

SEED DORMANCY OF *CORISPERMUM PATELLIFORME* LIJIN (CHENOPODIACEAE): A WILD FORAGE DESERT SPECIES OF NORTH CHINA

YOUJUN LIU^{1,2,3*}, SHIZENG LIU², YONGFU JI², FANG CHEN² AND XINWEN XU^{1,3*}

¹National Engineering Technology Research Center for Desert-oasis Ecological Construction, Xinjiang Institute of Ecology and Geography, Chinese Academy of Science, Urumqi, Xinjiang, 830011 China

²State Key Laboratory Breeding base of Desertification and Aeolian Sand Disaster Combating, Gansu Desert Control Research Institute., Lanzhou, Gansu, 730000, China.

³University of Chinese Academy of science, Beijing, 100049, China

*Corresponding authors E-mail: lyjsmallgrass@163.com; sms@ms.xjb.ac.cn

Abstract

The aim of this study was to investigate the mechanism by the comparison with germinability and water imbibition between non-scarified and scarified seeds. The seed structure was studied illustrated through SEM and the effect of pre-soaking and soaked solution on seed germination was determined with GA₃. Germination percentage was significantly improved (98.5%) in scarified seeds on 25°C in dark, whereas the value in non-scarified seeds was only 27.5%. Water absorption percentage in scarified seeds was higher (41.55%) than that of non-scarified seeds (36.68%). From seed illustration, the micropyle was jammed by some waxy substance which impeded water into seeds and the hard seed coat made up of collenchyma and palisade tissue which led to the barrier to water permeability. Germination percentage wasn't significantly improved (from 27.5% to 48.5%) by pre-soaking with the solution of 500mg/l GA₃, whereas significantly improved (from 27.5% to 98.5%) in the same concentration of GA₃. Our results showed that seed dormancy in *Corispermum patelliforme* Ijlin seeds was caused by the hard seed coat, the jammed micropyle and the inhibitory compounds from the "bubble structure". It can be concluded that dormancy-releasing requires removing or destroying seed coat.

Key words: *Corispermum patelliforme*, Chenopodiaceae, Wild forage, germination.

Introduction

Corispermum patelliforme Ijlin was an annual plant species belonging to Chenopodiaceae and Corispermium, and extensively distributed in the deserts of North China, occasionally found in Mongolia (Liu, 1985); In these habitats, the average annual rainfall was only between 200 and 80mm, or even less (Fu, 1989), *C. patelliforme* has thus developed the strategies to resist drought, poor soil nutrition, strong wind, sand-burying, high light intensity, heavy salinity and moisture stress, hence it plays an important ecological implication in wind-breaking and sand fixation, environment improvement and ecosystem functioning in arid areas. In addition, seeds, leaves and tender branches from *C. patelliforme* are delicious forage for sheep and camels, particularly in winter. Therefore, it was an important economic and ecological plant species in desert areas.

In desert habitats, the propagation of *C. patelliforme* seeds appear to more promising and cost effective in term of mass production of seedlings, however, the high dormancy percentage (72%) hindered the propagation (Liu, 2010) and thus establishment in artificial population of *C. patelliforme* was widely constrained. Liu *et al.*, obtained an effective method for breaking dormancy by pre-germination with 1000mg/L GA₃ in incubator in 25°C before sowing, but high mortality appeared after seed soaked in field experiments. In order to obtain the more effective methods for breaking dormancy, it was inevitable for us to investigate the dormancy mechanism of *C. patelliforme* seeds.

Various research works were performed in seed dormancy mechanism and the main results were included as follows:

(1) Mechanical dormancy, which was due to a water-impermeable seed coat and led to the obstacle for

moisture and gas. Occurs in seeds of 18 plant families angiosperms (no gymnosperms) including the Fabaceae (Baskin *et al.*, 2000, 2006; Morrison *et al.*, 1992, 1998), and the others, For instance, *Calligonum junceum* (Ren & Tao 2004), *Astragalus hamosus* and *Medicago orbicularis* (Patane *et al.*, 2006), *Tylosema esculentum* (Travlos *et al.*, 2007), *Astragalus arpilobus* (Long *et al.*, 2012), *Acacia aroma* (Venier *et al.*, 2012;), *Acacia nilotica*, *Prosopis juliflora* and *Dodonaea viscosa* (Nasr *et al.*, 2013) so on.

(2) Embryo dormancy, which was caused by the immaturity of embryo in the development when seed collected and broken by after-ripening, such as: *Dioscorea villosa* (Albrecht & McCarthy 2006), *Aconitum lycoctonum* (Vandelook *et al.*, 2009), *Hordenum spontaneum* (Yan *et al.*, 2012), *Narcissus alcaracensis* (Herranz *et al.*, 2013).

(3) Hormone dormancy, which was usually caused by some inhibitory compounds, e.g. abscisic acid and alkaloid so on, and finally led to seed dormancy. For instances, *Nicotiana plumbaginifolia* and *Arabidopsis* (Raz *et al.*, 2001; Frey *et al.*, 2004). *Atropa belladonna* (Abdel-Hady *et al.*, 2008), tallow tree (Li *et al.*, 2012), *Terminalia laxiflora* (Hassan *et al.*, 2013), etc. (4) Physical dormancy, which was mainly caused by environmental factors (Turner *et al.*, 2005), e.g. light, temperature and gas son on. For instances, *Dodonaea viscosa* (Baskin *et al.*, 2004), *Olimarabidopsis pumila* (Tang *et al.*, 2008), *Artemisia sphaerocephala* and *Artemisia ordosica* (Lai *et al.*, 2012), *Stipa bungeana* (Hu *et al.*, 2013), etc. In some case, seed dormancy was a complicated phenomenon caused by not single but several types mixed together, namely synthesis dormancy. The dormancy is known to occur only in seeds of Fabaceae

and seven other angiosperm families. For instance, *Prunus persica* cv.GF305 (Martínez-Gómez & Dicenta 2001). In recent years, a lot of research jobs on dormancy mechanism was involved in the following aspects, seed viability testing, seed permeability and histological feather (Long *et al.*, 2012; Venier *et al.*, 2012), seed soaked by sulphuric acid, gibberelin acid, hot water, nitrate, abscisic acid, (Ren & Tao, 2004; Nadiafi *et al.*, 2006; Bhattarai *et al.*, 2008; Nambara *et al.*, 2010), mechanical scarification (Duan *et al.*, 2004; Mandujano *et al.*, 2005; Patané & Gresta 2006), gibberellin treatment (Conversa *et al.*, 2010), storage and stratification (Conversa & Elia 2009; Venier *et al.*, 2011) plant allelochemicals (Jefferson & Pennacchio 2003; Sun *et al.*, 2006; McEwan *et al.*, 2010) gamma radiation (Abdel-Hady *et al.*, 2008) and so on. Various methods or measurements above were taken to investigate the dormancy mechanism by the effects of these on seed germination.

Despite the number of extensive studies reported that the effects of seed viability, seed permeability, seed soaked by concentrated sulfuric acid and hot water on seed germination of annual species. However, dormancy mechanism has been no report on seeds of *C. patelliforme* so far. The primary objective of this study was to investigate the dormancy mechanism of *C. patelliforme* seeds by determining the effects of seed coat scarification with a razor blade, pre-soaking by GA₃, seed soaking solutions of *C. patelliforme* seeds in distilled water and GA₃ on the seed germination, and comparing with the difference between water imbibition in scarification and non-scarification seeds, between seed coat bubble structure by soaked with distilled water or not and analyzing seed microstructure illustration by SEM, and finally provide some theoretical basis for the more effective methods for breaking dormancy-releasing of *C. patelliforme* seeds.

Materials and Methods

Seed collection: Mature and desiccated seeds of *C. patelliforme* were collected from the dry plants of natural population in the vicinity of Gansu Minqin National Studies Station for Steppe Ecosystem (CEEN) located in the desert area of Minqin County in Gansu Province of China in October 2005, and were stored in cotton bags in a shaded and ventilated room at room temperature (13–30°C, RH, 30–40%) until used in 2012. The seeds for germination were treated by soil sieve wiping the big other seeds, eggs and branches off and sterilized by fungicide Pylon before the germination process. The weight, viability and moisture content of seeds were measured by 1000 seed weight, Tetrazolium test (Baskin & Baskin, 1998) and oven drying test, respectively. The 1000 seed weight, seed viability and seed moisture content in storage were (1.72±0.02) g; (100.00±0.00) %; 8.95%mc, respectively.

Effect of seed coat scarification on seed germination: After soaked in distilled water for 4 hours, seeds were taken off and then the moisture on seed surface was wiped off by filter paper. The soaked seeds were pretreated by

the following methods: A. a little seed coat round micropyle was scarified with a razor blade under undamaged embryo; B. Seed coat wasn't scarified as control. Four replicates of 50 seeds in each were used and the germination experiment was carried out in illumination incubator at 25°C in complete dark.

Seed microstructure observation: The intact seeds were pasted in objective table and scanned by electronic microscope of JSM-6380LV (JEOL, Japan) after sprinkling gold and coating film, and finally some photos of seed surface microstructure, cross section and seed coat structure were taken, respectively.

Imbibition of water: To determine if the seed coat is permeable or impermeable to water, imbibition of water was compared in scarified and non-primed seeds. Seeds were scarified individually with a razor blade (mechanical scarification), and four replicates of 25 scarified and non-scarified seeds was used. Each replicate of treated and non-treated seeds was weighed to the nearest 0.0001g using a Sartorius electronic balance (Sartorius Co., Goettingen, Germany) and place a filter paper moistened with distilled water in Petri dishes in the laboratory. At time zero and at the time interval shown in Fig. 2, seeds were removed from the filter paper, blotted dry and weighed. Percentage of increase in seed mass was calculated by the following equation (Baskin *et al.*, 2004): % increase in mass = $[(W_i - W_d)/W_d] \times 100$, W_i = mass of imbibed seeds and W_d = mass of dry seeds.

Effect of pre-soaking by GA₃ on seed germination: After soaked in GA₃ solution of 500mg/l for 0, 4, 8, 12, 16, 20 and 24h, respectively, seeds were repeatedly rinsed by distilled water to keep the residual GA₃ solution off the surface of seeds. And then the germination experiment was performed in incubator in complete dark at 25°C and the filter paper was moistened in Petri dish. Four replicates of 50 in each were hired. The germinated seeds were counted in every day. The criterion for germination was germ stretching out seed coat and the experiment was finished after 20 days

Effect of GA₃ on seed germination: In the experiment, the filter paper was moisten by 0, 50, 100, 200, 500 and 1000mg/l GA₃ solution, the other process and criterion was the same as the above.

Effect of soaking solution of *C. patelliforme* seeds on seed germination: After 200 seeds was soaked in 50 ml distilled water of 35°C for 72h, and then the soaked seed was repeatedly rinsed by distilled water, and the soaked solution was saved for germination. The five different treatments were used in the present germination. A. The seed unsoaked germinated in soaked solution on filter paper; B. The seed unsoaked germinated in distilled water on filter paper; C. The seed soaked germinated in distilled water on filter paper. The design, process and criterion of germination were the same as the above. Finally the microstructure of soaked and unsoaked seed was scanned by electron microscope (JEOL, Japan) to compare the change between them in seed coat.

Data analysis: All data of germination percentage in the experiments were arcsine transformed prior to analyze 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. Significant level was 0.05 and 0.01, respectively; the curve was drawn by Microsoft Excel2003.

Results

Effects of seed coat scarification on seed germination: The results showed that the germination in scarified seeds was significant faster than that in non-scarified seeds from the process (Fig. 1-A), and the germination percentage in scarified seeds quickly reached 83% at the first day after germination, 97% in a week and 98.5% in 15 days. Whereas the value in non-scarified seeds only reached 1.5% at the first day after germination, 22% in a week and 27.5% in 12 days. The final germination percentage in scarified seeds was significantly higher than that in non-scarified seeds (Fig.1-B) ($F=449.8020$, $P=0.0001$).

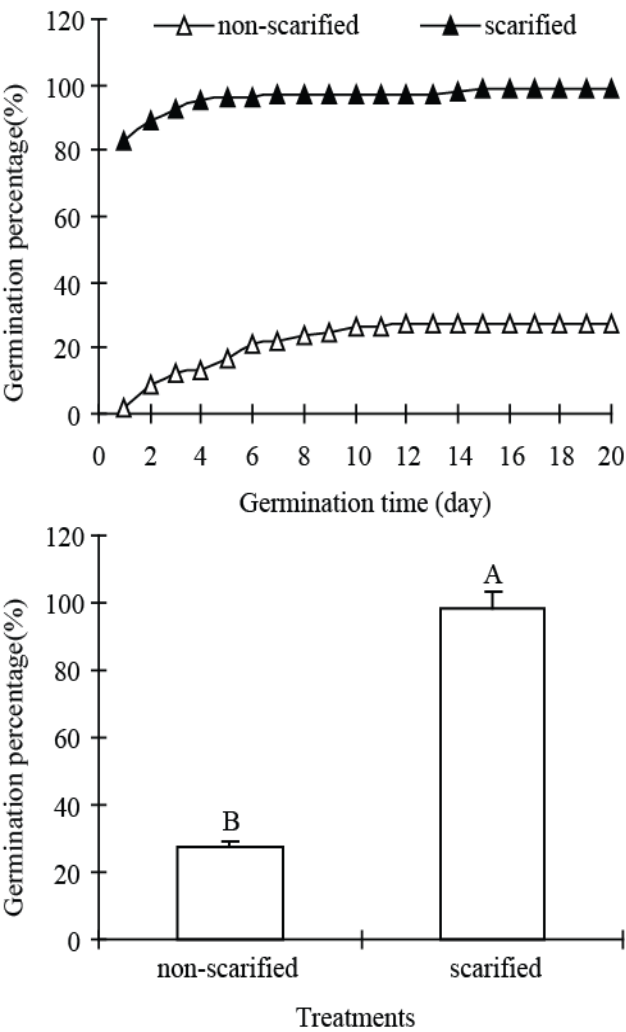


Fig. 1. Comparison with germination process and germination percentage by non-scarified (△) and scarified (▲) seeds of *C. patelliforme* in illumination incubator at 25°C in complete dark (A. Germination processes; B. Germination percentage).

Seed coat structure documented using SEM: The seed of *C. patelliforme* was like an ellipse in shape, the seed beak and micropyle was on the opposite sides of seeds (Fig. 2-A) and the micropyle was jammed with a lot of waxy substance (Fig. 2-B). The seed was made up of seed coat, embryo and endosperm and the embryo was like a circle in shape (Fig. 2- C). Seed coat was made up of “bubbles structure”, collenchyma and palisade tissue (Fig. 2-D).

Imbibitions of water: The results showed that there was an obvious difference between the scarified and non-scarified seed coat in water imbibition (Fig. 3). The scarified seeds quickly absorbed water within one hour, slowly within 2-6h and nearly stopped after 6h. Just in the moment when water absorption reached 41.3%. the seeds began to germinate and the germination percentage was increasing with the duration of water soaking. Whereas the non-scarified seeds slowly absorbed water within 0-48h, the water absorption reached 36.68 % within 48h and didn't increase from the moment.

Effect of pre-soaking by GA₃ on seed germination: The results showed that the germination percentage was not significant difference among soaking time (0, 4, 8, 12, 16, 20 and 24h) after seeds soaked by 500mg/l GA₃ ($F=1.8710$, $P=0.1316$), but seed germination was improved within 0-24h after seed soaked by GA₃ solution and the germination percentage was less than 50%. According to the seed germination process (Fig. 4-1), seed germination was very slow within 8d among the different soaking time and began to increase after 8d. However, the germination percentage was not significant change within 20d among the different soaking time.

Effect of different concentration GA₃ on seed germination: The results showed that seed germination was significantly improved in different GA₃ solutions ($F=35.5680$, $P=0.0001$). When the concentration of GA₃ was more than 200mg/l, the germination percentage was more than 90% in 20d. Although there was no significant difference among 200mg/l, 500mg/l and 1000mg/l GA₃, the germination percentage reached 98.5% in 500mg/l and 1000mg/l in 20d (Fig. 5-2). According to the seed germination process in different GA₃, the germination percentage was gradually improved with the duration of germination time and the increase of GA₃. When the germination time was more than 12d, seed germination began to become slow and stopped germinating after 18d.

Effect of soaking solution of *C. patelliforme* seeds on seed germination: According to experimental design, the concentrations of soaking solutions were A>B>C when seed germination. The germination percentage was significantly reduced with the increase of soaking solution concentration ($F=6.1390$, $P=0.0208$) (Fig. 6-1). Similarly, the germination velocity was also reduced with the increase of soaking solution concentration (Fig. 6-1). It showed that soaking solution had the ability to inhibit the germination of *C. patelliforme* seeds and the inhibitory substance was partly dissolved in water, and it derived from the solution of the “bubble structure” on seed surface in distilled water (Fig. 6-3 and Fig. 6-4).

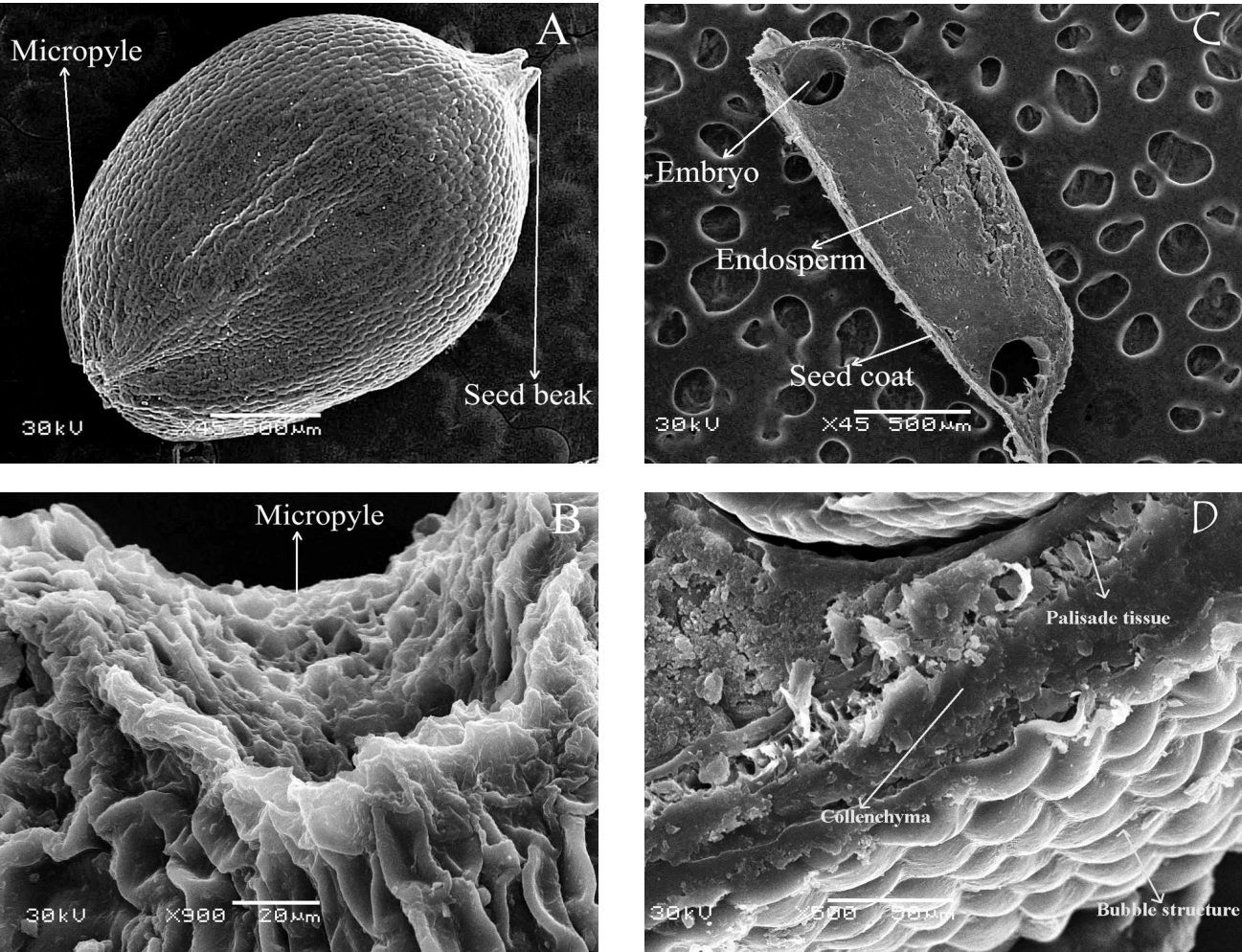


Fig. 2. Seed external morphology, anatomical structure and seed coat structure of *Corispermum patelliforme* using SEM. A: Seed morphological structure; B: Micropyle magnified structure; C: seed longitudinal section; D: Seed coat structure.

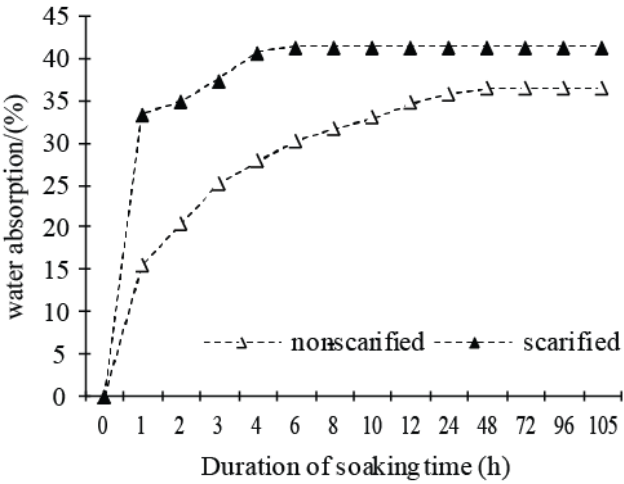


Fig. 3. Comparison with water absorption between the non-scarified and scarified seeds.

Discussions

Seed dormancy was a common phenomenon at the maturity of seeds in desert environments (Liu *et al.*, 2013). Available evidence suggests that there are at least two locations for dormancy mechanism in primary dormancy seeds: mechanism based within the embryo covering structures and mechanism based within the

embryo. The mechanism with the covering structure may involve mechanical, permeability and chemical barrier to germination (Adkins *et al.*, 2002). The present research demonstrated that the germination of *C patelliforme* seeds of was significantly improved (from 27.5% to 98.5%) by seed coat scarification at micropyle within 15 days and the germination percentage has already reached 83% in the second day after sowed. The results showed the dormancy in *C patelliforme* seeds was caused by seed coat, but which reason was it caused by? Mechanical, permeability, chemical barrier or the combination with two or three from the coat?

In general, the micropyle was considered as one of the most important entrances to outside water into embryo or endosperm. However, our illustrations of SEM on seed structure (Fig. 2) showed that the micropyle was jammed by some waxy substance which became a permeability barrier preventing water imbibition and it was testified by seed water imbibition course in non-scarified and scarified seeds (Fig. 3), and seed coat was made up of thickened cuticle and palisade tissue which offered to some resistance to the expansion to the embryo, the emergence to radicle and it was explained by the germination in non-scarified and scarified seeds (Fig. 1), and the seed coat was also a source for germination inhibitive compounds, it was testified by the inhibition of soaking solution from the “bubble structure” on the surface of seed coat.

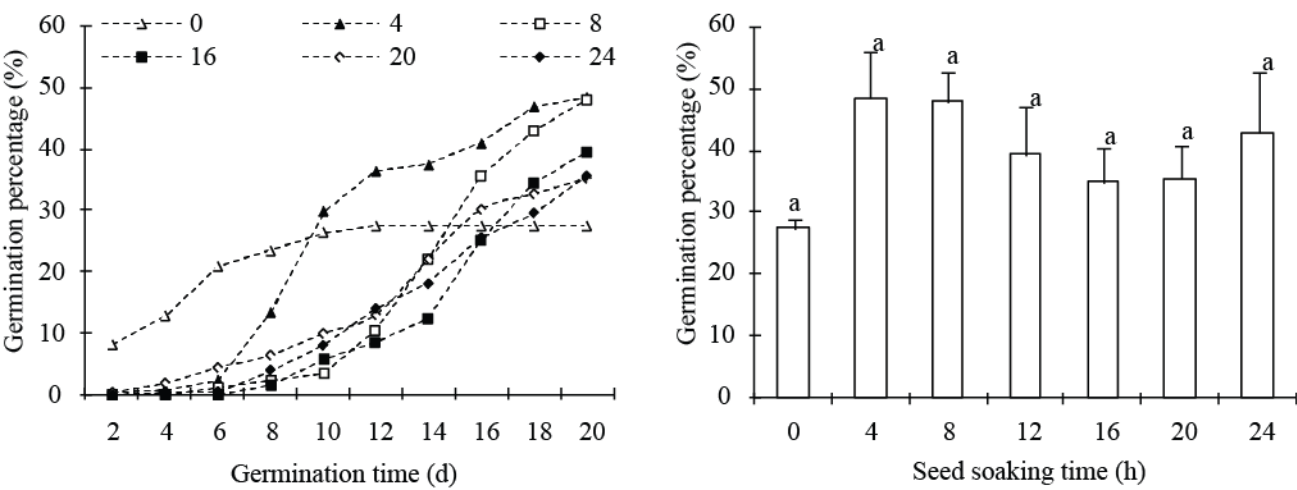


Fig. 4. Comparison with germination process and germination percentage after seeds of *C. patelliforme* pre-soaking by GA₃ (△0d, ▲, 4d, □, 8d, ■, 16d, ◇, 20d, ◆24d), 4-1. Germination process. 4-2 . Germination percentage.

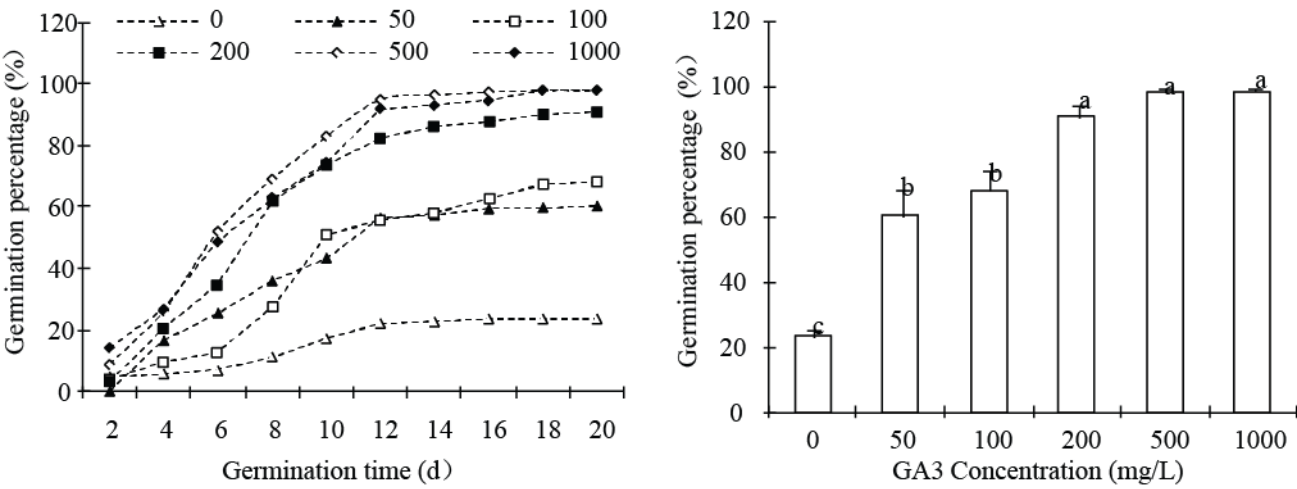


Fig. 5. Comparison with germination process and germination percentage after seeds of *C. patelliforme* pre-soaking by GA₃ (△ 0, ▲, 50, □, 100, ■, 200, ◇, 500, ◆1000 mg/l). 5-1. Germination process. 5-2 . Germination percentage.

The prevention of water imbibition was a common dormancy mechanism found in seeds of many dicotyledonous genera, but it wasn't so common for the grasses (Adkins *et al.*, 2002). However, the present study on seed water imbibition showed that the hard seed coat tissue impeded the water into embryo and endosperm and hence affect the germination of *C. patelliforme* seeds. According to Khan A. A. theory (Khan, 1989), it was necessary for seed germination to reached 40% water absorption percentage, at present experiment, the water absorption in non-scarified seeds was extremely slow (36.5% in 48h) and water absorption didn't change with the time extended after the moment (Fig. 2). These showed that the ability to absorb water in non-scarified seeds was very poor during germination even if the water was enough. On the contrary, Water absorption in scarified seeds reached 33.55% in 1h, 41.53% in 6h, didn't increase and a few of seeds began to germinate from the moment. It showed that water imbibition in scarified seeds was higher than that in non-scarified seeds and quickly began to germinate (Fig. 3). After seed coat scarified, the water imbibition was obviously increased and seed germination was greatly improved, the results

showed that the seed coat was impermeable and one of the causes of seed dormancy. Further, when combined with the seed coat microstructure together, the water impermeability was caused by the jammed micropyle, strong thickened cuticle and palisade tissue. The seed coat structure had the important ecological implication. In the habitat of *C. patelliforme*, the precipitation was rare and unpredictable and it mainly occurred between 7-9 month every year, whereas the optimal temperature for germination (20°C) occurred between 4-5 month, when seeds began to germination, the slow water imbibition, rare rain and intensive evaporation made the germination inhibited and imposed into dormancy. The trait played an important ecological implication in avoiding of high morality for rare rain after germination. Furthermore, due to chronically grow in mobile sand dunes, the seeds buried in sands frequently suffered from the friction by moving sands year after year. Consequently, the seed coat became thinner and thinner, which made the germination become easier due to be easier water imbibition and weaker resistance to the expansion to embryo and the emergence to radicle, moreover, seed viability was still kept in 100% after 7 years, therefore, the seedling

watched in field investigation derived from the seeds stored for several years in soil seed bank. These seeds with the different friction levels in soil seed bank were a powerful evidence for persistent soil seed bank. Moreover, there was a special “bubble structure” on the surface of seed coat and the special structure was partly dissolved in water and formed into some chemical compounds inhibited the germination of *C patelliforme* seeds (Fig. 6). The special structure had a regulation function for seed germination in precipitation unpredictable desert habitats. When the precipitation was very rare in seed germination, the concentration of inhibitory compounds from the special structure was so big that seed germination was prevented, and when the precipitation was so enough that the special structure was dissolved in soil and with the succession of precipitation, the concentration of inhibitory compounds became

smaller and smaller so that didn’t prevent from seed germination. In the case, the seeds with the thinner seed coat began to germinate and kept the high seedling emergence once enough soil moisture. Hence the “bubble structure” was like a sensitive probe of soil moisture status, when the moisture content in soil was so enough that can maintain the requirement for seed germination and seedling emergence, the special structure was dissolved completely. Similarly, when water in soil was too rare to maintain the requirements, the special structure was dissolved partly and made seed in dormancy to avoid high seedling morality for plenty of germination. Therefore, the special structure was a powerful explanation for bet-hedging strategy (Venable, 1985; Venable *et al.*, 1987, 1995) by chemical inhibitory compounds from seed coat and more and more thin seed coat friction by frequently moving sands.

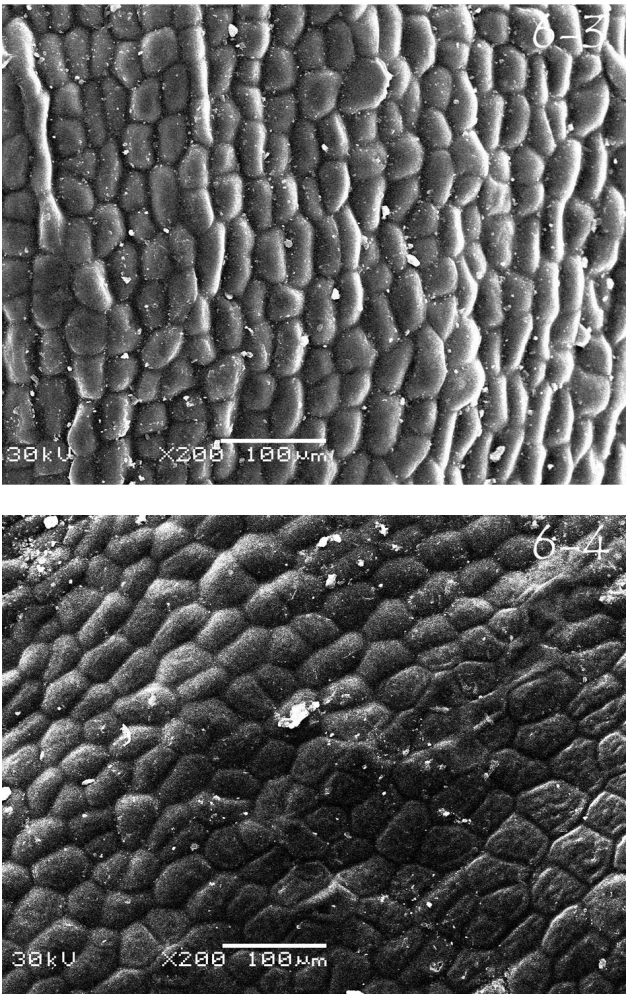
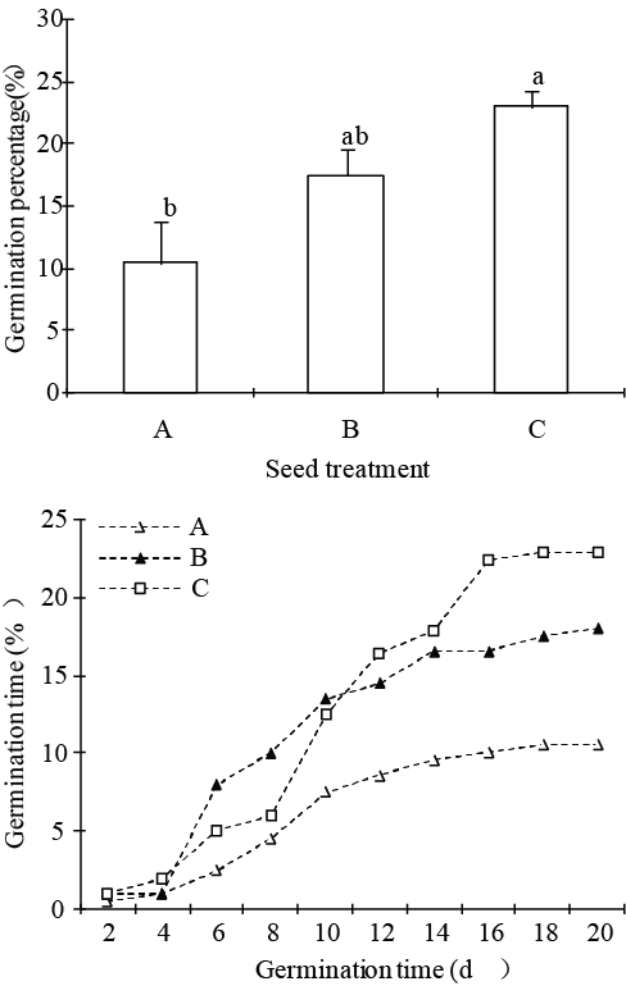


Fig. 6-1. Comparison with germination process and germination percentage in the three soaking solutions (A: unsoaked seeds germinated in soaking solutions; B: unsoaked seeds germinated in distilled water; C: soaked seeds germinated in distilled water), 6-1: germination percentage, 6-2: germination process 6-4: seed coat illustration comparison with the soaked (6-4) and unsoaked (6-3) seeds by SEM.

The dormancy-releasing was correlated with the disappearance of inhibitor, the formation of promote and the rate of promote and inhibitor. The different seeds and the same seeds maybe had the different rates of promote and inhibitor and the rates determined to the depth of dormancy. Just as the view of classical theory (Villiers &

Wareing, 1960; Amen, 1968) that seed dormancy was controlled by the rate of inhibitor and promote. If the rate was changed from the dominant inhibitor to the dominant promote, the dormancy would be gradually broken. The parts of producing inhibitory compounds in fruits/seeds in different species were various and mainly distributed in

pericarp, seed coat, embryo and endosperm. For instance, pulp in tomato and seed coat in sugar beet and American ginseng. The present study showed that the inhibitory compounds in *C. patelliforme* seeds were mainly distributed in the “bubble structure” and the inhibitory compounds were dissolved in water to prevent from seed germination. Although the germination percentage wasn’t significantly differences between the soaked seeds and the unsoaked seeds in 25°C, yet increased in soaked seeds, it suggested that the rate of inhibitor and promote was shifting towards seed germination and the inhibitor content would gradually increased with the duration of soaking time, and finally the balance began to shift towards inhibition germination. The result was explained by the germination percentage with 4 and 8h soaking time higher than those with 12, 16, 20 and 24h soaking time (Fig. 3-2). On the other hand, the germination percentage was so high in short time (4 to 8h) before 40% water absorption didn’t reach and the phenomenon was a point in case of the balance towards seed germination. The seed germination in GA₃ solutions was improved and the bigger GA₃, the higher germination percentage. The results were also a powerful explanation for the balance between inhibitor and promote shifting towards seed germination (Fig. 4-2).

Conclusions

To summarize, the dormancy of *C. patelliforme* seeds was caused by the hard seed coat that led to the barrier to expansion to embryo or radicle during seed germination, the jammed micropyle that led to the barrier to water permeability during seed water imbibition and the inhibitory compounds from the “bubble structure”. Hence the effective methods for dormancy-releasing were to remove or destroy seed coat, for instance, scarifying seed coat, rubbing seed coat by sand paper, soaking seeds by GA₃ after seed coat scarified and so on.

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