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Changes in Seed Germination Ability, Lipid Peroxidation and Antioxidant Enzyme Activities of *Ginkgo biloba* Seed during Desiccation

Jing Feng [1,2], Yongbao Shen [2,3,4,*], Fenghou Shi [2,3,4] and Chengzhong Li [5]

1 College of Landscape Architecture, Nanjing Forestry University, Nanjing 210037, China; fengjing9520@yeah.net
2 Collaborative Innovation Center of Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China; fhshi406@163.com
3 Southern Tree Seed Inspection Center, National Forestry Administration, Nanjing 210037, China
4 College of Forestry, Nanjing Forestry University, Nanjing 210037, China
5 Department of Horticulture, Jiangsu Agri-animal Husbandry Vocational College, Taizhou 225300, China; chengzhongli11@126.com
* Correspondence: ybshen@njfu.edu.cn; Tel.: +86-25-8542-7403; Fax: +86-25-8542-7402

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Abstract: With the aim of investigating the antioxidant system and germinability in response to the desiccation of *Ginkgo biloba* seeds, they were put in a drying room (25 ± 2% relative humidity, 25 °C) for 67 days. Results showed that the germination rate remained constant when seed moisture content (MC) decreased from 48% (fresh seeds) to 45.1%. However, when MC reached 40.1%, the germination percentage decreased from 92% to 50%. A significant positive correlation was observed between the MC and seed germination percentage (r = 0.910). The electrical conductivity was significantly increased during the initial desiccation (48–45.1%). Furthermore, both the superoxide dismutase (SOD) and peroxidase (POD) activity first reduced, then elevated to peak values before they declined again. POD activity rose earlier than SOD activity, indicating that the POD reaction was more desiccation-sensitive than the SOD. Significant negative correlations were observed between the MC and malondialdehyde (MDA) content (r = −0.619) and electrical conductivity (r = −0.745). Our collective results suggest that *G. biloba* seeds are highly sensitive to desiccation. Excessive desiccation could reduce the antioxidant enzyme activity of *G. biloba* seeds and intensify membrane lipid peroxidation, which causes the consequent reduction—or even the complete loss—of seed germinability.

Keywords: desiccation sensitivity; seed germination percentage; recalcitrant seed; lipid peroxidation

1. Introduction

Ginkgo (*Ginkgo biloba*) is an ancient relict species of gymnosperms that originated 280 million years ago [1]. It is native to China, and is commonly referred to as a living fossil. *Ginkgo biloba* is a deciduous tree belonging to Ginkgoaceae, Ginkgo, which is distributed in temperate and subtropical climate zones [2]. Englbert Kaempfer suggested the name Ginkgo in 1772, while Linnaeus had termed it *Ginkgo biloba* in 1771 [3]. *Ginkgo biloba* is one of the most important economic tree species in China, and its leaves are also valuable medicinal herbs. The seeds are edible as traditional health foods and are commonly known as ginkgo nuts in China.

In the wild, pollen matures in late April and begins pollination; however, fertilization does not occur until mid-August. The process from pollination to fertilization takes approximately 120 days [4–6]. Mature seeds have a large thousand kernel weight, and are composed of fleshy sarcotesta

(orange-yellow and glaucous), a hard, stony sarcoderm, (white, with two or three longitudinal ridges), a membranous endopleura (reddish brown), and an embryo surrounded by female gametophyte.

Like most tropical plants, subtropical plants produce seeds with a high moisture content (MC), for example, the seed MC of *Mangifera indica*, *Calophyllum polyanthum*, and *Persea americana* is 38%, 45%, and 52.5% respectively [7,8]. Ginkgo seeds also have a high moisture content (41%) [9] and active metabolism after shedding. Seed viability can be preserved at 4 °C for one year, but their capability to germinate decreases after six months [10]. The high moisture content of fresh seeds suggests that it may be recalcitrant [11]. In this study, *G. biloba* seeds could not be directly germinated after collection, and there were some inembryonate seeds. Generally, the main reason for this is due to the not-fully-developed embryo, which has the ability to germinate only when it continues to grow under certain conditions. The mechanism of embryo developmental-delay may be to prevent seeds from premature germination in the fall and survive winter [12]. Many studies have confirmed that either constant warm or cold stratification can generate continuous growth in the embryo and accelerate germination. For instance, the embryo of *G. biloba* significantly increased by 34% under 2–5 °C stratification; when placed in a warm greenhouse environment, the embryo will grow to its full size and begin germination. Nevertheless, the embryo development of *G. biloba* is related to the seed collecting period, and most seed embryos are fully developed (reach two-thirds of seed length) on the tree until abscission (in mid-December) [13].

Roberts [14] classified seeds according to their physiological behavior. Orthodox seeds obtain dehydration tolerance during maturation. This change plays a key role in the seed state from developmental stages to germination. Usually, the MC of seeds is low at the time of abscission, and can be further dried to 1% to 5% MC without injury. Germination only occurs when water and other environmental conditions are satisfied. In contrast, recalcitrant seeds do not have a dehydration process, so when seeds disperse from the parent plant, they have a high MC and quickly lose their germination ability within a few days to several weeks when stored below a threshold moisture level [15]. Desiccation sensitivity is the most important characteristic of recalcitrant seeds, and different seeds display various degrees of desiccation sensitivity [16]. The antioxidant system plays a key role in scavenging excess reactive oxygen species (ROS) during seed desiccation [17]. Under normal water content, the biological production and elimination of free radicals maintain dynamic equilibrium, but when seeds are subjected to water stress, the electron transfer of cells is blocked, so stress can generate and cause excessive accumulation of reactive oxygen species (O^{2-}, H_2O_2, ·OH, and $^1O_2$) [18]. When the balance is broken, lipid peroxidation is induced and exacerbated, resulting in the denaturation of nucleotides and proteins, finally leading to cell death [19]. Superoxide dismutase (SOD), peroxidase (POD), and other antioxidant enzymes play an extremely important role in scavenging excess ROS [20]. Recently, several studies have reported that reasons for the loss of viability in recalcitrant seeds were closely related to the damage of the antioxidant system [21–23]. Bailly [24] reported that seed desiccation tolerance may be related to the protective mechanism of intracellular scavenging oxidation reactions. Desiccation sensitivity of the *Osmanthus fragrans* ZibingZiyingui seed was closely related to a rapid decrease in the activity of antioxidantenzymes [25]. The loss of viability of *Quercus robur* embryos was associated with the increase in damaged free radical and cell membrane permeability [26]. Pukacka [27] found that the rapid dehydration of *Quercus robur* seeds led to the mobilization of the antioxidant system in embryonic axes—particularly increased levels of POX and SOD activities.

To our knowledge, no studies have reported on the recalcitrance of *G. biloba* seeds. Thus, the aim of this work was to examine the desiccation sensitivity of *G. biloba* seeds where physiological and biochemical changes were evaluated to elucidate the relationship between seed viability, germination ability, and antioxidant metabolism to provide technical guidance for the preservation and storage of its germplasm resources.

2. Materials and Methods

2.1. Plant Materials and Experimental Designs

On 3 December 2014 at the beginning of natural dispersion, mature fruits were collected from ten trees in Jiangsu Agri-animal Husbandry Vocational College, Taizhou City, Jiangsu Province. The mixed mature fruits were transported to the laboratory of Nanjing Forestry University on the following day. Seed viability was 98 ± 1% (mean ± SE), as confirmed by a positive tetrazoliumtest [28]. In this experiment, 85 ± 3% (mean ± SE) seed embryos were fully developed (reaching two-thirds of seed length) on the tree (3 × 100 seeds were checked). Immediately after removing the sarcotesta, five thousand seeds were sampled at random for this experiment and stored at 4 °C. On 9 December 2014, seed initial MC was determined.

Ginkgo biloba seeds were evenly placed on a plastic film on the floor in a drying room (25 ± 2% relative humidity, 25 °C). Three × 100 seeds were taken out and put in three open plastic boxes beside the plastic film, and these seeds were reweighed at 12:00 pm every day in order to estimate the relative MC of all *G. biloba* seeds. Seed relative MC (R) was calculated with Equation (2). When seed relative MC was monitored at roughly 5% intervals, Equation (1) was used to determine seed MC. Thus, samples were randomly taken for germination testing and enzyme activity determination after about 5% decrease in MC was found each time (from 48.0% to 15%).

2.2. Measurements

2.2.1. Determination of Moisture Content (MC)

According to the International Seed Testing Association [28], seeds were sliced into small pieces and spread evenly in an aluminum case. The aluminum case with the lid (W1) was weighed before placing the sample inside; afterwards, the sample and the aluminum box (together with the lid, W2) was weighed. An aluminum lid was placed on the aluminum box and dried at 130 ± 2 °C for 4 h in an oven, before quickly covering with an aluminum lid and drying for 30–45 min before the sample and the aluminum box (together with the lid, W3) was weighed again. Three replications of five seeds were used to determine the seed MC. Seed MC was calculated by the following formula:

$$\mathrm{MC} = \frac{\mathrm{W2} - \mathrm{W3}}{\mathrm{W2} - \mathrm{W1}} \times 100\% \quad (1)$$

Three × 100 seeds were chosen at random, according to the measured initial seed MC (A), the weight of seed before (M1) and after (M2) desiccation, and seed relative MC (R) was calculated by the following formula:

$$R = \frac{\mathrm{M2} - \mathrm{M1} \times (1 - \mathrm{A})}{\mathrm{M2}} \times 100\% \quad (2)$$

2.2.2. Germination Test

A seed germination test was performed as per the International Seed Testing Association [28]. Four × 50 seeds were sampled for each test when MC was at 45.1%, 40.1%, 34.9%, 30.0%, 25.0%, 20.0%, and 15.0%. After removing the seed coat, the de-coated seeds were put on moistened cotton wool with deionized water in plastic germination boxes and germinated in a growth chamber at a constant temperature of 25 °C (Figure 1). The seeds were recorded when their radicle elongated to 2 mm (n). The germinated seeds were counted daily over the 20-day incubation period. *G. biloba* seeds have an inembryonate phenomenon, where to eliminate the effect of embryoless seeds (NE) on the tests and obtain an accurate germination rate, both germination rate and embryoless seeds were counted. The germination rate (GR) was calculated by the following formula:

$$GR = \frac{\mathrm{n}}{N - NE} \times 100\% \quad (3)$$

where *GR* is the germination rate; n is the seeds that normally germinate; *N* is the tested seeds (50); and *NE* is the embryoless seeds.

Figure 1. Germination test of *G. biloba* seeds during desiccation. (**a**) De-coated seed; (**b**) cotton with deionized water; (**c**) plastic germination boxes (cover not shown).

2.2.3. Electrical Conductivity Assay

Electrical conductivity was assayed according to Xu [29] and Bailly [24]. Three samples of 20 seeds were dehydrated to different MC with uniform size, and no mechanical damage was used for testing. The seeds were first washed three times with water, then rinsed with deionized water several times, and soaked in a 500-mL conical flask with 200 mL deionized water (Milli-Q; Billerica, MA, USA). After being soaked at 25 °C for 12 h, electrical conductivity (S1) was measured at regular intervals with a conductivity meter (Model DDS–IIA; Shanghai, China). The bottle was sealed with a plastic film, boiled for 30 min, and cooled again to 25 °C before the absolute electrical conductivity (S2) was measured. Deionized water without seeds was used as blank control (E). In order to eliminate the influence of interindividual variation in seeds, electric conductivity was presented in percentage, and that was electric conductivity at different soaking times relative to the absolute conductivity. All measurements were performed in triplicate. Electrical conductivity (*L*) was calculated by the following formula:

$$L = \frac{\mathrm{S1} - \mathrm{E}}{\mathrm{S2} - \mathrm{E}} \times 100\% \quad (4)$$

where *L* is the electrical conductivity; S1 is the initial electrical conductivity; S2 is the absolute electrical conductivity; and E is the electrical conductivity of deionized water.

2.2.4. Determination of Anti-Oxidation System (SOD, POD Activity, and Malondialdehyde (MDA) Content)

Twenty *G. biloba* seeds were randomly selected. The embryos were stripped out, cut into small pieces, and mixed well. Next, three samples of 0.3 g embryo tissues (which were randomly selected)

were ground and extracted using a pre-chilled mortar and pestle in 6 mL of 50 mM phosphate buffer (pH 7.8). The extract was centrifuged at 3000 $\times g$ for 20 min and stored at 4 °C.

The total SOD activity was assayed by the capacity to reduce nitro blue tetrazolium (NBT) as described by Giannopolitis and Ries [30]. A 3 mL assay mixture contained 0.05 mL extract (0.05 mL phosphate buffer was used as the control), 1.5 mL 50 mM phosphate (pH 7.8), 0.3 mL 130 mM methionine, 0.3 mL 750 µM nitroblue tetrazolium, 0.3 mL 20 µM riboflavin, 0.3 mL 10 µM EDTA-Na_2, and 0.25 mL deionized water. The photo-reduction of NBT (formation of purple formazan) was measured at 560 nm, and an inhibition curve was made against different volumes of extract. One unit of SOD was defined as the amount of enzyme that caused SOD-inhibition of the photo-reduction of NBT by 50%.

POD activity was measured by the method described by Kochba [31]. The reaction mixture consisted of 2.9 mL 50 mM phosphate buffer (pH 5.5), 1.0 mL 2% H_2O_2, 1.0 mL 50 mM guaiacol, and 0.1 mL enzyme extract for 15 min at 37 °C. The same reaction mixture was boiled for 5 min as a control, then cooled with ice water immediately. The reaction was terminated with the addition of 2.0 mL 20% trichloroacetic acid (TCA). The increase in the absorbance due to the oxidation of guaiacol was measured at 470 nm.

The level of lipid peroxidation was expressed as MDA content and was determined according to Cakmak and Horst [32]. The reaction mixture in 3 mL contained 50 mM phosphate buffer (pH 7.8) and 0.5% 2-thiobarbituric acid (TBA). The extract was heated at 100 °C for 10 min and then immediately cooled on ice. After centrifugation at 3000 gn for 10 min, the absorbance of the supernatant was measured at 532 nm and subtracted from the absorbance at 600 nm as the correction of non-specific turbidity.

2.3. Statistical Analysis

The data were analyzed by SPSS (Version 23.0; IBM Corp., Armonk, NY, USA) and EXCELL (Version 2013; Microsoft Corp., Redmond, WA, USA) software. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). A correlation analysis was performed estimating Pearson's correlation coefficients. All reported values in this paper were mean ± SD for three replicates in each group. p-values < 0.05 were considered significant.

3. Results

3.1. Germination Changes and Electrical Conductivity

The MC of fresh *G. biloba* seeds was 48%. During desiccation, the MC of *G. biloba* seeds decreased gradually (Figure 2), and the seed germination percentage changed greatly. The initial desiccation process was slow, and the time required for the MC from 48–45.1% and 45.1–40.1% was 16 d and 13 d, respectively. However, it then sped up, and the time required for each 5% MC decrease was only about a week. Seed germination remained fairly constant when seed MC decreased to 45.1% (16 d). As desiccation continued, the seed germination percentage decreased (Figure 3); however, when the seed MC decreased to 40.1% (29 d), the germination percentage decreased from 92% to 50%. When the MC declined to 15% (67 d), the germination percentage was only 11%. The ANOVA results showed that there was a significant difference between seed MC and other indices during desiccation (Table 1). The analysis of variance showed that the MC of *G. biloba* seeds had a significant effect ($p < 0.01$) on seed germination percentage (Figure 3). These data indicate that *G. biloba* seeds are highly sensitive to desiccation.

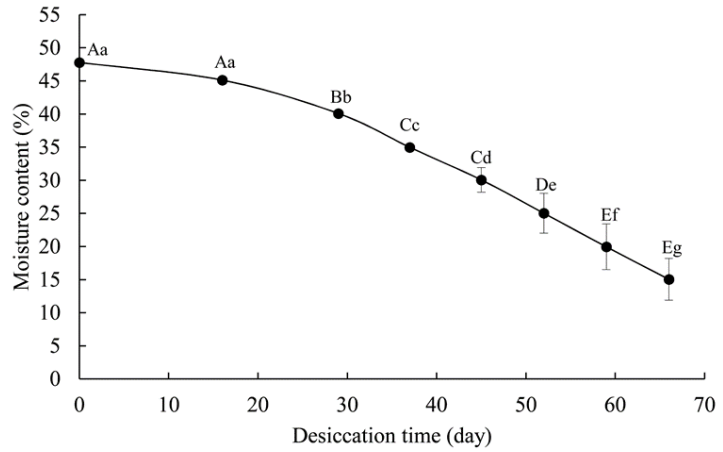

Figure 2. Changes to moisture content (MC) in *G. biloba* seeds during desiccation.

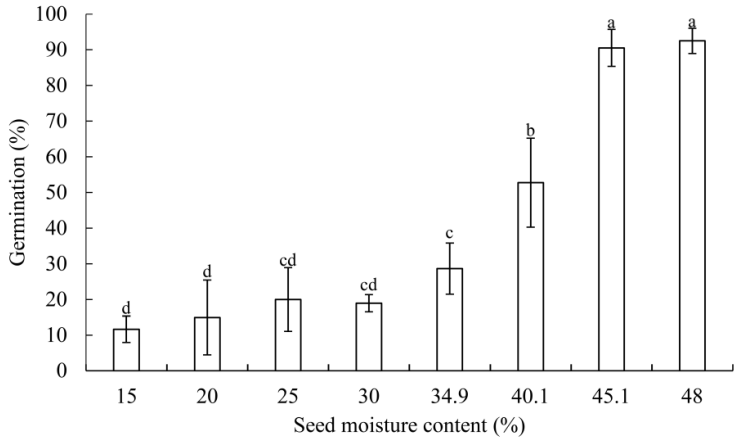

Figure 3. Changes of germination in *G. biloba* seeds during desiccation. Data are means of three replicates ± SD, and bars labeled with the same lower-case letter do not differ significantly at the 0.05 probability level.

Table 1. Results of a one-way ANOVA for physiological changes in *G. biloba* seed during desiccation.

Source		Sum of Squares	df	Mean Square	F	*p*
Germination percentage	Between Groups	2.359	7	0.337	116.831	<0.001
	Within Groups	0.046	16	0.003		
	Total	2.405	23			
Electrical conductivity	Between Groups	0.269	7	0.038	39.305	<0.001
	Within Groups	0.016	16	0.001		
	Total	0.284	23			
SOD activity	Between Groups	279,608.451	7	39,944.064	7.252	0.001
	Within Groups	88,130.424	16	5508.152		
	Total	367,738.876	23			
POD activity	Between Groups	553.311	7	79.044	15.173	<0.001
	Within Groups	83.350	16	5.209		
	Total	636.661	23			
MDA content	Between Groups	996.525	7	142.361	3.085	0.029
	Within Groups	738.356	16	46.147		
	Total	1734.880	23			

MDA: malondialdehyde; POD: peroxidase; SOD: superoxide dismutase.

Data obtained for *G. biloba* seed at 25 °C, moisture content (MC) at 45.1%, 40.1%, 34.9%, 30.0%, 25.0%, 20.0%, and 15.0% during desiccation.

Seed electrical conductivity increased gradually with seed desiccation, as shown in Figure 4. When the seed MC went down from 48% to 40.1%, the electrical conductivity of seeds significantly increased to 57% ($p < 0.01$). The seed electrical conductivity rose to a maximum 80.2%, and seeds almost lost germinability when the MC decreased to 30%, but maintained stability and had no significant difference ($p > 0.05$) as the MC decreased from 30% to 15%. In conjunction with Figure 3, changes of seed germination percentage from different MC showed that when the MC of *G. biloba* seeds dropped to about 40%, the membrane system had been seriously damaged.

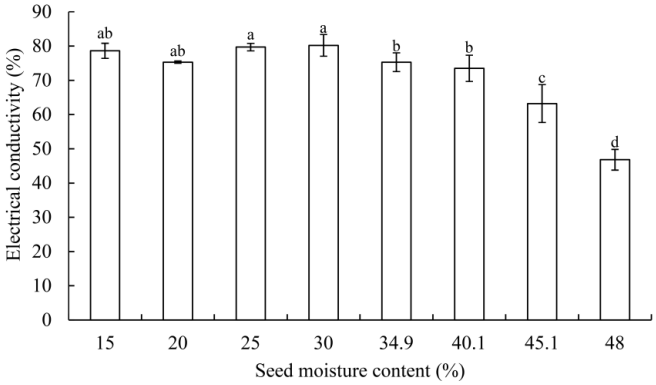

Figure 4. Changes of electrical conductivity in *G. biloba* seeds during desiccation. Data are means of three replicates ± SD, and bars labeled with the same lower-case letter do not differ significantly at the 0.05 probability level.

3.2. SOD, POD Activity, and MDA Contents

Figure 5 shows that the SOD activity in fresh embryos without desiccation was 739 $U \cdot g^{-1}FW \cdot h^{-1}$. At early stages of desiccation, SOD activity decreased significantly, and when the MC dropped to 40.1%, SOD activity decreased to 671 $U \cdot g^{-1}FW \cdot h^{-1}$. However, due to the protective mechanisms of the body, SOD activity increased significantly with continued desiccation. When the MC decreased to 25%, SOD activity reached a maximum of 920 $U \cdot g^{-1}FW \cdot h^{-1}$—far more than the initial SOD activity. However, this seed protection was very limited. As desiccation stress increased, SOD activity decreased significantly, so the seed lost the protection mechanism. Changes of the POD activity were similar to those seen for SOD (as shown in Figure 6), and only rose earlier than the SOD. In other words, after seed MC decreased to 45.1%, POD activity increased significantly, then reached a maximum of 31.64 $\mu g \cdot g^{-1}FW \cdot min^{-1}$ and declined sharply when MC was less than 30.0%.

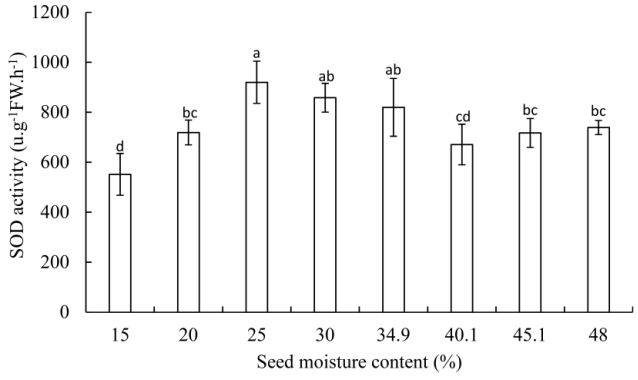

Figure 5. Changes of superoxide dismutase (SOD) activity in *G. biloba* seed embryos during desiccation. Data are means of three replicates ± SD, and bars labeled with the same lower-case letter do not differ significantly at the 0.05 probability level.

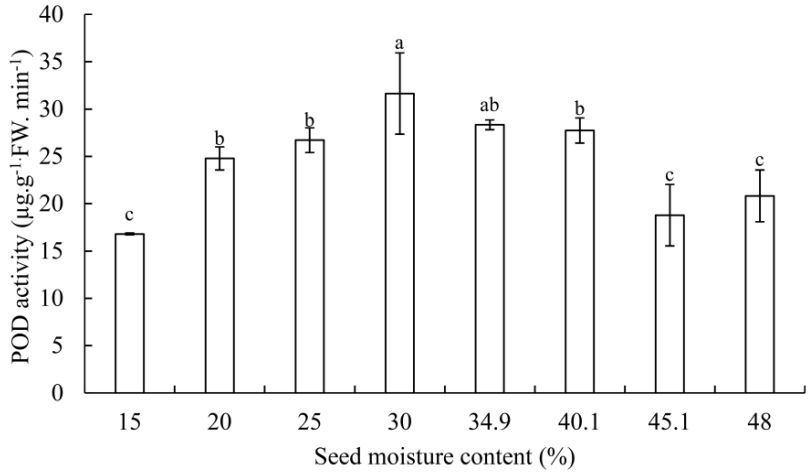

Figure 6. Changes of peroxidase (POD) activity in *G. biloba* seed embryos during desiccation. Data are means of three replicates ± SD, and bars labeled with the same lower-case letter do not differ significantly at the 0.05 probability level.

G. biloba seeds had a significant membrane peroxidation reaction during desiccation, where the content of reaction product MDA decreased from an initial desiccation of 19.02 mmol·g^{-1}FW to 14.01 mmol·g^{-1}FW (45.1% MC). With the change of SOD and POD, we found that the *G. biloba* seed MC decreased slightly, and the seed did not have significant damage. Desiccation damage occurred after the MC deceased to 45.1%, as shown in Figure 7. When the MC declined to 15%, MDA content increased to 34.43 mmol·g^{-1}FW, rising by 81.0%.

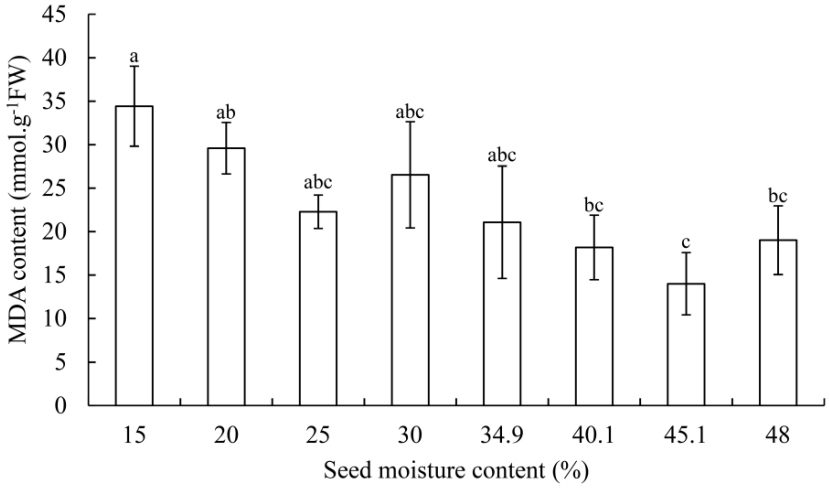

Figure 7. Changes of malondialdehyde (MDA) content in the *G. biloba* seed embryos during desiccation. Data are means of three replicates ± SD, and bars labeled with the same lower-case letter do not differ significantly at the 0.05 probability level.

3.3. Correlation Analysis

A significant correlation was observed between the seed MC and other indices during desiccation (Table 2), where there was a significant positive correlation between seed germination percentage and MC. According to Figure 2, the decrease in MC had a serious effect on the seed germination percentage. In addition, MC had no significant correlation between SOD and POD activity, but a significant positive correlation was observed with SOD and POD activity. This indicated that the two important protective enzymes SOD and POD had a synergistic effect, and had stronger activity in

the middle stage of desiccation (Figures 5 and 6). In contrast, a significant negative correlation was observed among the MC, MDA content, and electrical conductivity. These data suggest that with an increase in desiccation, membrane peroxidation was enhanced and the accumulation of MDA content was increased, resulting in the loss of seed germinability.

Table 2. Correlation coefficients (*r*) for physiological changes in *G. biloba* seed during desiccation.

Physiological Indicators	MC	SOD Activity	MDA Content	POD Activity	Electrical Conductivity	Germination Percentage
MC	1					
SOD activity	0.118	1				
MDA content	−0.619 *	−0.373	1			
POD activity	−0.002	0.595 *	−0.065	1		
Electrical conductivity	−0.745 *	0.092	0.421 *	0.439 *	1	
Germination percentage	0.910 *	−0.107	−0.545 *	−0.364	−0.864 *	1

* Significant at 0.05 *p* level; others are non-significant.

4. Discussion

4.1. Changes of Germination in G. biloba *Seeds during Desiccation*

King and Roberts [33] believed that the response of recalcitrant seeds and decreased MC was a non-linear relationship where an inflection point (critical moisture content) existed in the MC–Seed viability curve. At this point, the influence of desiccation on seed viability is very small, and beyond this limit, seed viability would decline sharply. Seeds have different levels of sensitivity to desiccation, suggesting that the critical moisture content is different and dependent on seed species. In this study, we found that when the MC dropped to 40.1% (29 d), it caused a serious impact on *G. biloba* seeds, where approximately half of the seeds did not germinate. Furthermore, each desiccation time interval from 40.1% to 15% MC was half as long as the interval from 48% to 40.1% MC, suggesting that the range of critical moisture content was between 45% and 40.1%, and only decreased by less than 8% when compared to fresh seeds. In this context, *G. biloba* seeds were still germinating even at 15% MC; this may be because these seeds were actually higher in MC than estimated, or because they were able to germinate at 15% MC, and further investigations are required. Investigating the MC and seed germinability by desiccation can be used to verify the recalcitrant seeds, and desiccation sensitivity was reported for several species. For example, a reduction in MC from 35% to 4% in *Campomanesia pubescens* seeds led to a decreased seed germination potential, rendering these seeds as recalcitrant [34]. Furthermore, germination percentage was zero after 30 days of desiccation when seed moisture content had decreased from 60.3% to 30.2%, suggesting that *Aesculus chinensis* seeds were also sensitive to desiccation and could be classified as recalcitrant [35]. Therefore, our results suggested that the *G. biloba* seeds were highly sensitive to desiccation and could be classified as recalcitrant.

4.2. Changes of Electrical Conductivity in G. biloba *Seeds during Desiccation*

The characteristics of the seeds that are intolerant to desiccation are reflected in the significant changes in both the cell membrane structure and damage to cell integrity. Seed desiccation sensitivity was as follows: when MC decreased to a certain extent, the structure of the seed cell changed significantly and the integrity of the cell was destroyed [36]. Furthermore, the electrolyte leakage rate could directly reflect the desiccation sensitivity of recalcitrant seeds [37]. The electrical conductivity of *G. biloba* seeds showed that the MC of *G. biloba* seeds decreased from 48% to 40.1%, and as water stress increased, the function and structure of the membrane system was severely damaged, causing a large leakage of electrolytes, resulting in a rapid increase in electrical conductivity. Similar trends were observed in *Campomanesia adamantium* seeds during desiccation [38]. In addition, the seed germination

percentage also decreased significantly. At this point, half of the seeds lost their germination ability, suggesting that the seeds were highly sensitive to desiccation. A negative correlation was found between electrical conductivity (EC) and germination, which resulted from high levels of leakage from non-germinating (dead) seeds.

Nevertheless, when compared to Figures 3 and 4, a difference was found where electrical conductivity increased significantly ($p < 0.05$), but germination rate remained constant ($p > 0.05$) during initial desiccation (48–45.1%). At this stage, *G. biloba* seeds leaked more than 70% of their cellular electrolytes, and analysis showed that there was no significant difference between 30% and 15% MC (Figure 4), indicating that the cell membranes of *G. biloba* seeds had been damaged. Thus, it is possible that the process of membrane injury may have begun in the embryonic axes [39], although it had yet to have any effect on seed germination.

4.3. Changes of SOD and POD Activity in G. biloba *Seeds during Desiccation*

Enzymatic antioxidant systems can scavenge reactive oxygen species that are produced under stress conditions. Many studies have confirmed that antioxidants such as SOD and POD were shown to have increased in response to desiccation [40,41], and may act as a "reservoir" and determine the period where a plant can remain desiccated before its viability is compromised [42]. During desiccation, the SOD and POD activity of *G. biloba* seed embryos were first reduced, then elevated to the highest value, before declining. This was in contrast to the trends observed in other recalcitrant seeds such as *Camellia sinensis* [43], and *Clausena lansium* [44] seeds, which increased first and then decreased. This indicated that seeds of tree species varied in the sensitivity of the antioxidant system to desiccation, and *G. biloba* seeds have their own characteristics where no such research has been previously reported. Specifically, germination rate declined sharply when MC decreased to 40.1%. However, the SOD and POD activity of embryos in *G. biloba* seeds first reduced slightly, but due to the protective mechanisms of the body, SOD and POD activity increased significantly as desiccation continued. Moreover, this protection was very limited. With desiccation stress, SOD and POD activity decreased significantly and the body lost its protection mechanisms. In this context, the two important protective enzymes SOD and POD in *G. biloba* seeds during desiccation showed strong activity in "mid-term" desiccation, where the POD reaction was more sensitive than the SOD.

4.4. Changes of MDA Content in G. biloba *Seeds during Desiccation*

The decreased activity of the antioxidant system and the enhancement of lipid peroxidation were the main reasons for the declined viability of recalcitrant seeds during desiccation [45,46]. The negative effects of seed desiccation were confirmed by the results of MDA content, where the highest values were obtained when MC was reduced from 48% to 15%. Furthermore, a significant correlation between MDA content and electrical conductivity ($r = 0.439$) was also observed. This may have been due to loss of the selective permeability of cellular membranes, suggesting that membrane integrity is vital, since any desiccation damage may have immediate consequences for the seeds' germinability. Recent studies have confirmed that decreased viability of recalcitrant seeds was closely related to the accumulation of membrane lipid peroxides, such as in *Camellia sinensis* [47]; *Quercus robur* [48]; *Trichilia connaroides* [49]; and other recalcitrant seeds. Thus, the desiccation of *G. biloba* seeds accompanied by a deepening degree of membrane lipid oxidation led to the severe damage of the membrane system, causing a large accumulation of MDA. This was consistent with seeds of *Azadirachta indica* [50] and *Pachira macrocarpa* [51]. However, it is important to mention that a decline from 30% to 25% MC was observed in Figure 7, where with respect to SOD and POD, the peak values were found at 25% and 30% MC, respectively (Figures 5 and 6), and the germination percentage kept constant. This indicates that the antioxidant system remained active in the middle of desiccation.

5. Conclusions

Slight water loss (8%) had a serious impact on the *G. biloba* seed germination rate during desiccation. The range of critical moisture content was between 45% and 40.1%, which suggests that this was the optimum moisture content for maintaining a high germination rate. Antioxidant systems were more highly mobilized at the beginning of desiccation. SOD and POD showed strong performances during "mid-term" desiccation, but the POD reaction was more sensitive than SOD. As previously stated, this protection was very limited; with desiccation stress, the metabolic balance was broken and caused excessive accumulation of reactive oxygen species, leading to the loss of membrane integrity and cell death. It should be noted that we have not described the seed development in relation to desiccation, and the relationship between different desiccation rates and the germination rate have yet to be fully elucidated. With respect to antioxidant systems, this study only concentrated on the antioxidant enzymes SOD and POD. Further experimentation is warranted to fully clarify the mechanisms for desiccation. Notwithstanding its limitations, this study indicated that *G. biloba* seeds are sensitive to desiccation and can be classified as recalcitrant.

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