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Study on Desiccation Tolerance and Biochemical Changes of *Sassafras tzumu* (Hemsl.) Hemsl. Seeds

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Abstract: The deciduous tree species *Sassafras tzumu* (Hemsl.) Hemsl., unique to China, holds significant economic and ecological value. However, its seeds exhibit poor storage tolerance and rapid decline in seed vigor. This study primarily investigates the desiccation tolerance of *S. tzumu* seeds. The results show that *S. tzumu* seeds have recalcitrant seed characteristics, with a semi-inactivation water content (at which point half of the seeds lose viability) of 20.7%. As desiccation progresses, seed viability decreases significantly; at a reduced water content of 11.93%, only 18.3% of the seeds remain viable, while most lose their viability completely. Relative electrolytic leakage (REC) and H_2O_2 content gradually increase during this process, while MDA content initially decreases before increasing again, exhibiting distinct trends compared to antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). SOD and POD activities exhibit an initial increase followed by a rapid decrease, whereas CAT activity shows a decline followed by a rapid increase. Dehydration to 15% water content in seeds is a key turning point in the process of seed desiccation in *S. tzumu*, and CAT is an enzyme key to maintaining seed viability. Both the accumulation of toxins and the decline in the activity of the antioxidant system contribute to the susceptibility of *S. tzumu* seeds to drought stress, a characteristic common to all recalcitrant seeds. To maintain high seed viability above 70% during storage, it is crucial to ensure water content above 23.58%.

Keywords: antioxidant system; recalcitrant seed; ROS; seeds viability; tree; water content

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1. Introduction

Sassafras tzumu (Hemsl.) Hemsl., a member of the genus *Sassafras* in the family Lauraceae, is a deciduous tree species with unique economic and ecological value in China. It can reach heights of up to 35 m and have a diameter at breast height (DBH) of 2.5 m. This native tree species occurs naturally in provinces such as Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, and Jiangsu [1]. The roots and bark of *S. tzumu* are utilized for their anti-inflammatory and wound-healing properties, while its wood is highly valued due to its hardness, beauty, durability, and innate resistance to corrosion [2]. It is particularly characterized by its rapid growth, with circular holes that enhance its aesthetic appeal and natural resistance to decay [3–5]. *S. tzumu* also possesses ornamental value through its yellow flowers blooming before leaf emergence in early spring followed by crimson foliage during autumn and the brown fruit ripening in October. Furthermore, various part, including stems, leaves, fruits, and petals, contain oil cells, making it an aromatic plant [6]. At present, *S. tzumu* is difficult to propagate from cuttings and tissue culture and therefore relies mainly on seed propagation [7]. However, the seeds of *S. tzumu* exhibit deep dormancy, and current production practices require an 8-month period of cold stratification to overcome this dormancy [8]. There has been limited research on *S. tzumu* seeds, and their storage characteristics remain unclear, while their long dormancy

period poses challenges for conservation and cultivation. In order to conserve resources and maximize the use of *S. tzumu* and related dormant seeds, it is critical to thoroughly investigate the desiccation tolerance of *S. tzumu* seeds.

Seeds can be classified according to their storage characteristics as orthodox seeds, recalcitrant seeds, and intermediate seeds [9,10]. Orthodox seeds can maintain seed viability at low water content after desiccation is completed prior to maturity. Recalcitrant seeds, on the other hand, have no pre-desiccation process during maturation and usually have high water content and metabolic levels when they fall off. Throughout development and postharvest storage, recalcitrant seeds of tropical origin are sensitive to desiccation and easily lose viability after desiccation [11]. In general, viability is significantly reduced when water content is less than 10%–15% [10]. Understanding the desiccation tolerance of seeds is the basis for long-term seed storage and utilization.

Desiccation sensitivity (tolerance) is a comprehensive attribute acquired during seed development [12], which refers to the adaptive changes made by cells in seeds at specific developmental stages to survive the immediate stress imposed on organelle and macromolecular structures when water is removed [13] and refers to their ability to tolerate desiccation and drying while maintaining viability in the later stage [14]. Late embryogenesis-abundant proteins (LEAs) are a very important feature in the acquisition of desiccation tolerance [15]. The interactions between metabolic shutdown, organelle dedifferentiation, and reduction of the membrane system elements limit the membrane targets of uncontrolled metabolism and the subsequent formation of free radicals and reactive oxygen species (ROS) [16]. In seeds, ROS are produced and removed in a dynamic equilibrium under typical conditions. This equilibrium is disrupted when seeds are exposed to water stress, which causes a significant accumulation of ROS, induces membrane lipid peroxidation, and leads to oxidation of membrane lipids, proteins, and nucleic acids, resulting in a variety of irreparable damages [17]. In this process, membrane lipid peroxidation and reduced function of the antioxidative system are closely linked to reduced seed viability [18]. At intermediate water contents, seed respiratory metabolism is uncontrolled, and seeds may be affected by free radicals at this stage. Water loss damage to recalcitrant seeds may also occur at this stage [19]. Reactive oxygen species are largely removed from recalcitrant seeds by antioxidant enzymes such as SOD, POD, and CAT. The decline in antioxidant function and the increase in membrane lipid peroxidation accelerate the decline in seed viability [18]. The accumulation of lipid peroxides occurs after cell death, suggesting that the loss of seed viability may actually be caused by the accumulation of lipid peroxides rather than the other way around [20].

S. tzumu is mainly propagated by seed, and its low seed yield, seed storage intolerance, and deep dormancy characteristics continue to cause numerous problems in resource utilization [21]. In this study, the desiccation tolerance of *S. tzumu* seeds was determined by comparing the storage characteristics of recalcitrant seeds and intermediate seeds, and the critical water content of *S. tzumu* seeds was established by measuring the changes in vitality and antioxidant system of *S. tzumu* seeds during natural desiccation. It provides a theoretical foundation for the long-term preservation of *S. tzumu* seeds and their postharvest handling. It also provides a reference for the storage characteristics of recalcitrant seeds and deeply dormant seeds in the north temperate zone.

2. Materials and Methods

2.1. Plants Materials

The flowering period of *S. tzumu* occurs in March and April, while the fruit reaches maturity in October. The *S. tzumu* seeds were collected from the Wulongshan State-owned forest farm (119°29′59″ E, 30°91′28″ N), Guangde City, Anhui Province. The seed collection area belongs to the northern subtropical climate zone, with an average altitude of 276.4 m. The average annual temperature is 15.4 °C, the average maximum temperature is 36.5 °C, with a mean maximum temperature of 36.5 °C and a mean minimum temperature of −4.3 °C. The annual rainfall is 1230 mm, and the average annual sunshine is 2162 h. The

climate is characterized by its mildness, abundant rainfall, and ample sunshine throughout the year, exhibiting distinct seasonal variations including hot and rainy seasons. Before testing, the pulp is washed, and the waxy layer is left on the seed surface. Fresh *S. tzumu* seeds have a viability rate of 81.3% ± 2.54%, a weight of 76.32 ± 0.55 g per 1000 seeds, and an initial water content of 29.98% ± 1.72%.

2.2. Seed Desiccation Treatment

The intact seeds were placed on the testing bench under controlled conditions of 24 ± 1 °C and 58% relative humidity. They were stored in a cool, well-ventilated area, with measurements taken every three days to monitor changes in seed water content. A total of 18 g of seeds were randomly selected and placed on small trays. The initial mass was calculated as M1, the initial water content as a%, and the mass after water loss as M2. When the relative water content of the seeds decreased by about 3%, random sampling was used to identify the relevant indicators, including seed viability, relative conductivity, POD activity, SOD activity, CAT activity, MDA content, and H_2O_2 content. This was conducted by monitoring the changes in seed water content in real time (indirect calculation method to determine seed water content) [22,23]. The relative water content is calculated as follows:

$$\text{Relative water content } (\%) = \frac{\text{M2} - \text{M1}(1 - \text{a}\%)}{\text{M2}}.$$

2.3. Determination of Seed Viability

Tetrazolium staining (TZ) method was used to determine seed viability [24]. From the dehydrated seeds at different desiccation times, 3 × 50 seeds were randomly selected. The hard seed coat was removed, but the seed embryo would remain intact. The vitality of the seeds was assessed by immersing them in 1% tetrazolium for 20 h at 35 °C after removing the seed shells. After staining, the results were observed and recorded. Seeds are considered viable when the radicle is stained red and most of the cotyledon is also stained red (Figure 1).

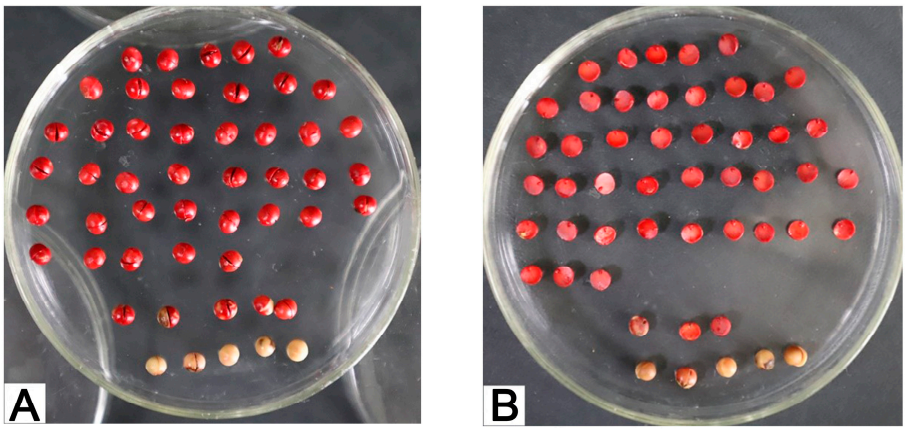

Figure 1. Viability of *S. tzumu* seeds obtained via TZ staining. (**A**) Cotyledon outer surface staining results. (**B**) Cotyledon inner surface staining results. Notes: Stained red means viable seed.

2.4. Determination of Relative Electrical Conductivity

Electrical conductivity was determined according to the method of Vieira et al. [25]. According to sampling method in Section 2.2, 20 seeds (of the same size, without mechanical damage) with different water content were tested in the following order: (1) rinse with deionized water 3 times; (2) soak in deionized water for 24 h; (3) determine the leaching solution conductivity (S1); (4) seal with plastic wrap; (5) boil in a boiling water bath for 30 min (killing the seeds); and (6) measure conductivity (S2) after natural cooling.

Deionized water without seeds was used as a blank control, and each treatment was repeated 3 times. The relative conductivity was calculated based on the following formula:

$$\text{Relative conductivity } (\%) = \frac{\text{S1} - \text{blank conductivity value}}{\text{S2} - \text{blank conductivity value}} \times 100\%$$

2.5. Determination of Antioxidant System Index

From the dehydrated seeds at different stages, 3 × 50 seeds were randomly selected and removed from the hard seed coat. After removing the seed shell, the seeds were grounded in liquid nitrogen then transferred to a 10 mL centrifuge tube, mixed well, and placed in a refrigerator at 5 °C for 10 min. The upper clarified liquid was centrifuged at 4 °C 8000 r·min^{-1} for 20 min. The supernatant was the enzyme extract. SOD activity was determined according to the method of Oberley and Spitz [26], 0.3 g seeds were extracted and 8 mL of extractants were added, which included 0.05 mol/L phosphate buffer (pH 7.0), 1.0 mmol·L^{-1} EDTA, 0.05% Triton-X-100 (v/v), 2% insoluble PVP (w/v), and 1 mmol·L^{-1} ASA. We took 5 test tubes and added reagents according to the following Table 1.

Table 1. SOD activity measurement reagent addition table.

Reaction Reagent	Measuring Tube			Light Pair Tube	Dark Pair Tube
Test tube number	1	2	3	4	5
50 mmol/L Phosphate buffer (mL)	1.5	1.5	1.5	1.5	1.5
130 mmol/L Met solution (mL)	0.3	0.3	0.3	0.3	0.3
750 umol/L NBT solution (mL)	0.3	0.3	0.3	0.3	0.3
100 umol/L EDTA-Na_2 (mL)	0.3	0.3	0.3	0.3	0.3
20 umol/L Riboflavin solution (mL)	0.3	0.3	0.3	0.3	0.3
enzyme extract solution (mL)	0.05	0.05	0.05	0	0
Distilled water (mL)	0.5	0.5	0.5	0.55	0.55

After the reagent is added, the dark pair tube (No. 5) immediately blocks the light, and the other test tubes are placed under 4000 lx fluorescent lamp for color development for 40 min (25 °C). After the end of the reaction, the OD value at 560 nm was measured and calculated (in order to zero the monitoring in the dark).

POD activity was determined according to the method of Pütter [27]. A total of 50 mL PBS (pH 6.0) +19 µL 30% H_2O_2 + 28 µL guaiacol + 0.5 mL Enzyme solution (with 0.5 mL of PBS added as blank control group) was added in sequence. The OD value at 470 nm was measured and recorded every 30 s. CAT activity was determined according to the method of Aebi [28]. A total of 0.2 mL enzyme extract (control was H_2O) +1.5 mL 10 mmol·L^{-1} PBS (pH 7.8) +1.0 mL H_2O (preheated at 25 °C) + 0.3 mL 0.1 mol·L^{-1} H_2O_2 was added in sequence. Each tube would be timed immediately after the reagents were added and quickly poured into the quartz colorimetric dish. The OD value was read at the wave length of 240 nm and recorded every 30 s for a total of 5 min. H_2O_2 and MDA content were determined according to the method of Zhou et al. [29] and Xu et al. [30]. The measurements were repeated three times for each parameter.

2.6. Data Processing and Analysis

Data processing and analysis were performed using tools such as Excel 2019, SPSS 24.0, Origin 2017, and other programs. For statistical analysis, Tukey's multiple comparison and one-way analysis of variance (ANOVA) were used for statistical analysis. Correlation analysis was performed using the Pearson correlation coefficient.

3. Results

3.1. Effects of Desiccation on Viability and Relative Electrical Conductivity of S. tzumu Seed

During the natural drying process, the water content of *S. tzumu* seeds gradually decreased to 7.6% after 24 h (Figure 2A,B). The equation between water content (%) and

desiccating time (d) of *S. tzumu* seeds is y = −3.56079 $x^{0.5504}$ + 27.05788 (y represents water content; x represents desiccating time). Within one day, the seeds lose 75% of their water content. As the water content decreased during desiccation, the viability of the seeds further decreased (Figure 2C). Seed viability decreased significantly to 49% ($p < 0.05$) when the water content decreased to 20.6%. The viability decreased to 40% when the water content decreased to 17.7%, and it further decreased to 18% when the water content decreased to 7.6%. Concluding that *S. tzumu* seeds were susceptible to desiccation, it was found that seed water content had a significant effect on growth vigor.

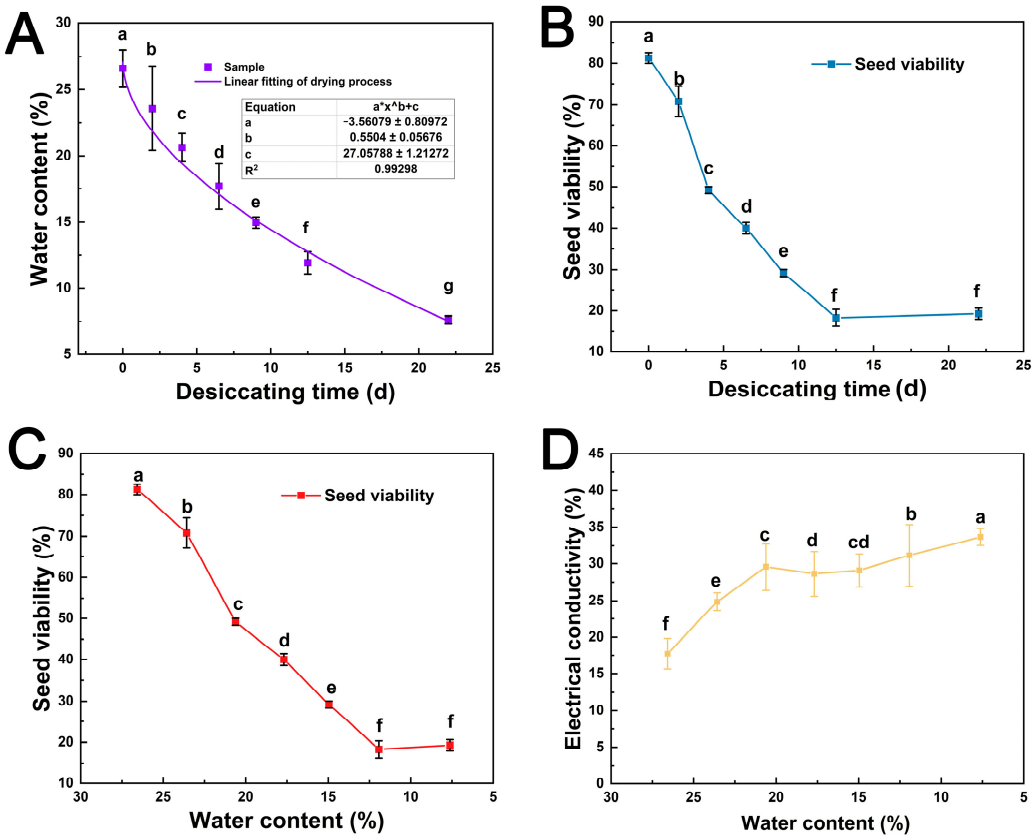

Figure 2. Changes on water content, viability, and electrical conductivity of *S. tzumu* seed during desiccation. (**A**) Changes in water content of seeds during desiccation; (**B**) changes in viability of seeds during desiccation; (**C**) changes of viability level in seeds during water content reduction; (**D**) changes of electrical conductivity in seeds during water content reduction. Note: The seed viability level was plotted with reference to the data of TZ staining. Different lower-case letters in the same column indicate significant difference at 0.05 level.

The relative electrical conductivity of *S. tzumu* seeds increased as a result of desiccation, as shown in Figure 2D. The relative electrical conductivity increased significantly to 24.87% when the water content decreased to 23.6%. After that, the relative electrical conductivity started to increase and reached 31.70% when the water content decreased to 7.6%. It was concluded that the cell membrane system was damaged throughout the seed desiccation process in addition to the changes in seed viability.

3.2. Effects of Desiccation on Antioxidant Enzyme System of S. tzumu Seed

S. tzumu seeds underwent drought stress in the early stages of desiccation. While SOD activity in the seed remained high, SOD activity decreased significantly as the water content decreased to 15% (Figure 3A). Although the responses of POD and CAT activity were slightly slower, POD enzyme activity increased rapidly during the early stages of desiccation and peaked at 251.33 $U \cdot g^{-1}$ FW when the water content decreased to 15.0% (Figure 3B), while CAT enzyme activity was not elevated until the water content decreased

to 15.0%. It was not until desiccation reached 7.6% that the CAT activity reached a maximum of 126.04 U·g^{-1} FW (Figure 3C). With the process of desiccation, the efficiency of the antioxidant system was significantly decreased when the water content decreased to 14.7%, and the activities of the other two antioxidant enzymes, except the CAT enzyme, were greatly reduced. The three antioxidant enzymes showed different response mechanisms. The SOD and POD protective enzymes had a good protective effect on *S. tzumu* seeds at the early stage of desiccation, while the CAT enzyme had a strong protective effect at the late stage of desiccation.

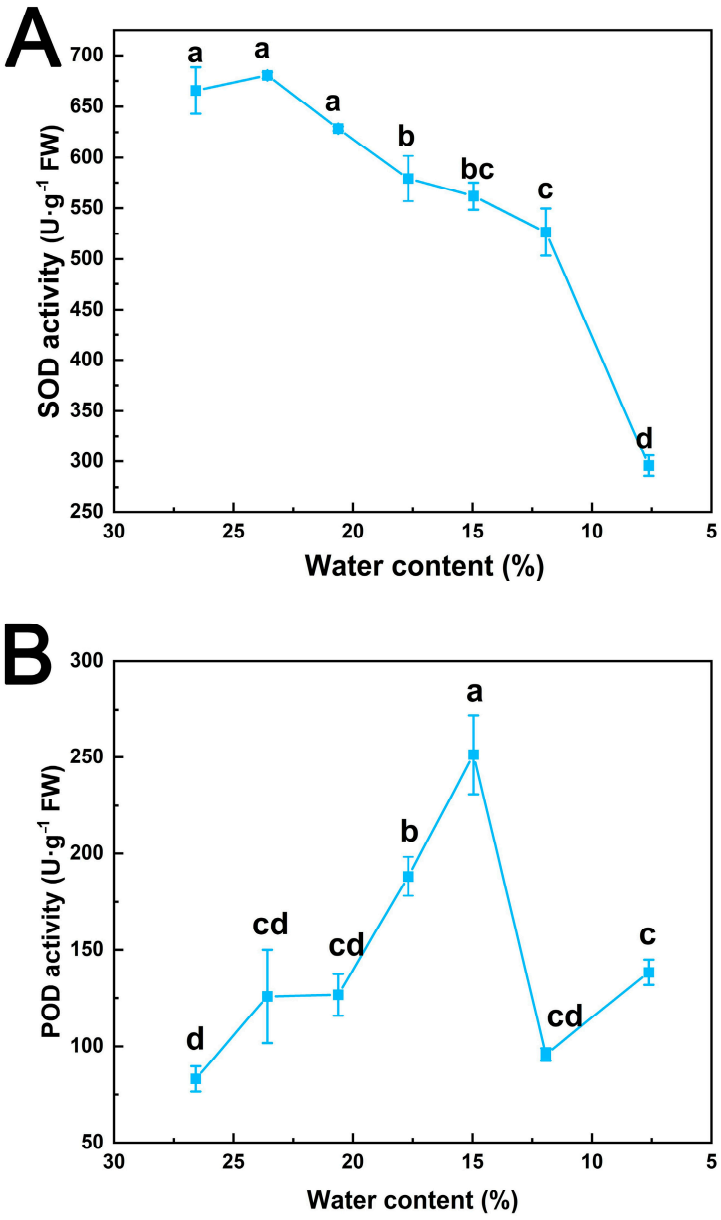

Figure 3. *Cont.*

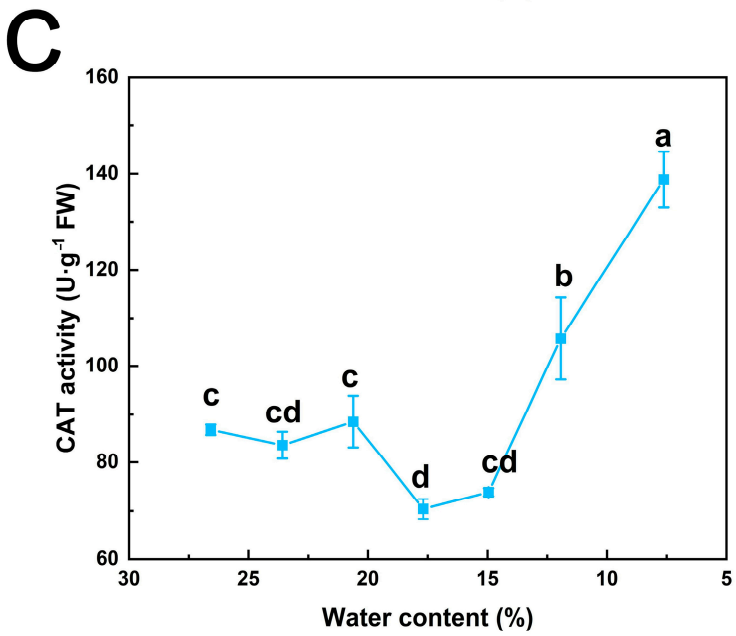

Figure 3. Changes on superoxide dismutase (SOD) activity (**A**), peroxidase (POD) activity (**B**), and catalase (CAT) activity (**C**) in *S. tzumu* seeds. Different lower-case letters in the same column indicate significant difference at 0.05 level.

3.3. Effects of Desiccation on Oxidative Stress of S. tzumu Seeds

The content of H_2O_2 fluctuated and increased from 95.61 μmol·g^{-1} to 216.25 μmol·g^{-1}, an increase of 266.2%. This indicates that in the desiccation process of *S. tzumu* seeds, H_2O_2 accumulates gradually with the decrease in water content and the deepening of seed damage (Figure 4A). The content of MDA, a lipid peroxidation product, decreased from 1.199 mmol·g^{-1}·FW to 0.898 mmol·g^{-1}·FW (water content 20.6%) in the early stage of desiccation. When the water content of seeds decreased from 20.6% to 15.0%, the MDA content increased rapidly to 1.623 mmol·g^{-1}·FW, with an increase of 80.7% ($p < 0.05$) (Figure 4B). Subsequently, the MDA content decreased significantly, while the H_2O_2 content increased significantly, which may be related to the increase in CAT activity in the organism.

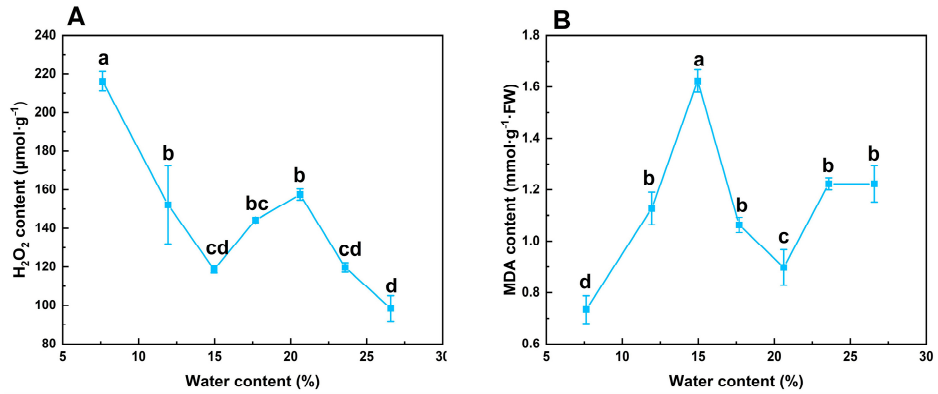

Figure 4. Changes on H_2O_2 content (**A**) and malondialdehyde (MDA) content (**B**) of *S. tzumu* seeds during desiccation. Different lower-case letters in the same column indicate significant difference at 0.05 level.

3.4. Correlation Analysis of Physiological Changes in S. tzumu Seed during Desiccation

The semi-inactivation water content of *S. tzumu* seeds is 20.7%. As the desiccation progressed, the seed viability decreased. Figure 5 shows that the water content of *S. tzumu* seeds was significantly correlated with other physiological indices during desiccation, while the water content of seeds significantly correlated with seed viability, relative elec-

trical conductivity, SOD activity, CAT activity, and H_2O_2 content. This suggests that CAT enzymes were activated after the water content of seeds decreased during desiccation. The cell membrane system was damaged, H_2O_2 accumulation increased, and seed viability decreased. Interestingly, there was a significant negative correlation between the viability level of *S. tzumu* seeds and SOD activity. The higher the SOD activity, the lower the seed vitality level. In addition, there was no significant correlation between POD enzyme activity and seed water content and seed vitality level, and it had no significant effect on the dehydration process of *S. tzumu* seeds.

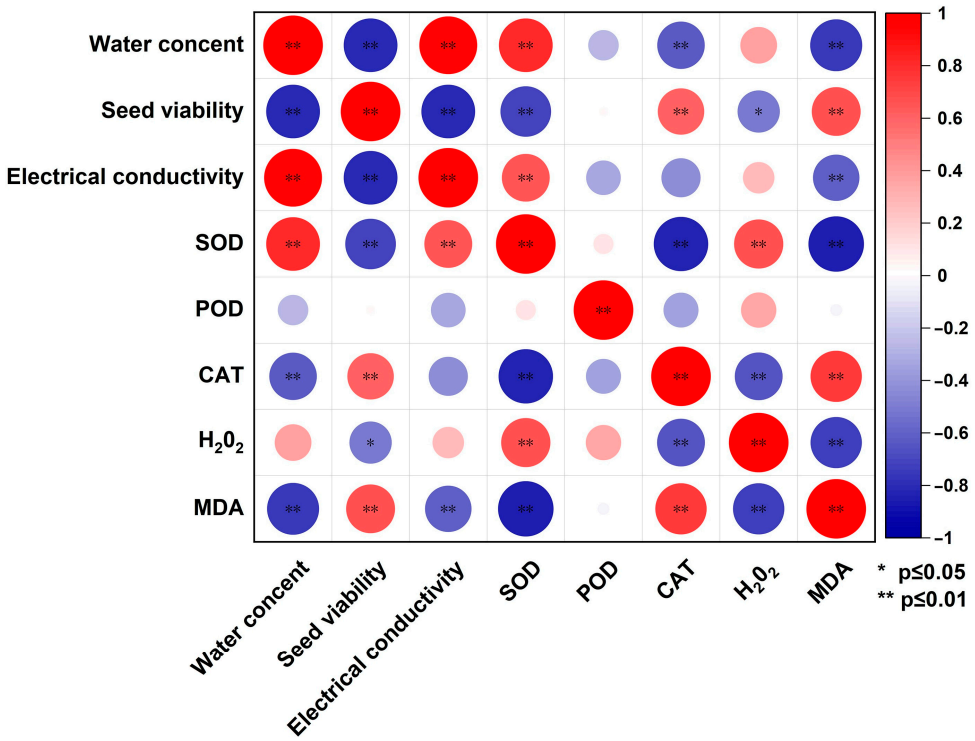

Figure 5. Correlation analysis for physiological changes in *S. tzumu* seeds during desiccation. Note: The seed viability level was plotted with reference to the data of TZ staining. Different colors represent the strength of the correlation between the two physiological indicators. Red represents positive correlation; blue represents negative correlation. The darker the color, the stronger the correlation. * The correlation was significant at the 0.05 level. ** The correlation was significant at the 0.01 level.

4. Discussion

4.1. Desiccation Tolerance of S. tzumu Seeds

Orthodox seeds can withstand water contents below 5%, but viability will not be affected, according to the current classification of seed storage types. Recalcitrant seeds have a desiccation tolerance that is quite complex and correlates with seed type, desiccation rate, and even origin. Recalcitrant seeds often become largely inactive when the water content drops to 15%–20% [31]. For example, when the water content drops to 15%, the germination rate of *Ginkgo biloba* L. seeds is only 11% [32]. The viability of *S. tzumu* seeds decreases with the decreasing water content. When the water content is reduced to 15%, the viability of *S. tzumu* seeds is reduced to 30%. Furthermore, when the water content of the seed is reduced to 10%, the viability of the seed is less than 20%, and most of the seeds are killed when dried to 15%–20% water content. *S. tzumu* seeds exhibit characteristics similar to recalcitrant seeds [31]. Simultaneously, the *S. tzumu* in the northern temperate zone confers enhanced chilling tolerance compared to plants in tropical climates. Exposure to low temperature stress can induce seed dormancy and inhibit germination while concurrently reducing water loss. Consequently, *S. tzumu* seeds have increased desiccation tolerance and prolonged seed longevity as a result of long-term evolution [33].

4.2. Changes and Interactions of Physiological Indexes during Dehydration of S. tzumu Seeds

The structural changes of seed cells, damage to cell integrity, and massive electrolyte exosmosis are the major manifestations of recalcitrant seed desiccation injury [23]. The basis of cell function is the integrity of the cell membrane. Cytoplasmic extravasation occurs when the structure of the cell membrane is destroyed [34,35]. The amount of cytoplasm that leaks out can indicate how badly the cell membrane has been damaged and, consequently, how well the cell is surviving [36]. Similar to recalcitrant seeds of *Ginkgo biloba* L. [32], *Camellia sinensis* O. Ktze., *Theobroma cacao* L., and *Artocarpus heterophyllus* Lam. [37], the relative conductivity increases throughout the desiccation process. The relative electrical conductivity of *S. tzumu* seeds increased during dehydration desiccation, which may have been caused by a significant exosmosis of dead seed cytoplasm. It is evident that the cell membrane of *S. tzumu* seeds eventually loses its integrity as water is lost.

The antioxidant enzyme system can effectively remove ROS produced under stress conditions. Loss of seed viability during desiccation may be caused by the imbalance of the antioxidant system and the accumulation of free radicals. Three antioxidant enzymes—SOD, POD, and CAT—are the primary defenses against cell damage from desiccation. They are all responsible for scavenging dangerous reactive oxygen species, which protects cells from toxic substances [38]. SOD is the first line of defence of the ROS scavenging system and plays an important role in the desiccation process. It was found that the SOD activity level of *S. tzumu* seeds remained high until the water content decreased to 15.0%, and then decreased significantly, which was not consistent with the trend of the recalcitrant seed *Clausena lansium* S. [39] but consistent with the trend in which the SOD activity of recalcitrant seed *Pachira glabra* Pasq. first maintained a stable level and then decreased [40]. With decreasing water content, POD activity first increased and then rapidly decreased to the highest value. The changes in POD activity were consistent with the change of *Panax notoginseng* F. H. Chen seeds [41]. The CAT activity initially decreased in response to the reduction in water content, followed by a rapid increase upon reaching its highest, which was consistent with the desiccation trend observed in wheat seeds [42].

Different seeds responded differently to desiccation [43]. In particular, the change of SOD activity in the early stage is relatively stable, and POD activity continues to increase, both of which play a protective role in the early stage of desiccation. The antioxidant system of *S. tzumu* seed in the process of desiccation has its own characteristics; in particular, the change of SOD activity in the early stage is relatively stable, and POD activity continues to increase in the early stage, both of which play a protective role in the early stage of desiccation. The efficacy of this defense system is significantly constrained and weakened as desiccation progresses. However, CAT activity was stable in the early stage and increased significantly in the late stage. When the water content of the seed decreased to 20.62%, the protection of CAT on the seed could be stimulated. Overall, the antioxidant system is crucial in minimizing the damage caused by seed desiccation [44].

H_2O_2 and MDA are two important indicators by which to measure the level of oxidative stress. H_2O_2 is highly aggressive and can denature various intracellular active components such as proteins and nucleic acids. MDA is the end product of lipid peroxidation and is highly cytotoxic [45,46]. The desiccation process of fresh *S. tzumu* seeds exhibited evident peroxidation. Enhanced membrane lipid peroxidation may be another major reason for the decreased viability of *S. tzumu* seeds during desiccation [19]. During the desiccation process of *S. tzumu* seeds, the MDA content first decreased, then increased significantly, and then decreased again at the later stage of desiccation, which was similar to the change trend of recalcitrant seeds of *Quercus palustris* Münchh. [47]. Before the water content of *S. tzumu* seeds decreased to 20.6%, the content of MDA was in a downward trend. Combined with the high level of SOD activity, the increased activity of POD, and the stable level of CAT activity, it could be seen that the activation of the antioxidant system slowed down the peroxidation of membrane lipids [48].

When the seed water content decreased from 20.6% to 15.0%, the seed viability decreased rapidly, and the antioxidant system in seeds was unbalanced, leading to a significant

increase in MDA content. In addition, 15% water content is a key turning point in the desiccation process of *S. tzumu* seeds, and the H_2O_2 content changes from a decreasing trend to an increasing trend when the water content of *S. tzumu* seeds decreases to 15%. Interestingly, significant changes were observed in the activities of CAT and POD when the seed water content reached 15%. Notably, these two enzymes exhibited contrasting trends in their activity alterations, with POD activity showing a rapid decrease, while CAT activity demonstrated a simultaneous rapid increase. Furthermore, during the desiccation process of *S. tzumu* seeds, it was found that MDA content was higher when POD and SOD activities were at a high level, which may be because the CAT enzyme plays a decisive role in the acquisition of desiccation tolerance [49]. SOD and POD activities were induced when the seeds were severely damaged. The two enzyme activities remained at high levels. A similar effect was found in *Boea hygrometrica* R. Br. and *LEA* 4 group genes were identified as regulating the antioxidant system [50]. The overall fluctuation of H_2O_2 content during the desiccation process was increased, which was significantly positively correlated with the relative conductivity and SOD activity, and significantly negatively correlated with CAT activity. This may be due to aggravated desiccation injury, which increases ROS in seed cells, while the delayed response of SOD and POD scavenging enzymes increases H_2O_2 levels [51]. This leads to a series of detrimental physiological and biochemical reactions that damage macromolecules in seed cells and ultimately reduce seed viability [19].

5. Conclusions

In general, *S. tzumu* seeds have the characteristics of recalcitrant seeds. The equation between the water content (%) and desiccating time (d) of *S. tzumu* seeds is $y = -3.56079\ x^{0.5504} + 27.05788$ (y represents water content; x represents desiccating time). As the seed water content is reduced to 20.62%, viability significantly decreases from 81.3% to 49.2%. Further reduction to 11.93% results in a meagre viability of 18.3%, with most seeds losing vitality accordingly. REC and H_2O_2 content gradually increase, while MDA content initially decreases and subsequently increases, exhibiting distinct trends compared to antioxidant enzyme activities: SOD and POD activities first increase and then rapidly decrease; whereas CAT activity shows an initial decline followed by rapid increase thereafter. The 15% water content is an important turning point in the desiccation process of *S. tzumu* seeds, with CAT being a key enzyme in maintaining seed viability. Both the accumulation of detrimental substances and the impairment of antioxidant systems contribute significantly to the susceptibility of *S.tzumu* seeds towards desiccation stress. During storage, it is crucial to maintain a safe water content above 23.58% in order to preserve seed viability above 70%. Storage below 50% water content is not recommended for *S.tzumu* seeds as it leads to a rapid decline in viability.

Author Contributions: C.P.: Conceptualization, Methodology, Investigation, Writing—Original draft preparation, Software. M.W.: Investigation, Visualization. Y.W.: Data curation, Project administration. Q.H.: Editing, Supervision. Y.S.: Supervision, Funding acquisition, Writing—Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All the data related to the study have been presented in the article.

Conflicts of Interest: All authors declare that there were no conflict of interest involved.

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