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Desiccation tolerance of intermediate pomelo (*Citrus maxima* 'Mansailong') seeds following rapid and slow drying

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Abstract

We investigated the effects of rapid and slow drying regimes on desiccation tolerance (DT) of developing pomelo seeds. Slow-dried seeds harvested at 130, 180, 210 or 235 days after anthesis (DAA) had greater DT than rapid-dried seeds ($P < 0.05$); However, when seeds were collected at 90 or 150 DAA, there was no significant difference in DT between the two drying treatments. Furthermore, the DT improvement from slow drying gradually decreased as seeds matured during the period from 180 to 235 DAA. It was concluded that the DT of intermediate *C. maxima* 'Mansailong' seeds following slow drying was higher than following rapid drying, but it depended on the seed developmental stage.

Keywords: *Citrus maxima* 'Mansailong', desiccation tolerance, rapid drying, seed development, seed moisture loss rate, slow drying

Introduction

Storing seeds is an important and effective way to preserve the genetic diversity of spermatophyte species. Based on the ability to survive desiccation and low temperature, seeds are generally divided into three categories: orthodox, intermediate and recalcitrant (Roberts, 1973; Ellis *et al.*, 1990). About 75-80%, 10-15% and 5-10% of the world's angiosperm species produce orthodox, intermediate and recalcitrant seeds, respectively (Dickie and Pritchard, 2002; Tweddle *et al.*, 2003; Berjak and Pammenter, 2008). During development, seeds gradually acquire desiccation tolerance (DT). Mature orthodox seeds are sufficiently DT to survive desiccation and prolonged preservation in conventional seedbanks (Wen *and* Song, 2007a; Li and Pritchard, 2009). However, recalcitrant seeds are always intolerant of desiccation (Pammenter and Berjak, 1999; Wen and Song, 2007b; Walters *et al*., 2013). Although mature intermediate seeds acquire partial DT, they lose viability relatively quickly during storage (Hong and Ellis, 1996; Malik *et al*., 2012; Zhang *et al*., 2014).

Drying rate is an important factor influencing the DT of seeds. However, the effect of drying rates on DT differs in orthodox and recalcitrant seeds. For immature orthodox seeds, slow-dried seeds had greater DT than rapid-dried ones (Blackman *et al*., 1992; Huang *et al*., 2009; Samarah *et al*., 2010). In contrast, recalcitrant seeds can be desiccated to lower moisture contents following rapid drying than following slow drying (Berjak *et al*., 1993; Pammenter *et al*., 1998; Wesley-Smith *et al*., 2001). Orthodox seeds accumulate abundant protective molecules during the later stages of development, which may contribute to the avoidance of desiccation injury (Wen *et al*., 2009; Chatelain *et al*., 2012; Delahaie *et al*., 2013). For immature orthodox seeds, it is possible that slow drying induces the expression and synthesis of protective substances associated with DT. On the contrary, recalcitrant seeds do not synthesise or rarely accumulate these substances (Berjak and Pammenter, 2008; Wen, 2011). The generally-accepted theory is that during rapid-drying, the recalcitrant seeds quickly transit through intermediate moisture contents, which can lead to less production of reactive oxygen species (ROS; Bailly, 2004; Berjak and Pammenter, 2008, 2013).

The effect of drying rate on the DT of intermediate seeds has rarely been studied (Magistrali *et al*., 2015). In this study, the effects of drying rate on the DT of pomelo seeds (intermediate storage category; Wen *et al*., 2010; Yan *et al*., 2014) at different developmental stages (DDS) were investigated.

Materials and methods

Seed materials

Seeds of pomelo cultivar (*Citrus maxima* 'Mansailong') used in this study were collected from Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Sciences (21° 41'N, 101° 25'E). They were extracted manually from newly-harvested fruits, with seed coat removed, surface-dried and stored for one day in polyethylene bags at 15°C before use. Days after anthesis were countered from the end of February, 2014, when the trees bloomed massively.

Desiccation method

Rapid drying was performed by placing fresh seeds in silica gel (15°C, 5% RH). For slow drying, seeds were dried in closed plastic boxes over a saturated solution of NaCl (15°C, 76% RH). Seeds were sampled at regular intervals for moisture content determination and germination testing. Seeds from 150 to 235 days after anthesis (DAA) were difficult to desiccate to low MC in the slow drying conditions. Therefore, when the MC of seeds harvested at 150 and 180 DAA was about 10%, they were transferred to a drying room (15°C, 50% RH) for further drying. For the seeds collected at 210 and 235 DAA and placed in the slow drying treatment, rapid drying conditions were applied when the MC reached about 20%.

Moisture content determination
According to ISTA (2004), seed moisture contents (MC; % fresh weight basis) were measured by determining the weight of eight individual seeds before and after oven-drying at 103 ± 2°C for 17 hours.

Seed moisture loss rate index
The seed moisture loss rate index (SMLR) was calculated to measure the dehydration rate according to the method of Samarah *et al*. (2009). The formula is as follows:

$$SMLR = \sum_{i=1}^{n} \frac{(SMC_i - SMC_{i+1})}{day_{i+1}}$$

n: number of times of seeds taken to measure the MC of the seeds.
SMC_i: Seed moisture content at the number i of days under drying.
day_{i+1}: number of days during dehydration.

In this study, the drying periods were measured in hours, which were then converted into days.

Seed germination assessment
To prevent imbibition injury, seeds were rehydrated at 25°C and saturated moist air for 24 hours upon removal from the slow or fast drying conditions. This was accomplished by placing seeds in a monolayer in an open Petri dish which was closed in another larger Petri dish with de-ionised water. Seeds were then sown on 1.0% plain agar at 25°C with a 14 hours day^{-1} photoperiod (20 µmol m^{-2} second^{-1}). Germination tests used five replicates of 20 seeds each, with those having intact roots and shoots were defined as emergence.

Statistical analysis
For the fresh weight, dry weight and moisture content, the analyses of significant difference was performed using analysis of variance (ANOVA) of SPSS 19.0 version. The critical moisture content quantifying DT was defined as the MC corresponding to 15% mortality rate meaning that the germination after drying was 85%, which was assessed by probit analysis in SPSS.

Results

Changes in essential characteristic during development
During seed development, there were significant changes in pericarp colour of the fruits, and seed fresh weight (FW), dry weight (DW) and MC (table 1). FW and DW quickly increased from 90 to 150 DAA and from 90 to 130 DAA, respectively. After 150 DAA, as the pericarp colour of the fruits changed from green to yellow green, the DW became stable, whereas, the FW gradually decreased. The decrease in MC was continuous before 180 DAA when it stabilised at approximately 40%.

Table 1. Changes in fruit pericarp color of fruits, seed fresh weight (FW), dry weight (DW) and moisture content (MC) of *Citrus maxima* 'Mansailong' at different stages of development.

Days after anthesis	Fruit pericarp colour	FW (g)	DW (g)	MC (%)
90	Green	0.149 ± 0.011a	0.039 ± 0.004a	73.97 ± 0.91a
130	Green	0.217 ± 0.007b	0.090 ± 0.004b	58.72 ± 0.73b
150	Green	0.230 ± 0.012b	0.119 ± 0.007c	48.56 ± 0.93c
180	Yellow-green	0.180 ± 0.012c	0.104 ± 0.009c	42.83 ± 1.98d
210	Yellow-green	0.180 ± 0.014c	0.109 ± 0.008c	39.63 ± 0.62d
235	Yellow	0.181 ± 0.019c	0.106 ± 0.010c	41.03 ± 0.89d

Note: Values are the means ± SE of eight individual seed replicates. Values labelled by the same letters within a column indicate no significant difference at $P = 0.05$.

Effects of rapid and slow drying on seed moisture loss rate index (SMLR)
Both drying conditions and stage of seed development influenced the SMLR (figure 1). For seeds from the same developmental stages, those rapidly dried had a higher SMLR than slowly dried. When the same drying method was used, the index of seeds from different developmental stages was also significantly different. The overall trend showed that the index gradually decreased during development. In this experiment, the index of seeds from 90 to 235 DAA during exposure to rapid drying and slow drying condition decreased from 36 to 11 and 10 to 4, respectively (table 2).

Effects of seed moisture loss rate on desiccation tolerance
Desiccation tolerance was assessed by the percentage of seed emergence after drying and critical moisture content (CMC). Irrespective of developmental stage, the DT of slow-dried seeds was either significantly higher or not significantly lower than the DT of rapid-dried seeds (figure 2). For seeds collected at 130, 180, 210 or 235 DAA, slow-dried seeds had greater DT compared with rapid-dried seeds. Meanwhile, the CMC of slow-dried seeds was lower than that of rapid-dried seeds at these developmental stages. For example, it was 14 and 6% for rapid-dried and slow-dried seeds from 180 DAA, respectively (table 2). However, there was no difference when seeds were harvested at 90 DAA and 150 DAA. Furthermore, for the seeds harvested between 180 and 235 DAA, the difference reduced as the seeds matured.

Discussion

In a continuum of seed storage behaviour, recalcitrant and orthodox seeds are at two extremes, and intermediate seeds are in-between these two seed categories (Ellis *et al*., 1991; Berjak and Pammenter, 1994, 2001). In this research, the physiological maturity of *C. maxima* 'Mansailong' seeds occurred at 180 DAA. At this stage, the MC was still high (table 1), indicating that there was no obvious maturation drying during the development

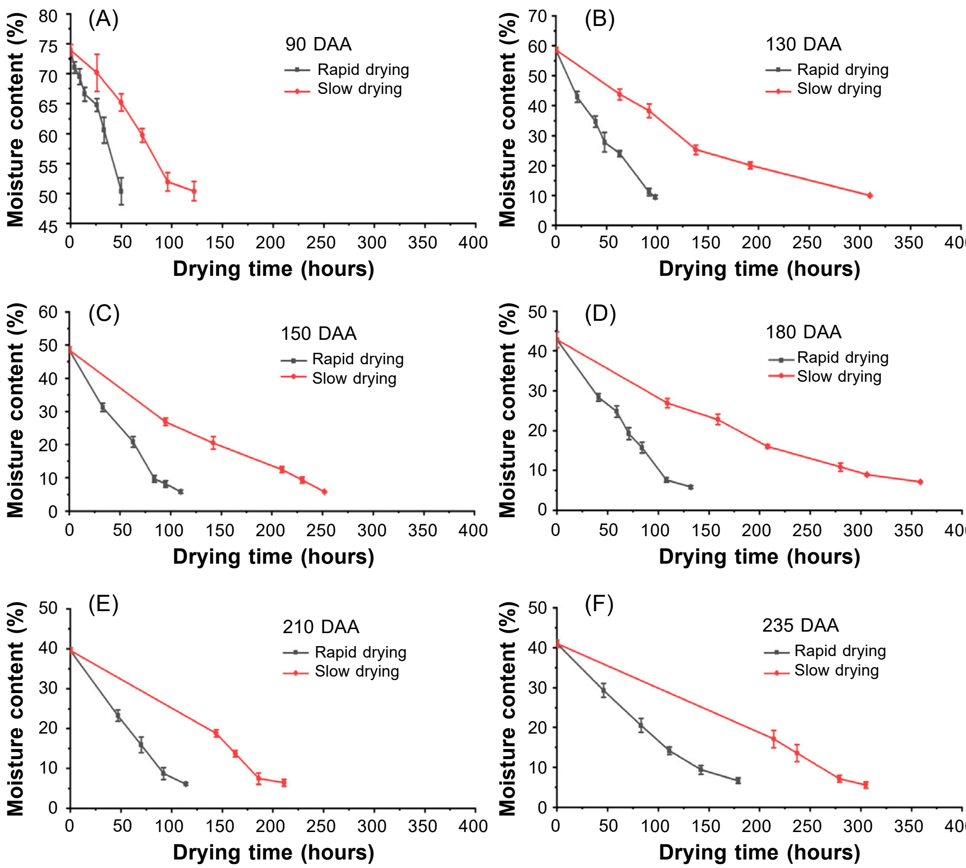

Figure 1. Changes in moisture contents of *Citrus maxima* 'Mansailong' seeds from different developmental stages (days after anthesis; DAA) during rapid and slow drying.

Table 2. Effects of rapid and slow drying on seed moisture loss rate index (SMLR) and the critical moisture content (CMC) of *Citrus maxima* 'Mansailong' seeds from different stages of development.

Days after anthesis	Rapid drying		Slow drying	
	SMLR	CMC (% fresh weight)	SMLR	CMC (% fresh weight)
90	36	69	10	67
130	32	31	11	23
150	21	20	8	19
180	15	14	6	6
210	13	11	5	6
235	11	10	4	5

Note: Based on the probit analysis, the CMC was identified as the MC corresponding to 15% mortality of the seeds. The variable of CMC quantified DT.

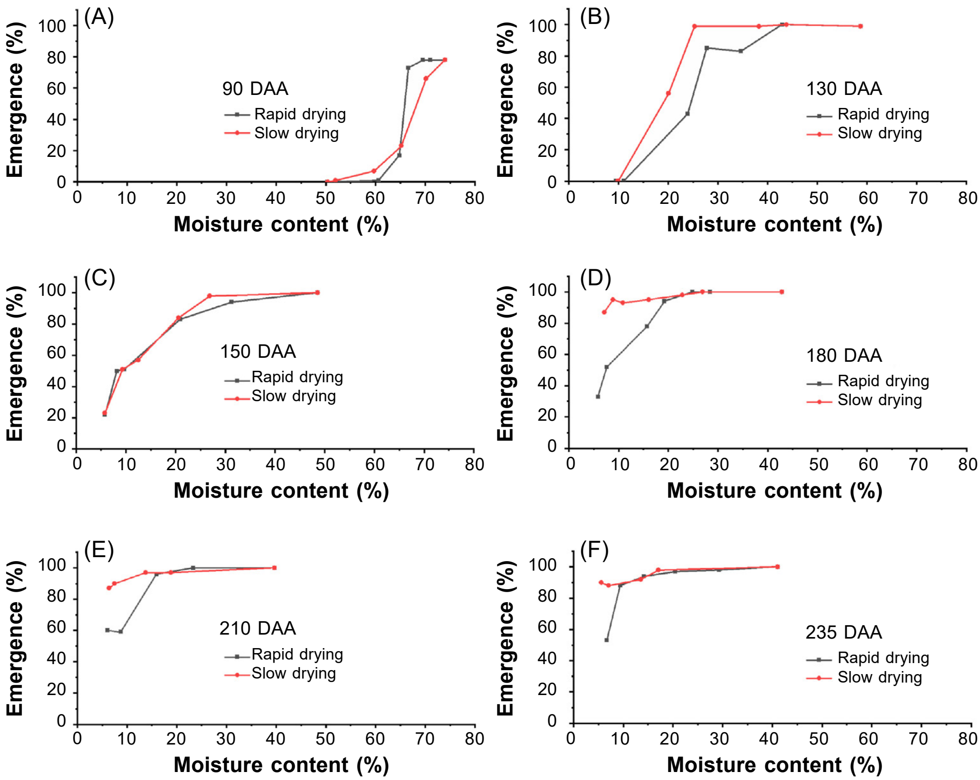

Figure 2. Changes in emergence of *Citrus maxima* 'Mansailong' seeds from different developmental stages (days after anthesis; DAA) following rapid and slow drying to different moisture contents (% fresh weight).

of *C. maxima* 'Mansailong' seeds, which is similar to the findings for recalcitrant seeds (Pammenter and Berjak, 1999; Wen and Song, 2007b). In addition, earlier reports showed that the response of immature orthodox seeds to slow and rapid drying was contrary to mature recalcitrant seeds (Blackman *et al*., 1992; Berjak *et al*., 1993; Pammenter *et al*., 1998; Wesley-Smith *et al*., 2001; Huang *et al*., 2009; Samarah *et al*., 2010). Like orthodox seeds, intermediate *C. maxima* 'Mansailong' seeds harvested at 130, 180, 210 and 235 DAA had higher DT after slow drying than after rapid drying (figure 2). The difference between slow-dried and rapid-dried *C. maxima* 'Mansailong' seeds probably resulted from desiccation-related proteins expression (Fu *et al*., 1997; Pammenter and Berjak, 1999; Berjak and Pammenter, 2004; Berjak, 2006; Dussert *et al*., 2018). On the other hand, even if there was no induction, according to the hypothesis of water replacement (Crowe, 2007), it was possible that original proteins and non-reducing sugars of *C. maxima* 'Mansailong' seeds had more time to stabilise the membranes and proteins during slow drying than rapid drying, resulting in less desiccation damage.

The effects of drying rate on DT not only depend on the seed storage category, but also on the seed developmental stage. In this research, for seeds collected at 90 and 150

DAA, no significant difference in DT was observed between slow- and rapid dried seeds (figure 2A, C). Moreover, from 180 to 235 DAA, the difference in DT between slow-dried and rapid-dried seeds reduced gradually as seeds matured (figure 2). This may be because that slow-drying can not induce the accumulation of protective compounds during very early development or that this accumulation is almost finished during late development (Kermode, 1973; Bradford and Chandler, 1992; Samarah *et al*., 2009; Wen *et al*., 2009; Wen, 2011). So, improvement of DT in response to slow drying only occurs under special conditions.

In summary, our results showed that in intermediate *C. maxima* 'Mansailong' seeds, DT following slow drying was better than DT following rapid drying. We recommend harvesting *C. maxima* 'Mansailong' seeds after 235 DAA and that they should be dried slowly to low MC before the preservation.

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