Botanical Studies (2010) 51: 465-472.

Impact of ultra-dry storage on vigor capacity and antioxidant enzyme activities in seed of *Ammopiptanthus mongolica*

Yi LI[1,2,*], Jian-Jun QU[1,2], Wei-Min ZHANG[1,2], Li-Zhe AN[3], Peng XU[4], and Yong-Cai LI[5]

[1]*Dunhuang Gobi and Desert Research Station, Cold and Arid Region Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou Gansu, 730000, P.R. China*
[2]*Key Laboratory of Desert and Desertification, Chinese Academy of Sciences, Lanzhou Gansu, 730000, P.R. China*
[3]*School of Life Science, Lanzhou University, Lanzhou Gansu, 730000, P.R. China*
[4]*Pratacultural College, Gansu Agricultural University, Lanzhou Gansu, 730070, P.R. China*
[5]*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou Gansu, 730070, P.R. China*

(Received July 7, 2009; Accepted May 14, 2010)

ABSTRACT. This research was to determine whether ultra-drying improves the vigor and storability of *Ammopiptanthus mongolica* seeds. Seeds of *A. mongolica* were dried to a 4.67%, 3.73%, and 2.24% moisture content (MC). After storage for 24 months, their level of vigor was measured. To determine whether these low MCs affect the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR), we evaluated the ability of dried seeds to germinate and to produce normal seedlings. Meanwhile, we also measured volatile aldehydes and malondialdehyde (MDA), lipid peroxidation production. Results indicated that the SOD, CAT, POD, APX and GR activities of ultra-dry seeds were higher than those of control seeds while volatile aldehydes and MDA were lower than in the control. The drying tolerance was related to a reorientation of the enzymatic antioxidant defence system. The electrical conductivity of ultra-dry seeds and controls showed obvious differences. All the results showed that ultra-dry storage is beneficial to maintaining the vigor of *A. mongolica* seeds. It is proposed that *A. mongolica* seeds may be stored in an ultra-dry state at ambient temperature for a long time.

Keywords: Antioxidant defence system; Seed longevity; Seed storability.

INTRODUCTION

Desiccation is that part of a seed's life programme that permits its storage and survival in various environment conditions (Leprince et al., 1993). The ability of seeds to withstand severe desiccation generally occurs during the phase of reserve accumulation, but this is dependent on the drying rate, which has been shown to affect seed survival after drying (Kermode, 1995; Pammenter and Berjak, 1999; Bailly et al., 2001). Many cellular and biochemical events appear associated with the desiccation tolerance of seeds. They include events that modify ultrastructural characteristics like vacuolation, synthesis of dehydrins, heat shock proteins, and activation of antioxidative defences (Galau et al., 1991; Vierling, 1991; Leprince et al., 1993; Vertucci and Farrant, 1995; Folkert et al., 2001, Bailly et al., 2001). Reactive oxygen species (ROS) are of increasing interest in seed physiology. Most contributions to date in this field have been concerned with the role of ROS in loss of vigor and viability during the storage of seeds (Bailly et al., 2004). Lipid peroxidation induced by ROS has been widely cited as a major cause of seed aging (Priestley, 1986; Mcdonald, 1999). Dehydration of seeds has been associated with the impairment of antioxidative mechanisms leading to oxidative damage and to numerous lethal lesions (Pammenter and Berjak, 1999; Li and Sun, 1999; Bailly et al., 2004). Tolerance to drought of vegetative tissues is thought to be related to antioxidant enzymes (Sherwin and Farrant, 1998). However, data on the role of antioxidant systems during the seed desiccation tolerance is lacking. The ability of seeds to bear desiccation could be associated with their ability to scavenge ROS in order to avoid deleterious events such as lipid peroxidation. These mechanisms might involve enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Bailly et al., 2001). However, seed germinability might be related to the efficiency of free radical scavenging because this scavenging may affect merely seed storability and vigor (Priestley, 1986; Bailly et al., 2000; Bailly et al., 2001).

*Corresponding author: E-mail: liyi2001@gmail.com.

Ultra-dry storage, also called low moisture content storage, is a technique for decreasing seed moisture content to under 5%. It can greatly reduce the cost of constructing and maintaining the genebank (Zheng et al., 1998). Some studies have confirmed that low moisture content storage can not only be used to maintain the quality of seeds, but also improve their storability (Wang et al., 1999; Wang et al., 2003; Li et al., 2007).

Ammopiptanthus mongolica is a relic of the Tertiary Period, distinctively distributed in the northwestern desert area of China, and it is a dominant plant in the stabilized sand fields. What is unique about *A. mongolicus* is that it remains evergreen for all four seasons in the desert area. It appears well suited to the desert and has a reputation for high tolerance to water deficiency (Wang et al., 2007). Although little is known about its uses, it has a great potential to provide materials to traditional medicine, to halt desert encroachment, and to stabilize sand dunes. The available data about this species include its botanical characteristics, its cultivation method, and brief descriptions of its habitat and geographical distribution (Ge et al., 2005).

The present study aims to determine whether ultra-dry induced loss of *A. mongolica* seed viability during storage is related to lipid peroxidation, as evaluated by volatile aldehydes and MDA and antioxidant enzyme activities, and also to determine whether antioxidant enzymes play a role in preventing lipid peroxidation and seed damage by ultra-dry storage.

MATERIALS AND METHODS

Seed material

Seeds of *Ammopiptanthus mongolica* were harvested from at least ten plants in October 2004 at Minqin Desert Plant Botanical Garden (38°34' N, 102°58' E) Gansu Province, China and represented the equilibrium moisture contents for seeds in open storage in the Lanzhou area in the summer (25°C, 75% relative humidity RH), the initial germination percentage and moisture content (MC) were 96.7% and 10.41%, respectively.

Seed treatment, storage and germination experiments

Seeds were packed in plastic net bags, and the ratio of the seeds to silica gel was 1:5 (w/w). Seed bags were buried into silica gel in a desiccator at 25°C for 15 days to reduce the moisture content of seeds to 4.67%, 3.73%, and 2.24%. The ultra-dry seeds were kept in sealed aluminum foil packages. To avoid imbibition injury, the ultra-dry seeds were hydrated for 48 h in a sealed desiccator containing saturated $CaCl_2$ solutions (RH is 35%). They were then hydrated for 48 h in a sealed desiccator containing saturated NH4Cl solutions (RH is 75%) at 25°C before the germination assessment (Huang et al., 2002). This brief hydration/dehydration treatment reduced the variation in rates of imbibition and germination due to differences in seed characteristics among individual seeds and did not affect seed longevity (Sun and Leopold, 1997; Narayana et al., 2003).

To investigate the storage longevity of the ultra-dry seeds over 24 months at ambient temperatures, the seed lots were stored for 12, 20 and 24 months. After each storage period, 100 seeds were germinated at constant 25°C with a 12 h daylight period during the warm temperature phase. The seed surfaces were sterilized using 10% Na-hypochlorite before the germination process. Four replicates of 100 intact seeds were sown on 1% (w/v) agar (Baoshengwu, Shanghai, China) in distilled water in 9 cm Petri dishes. Seeds were scored twice a week for up to seven weeks. A seed was considered as normally germinated when the radicle protruded by 2-3 cm without infection and the first leaves were visible (ISTA, 1999). A seed vigor index (VI) was determined according to the following equations: $VI=G_I \times S_x$, $GI=\sum(G_t/D_t)$, where G_I was the germination index, S_x was the radicle mean length × days after germination, and G_t was the germination percentage after t days, D_t is days of germination. Each treatment combination of MC, temperature, and storage duration was represented by samples in four sachets and was used to determine the percentage of germinated seeds.

Solute leakage of seeds previously exposed to 45°C at 76 or 100% RH was estimated by placing 3 replicates of 5 naked seeds each in 10 ml of distilled water at 25°C, and measuring the conductivity of the medium with a conductivity meter (model DDS SJ-308A, Shanghai) after a 1.5 h incubation period. To determine electrolyte leakage from seeds held in water at 45°C, 5 naked seeds (3 replicates) were placed for different periods up to 7 days in 10 ml of water at 45°C and the conductivity of the medium was measured at 25°C, Results are expressed as % of total leakage from naked seeds boiled for 30 min in water and represent the means of 4 replicates ± SD.

The moisture content (MC) of four samples of 100 seeds was determined gravimetrically by the oven method (8 h at 110°C ± 1°C) and was expressed on a wet basis (%, w.b.).

Seeds accelerated ageing

The ultra-dry seeds and the control seeds (initial MC) underwent accelerated ageing at 50°C for 1 month in an oven. Afterward, the seeds were put into nylon bags.

Seed volatile aldehydes and malondialdehyde test

Seeds of 5 g were soak in distilled water at 20°C for 12 h. Seeds were homogenized in cold 50 mM, phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 ×*g* at 4°C for 20 min and the supernatant was extracted (Zhu et al., 2001). The contents of volatile aldehydes were determined by the method of Wilson and McDonald (1986). The malondialdehyde (MDA) content was assayed according to Bailly et al. (1996).

Enzyme extraction and assays

The enzyme extraction: 1.0 g seeds were soaked in distilled water at 25°C for 12 h. Seeds were homogenized in ice cold water with 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 ×*g* for 20 min and the supernatant fluid was used in the enzyme assay (Zhu et al., 2001).

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of blue tetrazolium reduction (Giannoplitis and Ries, 1977). Measured inhibition of the photochemical reduction of nitroblue tetrozulium (NBT) was 560 nm. The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic-acid (EDTA), 13 mM methionine, 75 μM NBT, 16.7 μM riboflavin, and enzyme extract. Riboflavin was added last, and the reaction was initiated by placing the tubes under two 9 W fluorescentlamps. The reaction was terminated after 15 min by removal from the light source. An illuminated blank without protein gave the maximum reduction of NBT, and the maximum absorbance was therefore at 560 nm. SOD activity is calculated as the absorbance of samples divided by the absorbance of blank, giving the percentage of inhibition. One unit of SOD is defined as the amount required to inhibit the photoreduction of NBT by 50%. The activity of SOD was expressed as unit/mg protein.

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

Peroxidase (POD) activity was determined by the measurement of Kalpana et al. (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 *O*-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 ΔA_{470}/min.

Ascorbate peroxidase (APX) activity was assayed by using the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 1 mM sodium ascorbate, 2.5 mML H_2O_2 and the enzyme source in a final volume of 3 mL. The absorbance decrease was recorded at 290 nm every 15 s. APX activity was defined using the coefficient of absorbance 2.8 mM/cm.

Glutathione reductase (GR) activity was assayed by the method of Goldberg and Spooner (1983). The reaction mixture contained 0.1 mL enzyme extract, 2.5 mL 120 mmol phosphate buffer (pH 7.2), 0.1 mL of both EDTA (0.015 mmol) and oxidized glutathione (0.065 mmol). After 5 min, the 0.05 mL reduced nicotinamide adenine dinucleotide phosphate (NADPH) (9.6 mmol) was added and mixed thoroughly. The result was monitored after every 15 s at 340 nm. One unit of GR was defined as 0.1 ΔA_{340}/min.

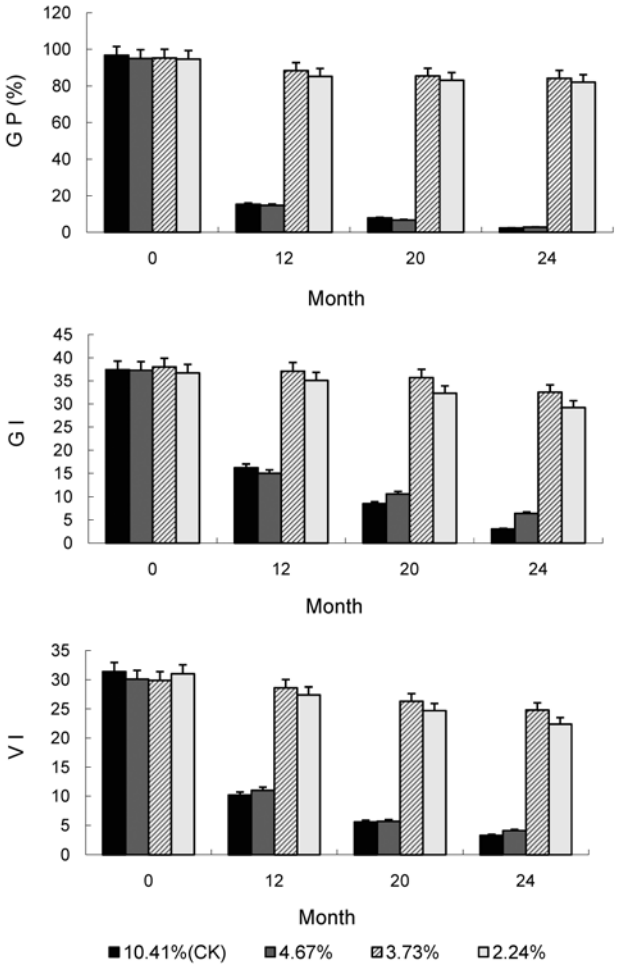
Statistical analysis

All data were based on a mean of four replicates. The data were subjected to an analysis of variance, and LSD values were calculated at P = 0.05.

RESULTS

Effect on seed vigor

After storage at 25°C for 24 months, the seed germination of 10.41% and 4.67% MC fell sharply, but germination for 3.73% and 2.24% MC only edged downward (LSD_{test} P<0.05, Figure 1). This suggests that ultra-dry conditions could improve storability.



GP: Germination Percent; GI: Germination Index; VI: Vigor Index

Figure 1. GP (%), GI and VI of *A. mongolica* seed with different MC stored at 25°C for 24 months. Date are means of four replicates ± standard deviation (SD). Where no bars are shown, the spread of SD is less than the size of symbols.

After accelerated ageing, the ultra-dry seeds maintained higher vigor levels than the control group (Table 1). They were highly tolerant to ageing with a low moisture content. After 1 month of accelerated ageing, the germination percentage (GP) and VI of the control (MC =10.41%) seeds fell sharply while those of the ultra-dried seeds (MC = 3.73% and MC = 2.24%) remained high. The effects of ultra-dry storage for *A. mongolica* seeds with a MC of 3.73% and 2.24% were almost the same as that of control. From Table 1 the electrical conductivity of ultra-dry seeds (MC = 3.73% and MC = 2.24%) was not significantly different from that of control. Thus, integrity of the membrane system in ultra-dry seeds can be maintained. These results suggest that ultra-drying of seeds within certain MC limits has no negative effects on *A. mongolica* seed vigor.

Ultra-dry storage effects on lipid peroxidation and antioxidative enzyme activities

The results of Figure 2 show that after accelerated ageing, the volatile aldehydes and malondialdehyde (MDA) contents of ultra-dry seeds were lower than those of the control (MC = 10.41%) (LSD_{test} $P<0.05$). This result is consistent with changes of germination and vigor (Table 1) and indicates that the deterioration of ultra-dry seeds was less than the control. In the attempt to check whether the antioxidant defense system was efficiently working to reduce lipid peroxidation, the reactive oxygen species scavenging enzymes, SOD, CAT, POD, APX, and GR, were assayed. After accelerated ageing, the activities of these enzymes in ultra-dry seeds (MC = 3.73% and MC = 2.24%) were much higher than those of the non-ultra-dry seeds (MC = 10.41%) (LSDtest P<0.05) (Figures 3 and 4).

DISCUSSION

Ultra-dry seed storage is simple and inexpensive. Seeds can be dried in a desiccator until the correct moisture content is reached (silica gel and quicklime are ideal desiccants). Seeds can be stored in sealed containers at ambient temperature (Zheng et al., 1998). A lot of important progress has been reported (Wang et al., 1999; Wang et al., 2003; Zheng, 2004; Li et al., 2007; Li et al., 2008).

Moisture content is the most important factor affecting the seed storage. The critical MC for seed storage is 5%, and seeds will die faster at 4% than at 6% MC (Harrington, 1973). Our experiment shows that under ultra-dry storage for 24 months, the GP remained very high. This means that the *A. mongolica* seeds can be stored at ambient temperature (25°C) with the relatively low MC, and their longevity decreased as seed MC increased. When the seeds of *A. mongolica* were dried to moisture contents of 3.73% and 2.24%, the viability and vigor were not statistically influenced, and the ageing-resistant capability was greatly enhanced (Figures 1, 2, 3, 4). After accelerated ageing, results showed that the 3.73% moisture content is more appropriate for *A. mongolica* seed under ultra-dry storage. GP, VI and mean radicle length stayed higher than in non-

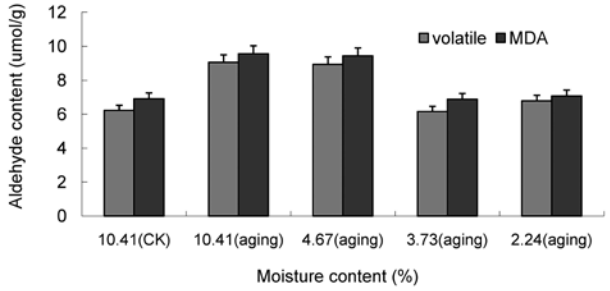


Figure 2. Volatile aldehydes and malondialdehyde contents after accelerated ageing at 50°C for 1 month. Date are means of four replicates ± standard deviation (SD).

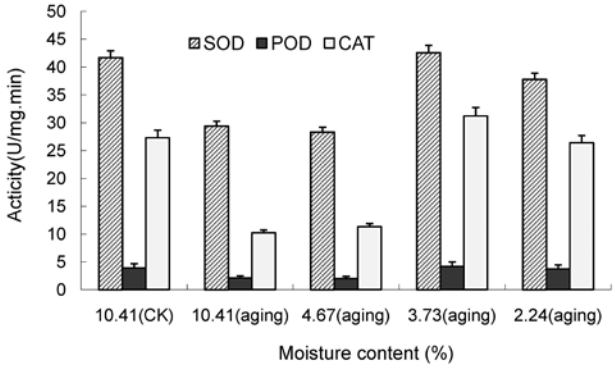


Figure 3. Changes of the activities of CAT, SOD and POD after accelerated ageing at 50°C for 1 month. Date are means of four replicates ± standard deviation (SD).

Table 1. Germination ability, vigor and electrical conductivity of ultra-dried seeds after accelerated ageing at 50°C for 1 month.

MC (%)	GP (%)	VI	Mean radicle length (cm)	Electrical conductivity (%)
10.41 (CK)	96.0±0.1a	31.5±0.1a	3.77±0.36a	35.33±0.11b
10.41 (aging)	33.2±0.2c	11.3±0.1c	1.11±0.03d	68.24±0.02c
4.67 (aging)	40.0±0.1b	9.3±0.1d	1.25±0.07d	70.31±0.17c
3.73 (aging)	96.2±0.2a	28.6±0.2b	3.70±0.24a	36.71±0.10a
2.24 (aging)	95.3±0.2a	27.7±0.1b	3.65±0.19b	37.00±0.04a

The values in a column with the same alphabetical letter are not significantly different (LSD_{test}, $P < 0.05$). All values are means ± SD of four replicates. MC, moisture content; VI, vigor index; GP, germination percentage.

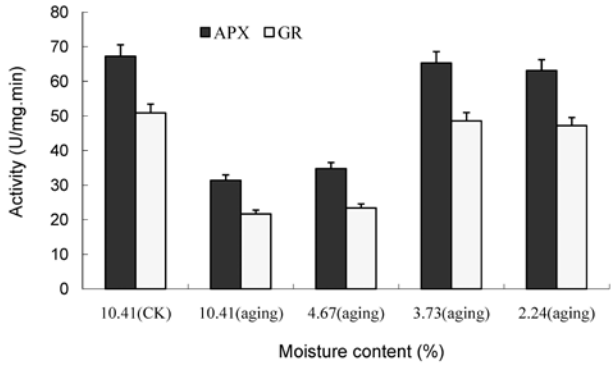


Figure 4. Changes of the activities of APX and GR after accelerated ageing at 50°C for 1 month. Date are means of four replicates ± standard deviation (SD).

ultra-dry seeds, implying that no biochemical or biophysical reaction occurred to injure the seed cells under low MC conditions (Table 1). Since maintaining seed viability during long-term storage is of the utmost importance, it appears that storage under low MC at ambient temperature is absolutely feasible. Our approach provides evidence showing that optimum moisture content for *A. mongolica* seed storage at ambient temperature is 3.73%.

In aged seeds, because of the destruction of the membrane system, many materials flow out of cells and in turn, the vigor of seed is reduced (Wang et al., 1999). Seed deterioration can be explained by the findings that the aging of seeds leads to lipid peroxidation that subsequently causes membrane perturbation (Goel and Sheoran, 2003). Because disintegration of cell membranes in the seed takes place during seed deterioration changes in the membrane of aged seeds lead to electrolyte leakage. The tolerance to desiccation, then, can be expressed by the electrolyte leakage rate (Berjak et al., 1993; Leprince et al., 1995). The change of electric conductivity during seed soaking is commonly used as an indicator for testing the integrity of the plasma membrane (Wang et al., 2003). In this study, the electrolyte leakage rate of *A. mongolica* increased after aging (Table 1), indicating a loss in membrane integrity. However, the electrical conductivity of ultra-dry seeds (MC = 3.73% and MC = 2.24%) was significantly lower than in the control (MC = 10.41%), indicating that ultra-drying can improve membrane function during *A. mongolica* seed desiccation. After high temperature ageing, the ultra-dry seeds showed strong storability. Compared with the control group, the electrical conductivity remained stable, which means that the integrity of the membrane system in the ultra-dry seeds was maintained during storage.

Based on our previous work dealing with the involvement of ROS in seed physiology (Bailly et al., 2008), we suggest that ROS content changes throughout all the stages of seed life. We propose that ROS, not only play a key role in the completion, they are also messengers of environmental cues during seed germination. Germination in non-optimal conditions is a stressful situation. It may enhance ROS generation and, in turn, prevent radicle emergence. However, under appropriate conditions, ROS must be present in sufficient levels to allow completion of the process (Bailly et al., 2008). The deleterious role ROS play in seeds is due to their high reactivity toward biomolecules like proteins, sugars, lipids, and nucleic acids. Two physiological processes that occur in seeds appear tightly linked to the deleterious role of ROS, seed desiccation and seed ageing. Oxidative processes and free radicals are usually considered to be involved in molecular and cellular damage induced by a wide range of stresses including dehydration (Smirnoff, 1993; Côme and Corbineau, 1996). Water loss is associated with an accumulation of free radicals and lipid peroxidation, and substantial damage to cell structure is then evident (Corbineau et al., 2004). The free radical damage hypothesis of desiccation injury postulates that various protective mechanisms become progressively unable to protect the cell structures against ROS generation during water loss and subsequent rehydration (Côme and Corbineau, 1996). For example the activity of the antioxidant enzymes, glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT), increases during dehydration in both tolerant and sensitive tissues (Farrant et al., 2004). Among these mechanisms, the antioxidant systems seem to play a key role, probably due to a reduction of mitochondrial activity, the main source of H_2O_2 generation, and to an antioxidant defense system efficient enough to control ROS accumulation during water loss. In our research, the changes of volatile aldehydes and MDA contents in seeds support the idea that loss of seed viability is associated with lipid peroxidation. The results of this experiment show that in the ultra-dried seeds, the activities of APX, CAT, GR, SOD and POD stayed high. Seed deterioration has been suspected to be associated with an accumulation of active forms of oxygen, including superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (.OH) (Noctor and Foyer, 1998). Antioxidant defense systems in plants include free radical and peroxide-scavenging enzymes (Song et al., 2004). During our research, the activities of SOD, CAT and APX significantly increased after accelerated aging (Figures 3 and 4). These results show that the activity changes of antioxidant enzymes are closely related to desiccation tolerance, and the ultra-drying process does not destroy the enzymes. It can prolong seed storage life by increasing SOD activity and decreasing the O^{2-} level. Reduced glutathione is an important non-enzymatic antioxidant that can act as a direct free radical scavenger (McDonald, 1999). Glutathione is a water-soluble antioxidant found in the cytoplasm that can eliminate hydroxyl radical and single oxygen radicals (Simth et al., 1989; Goel and Sheoran, 2003). In this study, the activity of GR increased under ultra-drying after accelerated aging (Figure 4). This increase in activity results in increased levels of reduced glutathione, a potent free radical scavenger. In this study, after ageing, a significant difference in the GR of the ultra-dry seeds (MC less than 5%) and non-ultra-dry seeds (MC more than 5%) was observed. Lipid peroxidation mediated by free radical and

peroxides is one of the probable reasons for seed viability loss during storage (Sung, 1996; Goel and Sheoran, 2003). Loss of viability and vigor were associated with increased peroxidation in rapidly aged seeds. The volatile aldehydes and MDA are the products of lipid oxidation and peroxidation. The contents of these products in seeds is therefore indicative of the degree of deterioration. Determination of the volatile aldehydes and MDA is a convenient method of quantifying the extent of lipid peroxidation. In our research, these contents decreased after accelerated ageing and were correlated with the increase in the activities of SOD, POD, APX, CAT and GR (Figures 2, 3, 4). The results of this experiment show that lipid peroxidation was greatly suppressed under ultra-drying conditions, implying that the enzyme systems were not destroyed and that highly active antioxidant enzymes remained in ultra-dry seeds. Our results establish a clear relationship between antioxidant enzymes, lipid peroxidation, and ultra-dry storage.

The present results have confirmed that ultra-dry treatment of *A. mongolica* seeds has not only not injured them, it has strongly enhanced their ageing resistant capability and storability. Ultra-dry storage at ambient temperature will be potentially useful for the preservation of *A. mongolica* germplasm.

LITERATURE CITED

Bailly, C., A. Benamar, F. Corbineau, and D. Côme. 2000. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Sci. Res. **10:** 35-42.

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

Bailly, C., H.E.M. Bouteau, and F. Corbineau. 2008. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. C. R. Biologies. **331:** 806-814.

Bailly, C., A. Catherine, L. Fabienne, H. Marie, C. Francoise, and C. Daniel. 2001. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. J. Exp. Bot. **52:** 701-708.

Bailly, C., J. Leymarie, A. Lehner, S. Rousseau, D. Côme, and F. Corbineau. 2004. Catalase activity and expression in developing sunflower seeds as related to drying. J. Exp. Bot. **55:** 475-483.

Berjak, P., C.W. Vertucci, and N.W. Pammenter. 1993. Effect of developmental status and dehydration rate on characteristics of water and desiccation-sensitivity in recalcitrant seeds of *Camellia sinensis*. Seed Sci. Res. **3:** 155-166.

Côme, D. and F. Corbineau. 1996. Metabolic damage related to desiccation sensitivity. *In* A.S. Ouédrago, K. Poulsen, and F. Stubsgaard (eds.), Intermediate/Recalcitrant Tropical Forest Tree Seeds. IPGRI, Roma, pp. 107-120.

Corbineau, F., P. Berjak, N. Pammenter, D. Vinel, M.A. Picard, and D. Côme. 2004. Reversible cellular and metabolic changes induced by dehydration in desiccation-tolerant wheat seedling shoots. Physiol. Plant. **122:** 28-38.

Farrant, J.M., C. Bailly, J. Leymarie, B. Hamman, D. Côme, and F. Corbineau. 2004. Wheat seedlings as a model to understand desiccation tolerance and sensitivity. Physiol. Plant. **120:** 563-574.

Folkert, A., A. Elena, Golovina, and B. Julia. 2001. Mechanisms of plant desiccation tolerance. Trends Plant. Sci. **6:** 431-438.

Galau, G.A., K.S. Jakobsen, and D.W. Hugues. 1991. The control of late dicot embryogenesis and early germination. Physiol. Plant. **81:** 280-288.

Ge, X.J., Y. Yu, Y.M. Yuan, C. Yun, and H.W. Huang. 2005. Genetic diversity and geographic differentiation in endangered *Ammopiptanthus* (Leguminosae) populations in desert regions of northwest China as revealed by ISSR analysis. Ann. Bot. **95:** 843-851.

Giannoplitis, C.N. and S.K. Ries. 1977. Superoxide dismutase purification and quantitative relationship with water-soluble protein in seedlings. Plant. Physiol. **59:** 315-318.

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

Goldberg, D.M. and R.J. Spooner. 1983. Glutathione reductase. *In* H.U. Bergmeyer (ed.), Methods of Enzyme Analysis. Vol. III. Verlag Chemie, Weinheim Deerfield Beach, pp. 258-265.

Harrington, J.F. 1973. Biochemical basis of seed longevity. Seed Sci. Technol. **1:** 453-461.

Huang, Z.Y., X.S. Zhang, and G.H. Zheng. 2002. Increased storability of *Haloxylon ammodendrom* seeds in ultradry storage. Acta Bot. Sin. **44:** 239-241.

ISTA. 1999. International Seed Testing Association. International rules for seed testing. Seed Sci. Technol. **27:** 27-35, 47-50.

Kalpana, R., Madhava, and K.V. Rao. 1995. On the aging mechanism in pigeonpea (*Cajanus cajan* L.) seed. Seed Sci. Technol. **23:** 1-9.

Kermode, A.R. 1995. Regulatory mechanisms in the transition from seed develop to germinate: interactions between the embryo and the seed environment. *In* J. Kigel and G. Galili (eds.), Seed development and germination. Marcel Dekker, New York, pp. 273-332.

Leprince, O., G.A.F. Hendry, and B.D. Mckersie. 1993. The mechanisms of desiccation tolerance in developing seeds. Seed Sci. Res. **3:** 231-246.

Leprince, O., C.W. Vertucci, G.A. Hengdry, and N.W. Atherton. 1995. The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature. Physiol. Plant. **94:** 233-240.

Li, Y., H.Y. Feng, T. Chen, and L.Z. An. 2007. Physiological responses of *Limonium aureum* seeds to ultra-drying. J. In-

tegr. Plant. Biol. **49:** 569-575.

Li, Y., J.J. Qu, Z. Dong, T. Wang, and L.Z. An. 2008. Storage behavior of *Zygophyllum xanthoxylon* (Bge.) Maxim seeds at low moisture contents. Acta Physiol. Plant. **30:** 651-656.

Li, C. and W. Sun. 1999. Desiccation sensitivity and activities of free radical-scavenging enzymes in recalcitrant *Theobroma cacao* seeds. Seed Sci. Res. **9:** 207-217.

McDonald, M.B. 1999. Seed deterioration: Physiology, repair and assessment. Seed Sci. Technol. **27:** 177-237.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. **22:** 867-880.

Narayana, U.M., P.K. Prakash, and Q.S. Wendell. 2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiate* (L.) Wilczke: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. J. Exp. Bot. **54:** 1057-1067.

Noctor, G. and C.H. Foyer. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. **49:** 13-37.

Pammenter, N.W. and P. Berjak. 1999. A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. Seed Sci. Res. **9:** 13-37.

Priestley, D.A. 1986. Seed Aging. Implication of seed storage and persistence in the soil. Cornell University Press, Ithaca.

Sherwin, H.W. and J.M. Farrant. 1998. Protection mechanisms against excess light in the resurrection plant *Craterostigma wilmsii* and *Xerophyta viscosa*. Plant. Growth. Regul. **24:** 203-210.

Simth, I.K., T.L. Vierheller, and C. Thorne. 1989. Properties and functions of glutathione reductase in plants. Physiol. Plant. **77:** 449-456.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. **125:** 27-58.

Song, S.Q., P. Berjak, and N.W. Pammenter. 2004. Desiccation sensitivity of *Trichiilla dregeana* axes and antioxidant role of ascorbic acid. Acta Bot. Sin. **46:** 803-810.

Sun, W.Q. and A.C. Leopold. 1997. Cytoplasmic vitrification and survival of anhydrobiotic organisms. Comparative Biochem. Physiol. **117A:** 327-333.

Sung, J.M. 1996. Lipid peroxidation and peroxide scavenging in soybean seeds during aging. Physiol. Plant. **97:** 85-89.

Vertucci, C.W. and J.M. Farrant. 1995. Acquisition and loss of desiccation tolerance. *In* J. Kigel and G. Galili (eds.), Seed development and germination. Marcel Dekker, New York, pp. 237-271.

Vierling, E. 1991. The roles of heat shock protein in plants. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. **42:** 579-620.

Wang, W., J.J. Chen, J. Li, Y.Z. Zhang, Z.Y. Shao, and B. Kuai. 2007. Extraordinary accumulations of antioxidants in *Ammopiptanthus mongolicus* (Leguminosae) and *Tetraena mongolica* (Zygophyllaceae) distributed in extremely stressful environments. Bot. Stud. **48:** 55-61.

Wang, X.F., X.M. Jing, and J. Lin. 2003. Studies on membrane function and sugar components of ultradried seeds. Acta Bot. Sin. **45:** 23-31.

Wang, X.F., G.H. Zheng, and S.J. Yang. 1999. Effect of ultradry storage on fluidity of plasma membrane of seeds. Chinese Sci. Bull. **44:** 1008-1013.

Wilson, D. and M. Mcdonald. 1986. A convenient volatile aldehydes assay for measuring soybean seed vigor. Seed Sci. Technol. **1:** 256-258.

Zheng, G.H. 2004. Ultradry seed storage: Improved strategy and technology for germplasm conservation. *In* G.H. Zheng (ed.), Seed Physiology Research. Science Press, Beijing, pp. 131-136.

Zheng, G.H., X.M. Jing, and K.L. Tao. 1998. Ultradry seed storage cuts cost of gene bank. Nature **393:** 223-224.

Zhu, C., G.W. Zeng, J.S. Hu, M.F. Zhang, and X.M. Jing. 2001. Aging-resistance of ultra-dried onion seeds and its free radical scavenging. J. Zhejiang. Univ. Sci. **27:** 139-144.

超乾保存對蒙古沙冬青（*Ammopiptanthus mongolica*）種子活力和抗氧化酶活性的影響

李 毅[1,2] 屈建軍[1,2] 張偉民[1,2] 安黎哲[3] 徐 鵬[4] 李永才[5]

[1] 中國科學院 寒區旱區環境與工程研究所 敦煌戈壁荒漠研究站

[2] 中國科學院 沙漠與沙漠化重點實驗室

[3] 中國甘肅蘭州大學 生命科學學院

[4] 中國甘肅農業大學 草業學院

[5] 中國甘肅農業大學 食品科技與工程學院

本文探討了超乾保存是否能夠提高蒙古沙冬青種子活力和種子保存穩定性。實驗中，採用矽膠乾燥法將蒙古沙冬青種子的含水量分別降至 4.67%、3.73%、2.24%，然後在 25°C 條件下貯藏 24 個月，測定其相關種子生理指標。種子進行超乾處理後，50°C 條件下進行人工老化處理 1 個月，測定生理指標。超乾種子 POD 酶、SOD 酶、CAT 酶、APX 酶、GR 酶的活性高於未經超乾處理的種子，而揮發性醛類物質和丙二醛的含量水準低於對照處理。電導率測定結果表明，超乾種子和未經超乾處理的種子存在顯著差異。以上結果說明，將蒙古沙冬青種子含水量降至 5% 以下，可以提高種子的抗老化能力，並且對於種質資源保存來說，超乾保存是一項簡單又經濟的方法。

關鍵詞：超乾保存；蒙古沙冬青；抗氧化系統酶。