Aquatic Botany 143 (2017) 18–24

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Hydrocharis dubia seeds maintain high seed vigor in ambient wet storage condition through scavenging hydrogen peroxide by antioxidant systems

Suting Zhao[a,c,1], Wenmin Huang[a,1], Hongsheng Jiang[a,c], Junyao Sun[a], Liyan Yin[b,**], Wei Li[a,d,*]

[a] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, 430074, China
[b] Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresources, Hainan University, HaiKou 570228, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China
[d] Hubei Key Laboratory of Wetland Evolution & Ecological Restoration, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

ARTICLE INFO

Keywords:
Hydrocharis dubia
Aquatic plants
Hydrogen peroxide
Antioxidant system
Seed vigor
Seed storage

ABSTRACT

To evaluate the mechanisms of seed vigor maintenance in an aquatic plant, *Hydrocharis dubia*, we studied the responses of germination ability, moisture content, H_2O_2 content, antioxidant enzyme activities, and antioxidant contents of the seeds under dry and wet storage conditions separately. Germination ability decreased significantly in dry environment, but was maintained at a high level in wet environment. Moisture content was significantly higher in wet treatment than in dry treatment. There was a significant positive relationship between germination ability and moisture content. The seeds in dry treatment accumulated significantly higher H_2O_2 than in wet treatment. There was a significant negative relationship between germination ability and H_2O_2 content. Superoxide dismutase activity in wet treatment was significantly higher than in dry treatment. Similar results were observed in guaiacol peroxidase, ascorbate peroxidase and dehydroascorbate reductase activities and proline content. No significant differences were found in ascorbic acid content. These results indicated that *H. dubia* seed vigor was associated with seed moisture and H_2O_2 content. The seeds in *H. dubia* maintained high seed vigor in wet environment through H_2O_2 scavenging by antioxidant systems. Based on our results, we suggest to store *H. dubia* seeds in wet condition.

1. Introduction

Deterioration of seeds during storage is of great concern since aged seeds result in poor seedling emergence, low yield, economic losses, and decreased genetic diversity (Pasquini et al., 2012). Seed deterioration is associated with a variety of cellular, biochemical and metabolic changes, such as loss of membrane integrity, reduction of energy metabolism, enzyme inactivation, RNA impairment, and DNA degradation (McDonald, 1999; Kibinza et al., 2006; Hu et al., 2012). Although the mechanisms of seed deterioration have not been fully understood, the accumulation of ROS, such as superoxide radical, hydroxyl radical and H_2O_2, is considered to be the major factor for seed deterioration in terrestrial seeds (Bailly et al., 1996; Lehner et al., 2008; Yao et al., 2012; Kong et al., 2015). As the most stable form of ROS, H_2O_2 is vital for ROS production and scavenging. Increased H_2O_2 accumulation can trigger oxidative stress, leading to the disruption of metabolic functions (Xia et al., 2015b). ROS is regulated by antioxidant enzymes and antioxidants (Wu et al., 2009; Morscher et al., 2015). Antioxidant enzymes, such as SOD, GPOD, and APX prevent ROS accumulation in order to minimize the damaging effects (Bailly et al., 2002; Kibinza et al., 2006; Yao et al., 2012). SOD, which represents the first defense enzyme against ROS, converts superoxide radical to H_2O_2. GPOD and APX scavenge the accumulated H_2O_2 to nontoxic substances (Hu et al., 2012). Moreover, antioxidants are essential for scavenging excess H_2O_2. ASA is a critical water soluble antioxidant with low molecular weight, and can scavenge H_2O_2 directly or be an electron donor in cooperation with APX. It can also be regenerated by DHAR (Wu et al., 2009). Proline, another H_2O_2 scavenger, plays an adaptive role in stress tolerance (Ozden et al., 2009; Kong et al., 2015).

Numerous researchers have investigated and extensively reported on seed storage and seed vigor maintenance in seeds of terrestrial plants (Kibinza et al., 2006; Li et al., 2007; Lehner et al., 2008; Wu et al., 2009;

Abbreviations: APX, ascorbate peroxidase; ASA, ascorbic acid; DHAR, dehydroascorbate reductase; GPOD, guaiacol peroxidase; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; SOD, superoxide dismutase

* Corresponding author at: Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, 430074, Wuhan, China.
** Corresponding author at: Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresources, Hainan University, Haikou, 570228, China.
E-mail addresses: lyyin@163.com (L. Yin), liwei@wbgcas.cn (W. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aquabot.2017.08.001
Received 21 March 2017; Received in revised form 10 August 2017; Accepted 13 August 2017
Available online 18 August 2017
0304-3770/

Kong et al., 2015). For aquatic plants, most studies focused on seed germination (Yin et al., 2009; Xiao et al., 2010; Yin et al., 2013; Kaldy et al., 2015; Wagner and Oplinger, 2017a, 2017b). Seed storage is still under investigation and little is known in regard to seed vigor maintenance mechanisms. Aquatic plants may possess special mechanisms to maintain seed vigor under natural conditions (Li, 2014). Based on storage characteristics, seeds may be classified into three categories including orthodox seeds, recalcitrant seeds, and intermediate seeds. Orthodox seeds are tolerant to desiccation and can survive under long-term storage. Recalcitrant seeds are desiccation sensitive and do not survive drying below a relatively high moisture content. Intermediate seeds will tolerate a higher degree of drying than recalcitrant seeds but will tolerate much less desiccation when compared with orthodox seeds. In seeds of aquatic plants, unorthodox storage characteristics are common. However, orthodoxy may be more widespread than previously thought (Hay et al., 2000). Aquatic plant populations have significantly declined throughout the world in recent years (Kauth and Biber, 2015). Seeds of aquatic plants are necessary for the re-establishment of populations following natural and anthropogenic disturbances. In particular, determining the appropriate storage condition is vital for ensuring seed vigor (Kauth and Biber, 2015).

Hydrocharis dubia is a floating-leaved plant with high seed yields. It can be used as fodder, vegetable, or green manure. More importantly, it is very useful in purifying water quality (Zhao et al., 2008). However, the storage characteristics of *H. dubia* seeds have not yet been investigated. In this study, we explored the physiological mechanisms of seed vigor maintenance in *H. dubia* seeds under two contrasting storage conditions: ambient dry (orthodox seeds storage condition) and ambient wet (unorthodox seeds storage condition). The aims of our study were (1) to investigate the appropriate seed storage methods, (2) to study the relationship between seed vigor and H_2O_2 content, and (3) to investigate the response of antioxidant systems to seed deterioration in an aquatic plant.

2. Materials and methods

2.1. Seed collection

Mature fruits of *H. dubia* were harvested in November 2015 from plants naturally growing in Wuhan Botanical Garden, Chinese Academy of Sciences (30°35′N, 114°17′E). The seeds are 1.4 ± 0.04 mm long and 0.7 ± 0.02 mm wide. The fresh weight of *H. dubia* seed are about 0.5 mg. The seeds with brown testa were extracted from the fruits and washed with distilled water.

2.2. Seed storage treatments

Seed samples were stored in two conditions: (1) ambient dry: the seeds were put in paper bags at ambient temperature, with mean relative humidity of 75%; (2) ambient wet: the seeds were put at the bottom of a plastic bucket (diameter 15 cm, height 20 cm) full of sand. The bucket was put at the bottom of a pool (60 × 40 × 80 cm) full of water at ambient temperature, with constant 100% relative humidity. There were approximately 6000 seeds (about 3 g) in each storage treatment with five replicates. The air temperature in dry storage was obtained from reports by the meteorological bureau. The water temperature in wet storage condition was recorded with a sensor. We randomly chose the subsamples to test seed germination ability, moisture content, H_2O_2 content, antioxidant enzyme activities, and antioxidant contents every 30 days. The physiological parameters were tested after moisture equilibration in distilled water for 24 h. This experiment was conducted from December 2015 and until June 2016.

2.3. Test of germination ability

The germination experiment was carried out according to the method applied by Yin et al. (2013) using 50 seeds each treatment with five replicates. In this study, germination ability was evaluated by germination percentage and germination index. Germination percentage was calculated by the following equation:

$$\text{Germination percentage} = \frac{n}{N} \times 100\%$$

where n is the total number of germinated seeds, and N is the total number of test seeds. Germination index was calculated by the following equation:

$$\text{Germination index} = \sum \frac{Gt}{Dt}$$

where Gt is the number of germinated seeds at t days and Dt is the number of days required for germination.

2.4. Test of seed moisture content

The seed moisture contents were determined according to (Xia et al., 2015a) with slight modifications. Briefly, 100 seeds (approximately 0.05 g) were placed in a glass sample container and weighed. They were oven-dried at 125 °C for 2 h (five replicates). After cooling for 24 h in a desiccator, seeds were weighed several times until constant final seed weight. The seed moisture content was calculated according to the percentage of fresh weight.

2.5. Determination of H_2O_2

H_2O_2 content was extracted with 5% trichloroacetic acid from 100 seeds with five replicates and assayed by the absorbance change of the titanium peroxide complex at 415 nm (Patterson et al., 1984).

2.6. Determination of antioxidant enzymes

The enzymes were extracted with a sodium phosphate buffer (0.1 M, pH 7.8, containing 2 mM dithiothreitol, 0.1 mM EDTA) from 100 seeds with five replicates. SOD (EC 1.15.1.1) activity was determined by measuring its capacity to inhibit the photochemical reduction of nitroblue tetrazolium at 560 nm (Beyer and Fridovich, 1987). POD (EC 1.11.1.7) activity was assayed using the guaiacol method at 470 nm as described by Chance and Maehly (1955). APX (EC 1.11.1.11) and DHAR (EC 1.8.5.1) activities were measured by the changes of ASA at 290 nm (Dalton et al., 1993).

2.7. Determination of proline and ASA

Proline and ASA contents were extracted with 5% sulfosalicylic acid from 100 seeds with five replicates. Proline content was measured by the reaction of proline and triketohydrindene hydrate at 520 nm (Demiral and Turkan, 2005). ASA content was determined by the reaction of ferric ion, ASA and bathophenanthroline at 525 nm (Arakawa et al., 1981).

2.8. Data analyses

Statistical analyses were conducted in IBM SPSS Statistics. *T*-test was used to analyse the differences between air temperature and water temperature. For each treatment, repeated-measures ANOVA was applied, with the storage duration as within-subjects variables and storage condition as between-subjects factors. Normality was confirmed by Shapiro-Wilk test and homogeneity of variance verified with levene test. Data of SOD, GPOD and APX were not normality/homogeneity even after transformed. These data were analyzed by Friedman test. Spearman correlation was used to test the correlation between germination ability and other parameters (seed moisture content and H_2O_2 content) according to the mean values. The significance level was set at