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Effects of Ultradry Storage on Fluidity of Plasma Membrane of *Haloxylon persicum* Seeds

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Abstract: The DPH fluorescent probe (1, 6-diphenyi-1, 3, 5,-hexatriene) was used to study the effects of ultradry seed storage on the fluidity of plasma membrane. Results indicated that the micro-viscosity of plasma membrane of ultradried seeds had no significant changes compared with the Haloxylon persicum seeds which were stored under 4°C condition. However, there was a little adverse effect on the seeds with extreme dehydration. The results were consistent with higher vigor level of ultradried seed. It indicated that ultradry seed storage could maintain the physiological function of seed, protect the integrity of the membrane and improve the storability of seeds.

Keywords: Haloxylon persicum seeds, plasma membrane, ultradry storage

INTRODUCTION

Today facing with the great loss of plant biodiversity around the world, low moisture content conservation (it also called ultradry seed storage) through longterm storage of seed is possible for a significant proportion of higher plants (Hsu *et al*., 2000; Tsou and Mori, 2002). Ultradry 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 ultradry seed storage not only can be used to maintain the quality of seeds but also improve the storability of seeds (Wang *et al*., 2005).

The relationship between the integrity of structure and function of biological membrane and seed vigor is a very important aspect that has been paid more attention to in seed aging and vigor field (Tao and Zheng, 1991). Biomembrane is a kind of barrier connecting with cell in-out. The fluidity of plasma membrane plays import roles in regulating many functional actions connecting with membrane. The studies on the relationship between plant chilling-resistive and the fluidity of membrane have been covered for many times (Murate and Los, 1997), but the effects about ultradry seed storage on the fluidity of membrane have not been reported.

Haloxylon persicum is a shrubby perennial plant distributed in many areas of deserts in the northwest China. Moreover, it is very important to fix the sand dune, prevent soil desertification, conserve water and soil and improve zoology environment (Tong and Han, 2009). However, seeds of *H. persicum* are short-lived seeds and only have 8-10 months storage longevity at non-controlled room temperature. As time goes on, the seed vigor significantly decrease and lose their productive value. Therefore, the current difficulty during production is prolonging the seeds longevity and improving the utilization. The objective of this study was to determine whether ultradry seed storage had any effect on the fluidity of plasma membrane of *H. persicum* seeds and find out the mechanism of seed tolerance of desiccation which would provide theorectical basis for practice.

MATERIALS AND METHODS

Plant materials: Mature seeds of *H. persicum* were collected from the dry inflorescences of the shrubs in November 2009, from natural populations in the desert area near Guanjia Lake, Xinjiang. The initial Germination Percentage (GP) of *H. persicum* was 93.3% and Moisture Content (MC) was 10.57%.

Seed ultradrying treatment and prehumidification: Seeds were packed in plastic net bags; the ratio of the seeds to silica gel was 1:10 (w/w). Seed bags were buried into silica gel in a desiccator at normal atmospheric temperature (25°C) for 28 d to reduce the moisture content of seeds to 5.23, 4.24, 3.25 and 1.01%. The ultradried seeds were kept in sealed aluminum foil packages and stored in a refrigerator at 4°C for experiment.

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To avoid imbibition injury of seeds, the ultradried seeds were put into a sealed containing saturated $CaCl_2$ solutions (relative humidity is 35%), then transferred to a sealed desiccator containing saturated NH_4Cl solutions (relative humidity is 70%) and finally transferred into a sealed desiccator containing water (relative humidity is 100%) at normal atmospheric temperature (20°C) before the germination assessment and the following experiment. Each step lasted for 24 h.

Measurement of seed MC, germination and vigor test: According to International Rules for Seed Testing (International Seed Testing Association, 1993), seed MC were determined 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 before germination. Four replicates with 100 seeds each were germinated at 25°C. Germination was checked every 24 h and GP and radicle weight were estimated on 7 d. Seeds Vigor Index (VI) was determined according to the following equation: VI = GI×Sx, GI = Σ (Gt/Dt), where GI is germination index, Sx is radicle mean length x days after germination, Gt is GP after t days, Dt is days of germination.

Ultradrying storage experiments and accelerated aging: After ultradrying, the ultradried and un-ultradried seeds (control) were accelerated aged at 45°C for 2 days in an oven. After accelerated aging, the seeds were put into nylon bags. The ultradried and un-ultradried *H. persicum* seeds were placed in the room temperature at 15-25°C for 12 months.

Measurements of relative electric conductivity: The relative electric conductivity of seed leachate was performed by soaking 100 seeds (uniform in size and without visual injury) in 200 mL of deionized distilled water at 25°C for 24 h (Zheng, 1980). The conductivity of the soaking water was measured by conductivity meter (model DDS SJ-308A, shanghai, China) at regular intervals.

Isolation and purification of plasma membrane: Plasma membrane wesicles were isolated by the aqueous polymer two-phase partitioning system by the method of Robot and sanche-nieto with some modification (Sanchez-Nieto *et al*., 1997).

Treatment of osmosis stress: 20 μL of plasma membrane suspension (containing about 50 μg protein) was added to the solution containing 10, 20, 30% PEG4000, 20% PEG4000+0.1 mol/L glucose and 20% PEG4000+0.1 mol/L sucrose separately, kept motionless for 40 min, the control was soaked with distilled water for 20 min, then rinsed with distilled water for three times.

Fluorescence analysis: Fluorescence intensity was determined by the method of Guo (1993) with some modification. 3 mL medium contained 0.5 mol/L mannit, 20 μL plasma suspension (about 50 μg protein), 20 μL 2×10^{-5} mol/L DPH (diluted when it was used, tetrahydrofuran as solvent, 2×10^{-3} mol/L stored liquid was prepared), agitated equally, when balanced for 40 mins.

Fluorescence intensity and fluorescence spectrogram was determined by a hitacht MPF-4 spectrofluori meter. Fluorescence deflection (P) and micro-viscosity ($\bar{\eta}$) were determined by the following equation, with exciting wave of 362 nm, emission wave of 531 nm, the plasma membrane suspension without DPH acting as control: $P = (I_{VV}-GI_{VH})/(I_{VV}+GI_{VH})$, $\bar{\eta} = 2P/(0.46-P)$, where P is fluorescence deflection, I_{VV} is the fluorescence intensity with the polarizer light axle and analyzer light axle all in vertical direction; I_{VH} is the fluorescence intensity with polarizer light axle in vertical direction and analyzer light axle in level direction; G is rectical factors. $G = I_{HV}/I_{HH}$, where I_{HV} is the fluorescence intensity with level polarizer light axle and vertical analyzer light axle; I_{HH} is the fluorescence intensity with polarizer light axle and analyzer light axle all in level direction.

Statistical analysis: Data were analyzed using SAS 8.0 system. If the F-test for a factor was significant in the ANOVA, at Least Significant Difference (LSD) was calculated to compare means.

RESULTS

Effect of ultradry storage on seed vigor: *H. persicum* seed which is rich in protein is a typical short-lived seed that can survive about ten months at the ambient temperature. Especially, it loses vigor rapidly during summer season with high temperature and moisture. After 12 months of storage at room temperature, the ultradried seeds could maintain high vigor level, while the un-ultradried seeds lost their vigor (Table 1). In comparison with the un-ultradried seeds stored at room temperature and low temperature, GP, GI as well as VI of seeds with different moisture contents of 3.25 and 5.23% of stored at room temperature showed significant changes, but there was a litter adverse effect on seed with 1.01% MC (Table 1). The results showed that the storability could be greatly improved under ultradry condition within certain MC limits.

Effect of ultradrying on the anti-aging ability of *H. persicum* seeds: After accelerated aging, the ultradried seeds (3.25% MC) still kept higher vigor levels in comparison with higher MC seeds stored in 4°C condition ((Table 2). After 2 days of accelerated aging, the GP and VI of the seeds with 10.87 and 1.01% MC decreased greatly. It meant that ultradried seeds

Table 1: Effects of different moisture contents on GP, GI and VI of *H. persicum* seeds at room temperature and low temperature (4°C) after storage of 12 months

Storage condition	MC (%)	GP (%)	GI	VI
4°C storage	10.57(CK)	85.3^{a}	33.62^{a}	115.19^{a}
Room temperature storage	10.57	0^{d}	0^{d}	0^{e}
Room temperature storage	5.23	67.7^{b}	17.98^{bc}	67.68^{c}
Room temperature storage	3.25	80.0^{b}	27.07^{b}	108.43^{b}
Room temperature storage	1.01	42.0^{bc}	9.86^{c}	11.68^{d}

The values in a column with the same alphabetical letter are not significantly different (LSD_{test}, p = 0.05)

Table 2: The vigor level of *H. persicum* seeds after accelerdted aging (45°C)

Storage condition	MC (%)	GP (%)	VI
4°C storage	10.57(CK)	93.3^{a}	225.16^{a}
45°C aging	10.57	59.3^{c}	98.7^{d}
45°C aging	5.23	79.1^{b}	103.5^{c}
45°C aging	3.25	91.2^{a}	165.4^{b}
45°C aging	1.01	47.8^{d}	75.2^{e}

The values in a column with the same alphabetical letter are not significantly different (LSD_{test}, p = 0.05)

Table 3: The effect of ultradry storage on polarization and micro-viscosity of plasma membrane from *H. persicum* seeds

	4°C 10.57%	Room temperature 10.57%	5.23% MC	3.25% MC	1.01% MC
p	0.121^{c}	0.299^{a}	0.147^{c}	0.139^{c}	0.170^{b}
$\bar{\eta}$	0.535^{c}	1.921^{a}	0.726^{c}	0.711^{c}	0.867^{b}

The values in a column with the same alphabetical letter are not significantly different (LSD_{test}, p = 0.05)

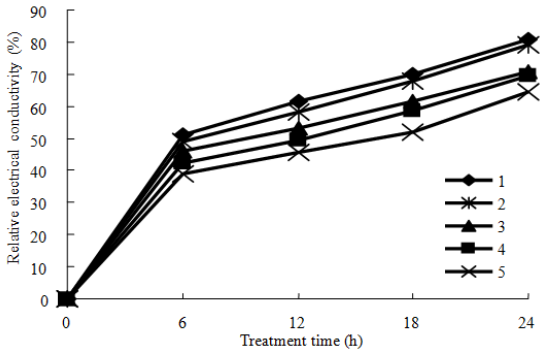

Fig. 1: Electrical conductivity change of *H. persicum* seeds after 12 months of room temperature storage
1: Control seed (MC 10.57%); 2: Ultradried seed (MC 1.01%); 3: Ultradried seed (MC 5.23%); 4: 4°C storage seed (MC 10.57%); 5: Ultradried seed (MC 3.25%)

within certain MC limits had no negative effects on *H. persicum* seed vigor and ultradried *H. persicum* seeds became more tolerant of aging at 45°C, however, the *H. persicum* seeds could not be dried too severely.

Effect of ultradry storage on the function of permeability plasma membrane: The changes of electrical conductivity of exosmosis liquid can illustrate the integrity of membrane. After 2 d of high temperature accelerated aging, the electrical conductivity of ultradried *H. persicum* seeds (3.25% MC and 5.23% MC) was significantly different from that of un-ultradried control (Fig. 1). In comparison with seeds in 4°C condition, the electrical conductivity of ultradried seeds (3.25% MC) had no significant difference, but the effect was weakened in the seeds with 1.01% MC. This indicated that the integrity of the membrane system in ultradried seeds within certain MC limits could be maintained and *H. persicum* seeds were sensitive to ultradry seed storage.

Effect of ultradry storage on micro-viscosity of plasma membrane: After labeling with DPH, the fluorescence deflection and the micro-viscosity of plasma membrane can reflect the fluidity of plasma membrane also. The higher the value of the micro-viscosity is, the weaker the fluidity of plasma membrane is; on the contrary, the stronger it is. The micro-viscosity of plasma membrane of ultradried seeds was much lower than that of seeds with higher MC at room temperature (Table 3). With exception in *H. persicum* seeds with 1.01% MC, as compared with the seeds in 4°C condition, the others had no significant difference in the micro-viscosity of plasma membrane. This suggested that ultradry seed storage kept the fluidity of plasma membrane. The results obtained above showed that if seed MC surpassed the safe lowest MC of seeds, its vigor level decreased and the micro-viscosity of plasma membrane increased, but the physiological function of plasma membrane was weakened. Though the ultradry seed storage effected of *H. persicum* seeds with 1.01% MC decreased, it still had advantage over the seeds with higher MC stored at room temperature.

DISCUSSION

In natural habitats, *H. persicum* seeds mature in November and germination starts from the following spring, the time when the snow starts to melt (Huang *et al*., 2001). If they germinated in the early spring, the seedlings would die in the low temperatures and the following drought season. If they do not germinate till the autumn, the un-lignified seedlings would be frozen to death in the following winter. Despite these negative factors, the short seed longevity is the main limitation for the germplasm preservation of this plant, for very few seeds can survive in the soil for longer than 10 months. The International Board for Plant Genetic Resources has recommended that 5% of seed moisture content (and-18°C) is the preferred condition for the germplasm conservation (Tao and Zheng, 1991) and it was suggested that the seed viability would decreased if the moisture contentfell below 5% (Priestley, 1986). However, our studies show that *H. persicum* seeds can be ultradry-stored by appropriate methods. The results confirmed that when MC of *H. persicum* seeds was reduced below 5% level, it did not induce any significant changes either in GP or in VI (Table 1). On

the contrary, their storage was improved greatly. The pretreatment of gradual moisture equilibration could be successfully used to avoid the imbibitional injury in ultradried seeds. This was consistent with the results of the previous work (Tong and Han, 2009; Tong *et al*., 2010).

The differential permeability of membrane is the main characteristic of biomembrane. Because of the destruction of the membrane system in aging seed, many materials flowed out of cells and the seed vigor reduced. Our studies showed that the changes of electrical conductivity of ultradried seed were in unity with the seeds stored in 4°C condition (Fig. 1). It meant that the integrity of the membrane system in the ultradried seeds was maintained during storage.

The fluidity of membrane is one of the fundamental characteristic of biomembrane. DPH is a kind of specific probe used to determine the fluidity of membrane. The data from different organs of living things confirmed that aging and stress must be followed with the significant increase in micro-viscosity of membrane. The results revealed that the micro-viscosity of membrane was almost the same both in ultradried seeds and seeds storied in 4°C condition (Table 3). We suppose that ultradry seed storage preserves the moving of hydrocarbon molecule, maintains the inherence of molecule arrange and prevents gelatination of membrane-lipid, so the fluidity of membrane can be maintained. However, when MC of *H. persicum* seeds was very low, the fluidity of membrane was weakened and the ability maintaining the integrity of membrane decreased. It is possible that extremely low seed MC made the loss of water of membrane in liquid crystalline state and then resulted in the decrease of structure and function of membrane.

The above conclusions have confirmed that the vigor level of seed is in accordance with the osmotic function and the fluidity of plasma membrane. Ultradry seed storage maintains the integrity of plasma membrane and ensures normal function of plasma membrane. So the ultradrying technique has not only caused less injury to the seeds but strongly enhanced the aging-resistant capability and storability of *H. persicum* seeds. This technique will be potentially useful for the preservation of *H. persicum* germplasm.

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