

Water loss and response to desiccation of *Castanopsis sclerophylla* (Fagaceae) seeds

Jiajin Li¹, Ganesh Jaganathan², Han Kang¹, and Baolin Liu¹

¹University of Shanghai for Science and Technology

²Affiliation not available

March 30, 2022

Abstract

In many -but not all- recalcitrant *Quercus* seeds, water loss during drying only occurs through scar. Consequently, embryo sitting on the opposite side of scar is well protected from desiccation. However, whether such mechanism is common throughout Fagaceae species is unexplored. Similarly, little is known about the desiccation response of other Fagaceae genera. Germination and desiccation tolerance of *Castanopsis sclerophylla* (Fagaceae) were studied by drying the seeds. Fresh seeds had a moisture content (MC) of 36% and germinated to 93% when incubated at 15/20°C. Drying seeds to 22.5 and 20.7% MC decreased germination to 27 and 0%, respectively; indicating seeds are recalcitrant. X-ray computed tomography and photomicrographs showed that embryo of *C. sclerophylla* sits just below scar. Regardless of drying occurring either through only scar or whole pericarp, embryo drying was deemed unavoidable. Nevertheless, germination percentage during drying showed exceptional similarities with the other Fagaceae species.

Introduction

Based on their ability to withstand drying, two broad groups of seeds have been recognised by Roberts (1973). One group includes nearly 90% of the known plant species that produces seeds which can survive drying to the low moisture content (MC), c. 3-10% on a fresh weight basis (f. wb), thus desiccation-tolerant, i.e. orthodox. Another group of species produces seeds that lose viability if dried to the moisture range of 20-30% f.wb, therefore referred to as desiccation-sensitive or recalcitrant (Roberts, 1973). The seeds of desiccation-sensitive species are not amendable to low temperature storage and storing them at higher temperatures in the range of 0-10 °C does not preserve viability for more than 1 year (Berjak and Pammenter, 2008). Ellis et al. (1990) described a third group of seed storage, which they referred to as intermediate, wherein seeds can tolerate desiccation to c. 9-12% moisture content, but further drying reduces seeds viability, and/or seeds lose viability during low temperature storage.

The family Fagaceae is widely distributed in the temperate rainforests and includes nine genera with approximately c. 1000 species (Manos and Zhou, 2001). Information on seed storage behavior and germination ecology of Fagaceae seeds are restricted to the largest genus *Quercus*, which includes c. 400 species (Bonner, 1996; Pritchard, 1991; Xia et al., 2012a). These studies suggest that most of the *Quercus* seeds are large and they do not withstand drying to moisture content less than 15-20% f. wb, thus recalcitrant. However, little is known about the seed storage behavior of species in other genera within Fagaceae. *Castanopsis* is the second largest genus in Fagaceae which includes approximately 120 species (Huang et al., 1999; Wu et al., 2014). Current knowledge about seed storage behavior of *Castanopsis* species are unclear. For example, Kew's seed information database (SID, 2020) reports seed storage behavior of 10 *Castanopsis* species, yet 6 of them are labelled as uncertain and 4 species (1 orthodox and 3 recalcitrant), are doubtfully assigned.

Such confusion is further complicated by previous studies lacking desiccation experiments or failing to report

the moisture content. Shopmeyer (1974) reported that seeds of *Castanopsis chrysophylla* collected from North America reduced viability to 5% following hermetic air-dry storage at 5°C for 5 years, but the moisture content of the seeds was not mentioned. Similarly, Campbell (1980) showed that seeds viability of *C. indica*, *C. purpurella* and *C. tribuloides* from Nepal can only maintain viability for 2 months in moist storage at room temperature, but did not provide any information about viability following desiccation. Tian and Tang (2010) found that fresh seeds of *C. fissa* with 42.7% moisture content had 100 % germination. However, drying seeds to 25.3% moisture content reduced the germination to 17%. Further desiccation to 17.3% moisture content within 72 hours resulted in complete seed death. However the quick drying rate of *C. fissa* seeds is contrasting with other Fagaceae species reported by various authors (Li et al., 2018; Pritchard, 1991; Xia et al., 2012b). In general, *Quercus* seeds dried in silica gel require about 20 days to reach approximately 20% MC. Recently, Basrudin et al. (2019) reported that the seeds *C. buruana* Miq. dried in the sunlight after washing reduced viability, neither the moisture content measurements nor any desiccation experiments were performed. Apparently, there are no other studies on seed desiccation sensitivity of *Castanopsis*.

Xia et al. (2012b) reported that seed anatomy plays an important role in controlling water loss of Fagaceae species. Using nine *Quercus* species, those authors reported that the water loss occurs (only) through the scar during desiccation in many -but not all- species, and the main pericarp being impermeable to water, protects the embryo by not allowing the water to leave seeds. In most *Quercus*, embryo sits on the opposite side of the scar (Baskin and Baskin, 2007; Martin, 1946; Nixon, 1985; Nixon, 1993), thus water loss occurring only through scar will dry the cotyledons and the embryo is kept in a hydrated state for long-time, (Finch-Savage and Blake, 1994). Consequently, this mechanism could protect the seeds under ecological drying due to drought. However, whether a similar mechanism operates in other genus of Fagaceae is unknown.

Castanopsis sclerophylla distributed in southern and eastern subtropical forests of China is one of the dominant species of evergreen broad-leaved forest (Huang et al., 1999). It is an important economic tree providing valuable timber for construction and water-soil maintenance. Further, the seeds of *C. sclerophylla* are rich in starch, and consumed as food (Xin et al., 2007). However, the population is shrinking with poor establishment of new trees and expansion of agricultural land area adds further pressure for regeneration. Clearly, we know little about the physiological and morphological changes during desiccation and nothing is known about the desiccation tolerance ability of seeds of this species. Thus, the first objective of this study was to define the germination characteristics and drying pattern of *C. sclerophylla* seeds. In particular, we wanted to identify the seed storage behavior.

X-ray computed tomography (CT) is a non-invasive approach which have 3-D visualization and can quantify biological structure (Burg et al., 1994; Dell'Aquila, 2007; Foucat et al., 1993; ISTA, 2020). In *Syagrus flexuosa* fruits, Stuppy et al. (2003) used high-resolution X-ray computed tomography (HRCT) to clearly distinguish all morphologically relevant parts and organs such as the endocarp with fibrovascular bundles and endosperm with embryo. Kunishima et al. (2020) used a laboratory-based X-ray microscope to study 3D-structural on the development of pansy seeds without any pretreatment. Therefore, the second objective of the present study was to use CT to scan the whole seed without any treatment, so as to show an intuitive view of the internal structure of seeds and better understand the effect of the morphological structure of seeds during drying.

Materials and Methods

Fully matured fruits (botanically acorns; but hereafter referred to as seeds) of *C. sclerophylla* were collected during the natural dispersal time at the last week of November 2018 from 11 trees in Thousand Island Lakes (TIL) of Zhejiang province, China (29°22' – 29°50' N, 118°34' – 119°15' E). The seed collection region of TIL has a temperate climate with a mean annual temperature of 17.6 °C between 2008 and 2017, ranging from 5.4 °C in January to 29 °C in July. The study site receives an annual rainfall of 1637.46 mm, with peak rainfall occurring between March and June. After collection, the seeds were brought to the University of Shanghai for Science and Technology, Shanghai, China, within three days via. road. After arrival, any insect infested seeds were removed by visual inspection. Seeds were soaked in sodium hypochlorite (NaOCl) solution for 30 minutes for surface sterilization and washed in sterile water thrice. The seeds were stored

at 4 °C until used in the experiments in plastic bags with holes for gas exchange. Seeds were used in the subsequent experiments within 5 days after collection.

The average weight of 100 seeds was determined by measuring the weight of three replicates of 100 seeds in a standard balance (0.001 g). Moisture content was determined by drying three replicates of five seeds at 103°C for 17 hours in a standard oven drying method, following the methods recommended by the International Seed Testing Association (ISTA, 2020). All moisture contents are expressed on a f. wb.

Germination test was conducted by incubating four replicates of 10 seeds on 1% agar-water in closed plastic boxes with small holes (l × b × w : 154 × 95 × 45 mm) at 15/20°C (12 hours dark / 12 hours light of 60 μ mol m⁻²s⁻¹ light provided by cool tungsten filament bulbs available in the germination chamber). Light was supplied only during the warm phase. Seed germination was defined as radicle emergence by at least 2mm and counted weekly until no germination for three consecutive weeks.

Our preliminary trails showed that seeds of *C. sclerophylla* dried slowly and lost viability around 25% moisture content. Therefore, in order to properly test the seed responses to desiccation, a total of 275 seeds were grouped into five sets each containing 55 randomly selected seeds and dried separately using freshly regenerated silica gel in a 5:1 ratio by weight. Seeds and silica gel were mixed, sealed in five different airtight polythene boxes (l × b × w : 170 × 115 × 45 mm) and placed at room temperature (22 ± 1 °C). After 3, 5, 7, 14 and 21 days, one set of seeds (i.e. one box) each were opened and three replicates of five seeds (15 in total) were used to determine the moisture content of the seeds. The remaining seeds (four replicates of 10) were used for germination as mentioned above. Germination data were arcsine transformed before analysis and tested for statistical significance using one-way ANOVA with Duncan test in SPSS, version 21.0.

In order to determine the morphology, full and cross-sections of the seeds were photographed by camera (EOS 60D, Canon, Japan) and the whole seeds were scanned by Industrial Computer Tomography (XTH 225 ICT, Nikon, Japan). The seeds were placed on the CT sample table and moved to the appropriate position. After scanning several times and to improve the clarity of the images, the parameters of CT scan were set as: tube voltage 90 kV, tube current 90 μ A, exposure time 500 ms, and the number of pictures taken 2000 times. Using the built-in image processing software (CT Pro 3D, Nikon, Japan), the imported scanned images were reconstructed in three dimensions (3D) to obtain the CT image of the seed, including the front view, the top view and the side view.

Results and Discussion

The average 100-seed weight was 134.1±6.8 g. Freshly collected seeds had a moisture content of 36.2±1.9% and 92.5±0.4% germination (Fig. 1). After 7-day drying, seed germination decreased significantly to 27.5% where moisture content declined to 22.5%. The viability of seeds dried to 20.7% moisture content (seeds dried for 14 days) was 5% and further drying to lower moisture contents resulted in complete loss of viability (Fig. 1). The sharp decline in germination when seeds moisture content reduced to c. 20% f. wb. indicates that the seeds are ‘recalcitrant’

Compared with other desiccation-sensitive seeds of Fagaceae species, e.g. *Q. robur* (Finchsavage, 1992), *Q. coccifera* L., *Q. pubescens* Willd. and *Quercus pedunculiflora* K. Koch (Ganatsas and Tsakalimi, 2013), *C. sclerophylla* seeds had a similar desiccation response, showing that the percent germination of seeds decreased with the progress of drying. The seeds lost their viability completely when the moisture content of the seeds dropped lower than 20% in 2-3 weeks. The result is also consistent with drying rates reported on *Quercus* seeds, where these studies reported that the drying rate of Fagaceae seeds is relatively slow. Further, it required 15-25 days to reach moisture levels that are lethal to seeds when dried in silica gel. However, Tian and Tang (2010) reported that *C. fissa* and *Q. fabri* seeds can be dried to approximately 20% moisture content within 72 hours, which is contradicting with our results. This is not surprising, as we found in our earlier work (Li et al., 2018) that *Q. fabri* seeds required a longer drying period of at least 20 days to reach approximately 20% when dried above silica gel, which is in agreement with Xia et al. (2012b). The contradicting result on the drying rate could be attributed to the differences in the drying method. Both whether or not the seeds were dried in a sealed environment and the amount of silica gel used was not

provided by Tian and Tang (2010).

In this study, we show that X-ray computed tomography (CT) can be a useful tool to study the internal structures of seeds without altering the intact structure of seeds. CT results show that the pericarp of *C. sclerophylla* is similar to other Fagaceae seeds, such as *Quercus* (Xia et al., 2012b). Pericarp in *Quercus* is divided into three areas: (1) the apex; (2) the scar; and (3) main pericarp (the remaining area between the apex and the scar), and our results indicate that these regions are clearly distinguishable in *C. sclerophylla* (Fig. 2.3a). Xia et al. (2012b) found that water loss during seeds desiccation of *Quercus* species occurs only through the scar instead of main pericarp or the apex in some species including *Q. nuttallii*, *Q. suber* and *Q. palustris*. Xia et al. (2012a) showed that the moisture content of *Q. franchetii* only reached 23% after 164 days of drying in silica gel. For this species, a comparatively small scar (ca. 6% of the pericarp area) might be the mechanistic basis of limited water loss through the pericarp (Xia et al., 2012b). For *Q. suber*, Eduardo and Belén (2000) studied the morphological and micro-morphological differences of the scar and main pericarp. The main pericarp provides a protective barrier in slowing down water loss and it is mainly contained of a layer of epidermal wax, a thick cuticle covered epidermal layer and a single layer of palisade cells. However, the scar lacks this protective structure, which is comprised by vascular strand presumably facilitating moisture loss. Thus, this feature was reported to have ecological advantage, because the embryos of *Quercus* seeds cannot reach lower water content during brief exposure to drought conditions in natural environment.

Whilst investigating the route of water loss in *C. sclerophylla* might be arguably an important area worth exploring, we believe the results of photomicrographs and CT scan suggested otherwise. Clearly, in *C. sclerophylla*, embryo is on the opposite side of the apex and sits just below the scar region (Fig. 2). This is in marked contrast with *Quercus* embryo, which is located on the apex side and far away from scar (Finch-Savage and Blake, 1994; Xia et al., 2012b). Thus, during desiccation no matter water loss only occurs through scar or intact pericarp, embryo of *C. sclerophylla* seed will be the first part within the seeds to lose water due to embryo position and continuous drying may kill the seeds, so there is no practical significance to study the water loss pathway during seed drying. However, our drying results indicate that *C. sclerophylla* seeds lose viability only when the moisture content drops to c. 20% f.wb.

There appears to be strong difference in the germination pattern between *Quercus* and *C. sclerophylla* seeds. Notably, in *C. sclerophylla* seeds, embryo elongates and grew thicker initially before protrude through the scar, then elongated into root and radicle (see Fig. 2.3). For *Quercus* seeds, the radicle protrudes through the apex region during germination (Bonner and Vozzo, 1987; Löf et al., 2019). It is possible to suggest that this difference could be due to the position of embryo between these two genera. However, this standpoint requires detailed investigation given that seeds of many *Quercus* have epicotyl physiological dormancy (Allen and Farmer Jr, 1977; Baskin and Baskin, 2014; Farmer Jr, 1977).

Data accesseibility

We do not have any data to be archived. The images and germination results are presented in the form of figures.

References

- Allen, R., and Farmer Jr, R. E. (1977). Germination characteristics of bear oak. *Southern Journal of Applied Forestry* **1**, 19-20.
- Baskin, C. C., and Baskin, J. M. (2007). A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. *Seed science research* **17**, 11-20.
- Baskin, C. C., and Baskin, J. M. (2014). "Seeds: ecology, biogeography, and evolution of dormancy and germination," Second/Ed. Elsevier.
- Basrudin, Budi, S. W., Achmad, and Sukarno, N. (2019). Characteristics of morphology, anatomy and dormancy breaks of *Castanopsis Buruana* Miq. Seeds as an endemic plant of Sulawesi, Indonesia. *Plant Archives*

19 , 4101-4108.

Berjak, P., and Pammenter, N. W. (2008). From Avicennia to Zizania: Seed Recalcitrance in Perspective. *Annals of Botany* **101** , 213-228.

Bonner, F., and Vozzo, J. (1987). "Seed Biology and Technology of *Quercus* ," U.S. Dept of Agriculture, Forest Service, Southern Forest Experiment Station.

Bonner, F. T. (1996). Responses to Drying of Recalcitrant Seeds of *Quercus nigra* L. *Annals of Botany* **78** , 181-187.

Burg, W. J. V. D., Aartse, J. W., Zwol, R. A. V., Jalink, H., and Bino, R. J. (1994). Predicting Tomato Seedling Morphology by X-ray Analysis of Seeds. *Journal of the American Society for Horticultural Science* **119** , 258-263.

Campbell, M. W. (1980). Plant Propagation for Reforestation in Nepal. *Lancet* **170** , 255-256.

Dell'Aquila, A. (2007). Towards New Computer Imaging Techniques Applied to Seed Quality Testing and Sorting. *Seed Science and Technology* **35** , 519-538.

Eduardo, S. V., and Belén, V. A. (2000). Pericarp Micromorphology and Dehydration Characteristics of *Quercus suber* L. Acorns. *Seed Science Research* **10** , 401-407.

Ellis, R. H., Hong, T. D., and Roberts, E. H. (1990). An Intermediate Category of Seed Storage Behaviour? I. *Coffee* . *Journal of Experimental Botany* **41** , 1167-1174.

Farmer Jr, R. E. (1977). Epicotyl dormancy in white and chestnut oaks. *Forest Science* **23** , 329-332.

Finch-Savage, W. E., and Blake, P. S. (1994). Indeterminate Development in Desiccation-sensitive Seeds of *Quercus robur* L. *Seed Science Research* **4** .

Finchsavage, W. E. (1992). Seed Development in the Recalcitrant Species *Quercus robur* L.: Germinability and Desiccation Tolerance. *Seed Science Research* **2** , 17-22.

Foucat, L., Chavagnat, A., and Renou, J.-P. (1993). Nuclear Magnetic Resonance Micro-imaging and X-radiography as Possible Techniques to Study Seed Germination. *Scientia Horticulturae* **55** , 323-331.

Ganatsas, P., and Tsakalimi, M. (2013). A Comparative Study of Desiccation Responses of Seeds of Three Drought-resistant Mediterranean Oaks. *Forest Ecology and Management* **305** , 189-194.

Huang, C. J., Zhang, Y. T., and Bartholomew, B. (1999). "Fagaceae (Vol. 4). Flora of China (FOC) " Science Press, Beijing, China.

ISTA (2020). "The International Seed Testing Association," Bassersdorf, CH-Switzerland.

Kunishima, N., Takeda, Y., Hirose, R., Kalasová, D., Šalplachta, J., and Omote, K. (2020). Visualization of internal 3D structure of small live seed on germination by laboratory-based X-ray microscopy with phase contrast computed tomography. *Plant Methods* **16** , 7.

Li, J., Jaganathan, G. K., Zhong, Y., Liu, B., and Yu, M. (2018). Exogenous application of hydrogen peroxide improves germination in slow drying *Quercus fabri* (Fagaceae) seeds, but does not improve longevity. *Seed Science and Technology* **46** , 377-383.

Löf, M., Castro, J., Engman, M., Leverkus, A. B., Madsen, P., Reque, J. A., Villalobos, A., and Gardiner, E. S. (2019). Tamm Review: Direct seeding to restore oak (*Quercus* spp.) forests and woodlands. *Forest Ecology and Management* **448** , 474-489.

Manos, P. S., and Zhou, Z. C. (2001). Systematics of Fagaceae: Phylogenetic Tests of Reproductive Trait-evolution. *International Journal of Plant Sciences* **162** , 1361-1379.

- Martin, A. C. (1946). The Comparative Internal Morphology of Seeds. *The American Midland Naturalist* **36** , 513-660.
- Nixon, K. (1985). A biosystematic study of *Quercus* series Virentes (the live oaks) with phylogenetic analyses of Fagales, Fagaceae and *Quercus*, Ph.D. Thesis.
- Nixon, K. C. (1993). Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. <http://dx.doi.org/10.1051/forest:19930701> **50** .
- Pritchard, H. W. (1991). Water Potential and Embryonic Axis Viability in Recalcitrant Seeds of *Quercus rubra* . *Annals of Botany* **67** , 43-49.
- Roberts, E. H. (1973). Predicting the Storage Life of Seeds. *Seed Science and Technology* **1** , 499-514.
- Shopmeyer, C. S. (1974). Seeds of Woody Plants in the United States. *Aspen Bibliography* .
- SID (2020). Seed Information Database, Royal Botanic Gardens Kew. (2020). Version 7.1. Available from: <http://data.kew.org/sid/> (May 2020).
- Stuppy, W. H., Maisano, J. A., Colbert, M. W., Rudall, P. J., and Rowe, T. B. (2003). Three-dimensional Analysis of Plant Structure using High-resolution X-ray Computed Tomography. *Trends in Plant Science* **8** , 2-6.
- Tian, M., and Tang, A. (2010). Seed Desiccation Sensitivity of *Quercus fabri* and *Castanopsis fissa* (Fagaceae). *Seed Science and Technology* **38** , 225-230.
- Wu, J.-Y., Ding, S.-T., Li, Q.-J., Zhao, Z.-R., Dong, C., and Sun, B.-N. (2014). A new species of *Castanopsis* (Fagaceae) from the upper Pliocene of West Yunnan, China and its biogeographical implications. *Palaeoworld* **23** , 370-382.
- Xia, K., Daws, M. I., Hay, F. R., Chen, W. Y., Zhou, Z. K., and Pritchard, H. W. (2012a). A Comparative Study of Desiccation Responses of Seeds of Asian Evergreen Oaks, *Quercus* subgenus *Cyclobalanopsis* and *Quercus* subgenus *Quercus* . *South African Journal of Botany* **78** , 47-54.
- Xia, K., Daws, M. I., Stuppy, W., Zhou, Z. K., and Pritchard, H. W. (2012b). Rates of Water Loss and Uptake in Recalcitrant Fruits of *Quercus* Species are Determined by Pericarp Anatomy. *Plos One* **7** , e47368.
- Xin, Z., Gaofu, X. U., Dongwei, S., Yongjie, G. U., Hui, G., Xiaohua, L., and Xiaoyong, C. (2007). Maintenance and natural regeneration of *Castanopsis sclerophylla* populations on islands of Qiandao Lake Region. *Acta Ecologica Sinica* **27** , 424-431.

Figure legends

Figure 1. Effect of drying on germination of *Castanopsis sclerophylla* seeds. Different lower-case letters indicate significant difference in germination between seeds dried to various moisture contents ($P < 0.05$).

Figure 2 Photomicrographs and CT images of *Castanopsis sclerophylla* seeds (a) half cotyledon with embryo; (b) growth of the embryo; (c) the top view; (d) the front view. SC, seed coat; COT, cotyledons; EMB, embryo.

Figure 3 Photographs of *Castanopsis sclerophylla* seeds at different germination states (a) the whole seed; (b) 0h; (c) 48h; (d) 96h; (e) 144h; (f) 240h. SC, seed coat; SR, scar; AP, apex; R, radicle, COT, cotyledons; S, stem, RT, root.

figures/fig1/fig1-eps-converted-to.pdf

