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Storage Time Affects the Viability, Longevity, and Germination of *Eriochloa villosa* (Thunb.) Kunth Seeds

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Abstract: The effects of storage time on *Eriochloa villosa* (Thunb.) Kunth seed longevity and germination were investigated. A number of physiological and biochemical indexes, such as germination indexes, seed viability, storage materials, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and α-amylase (AMS) activity, were determined to investigate the mechanisms behind *E. villosa* seed longevity. The longevity of *E. villosa* seeds under indoor dry storage conditions was more than eight years. The vitality of *E. villosa* seeds decreased dramatically with increasing storage time. The malondialdehyde (MDA) concentration of the seeds increased dramatically with an increase in the storage period. The SOD, POD, CAT, and AMS activities significantly decreased over time, while the soluble sugar content first increased and then decreased. Storage duration significantly affected the soluble protein content of *E. villosa* seeds. The germination index of the seeds correlated with their physiological and biochemical indices and showed a significant positive correlation with the soluble sugar content, SOD, POD, and AMS activity, and a significant negative correlation with the MDA concentration. This study is of great importance for understanding the characteristics of the field seed bank of *E. villosa* and for implementing integrated weed control measures to contribute to sustainable agricultural development.

Keywords: *Eriochloa villosa*; storage year; seed viability; seed longevity

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1. Introduction

Eriochloa villosa (Thunb.) Kunth. is an annual grass belonging to the Gramineae family of weeds, flowering and fruiting from July to October. It reproduces from seeds and is distributed in northeast, central, and western China. *Eriochloa villosa* is also found in Japan, India, the United States, Canada, and Eastern Europe [1]. It is a difficult-to-control grass in China and other parts of the world. *Eriochloa villosa* seeds are larger than other grass weed seeds and have a greater emergence depth, and most of the seeds generally fall to the plant's periphery after fruiting and can be disseminated by wind across considerable distances with little ability. In addition, *E. villosa* seeds have innate dormant characteristics that lead to dense local growth in fields in subsequent years. Their tillering ability is powerful, making it difficult to prevent and eradicate them during the growing season [2–4]. *Eriochloa villosa* is a C4 plant that produces over 28,000 seeds per plant, or more than 10,000 seeds/m^2, and impairs corn, soybean, potato, and wheat cultivation areas [5]. Yu Wen [6] reported that in China, *E. villosa* has been a problem in farms for years, spreading over 13,000 hm^2 and severely infesting fields with more than 3000 plants/m^2, seriously interfering with average crop growth and causing possible crop yield losses of more than 70% [7].

Seed longevity is a complex trait that is influenced by various factors and can vary greatly between species and even among populations of the same species [8,9]. Seeds from different storage years show differences in morphological characteristics and germination properties, as

observed in some tropical species [10,11]. When seeds reach physiological maturity, they begin the process of senescence, and seed vigor gradually decreases until death. Measuring and analyzing changes in seed vigor can provide a theoretical basis for elucidating the mechanisms of seed longevity. Various methods are available for determining seed vigor, and a suitable and accurate method should be chosen for each research subject. For instance, seedling growth tests [12] are commonly used to determine the seed vigor of wheat [13] and soybean [14]. In maize, Du et al. [15] determined physiological and biochemical indicators, including seed germination, leaching solution conductivity, staining by the triphenyl tetrazolium chloride (TTC) method, and malondialdehyde (MDA) content.

Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are crucial for regulating seed generation, maturation, germination, dormancy, seedling establishment, and aging [16]. The interactions among multiple factors, including the environment and the seed itself, cause deterioration during seed storage. As a result, alterations occur in the stored organic matter, enzyme activity, and genetic material of the seed [17]. Basavarajappa et al. [18] showed that the activity of POD decreased, while the level of MDA increased in corn seeds during seed aging. He analyzed the reasons for the existence of overall membrane lipid peroxidation in aged seeds. Zhang [19] found that the germination rate, dehydrogenase activity, and SOD activity of onion seeds decreased with a gradual increase in storage time. Wei et al. [20] reported that wheat seed viability positively correlated with changes in α-amylase (AMS), POD, CAT, and ascorbate peroxidase activities as the storage period increased and that high seed viability was associated with high activity of these enzymes. Ji [21] and Sui et al. [22] found that SOD, POD, and CAT activities decreased while the MDA content and production rate increased in cotton seeds and western ginseng seeds treated with artificial aging. Proteins and sugars are the main storage materials in seeds. Soluble sugars are important respiratory substrates, nutrients, and energy sources for seed embryo growth and development, and sugar molecules themselves can be involved in regulating seed germination and seedling growth as signaling molecules [23]. Soluble proteins in seeds comprise a class of functional proteins necessary to maintain cell survival, and their content is an important factor related to seed viability, such as nitrogen supply for seed germination and seedling growth [24]. Usually, when seeds begin to age, the stored material in the seeds changes gradually, with more severe aging resulting in lower stored material contents [25,26]. The soluble protein contents of alfalfa seeds increase and then decrease with increasing storage age, reaching a maximum content in the second year of storage [27]. With increasing storage time, the soluble protein contents of glutinous maize seeds decrease, while the content of soluble sugar increases [28].

E. villosa is an aggressive weed that is difficult to manage and severely reduces the maize yield in Heilongjiang Province, China. To date, *E. villosa* has been studied mainly in terms of dormancy and control; however, there is little research on *E. villosa* seed longevity. Because the longevity of seeds affects the weed's dispersal and seedling establishment, it also affects its ability to compete with crops such as maize. Storage time can be employed to simulate the storage of weed seeds in field soil, which can be used to predict the occurrence and damage caused by aboveground weeds. In this study, we investigated the longevity and germination of *E. villosa* seeds after numbers of different storage years to determine changes in the stored material in the seeds, which can provide a scientific basis for timely and sustainable weed control measures.

2. Materials and Methods

2.1. Materials

Eriochloa villosa seeds were collected in the years 2012, and 2014 to 2020 from maize fields in Xiangyang Township, Xiangfang District, Harbin City, Heilongjiang Province, China. All *E. villosa* seeds were collected in cloth bags and stored at 15–20 °C in the seed storage room of the College of Plant Protection, Northeast Agricultural University, Harbin. The experiments were conducted in 2020.

2.2. Methods

2.2.1. Germination Index Determination

The seeds of *E. villosa* were placed in a conical flask filled with an appropriate amount of 3% sodium hypochlorite to disinfect the seeds. Following disinfection for 30 min, the seeds were rinsed thrice with sterile water. Thirty seeds from different storage years were placed into a sterile Petri dish (100 mm) lined with sterile filter paper, and 15 mL of sterile water was added. Then the Petri dish was covered and placed in a light incubator for testing. The incubation conditions were 25 °C, 12 light:12 dark photoperiod, and 4000 lx light intensity. Three replicates were performed for each storage year. The filter paper was replenished to prevent it from drying out during the experiment. Seed germination was observed and recorded daily, with the radicle exposed to the seed coat as the standard vertebra, and the relevant germination indices were calculated at the end of the 15-day experiment [29].

$$GP(\%) = \frac{N6}{N} \times 100$$

$$GR(\%) = \frac{\sum Ni}{N} \times 100$$

$$GI = \frac{\sum Gt}{Dt}$$

where GP is germination potential; GR is germination rate; Ni is number of seeds germinated on the day of "i"; N is number of seeds; GI is germination index; Dt is corresponding germination days; and Gt is the number of germination at different times corresponding to Dt.

2.2.2. Seed Viability Determination

The viability of the *E. villosa* seeds was measured using the TTC method. A 1.0% TTC solution was configured with a phosphate buffer solution (pH = 7.0) and 2,3,5-triphenyl tetrazolium chloride powder. To remove impurities from the epidermis, the seeds were soaked in sterile Petri dishes containing sterile water for 5 h. After soaking, the seeds were evenly placed on filter paper, their seed coat was peeled off, the seed embryo was cut with a razor blade, 10 mL of the prepared TTC solution was added, and the seeds were then incubated at 37 °C for 2 h. Thirty seeds were randomly selected from different storage years, and each treatment was repeated thrice, and parallel experiments were performed three times [30]. After staining, the seeds were rinsed with water three times, and the staining sites and numbers of *E. villosa* seeds of different years were observed and recorded. Identification was based on three criteria: (1) all seed embryos were stained; (2) the tips of the embryo roots were not stained; and (3) all seed embryos were not stained. Both (1) and (2) indicated viable seeds, while (3) indicated non-viable seeds [31].

2.2.3. Electrical Conductivity Determination

The conductivity was determined using the conductivity meter method. From each storage year, 0.5 g of *E. villosa* seeds was sampled, rinsed with sterile water to remove impurities on the epidermis, and put into conical flasks with 100 mL of deionized water. The control was a conical flask containing only 100 mL of deionized water. All flasks were sealed with sealing film and placed in a water bath at 25 °C. The conductivity was measured every two hours, and the test was concluded when the reading on the conductivity meter was stable. The conductivity of the *E. villosa* seed extract was calculated as the final reading minus the blank conductivity. Three replicates were performed for each storage year. The results were averaged.

2.2.4. Effects of Storage Age on Physiological and Biochemical Indices of *E. villosa* Seeds Malondialdehyde Determination

The thiobarbituric acid method was used for MDA determination [32]. From each storage year, 0.5 g of *E. villosa* seeds was sampled and put into a conical flask with 25 mL

of distilled water, and the mouth of the flask was sealed with sealing film, then placed in a boiling water bath for 30 min, after which the samples were filtered through gauze into a beaker and then transferred into a 50 mL volumetric flask and fixed with distilled water. The extract (1.0 mL) was pipetted into a 10 mL graduated test tube, and 5 mL of anthrone reagent (1 g of anthrone dissolved in 1000 mL of dilute sulfuric acid) was added. The sample was placed in a boiling water bath for 10 min, removed, and cooled to room temperature. The absorbance was measured at 620 nm. The soluble sugar content in the seeds was obtained using the regression equation of the standard curve. The results were averaged from three replicates per year.

$$C\ (\mu mol/L) = 6.45(A_{532} - A_{600}) - 0.56\ A_{450}$$

Soluble Sugar Determination

Soluble sugar was measured by the anthrone colorimetric method [33]. First, 0.5 g amounts of *E. villosa* seeds of different years were weighed and each put into a conical flask with 25 mL of distilled water, and the mouth of the conical flask was sealed with sealing film. The flask was then put into a boiling water bath and boiled for 30 min, and then the seeds were filtered through gauze into a beaker and then transferred into a 50 mL volumetric flask and fixed with distilled water. Then 1.0 mL of the extract was pipetted into a 10 mL graduated test tube, and 5 mL of anthrone reagent (1 g anthrone dissolved in 1000 mL dilute sulfuric acid) added. The test tube was put into a boiling water bath, reacted for 10 min, removed, and cooled down to room temperature, after which the absorbance was measured at 620 nm. The content of soluble sugars in the seeds of *E. villosa* of different years was obtained according to the regression equation of the standard curve. The results were averaged in three replicates for each year.

$$\text{Soluble sugar content (mg/g)} = C \cdot V \cdot D / 1000\ W$$

where C is the soluble sugar content from the standard curve (μg); V is the volume of extraction solution (mL); D is dilution times; and W is sample fresh weight (g).

Soluble Protein Determination

The standard solution was configured, and a standard curve was made by the Kormas Brilliant Blue G-250 method [34]. First, 0.5 g of *E. villosa* seeds of different years were weighed and ground in a mortar, then transferred to a centrifuge tube after grinding. Then 5 mL of phosphate buffer (pH = 7.0) was added, and the tube was mixed with a vortex shaker and then centrifuged at 4500 rpm for 25 min. Then 0.1 mL of the supernatant was aspirated and mixed with 5 mL of Kaumas Brilliant Blue G-250 reagent and reacted for 5 min. The absorbance was measured at 595 nm with a blank as the control. The content of soluble protein in *E. villosa* seeds of different years was determined according to the regression equation of the standard curve. The results were averaged from three replicates for each year.

$$\text{Soluble protein content (mg/g)} = C \cdot V_T / 1000 Vs \cdot W_F$$

where C is soluble protein content from the standard curve (μg); V_T is total volume of sample extracts (mL); V_s is sample volume for measurement (mL); and W_F is sample quality (g).

Superoxide Dismutase Activity Determination

The nitrogen blue tetrazolium assay was used for the determination of SOD activity [35], where 0.5 g of *E. villosa* seeds from each storage year were rinsed with distilled water, dried with filter paper, and ground using a mortar and pestle. After grinding, 5 mL of phosphate buffer (pH = 7.8) was added, and the sample was thoroughly mixed with a vortex shaker and then centrifuged at 6000 rpm for 20 min to obtain the supernatant. One control tube was shaded, and 1.5 mL of 0.05 mol/L phosphate buffer, 0.3 mL of 130 mmol/L Met solution, 0.3 mL of 750 mmol/L NBT solution, 0.3 mL of 100 μmol/L

EDTA-Na2 solution, 0.3 mL of 20 μmol/L riboflavin, and 0.1 mL of enzyme solution were added to each tube, including the control; in addition, 0.5 mL of distilled water was added to the control tube. All tubes were subjected to a photochemical reduction reaction in a light incubator for 10 min and then removed and placed in the dark to terminate the reaction. The control tube was blanked and zeroed, and the colorimetric reaction was performed at 560 nm. The SOD activity was calculated using the following equation:

$$\text{SOD activity } (\mu/g) = (A_{CK} - A_E)V/0.5\ A_{CK} \cdot Vt \cdot W$$

where A_{CK} is absorbance value of the control tube; A_E is absorbance value of the sample tube; V is total sample volume (mL); V_t is volume of enzyme supernatant added for the assay (mL); and W is sample quality (g).

Peroxidase Activity Determination

The POD activity was measured using the guaiacol oxidation colorimetric assay [35]. Here, 0.5 g quantities of *E. villosa* seeds from different storage years with sufficient water absorption were sampled, rinsed with distilled water, blotted with filter paper, and ground using a mortar and pestle, and then 5 mL of phosphate buffer solution (pH = 5.5) was added, mixed thoroughly with a vortex shaker, and centrifuged for 20 min at 4000 rpm. The reaction system was 2.9 mL of 0.05 mol/L phosphate buffer solution (pH = 5.5), 1 mL of 2% H_2O_2, 1 mL of 0.05 mol/L guaiacol, and 0.1 mL of enzyme solution. For the control, the enzyme solution was boiled for 5 min and used. After the enzyme solution was added, the test tube was quickly put into a water bath at 37 °C for 15 min and immediately placed into an ice bath. Finally, 2 mL of 20% TCA was added to terminate the reaction, and the absorbance value was measured at 470 nm. The POD activity was calculated using the following equation:

$$\text{POD activity } (\mu \cdot g{-}1 \cdot min{-}1) = \Delta A470 \cdot V_t/0.01\ V_S \cdot t \cdot W$$

where ΔA_{470} is variation of absorbance value during reaction time; V_t is total volume of enzyme extraction solution (mL); t is reaction time (min); V_S is volume of enzyme solution used in the assay (mL); and W is dry weight of seeds of the sample to be tested (g).

Catalase Activity Determination

The CAT activity was determined using the hydrogen oxide reduction method. Here, 0.5 g of *E. villosa* seeds from different storage years with sufficient water absorption was sampled, rinsed, and dried. Seeds were then ground and transferred into a 25 mL volumetric flask, and the volume was fixed with phosphate buffer (pH = 7.8), then stored in a refrigerator at 4 °C for 10 min, before 1.5 mL of the sample was aspirated into a test tube for centrifugation. The sample was centrifuged at 8000 rpm for 20 min. To the supernatant, 0.2 mL of enzyme extract (0.2 mL of boiled inactivated enzyme solution was added to the control tube), 1.5 mL of 0.2 mol/L phosphate buffer (pH = 7.8), and 1.0 mL of distilled water were added. The four tubes were placed in a water bath at 25 °C for 3 min, and 0.3 mL of 0.3 mol/L H_2O_2 was added. After a set time, the contents of each tube were immediately poured into a quartz cuvette, and the absorbance was measured at 240 nm and read every 1 min for a duration of 4 min. The CAT activity was calculated according to the following equation:

$$\text{CAT activity } (\mu \cdot g^{-1} \cdot min^{-1}) = \Delta A_{240} \cdot V_t/0.1\ V_S \cdot t \cdot W$$

$$\Delta A_{240} = A_0(A_1 + A_2 + A_3)/3$$

where ΔA_{240} is absorbance value of the control tube with boiling inactivated enzyme solution; A_1, A_2, A_3 are sample absorbance values; t is reaction time (min); V_t is total volume of enzyme extraction solution (mL); V_S is volume of enzyme extraction solution at the time of measurement (mL); and W is dry weight of seeds of the sample to be tested (g).

α-Amylase Activity Determination

For determining the amylase activity, the test method proposed by Li [36] with modifications was used. Standard curves were plotted after colorimetric determination at 540 nm using different volumes of maltose standard solution. Here, 0.5 g of sprouted seeds were weighed in a mortar, to which a small amount of quartz sand and 2 mL of distilled water were added; this was ground and transferred to a centrifuge tube, washed with distilled water in stages, mixed using a vortex shaker, and centrifuged at 3000 rpm for 10 min. The supernatant was then fixed to 50 mL using the amylase stock solution and analyzed for AMS activity. Amylase stock solution (5 mL) was fixed with 50 mL of amylase diluent for the determination of the total amylase activity using three clean and numbered test tubes. To the control tube, 1.0 mL of amylase stock solution, 1.0 mL of 1% starch solution, and 2.0 mL of DNS reagent were added before holding the tube at 40 °C for 10 min. Each tube was shaken after adding the reagent; then, 2 mL was taken separately, and 2 mL of DNS reagent was added. This was mixed well, placed in a boiling water bath for 5 min, removed, and cooled, and distilled water was added to a final volume of 20 mL. Finally, the optical density was measured at 540 nm. The maltose content was determined using a standard curve.

$$\alpha\text{-amylase activity } (mg \cdot g^{-1} \cdot 5min^{-1}) = C_{\alpha} \cdot V_t / V \cdot t \cdot W \quad (1)$$

where C_{α} is the content of maltose found from the standard curve; Vt is the total volume of sample dilution; W is sample quality; t is enzyme action time; and V is the volume of enzyme liquid used for color development.

2.2.5. Correlation of Germination Indices with Physiological and Biochemical Indices

The *E. villosa* seed germination indices were correlated with the physiological and biochemical indices (MDA, soluble sugar content, soluble protein content, SOD, POD, CAT, and AMS activity).

2.3. Data Processing and Analysis

Origin2019 was used to organize and graph the data, and SPSS software (version 22.0) was used for the statistical analysis.

3. Results

3.1. Effects of Storage Years on Germination of E. villosa Seeds

The germination potential (Figure 1A), germination rate (Figure 1B), and germination index (Figure 1C) of the freshly collected *E. villosa* seeds were significantly lower than after one year of storage, indicating that the *E. villosa* seeds were dormant upon collection. The germination indices of the seeds stored for one year were significantly higher than those of seeds stored for longer. All three germination indices showed significant decreasing trends from two to eight years of storage. The greatest decrease was observed after five years of storage, when the germination potential and germination rate were significantly lower than those of seeds stored for four years. Figure 1B shows that more than 50% of *E. villosa* seeds can still germinate after eight years of storage, with the seed life exceeding eight years.

3.2. Effects of Storage Years on the Viability of E. villosa Seeds

The results of seed staining are shown in Table 1. Overall, the viability of the *E. villosa* seeds decreased with increasing storage time. These results were consistent with the germination rate (Figure 1).

3.3. Effects of Storage Years on the Electrical Conductivity and MDA Content of E. villosa Seeds

Storage year had a significant effect on the electrical conductivity and MDA content of the *E. villosa* seeds (Figure 2). The electrical conductivity of the leachate first decreased and then increased with an increase in storage time. The conductivity of seeds stored for one year decreased significantly by 31.45% relative to that of newly collected seeds. After

two years of storage, the conductivity of the seeds began to increase significantly and was more than three times higher than that after one year of storage. Changes in conductivity were lower after three and four years of storage. The MDA content in the seeds tended to increase significantly with increasing storage time (Figure 2). The MDA content in seeds stored for 1–6 years was significantly higher than that in newly collected seeds, and the differences among storage years were also significant.

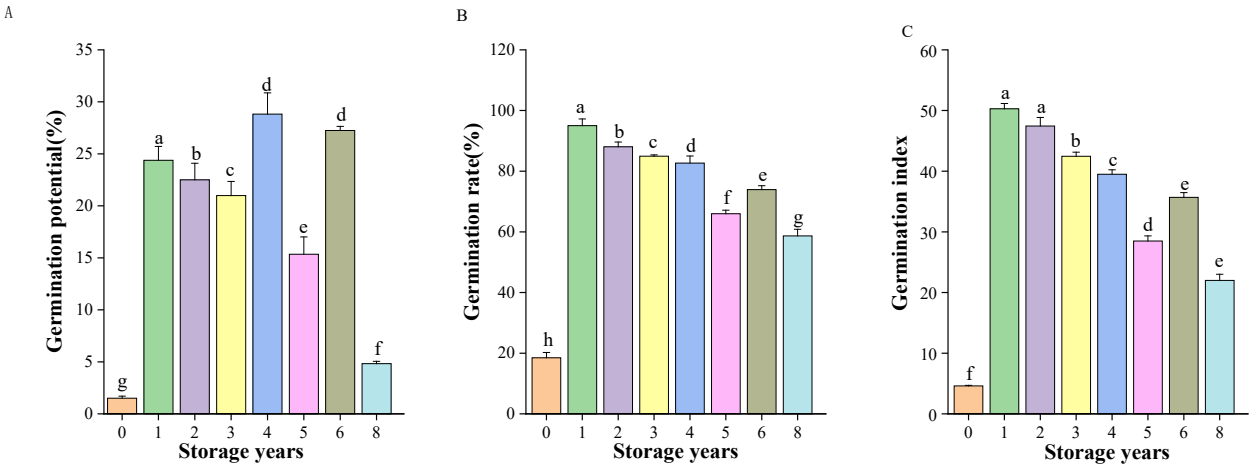

Figure 1. Effect of storage years on the germination of *E. villosa* seeds. (**A**–**C**) represent the changes in germination potential, germination rate, and germination index with increasing storage years, respectively. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

Table 1. *E. villosa* seed viability results after TTC training.

Storage Years	(1) Grain Number	(2) Grain Number	(3) Grain Number	Vigorous (%)
0	27	23	25	83.88 ± 1.73 b
1	30	26	28	93.34 ± 2.12 a
2	22	23	17	70.00 ± 1.56 cd
3	22	22	19	70.00 ± 1.69 cd
4	18	20	23	66.67 ± 3.44 de
5	17	14	16	53.33 ± 2.58 f
6	18	21	19	63.33 ± 3.05 e
8	11	12	9	36.67 ± 2.81 g

For each column, mean values followed by the same letters indicate no significant differences between the treatments.

3.4. Effects of Storage Years on the Soluble Sugar Content, Soluble Protein Content, and AMS Activity of E. villosa Seeds

The soluble sugar content in the *E. villosa* seeds increased and then decreased with increasing storage years, and the difference was significant (Figure 3). The soluble sugar content increased significantly by 15.54% within one year of storage. Subsequently, the soluble sugar content decreased significantly after four years of storage. The soluble sugar contents of the seeds in the fifth and sixth years of storage were not significantly different from those in the fourth year.

The effect of storage duration on the soluble protein contents of the seeds was not significant (Figure 3). During storage, the soluble protein contents did not significantly fluctuate.

With an increase in the storage year, the AMS activity in the seeds decreased and then leveled off (Figure 3). The AMS activity was highest in the year of collection and significantly differed from that of the seeds stored for 1–6 years. The AMS activity leveled off after three years of storage and did not significantly change after four, five, or six years. The lowest AMS activity in year six was 29.70% lower than that in seeds when they were first collected.

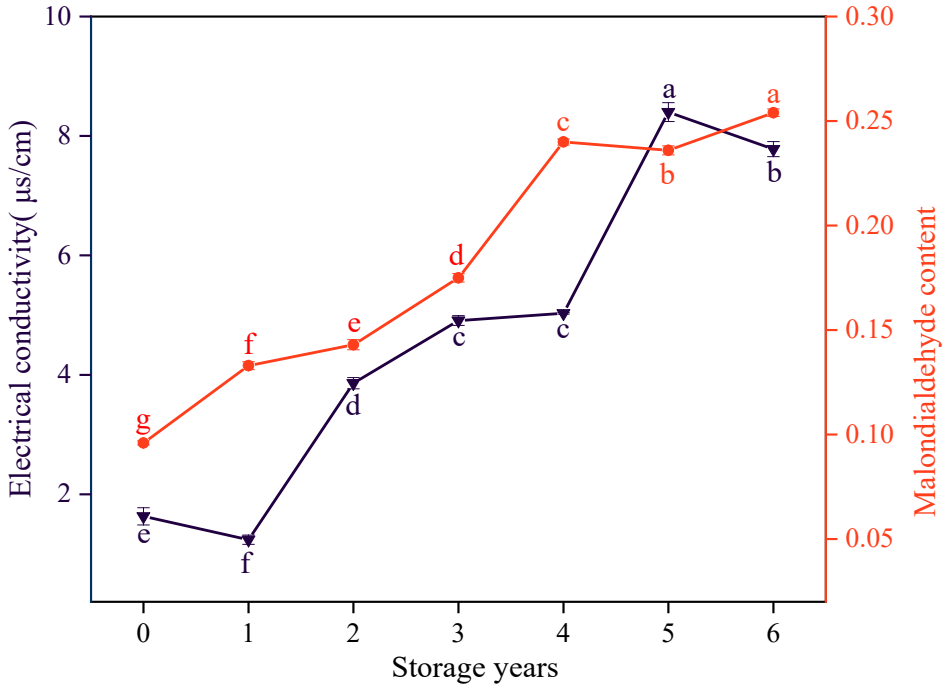

Figure 2. Effects of storage years on the seed conductivity and malondialdehyde content of *E. villosa.* Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

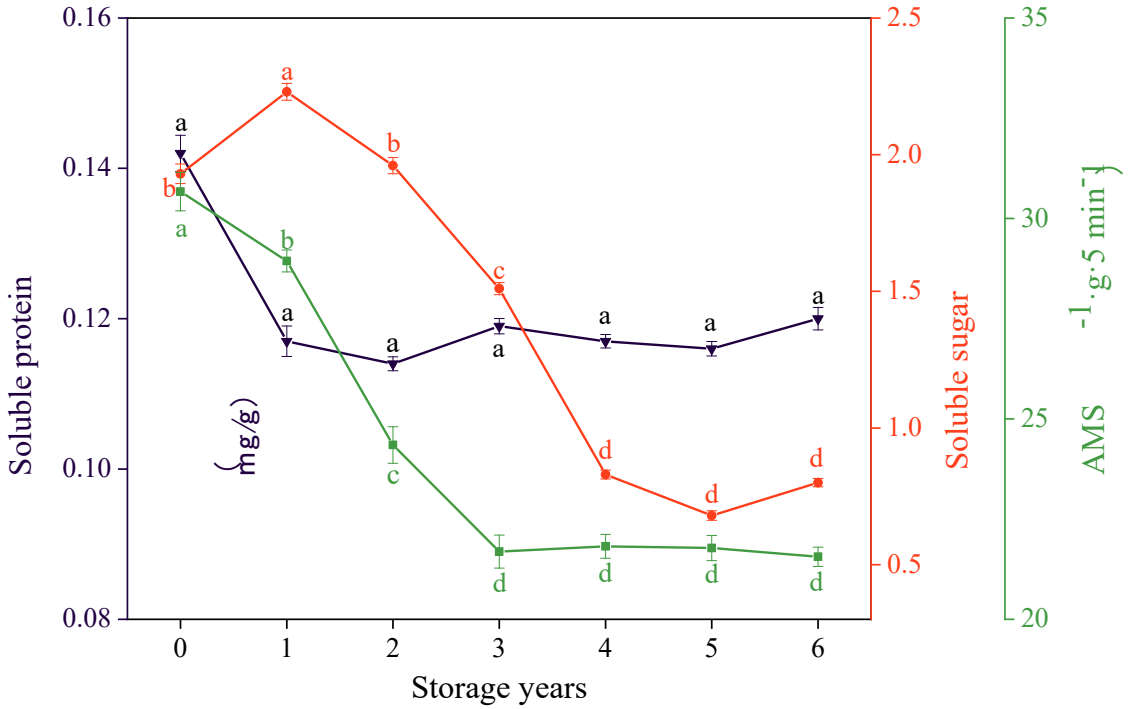

Figure 3. Effects of storage years on the seed soluble sugar, soluble protein content, and AMS of *E. villosa.* Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.5. Effects of Storage Years on the SOD, POD, and CAT Activity of E. villosa Seeds

With increasing storage time, the SOD activity in the *E. villosa* seeds significantly decreased. There was no significant difference between the SOD activity in newly collected seeds and those stored for one year. Thereafter, the SOD activity decreased significantly. After two years of storage, the SOD activity decreased by 2.65% compared with newly collected seeds. The SOD activity after six years of storage was significantly lower than

the SOD activity in those that were newly collected ($p < 0.05$). The POD activity in the newly collected *E. villosa* seeds increased significantly by 14.33% after one year of storage. Activity then significantly decreased in seeds stored for 2–6 years compared with levels in newly collected seeds. The lowest POD activity was observed after five years of storage, while differences in POD activity were not significant in seeds stored for four to six years (Figure 4).

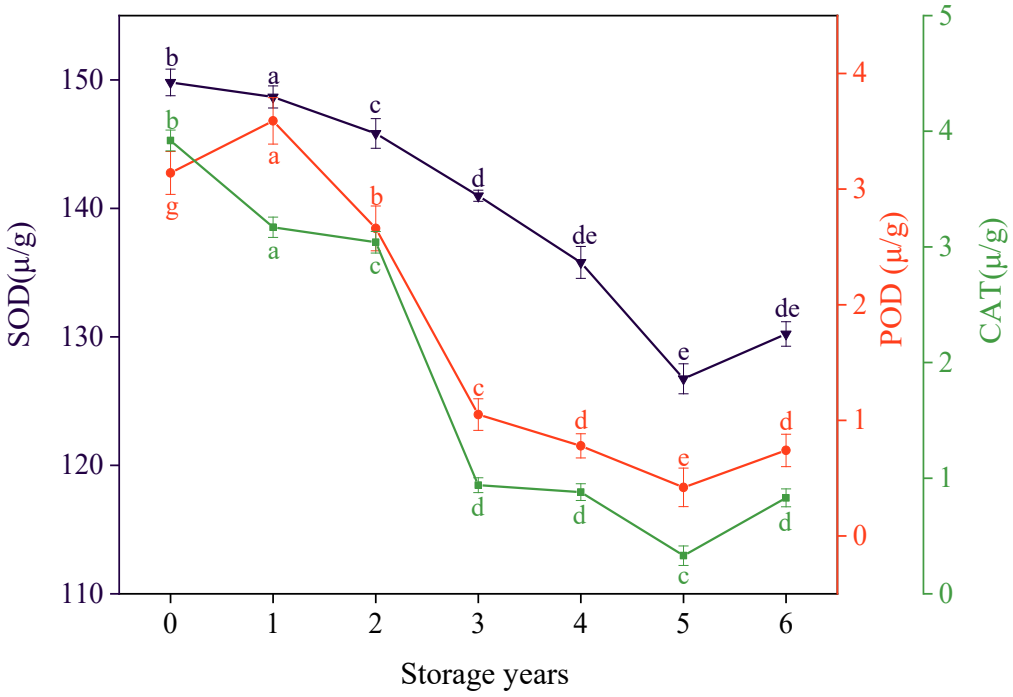

Figure 4. Effects of storage years on the activities of SOD, POD, and CAT in *E. villosa* seeds. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

The storage years had a significant effect on the CAT activity in the *E. villosa* seeds (Figure 4). The CAT activity first increased with storage time and then significantly decreased. The CAT activity after one year of storage was significantly greater than when the seeds were first collected. It then sharply decreased from two to three years of storage, with non-significant differences between three and four years of storage. The lowest CAT activity was observed after five years of storage, with a 76.01% decrease in the CAT activity in year six compared to that in freshly collected seeds.

3.6. Correlation of the Seed Germination Indexes of E. villosa with Its Physiological and Biochemical Indexes

There were significant correlations between the seed germination indices and the physiological and biochemical indices (Table 2). The germination rate and germination potential had significant positive correlations with the soluble sugar content and AMS activity ($p < 0.05$), stronger significant positive correlations with the SOD and POD activities ($p < 0.01$), and significant negative correlations with MDA ($p < 0.01$). The germination index had strong significant positive correlations with SOD, POD, and AMS activity ($p < 0.01$), and was negatively correlated with MDA ($p < 0.05$). All germination indices of seed longevity decreased after years of storage, and the correlation analysis showed significant correlations between these and the MDA contents and POD enzyme activity. During *E. villosa* seed aging, free radical production and scavenging are unbalanced, and the membrane is severely peroxidized, which increases the MDA concentration and decreases POD enzyme activity, ultimately resulting in a decrease in seed viability.

Table 2. The relationship between seed germination and physiological and biochemical indexes.

Index	MDA	Soluble Sugar	Soluble Protein	POD	CAT	AMS
Germination rate	−0.991 **	0.910 *	0.044	0.851	0.843	0.851 *
Germination potential	−0.984 **	0.944 *	−0.003	0.882 **	0.872	0.902 *
Germination index	−0.941 *	0.483	−0.645	0.886 **	0.875	0.972 **

Note: * means significant difference at 0.05 level, ** means significant difference at 0.01 level.

4. Discussion

Seed longevity decreases with storage [37] and can usually be judged by the color of the seed coat, which loses its luster, while the germination potential, germination rate, and germination index also decrease when seeds are stored for a long time [38,39]. In this study, we found that the germination potential, germination rate, and germination index of *E. villosa* seeds decreased significantly during eight years of storage, and the determination of *E. villosa* seed vigor by the TTC method was consistent with germination test results. This agreed with the results of Hidalgo et al., who found the seeds of Phalaris spp. varied greatly in their germination rates after storage for different years, and the longer the storage year, the lower the germination rate [40]. It is also consistent with the results of studies on dogwood [41], alfalfa [27], and tomato [42] seeds. The germination rate of the freshly collected *E. villosa* seeds was lower compared with that of seeds stored for one year because of the dormant nature of the freshly collected seeds [43]. This result agrees with that of Rodríguez et al. [44] and is identical in other seeds, such as *Amaranthus* [45] and *Arabidopsis thaliana* [46], and other characteristics. As seeds age, cell membrane integrity is lost, and changes in membrane permeability are responded to by changes in conductivity; therefore, conductivity measurements have been used to indicate seed viability and are a basic method to detect seed longevity [24]. Seeds of *Brassica alboglabra* Bailey [47], *Toona sinensis* [48], mustard [49], and American ginseng [22] were treated with artificial aging methods, resulting in decreased seed viability, while the conductivity increased significantly with increasing aging time, which was consistent with the results of the present study.

In this study, the soluble sugar content of the *E. villosa* seeds stored for one year was higher than that of freshly collected seeds, after which it gradually decreased with increasing storage time. However, the soluble protein content decreased within one year of storage, after which it did not change significantly. This is consistent with the results of Jin [50] and Cai et al. [51] who studied changes in the soluble sugar and protein contents of sand mustard and rice seeds with storage years. While some researchers found that the soluble protein contents of cucumber seeds [52], pepper seeds [53], and rape seeds [54] decreased with increasing storage time, the soluble sugar contents also underwent similar changes, which differed from what was observed in *E. villosa*. This shows that the soluble sugar contents of *E. villosa* seeds undergo a period of accumulation after collection to achieve the normal vital activities of the seeds while avoiding damage caused by aging, and with time, the *E. villosa* seeds begin to age, and their soluble sugar contents gradually decrease. The soluble protein content of the *E. villosa* seeds did not change significantly between storage years, probably because the main activity within the dormant *E. villosa* seeds was to provide energy using soluble sugar.

The decrease in viability during seed aging is closely related to membrane lipid peroxidation, of which MDA is the end product. Peroxidation of unsaturated fatty acids resulting from decreased antioxidant enzyme activity is thought to be one of the main reasons for the loss of seed viability during storage [55,56]. Normal seed activity generates free radicals and reactive oxygen species, which can be scavenged by SOD, POD, and CAT [57]. Among these enzymes, SOD scavenges O^{2-} to generate hydrogen peroxide, but in an adverse environment, many free radicals are generated due to biochemical reactions, causing the membrane mass to be peroxidized and leading to the destruction of the seed membrane system; however, POD and CAT within the seeds scavenge O^{2-} and H_2O_2 [58], preventing the accumulation of free radicals in the seeds and delaying seed aging. Therefore,

MDA, SOD, POD, and CAT levels can change in response to membrane lipid peroxidation. Many studies have indicated that seed aging is associated with antioxidant enzymes, MDA, and amylase activity, which is consistent with studies on *E. villosa* seeds. Zheng [59] showed that the AMS activity of poplar seeds tended to decrease to different degrees during storage. Hao [60] and Chang et al. [61] showed that the POD activity of *Pinus oleifera* seeds decreased significantly, while the MDA content increased significantly with increasing storage time, and the SOD and CAT activities of sand onion seeds increased and then decreased with increasing storage time and were positively correlated with their germination indexes. Han et al. [62] and Zacheo et al. [63] found that the germination index of artificially aged wheat mango and almond seeds was positively correlated with SOD, POD, and CAT activities, and the MDA content in the seeds increased with increasing aging time. In the present study, the SOD activity of *E. villosa* seeds gradually decreased with increasing storage years, but the decrease was slow, while the POD and CAT activities were highest in the first year of storage and then sharply decreased, the AMS activity gradually decreased over time and finally leveled off, and the MDA concentration gradually increased with storage time. The *E. villosa* seed germination indexes had significant positive correlations with SOD, POD, and AMS, and a negative correlation with MDA. This indicated that aging of the *E. villosa* seeds during storage reduced enzymatic activity, decreased antioxidant capacity, and increased toxic substances, resulting in decreased seed viability.

In the field, competition for resources occurs between weeds and maize. *E. villosa* is a troublesome grass that has similar resource requirements as maize and is more tolerant to some herbicides, making it difficult to control. The presence of large numbers of *E. villosa* can reduce ventilation and light penetration in a field, thereby affecting crop growth and yield. Therefore, studying *E. villosa* seed longevity and germination characteristics is crucial for the future scientific management of weeds. In this study, we examined the germination rate of *E. villosa* seeds stored for six years, which reached more than 70%, while the germination rate of seeds stored for eight years was still over 50%, which is a medium life according to the seed life classification [50]. *E. villosa* has a high fruit set, poor dispersal and diffusion capacity, easy formation of a persistent soil seed bank, deep soil confinement, and tolerates low temperatures and oxygen content, helping seeds to remain viable. Agricultural soil is susceptible to compaction by natural processes and production activities; therefore, it is periodically tilled by crushing, turning, suppression, rototilling, tilling, and deep loosening [64]. These operations will turn new *E. villosa* seeds into deeper soils and expose *E. villosa* seeds that have been buried deep underground for many years, allowing these exposed seeds to germinate and sprout, thus harming the crop.

5. Conclusions

This study revealed that the longevity of *E. villosa* seeds under indoor, dry storage conditions was greater than eight years. This means that *E. villosa* seeds can be stored in the soil seed bank in the field and still have the ability to germinate, causing continuous damage to the crop. However, the germination tests showed that the vitality of the seeds decreased dramatically with increasing storage time. The MDA concentrations of the seeds increased dramatically with increasing storage periods, while the levels of various antioxidant enzymes (SOD, POD, CAT, and AMS) significantly decreased over time. In contrast, the soluble sugar contents first increased and then decreased. The effect of storage time on the soluble protein contents of *E. villosa* seeds was not significant. The germination index of the seeds was positively correlated with most physiological and biochemical indices (soluble sugar content, SOD, POD, and AMS activity) and negatively correlated with the MDA concentration.

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