments (silica)
Dryroom
15°C
30°C
45°C
60°C
0
1
2
3
10
12
14
Drying period (days)

Figure 1

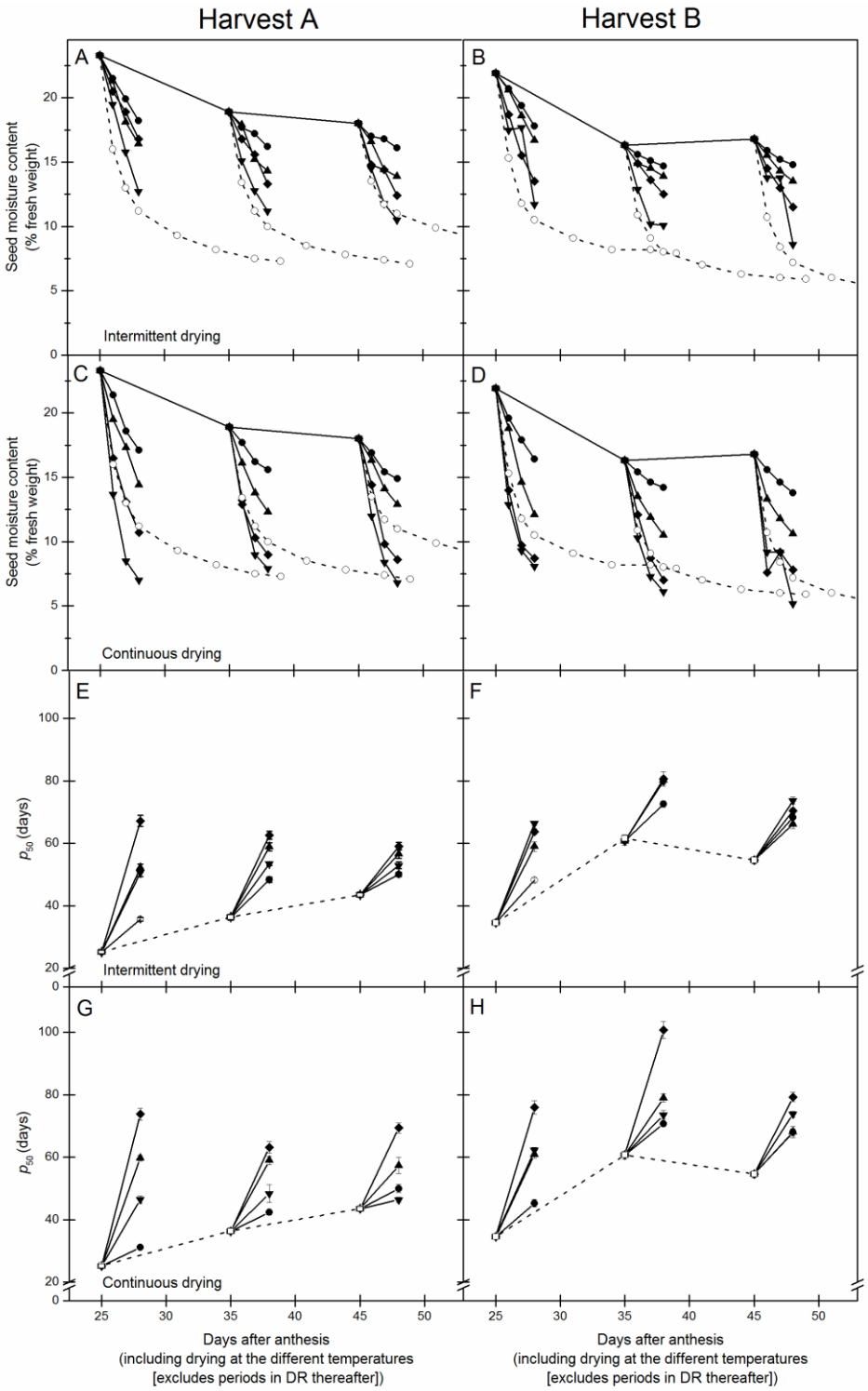
Harvest A
Harvest B
A
B
C
D
E
F
G
H
Seed moisture content (% fresh weight)
Intermittent drying
Continuous drying
p_{50} (days)
Days after anthesis
(including drying at the different temperatures
[excludes periods in DR thereafter])

Figure 2

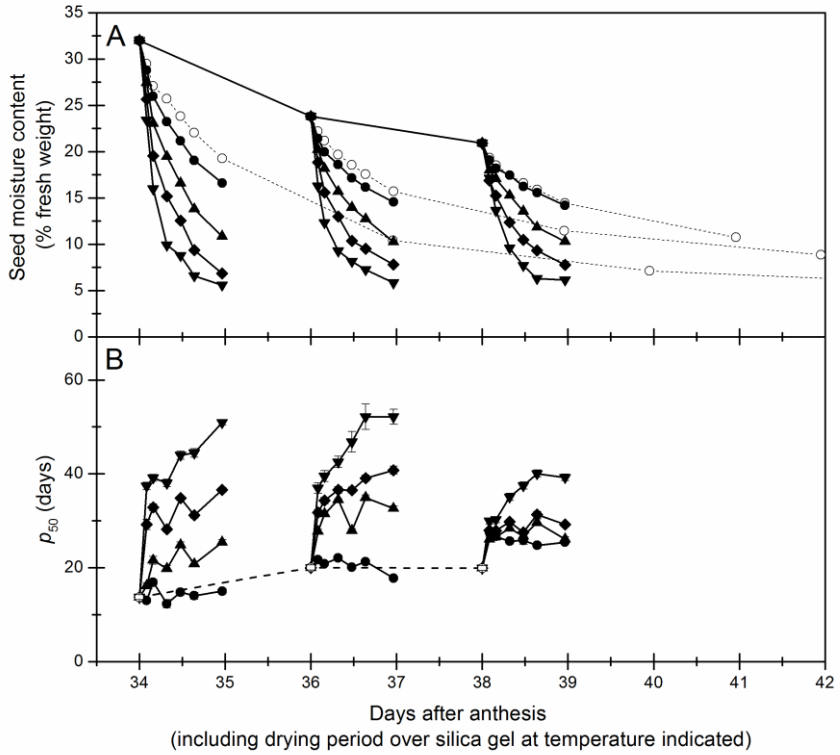
A
B
Seed moisture content
(% fresh weight)
p_{50} (days)
Days after anthesis
(including drying period over silica gel at temperature indicated)

Figure 3

Table S1. The results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for seeds of rice accession IRGC 117265 harvested after 25, 35 and 45 days after 50% anthesis (DAA) which were dried either immediately after harvest in the drying room (DR; 15°C /15% RH) or were subjected to 3 d of continuous (Con) or intermittent (In) drying at 15, 30, 45 or 60°C and 30% RH (maintained by a saturated $MgCl_2$ solution) prior to final drying in the drying room. For those seed lots which showed a complete loss in dormancy the viability model (Ellis and Roberts, 1980) was applied, and for seeds which showed a reduced initial viability an additional parameter* was applied to determine the proportion of responding seeds within the population (Mead and Gray, 1999). The parameters shown are for the best-fit model.

			Harvest A					Harvest B				
Maturity (DAA)	Treatment	Duration	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (s.e.) (days)	K_d (s.e.) (NED)	β_1 (s.e.) (days)	K_i (s.e.) (NED)	σ^{-1} (s.e.) (days^{-1})	p_{50} (s.e.) (days)
25 DAA	DR		0.43 (0.17)	0.12 (0.03)	3.64 (0.36)	0.14 (0.01)	25.2 (0.64)	0.08 (0.16)	0.20 (0.04)	3.35 (0.24)	0.10 (0.01)	34.6 (0.93)
	15°C /30% RH	In	0.58 (0.17)	0.13 (0.03)	4.80 (0.31)	0.13 (0.01)	35.7 (0.57)	0.38 (0.16)	0.17 (0.03)	4.30 (0.25)	0.09 (0.01)	48.3 (0.72)
		Con	0.26 (0.16)	0.22 (0.04)	3.69 (0.27)	0.12 (0.01)	31.1 (0.60)	0.09 (0.21)	0.21 (0.05)	3.86 (0.32)	0.09 (0.02)	45.3 (1.18)
	30°C /30% RH	In	0.44 (0.18)	0.16 (0.03)	4.54 (0.40)	0.09 (0.01)	52.1 (1.20)	0.03 (0.23)	0.17 (0.04)	4.58 (048)	0.08 (0.01)	58.9 (1.55)
		Con	0.79 (0.18)	0.09 (0.02)	6.59 (0.63)	0.11 (0.01)	59.7 (0.98)	0.71 (0.19)	0.13 (0.03)	4.58 (0.31)	0.08 (0.01)	60.9 (1.11)
	45°C /30% RH	In	0.35 (0.25)	0.19 (0.05)	5.19 (0.63)	0.08 (0.01)	67.2 (1.77)	1.23 (0.18)	0.05 (0.01)	5.47 (0.38)	0.09 (0.01)	63.8 (0.89)
		Con	0.59 (0.26)	0.19 (0.06)	5.06 (0.61)	0.07 (0.01)	73.5 (1.94)	0.29 (0.24)	0.18 (0.05)	4.01 (0.37)	0.05 (0.01)	76.0 (2.10)
	60°C /30% RH	In		*	2.77 (0.24)	0.06 (0.00)	50.5 (1.17)		*	5.50 (0.34)	0.08 (0.00)	66.5 (0.69)
		Con		*	3.24 (0.43)	0.07 (0.01)	46.5 (1.23)		*	3.80 (0.25)	0.06 (0.00)	62.3 (0.88)
35 DAA	DR		0.23 (0.18)	0.18 (0.04)	4.12 (0.32)	0.11 (0.01)	36.4 (0.87)	0.98 (0.24)	0.18 (0.07)	4.53 (0.38)	0.07 (0.10)	60.7 (1.21)
	15°C /30% RH	In	0.14 (0.17)	0.48 (0.09)	4.20 (0.26)	0.09 (0.01)	48.4 (0.84)	1.13 (0.26)	0.14 (0.05)	4.94 (0.43)	0.07 (0.01)	72.6 (1.00)
		Con	0.33 (0.19)	0.28 (0.05)	3.58 (0.25)	0.08 (0.01)	42.4 (0.91)	1.42 (0.15)	0.07 (0.02)	5.50 (0.30)	0.08 (0.00)	70.7 (0.82)
	30°C /30% RH	In	0.27 (0.17)	0.20 (0.04)	4.69 (0.35)	0.08 (0.01)	58.9 (1.28)	1.80 (0.20)	0.03 (0.02)	4.77 (0.38)	0.06 (0.00)	80.2 (1.30)
		Con	0.62 (0.19)	0.08 (0.02)	5.90 (0.71)	0.40 (0.01)	59.0 (1.38)	1.58 (0.20)	0.05 (0.02)	4.63 (0.35)	0.06 (0.00)	79.0 (1.24)
	45°C /30% RH	In	0.08 (0.18)	0.22 (0.04)	4.31 (0.30)	0.07 (0.02)	62.6 (1.30)	0.98 (0.31)	0.26 (0.13)	4.04 (0.40)	0.05 (0.01)	80.7 (2.30)
		Con	0.31 (0.27)	0.27 (0.09)	4.24 (0.42)	0.07 (0.02)	63.2 (1.80)	2.25 (0.19)	0.00 (0.01)	4.80 (0.66)	0.05 (0.01)	100.8 (2.68)
	60°C /30% RH	In		*	5.63 (0.34)	0.11 (0.01)	53.5 (0.58)		*	4.64 (0.28)	0.06 (0.00)	80.0 (0.87)
		Con	0.83 (0.40)	0.38 (0.31)	2.80 (0.40)	0.06 (0.01)	48.3 (2.81)	1.72 (0.21)	0.03 (0.02)	3.38 (0.25)	0.05 (0.00)	73.6 (1.44)

	DR		1.22 (0.17)	0.08 (0.02)	4.60 (0.32)	0.11 (0.01)	43.5 (0.74)	0.34 (0.15)	0.13 (0.03)	4.31 (0.28)	0.08 (0.01)	54.6 (1.11)
	15°C /30% RH	In	0.67 (0.13)	0.22 (0.04)	0.40 (0.19)	0.08 (0.00)	50.1 (0.77)	1.18 (0.26)	0.08 (0.03)	4.24 (0.43)	0.06 (0.01)	68.3 (1.67)
		Con	0.34 (0.20)	0.25 (0.06)	4.05 (0.34)	0.08 (0.01)	50.0 (1.33)	1.06 (0.20)	0.26 (0.09)	4.13 (0.24)	0.06 (0.00)	68.0 (1.06)
	30°C /30% RH	In	0.25 (0.22)	0.25 (0.06)	4.29 (0.38)	0.08 (0.01)	56.6 (1.44)	1.21 (0.22)	0.05 (0.02)	4.86 (0.53)	0.07 (0.01)	66.2 (1.42)
45 DAA		Con	0.17 (0.35)	0.28 (0.10)	3.87 (0.57)	0.07 (0.01)	57.4 (2.60)	1.14 (0.32)	0.19 (0.11)	3.72 (0.35)	0.05 (0.01)	68.1 (1.82)
	45°C /30% RH	In	0.10 (0.21)	0.40 (0.09)	4.32 (0.33)	0.07 (0.01)	59.8 (1.23)	1.66 (0.16)	0.02 (0.01)	4.82 (0.31)	0.07 (0.00)	70.4 (0.88)
		Con	0.53 (0.19)	0.19 (0.05)	3.80 (0.29)	0.05 (0.00)	69.4 (0.60)	0.77 (0.21)	0.30 (0.09)	3.89 (0.26)	0.05 (0.00)	79.3 (1.46)
	60°C /30% RH	In	1.31 (0.21)	0.10 (0.04)	4.10 (0.30)	0.08 (0.01)	53.0 (1.10)	1.80 (0.24)	0.03 (0.02)	4.66 (0.35)	0.06 (0.00)	73.7 (1.14)
		Con		*	2.84 (0.19)	0.06 (0.00)	46.4 (0.88)		*	3.79 (0.21)	0.05 (0.00)	73.8 (0.87)

*Immunity values were 0.09 (0.02), 0.24 (0.02), 0.03 (0.01) and 0.06 (0.01) for seeds from harvest A dried at 60°C/30%RH In (25DAA), Con (25DAA), In (35DAA) and Con (35DAA), respectively. Immunity values were 0.05 (0.01), 0.09 (0.01), 0.01 (0.00) and 0.04 (0.01) for seeds from harvest B dried at 60°C/30%RH In (25DAA), Con (25DAA), In (35DAA) and Con (45DAA), respectively.

Table S2. The results of fitting the combined loss in dormancy and loss in viability model (Kebreab and Murdoch, 1999) for seeds of rice cv. 'Macassane' harvested after 34, 36 and 38 days after 50% anthesis (DAA) which were dried either immediately after harvest in the drying room (DR; 15°C /15% RH) or were subjected to between 2 and 24 h of drying over silica gel at 15, 30, 45 or 60°C prior to final drying in the drying room. For those seed lots which showed a complete loss in dormancy the viability model (Ellis and Roberts, 1980) was applied, and for seeds which showed a reduced initial viability an additional parameter* was applied to determine the proportion of responding seeds within the population (Mead and Gray, 1999). The parameters shown are for the best-fit model.

Maturity	Drying temperature	Drying duration	K_d (s.e.)	β_1 (s.e.)	K_i (s.e.)	σ^{-1} (s.e.)	p_{50}
(DAA)	(°C)	(h)	(NED)	(days)	(NED)	(days^{-1})	(days)
34 DAA	DR		0.11 (0.19)	0.37 (0.10)	3.50 (0.41)	0.26 (0.03)	13.7 (0.40)
	15°C	2	0.60 (0.45)	0.16 (0.24)	2.88 (0.98)	0.22 (0.07)	13.0 (0.53)
		4	0.57 (0.40)	0.11 (0.20)	2.69 (0.86)	0.16 (0.06)	16.9 (0.62)
		8	0.21 (0.74)	0.35 (0.44)	2.18 (0.15)	0.18 (0.10)	12.3 (0.87)
		12	0.30 (0.57)	0.18 (0.29)	2.99 (1.23)	0.20 (0.08)	14.8 (0.67)
		16	0.29 (0.55)	0.10 (0.27)	4.37 (1.65)	0.31 (0.11)	14.0 (0.72)
		24	0.59 (0.42)	0.07 (0.21)	4.09 (1.21)	0.27 (0.08)	15.0 (0.63)
	30°C	2	0.47 (0.43)	0.20 (0.15)	2.11 (1.57)	0.13 (0.10)	16.1 (0.66)
		4	0.47 (0.41)	0.08 (0.10)	2.81 (1.63)	0.13 (0.10)	21.6 (0.86)
		8	0.66 (0.39)	0.07 (0.08)	3.40 (1.07)	0.17 (0.05)	19.7 (0.66)
		12	0.49 (0.40)	0.08 (0.08)	3.76 (0.89)	0.15 (0.03)	24.8 (0.61)
		16	0.61 (0.16)	0.08 (0.03)	3.92 (0.45)	0.19 (0.02)	20.7 (0.55)
		24	0.59 (0.17)	0.10 (0.03)	3.54 (0.35)	0.14 (0.01)	25.4 (0.58)
	45°C	2	0.68 (0.38)	0.03 (0.04)	3.78 (0.96)	0.13 (0.03)	29.2 (1.10)
		4	0.64 (0.32)	0.04 (0.04)	4.40 (0.92)	0.13 (0.02)	32.9 (0.71)
		8	0.03 (0.33)	0.19 (0.05)	2.97 (0.72)	0.11 (0.02)	28.2 (0.55)
		12	0.58 (0.32)	0.07 (0.04)	4.65 (0.81)	0.13 (0.02)	34.8 (0.49)
		16	0.33 (0.32)	0.10 (0.04)	3.13 (0.75)	0.10 (0.02)	31.1 (0.60)
		24	0.70 (0.13)	0.06 (0.01)	4.65 (0.33)	0.13 (0.01)	36.6 (0.51)

		2	1.27 (0.38)	0.01 (0.04)	5.65 (1.38)	0.15 (0.03)	37.4 (0.75)
		4	0.90 (0.37)	0.04 (0.04)	4.67 (1.25)	0.12 (0.03)	39.1 (0.67)
		8	0.65 (0.37)	0.04 (0.04)	4.20 (1.22)	0.11 (0.03)	38.0 (0.74)
	60°C	12	1.01 (0.16)	0.03 (0.02)	4.17 (0.53)	0.09 (0.01)	43.9 (0.85)
		16	1.10 (0.16)	0.03 (0.02)	3.75 (0.49)	0.08 (0.01)	44.4 (0.88)
		24		*	5.29 (0.42)	0.10 (0.01)	50.9 (0.56)
	DR		0.75 (0.16)	0.09 (0.04)	3.27 (0.32)	0.16 (0.01)	20.0 (0.54)
	15°C	2	0.51 (0.38)	0.18 (0.10)	3.62 (0.74)	0.17 (0.03)	21.7 (0.41)
		4	0.87 (0.40)	0.16 (0.11)	3.49 (0.74)	0.17 (0.03)	20.8 (0.41)
		8	0.63 (0.39)	0.21 (0.13)	2.79 (0.70)	0.13 (0.03)	22.0 (0.48)
		12	1.05 (0.40)	0.08 (0.09)	4.26 (0.82)	0.21 (0.04)	20.1 (0.41)
		16	0.64 (0.39)	0.15 (0.10)	3.92 (0.76)	0.18 (0.03)	21.3 (0.40)
		24	0.94 (0.49)	1.12(***)	3.30 (0.88)	0.19 (0.04)	17.8 (0.38)
	30°C	2	1.03 (0.43)	0.10 (0.07)	3.93 (0.63)	0.14 (0.02)	27.7 (0.46)
		4		*	5.04 (0.31)	0.16 (0.01)	31.5 (0.36)
		8	1.33 (0.40)	0.01 (0.05)	26.68 (3.80)	0.78 (0.11)	34.4 (0.23)
		12	0.76 (0.42)	0.09 (0.06)	5.38 (0.74)	0.19 (0.02)	27.9 (0.40)
		16	0.90 (0.42)	0.07 (0.06)	4.14 (0.67)	0.12 (0.02)	34.9 (0.57)
36 DAA		24	0.82 (0.17)	0.09 (0.03)	3.81 (0.26)	0.12 (0.01)	32.6 (0.52)
	45°C	2	0.72 (0.50)	0.21 (0.09)	3.86 (2.01)	0.12 (0.05)	31.7 (0.69)
		4	1.20 (0.50)	0.02 (0.03)	6.01 (2.01)	0.17 (0.05)	34.9 (0.67)
		8	1.25 (0.49)	0.02 (0.03)	6.31 (2.21)	0.17 (0.05)	36.6 (0.69)
		12	0.99 (0.49)	0.06 (0.04)	5.20 (2.08)	0.14 (0.05)	36.5 (0.61)
		16	1.20 (0.49)	0.03 (0.03)	5.61 (2.13)	0.14 (0.05)	39.1 (0.64)
		24	1.30 (0.20)	0.01 (0.01)	6.53 (0.98)	0.16 (0.02)	40.8 (0.81)
	60°C	2	0.49 (0.66)	0.07 (0.05)	5.47 (4.82)	0.15 (0.10)	37.0 (1.05)
		4	0.73 (0.69)	0.08 (0.06)	4.71 (4.77)	0.12 (0.09)	39.4 (1.23)
		8	0.53 (0.65)	0.07 (0.04)	5.54 (4.85)	0.13 (0.10)	42.5 (1.22)
		12	0.57 (0.67)	0.08 (0.05)	3.91 (4.76)	0.08 (0.09)	46.8 (2.16)
		16	0.60 (0.63)	0.04 (0.04)	5.37 (5.10)	0.10 (0.10)	52.2 (2.65)

		24	1.14 (0.27)	0.01 (0.01)	7.56 (2.32)	0.15 (0.04)	52.1 (1.62)
38 DAA	DR		0.64 (0.23)	0.19 (0.08)	3.44 (0.37)	0.17 (0.02)	19.9 (0.58)
	15°C	2	0.83 (0.55)	0.15 (0.17)	3.95 (0.89)	0.14 (0.04)	25.5 (0.63)
		4	1.00 (0.52)	0.01 (0.15)	6.63 (1.63)	0.25 (0.06)	25.7 (0.74)
		8	0.97 (0.55)	0.08 (0.11)	4.38 (1.01)	0.17 (0.04)	25.7 (0.62)
		12	1.05 (0.54)	0.03 (0.16)	4.52 (1.17)	0.18 (0.05)	25.8 (0.84)
		16	0.52 (0.54)	0.17 (0.17)	4.21 (0.96)	0.17 (0.04)	24.8 (0.58)
		24	1.19 (0.57)	0.02 (0.16)	5.31 (1.42)	0.21 (0.06)	24.9 (0.81)
	30°C	2	0.92 (0.48)	0.06 (0.08)	4.78 (1.41)	0.18 (0.05)	26.1 (0.60)
		4	1.16 (0.49)	0.02 (0.07)	5.83 (1.71)	0.22 (0.06)	26.5 (0.72)
		8	1.14 (0.48)	0.04 (0.07)	6.16 (1.54)	0.22 (0.05)	28.4 (0.57)
		12	1.38 (0.49)	0.00 (0.07)	7.47 (1.87)	0.28 (0.07)	26.7 (0.62)
		16	1.48 (0.51)	0.01 (0.08)	5.77 (1.54)	0.19 (0.05)	29.6 (0.72)
		24	0.88 (0.20)	0.09 (0.03)	4.94 (0.55)	0.19 (0.02)	26.1 (0.54)
	45°C	2	1.30 (0.47)	0.03 (0.04)	5.74 (2.28)	0.21 (0.07)	28.0 (0.55)
		4	1.00 (0.45)	0.05 (0.05)	4.86 (2.17)	0.17 (0.07)	27.9 (0.57)
		8	1.14 (0.45)	0.04 (0.04)	6.19 (2.27)	0.21 (0.07)	29.7 (0.51)
		12	1.39 (0.47)	0.02 (0.05)	5.70 (2.28)	0.21 (0.07)	27.5 (0.59)
		16	1.38 (0.19)	0.01 (0.02)	6.83 (1.02)	0.22 (0.03)	31.3 (0.65)
		24	1.12 (0.22)	0.07 (0.03)	4.74 (0.49)	0.16 (0.02)	29.2 (0.49)
	60°C	2	0.91 (0.56)	0.12 (0.07)	4.41 (1.89)	0.15 (0.05)	29.9 (0.66)
		4	1.19 (0.55)	0.03 (0.05)	5.74 (2.11)	0.19 (0.06)	30.2 (0.67)
		8	1.36 (0.54)	0.01 (0.04)	7.36 (2.33)	0.21 (0.06)	35.1 (0.75)
		12	1.48 (0.55)	0.01 (0.04)	6.37 (2.15)	0.17 (0.05)	37.2 (0.78)
		16	1.14 (0.52)	0.01 (0.04)	8.40 (2.52)	0.21 (0.06)	40.3 (0.77)
		24	1.51 (0.23)	0.02 (0.02)	6.84 (0.89)	0.17 (0.02)	39.3 (0.69)

*Immunity values were 0.08 (0.01) for seeds harvested after 34 DAA and dried at 60°C for 24 h and 0.05 (0.01) for seeds harvested after 36 DAA and dried at 30°C for 4 h.