

Full Length Research Paper

# Increasing storability of *Ceratoides arborescens* seeds in ultradry storage

Tong Li-rong<sup>1,2</sup>, Han Jian-guo<sup>1\*</sup> and Dong Kuan-hu<sup>2</sup>

<sup>1</sup>Department of Grassland Science, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China.

<sup>2</sup>College of Animal Science and Technology, Shanxi Agricultural University, Taigu, Shanxi Province 030801, China.

Accepted 18 March, 2010

**This research was designed to determine whether ultradry storage improves the longevity of *Ceratoides arborescens* seeds. Ultradry *C. arborescens* seeds were obtained in a desiccating container with silica gel, of which seed moisture content was from 10.87 to 3.78%, and stored at 4°C and room temperature (15 - 20°C) for 12 months. The results indicated that ultradrying treatment did not induce any significant change in seed germination and vigor index. After ultradrying, aging was accelerated in the seeds (45°C, 2 days), and some physiological indices were thereafter tested. From the results, it was clear that the ultradried seeds were much more tolerant to ageing treatment than the control seeds as shown by germination percentage, vigor index and electrical conductivity. Our results also showed that dehydrogenase activity of ultradried seeds was higher than that of the control seeds. The results indicated that the moisture content of seed was a key index for storage at room temperature and 3.78% seem to be the best moisture content for ultradried seeds of *C. arborescens* in our research. From these results, we suggested that seed moisture content less than 5% enhances longevity and ultradry storage could be a potentially useful and cost effective technology for conservation of the plant genetic resource.**

**Key words:** *Ceratoides arborescens*, ultradry storage, moisture content, physiological indices.

## INTRODUCTION

The International Board for Plant Genetic Resources (IBPGR) was founded in 1974. One of its first acts was to recommend hermetic storage at temperatures of -18°C or less with 5 ± 1% seed moisture content (fresh weight basis, f.wt) for the long-term storage of orthodox seeds (Roberts, 1973) for genetic resources conservation (IBPGR, 1976). Although, cold storage of seeds at -18°C or less is presently the most common method for conservation germplasm in gene banks, its use still pose a problem for many gene banks in the developing countries because of the high cost for building and operating (Zheng and Jing, 1998). It is obvious that mankind today is faced with a challenge for urgent and efficient conservation of biodiversity. Hence cheaper alternatives to expensive cold storage of germplasm seed are needed. One of the possible methods is an ultradry seed storage,

based on sound scientific principles.

*Ceratoides arborescens* is a perennial plant of Chenopodiaceae ceratoides, which is distributed in northeast, north, northwest and the qinghaitibet plateau of China. It is our specialty plant of xerophytic shrub. They appear to be suitable for cold, barren and have a reputation for high tolerance to water deficiency. Moreover, it has the important ecological and economic value. However, seeds of *C. arborescens* 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.

Seed moisture content (MC) and storage temperature are the most important factors affecting seed longevity and vigor during storage. Previous work (Zheng, 1980) showed that low MC could substitute for low temperature alternatively during storage of some orthodox seeds. However, the viability and genetic stability after storage of seeds dried below 4 - 5% have not yet been affirmed

\*Corresponding author. E-mail: [jianguohan2058@126.com](mailto:jianguohan2058@126.com). Tel: 86-10-62734746. Fax: 86-10-62731264.

(Harrington, 1973; Roberts, 1972; Tao and Zhang, 1991). The results of IBPGR supported research projects undertaken by University of Reading (Ellis et al., 1986, 1988, etc) and Beijing Botanical Garden (Cheng et al., 1992b; Zheng and Cheng 1990b, Zheng et al., 1993) have consistently indicated that longevity of seeds of many crops, ornamentals and trees could be greatly increased by storing them under ultradry conditions (<5% MC). However, the storability about grass seed under ultradry conditions has not been reported. This paper describes the effects of ultradrying storage on subsequent seed vigor. The present work aims at finding an efficient way for this means of germplasm conservation of *C. arborescens*, as well as to sustain the biodiversity and conservation of other plants that inhabit these desert areas.

## MATERIALS AND METHODS

### Materials

Seeds of *C. arborescens* were provided by Professor Yijin of Inner Mongolia Agricultural University in November 2007. The initial germination percentage of *C. arborescens* was 93.0% and moisture content (MC) was 10.78%.

### Ultradrying treatment

The ultradried seeds were obtained by putting seeds over silica gel in desiccators at normal atmospheric temperature (25°C) for 42 days. The MC was reduced to 5.89 and 3.78%. The ultradried seeds were kept in sealed aluminum foil packages for experiment.

### Prehumidification

To avoid imbibition injury of seeds, the ultradried seeds were put into a sealed vessel containing saturated CaCl<sub>2</sub> solutions (relative humidity is 35%), then transferred to a sealed desiccator containing saturated NH<sub>4</sub>Cl 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 moisture content (MC), germination and vigor test

According to International Rules for Seed Testing (ISTA, 1993). Seed moisture content was determined by the oven method (8 h at 110 ± 1°C) and could be expressed on the wet basis (% w.b.). The seed surfaces were sterilized using 10% Na-hypochlorite before the germination process. Four replicates with 100 seeds each were germinated at 25°C. Germination was checked every 24 h, and germination percentage and radicle weight were estimated at 7 days. Seeds vigor index (VI) was determined according to the following equation:  $VI = GI \times Sx$ ,  $GI = \sum(Gt/Dt)$ , where GI is germination index, Sx is radicle mean length x days after germination, Gt is germination percentage (GP) after t days and Dt is days of germination.

### Conditions of accelerated aging and nature aging

After ultradrying, the ultradried and un-ultradried seeds (control) of

*C. arborescens* were placed in the room temperature at 15 - 20 and 4°C, respectively, for 12 months. The ultradried and un-ultradried seeds (control) were put at a constant temperature of 45°C for 2 days to accelerate aging.

### Measurements of relatively electrical conductivity

The relatively electrical 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.

### Measurements of dehydrogenase activity

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

### Statistical analysis

Data were subjected to analysis of variance using SAS system (SAS Institute, 1996). If the F-test for a factor was significant in the ANOVA, a least significant difference (LSD) was calculated to compare means.

## RESULTS

### Effect of ultradry storage on seed vigor

*C. arborescens* seed which is rich in protein is a typical short-lived seed which 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). Germination percentage, germination index as well as vigor index of seeds with different moisture contents of 3.78 and 5.89% stored at room temperature showed significant changes when compared with the un-ultradried seeds stored at room temperature and low temperature (Table 1). Moreover, the lower the MC that was maintained in seeds, the better was the germination. The results showed that the storability could be greatly improved under ultradry condition.

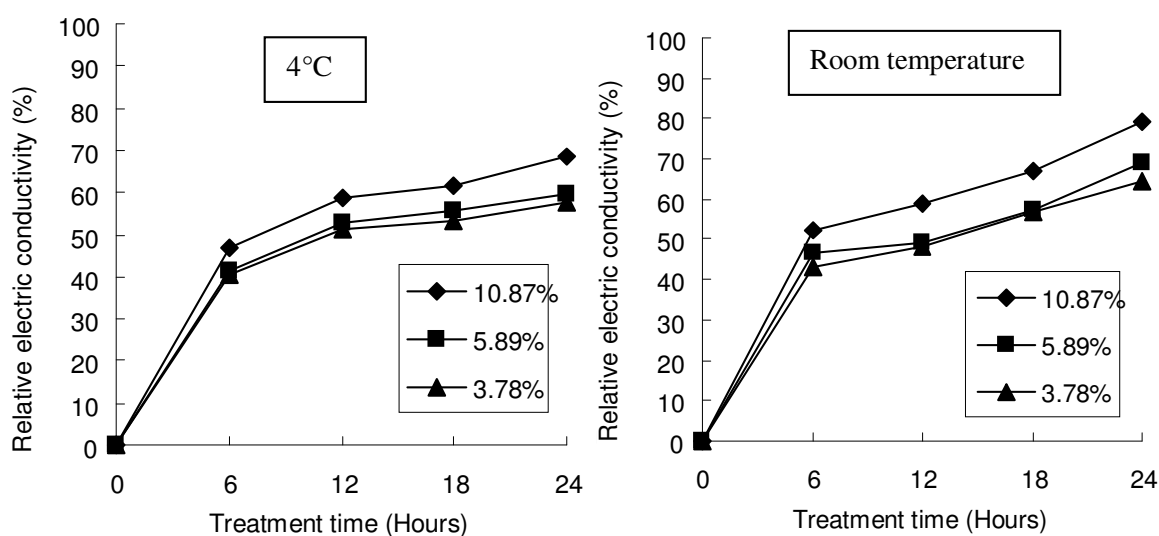
### Effect of ultradry storage on the function of permeability plasma membrane

The changes of relative electric conductivity of exosmosis liquid can illustrate the integrity of membrane. A large amount of electrolytic exosmosis is an important sign of biomembrane being destroyed during the process of seed vigor decreasing. We had found that with the extension of storage time, the relative electric conductivity of exosmosis liquid of *C. arborescens* seeds increased gradually (Figure 1). The changes in relative electric

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

Storage condition	MC (%)	GP (%)	GI	VI
room temperature storage	3.78	89.3±3.36a	43.20±5.43a	160.52±7.65a
room temperature storage	5.89	82.7±3.78b	40.14±6.11b	132.54±7.30b
room temperature storage	10.87	72.8±1.69c	37.12±3.66c	114.11±8.31c
4 °C storage	10.87	80.6±3.07b	39.34±5.20b	132.17±7.28b

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



**Figure 1.** The relative electric conductivity change of different MC on *C. arborescens* seeds at different temperature after 12 months (%).

conductivity of ultradried seeds after 12 months at 4 °C and room temperature were lower than those of the control (10.87% MC). There were no significant changes of relative electrical conductivity between 5.89 and 3.78% MC. It indicated that the physiological function of membrane system could be maintained better in ultradried seeds than in control. This result is consistent with changes of germination and vigor (Table 1).

#### Effective of ultradrying on the anti-aging ability of *C. arborescens* seeds

After accelerated aging, the ultradried seeds still kept higher vigor levels in comparison with higher MC (control). Table 2 showed that the *C. arborescens* seeds were tolerant to dehydration. They were highly tolerant to aging with low MC (MC less than 5%). After 2 days of accelerated aging, the GP and VI of the control (MC 10.87%) seeds decreased greatly, meanwhile those of the ultradried seeds (3.78% MC) were remained at a high level. However, for the seeds with a MC of 5.89%, the vigor began to

decline. The relative electrical conductivity of ultradried seeds (3.78% MC) was significantly different from that of 10.87 and 5.89% MC (Table 1). This suggested that the integrity of the membrane system in ultradried seeds could be maintained. These results suggested that ultradried seeds within certain MC limits had no negative effects on *C. arborescens* seed vigor, however the *C. arborescens* seeds could not be dried too severely.

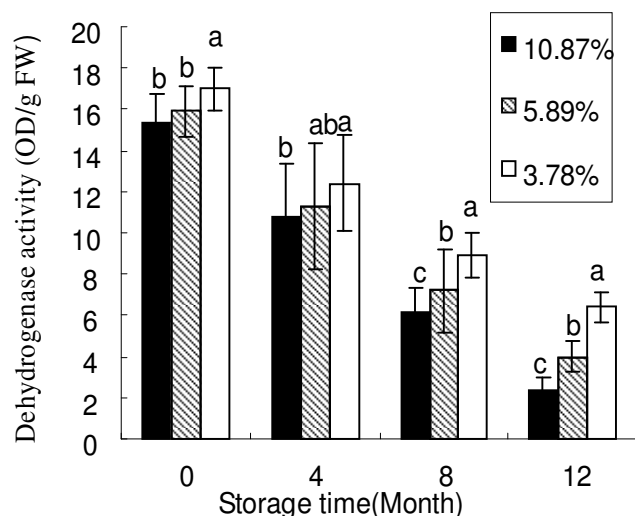
#### Effect of ultradry storage on dehydrogenase activity

Dehydrogenase plays the leading role in the metabolism and is influenced by aging. Dehydrogenase activity and the seed viability is positively correlated (Ellis et al., 1986). Dehydrogenase activity is closely related to the seed vigor, and dehydrogenase activity which can reflects the reduction ability of cell metabolism and the damage degree of embryos is the most important physiological indexes of seed viability and vigor (Zeng et al., 1998). It also reflects the reduction ability of cell metabolism and the damage degree of embryos. Dehydrogenase activity of *C.*

**Table 2.** The anti-aging ability of *C. arborescens* seeds after accelerated aging (45°C).

MC (%)	GP (%)	VI	Electrical conductivity (%)
10.87(CK)	17.6±2.62c	9.04±1.02c	53.10±3.44a
5.89	67.5±2.86b	43.9±1.77b	51.09±3.73a
3.78	91.0±3.41a	121.11±3.05a	45.82±2.68b

The values in a column with the same alphabetical letter are not significantly different (LSD<sub>test</sub>, P = 0.05).

**Figure 2.** Effects of ultradrying treatment on dehydrogenase activity of *C. arborescens* seeds at room temperature.

*arborescens* seeds reduced gradually with the extension of storage time, but its activities of 3.78% MC were significantly higher than that of control, which meant that dehydrogenase of ultradried seeds was more resistant to aging than that of the non-ultradrying control (Figure 2).

## DISCUSSION

It is well known that a decrease in seed vigour precedes a decrease in viability during seed deterioration (Delouche and Caldwell, 1960). There is a recommendation to store seeds of MC in gene banks (IBPGR, 1993). IBPGR has recommended that  $5 \pm 1\%$  of seed moisture content (and  $-18^\circ\text{C}$ ) is the preferred condition for the germplasm conservation, and it was suggested that the seed viability would be decreased if the moisture content fell below 5%. However, our studies show that *C. arborescens* seeds can be ultradry-stored by appropriate methods. The natural moisture content of *C. arborescens* seed at shedding is 10.87%, and this can be reduced to 5.89 or 3.78% within 42 days using the method of silica gel and then stored in sealed aluminium packets.

The results from 12 months storage show unequivocally

that at room temperature ultradried seeds storage of *C. arborescens* results in less rapid deterioration than that of storage with un-ultradried seeds (control). Moreover, the experimental data indicated that ultradried seeds became more tolerant of aging under accelerated aging condition and more storable under the condition of air open storage.

At low temperature ( $4^\circ\text{C}$ ) the aging of seeds is quite slow (Figure 1). In our experiment, GP and VI of 3.78% MC remained at very high level after 12 months of storage (Table 1). In contrast, the relative electric conductivity changed and dehydrogenase activity of ultradried seeds was more resistant to natural aging than those of undried controls. This means *C. arborescens* 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 and 2). 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 room 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. Further studies in the physiological and ultrastructural aspects of embryo cells are required to affirm the viability and genetic stability of *C. arborescens* seeds after ultradried storage.

## ACKNOWLEDGEMENTS

This research was supported by the Research Fund for the Doctoral Program of Higher Education of the Ministry of Education of China (200800190001) and the project of the key technology research of Vegetation Restoration and Reconstruction of Degraded Grassland which belongs to the National Eleventh Five-Year Research Program of China (2007BAD56B01-11). We also thank Professor Yijin of Inner Mongolia Agricultural University for providing *C. arborescens* seeds.

## REFERENCES

- Cheng HY, Zheng GH, Jing XM (1992b). Effect of ultradrying on storability of elm seeds. *Plant Physiol. Commun.* 28(5): 340-342 (in Chinese).
- Delouche JC, Caldwell WP (1960). Seed vigour and vigour tests. *Proc. Assoc. Off. Seed Anal.* 50: 124-129.
- Ellis RH, Hong TD, Roberts EH (1986). Logarithmic relationship between

- moisture content and longevity in sesae seeds. *Ann. Bot.* 57: 499-503.
- Ellis RH, Hong TD, Roberts EH (1988). A low moisture content limit to logarithmic relation between seed moisture content and longevity. *Ann. Bot.* 61: 405-408.
- Harrington JF (1973). Biochemical basis of seed longevity. *Seed Science and Technology*. 1: 453-461.
- Kun E, Abood L (1949). Colorimetric estimation on succinic dehydrogenase by TTC. *Science*, 109: 144-146.
- International Board for Plant Genetic Resources (1976). Report of IBPGR working group on engineering, design and cost aspects of long-term seed storage facilities. International Board for Plant Genetic Resources, Rome.
- International Seed Testing Association (ISTA) (1993). International Rules for Seed Testing. *Seed Sci. Technol.* 21(Supplement).
- Roberts EH (1972). *Viability of Seeds*. London: Chapman and Hill.
- Roberts EH (1973). Predicting the storage life of seeds. *Seed Sci. Technol.* 1: 499-514.
- SAS Institute (1996). *The SAS System for Windows 6.12*. SAS Inst Cary, NC, USA.
- Tao KL, Zhang GH (1991). *Seed Vigor*. Beijing: Science Press (in Chinese).
- Zeng XY, Chen RZ, Fu JR (1998). The effects of water content during storage on physiological activity of cucumber seeds. *Seed Sci. Res.* 8: 65-68.
- Zheng GH, Cheng HY, Jing XM (1993). Studies on seed germplasm conservation by ultradry storage. An abstracts presented at the 60<sup>th</sup> anniversary of the Botanical Society of China. Beijing (in Chinese).
- Zheng GH, Cheng HY (1990b). Current progresses in research of ultradrying seeds. *Plant J.* 6: 22, (in Chinese).
- Zheng GH, Jing XM (1998). Ultradry seed storage cuts cost of gene bank. *Nature*, 393: 223-224.
- Zheng GH (1980). Studies on control of viability of citrus seeds. *Scientia Agricultural Sinica*, 13(2): 37-43.