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Post desiccation germination of mature seeds of tea (*Camellia sinensis* L.) can be enhanced by pro-oxidant treatment, but partial desiccation tolerance does not ensure survival at −20 °C

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ABSTRACT

The maximal potential desiccation tolerance (MPDT) of tea (*Camellia sinensis*) seeds has been a matter of debate for decades. Here we assessed the ability of tea seeds from three sites in China to germinate after desiccation. Desiccation tolerance was greatest in Kunming, followed by Puer and Lincang, with Kunming seeds tolerating drying to 8% moisture content (MC), or ~0.5 water activity (a_w). Such tolerance was observed in Lincang seeds only when hydrogen peroxide (H_2O_2) at 0.5 or 1 M was applied to seeds, indicating a stimulatory role for H_2O_2 in post-desiccation germination. Puer seeds exhibited MPDT of 16% MC (~0.7 a_w). Therefore, seeds from all three sites were not recalcitrant. The length of the dry season after dispersal and the high ratio of seed coat to seed mass (>0.3) support the observation of non-recalcitrant behaviour. The seeds were not immature, as the lipid signal in embryonic axes mirrored that of the cotyledons (30% oil). Even after high survival [>60% total germination (TG)] on drying to 10–13% MC, no Kunming seeds tolerated 1 month storage at −20 °C coinciding with lipid transitional changes at this temperature. The results indicate that tea seeds from China are neither recalcitrant nor storable at −20 °C.

1. Introduction

Camellia is the largest and economically most important genus in the family Theaceae, with >200 species and a centre of diversity in China, which possesses 80% of the species [1]. *Camellia sinensis* (L.) O. Kuntze (Theaceae), tea, is the most widely consumed beverage plant in the world with commercial production in over 40 countries yielding ~3.6 million tonnes of tea leaves annually. The leaves are rich in chemicals of importance for the human diet, including amino acids, caffeine, catechins, flavonoids, polysaccharides and vitamins (C, E, K) [2]. China was one of the earliest countries to widely produce and use tea, possibly as early as 2750 B.C. in Southwest China [2]. The Yunnan Province has been producing tea for 1700 years [3] and plantations from the Ming Dynasty (1368–1644 A.D.) and the Qing Dynasty (1644–1912 A.D.) are still found today in the regions of Lincang and Puer. Similarly, *Camellia* has a long history of ornamental use with cultivation in China since 1300 years ago [1]. The third use of *Camellia* as a commodity is in the oil industry, especially *C. oleifera*. Several species produce edible seed oil, which is used extensively in China for cooking. In China, ~650,000 tonnes of seeds are harvested each year, yielding ~165,000 tonnes of oil [1,4].

C. sinensis has a native habitat, and is generally cultivated, at altitudes between 1000 and 1500 masl (metres above sea level), although some varieties extend down to ~200 and up to ~2000 masl. The main four varieties of tea are: *C. sinensis* var. *sinensis*, which is grown in vast areas south of the Yangtse River from Tibet in the west to Japan in the East; *C. sinensis* var. *assamica*, which is cultivated from Northeast India (Assam) to Southwest China, Vietnam and Thailand; *C. sinensis* var. *dehungensis* mainly occurs in Yunnan; and *C. sinensis* var. *pubilimba* is mainly found in South China [5]. Altitude is a key determinant of the species' autecology. Firstly, altitude affects fatty acid composition of seed oils, and thus thermal properties. A species growing at elevated altitudes is likely to have lower amounts of saturated fats in the seed which has been shown to impact on temperatures for germination, based on macro-evolutionary biogeographical evidence on a broad spectrum of plants, including *Helianthus* [6]. Secondly, as biotic stresses increase with elevation, e.g. UV light levels and temperature minima, the plant should be adapted to cope with numerous stresses,

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including seed traits that enable overwintering under relatively dry and cool conditions.

Because of the likely presence in wild species of traits desirable for plant breeding, it is important to conserve the genetic resources in the *Camellia* genus for future exploitation. Ten species are considered to be vulnerable and one species, *C. hengchunensis*, has been classified as endangered according to the International Union for Conservation of Nature in 2011 (http://www.iucnredlist.org). However, the seed storage biology is unknown for the majority of *Camellia* species. Of the only four species listed on the Seed Information Database (Release 7.1: http://data.kew.org/sid/), three are tentatively classified as non-orthodox, and information is generally meagre. However, for tea (*C. sinensis*) the assignment of seed storage biology has been the subject of dispute as the response to desiccation has varied with seed lot [7–11], although the reasons for such variability have not been resolved. Whilst some tea seed lots from India and South Africa have been shown to be highly desiccation sensitive, i.e. recalcitrant [8,9,11], seeds collected from their native habitat in China have displayed tolerance of drying to ~9% MC and subsequent submersion in liquid nitrogen [10]. This supports an earlier report that tea seeds can tolerate desiccation [7].

Sub-tropical and tropical oilseeds tend to be partially desiccation tolerant to varying degrees within sorption zone II of the water sorption isotherm (from ~20 to 75% RH), and have been referred to as Type II seeds, i.e. they do not fit the definition of either orthodox (Type I seed) or recalcitrant (Type III seeds) [12]. Many of these species are major world commodities, for example citrus [13,14], coffee [15], neem [16] and papaya [17]. Limited long-term storage potential at conventional seed bank temperature (−20 °C) appears relatively common in such seeds, possibly as a consequence of the thermal behaviour of the seed lipids [e.g. 18]. A significant feature of these seeds is that the low temperature sensitivity, whilst in the dry state, is species- (or seed lot-) specific, thereby compromising storage at conventional seed bank temperature (−20 °C). Tea seeds typically contain ~31–37% oil [19] and thus could show Type II behaviour when dried and stored at sub-zero temperatures. We explored this possibility by relating the storage response to the thermal behaviour, specifically the transition enthalpy of the seed lipids.

The debate about inter-species and inter-seasonal variation in seed storage responses for non-orthodox seeds has advanced recently with the realisation that environmental conditions during seed development can enable seeds to 'jump' seed storage classes. *Acer pseudoplatanus* from northern Europe was shown to display phenotypic recalcitrance whilst southern populations, from the species' native distribution range, exhibited much higher levels of desiccation tolerance [20,21]. Therefore, we assessed the desiccation responses of tea from its native distribution in China and from three sites with varying environmental conditions.

Poor survival of desiccation in seeds has been associated with oxidative damage caused by reactive oxygen species (ROS) [22–24]. Conversely, ROS also play essential roles in development [22]. For example, hydrogen peroxide (H_2O_2) treatment can promote germination, e.g. in *Zinnia elegans* [25]. Studies of the role of H_2O_2 during desiccation of recalcitrant seeds are rare, but there is some evidence that exogenous H_2O_2 may enhance germination in desiccation-stressed seeds of *Castanea sativa* [22].

The aim of this paper was to study the storage behaviour of tea seeds from three sites in China, to relate storage performance at −20 °C to the thermal properties of the lipids and to establish whether pro-oxidant treatment could alleviate desiccation stress. Overall, we wished to identify the MPDT of mature tea seeds.

2. Materials and methods

2.1. Seed material

Fruits of *C. sinensis* var. *sinensis* from Kunming and *C. sinensis* var. *assamica* from Lincang and Puer, China, were collected at the point of natural dispersal in the autumn of 2007 and 2010, and used within six weeks of harvest. The seed lots were given the notation K, L and P for the provenances and 1 and 2 for the respective collecting years (see Table 1 for seed lot details). Meteorological data were obtained from the Meteorological Bureau of the Yunnan Province, which provided monthly average temperature and rainfall data recorded from 1979 to 2008 for each location.

Fruits were collected from a minimum of 10 individual shrubs per location, subsequently pooled per provenance per year and air freighted to the UK. On arrival, pericarps were removed and the cleaned seeds with intact seed coats – botanically called the sclerotic outer mesotesta [26] – were stored in plastic bags at 5 °C for around 4 weeks as the experiments were being initiated. The bags were opened at least once a week to allow exchange of air. Only seeds with a healthy appearance (i.e. no apparent fungal or bacterial infection, discoloration and insect damage) were used in the experiments.

2.2. Desiccation and germination treatments

Seeds were dried over silica gel (5:1 to seed) in sealed plastic bags at 15 °C for up to 12 d. The silica gel was replenished every 24 h and the bags ventilated. At each sampling time, five replicates of 15 seeds were withdrawn, four of which were used for the germination test and the remaining 15 seeds used for moisture content (MC) determination of the seed coat, embryonic axes and cotyledons individually.

To reduce any risk of cross contamination during the germination tests, individual seeds were placed in 40-mm diameter plastic pots containing 20 mL 1% agar water. The test was run at 25 °C under warm white fluorescent light at an irradiance of 15 μmol m^{-2} s^{-1} on a day/night cycle of 8/16 h. Germination was defined as radical emergence by at least 5 mm and scored regularly. Total germination (TG) was monitored for 61 d from the onset of imbibition. As no further germination occurred in the preceding 7 d period, the TGs observed at 61 d were taken as final. The vast majority of the remaining ungerminated seeds were cut-tested and found to be soft and unviable. Seed germination was expressed as a percentage of the total number of seeds sown.

2.3. Water sorption experiments

Three individual seeds each from K2, L2 and P2 seed lots were used to determine the relationship between the tissue MC and relative humidity (RH), i.e. the sorption isotherm. Various concentrations of non-saturated lithium chloride (LiCl) solutions were prepared to yield equilibrium RHs between 15 and 100%. The solutions, in chambers, were maintained in a temperature-controlled room at 20 ± 2 °C. Seeds were cut into half to accelerate equilibration to the desired RH. The water activity (a_w) was calculated as RH/100. After equilibration, axes, cotyledons and seed coats were separated and the MCs determined following drying at 103 °C for 17 h [27] and expressed on a fresh mass basis.

2.4. Estimation of low temperature sensitivity

Six replicates of 20 seeds from each of the K2, L2 and P2 seed lots were dried over silica gel in sealed plastic bags at 15 °C, as specified above. After desiccation for 3 d, the MC decreased to 10–13%. Samples were then transferred to sealed plastic bags and stored at

Table 1
Details of the *C. sinensis* seed lots studied. Seed lots were harvested in November in 2007 or 2010. The seed coat ratio was calculated from the dry mass of the axes, cotyledons and coats, as the ratio of the coat against whole seed mass. Data represent mean ± SE for 9–15 individual seeds/tissues. Values in the same row with a different letter are significantly different ($P < 0.05$). ND – seed coat dry mass was not determined.

Taxon	Seed lot code	Harvest			Seed characteristics		
		Date	Location	Altitude (masl)	Embryonic axis dry mass (mg)	Cotyledon dry mass (g)	Seed coat ratio
C. sinensis var. *sinensis*	K1	2007	Kunming	1895	1.11 ± 0.1b	0.45 ± 0.05bc	0.32 ± 0.02b
C. sinensis var. *sinensis*	K2	2010	Kunming	1895	1.84 ± 0.81a	0.41 ± 0.06bc	0.31 ± 0.03b
C. sinensis var. *assamica*	L1	2007	Lincang	1280	2.1 ± 0.26a	0.53 ± 0.08bc	ND
C. sinensis var. *assamica*	L2	2010	Lincang	1280	1.69 ± 0.16ab	0.39 ± 0.04c	0.39 ± 0.04a
C. sinensis var. *assamica*	P1	2007	Puer	1200	1.14 ± 0.09b	0.57 ± 0.05b	0.31 ± 0.02b
C. sinensis var. *assamica*	P2	2010	Puer	1200	2.55 ± 0.4a	0.79 ± 0.11a	0.38 ± 0.03ab

−20 °C for 1 week (3 replicates) and 1 month (3 replicates) and subsequently germinated as specified above. Non-desiccated, unfrozen seeds were used as controls.

2.5. Oil content determination

Seed oil was extracted with supercritical fluid carbon dioxide using an ISCO SFX 3560 fat analyser (ISCO Inc., Lincoln, NE, USA) as described by Seal et al. [28]. For K2, L2, P2 seed lots, four replicates of individual seeds were cut into quarters and dried at 70 °C until constant mass. After separation of the seed coat, the embryos were ground with diatomaceous earth (1 part seed to 3 parts earth) in an IKA-WERKE A11 basic grinding mill (Staufen, Germany) to a particle size of ≤1 mm. Ground samples were analysed for oil content using a two step extraction at 6000 psi and 80 °C. Sunflower oil was used as a control with every sample preparation. Oil was collected on glass wool and dried to constant mass under vacuum at 70 °C for 1 h to remove residual carbon dioxide and water, and the percentage oil content (w/w) was calculated on a dry mass basis.

2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to assess the properties of oil in the cotyledons and embryonic axes from lots K2, L2 and P2. A DSC (Perkin-Elmer, UK) was used controlled by a Perkin Elmer TAC-7 (Pyris 7 software) and calibrated using indium, zinc and pure water as standards. The three replicates of dry tissue samples had average MCs of approximately 7% and average dry masses of about 8 mg and 37 mg for axes and cotyledons, respectively. Samples were pre-weighed, sealed in aluminium pans and cooled from 25 °C to −100 °C and then warmed to 25 °C at a cooling/warming rate of ±10 °C min^{-1}. Transition enthalpies in the tissues were estimated on the basis of the areas under the peaks. For the warming thermograms, areas were integrated from approximately −35 °C to 5 °C. The main peaks occurred between −70 and 10 °C and representative cooling and warming thermograms are presented for this temperature range.

2.7. Effects of H_2O_2 on seed germination after desiccation

Seeds of the Lincang seed lots (L1 and L2) were used to study the effects of exogenous H_2O_2 (0.1–1 M) on germination following desiccation. The seed coats of L1 were removed prior to desiccation (i.e. embryos) whilst the L2 material was dried as seed. After desiccation for 1–12 d, four replicates of 15 seeds/embryos per time interval were imbibed for 1 h in H_2O or in 0.1, 0.5 or 1 M H_2O_2 at room temperature, then thoroughly rinsed, blotted dry and germinated as described above.

2.8. Statistical analysis

Data were tested for significance using one-way analysis of variance (ANOVA) in combination with least significant difference (LSD) post hoc comparison of means and also with a Fisher-pair wise comparison. Arcsine transformation was applied to the TG data. Significant differences are shown for $P < 0.05$.

3. Results

3.1. Environmental conditions and seed characteristics at the three collection sites

The seeds of *C. sinensis* collected from three locations developed under slightly different environmental conditions (Fig. 1), partly as a result of variable altitudes from 1200 masl to 1895 masl (Table 1). The temperature differences between Puer and Kunming, and between Lincang and Kunming were approximately 4 °C and 2 °C, respectively, in the autumn to spring (September–April) and 2 °C and 1.5 °C, respectively, from April to August (Fig. 1b). The temperature difference between Puer and Lincang was <1.5 °C throughout the whole year, and both locations were slightly warmer than Kunming. All sites had a ∼5 month dry season (December–April) after seeds were dispersed naturally in early November. During the dry season rainfall per month was 14–24 mm in Kunming, 12–35 mm in Lincang and 21–51 mm in Puer. Throughout the year precipitation was greatest in Puer, followed by Lincang and Kunming (Fig. 1c). In summary, Kunming is the coolest site, receives the least rainfall and is located at the highest altitude, whilst Puer is the wettest and hottest location and at the lowest altitude.

Dry seed mass varied from 597 mg for Kunming K2 to 1279 mg for Puer P2. The characteristics of the tissue components were investigated, specifically the dry mass of the axis, cotyledon and seed coat. The dry mass of the axes ranged from 1 to 2.6 mg, and that of the cotyledons from 390 to 790 mg. The mass of the coat was compared with the mass of the whole seed to determine the seed coat ratio, which varied from 0.31 to 0.39 (Table 1).

3.2. Loss of water and viability on desiccation

The initial MCs for tissues of seed lots K1, L2, P1 were 59–69% for the embryonic axes, 46–57% for the cotyledons and 40–51% for the whole seeds (Fig. 2). The initial MCs for tissues of seed lots K2, L1, P2 were 57–66% for the embryonic axes, 49–55% for the cotyledons and 41–45% for the whole seeds (data not shown), which indicated considerable similarity in MC between the seeds collected in two separate years.

Desiccation led to a decline in TG in all seed lots (Fig. 2), but Kunming lot K1 showed the highest relative desiccation tolerance (Fig. 2a), compared with Lincang L2 (Fig. 2b) and Puer P1 (Fig. 2c). After 3 d of drying to 9% MC, TG in Kunming K1 was still 67% (Fig. 2a). However, Lincang L2 at 18% MC and Puer P1 at 10% MC had only 25% TG (Fig. 2b) and 16% TG (Fig. 2c), respectively. When all seed lots were dried for 12 d to 5–6% MC the Kunming seed lot K1 had 15% TG (Fig. 2a), whereas the other two seed lots had died (Fig. 2b and c). A co-plot of TG against whole seed MC revealed mid-points for lethal desiccation (LD_{50}) of ∼8%, 16% and 28% MC for K1, P1

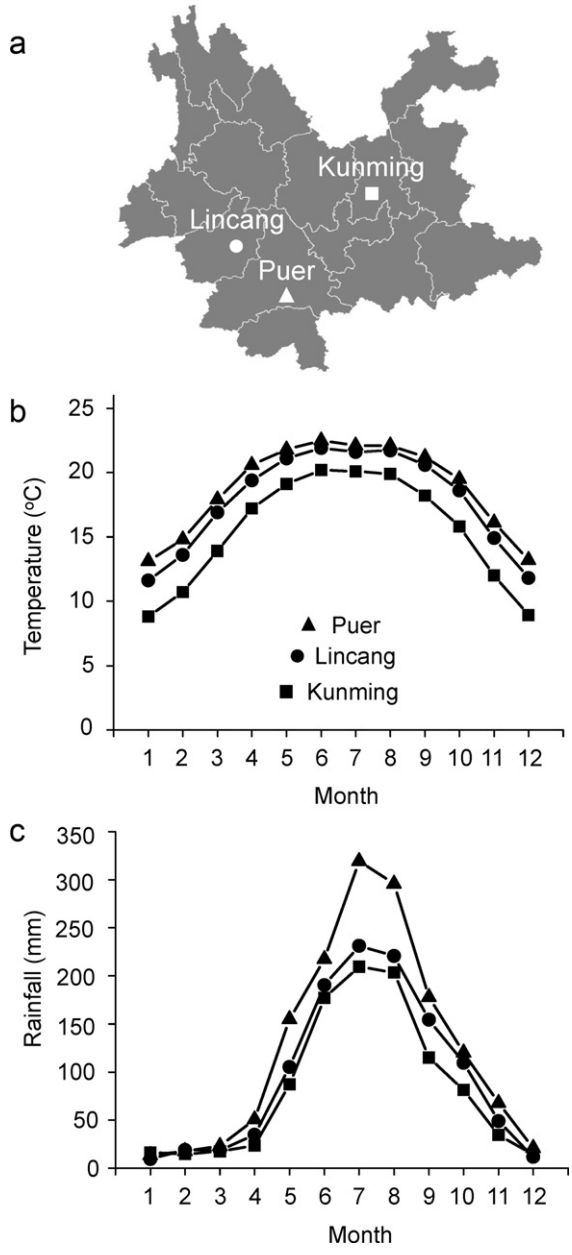

Fig. 1. *C. sinensis* seed lot site details. (a) Map of Yunnan with the locations of the collection sites in Kunming, Lincang and Puer. (b) Average temperature and (c) rainfall recorded from 1979 to 2008 for Kunming (■), Lincang (●) and Puer (▲).

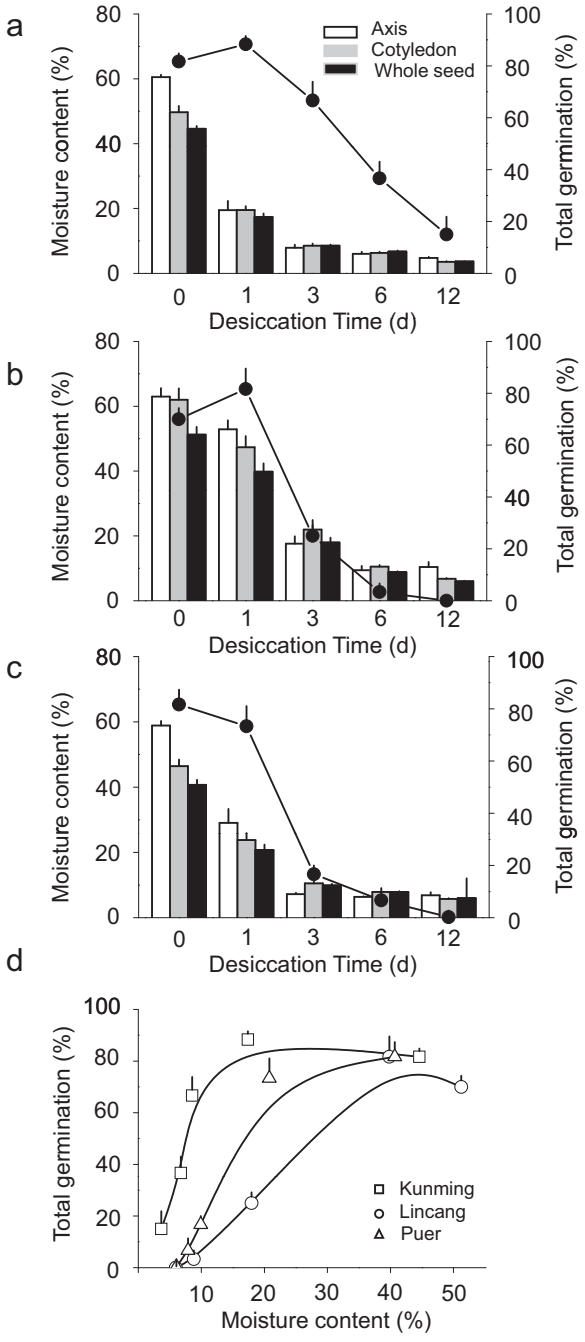

Fig. 2. Effect of drying on *C. sinensis* seed germination. (a–c) Moisture content (MC, bars) and total germination (TG, ●) at intervals of desiccation for seeds from: (a) Kunming K1; (b) Lincang L2; and (c) Puer P1 ($n = 4$ biological replicates of 15 seeds each). The columns represent the MCs of different seed tissues ($n = 15$ individual seeds): axis (white); cotyledons (grey); and whole seeds (black). (d) Relationship between MC and TG for Kunming K1 (□), Lincang L2 (○), Puer P1 (△). Data represent mean ± SE.

and L2 seed lots, respectively (Fig. 2d). There was little difference between axes and cotyledon MCs after 3 d of drying (Fig. 2a–c). In the second set of desiccation experiments performed on decoated seeds from seed lots K2, P2 and L1, the LD_{50} values were estimated to be 11%, 18% and 27% MC for K2, P2 and L1 seed lots, respectively (data not show). Those results confirmed the same basic trend in desiccation tolerance among six studied seed lots collected in two separate years.

3.3. Sorption isotherms

The sorption isotherms of different tissues (axes, cotyledons) and seeds at 20 ± 2 °C (Fig. 3) were similar to those previously reported for tea [29]. All isotherms belonged to a Type II isotherm according to Brunauer's classification [30]. The higher the a_w, the higher was the equilibrium MC. At the same a_w, the MC of the different seed tissues was similar. At the point of LD_{50} (8% MC for whole seeds) for K2, the a_w was ~0.5. Alternatively, at an a_w of 0.7, the axes, cotyledons and whole seeds of the three lots (K2, L2, P2) were ~15% MC (Fig. 3).

3.4. Oil content and thermal analysis

Seed from all three lots had high oil contents (Table 2); Kunming K2 with 30.6% oil was the highest, but this was not significantly ($P > 0.05$) higher than Lincang L2 (28.3%) or Puer P2 (27.7%).

Table 2
Oil contents (% dry mass basis), measured after supercritical fluid extraction, of different seed lots ($n = 4$ individual seeds) and enthalpies for the lipid melting during warming ($J\,g^{-1}$ dry mass of seed tissue) based on DSC thermal analysis. Data represent mean ± SE ($n = 3$). Values in the same column with a different letter are significantly different ($P < 0.05$).

Taxon	Location	Embryo* oil content (%)	Lipid transition in the cotyledon ($J\,g^{-1}$)	Lipid transition in the embryonic axis ($J\,g^{-1}$)
C. sinensis var. *sinensis*	Kunming K2	30.6 ± 4.0a	11.8 ± 0.2b	14.7 ± 0.6a
C. sinensis var. assamica	Lincang L2	28.3 ± 3.6a	10.6 ± 0.3b	15.8 ± 3.9a
C. sinensis var. assamica	Puer P2	27.7 ± 0.7a	16.3 ± 0.9a	16.0 ± 0.7a

* When removed from the sclerotic mesotesta the embryo sometimes adheres to the thin endotesta.

Embryonic axes and cotyledons that had been desiccated for 12 d were analysed by DSC to establish the thermal properties of the lipid component. Thermal analysis revealed no sharp exothermic peak during cooling, usually associated with the freezing of water, but main transitions centred on −30 °C and −65 °C (Fig. 4a and c). Similarly, two melting peaks occurred during warming centred on −25 °C with a shoulder at −15 °C and a smaller peak at 0 °C. There was considerable coincidence between the thermal events in the axes (Fig. 4b) and cotyledons (Fig. 4d).

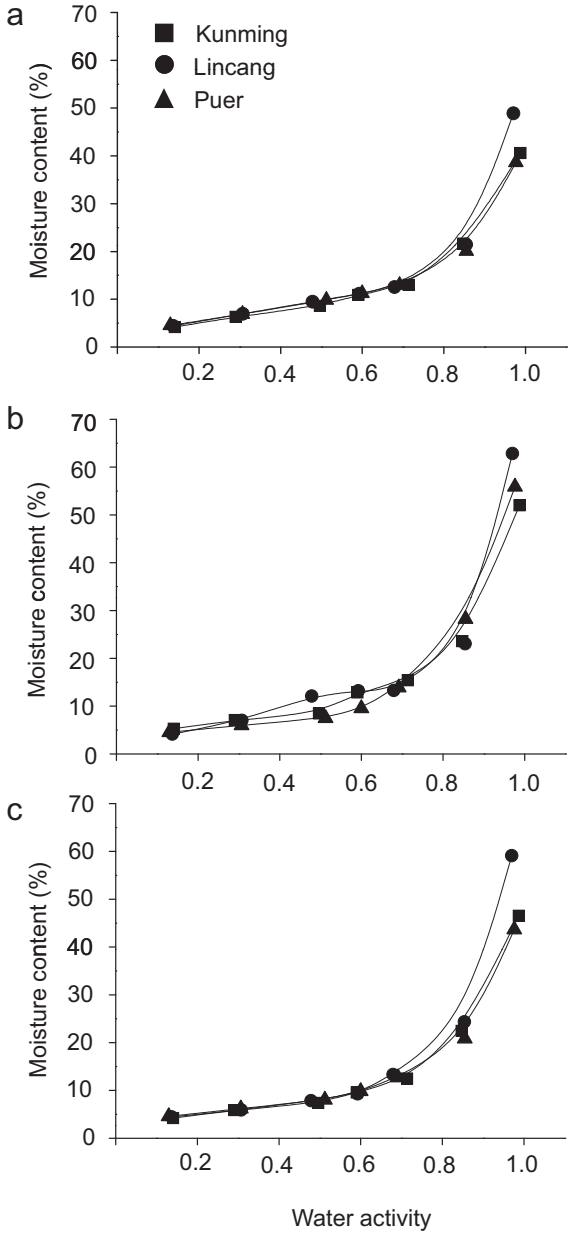

Fig. 3. Relationship between *C. sinensis* seed/tissue equilibrium moisture content (MC) and water activity (a_w) at 20 ± 2 °C for Kunming (■), Lincang (●) and Puer (▲) whole seeds (a), axes (b) and cotyledons (c). Data represent mean ± SE for three individual seeds each.

The total oil contents of the seeds (composed mainly of the cotyledons) from all three locations were similar (Table 2) and the DSC data suggested that the oil contents of axes were similar to those of the cotyledons. The enthalpy of the lipid melting was between 10 and 16 $J\,g^{-1}$ for the cotyledons and between 15 and 16 $J\,g^{-1}$ for the axes (Table 2). The thermal analysis showed that Puer seeds had significantly ($P < 0.05$) larger transition enthalpies in the cotyledons compared to Lincang and Kunming seeds (Table 2). However there was no significant difference in the lipid transition enthalpies of the embryonic axis. Overall, the thermal properties of the oils in the tissue of three provenances were similar.

3.5. Low temperature sensitivity following desiccation

Seed storage at a conventional seed bank temperature (−20 °C) was assessed for partially dried seeds of three seed lots K2, L2 and P2 (Fig. 5). After 3 d of desiccation, when seed MC was 10–13% ($a_w \sim 0.55$–0.65, Fig. 3), the TG of K2 seeds significantly decreased ($P < 0.05$) from 83% to 66%; the other two seed lots L2 and P2 fell to 25% and 16% TG, respectively. Following one week of storage at −20 °C after desiccation, TG significantly decreased ($P < 0.05$) in both K2 and L2 seeds to 10% and 2%, respectively. No seeds of P2 survived one week storage at −20 °C. No seeds survived one month storage at −20 °C (Fig. 5).

3.6. Exogenous H_2O_2 partly alleviates desiccation-induced damage

Hydrogen peroxide is known to stimulate seed germination [31], and the Lincang seed lots (L1 and L2) were used to explore whether exogenous H_2O_2 could alleviate the detrimental effects of partial desiccation. The treatment was applied following different desiccation treatments with and without seed coat (Fig. 6). Soaking seeds increased the MCs of both the embryonic axes and the cotyledons and no significant differences were found for imbibition in H_2O or H_2O_2 (data not shown). Treatment of non-desiccated seeds and seeds that had been desiccated for 1 d without (Fig. 6a and b) or with (Fig. 6f and g) the seed coat with H_2O, 0.1 M, 0.5 M or 1 M H_2O_2 did not change TG significantly ($P > 0.05$). In seeds in which TG had dropped to 53% with the seed coat removed (Fig. 6c) and to 25% in seeds with the seed coat present (Fig. 6h) after desiccation for 3 d, all H_2O_2 concentrations increased TG. In the seeds dried without the seed coat, H_2O_2 treatment increased TG in a concentration-dependent manner with 0.5 M H_2O_2, and 1 M H_2O_2 increasing TG significantly ($P < 0.05$) to 78% and 90%, respectively (Fig. 6c). In seeds desiccated within their seed coats, 0.5 M H_2O_2 improved TG more than 1 M H_2O_2 (Fig. 6h); TG increased significantly ($P < 0.05$) from 25% to 83% when 0.5 M H_2O_2 was used, and to 72% when 1 M H_2O_2 was used. When material was desiccated for 6 d without the seed coat, TG dropped to 38% and did not benefit from H_2O_2 treatment and soaking in H_2O decreased TG to 10% (Fig. 6d). No seed germinated after desiccation for 12 d without the seed coat (Fig. 6e)

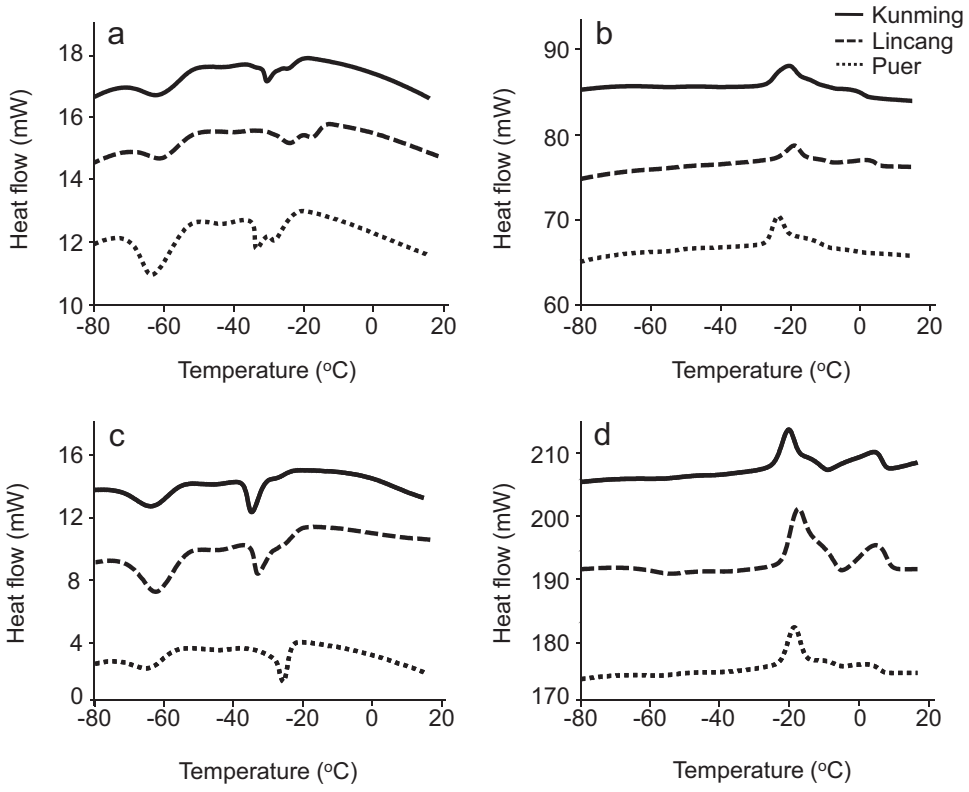

Fig. 4. DSC cooling (a and c) and warming (b and d) thermograms showing lipid phase changes in the axis (a and b) and cotyledons (c and d) of *C. sinensis* seeds from three provenances (K2, L2, P2). Samples were held at 25 °C for 1 min cooled to −100 °C, held for 1 min and re-warmed to 25 °C; cooling and rewarming were at a rate of ±10 °C per min.

and 6 and 12 d with seed coat, irrespective of H_2O_2 treatment (Fig. 6i and j).

4. Discussion

This study shows that *C. sinensis* produces seeds that are not necessarily recalcitrant, as seeds from Kunming K1 had 67% TG after drying to 9% MC, equivalent to an a_w of ~0.5 (Figs. 2a and 3), showing non-recalcitrant seed storage behaviour. However, the two other provenances displayed much lower levels of desiccation tolerance, particularly Lincang L2 with a LD_{50} of 28% MC. The latter's level of desiccation intolerance is typical of many recalcitrant seeds, and other previously investigated seed lots of tea, for example from Korea [32], Bengal [8] or India [11]. In all these studies [8,11,32], seeds died when desiccated to MCs between 12 and 20%. Hence, they were even more desiccation sensitive than the Lincang and Puer seed lots investigated here (Fig. 2b and c).

The Kunming K1 seed lot had a desiccation tolerance level similar to that reported by Visser and de Waas Tillekeratne [7], who stored seeds in an environment of 0 °C and 10% RH for 20 weeks with 50–70% TG. The MC was not reported and with such a large seed mass, there is the possibility that the seeds did not dry fully. However, we have no evidence for the seed material used here of any resistance to drying, as seeds of all seed lots dried to below 6% MC in 12 d (Fig. 2). Desiccation of other (sub)tropical seeds resulted in reduced water permeability (*Citrus limon* [33] and *Hyophorbe lagenicaulis* [34]), induction of dormancy [35] or specific temperature sensitivity on subsequent rehydration (*Azadirachta indica* [16], *Carica papaya* [35] and *Cuphea* sp. [36]). However, we found no evidence of reduced germination rate (i.e. speed of germination) after the drying of tea seeds, which would be indicative of induced seed coat impermeability or dormancy (Fig. 6). The benefits from removing the seed coat from tea at ~10% TG (Fig. 6a) were relatively minor and likely a consequence of removing a physical barrier to the emerging radicle. Similarly, seed coat removal and pre-soaking in water were seen to increase the germination speed in fresh tea seeds [7].

Nevertheless, tea seed TG, of all seed lots, reduced gradually with increasing desiccation (Fig. 6). Chen et al. recently suggested that high concentrations of H_2O_2 that accumulated during the time course of desiccation could be involved in seed death in tea [37].

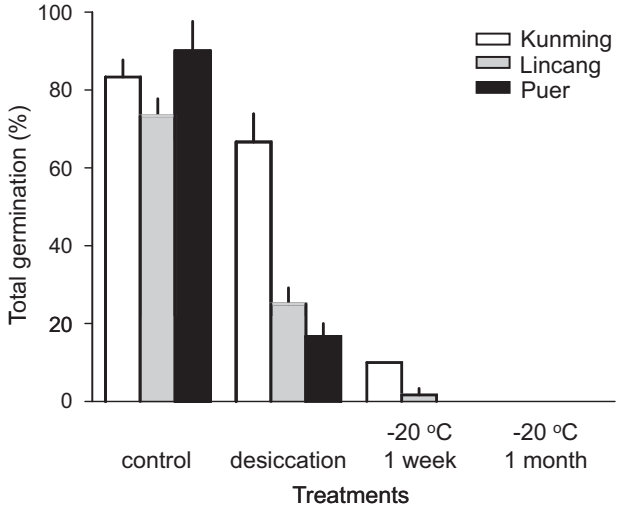

Fig. 5. Total germination (TG) for *C. sinensis* seeds from three provenances without desiccation and storage ('control'), desiccation without storage ('desiccation'), and stored at −20 °C for one week or one month following desiccation treatment. White columns represent the Kunming K2 seeds; grey columns are for Lincang L2; black columns for Puer P2. $n = 3$ replicates of 20 seeds each. Data represent mean ± SE.

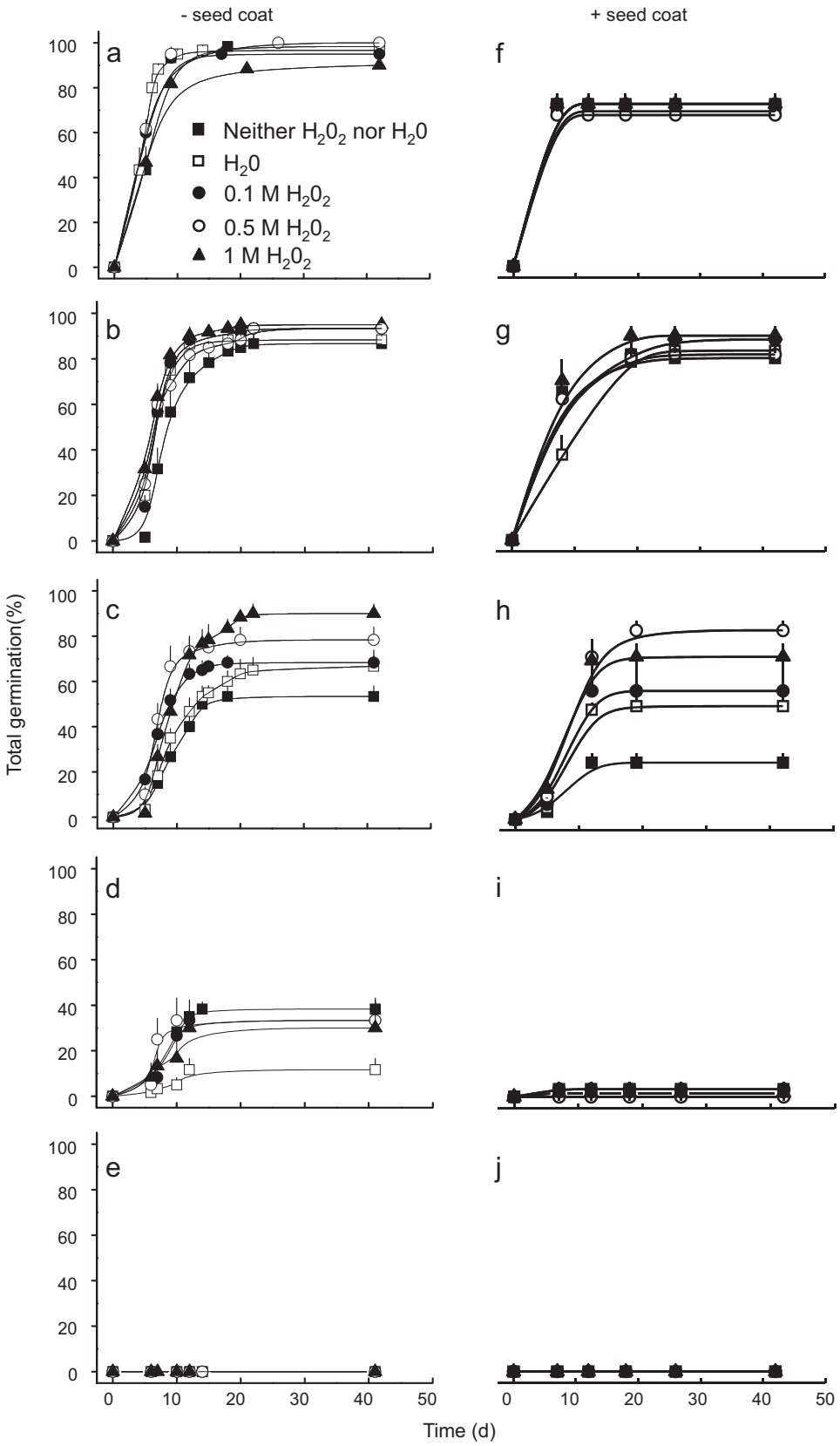

Fig. 6. Germination of *C. sinensis* seeds from Lincang treated with different concentrations of H_2O_2, H_2O, or untreated, after desiccation for 0, 1, 3, 6, 12 d without (a–e) or with (f–j) the seed coats present. Treatments: (■) seeds that were not desiccated (a and f) or desiccated (b–e, g–j) but not treated with either with H_2O or H_2O_2; (□) seeds treated with H_2O; (●) seeds treated with 0.1 M H_2O_2; (○) seeds treated with 0.5 M H_2O_2; (▲) seeds treated with 1 M H_2O_2 ($n = 4$ biological replicates of 15 seeds/embryos each). Data represent the mean ± SE.

However, ROS have multiple roles in metabolism and are important signalling molecules generally [38,39]. Previously, we have observed enhanced TG in recalcitrant seeds of sweet chestnut (*Castanea sativa*) after a one-off, short-term exogenous application of H_2O_2 [22]. Similarly, a short, 1 h treatment of Lincang L1 and L2 seeds desiccated for 3 d with 0.5–1 M H_2O_2 enhanced TG up to 25% more than treatment with water alone (Fig. 6c and h). Compared to the dried samples, TG was enhanced by 37–58% after H_2O_2 treatment. H_2O_2 could have acted to surface-sterilize the seeds and protect against the growth of seed-decomposing micro-organisms in the germination test. As H_2O_2 is involved in developmental processes, H_2O_2 treatment may also have up-regulated defence-related genes, stimulated cell division and differentiation and contributed to the removal of cells that are damaged beyond repair by initiating programmed cell death [38,39]. The second set of explanations for the beneficial role of H_2O_2 is perhaps more likely, as it was previously shown that surface-sterilised, partially desiccated sweet chestnut seeds still benefited from H_2O_2 treatment [22]. Alternatively, H_2O_2 could have been involved in the alleviation of seed dormancy. Induction of seed dormancy by desiccation has been reported in *Carica papaya* [35]. However, there is no evidence in the literature that desiccation can induce secondary dormancy in tea seeds. By contrast, all evidence suggests that desiccation causes seed death, so that we consider the alleviation of desiccation-induced secondary dormancy an unlikely explanation for the beneficial role of H_2O_2. In addition, Fig. 6e and j shows that H_2O_2 did not improve TG of Lincang seeds that had been desiccated for 12 d. If H_2O_2 would have acted as a dormancy-alleviating agent, the seeds should have germinated, which they did not do. H_2O_2 was most effective for tea seeds that had been desiccated for 3 d. The precise mechanism of seed 'rescue' by H_2O_2 in these seed lots is not known. However, without H_2O_2 treatment a large proportion of the desiccated seeds would have been classified, erroneously, as dead in the germination test.

The level of desiccation tolerance for Kunming could be indicative of seeds adapted to cope with the more challenging environmental conditions at this site, although we do not rule out a genetic component to the relatively higher level of desiccation tolerance. It has been shown previously for African trees that recalcitrant behaviour is more prevalent in species with large seeds (>500 mg) that are shed in months with >50 mm precipitation [40]. This association between dispersal and rainfall in trees is broadly supported by the responses of tea, as precipitation at seed fall was <50 mm for Kunming (Fig. 1) which showed the highest desiccation tolerance among the three provenances (Fig. 2). In addition, as reported for another woody commodity species, coffee, the months after dispersal can be used as a marker for relative desiccation tolerance [41]. Coffee species adapted to a dry season of ~4–5 months have LD_{50}s of about 8–10% MC. Tea seeds from Yunnan tolerated a similar length of dry season (Fig. 1) and for Kunming had a similar potential for desiccation tolerance. In contrast, the Lincang and Puer seeds grew in an environment with a slightly shorter, less intense dry season and they had slightly higher desiccation sensitivity.

Subtle differences in desiccation tolerance have been related to developmental heat sum, e.g. in *Aesculus hippocastanum* (horse chestnut) [20] and *A. pseudoplatanus* (sycamore) [21]. For *A. pseudoplatanus*, seed lots harvested from outside the species' native distribution range displayed high levels of desiccation sensitivity consistent with recalcitrancy. However, sycamore seeds from the native range in southern Europe, having accumulated more developmental heat sum, were tolerant of drying to ~15% MC and storage for one week at −20 °C and in liquid nitrogen. Consequently, we were concerned as to the developmental status of the tea seeds used in this investigation, as it is extremely difficult to judge seed maturity in terms of the physiology of the embryonic axis when it constitutes <1% of the mass of the seed, as is the case in many seeds. Surrogates of maturity that have been commonly used in studies on woody species include seed or tissue mass [20,21] and lipid content, which continues to accumulate late in oilseed development, as observed using DSC [42]. In the present study, the axis dry mass in tea varied from 1 to 2.6 mg (Table 1), somewhat heavier than material grown in South Africa, which weighed 0.8–1.1 mg [9]. In addition, DSC revealed strong signs of lipid melts in the axes as well as the cotyledons (Table 2 and Fig. 4), in contrast to the thermal traces for South African material [9]. This evidence suggests that in the present study, seeds from all three provenances were well developed. Nonetheless, Kunming seeds were more desiccation tolerant than those from the other two provenances. There is potentially a genetic difference between the various seed lots used as they represent two varieties, var *sinensis* (from Kunming) and var *assamica* (from Lincang and Puer). In inter-species crosses of coffee, desiccation tolerance was shown to be heritable [43]. Thus genetic background may have contributed to the differences observed and current efforts to sequence the tea genome will facilitate future seed biology studies considerably.

Our final consideration was whether the seeds could be banked, given the relative level of desiccation tolerance, below the unfrozen water content which is predicted to be about 14% MC for tea based on oil content [13,44]. However, seeds of all three provenances at 10–13% MC failed to last 1 month, and a significant proportion of seeds from Kunming only survived 1 week at −20 °C (Fig. 5). The thermal fingerprints and thus the lipid properties of tea have similarities to two other oilseed groupings that are difficult to store, viz. citrus and coffee. Fats with melting temperatures close to 0 °C appear to compromise dry storability [45]. In tea, the lipids finished melting by 5 °C (Fig. 4). Tea contains three main fatty acids: oleic (18:1), 62.5% of mass; linoleic (18:2), 18.1%; and palmitic (16:0), 10.4% [19]. Accordingly, we observed three cooling and melting transitions (Fig. 4). Similarly, citrus has two melting peaks at −20 °C and (peaks or shoulders) at 0 °C [13] reflecting the main oils: linoleic (+oleic) and palmitic acid respectively [46]. Coffee has two main lipids, oleic and palmitic acid, and melting peaks around −20 °C and 5 °C, respectively [15]. Thus we can provisionally assign the thermal peaks in tea seed oil to its main constituents. How the phase behaviour of the oil contributes to loss of viability at −20 °C remains to be resolved; although the suggestion has been made for oilseeds with MCs close to the unfrozen water content that the oil (bodies) facilitate(s) ice crystal growth [47].

For a number of reasons the response of tea seeds does not fit the intermediate category of sensitivity to −20 °C and 0 °C, which has been interpreted as sensitivity to all sub-zero temperatures [48]. Firstly, whilst partially desiccated seeds died at −20 °C the response was quicker than that of an intermediate seed. Secondly, earlier studies have shown the ability of tea seeds to tolerate storage of many months at temperatures close to 0 °C. Finally, some tea seed lots clearly survive storage in liquid nitrogen [10]. Thus we define tea seeds as Type II after the classification system of Pritchard [12]; that is, seeds of species with varying tolerance to the withdrawal of water in the region of zone II of the sorption isotherm (ca. 20–75% RH), but with sensitivities to specific low temperatures dependent on the composition of the seed lipids. In summary, tea seeds are not necessarily recalcitrant, but long term storage *ex situ* may be limited to cryopreservation.

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