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A report on ultra-dry storage experiment of *Zygophyllum xanthoxylon* seeds

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ABSTRACT. This research aimed to determine whether ultra-dry storage improves the longevity of *Zygophyllum xanthoxylon* seeds. Moisture content of *Z. xanthoxylon* seeds was dried to 4.81%, 3.81%, 2.41% and 1.99% (w.b.) in a desiccating container with silica gel, and stored at 45°C, 25°C and 15°C for 24 months. The data from 24 months showed that the optimum moisture content for storage varies with temperature. Our results found that optimum moisture can not be considered independently of temperature. After ultra-drying the seeds were accelerated aged (50°C, 1 month), some physiological indices were tested. The results indicated that Dehydrogenase, POD, SOD and CAT activities of the ultra-dry seeds were higher than those of the control seeds, while volatile aldehydes and malondialdehyde were lower than the control group. The results indicate that moisture content of seed was a key index for storage at ambient temperature (25°C) and 3.81% seem to be the best moisture content for ultra-dry seeds in our research. RAPD markers were also used to evaluate the genetic fidelity of seeds, all RAPD profiles from ultra-dry seeds were monomorphic and similar to non-ultra-dry seeds, we conclude that variation is almost absent in ultra-dry storage. From these results, we suggest that seed moisture content less than 5% enhances longevity and ultra-dry could be an economical way for conservation of the plant genetic resource.

Keywords: Moisture content; Physiological indices; RAPD; Seed storage; Ultra-dry; *Zygophyllum xanthoxylon*.

INTRODUCTION

Today facing with the great loss of plant biodiversity around the world, one way to protect is to store the plant seeds in gene bank. The effective preservation of seeds depends on their moisture content and the store temperature (Hsu et al., 2000; Tsou and Mori, 2002), but in developing countries where the costs of cold storage are prohibitive (Zheng and Jing, 1998). Low moisture content conservation (it also called ultra-dry seed storage) through long-term storage of seed is possible for a significant proportion of higher plants. Where feasible, long-term seed storage serves as a safe and relatively inexpensive method of plant genetic resources conservation (Hong and Ellis, 1996). Ultra-dry seed storage is a technique for decreasing the seed moisture content to less than 5% and stored at ambient temperatures, it can reduce the cost for constructing and maintaining the genebank and has brought worldwide attention because of its potential economic effect and promising application in germplasm conservation. A lot of studies have been confirmed that ultra-dry seed storage not only can be used to maintain the quality of seeds but also improve the storability of seeds (Wang et al., 2003). Positive results of ultra-dry storage to improve storability have been reported (Eills et al., 1989, 1990a, 1992, 1993, 1994, 1995; Cheng et al., 1991; Zheng and Jing, 1998; Wang et al., 1999; Zhu et al., 2001; Huang et al., 2002; Wang et al., 2005; Li et al., 2007).

Seeds during long-term storage at last lost their ability to germinate. There are some papers have identified lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic integrity as major cause (Priestly, 1986; Smith and Berjak, 1995; Walters, 1998; McDonald, 1999; Narayana Murthy et al., 2003). Under accelerated aging conditions such as high temperature and high seed water moisture lead to biochemical deterioration during seed aging (McDonald, 1999). In these cases, lipid peroxidation and the loss of membrane phospholipids are major cause of seed aging under accelerated aging conditions; the consequence of

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formation of an increase amount of free oxygen radicals (Goel and Sheoran, 2003). Several protective mechanisms including free radical and peroxide scavenging enzymes, for example superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been evolved within seeds (McDonald, 1999). SOD is a key enzyme in the regulation of the amount of superoxide radicals and peroxides. Hydrogen peroxide can react in the Haber-Weiss reaction forming hydroxyl radicals (Bowler et al. 1992) that cause lipid peroxidation. CAT and POD are implicated in the removal of H_2O_2 (Goel and Sheoran, 2003). The removal of H_2O_2 through a series of reactions is known as an ascorbate-glutathione cycle in which ascorbate and glutathione participate in a cyclic transfer of reducing equivalents resulting in the reduction of H_2O_2 to H_2O using electrons derived form nicotinamide adenine dinucleotide phosphate (NADPH) (Goel and Sheoran, 2003).

Zygophyllum xanthoxylon is a dominant plant in the stabilized sand fields in the northern desert of China. They appear to be suitable for desert and have a reputation for high tolerance to water deficiency (Zhao and Zhu, 2003; Zhou et al., 2006). Although little is known about their uses, they have great potential to provide different services such as traditional medicine, halting desert encroachment and stabilizing sand dunes (Zhou et al., 2006). The available data about this species are its botany characteristics, cultivation method, brief descriptions of its habitat condition, and the range of its geographical distribution (Zeng et al., 2004; Zhou et al., 2006). However, reports on the protection germplasm resource and biochemical basis of seed storage in *Z. xanthoxylon* are very few. Hence, our aim was to investigate seed germination ability and viability in *Z. xanthoxylon* seeds after ultra-drying and to explore the physiology mechanism of ultra-dry storage.

MATERIALS AND METHODS

Plant material

Seeds of *Zygophyllum xanthoxylon* were harvested from at least 10 plant species 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% RH), there an initial germination percentage of 90.9% and moisture content (MC) of 11.43% were determined.

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 desiccator at normal atmospheric temperature (25°C) for 15 d to reduce the moisture content of seeds to 4.81%, 3.81%, 2.41% and 1.99%. The ultra-dried seeds were kept in sealed aluminum foil packages for experiment.

The rapid uptake of water by dry seeds can result in imbibition injury (Powell and Matthews, 1978). Seeds are more likely to be damaged the lower their initial moisture content (Ellis et al., 1990b) at which they imbibe water. Imbibition injury can be avoided by conditioning (humidifying) the seeds in a moist atmosphere (close to 100% RH) in order to raise seed moisture contents before the seeds are set to germinate in contact with liquid water (Ellis et al., 1985). In our research to avoid the imbibition injury, the ultra-dried seeds were hydrated for 48 h in a sealed desiccator containing saturated $CaCl_2$ solutions (RH is 35%) and then they were hydrated for 48 h in a sealed desiccator containing saturated NH_4Cl solutions (RH is 75%) at normal atmospheric temperature (25-30°C) (Huang et al., 2002) before the germination assessment and the following experiment.

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

According to International Rules for Seed Testing (ISTA, 1993). Moisture content (MC) of three samples of 100 seeds was determined gravimetrically by the oven method (8 h at 110°C ± 1°C) and could be expressed on the wet basis (%, w.b.). The seed surfaces were sterilized using 10% Na-hypochlorite the 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°C ±1°C. Four 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: $VI = G_I \times S_x$, $G_I = \sum(G_t/D_t)$, 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 24 months at three different temperatures, the seed lot was split into three sub-samples. Seeds were stored at 45, 25 and 15°C, 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°C 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°C for 12 h (Zheng et al., 1988). The conductivity of the soaking water was measured by conductivity meter (model DDS SJ-308A,

made in Shanghai). Leakage rate was expressed in l/μs.cm.

Seed volatile aldehydes and malondialdehyde test

5 g seeds were soak in distill water at 20°C 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°C for 20 min and the supernatant was collected (Zhu et al., 2001). The content of volatile aldehydes was 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

For enzyme extraction, 1.0 g seeds were soaked in distilled water at 25°C 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).

Superoxide dismutase (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 mmol/L ethylenediaminetetra-acetic-acid (EDTA), 13 mmol/L methionine, 75 μmol/L NBT, 16.7 μmol/L riboflavin and enzyme extract. Riboflavin was added at last and the reaction was initiated by placing the tubes under two 9W fluorescent lamps. The reaction was terminated after 15 min by removal from the light scource. 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 of 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 *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 $\Delta A_{470}\,min^{-1}$.

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 $\Delta A_{240}\,min^{-1}$.

RAPD marker

DNA of seeds derived from radicel and the method described by Hanania et al. (2004).

For PCR amplification, eight arbitrary 10-base primers were selected for PCR amplification. Amplification reactions were performed with 25 dm^3 of 10×assay buffer, 2.0 of 1.25 mM each of dNTP's, 15 ng of the primer, 1× Taq polymerase buffer, 0.5 units of Taq DNA polymerase (TaKaRa), 2.5 mM $MgCl_2$, and 30 ng of genomic DNA. DNA amplification was performed in a Perkin Elmer Cetus 480 DNA Thermal Cycler programmed for 45 cycles as follows: 1st cycle of 3.5 min at 92°C, 1 min at 35°C, 2 min at 72°C; followed by 44 cycles each of 1 min at 92°C, 1 min at 35°C, 2 min at 72°C followed by one final extension cycle of 7 min at 72°C. The amplification products were separated by electrophoresis in 1.2% (w/v) agarose gels with 0.5×TBE buffer, stained with 0.2 mg dm^{-3} ethidium bromide. A 1 kb DNA ladder was used as molecular standards and the bands were visualized and analyzed by JD-801 Gel Electrophoresis Image analytic system (Jiangsu, China). All the reactions were repeated at least twice.

Statistical analysis

Student's t-test was used to statistically test the difference between two means. For comparison of means multiple treatments, Tukey's test was used. To fit the normality, the percentage values were arcsine transformed prior to statistical analysis. Significance lecel was at P=0.05.

RESULTS

Germination of seeds at different temperature stored for 24 months

Seeds stored at 45°C, the lower MC that was maintained in seeds, the better was germination (Figure 1). Seeds at 1.99% MC retained higher viability after 24 months, whereas seed viability was reduced gradually if moisture content was maintained at original MC or higher than 1.99%. These results showed that *Z. xanthoxylon* seed stored at high temperature (45°C) the MC had a significant effect on viability, but after ultra-dry treatment, the seed storability could be improved. Results suggested that seeds stored at 45°C the optimum MC was 1.99%. When the storage temperature was 25°C, MC of 11.43%, 4.81% and 1.99% reduced the germination percent greatly, but for MC 3.81% and 2.41%, only a little decreased. This suggested that the storability could be improved by ultra-dry condition. Stored at 25°C the optimum MC was 3.81%-2.41% (Figure 2). Storing seeds at 15°C, however, could extend seed storage life and good viability for seeds with all MC retained for at least 24 months, except for the control (MC 11.43%) (Figure 3).

Seed vigor

After accelerated aging, the ultra-dry seeds still kept higher vigor levels in comparison with the control group. Table 1 shows that the *Z. xanthoxylon* seeds are tolerant to dehydration. They were highly tolerant to aging with low moisture content (MC) (MC less than 5%). After 1 month of accelerated aging, the germination percentage (GP) and VI of the control (MC 11.43%) seeds decreased greatly, meanwhile those of the ultra-dried seeds (MC 3.81% and MC 2.41%) remained at a high level. The effect of ultra-dry storage for *Z. xanthoxylon* seeds with a MC of 3.81% was almost the same as that of low temperature storage (-18°C). However, for the seeds with a MC of 2.41%, the vigor began to decline. From Table 1 the electrical conductivity of ultra-dry seeds (MC 3.81%) was not significantly different from that of MC 11.43% stored at -18°C. This suggests that the 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 *Z. xanthoxylon* seed vigor, however the *Z. xanthoxylon* seeds can not be dried too severely.

Physiologlcal indices

We have found that after accelerated aging, volatile aldehydes and malondialdehyde (MDA) contents of ultra-dry seeds were lower than those of the control (MC 11.43%). 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 degree of lipid oxidation and lipid peroxidation lower (Figure 4). After accelerated aging, the activities of dehydrogenase (Figure 5) was higher than those of the non-ultra-dry seeds (MC 11.43%). This result is consistent with changes of germination and vigor (Table 1).

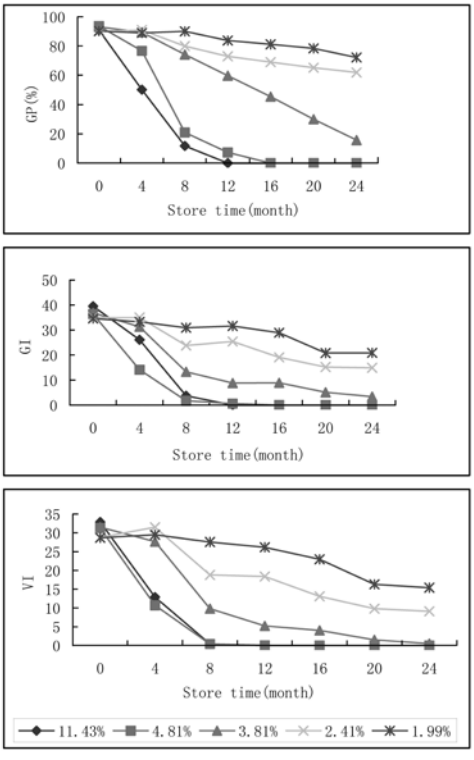

Figure 1. GP (%), GI and VI of *Z. xanthoxylon* seed stored at 45°C for 24 months.

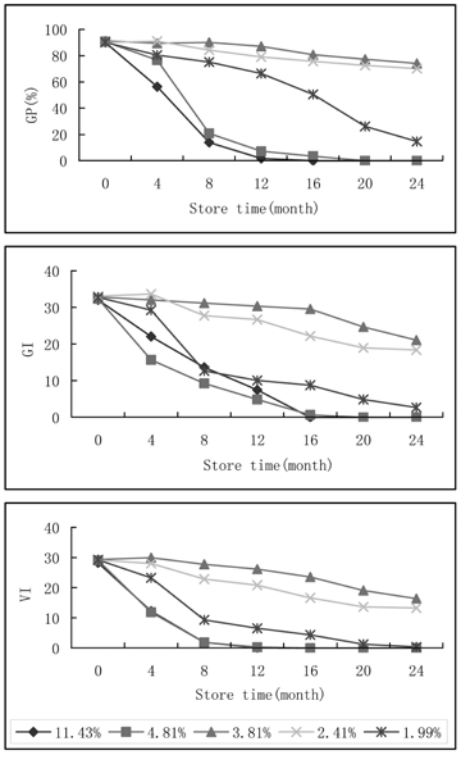

Figure 2. GP (%), GI and VI of *Z. xanthoxylon* seed stored at 25°C for 24 months.

Table 1. Germinating ability, vigor and electrical conductivity of ultra-dry seeds after accelerated aging for 1 month.

Treatment	MC (%)	GP (%)	Mean radicle length (CM)	VI	Electrical Conductivity (μS·cm)
-18°C storage	11.43	90.93 a	6.51±0.36 a	32.88 a	38.39 a
50°C aging	11.43	30.12 c	0.14±0.02 c	1.56 c	75.43 c
50°C aging	3.81	90.55 a	6.57±0.31 a	33.00 a	38.01 a
50°C aging	2.41	68.88 b	5.35±0.24 b	14.15 b	52.11 b

The values in a column with the same alphabetical letter are not significantly differen. All values are means ± SD of three replicates.

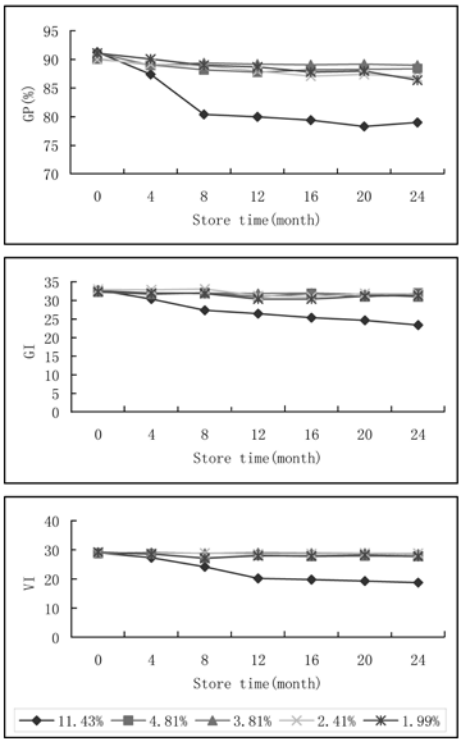

Figure 3. GP (%), GI and VI of *Z. xanthoxylon* seed stored at 15°C for 24 months.

Monitoring of genetic fidelity by RAPD

In order to confirm genetic fidelity (at molecular level) of seeds after ultra-dry treatment, the seeds were screened with the 8 random RAPD primers, one primer that produced distinct amplification profiles. The representative profile of the ultra-dry seeds and the control (non-ultra-dry) with primer is shown in Figure 7. It was obvious that the ultra-dry seeds showed identical RAPD profiles (i.e. no polymorphism was observed). These results confirmed the genetic fidelity of the ultra-dry seeds.

DISCUSSION

At low temperature (15°C) the aging of seeds is quite slow (Figure 3). In our experiment GP remained at very high even after 24 months of storage. We showed that optimum MC was observed for three temperatures study and the critical value decreased with temperature increased (Figures 1-2). This means *Z. xanthoxylon* seeds can be stored over a wide range of temperatures at the relatively low MC (MC <5%), their longevity begins to vary greatly as seed MC is increased (Figures 1-3). Since maintaining seed viability during long-term storage is of the utmost importance, it appears that storage at this low MC (MC <5%) and at ambient temperature, is absolutely necessary. So, it is very important to be able to store seeds without use of low temperature if seed longevity and vigor can be maintained. But reports using numerous species (Vertucci et al., 1994; Ellis et al., 1995; Chai et al., 1998; Hu et al., 1998a, b; Kong and Zhang, 1998; Shen and Qi,

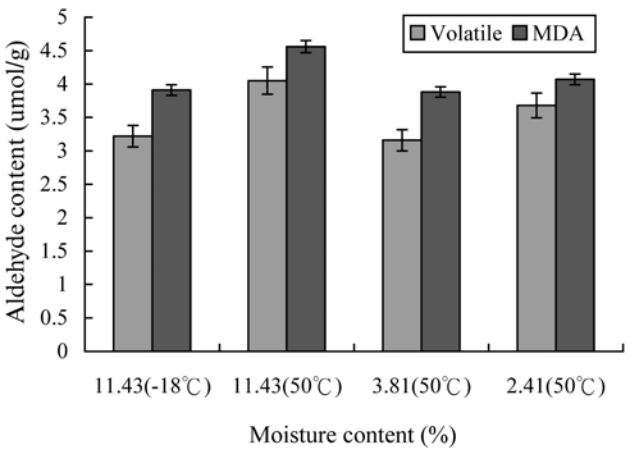

Figure 4. Volatile aldehydes and malondialdehyde contents. All values are means ± SD of three replicates.

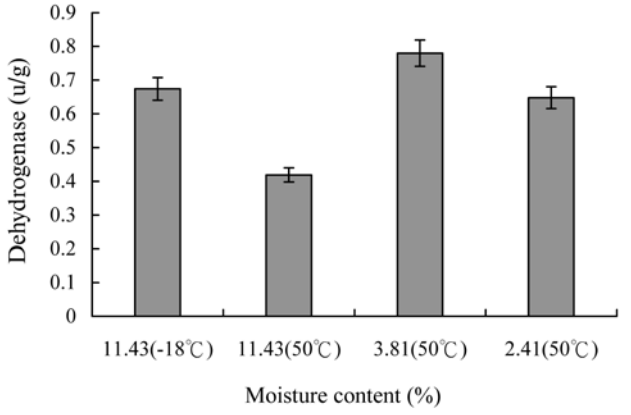

Figure 5. Effects of ultra-dry storage on dehydrogenase of *Z. xanthoxylon* seed. All values are means ± SD of three replicates.

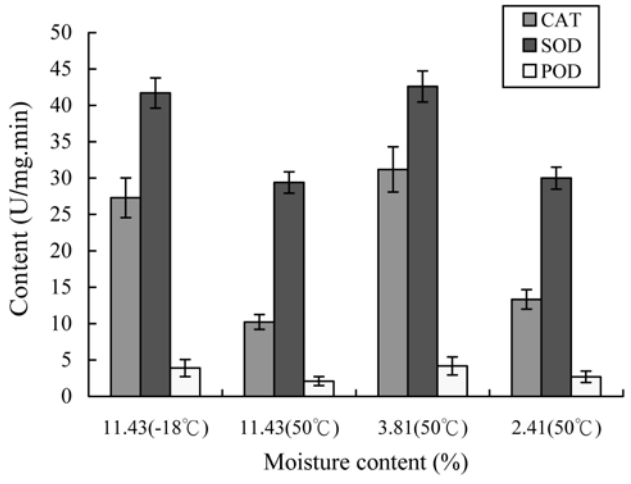

Figure 6. Changes of the activities of CAT, SOD and POD after accelerated aging at 50ºC for 1 month. All values are means ± SD of three replicates.

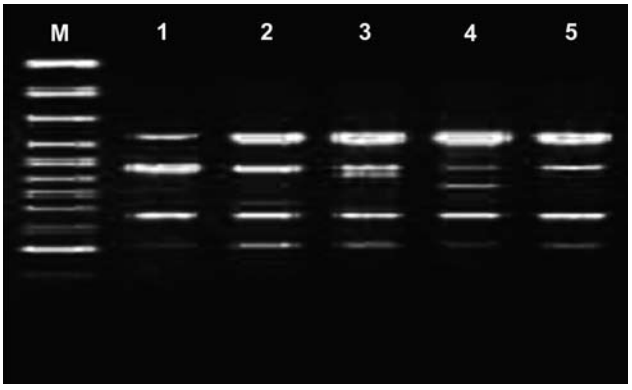

Figure 7. RAPD bands of *Z. xanthoxylon* seeds. M~DNA Marker; 1~MC 11.43%; 2~MC 4.81%; 3~MC 3.81%; 4~MC 2.41%; 5~MC 1.99%.

1998) have demonstrated that the seeds aged more rapidly under extremely dry conditions. So we can conclude that drying to extremely low MC may shorten seed longevity. Our data confirmed this viewpoint (Figure 2), at ambient temperature (25°C) the optimum MC of *Z. xanthoxylon* seeds is 3.81% and 2.41%, not 1.99%. However, for ex situ genetic resource conservation, which is typically considered as more than 10 years, further storage testing for this species will be required (our trials only extended 2 years).

In our research, the seeds of *Z. xanthoxylon* were dried to a moisture content of less than 5%, the viability and vigor were not statistically significantly influenced; on the contrary, the aging-resistant capability was greatly enhanced (Figures 4-6). After accelerated aging, the results showed that the 3.81% moisture content was more appropriate for *Z. xanthoxylon* seed ultra-dry storage. GP, VI and mean radicle length were kept higher than non-ultra-dry seeds, implying that no biochemical and biophysical reaction might have occurred to injure the seed cells under the conditions of low MC (Table 1). The conductivity test was based on the assumption that a disintegration of cell membranes in the seed takes place during seed deterioration. Seed deterioration can be explained by the findings that the aging of seeds leads to lipid peroxidation that subsequently causes membrane perturbation (Ponquett et al., 1992; Chang and Sung, 1998; Goel and Sheoran 2003). Such changes in the membrane of aged seeds lead to electrolyte leakage. So tolerance of desiccation can be expressed by the electrolyte leakage rate (Berjak et al., 1993; Leprince et al., 1995). In this study, the electrolyte leakage rate of *Z. xanthoxylon* increased after aging (Table 1, MC 11.43%), indicating a loss in membrane integrity. However, the electrical conductivity of ultra-dry seeds (MC 3.81%) significantly decreased compared with the control (MC 11.43%), indicating that ultra-drying could improve the membrane function during *Z. xanthoxylon* seed desiccation. After high temperature aging, the ultra-dry seeds showed strong storability. Compared with the control group (seeds stored at -18°C), the electrical conductivity remained stable, which means that the integrity of the membrane system in the ultra-dry seeds was maintained during storage.

The imbibition injure occurred in a wide range of crops when dried seeds were dipped directly in the water. So the imbibition injure is inevitable to ultra-dried seeds (Zheng, 1994). Even if there is imbibition injure, the pre-humitification of ultra-dried seeds can be repaired. So we could suppose that the water depletion induces the structural changing of a seed's cell membrane, which make ultra-dried seeds lose vigor. The effects of pre-humidification were correlated to the recovery of the membrane and enzyme, which improve the aging-resistant capability of ultra-dried seeds. The results of this experiment show that in the ultra-dried seeds, high activities of dehydrogenase, CAT, SOD and POD were kept. Free radical-induced damage plays a key role in seed deterioration during aging (Pinhero et al., 1998). Seed deterioration has been suspected to be associated with an accumulation of active forms of oxygen, for example superoxide radical (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$). The chief toxicity of O_2 and H_2O_2 is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radical and other destructive species, such as lipid peroxides (Noctor and Foyer, 1998). Efficient destruction of O_2 and H_2O_2 requires the action of several antioxidant enzymes acting in synchrony (Song et al., 2004). Antioxidant defense systems in plants include free radical and peroxide-scavenging enzymes. Superoxide produced in the different compartments of plant cells is rapidly converted to H_2O_2 by the action of SOD. CAT converts part of H_2O_2 to water and O_2. SOD activity is involved in the regulation of intracellular concentration of superoxide radical and H_2O_2. During our research, the activities of SOD and CAT significantly increased after accelerated aging (Figure 6). These results show that the changes of activities of antioxidant enzymes are closely related to desiccation tolerance and the ultra-drying does not destroy the enzymes. The ultra-drying treatment can prolong the seed storage life by increasing SOD activity.

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 is the products of lipid oxidation and peroxidation. So, the contents of these products in seeds can tell its deterioration degree. So determination of the volatile aldehydes and MDA is a convenient method of quantifying the extent of lipid peroxidation. In our research, the contents of the volatile aldehydes and MDA decreased after accelerated aging and were correlated with the increase in the activities of SOD, POD and CAT (Figures 4-6). The results of this experiment show that the lipid peroxidation was greatly suppressed under the ultra-dried condition. This implied that the enzyme systems were not destroyed and high activities of antioxidant enzymes were kept in ultra-dry seeds.

Somaclonal variation can either bring changes at the DNA level or it may induce changes in chromosome numbers. In general, morphological markers, chromosome analysis, isoenzyme or DNA markers may be used to detect somaclonal variation. As found in the present study, various investigators have observed the absence of variations. Cheng et al. (1997) and Meng et al. (2003) used RAPD markers to evaluate genetic stability of peanut seeds and three vegetable seeds, respectively. In a similar way, another author using RFLP to observe somaclonal variations in various crops (Hu, 2005). In our study, RAPD was chosen to detect somaclonal variation. No genetic instabilities were detected between the ultra-dry seeds and the control seeds (non-ultra-dry seeds). We conclude that somaclonal variation is almost absent in our ultra-dry seeds, the similarity in RAPD banding may suggest genetic fidelity, and therefore we believe that the methodology used to dry the seeds did not induce major genetic changes. Although in the present experiment the analysis of somaclonal variation was based on RAPD markers, it will be required to obtain consistently seedlings and to analyze relative differences in seedling characteristics. In future, it would be surely useful to extent the spectrum of DNA markers for genetic fidelity studies in ultra-dry storage.

The above conclusions have confirmed that the ultra-drying technique has not only caused less injury to the seeds but strongly enhanced the aging-resistant capability and storability of *Z. xanthoxylon* seeds. This technique will be potentially useful for the preservation of *Z. xanthoxylon* germplasm.

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霸王（*Zygophyllum xanthoxylon*）種子超乾保存研究報告

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本文探討了超乾保存是否能夠延長霸王種子壽命。實驗中，採用矽膠乾燥法將霸王種子的含水量分別降至 4.81%、3.81%、2.41%、1.99%，然後分別貯藏在 45°C、25°C、15°C 條件下 24 個月。實驗結果表明，種子最適含水量隨貯藏溫度的改變而變化。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。 超乾種子過氧化氫酶、POD 酶、SOD 酶、CAT 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。室溫條件下貯藏，霸王種子含水量是一個關鍵因素，在本實驗中含水量 3.81% 是最適含水量。RAPD 實驗結果表明，超乾種子和未經超乾處理的種子在分子水準沒有差異，超乾處理並沒有產生 DNA 變異。以上結果說明，將霸王種子含水量降至 5% 以下，可以提高種子壽命，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；含水量；霸王；生理指標；種子保存；RAPD。