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SEED GERMINATION OF *CORISPERMUM PATELLIFORME* IN DIFFERENT STORAGE LENGTH AT ROOM TEMPERATURE: A DOMINANT ANNUAL SPECIES IN THE DESERTS OF NORTHERN CHINA

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Abstract

Seed dormancy is a major constraint in seedling establishment in desert habitats, Seeds of *C. patelliforme* were collected from natural population in desert areas. dried and then stored for 2 and 5-years at room temperature, The experiments of seed viability and germination at different temperatures (15, 20, 25, 30, 25/15, 35/15, 25/5ºC), light (0h, 14h, 24h), moisture (0, 10.6, 16.5, 21.3, 25.5%) and salinity (0, 50, 100, 200, 300 mM) stress was conducted to evaluate the change of seed viability and germination in seed storage. The seed viability remained to be 100%. The optimal temperature for germination was changed from 25/15ºC (28.00±2.31%) to 25/5ºC (82.67±7.05%%) with the storage length increased from 2 to 5-years. and FGP at 5-year storage were higher than those at 2-year storage, but the differences were significant only at 25ºC and 25/5ºC. Difference between light treatments were not significant All the germination values in PEG-6000 and NaCl concentrations at 5-year storage were higher than those at 2-year storage. The maximum of IGP and FGP were obtained at 10.6% PEG-6000 or 50mM NaCl concentrations at 5-year storage, the RGP in seed storage were increased with the increased PEG-6000 and NaCl concentrations. A 5-year storage at room temperature significantly enhanced the germination percentage of selected species by breaking dormancy and improved the germinability in moisture and salinity stress. This is beneficial to understand the formation mechanism of persistent soil seed-bank and establishing the artificial vegetation in desert areas.

Introduction

Corispermum patelliforme Iljin (*Chenopodiaceace*), a dominant annual species, is mainly found in the desert areas of the Northern China, including Yike Zhao League and Ba Yan Noir League of Inner Mongolia. It is also found in Mongolia (Liu, 1985), In these areas, the average annual rainfall is between 200 and 80mm, or even less (Fu, 1989). *C. patelliforme* has thus developed adaptive strategies to resist drought, poor soil nutrition, strong wind, sand-burying, high light intensity, heavy salinity and moisture stress; hence it plays an important role in wind-breaking, sand fixation, ecological environment improvement, and ecosystem functioning in arid areas. Moreover, *C. patelliforme* seeds, leaves and tender branches are palatable fodders for sheep and camels. Therefore, it is an important economic and ecological plant species in desert areas.

In deserts habitats, the propagation of *C. patelliforme* seeds appears to be more promising and cost effective in term of mass production of seedlings. However, the major problem in the propagation of *C. patelliforme* seeds is the long period of seed dormancy and the less than 30% seed germination percentage (Liu *et al*., 2010). The majority of plant species from desert environments produces seeds that is dormancy at maturity, and the mostly physiological dormancy (Baskin & Baskin, 1998). which can be broken by different storage methods, and increase germination percentage and rate and change the light, temperature and salinity requirements. For instance, cold stratification (Bewley & Black,1982; Huang *et al*., 2004), dry storage at low temperature (Wang *et al*., 2010), and low temperature stratification (Zhou *et al*., 2003).

Despite the number of extensive studies reported that the effects of seed size, light, temperature and salinity on seed germination of annual species (Wu *et al*., 2011: Khan *et al*., 2011: Zahir Farrukh,2012;Tobe *et al*., 2005; Baskin *et al*., 2004; Wei *et al*., 2008:Ahmed Debez *et al*., 2004; Nichols *et al*., 2009), However, what changes would happen in seed germination requirements to temperature, light, moisture and salinity after stored for a few years at room temperature since harvested? There is no report on seeds of *C. patelliforme* so far.particularly,The aim of this study was to evaluate: (1) seed viability change of *C. patelliforme* from Minqin County in Gansu province of China stored at room temperature. (2) The change of seed germination requirements to temperature, light, moisture and salinity stress at different storage length and (3) finally provide a theoretical basis for the establishment of artificial vegetation of *C. patelliforme* .

Materials and Methods

Materials: Seeds of *C. patelliforme* were collected from the plants of natural population in Minqin County of Gansu Province of China in October 2005, and were stored in cotton bags in a shaded and well-ventilated room at room temperature (13-30ºC, RH, 30-40%) until used in 2010 and 2007. The seeds were sieved to take off other big seeds,branches and insect eggs and sterilized by fungicide Pylon before the germination experiment. The weight, viability and moisture content of seeds stored for 2 years and 5-year were measured, respectively,by 1000 seed weight, tetrazolium test (Hendry & Grime, 1993; Baskin & Baskin, 1998) and oven drying test. The 1000 seed weight, viability and seed moisture content in storage were respectively (1.72±0.02)g and (1.70±0.02)g; (100.00±0.00)% and (100.00±0.00)%; 9.2% mc and 8.3% mc, respectively.

Methods: Three replicates of 25 seeds in each were used for 2-year and 5-year storage seeds. The seeds were incubated in 9cm diameter Petri-dishes. Bottomed With 2 layers of Whatman 1 filter paper and moistened with 2ml of distilled water. The criterion for germination was radicle appearance (1mm) from the seed coat. For temperature experiment, The Petri plates were wrapped in aluminum foil, covered with a dark cloth and transferred to incubator set at 15, 20, 25, 30, 25/15ºC, 35/15 and 25/5ºC (14h/10h). For light experiments, light time was devised into 3 different levels, 0h, 14h and 24h at the light intensity of 3000lx, and incubated at 25/15ºC for 20 days, Germination in light was checked every 24h, and distilled water was added when filter paper was a little dry. and the germinated seeds were removed. For moisture and salinity stress experiments, seeds stored for 2 years and 5-year were initially incubated at 25/15ºC in dark for 14 days, and then seeds germinated were counted and removed from the dishes, the initial germination percentage was calculated after 14 days pretreatment, and seeds not germinated from the pretreatment were rinsed 3 times in distilled water and then incubated in distilled water, seeds germinated in distilled water was calculated after 6 days, Finally the recovery and final germination percentage were calculated. In these 2 experiments, PEG-6000 and NaCl were employed to act as seed germination moisture and salinity stress induction, and their concentrations were 0, 10.6, 16.5, 21.3, 25.5% and 0, 50, 100, 200 and 300 mM respectively.

Germination of *C, patelliforme* seeds was described by three indexes of initial germination percentage (IGP), recovery germination percentage (RGP) and final germination percentage(FGP), The number of seeds germinated is estimated by FGP. In salinity and moisture stress experiments, the IGP was recorded as (A/C) ×100, the RGP as (B/C) ×100, the FGP after recovery as [(A+B)/C]×100, where A is the number of seeds germinated in initial salt solutions, B is the number of seeds recovered to germination in distilled water and C is the number of seeds tested (Khan & Ungar, 1984).

Data analysis: All data of germination percentage in the experiments were arcsine transformed prior to analysis in order to ensure homogeneity of variance**,** The means and standard errors and significant levels for all the treatments were compared using analyzed with Spss13.0 software.

Results

Effects of different temperatures and light times on seed germination: The results revealed that FGP of *C. patelliforme* was significantly affected by different temperatures at either 2-year or 5-year storage length under room temperature (Fig. 1A and B), For the 2-year storage stage, the FGP was less than 28% at all temperatures, the optimal temperature for seed germination was 25/15ºC (28.00±2.31%) (Fig. 1A). For the 5-year storage length, the optimal temperature for seed germination was 25/5ºC (82.67±7.05%) (Fig. 1B).and the FGP at the 5-year storage length was higher than those at the 2 years storage at all temperatures, however, the differences were significant only at 25 and 25/15ºC, and the FGP were increased by 17.33% and 74.67%. This means that seed deep dormancy can be broken and the FGP can be improved by prolonged storage (5-year storage) at room temperature (C).

For light time experiment, the FGP was reduced with the extension of light time and the FGP was very low (less than 3.0%) no matter the seeds were stored for 2 years or 5-years. The FGP in dark was significantly higher than those in light either seeds stored for 2 years or 5-years (D and E), Although seed germination at a 5-year storage length in dark was higher than that at 2-year storage length in dark, yet the difference was not significant at 0.05 level (F). The FGP was increased by 9.33%, This increase would be improved with the extension of seed storage length at room temperature.

Effects of moisture and salinity stress on seed germination: The results showed that the IGP was significantly affected by PEG-6000 concentration at both 2-years and a 5-years storage length, and firstly it is increased and then reduced with the increase of PEG-6000 concentration, and the values at 5-year storage stage were higher than those at 2 years storage length, but the difference was significant only at 21.3% PEG-6000 concentration. In addition, the maximum values were obtained at the same PEG-6000 concentration (10.6%) for the two different storage lengths (A). This means that the proper moisture stress can promote the germination of *C. patelliforme* seeds and obtained the higher FGP with the increase of seed storage length.

The RGP was significantly affected by PEG-6000 concentration at either 2-year or 5 year storage, and basically increased with the increase of PEG-6000 concentration, and the increase at 5 year storage was higher than that at 2 years storage. But the difference was significant only at 21.5 and 25.5% PEG-6000 concentration. The maximum was obtained at 21.5% PEG-6000 concentration for both of 2 years and 5 years storage length (18.67 and 22.67%, respectively) (B).

For moisture stress experiment, the FGP of 5 years storage was higher than that of 2 years storage at all temperatures. but the differences were not significant except at 25.5% PEG-6000 concentrations. The maximum was obtained at 10.6% PEG-6000 concentration, respectively. (38.67% and 45.33%). the FGPs either at 2-year or 5-year storage were significantly affected by PEG-6000 concentrations, and the values at a 2-year storage was firstly increased and then decreased with the increase of PEG-6000 concentrations. While the values at 5-year storage was firstly increased, and then decreased, and then increased again (C). All data showed that the storage time could improve the germinability of *C. patelliforme* seeds at room temperature and the moisture stress at certain concentration could also enchance the FGP (Fig. 2).

For salinity stress experiments, the IGP and FGP for 2-year and 5-year storage trrreatments were significantly affected by NaCl concentrations. And firstly increased and then decreased with the increase of NaCl concentrations. The maximum was obtained at 50mM NaCl concentrations, respectively (34.67% and 42.67%) and the values at 5-year storage length were higher than that of at 2-year storage length (A and C). For 2-year storage length, the IGP difference was significant only at 100 and 300mM NaCl concentrations, the FGP difference was significant only at 100mM NaCl concentrations. The RGPs in both 2-year and 5-year storage treatments were significantly affected by NaCl concentrations, and increased with the increase of NaCl concentrations, The values at 5-year storage length was higher than that at 2-year storage length at all NaCl concentrations, but the differences was not significant at every NaCl concentrations This means that seed storage at room temperature could improve the germinaability of *C. patelliforme* seeds. While salinity affected seed germination.

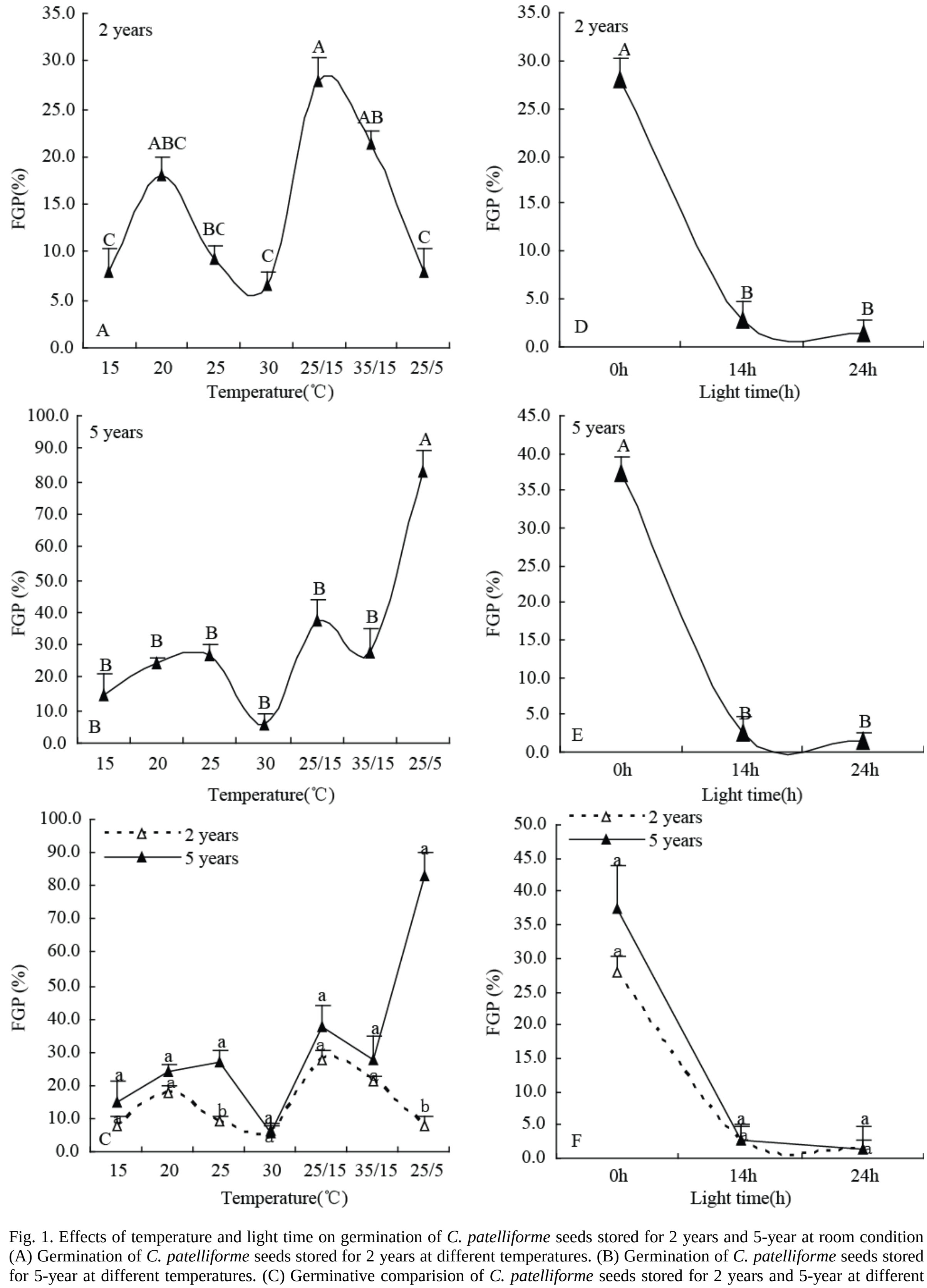

Fig. 1. Effects of temperature and light time on germination of *C. patelliforme* seeds stored for 2 years and 5-year at room condition (A) Germination of *C. patelliforme* seeds stored for 2 years at different temperatures. (B) Germination of *C. patelliforme* seeds stored for 5-year at different temperatures. (C) Germinative comparision of *C. patelliforme* seeds stored for 2 years and 5-year at different temperatures (D) Germination of *C. patelliforme* seeds stored for 2 years at different light time (E) Germination of *C. patelliforme* seeds stored for 5-year at different light time. (F) Germinative comparision of *C. patelliforme* seeds stored for 2 years and 5-year at different light time

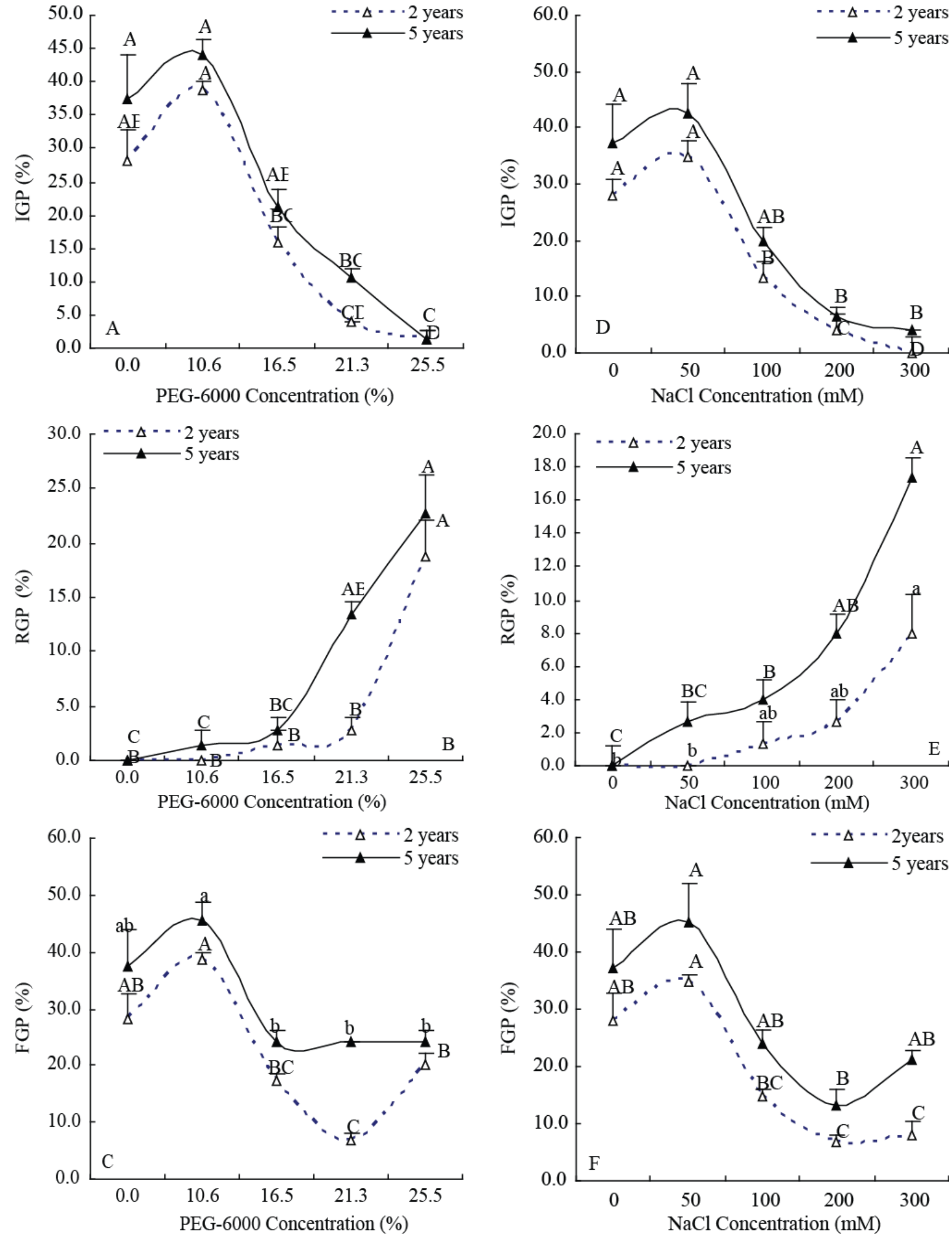

Fig. 2. Effects of PEG-6000 and NaCl on germination of *C. patelliforme* seeds stored for 2-year and 5-year at room condition. (A) IGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year at different PEG-6000 concentrations. (B) RGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year PEG-6000 concentrations. (C) FGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year at different PEG-6000 concentrations. (D) IGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year at different NaCl concentrations. (E) RGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year at different NaCl concentrations. (F) FGP comparision of *C. patelliforme* seeds stored for 2-year and 5-year at different NaCl concentrations.

Discussion

Seed dormancy is a common phenomenon at the maturity of seeds in desert environments. Physical dormancy is one of the most common types. For many species, seed dormancy can be broken by different seed storages pre-treatment, for instance, cold stratification, dry storage at low temperature, stratification at low temperature and storage at room temperature, and many valuable results were obtained from them, Huang *et al.*, (2004) reported that cold stratification could increase the seed germination percentage and rate, by change of temperature, light intensity and increase of salinity Zhou *et al.*, (2003) reported that the deep dormancy of *Paris polyphylla* var. *yunnanensis* was broken by an interval of 14 days at 4ºC and 14 days at 22ºC stratification, and Wang & Baskin (2010) reported that the germination percentage and rate of *P. incanus* and *B. vernae* were enhanced by cold dry storage. Likewise, Wang *et al.,* (2009) reported that the germination rate of *Ligularia virgaurea* seeds was decreased with the increasing storage length at room temperature and increased with decreasing temperature when seed stored for either 6 or 12 months.

The results of this research showed that the germination of *C. patelliforme* seeds could be markedly improved by a 5-year storage at room temperature and the optimal temperature for germination was also changed from 25/15ºC to 25/5ºC, the FGP was increased from 28.00% to 82.67%. The IGP, RGP and FGP at different PEG-6000 and NaCl concentration at stored for 5-year storage were higher than those at the 2 year storage. The maximums of IGP and FGP were obtained at 10.6% PEG-6000 or 50mM NaCl concentrations. This indicates that deep seed dormancy seeds of *C. patelliforme* seeds can be broken by the 5-year storage at room temperature The germinability affected by moisture and salinity at 25/15ºC could be improved by the increase of storage length at room temperature. it means that the current results is accorded with the research by Huang *et al.,* (2004). Although seed is pretreated by the same method of seed storage at room temperature, our results is different from Wang *et al.,* (2009), This may be relative to the habitat conditions.Therefore we suggested that the combination of seed storage length at room temperature, germination temperature and proper concentration of moisture and salinity should be considered in improving the germination of *C. patelliforme* seeds. Moreover, the germination percentage and rate enhanced may be closely related to seed storage temperature, seed moisture content and storage RH (Baskin & Baskin, 1979; Steadman *et al*., 2003; Walters, 1998; McIvor & Howden, 2000. Sharif-Zadeh & Murdoch, 2001),

This research would be also helpful to deeply understanding of the formation mechanism of persistent soil seed-bank of annual species to adapt to the unpredictable environmental condition and establishing artificial vegetation in desert habitats. On the one hand, most annual species from desert areas have developed seed dormancy traits adaptive to the persistent soil seed-bank. When seeds got matured, a plenty of seeds can't germinate and come into soil and formed into soil seed bank and become part of the soil seed bank with the different dormancy levels, and then the seeds will go though a series of temperature, light, moisture, salinity stress and other environmental changes (including the range of room temperature and the different salinity and moisture stress), Seed dormancy were broken slowly with the increase of stored length in soil, and the seedling mortality was decreased even when deadly environmental conditions occurs at seedlings stage, for instance, extreme temperature change, strong winds, severe drought. On the other hand, the 5-year storage at room temperature would be applied to artificial vegetation restoration in desert areas due to breaking the dormancy of *C. patelliforme* seeds and markedly enhancing the germination percentage;However, the 5-year storage was too long to be easily realized, Therefore, the effects of seed storage length, temperature, moisture, salinity and their interaction on germination of *C. patelliforme* seeds should be studied in the future Moreover, the experiment of seed storage length, seed moisture content, seed viability, relative air humidity in seed storage and their interaction should be conducted to investigate the optimal combination of seed storage condition.

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