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Full Length Research Paper

Effect of ultradrying on germination and physiological and biochemical characteristics of *Haloxylon persicum*

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This study was to determine whether the vigor of the *Haloxylon persicum* seeds can be maintained at a higher level and their storage characteristics can be improved by ultra-dry storage. Moisture content of *H. persicum* seeds was dried to 7.62, 6.73, 5.23, 4.24, 3.24 and 1.02% in a desiccating container with silica gel, and stored at 25, 4 and -20℃ for 12 months. The results showed that the *H. persicum* seeds became more tolerant to aging condition if they could be dried to below 3.24% of moisture content. Under the same aging condition, the germination of seeds with 3.24% of moisture content decreased greatly, while that of ultra-dry seeds still kept a high level. The dehydrogenase activity of ultra-dry seeds was higher than that of the control (moisture content 3.24%, aging treatment). The results indicated that dehydrogenase, guaiacol peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities of the ultra-dry seeds were higher than those of the control seeds. The results demonstrated that an appropriate ultradried *H. persicum* seeds could persist and ultradrying treatment was an ideal method of seed storage.

Key words: Seeds of *Haloxylon persicum*, ultra-dry storage, germination, physiological characteristics.

INTRODUCTION

Haloxylon persicum is a shrubby perennial plant distributed in many areas of deserts in the northwest China. From the economic view, it is very important to fix the sand dune, prevent soil desertification, conserve water and soil and improve zoology environment. However, it is a vulnerable plant species due to the harvesting for fuel by man and many other reasons. Therefore, a method for germplasm preservation for *H. persicum* is greatly required. As is well known, seed is one of the principal germplasm resources, and has the highest resistance to the extreme environment in plant's life cycle. Therefore, the gene bank of seed is one of the most efficient means for germplasm preservation *for H. persicum*.

Before the 1950s, seed was stored openly and its longevity was very short because the temperatures and moisture contents varied along with storage conditions. Ellis et al. (1986) found that if the moisture content of Gingli is reduced from 5 to 2%, the longevity would increase 39 times. From then on, the research on ultra-drying storage of seed has made progress. This paper described the effect of ultra-drying storage on subsequent seed vigor. The objective of this study was to establish a suitable method for storing the seeds of *H. persicum*, providing a theoretical base for sustaining the biodiversity and conserving the other plants which inhabit these desert areas.

MATERIALS AND METHODS

Materials

Mature seeds of *H. persicum* were collected from the dry inflorescences of the shrubs in November 2006, from natural populations in the desert area near Guanjia Lake, Xinjiang.

Seed ultra-drying treatment and pre-humidification

Seeds were packed in plastic net bags; the ratio of the seeds to silica gel was 1:5 (w/w). Seed bags were buried into silica gel in a

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desiccator at normal atmospheric temperature (25℃) for 15 d to reduce the moisture content of seeds to 7.62, 6.73, 5.23, 4.24, 3.24 and 1.02%. The ultra-dried seeds were kept in sealed aluminum foil packages for experiment.

Measurement of seed moisture content (MC), germination percentage (GP), germination index (GI) and vigor index (VI)

The measurements were done according to International Rules for Seed Testing (ISTA, 1985). Moisture content (MC) of three samples of 100 seeds was determined gravimetrically by the oven method (8 h at 110 ± 1℃) and could be expressed on the wet basis (%, w.b.). The seed surfaces were sterilized using 10% Na-hypochlorite before the germination process. Seeds were tested for germination on top of three piece of filter paper moistened with 4 cm^3 distilled-deionized water in 6-cm-diameter Petri dishes at 20 ± 1℃. Three replicates of 50 seeds were used for each treatment. Emergence of the radicle was the criterion used to assess germination. Germination was counted for 7 days. Seeds vigor index (VI) was determined according to the following equation: $VI = G_I \times S_x$, $GI = \Sigma(G_t/D_t)$, where G_I is germination index, S_x is radicle mean length x days after germination and G_t is germination percentage after t days; D_t is days of germination.

Ultra-dry storage experiments and accelerated aging

To investigate the storage longevity of the ultra-dry seeds over 12 months at three different temperatures, the seeds lot was split into three sub-samples. Seeds were stored at 25, 4 and -20℃, respectively. Each treatment combination of MC, temperature and storage duration was represented by a sample in one sachet, which was used to determine the percentage of germinated seeds.

The ultra-dried seeds and non-ultra-dried seeds (control) were accelerated aged at 50℃ for 1 month in an oven (Wang et al., 2005). After accelerated aging, the seeds were put into nylon bags.

Seed conductivity test

Seed conductivity tests were performed by soaking 100 seeds (uniform in size and without visual injury) in 300 ml of deionized distilled water at 25℃ for 12 h (Zheng et al., 1988). The conductivity of the soaking water was measured by conductivity meter (model DDS SJ-308A). Leakage rate was expressed in 1/us.cm.

Seed malondialdehyde test

5 g seeds were soak in distill water at 20℃ for 12 h. Seeds were homogenized in cold 50 mmol/L phosphate buffer (pH = 7.0). The homogenate was centrifuged at 15000 × g under 4℃ for 20 min and the supernatant was collected (Zhu et al., 2001). The malondialdehyde (MDA) content was assayed according to Bailly et al. (1996).

Enzyme extraction and assays

For enzyme extraction, 1.0 g seeds were soaked in distilled water at 25℃ for 12 h and then homogenized on ice with 50 mmol/L phosphate buffer (pH = 7.0). The homogenate was centrifuged at 15,000 g for 20 min and the supernatant was used for enzyme assay (Zhu et al., 2001).

Dehydrogenase activity was determined by triphenyl tetrazolium chloride (TTC) method (Kun and Abood, 1949).

SOD activity was determined by measurement of inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm (Giannoplitis and Ries, 1977). The 3 mL reaction mixture contained 50 mmol/L phosphate buffer (pH=7.8), 0.1 mmolg/L ethylenediaminetetra-acetic-acid (EDTA), 13 mmol/L methionine, 75 umol/L NBT, 16.7 umol/L riboflavin and enzyme extract. Riboflavin was added at last and the reaction was initiated by placing the tubes under two 9 W fluorescent lamps. The reaction was determined after 15 min by removal from the light source. An illuminated blank without protein gave the maximum reduction of NBT, therefore, the maximum absorbance at 560 nm. SOD activity is present as absorbance of sample divided by absorbance for blank, giving the percentage of inhibition. 1 unit of SOD is define as the amount required to inhibit the photoreduction of NBT by 50%. The activity of SOD was expressed as unit/mg protein.

POD activity was determined by measurement of Kalpana and Madhava Rao (1995). The reaction mixture contained 0.1 mL enzyme extract, 2 mL 0.1 mol sodium-acetate buffer (pH = 4.5) and 0.5 mL 0-dianisidine solution (0.2% in methanol, freshly prepared). The reaction was initiated with the addition of 0.1 mL of 0.2 mol H_2O_2. The change of absorbance was recorded at 470 nm at an interval of 15 s for 2 min. One unit of POD was defined as 0.1 ▲A470 min^{-1}.

CAT activity was estimated by the method of Goel and Sheoran (2003). The reaction mixture contained 0.6 mL enzyme extract, 0.1 mL of 10 mmol H_2O_2 and 2 mL 30 mmol phosphate buffer (pH = 7.0). The absorbance was recorded at 240 nm immediately after addition of enzyme extract at an interval of 15 s for 2 min. The blank was without enzyme extract. One unit of CAT was defined as 0.1 ▲A240 min^{-1}.

RESULTS

Germination of seeds at different temperatures stored for 12 months

When seeds were stored at 25℃, the lower MC in seeds was maintained and the germination was better (Figure 1A). Seeds with 3.24% MC retained the highest viability after 12 months. These results showed that if *H. persicum* seed was stored at room temperature (25℃), the MC had a significant effect on viability. While after ultra-dry treatment, the seed storability could be improved. Results suggested that under the temperature of 25℃, the optimum MC of seed to be stored was 3.24%. When the storage temperature was switched to 4℃, MC of 7.62 and 6.73% reduced the germination percent slightly, but for MC 5.23, 4.24, 3.24 and 1.02%, germination greatly decreased. At 4℃ the optimum MC was 6.73% (Figure 1B). Storing seeds at -20℃, however, could extend seed storage life and good viability for seeds with all MC retained for at least 12 months (Figure 1C). When the seed contains 3.24% moisture, the ultra-drying treatment preserves the seed vigour, germination viability and percentage.

Effects of desiccation on seed conductivity of *H. persicum*

The integrality of cell membrane is key to maintaining seed vigor. After 12 months, the electric conductivity of MC (3.24%) of seeds is lower than other MC's at 25℃

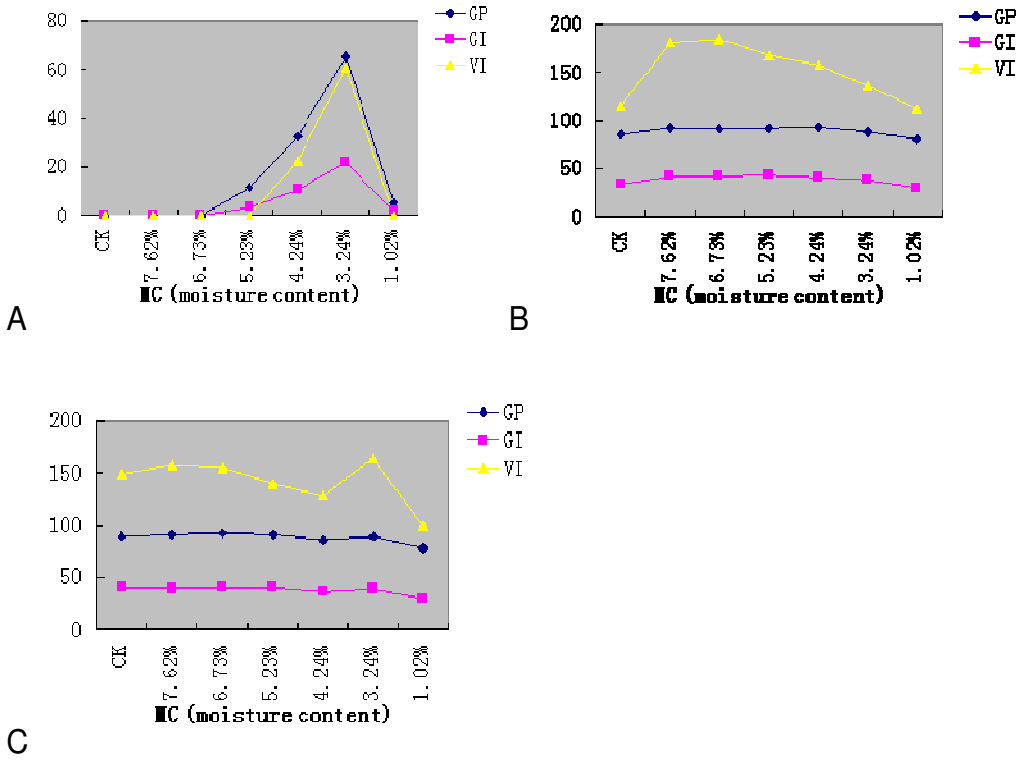

Figure 1. Germination percentage (GP, %), germination index (GI) and vigor index (VI) of *H. persicum* seed stored for 12 months (A. 25℃; B. 4℃; C. -20℃).

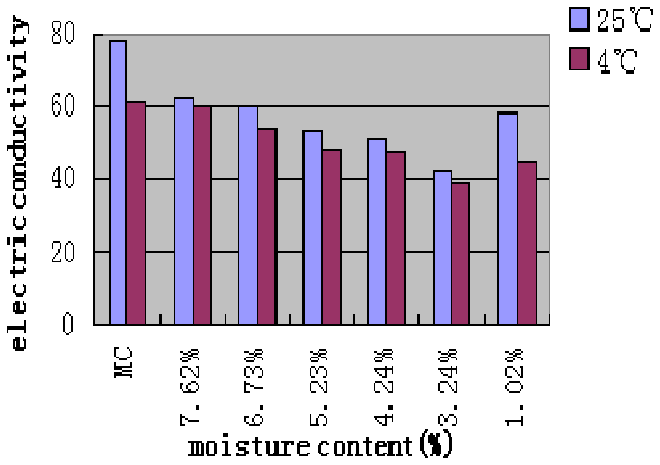

Figure 2. Effects of ultradrying treatment and storage temperature on the electric conductivity of *H. persicum* seeds after 12 months storage.

(Figure 2), whereas the electric conductivity remain the same at 4℃. The stability of different moisture content of ultra-dry *H. persicum* seed's cell membrane is not affected at 25℃ and lower temperatures. In this study, the effect is lowest when the moisture content is 3.24%. MC (3.24%) is fitting moisture content index of ultra-dry *H. persicum* seed.

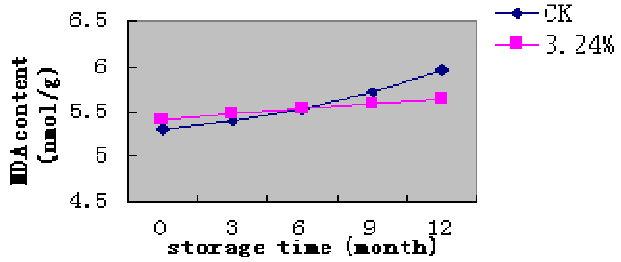

Figure 3. Change of MDA content in ultra-dry storage *H. persicum* seeds.

Physiological indices

The malondialdehyde (MDA) contents of ultra-dry seeds were lower than those of the control (Figure 3). During the normal storage process, the SOD, POD and CAT activities will decrease with storage time. After 12 months in normal storage, the SOD, POD and CAT activities reduced evidently (Figure 4). The activity varied slightly through seeds ultra-drying treatment. This shows that ultra-dry storage can prevent the activities from falling. It indicates that the deterioration of ultra-dry seeds was less than the control. Perhaps the ultra-dry seeds had an efficient antioxidant defense system that made the degrees of lipid oxidation and lipid peroxidation lower. This result is consistent with changes of germination and vigor.

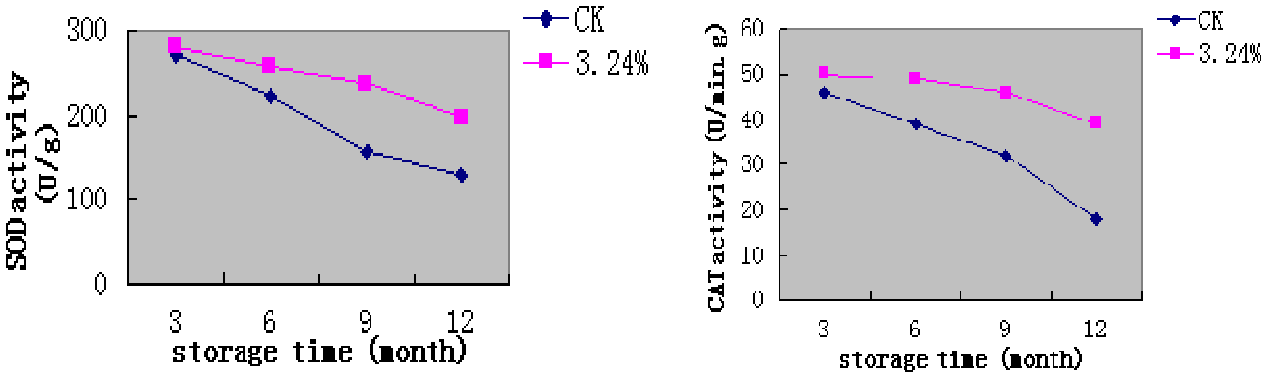

Figure 4. Changes of SOD and CAT of *H. persicum* seeds with different moisture content.

DISCUSSION

The seed vigor is affected by storage time, storage temperature, seed moisture content and environmental humidity. Seed moisture content and storage temperature are the most important factors. The seed's physiological characteristics, which include germination percentage, electric conductivity and the activities of SOD, POD and CAT, varies with different moisture contents of seed. The appropriate moisture content of seed will improve seed's vigor.

H. persicum seeds, with five levels of moisture content (%) were stored for one years at three temperatures (25, 4 and -20℃) and their viability in each combination were measured in this experiment. The results indicated that the activities of dehydrogenase, POD, SOD and CAT of the ultra-dry seeds were higher than those of the control seeds. *H. persicum* seeds which have weak tolerances to storage conditions may increase their aging tolerances by ultra-drying treatments to lengthen their vigor and viability to a certain extent. This research showed that the ultra-drying technique could enhance the aging-resistant capability and storability of *H. persicum* seeds. This technique would be potentially useful for the preservation of *H. persicum* germplasm.

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REFERENCES

Bailly C, Benamar A, Corbineau F (1996). Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase actibities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant. 97: 104-110.

Ellis RH, Hong TD, Roberts EH (1986). LongarithInic relationashiP between Inoisture content and longevity in sesae seeds. Ann. Bot. 57: 499-503.

Giannoplitis CN, Ries SK (1977). Superoxide dismutase purification and quantitative relationship with water-soluble protein in seedlings. Plant Physiol. 59: 315-318.

Goel A, Sheoran IS (2003). Lipid peroxidation and peroxide-scavenging enzyme in cotton seeds under natural ageing. Biol. Plant. 46: 429-434.

International Seed Testing Association (ISTA) (1985). International Rules for Seed Testing. Seed Sci. Technol. 13: 299-355.

Kun E, Abood L (1949). Colorimetric estimation on succinic dehydrogenase by TTC. Science, 109: 144-146.

Wang XF, Jing XM, Lin J (2005). Starch mobilization in ultradried seed of maize (*Zea mays* L.) during germination. J. Integr. Plant Biol. 47: 443-451.

Zheng GH, Yan YT, Zhang QC (1988). Studies on the imbibitional chilling injure and the reparation of damaged membrane systems in soybean seeds. Sci. Sin. 31: 45-53.

Zhu G, Zeng GW, Hu JS, Zhang MF, Jing XM (2001). Aging-resistance of ultra-dried onion seeds and its free radical scavenging. J. Zhejiang Univ. Agric. Life Sci. 27: 139-144.