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Super absorbent polymer seed coatings promote seed germination and seedling growth of *Caragana korshinskii* in drought*

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Abstract: Coating seeds with water absorbent materials can improve their survival, especially for those planted in drought or barren areas. In this study, effects of five kinds of super absorbent polymers (SAPs) on seed germination and seedling growth of *Caragana korshinskii* under drought conditions were investigated. Our results showed that SAP coatings could significantly improve the percentage and energy of seed germination, as well as reduce the relative electrical conductivity (REC), proline, malondialdehyde (MDA), H_2O_2 content, and peroxidase (POD) activity during germination. These results implied that seeds could uptake moisture from SAP coatings to alleviate drought-induced oxidative stress and membrane damage, thus exhibiting a better vigor and germination performance. After coating *C. korshinskii* seeds with SAPs, more seedlings emerged and grew better. Under the combined influence of the water absorption capacity of SAP and other factors, the efficiencies of five SAP coatings are in the sequence D>E>B>A>C. The function of the SAP coating on promoting seedling survival was confirmed in Mu Us Sandy Land in Ordos, Inner Mongolia Autonomous Region, China. The average seedling number of SAP D-coated seeds increased twofold on that of naked seeds. Our results are expected to be helpful in understanding and utilizing SAP seed coatings in improving plant survival under drought conditions.

Key words: Super absorbent polymer; Seed coating; *Caragana korshinskii*; Seed germination; Seedling; Drought
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1 Introduction

Desertification control has made significant strides in recent years. However, desertification is still a threat in China and other countries, so it is urgent to improve the prevention system (Wang *et al.*, 2002; Cui and Lu, 2012). Aerial seeding is a rapid and effective measure for afforestation, and it has been applied to vegetation restoration and reconstruction of hinterland of Mu Us Sandy Land in Ordos, Inner Mongolia Autonomous Region, China for forty years (Li *et al.*, 2009). Coating can increase the weight of small seeds such that they are deeply buried in sands, preventing them from being blown away by wind during aerial seeding. In addition, nutrients added in the seed coating can improve seed germination and seedling survival rate (Moussa and Khodary, 2003). Low survival rate after aerial seeding is an outstanding issue, which might be attributed to inappropriate conditions of temperature, soil moisture, burial depth of the seeds, etc. (Zheng *et al.*, 2005). Water is extremely deficient in deserts, making it a vital factor for seedling survival in those areas. During a year, the period from late May to middle June is the optimal time to carry out aerial seeding in Mu Us Sandy Land in Ordos, but the average precipitation was only 33.6 mm from 1981 to 2000 (http://www.cma.gov.cn).

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How to maximize the utilization of the limited water resource to enhance the survival rate of plants is an essential issue in afforestation and sand control.

Super absorbent polymer (SAP), a high water-absorbent resin, can quickly absorb hundreds of times its own weight of pure water. In order to produce SAP, monomers like acrylamide, acrylate, starch, and others were highly cross-linked with an adjuvant, forming a reticular structure with plenty of hydrophilic hydroxyl and carboxylic groups. In agriculture, the SAPs' amendment effectively increases the water storage capacity of soil (Johnson, 1984), leading to improvement of seed germination and survival rate of crop plants. In a recent study, 25% of carbonyl amide polymer could absorb and hold 80–180 times its volume of water. Application of this hydrogel improved soil moisture content, the number of germinated seeds, and the yield of rice (Rehman *et al.*, 2011). The responses of plants to SAP varied in different species. For instance, seedling growth of wheat and barley was improved by 0.1%–0.3% SAP (mixture of polyacrylamide and acrylate) amendment, but the seed germination of neither species was affected. However, seed germination of chickpea was significantly increased with 0.2% SAP, but seedling growth was not changed (Akhter *et al.*, 2004). The efficiency of hydrophilic polymer was also affected by the type and salinity of soil it amended. The use of hydrophilic polymer (Superab A200) increased available water content, biomass, and water use efficiency of corn, especially in sandy soils with lower salinity (Dorraji *et al.*, 2010).

The concept of coating small seeds was originally raised by Blessing in the 1960s (Wang *et al.*, 1998), in order to make them larger and therefore easier to handle and plant in agricultural production. From then on, different coating agents, with pesticides, aquasorb, plant hormone, inorganic salt, and others have been developed and extensively used, protecting plants from disease, drought stress, and nutrient deprivation. Due to its tremendous capacity in water absorbance, SAPs were used as a new formula for seed coating agents in aerial seeding and vegetation restoration in China. Different from application of hydrogel directly in soils in agricultural production, it is more suitable for sowing SAP pelleted seeds in large areas, especially for aerial seeding in barren areas. In this way, as imbibed SAP is centralized around seeds, less SAP is required and moisture evaporation is reduced, and at the same time the weight of small seeds could be increased by coating. It was found that coating agent containing 5% SAP could significantly improve seed germination and seedling emergence of legume herbage *Medicago sativa* and *Astragalus adsurgens* in drought (Wang *et al.*, 2002). The effects of different SAPs on disintegration time of *Calligonum alaschanicum* seeds for aerial sowing in desert were studied. Among four types of SAPs, seeds coated with polyacrylamide- or starch-type SAP had proper disintegration time and strength (Liu *et al.*, 2004). *Haloxylon ammodendron* and *Haloxylon persicum* are very sensitive to the concentration of SAP treatment, i.e. 0.05% SAP promotes seed germination and 0.1% SAP enhances root growth, but 0.2% SAP inhibits both (Li *et al.*, 2012). These SAP coatings on various psammophytes were effective in improving available soil moisture and thus could increase plant establishment in arid and semi-arid regions.

Caragana korshinskii is a wild legume woody species of importance for sand control in Northwest China. In this study, we selected five kinds of SAPs with different absorbing capacities, and pelleted the *C. korshinskii* seeds with coating agent containing these SAPs. Then we comparatively studied their influence on seed germination and seedling growth, detected biochemical changes in seeds during germination, and analyzed the relationship of these phenotypes with water absorbance capacity of SAPs. The conclusion was confirmed finally in Mu Us Sandy Land. We hope that our study might help understand the function of SAP seed coatings in improving plant survival under drought conditions.

2 Materials and methods

2.1 Materials

C. korshinskii seeds were obtained from the Forestry Science Research Institute at Ordos, Inner Mongolia Autonomous Region, China. The seeds, collected in the autumn of 2011, were dried under natural environmental conditions to moisture content of 7.2% (wet weight basis) and then sealed in plastic bags and stored at −80 °C.

Five kinds of SAPs are: A, polyacrylamide (Sinopharm Group Co., Ltd., Beijing, China); B, sodium polyacrylate (Sinopharm Group Co., Ltd.); C, Balite™ efficient poly agent (Heze Tianling Agrochemicals Co., Ltd., Shandong, China); D, Hankeshu™ aquasorb (Beijing Sangsong Eco-Technology Co., Ltd., Beijing, China); E, Zhengyuan™ aquasorb (Henan Zhengyuan Bio-technology Co., Ltd., Henan, China). The active constituent of SAP C is denatured polyvinyl alcohol. The active constituent of SAP D is acrylamide and potassium acrylate copolymer. The active constituent of SAP E is acrylamide and sodium acrylate copolymer. Based on our previous data, we chose ethylene cellulose (1.5%) as the adhesive. The attapulgite clay and talcum powder were mixed as the filling materials with a mass ratio of 2:1. The experimental coating machine XT-86, made in Shanghai, China, was used in this study.

Table 1 Formulae of different coating agents

Group	Formula (per 100 g coating powder)		
	Clay (g)	Talcum powder (g)	SAP (g)
CK1	0	0	0
CK2	67	33	0
A	60	30	10
B	60	30	10
C	60	30	10
D	60	30	10
E	60	30	10
1% D	66	33	1
4% D	64	32	4
7% D	62	31	7
10% D	60	30	10
13% D	58	29	13

2.2 Determination of water absorption capacity of SAPs

One gram of SAP powder was placed in a beaker with a sufficient amount of distilled water, and it was left to stand for 24 h. After the SAP powder was imbibed to be saturated, we filtered out the free water and weighed the wet aquasorb. Then the weight of the saturated aquasorb is the absorption fold of the SAP (Huang *et al.*, 2002).

2.3 Preparation of the *C. korshinskii* seeds coated with SAP

Clay, talcum powder, and SAP were ground over a 200-mesh sieve, and then mixed evenly in the indicated proportion listed in Table 1 to make 250 g seed coating powder. Ethylene cellulose was dissolved in alcohol to make 150 ml 1.5% adhesives. Five hundred grams of plump and uniform *C. korshinskii* seeds were selected, and placed in the coating machine and kept rolling. Adhesive solution was sprayed on the seeds, and it was ensured that the outsides of the seeds were moistened with adhesive. Then, the powder mixture was sprayed on the seeds, so that the seeds were coated with powder evenly. The adhesive was then constantly sprayed alternated with the mixed coating powder for about 15 cycles until the coated seeds reached 1.5 times weight of naked seeds. The naked seeds and seeds coated with clay and talcum powder only were set as controls.

2.4 Germination test in the laboratory

Seed germination tests were conducted in a pot, 56 mm in diameter and 11 cm in height, filled with sand from Mu Us Sandy Land of Ordos. The sand was sieved over 40 mesh after drying at 140 °C for 6 h. The temperature of germination is 25 °C, and the buried depth of seeds in sand was about 5 mm. Three replications of germination tests with 100 seeds each were conducted. Water was supplied every three days. To study the influence of water supply on seed germination, 38, 50, 62, 74, 86, or 98 ml of water was supplied each time, corresponding to precipitation levels of 16, 21, 26, 31, 36, and 41 mm (Nie and Zheng, 2005; Zheng *et al.*, 2005). To investigate the effect of SAPs on seed germination and seedling growth, 50 ml of water was supplied to mimic the drought condition.

A seed was considered as germinated when the radicle had elongated to 2–3 mm. Germination number was counted daily for seven days. Germination (%) and germination energy (%) were the percentages of germinated seeds three and seven days, respectively, after imbibition relative to the total number of seeds tested. Germination index (GI) and vigor index (VI) were calculated according to Hu *et al.* (2005). GI=$\sum(G_t/T_t)$ (Guan *et al.*, 2009), where G_t is the number of the newly germinated seeds on Day T_t (T_t=1, 2, 3, …). VI=GI×S, where S represents the average length of radicle (mm) at the end of germination.

2.5 Pro accumulation and membrane permeability

Proline (Pro) accumulation was measured after the seeds were imbibed for five days (Zou, 2000). Membrane permeability could be represented by relative electrical conductivity (REC), and it was determined according to Li *et al.* (2014).

2.6 Determination of MDA and H_2O_2 content

After the seeds were ground to powder in liquid nitrogen, the malondialdehyde (MDA) content was measured using the thiobarbituric acid reaction method (Gao *et al.*, 2009). The content of H_2O_2 was determined at 590 nm with spectrophotometer using a peroxidase (POD)-based assay (O'Kane *et al.*, 1996).

2.7 Measurement of peroxidase activity

The seeds were ground in an ice-bath and extracted with 10 ml of 0.1 mol/L phosphate buffer (pH 7.8) containing 0.2 g polvinylpyrrolidone, 10 mmol/L β-mercaptoethanol, and 0.2 mmmol/L ethylenediaminetetraacetic acid (EDTA). After being filtered, the homogenate was centrifuged at 15 000*g* at 4 °C and the supernatant was used for the guaiacol oxidation assay (Chance and Maehly, 1955). One unit of POD activity was defined as an increase of 0.01 in absorbance per minute at 470 nm. The activity is expressed as U/g fresh weight (FW).

2.8 Seedling growth of the seeds

Seedling emergence (%) was the percentage of seedling protruding through sands after 21 d of imbibition relative to the total number of seeds tested. Seedling emergence rate=$\sum(100G_i/(nT_i))$, where G_i is the number of the protruded seedlings on Day T_i (T_i=1, 2, 3, …), and n is the total number of seeds tested (Zheng *et al.*, 2005). The height of the seedling and the length of the lateral root were measured by ruler after 21 d of imbibition, and the number of primary lateral roots was counted. The dry weight of the seedling was measured after drying at 80 °C for 24 h (Zhang *et al.*, 2007).

2.9 Survival rate of the seeds in Mu Us Sandy Land in Ordos

The naked seeds and SAP-coated seeds were sown on the soil surface through spray seeding machines in early June in Mu Us Sandy Land of Ordos. For each group of seeds, the test region is 46 690 m^2, and the central location is N 38°04′25.26″, E 107°36′ 48.30″. In total 2000 g naked seeds (CK1) or 3000 g coated seeds were sown on their test regions. Five quadrats of 5 m×5 m each were random set for each test region. On average 21 seeds were sown in each quadrat. The seedling number in each quadrat was counted in October.

The soil is Aeolian sandy soil. The particle size distribution of samples was analyzed with S3500 Tri-Laser particle analyzer (Microtrac Inc., Montgomeryville, PA, USA). The volumetric composition of the four particle size groups was (5.9±1.2)%, (46.6±1.6)%, (42.5±1.6)%, and (5.0±1.2)%, for <1, 1–50, 50–250, and >250 μm particles, respectively. There is no artificial water supply. The precipitation was 16.9 mm during June after sowing, and 13.5 mm in July.

2.10 Statistical analysis

All values were expressed as mean±standard deviation (SD) of no less than three replicates. Data were analyzed by analysis of variance (ANOVA), and the least significant difference (LSD) tests were adopted for multiple comparisons at P<0.05. The data presented as percentages were transformed before analysis according to $\hat{y}=\arcsin\sqrt{x/100}$ (Guan *et al.*, 2013), where x is raw data presented as percentage, and $\hat{y}$ is transformed data.

3 Results

3.1 Influence of water supply on germination

The influence of water supply on germination is first explored. Water supply of 38, 50, 62, 74, 86, or 98 ml corresponds to precipitation levels of 16, 21, 26, 31, 36, and 41 mm. The result showed that amount of water supply has a significant effect on germination of *C. korshinskii* seeds (Fig. 1). At a water supply less than 74 ml, additional 12 ml water or 5 mm precipitation could improve the germination dramatically. When more than 74 ml water was supplied, the increase of moisture has little effect on germination. Considering the germination was significantly retarded when the amount of water was 50 ml, we chose 50 ml of water supply to mimic drought condition in the following research.

3.2 Water absorption capacity of different SAPs

The water absorption capacity, defined by weight of the saturated aquasorb relative to dry powder, is the most important characteristic of aquasorb. As shown in Fig. 2, various SAPs have a visible difference in absorption ability in pure water. SAP D has the greatest absorption capacity and the weight of saturated SAP

D has increased 460 times that at its initial state. SAP E exhibits a comparative water absorbing capacity with SAP D. SAP A is the least efficient aquasorb with only 25% water absorbing capacity of D.

3.3 Effects of SAP coatings on seed germination

Seeds need a certain amount of water during germination, and 50 ml of water supply every three days is not sufficient for *C. korshinskii* seeds. To improve the ability of water absorbency and retention, we prepared coating reagents according to the formula in Table 1 and made seed pellets with various SAP coatings. The pelleted seeds with any SAP coating have a better germination ability than the naked (CK1) or pelleted (CK2) seeds without SAP (Fig. 3), suggesting that the SAP coating could ameliorate the moisture condition and remarkably promote germination. The SAP D and SAP E are two most efficient agents to improve seed germination, which is consistent with their higher water absorption ability than the other three SAPs. Compared with naked seeds, coating with SAP D resulted in a 244% increase on the percentage of germination (Fig. 3a), a 636% increase on germination energy (Fig. 3b), and a 401% increase on GI (Fig. 3c). These results suggested that SAP D coating not only enhanced the final germination percentage, but it was also much more efficient in speeding up the germination process, as more seeds germinated during the early stage. Considering the longer radicle in SAP D coated seeds, the VI increased 15.9 times that of the control seeds (Fig. 3d), implying dramatic improvement of the seed vigor by SAP coating. However, the role of SAP on germination improvement and its water absorption capacity are not strictly correlated. Though water absorption capacity of SAP C is higher than that of A, germination ability of SAP C-coated seeds is lower than that of A. This result implied that other characteristics of SAP coatings might affect seed germination. Under the combined influence of water absorption capacity of SAP and other factors, the efficiency of five SAP coatings are in the sequence of D>E>B>A>C.

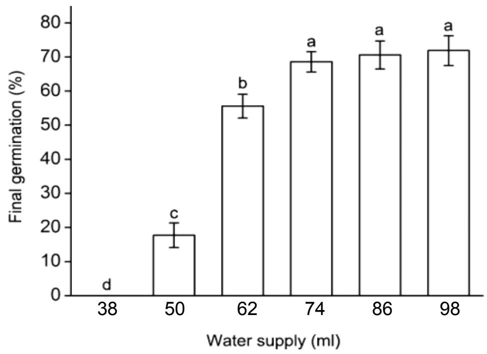


Fig. 1 Effect of water supply on germination of *C. korshinskii* seeds

C. korshinskii seeds were sown in sands with a burial death of 5 mm, and 38, 50, 62, 74, 86, or 98 ml of water was supplied every three days. The numbers of germinated seeds were counted after seven days of imbibition. The data are expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

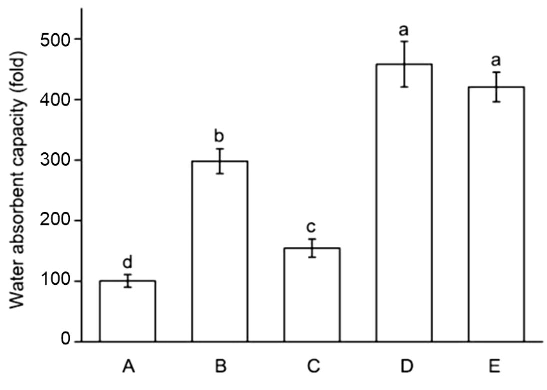


Fig. 2 Water absorbent capacities of different SAPs

Groups A, B, C, D, and E represent five kinds of SAPs. The weight of the saturated aquasorb relative to dry SAP powder is the absorption fold of the SAP. The data are expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

3.4 Effects of SAP coatings on proline content in germinating seeds

There is a close relationship between proline accumulation and resistance of plants to osmotic stress. Proline is one of the cytoplasmic osmotic regulators working to resist dehydrating (Meier *et al.*, 1992), so its accumulation probably indicates that the plant is under water stress or salt stress (Smirnoff, 1993). The proline content in control groups CK1 and CK2 was higher than that in the seeds coated with SAPs, indicating that seeds in the control groups have suffered from serious drought stress (Fig. 4a). Among coated seeds, seeds with SAP B, D, or E coatings had less proline accumulation than A or C, which demonstrated that these seeds might obtain much water from their coatings and experience with milder drought stress. According to our data, proline content is negatively correlated with the water absorption capacity of SAPs with statistical significance at the $P<0.01$ level (Table 2).

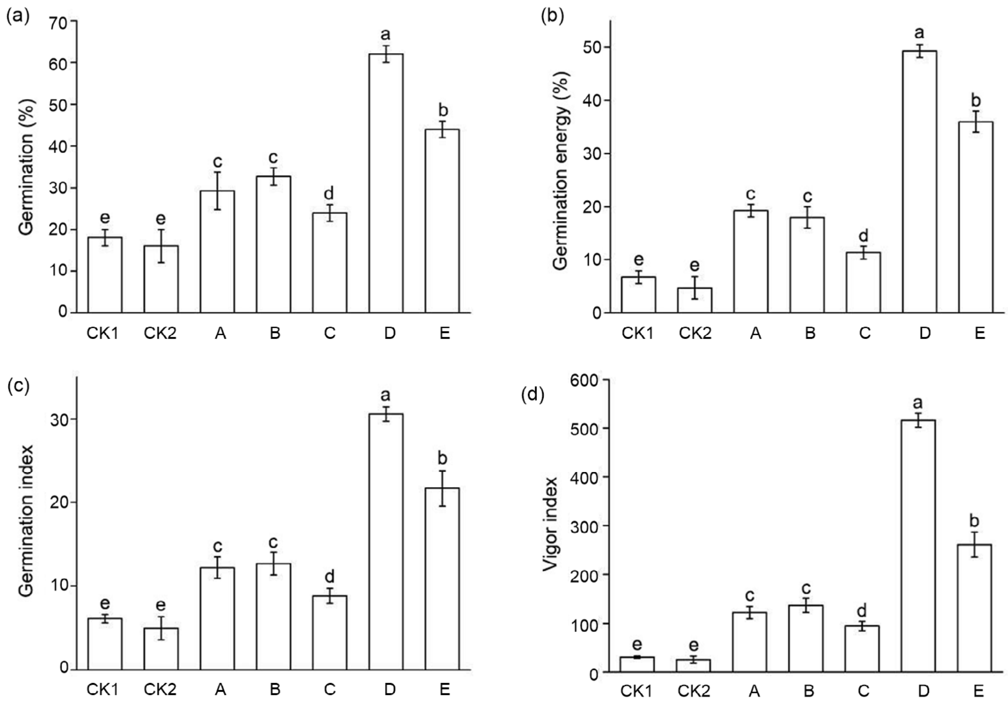
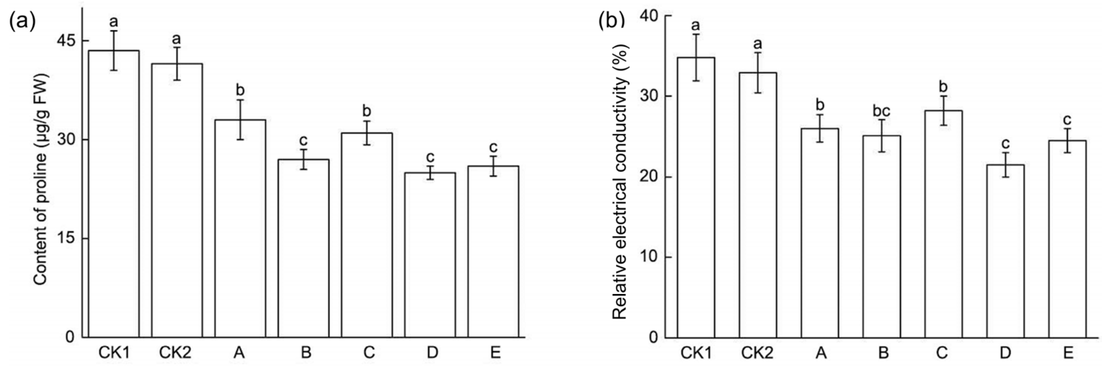


Fig. 3 Effects of SAP coatings on seed germination of *C. korshinskii*

Seed germination (a), germination energy (b), germination index (c), and vigor index (d) in seeds with different coatings. Germination (%) and germination energy (%) were the percentages of germinated seeds three and seven days after imbibition, respectively. CK1 and CK2 represent naked seeds and seeds coated with clay and talcum powder, respectively. Groups A, B, C, D, and E represent seeds coated with corresponding SAP. The data are expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the *P*<0.05 level



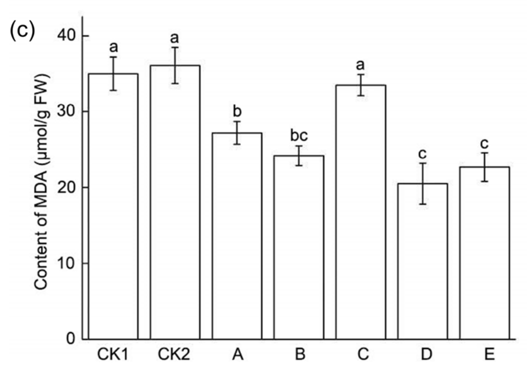


Fig. 4 Effects of different SAP coatings on proline content and membrane in germinating *C. korshinskii* seeds

Proline content (a), relative electrical conductivity (REC) (b), and malondialdehyde (MDA) content (c) in seeds with different coatings after five days of imbibition. CK1 and CK2 represent naked seeds and seeds coated with clay and talcum powder, respectively. Groups A, B, C, D, and E represent seeds coated with corresponding SAP. The data are expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the *P*<0.05 level

Table 2 Effect of concentration of Hankeshu™ aquasorb (D) on seeding growth

Group	Percentage of seedling emergence (%)	Seedling emergence rate	Root length (cm)	Seedling height (cm)	Dry weight (mg)
CK1	15.0±3.0^{d}	2.5±0.2^{e}	5.2±0.6^{d}	3.2±0.2^{c}	19.1±1.4^{c}
CK2	16.0±2.0^{d}	2.6±0.1^{e}	5.4±0.5^{d}	3.3±0.2^{c}	21.1±1.5^{c}
1% D	18.0±2.0^{d}	2.9±0.1^{e}	5.7±0.3^{d}	3.6±0.2^{c}	22.3±1.8^{b}
4% D	29.3±4.6^{c}	3.5±0.3^{d}	9.3±0.4^{c}	5.2±0.2^{b}	42.4±2.0^{b}
7% D	52.7±2.3^{b}	5.6±0.2^{c}	14.5±0.9^{b}	6.6±0.1^{a}	47.7±1.8^{a}
10% D	60.0±2.0^{a}	7.1±0.3^{a}	16.9±0.4^{a}	6.8±0.2^{a}	48.9±2.0^{a}
13% D	50.0±3.0^{b}	6.1±0.3^{b}	13.5±0.3^{b}	6.8±0.4^{a}	46.3±1.3^{a}

These data were obtained 21 d after seed imbibition, and expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

3.5 Effects of SAP coatings on cell membrane damage of seeds

Cell membrane is always susceptible to water deprivation stress. REC was measured to explore the integrity of the membrane during germination. The conductivity of control seeds (CK1, CK2) was significantly higher than that of SAP-coated seeds, which declared that the damage to their membrane system was more serious (Fig. 4b). In addition, the seeds coated by SAPs with higher water absorption ability have a relatively low conductivity. REC in seeds treated by aquasorb D or E is lower than that in seeds treated with A or C, so membrane integrity was better in D- or E-coated seeds. MDA is often regarded as a marker of membrane lipid peroxidation and the strength of resistant response to the stress (Sharma and Dubey, 2005). The MDA content in seeds coated with D was the lowest, but there was no significant difference among D, E, and B. SAP A-coated seeds had a relatively lower MDA content than SAP C-coated seeds, which is probably an explanation of the higher germination in SAP A-treated seeds. These data indicated that SAP could protect seeds from drought-induced membrane lipid peroxidation and permeability injury during germination (Fig. 4c).

3.6 Effects of SAP coatings on H_2O_2 content and POD activity

Water deprivation can lead to oxidative stresses in plant cells, and increase the generation of reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2). In order to survive in stresses such as drought, plants would activate their anti-oxidation system to make protective responses (Pastori and Foyer, 2002). POD is one of the important protective enzymes to remove ROS in the early stage of stress, and it would be activated when subjected to stress (Pinhero *et al.*, 1997). As shown in Fig. 5a, seeds in CK1, CK2, and SAP C-treated groups had the largest amount of H_2O_2, and the seeds coated with SAP D or E showed the lowest content of H_2O_2. These results indicated that SAP D or E efficiently reduced accumulation of ROS, herein H_2O_2, while SAP C failed to do the same. POD activity is consistent with the H_2O_2 and MDA content, in response to ROS production and lipid peroxidation of the cell membrane. When ranged sequentially, the POD activity is the highest in the control groups CK1 and CK2, moderate in groups A, B, and C, and the lowest in seeds coated with SAP D or E (Fig. 5b).

3.7 Effects of SAP coatings on seedling growth

When a seed germinates, the coating will crack and scatter in the soil, which can reduce the evaporation of the soil moisture and promote the growth of seedlings. It was shown that seeds coated with SAPs significantly grew better than controls during the seedling stage, especially those coated with SAP D or E (Table 3). Among the seedling growth parameters measured, values of SAP D or E group are 2–3-fold higher than the controls. There is no significant difference between D and E, except for seedling emergence. Effects of SAP A and C on seedling growth are similar, while B has a small but significant advantage for root growth compared with A and C. It was worth mentioning that the majority of seedling growth parameters detected in different coating groups were significantly positive-correlated with water absorption capacity of SAPs, with the only exception of root length (Table 4).

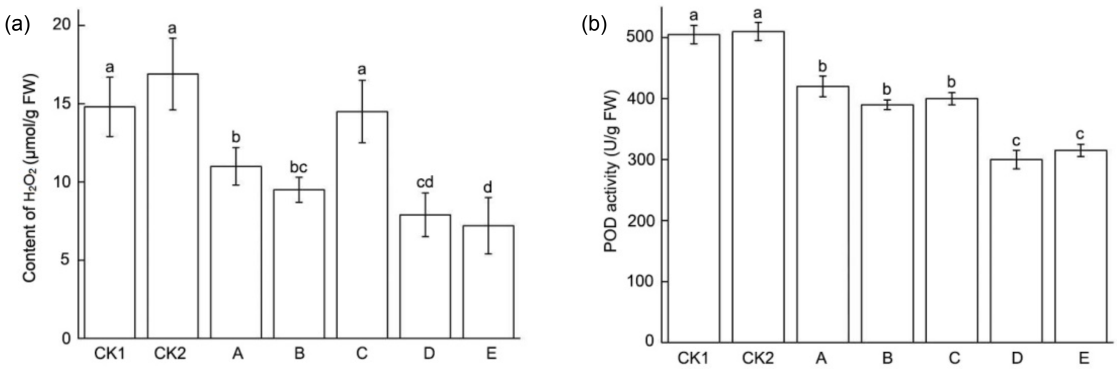


Fig. 5 Effects of different SAPs on H_2O_2 content and POD activity in germinating seeds

H_2O_2 content (a) and POD activity (b) in seeds with different coatings after five days of imbibition. CK1 and CK2 represent naked seeds and seeds coated with clay and talcum powder, respectively. Groups A, B, C, D, and E represent seeds coated with corresponding SAP. The data are expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

Table 3 Effect of different SAP coatings on seeding growth of *C. korshinskii*

Group	Seeding emergence rate	Root length (cm)	Lateral root number	Seedling height (cm)	Dry weight (mg)
CK1	$2.4±0.1^e$	$5.4±0.2^d$	$3.5±0.2^d$	$3.5±0.3^d$	$22.2±1.7^d$
CK2	$2.5±0.2^e$	$5.2±0.6^d$	$2.9±0.2^d$	$3.2±0.2^d$	$19.6±1.8^d$
A	$3.5±0.3^c$	$9.3±0.4^c$	$6.8±0.4^c$	$5.2±0.2^{bc}$	$42.4±2.0^{bc}$
B	$3.6±0.2^c$	$10.5±0.9^b$	$8.9±0.4^b$	$5.4±0.1^b$	$45.7±1.8^b$
C	$3.1±0.1^d$	$8.7±0.3^c$	$6.0±0.4^c$	$4.9±0.2^c$	$37.3±1.3^c$
D	$7.3±0.2^a$	$17.1±0.3^a$	$11.6±0.8^a$	$6.9±0.3^a$	$49.4±1.9^a$
E	$6.4±0.3^b$	$16.5±0.3^a$	$10.6±0.8^a$	$6.8±0.4^a$	$49.3±1.3^a$

These data were obtained 21 d after seed imbibition, and expressed as mean±SD from three replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

Table 4 Correlations between water absorption capacity of SAPs and seedling growth parameters or biochemical markers

Variate	Pearson correlation coefficient	*P*-value (two-tailed)
Seedling emergence rate	0.902^*	0.036
Lateral root number	0.969^{**}	0.006
Seedling height	0.925^*	0.024
Dry weight	0.881^*	0.048
Proline content	-0.973^{**}	0.005
POD activity	-0.957^*	0.011

* Significant correlation at the $P<0.05$ level; ** Significant correlation at the $P<0.01$ level

In order to obtain the optimal formula of coating reagent, we adjust the proportion of SAP D in a gradient of 1%, 4%, 7%, 10%, and 13%. As the content of SAP D rose from 1% to 10%, its promoting effect on seedling growth increased accordingly. Compared with naked seeds, these seedling growth parameters at least doubled in 10% SAP D-coated seeds, among which seedling emergence even tripled. However, once the content of SAP D was increased to 13%, seedling emergence and root length were significantly inferior to those under 10% SAP D, showing 10% SAP D was the best for seedling growth (Table 2).

3.8 Effect of SAP coatings on seedling survival in Mu Us Sandy Land

The effect of SAP coatings on seedling survival was finally tested in Mu Us Sandy Land. Seeds were sown in June, and the seedling number in quadrats of 5 m×5 m was recorded in October. The field trial showed that in the arid place, control seedlings had a low survival rate for deprivation of water, and SAP coatings were helpful to improve seedling survival. Consistent to our previous data, SAP D is still the best aquasorb, which enables the average seedling number per quadrat to increase by twofold on that of naked seeds (Fig. 6). It was also noticed that coating agent only with adhesives and support materials in CK2 showed a slight but not significant inhibition on seedling survival.

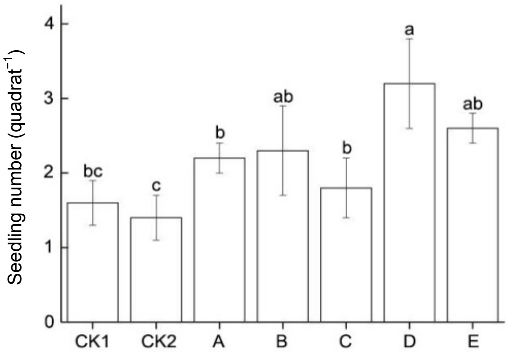


Fig. 6 Seedling survival of *C. korshinskii* seeds with different coatings in quadrats in Mu Us Sandy Land

Seeds were sown in June, and the seedling number was recorded in quadrats of 5 m×5 m in October. CK1 and CK2 represent naked seeds and seeds coated with clay and talcum powder, respectively. Groups A, B, C, D, and E represent seeds coated with corresponding SAP. The data are expressed as mean±SD from five replicates. Different superscript letters indicate significant difference at the $P<0.05$ level

4 Discussion

SAP coatings can ensure water supply for both seeds and seedlings. During imbibition, the intact coating, which serves as a "mini-reservoir" around the seeds, absorbs enough water for germination. Once the coating disintegrated, the pieces containing SAP will disperse in the soil, which is still able to maintain water and reduce soil moisture evaporation and seepage losses, thus improving water use efficiency and playing an indirect role in promoting plant growth (Chirino *et al.*, 2011). According to our results, the use of SAP in *C. korshinskii*-pelleted seeds could significantly promote seed germination and seedling growth under drought conditions. Water absorption capacity of SAPs determined the amount of water storage in the soils, and thus affected plant establishment in them. In our study, SAP D was the optimal hydrogel to promote seed germination and seedling growth, which is consistent with its greatest water absorption capacity among the five SAPs measured.

Water absorption capacity is the essential characteristic of SAP when considered as a coating agent. According to our results, the water absorption capacity of SAP is positively correlated with the majority of seedling growth parameters, while negatively correlated with proline content and POD activity with a measure of statistical significance. However, the effect of SAP on seed germination is not strictly correlated with its water absorption capacity. For instance, though water absorption capacity of SAP is ranged as A<C<B, germination ability of seeds with SAP A coating is better than that with C, even comparable with B. It is inferred that seed germination is probably sensitive to other unknown features of SAPs. Under the combined influence of several factors, the efficiencies of five SAP coatings are in the sequence of D>E>B>A>C.

The cell membrane is an important structure for exchange of molecules and ions. When the plants are subjected to environmental stress, along with the loss of selective permeability, intracellular electrolyte would leak out and increase the electrical conductivity of the surrounding solution. Results of REC detection showed that the membrane integrity of the SAP-coated seeds is significantly better, presenting a protection from membrane damage. When plants are suffering from drought, the balance of redox is broken.

The cells would produce large amounts of H_2O_2 and other ROS, directly or indirectly, resulting in a series of physiological and biochemical disorders (Smirnoff, 1993). When excessive ROS could not be cleared, membrane lipid peroxidation was likely to be triggered, giving rise to the increase of MDA content. Therefore, MDA is usually used as an indicator of cell membrane lipid peroxidation. Under drought stress, more H_2O_2 and MDA accumulated in CK1, CK2, and SAP C-coated seeds, suggesting severe membrane lipid peroxidation in them during seed germination. However, coating with SAP D, E, B, or A was able to significantly reduce H_2O_2 and MDA levels, and thus alleviate membrane oxidative damage.

The antioxidant system of plants consists of a non-enzymatic antioxidant system and an antioxidant enzyme system. Proline is not only an osmotic regulator, but also a non-enzymatic antioxidant, and thus an indicator of drought stress. When subjected to drought stress, plants will produce more proline, and increasingly accumulate this as the stress proceeds to reduce osmotic damage to cells (Delauney and Verma, 1993). At the same time proline can scavenge free radicals, such as singlet oxygen and HO•, helping cells to overcome the oxidative damage caused by water deficit (Mohanty and Matysik, 2001). Our results showed that seeds with SAP coatings produced less proline, which implied that SAPs probably reduced drought stress suffered by the *C. korshinskii* seeds. The stronger the water absorption ability of

SAP occupied, the less proline in seeds was produced. POD shows a dual function in stress response. In the early stage of adversity, as a component of the antioxidant enzyme system, POD removed hydrogen peroxide and showed a protective effect (Sofo *et al.*, 2005). However, in late adversity, POD participated in the active oxygen production and chlorophyll degradation, leading to membrane lipid peroxidation. After imbibition for five days, POD activity was higher in naked seeds than in SAP-coated seeds (Fig. 5b), ready to activate the antioxidant system under worse water supply in the control groups. In SAP-coated seeds, POD is less responding, in agreement with better moisture supply from these "mini-reservoirs" around seeds. Moreover, POD activity has significant negative correlation with water absorption capacity of SAPs (Table 4).

SAP has great water holding capacity but a limited air permeability. On the contrary, sands possess better air permeability but worse water holding capacity. An inappropriate type or amount of SAP will cause uneven distribution of air and liquid in seeds. Furthermore, it is possible that SAP could sometimes compete with plants for moisture, which is harmful to the growth of seedlings. The components and proportion of inactive support materials in seed coating are also important. Grellier *et al.* (1999) found that high adhesive concentration had a negative effect on water transfer in pelleted seeds. As shown in Fig. 6, control-coated seeds in CK2 group showed less seedling survival in sands than naked CK1 seeds. It is likely to be because clay, talcum powder, or ethylene cellulose in coating exerts an adverse influence on seedling growth. Moreover, when determining the components of seed coating, the feature of the soil, precipitation, and other environmental factors should also be taken into account to gain a better efficiency of plant establishment in arid regions.

5 Conclusions

Our results showed that SAP coatings could significantly improve the vigor and germination of *C. korshinskii* seeds. According to changes of biochemical markers in germinating seeds, SAP coatings seem to function by alleviating drought-induced oxidative stress and membrane damage. After coating seeds with SAPs, more seedlings emerged and grew better either in the experimental environment or in Mu Us Sandy Land. The efficiency of SAP coatings on enhancing seed germination and seedling growth was positively correlated with their water absorbent capacity. Our results are expected to be helpful on understanding and utilizing SAP seed coatings in improving plant survival under drought conditions.

Compliance with ethics guidelines

Li-qiang SU, Jia-guo LI, Hua XUE, and Xiao-feng WANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Akhter, J., Mahmood, K., Malik, K.A., *et al.*, 2004. Effects of hydrogel amendment on water storage of sandy loam and loam soils and seedling growth of barley, wheat and chickpea. *Plant Soil Environ.*, **50**(10):463-469. http://dx.doi.org/10.17221/202/2004-PSE

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. *Methods Enzymol.*, **2**:764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

Chirino, E., Vilagrosa, A., Vallejo, V.R., 2011. Using hydrogel and clay to improve the water status of seedlings for dryland restoration. *Plant Soil*, **344**(1-2):99-110. http://dx.doi.org/10.1007/s11104-011-0730-1

Cui, X., Lu, Q., 2012. Development status and prospect of standardized desertification combating in China. *Arid Zone Res.*, **29**(5):913-919 (in Chinese).

Delauney, A.J., Verma, D.P.S., 1993. Proline biosynthesis and osmoregulation in plants. *Plant J.*, **4**(2):215-223. http://dx.doi.org/10.1046/j.1365-313X.1993.04020215.x

Dorraji, S.S., Golchin, A., Ahmadi, S., 2010. The effects of hydrophilic polymer and soil salinity on corn growth in sandy and loamy soils. *Clean-Soil Air Water*, **38**(7):584-591. http://dx.doi.org/10.1002/clen.201000017

Gao, C., Hu, J., Zhang, S., *et al.*, 2009. Association of polyamines in governing the chilling sensitivity of maize genotypes. *Plant Growth Regul.*, **57**(1):31-38. http://dx.doi.org/10.1007/s10725-008-9315-2

Grellier, P., Riviere, L.M., Renault, P., 1999. Transfer and water-retention properties of seed-pelleting materials. *Eur. J. Agron.*, **10**(1):57-65. http://dx.doi.org/10.1016/S1161-0301(98)00050-1

Guan, Y., Jin, H., Wang, X., *et al.*, 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **10**(6):427-433. http://dx.doi.org/10.1631/jzus.B0820373

Guan, Y., Wang, J., Tian, Y., *et al.*, 2013. The novel approach to enhance seed security: dual anti-counterfeiting methods applied on tobacco pelleted seeds. *PLoS ONE*, **8**(2): e57274. http://dx.doi.org/10.1371/journal.pone.0057274

Hu, J., Zhu, Z.Y., Song, W.J., *et al.*, 2005. Effects of sand

priming on germination and field performance in direct-sown rice (*Oryza sativa* L.). *Seed Sci. Technol.*, **33**(1): 243-248.
http://dx.doi.org/10.15258/sst.2005.33.1.25
Huang, Z., Zhang, G., Li, Y., *et al.*, 2002. Characteristics of aquasorb and its application in crop production. *Trans. Chin. Soc. Agric. Eng.*, **18**(1):22-26 (in Chinese).
http://dx.doi.org/10.3321/j.issn:1002-6819.2002.01.006
Johnson, M.S., 1984. The effects of gel-forming polyacrylamides on moisture storage in sandy soils. *J. Sci. Food Agric.*, **35**(11):1196-1200.
http://dx.doi.org/10.1002/jsfa.2740351110
Li, J., Wang, Y., Pritchard, H.W., *et al.*, 2014. The fluxes of H_2O_2 and O_2 can be used to evaluate seed germination and vigor of *Caragana korshinskii*. *Planta*, **239**(6):1363-1373.
http://dx.doi.org/10.1007/s00425-014-2049-7
Li, W., Yan, W., Liu, Z.X., *et al.*, 2009. Technical measures of enhancing aerial-seeding effect in Mu Us Sandy Land. *J. Desert Res.*, **29**(1):114-117 (in Chinese).
Li, X., Jiang, J., Song, C.W., *et al.*, 2012. Effect of super absorbent polymer on seed germination and seedling roots of *Haloxylon ammodendron* and *H. persicum*. *Arid Zone Res.*, **29**(5):797-801 (in Chinese).
Liu, R., Zhang, K., Zong, L., *et al.*, 2004. Study on pelletizing of *Calligonum alaschanicum* for aerial sowing in desert. *Inn. Mong. Forest. Sci. Technol.*, **2004**(3):3-6 (in Chinese).
Meier, C.E., Newton, R.J., Puryear, J.D., *et al.*, 1992. Physiological responses of loblolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustment and tissue elasticity. *J. Plant Physiol.*, **140**(6):754-760.
http://dx.doi.org/10.1016/S0176-1617(11)81034-5
Mohanty, P., Matysik, J., 2001. Effect of proline on the production of singlet oxygen. *Amino Acids*, **21**(2):195-200.
http://dx.doi.org/10.1007/s007260170026
Moussa, H.R., Khodary, S.E.A., 2003. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int. J. Agric. Biol.*, **35**(1):179-187.
Nie, C., Zheng, Y., 2005. Effects of water supply and sand burial on seed germination and seedling emergence of four dominant psammophytes in the ordos plateau. *Acta Phytoecol. Sin.*, **29**(1):32-41 (in Chinese).
O'Kane, D., Gill, V., Boyd, P., *et al.*, 1996. Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta*, **198**(3):371-377.
http://dx.doi.org/10.1007/BF00620053
Pastori, G.M., Foyer, C.H., 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of “redox” and abscisic acid-mediated controls. *Plant Physiol.*, **129**(2):460-468.
http://dx.doi.org/10.1104/pp.011021
Pinhero, R.G., Rao, M.V., Paliyath, G., *et al.*, 1997. Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings. *Plant Physiol.*, **114**(2):695-704.
http://dx.doi.org/10.1104/pp.114.2.695
Rehman, A., Ahmad, R., Safdar, M., 2011. Effect of hydrogel on the performance of aerobic rice sown under different techniques. *Plant Soil Environ.*, **57**(7):321-325.
Sharma, P., Dubey, R.S., 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regul.*, **46**(3): 209-221.
http://dx.doi.org/10.1007/s10725-005-0002-2
Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.*, **125**(1):27-58.
http://dx.doi.org/10.1111/j.1469-8137.1993.tb03863.x
Sofo, A., Dichio, B., Xiloyannis, C., *et al.*, 2005. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. *Funct. Plant Biol.*, **32**(1):45-53.
http://dx.doi.org/10.1071/FP04003
Wang, B., Sun, B., 1998. Current situation and prospect of seed-coating chemical in China. *Crop*, **62**(2):19-20 (in Chinese).
Wang, T., Zhu, Z., Wei, W., 2002. Sandy desertification in the north of China. *Sci. China Earth Sci.*, **45**(S1):23-34.
http://dx.doi.org/10.1007/BF02878385
Zhang, C.F., Hu, J., Lou, J., *et al.*, 2007. Sand priming in relation to physiological changes in seed germination and seedling growth of waxy maize under high-salt stress. *Seed Sci. Technol.*, **35**(3):733-738.
http://dx.doi.org/10.15258/sst.2007.35.3.19
Zheng, Y., Xie, Z., Yi, Y, *et al.*, 2005. Effects of burial in sand and water supply regime on seedling emergence of six species. *Ann. Bot.*, **95**(7):1237-1245.
http://dx.doi.org/10.1093/aob/mci138
Zou, Q., 2000. Plant Physiology Experiment Guidance. China Agriculture Press, Beijing, p.96-97 (in Chinese).

中文概要

题　目：高吸水性聚合物种子包衣促进干旱条件下柠条种子萌发及幼苗生长

目　的：研究干旱条件下五种高吸水性聚合物（SAP）种衣剂对柠条种子萌发及幼苗生长的影响。

创新点：在模拟干旱和沙区条件下，阐明了保水剂包衣促进柠条种子萌发及幼苗生长的作用及其与吸水能力的关系。

方　法：以不同 SAP 为种衣剂，制成丸化倍数为 0.5 的柠条包衣种子。在沙土中模拟干旱条件进行萌发实验，计算萌发率和萌发势，并测定萌发种子中丙二醛和过氧化氢含量等生化指标。在幼苗期测算出苗率、根长、苗高和干重。测定 SAP 的吸水倍数，分析其与包衣效果的相关性。最后在毛乌素沙漠中播种，记录成苗数。

结　论：SAP包衣能显著提高柠条种子的萌发和幼苗生长，同时降低种子中丙二醛和过氧化氢的含量，以及膜相对电导率和过氧化氢酶的活性，缓解干旱诱发的氧化胁迫和膜损伤。五种 SAP 包衣的作用效果为 D>E>B>A>C，其中 SAP 的吸水性能具有决定作用。10% SAP D 能够将发芽率提高 244%，并将毛乌素沙区中的平均成苗数提高一倍。本研究得到的 SAP 种子包衣促进干旱地区植物存活的结论，有助于相关理论和实践研究。

关键词：高吸水性聚合物；种子包衣；柠条；种子萌发；幼苗；干旱