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不同种子预处理对10种沙拐枣植物萌发的影响*

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摘 要:为了确定沙拐枣植物种子的萌发特性及最优播前预处理方法,在实验室条件下,对10种沙拐枣植物的种子进行了磨砺、硫酸和热水浸泡、冷藏、种子浸出液处理,然后进行发芽实验研究. 萌发实验的结果表明,10种沙拐枣植物对于不同的种子预处理,均表现出相似的萌发反应. 磨砺、硫酸浸泡和冷藏处理对种子萌发有明显地促进作用. 与对照相比,种子浸出液处理对种子的发芽率、发芽速度均具有明显地抑制作用,并能增强种子的休眠. 冷藏处理具有打破有活力的种子休眠、促进种子萌发的作用,但它与热水浸泡处理一样,对有活力种子表现出一定的致死作用. 沙拐枣植物的萌发模式在不同种子预处理间均表现出明显的差异性. 机械磨擦和硫酸处理能够促进种子的萌发率及发芽势. 泡果沙拐枣(*Calligonum junceum*)在本项实验中表现出很强的萌发能力.

关键词:沙拐枣;种子预处理;种子萌发;种子休眠

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Effects of different seed pretreatments on germination of ten *Calligonum* species

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Abstract: In order to determine the optimum seed pre-sowing treatments and patterns of germination, a greenhouse experiment was conducted to study the effects of abrasion, sulphuric acid, boiling water, cold stratification and seed exudate treatments on the germination of ten *Calligonum* species. The results showed that the response of seed germination to the different pretreatments was similar for all ten *Calligonum* species. The abrasion, sulphuric acid and cold stratification treatments significantly promoted overall germinability. Compared with the control, the exudate treatment significantly decreased the percent germination, hampered the rate of germination and bolstered dormancy for almost all species. The cold stratification treatment can break the dormancy of viable *Calligonum* seeds and increased the germination, but it

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has a little lethal effect on viable seeds probably as well as the boiling water treatments The germinaton patterns performed significant difference between the pre-sowing treatments for all ten *Calligonum* species, and the speed and percent germination of the *Calligonum* species can be greatly increased by mechanical scarification or sulphuric acid treatments The results showed that seeds of *C. junceum* have strong ability of germinating.

Key words: *Calligonum*; seed pretreatments; seed germination; seed dormancy

Calligonum species are dominant perennial shrubs in active sand dunes and stabilized sand field in the northern desert of China[1,2]. They can exist in mobile sand dunes in conditions of extreme drought[2,3]. They have a reputation to high tolerance to water deficit. They appear to be suitable for revegetating of desert[1,2,4,5]. They have great potential to provide different products and services such as forage, traditional medicine, halting desert encroachment and stabilizing sand dune[2,3]. As their great importance in providing many uses and services, they have attracted some attention. However, there has been little experimental research dealing with the seed germination and seedling emergence of them[6]. The available information about these species is their botany, cultivated method, taxonomy, genetic diversity, brief descriptions of their habitat condition and the range of their geographical distribution[1~8].

One of the problems related to the neglect of these is the difficulty of raising seedling from seeds The seeds have a hard impermeable seed coat which prevents seeds from imbibing of water and germination[2,6,8]. Therefore, the seed treatments before sowing are required to obtain rapid, uniform and high germination[9~14]. The purpose of the present study was to investigate the germination responses and speed of ten *Calligonum* species to different pre-sowing treatments, as an understanding of these factors is crucial for the successful regeneration and recruitment of these long-lived desert plant species.

1 Materials and methods

1.1 Seed collection and preparation

Seeds of ten *Calligonum* species (*C. junceum*, *C. leucocladum*, *C. rubicundum*, *C. densum*, *C. mongolicum*, *C. chinense*, *C. caput-medusae*, *C. arborescens*, *C. alaschanicum* and *C. potaninii*) were collected from at least 10 plants per species in August and September, 1998 at Shapotou Desert Research and Experimental Station of Chinese Academy of Sciences (37°32′N, 105°02′E, 1 339 m a. s. l.), Ningxia province, China. Seeds were allowed to air-dry and stored at room temperature (23℃~26℃) until May 1999, when experimental pretreatments were initiated (storage in this manner did not affect the dormancy or viability of the seeds). For each species, seeds were mixed and then allocated at random. The aborted and predated seeds were discarded. Intact plump seeds were surface sterilized with Na-hypochlorite prior to any experimental usage. Seed viability is variable, but is generally between 30%~50%[2,6].

1.2 Pre-sowing seed treatment experiments

1.2.1 Abrasion of seed coat The mechanical scarification treatment was carried out by grinding seeds in a mortar with a pinch of clean silica sand until all seta of seed coat was removed and the seed coat was broken.

1.2.2 Sulphuric acid immersion Since seeds from the ten species apparently were different in the size (Table 1) and the degree of hardness of their seed coats (based on visual inspection), but they received same sulphuric acid treatments. Seeds were immersed in 96% sulphuric acid for 30 min, and then rinsed thoroughly in running water for 45 min.

Table 1 Date of seed collection, as well as seed size and dry mass of ten *Calligonum* **species. Seed size and seed dry mass are means** (±sd) **of ten randomly selected seeds from each species** (Tao, 2000)

Species	Date of seed collection	Length of seed (mean±sd) (mm)	Diameter of seed (mean±sd) (mm)	Seed mass (mean±sd) (mg)
C. junceum	24-Aug.-98	11.07(±0.76)	9.24(±0.99)	0.08(±0.01)
C. leucocladum	13-Aug.-98	12.53(±0.97)	9.95(±1.81)	0.07(±0.01)
C. rubicundum	3-Sep.-98	16.70(±1.62)	14.17(±1.43)	0.16(±0.02)
C. densum	3-Sep.-98	16.09(±2.05)	14.77(±1.83)	0.10(±0.01)
C. mongolicum	24-Aug.-98	13.32(±1.35)	9.32(±1.48)	0.10(±0.02)
C. chinense	29-Aug.-98	13.11(±0.90)	11.85(±1.12)	0.12(±0.01)
C. caput-medusae	10-Aug.-98	21.66(±2.31)	18.18(±3.05)	0.15(±0.01)
C. arborescens	10-Aug.-98	20.32(±4.42)	16.34(±4.01)	0.15(±0.03)
C. alaschanicum	1-Sep.-98	19.78(±2.44)	16.27(±2.17)	0.11(±0.02)
C. potaninii	1-Sep.-98	14.73(±1.93)	12.23(±2.12)	0.11(±0.01)

1.2.3 Hot water treatment Each replicate of seeds was enclosed in a coffee filter bag which was then folded and fastened with paper clips to prevent seed loss. The seeds were then immersed in boiling water for 10 min. After immersion, they were removed from the boiling water and left to cool on a table for about 5 min.

1.2.4 Cold stratification Seeds were soaked in distilled water for 2 d, wrapped in paper bags, and then stored in plastic bags in a refrigerator (3℃), for 25 d. During the period of imbibition, water was replaced twice a day.

1.2.5 Exudate treatment During preliminary studies it had been noted that a yellow, water-soluble material was exuded from soaking seeds, it may be contained water soluble inhibitors from the seed coat[6]. To examine the possible effect of this exudate on seed germination, a group of seeds was exposed to the exudate solution during the germination period, instead of clean distilled water.

1.3 Germination experiments

The germination experiments were conducted during May-June 1999. Controlled seeds and seeds that had undergone the pretreatments described above were placed on wet filter paper in glass petri dishes (11.5 cm diameter × 2 cm depth). These were then placed into the temperature-controlled chambers for germination, set at 14 h of daylight at 25℃ and 10 h of darkness at 12℃, to approximate general springtime field conditions. To ensure no systematic effects due to position within the chamber, petri dishes were re-arranged at random every 2 d. All pre-sowing seed treatments consisted of five replicates of 40 seeds for each species. Visible radicle growth was used to define germination. Germination was recorded every 5 d and allowed to proceed for 9 weeks except where specified. Ungerminated seeds were soaked in water at 30℃ for 24 h. Seed coats were cut and the embryo was soaked in 1% tetrazolium chloride for 24 h at 30℃. Pink embryos were scored as alive. Germination was expressed as percentage of viable seeds germinated.

1.4 Data analysis

The results of the germination experiments were analyzed for statistical significance (ANOVA) with the STATISTICA software package for personal computer[15]. All percent germination data were arcsine-square-root transformed prior to analysis. Means and standard deviations were used in presentation of tables and graphs. Multiple comparisons of means were made with Duncan's tests at 95%. The following parameters were determined[13].

Germinability (%) = (number of germinating seeds/number of seeds initiated) × 100

Relative germinability (%) = (number of germinating seeds/number of viable seeds initiated) × 100

Dormancy (%) = (number of ungerminated but viable seeds/number of seeds initiated) × 100

Relative dormancy (%) = (number of ungerminated but viable seeds/number of viable seeds initiated) × 100

Mortality (%) = (number of inviable seeds/number of seeds initiated) × 100

Index of germination rate: IGS = $\sum G/t$,

where G is the relative germinability percentage, at 5 d intervals, and t is total germination period. This parameter characterizes the pace of germination for particular seed replicates.

2 Results

2.1 Seed quality of germination ecology

Table 2 summarizes mean values for germinability, relative germinability, dormancy, relative dormancy, mortality and IGS, prior to any pretreatment. The germinability and relative germinability both were significantly different among ten species (one-way ANOVA: $F_{9,40}$ = 14.94, P < 0.000 1 for the former; $F_{9,40}$ = 10.52, P < 0.000 1 for the latter). *C. junceum* had the highest level of germinability and relative germinability (47% and 69.3%). *C. leucocladum* and *C. mongolicum* had significant lower level of germinability and relative germinability (11.5% and 28.8% for the former, 14.5% and 31.3% for the latter) than other species. Inversely, *C. leucocladum* and *C. mongolicum* had significant higher level of relative dormancy (71.2% and 68.7%). The one-way ANOVA showed that the percentage of dormant seeds was not affected by species significantly ($F_{9,40}$ = 2.12, P = 0.53). There was significant difference among species in seed mortality and IGS (one-way ANOVA: $F_{9,40}$ = 7.70, P < 0.000 1 for mortality; $F_{9,40}$ = 6.87, P < 0.000 1 for IGS). *C. leucocladum*, *C. mongolicum* and *C. alaschanicum* had significantly higher level of mortality (61%, 55.5% and 54.5%). The IGS were lower for *C. leucocladum* (4.2) and *C. mongolicum* (4.5; Table 2).

Table 2 Overall seed germination qualities for ten species of Calligonum **following storage for 8 months at room temperature. Different superscript letters indicated significant difference between the values of pairs of species in the same column** (Duncan's multiple comparison test, P < 0.05)

Species	Germinability (±sd) (%)	Relative germinability (±sd) (%)	Dormancy (±sd) (%)	Relative dormancy (±sd) (%)	Mortality (±sd) (%)	IGS (±sd)
C. junceum	47.0±7.4 a	69.3±13.4 a	21.5±10.2 a	30.7±13.4 a	31.5±6.8 a	9.8±2.4 a
C. leucocladum	11.5±5.2 b	28.8±8.1 b	27.5±7.7 a	71.2±8.1 b	61.0±11.0 b	4.2±1.5 bd
C. rubicundum	37.5±7.7 ac	58.9±14.7 a	27.0±11.0 a	41.1±14.7 a	35.5±6.9 a	7.6±2.7 abc
C. densum	42.5±3.1 ac	64.1±9.6 a	24.5±8.7 a	35.9±9.6 a	33.0±6.5 a	8.6±1.5 a
C. mongolicum	14.5±8.2 b	31.3±8.7 b	30.0±10.3 a	68.7±8.7 b	55.5±16.6 b	4.5±0.8 cd
C. chinense	35.5±4.1 ac	61.3±6.2 a	22.5±4.7 a	38.7±6.2 a	42.0±5.1 ab	7.6±1.0 ac
C. caput-medusae	34.0±8.4 ac	52.2±12.9 a	31.5±9.9 a	47.8±12.9 a	34.5±9.7 a	6.4±1.9 ab
C. arborescens	29.5±4.5 ac	58.5±2.9 a	21.0±3.8 a	41.5±2.9 a	49.5±7.8 ab	7.0±1.0 abc
C. alaschanicum	26.0±8.2 c	58.2±12.6 a	19.5±8.9 a	41.8±12.6 a	54.5±13.9 b	7.4±2.3 a
C. potaninii	34.0±4.5 ac	64.2±7.1 a	19.0±4.2 a	35.8±7.1 a	47.0±4.5 ab	8.0±1.5 ac

2.2 Effect of pretreatments on germination speed

After 5 days of wetting, almost all species began to germinate at all pretreatments and the control. Their relative germinability exceeded 10% at the abrasion, sulphuric acid and boiling water treatments, but decreased 10% at the cold stratification, exudate and the control. For all species, the abrasion and sulphuric acid treatments tended to quicken the course of germination somewhat, inversely, the cold stratification and exudate treatments slowed the germination. Especially, the exudate treatment greatly inhibited the course of germination even the final relative germinability for *C. leucocladum*, *C. densum*, *C. mongolicum*, *C. caput-medusae*, *C. arborescens*, *C. alaschanicum* and *C. potaninii*. In the period of 10 days to 20 days, *C. rubicundum*, *C. densum*, *C. chinense*, *C. caput-medusae*, *C. arborescens*, *C. alaschanicum* and *C.*

potaninii all showed the faster germination speed at all pretreatments and the control (Fig. 1).

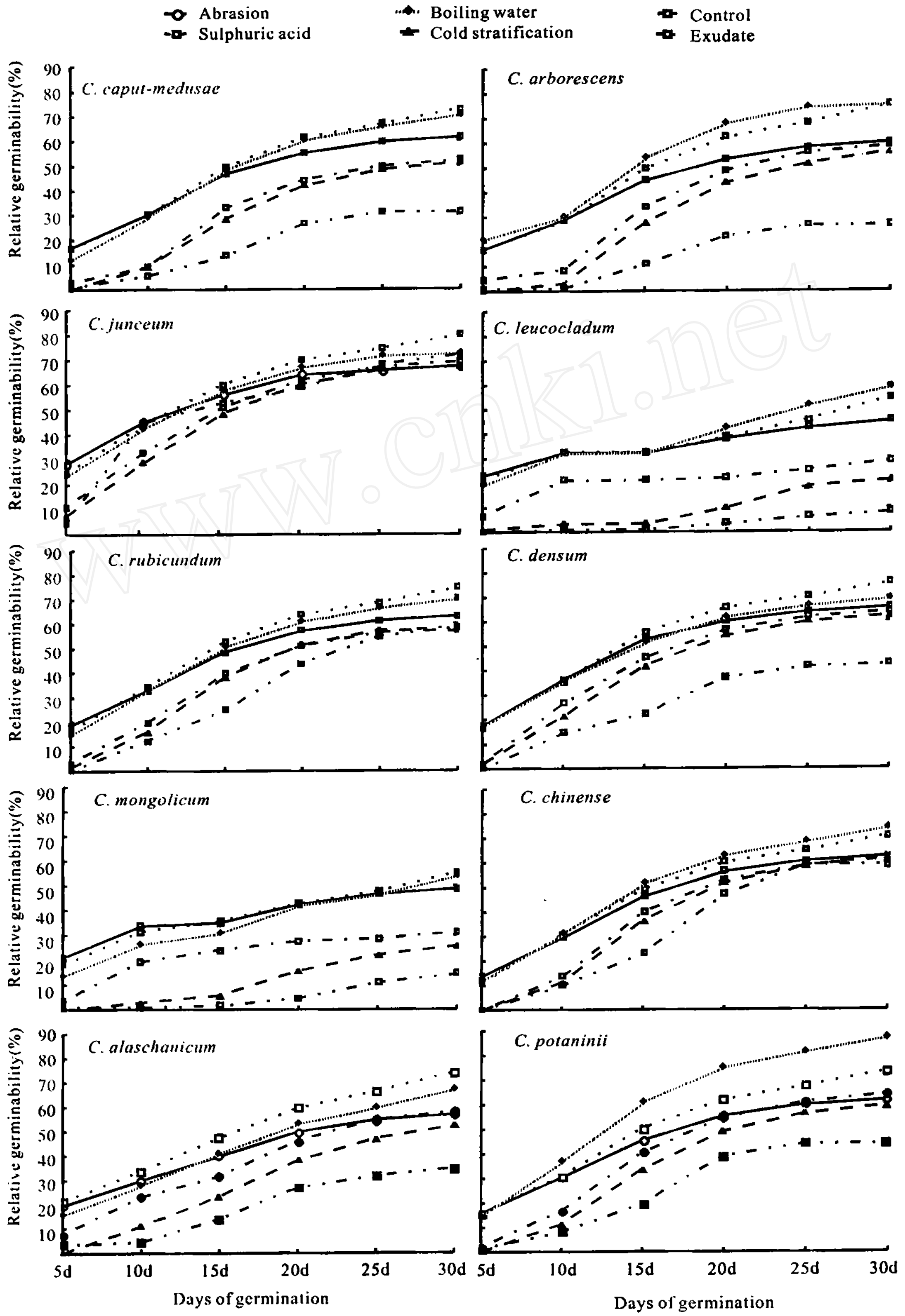

Fig. 1 Comparison of germination period for ten *Calligonum* seeds among different pretreatments

2.3 Effect of pretreatments on germination

The final relative germinability was significantly different between pretreatments in all species (one-way ANOVA: $F_{5,24}=5.03$, $P=0.0038$ for *C.*

junceum; $F_{5,24}$ = 24.50, P < 0.000 1 for *C. leucocladum*; $F_{5,24}$ = 6.85, P = 0.000 7 for *C. rubicundum*; $F_{5,24}$ = 12.76, P < 0.000 1 for *C. densum*; $F_{5,24}$ = 32.22, P < 0.000 1 for *C. mongolicum*; $F_{5,24}$ = 3.32, P = 0.024 0 for *C. chinense*; $F_{5,24}$ = 38.36, P < 0.000 1 for *C. caput-medusae*; $F_{5,24}$ = 26.58, P < 0.000 1 for *C. arborescens*; $F_{5,24}$ = 10.69, P < 0.000 1 for *C. alaschanicum*; $F_{5,24}$ = 12.68, P < 0.000 1 for *C. potaninii*). Seeds from all species showed an increase in relative germinability after sulphuric acid and boiling water treatments than the control and other treatments. The relative germinability was significantly decreased by exudate treatment for *C. leucocladum*, *C. densum*, *C. mongolicum*, *C. caput-medusae*, *C. arborescens*, *C. alaschanicum* (Fig. 2).

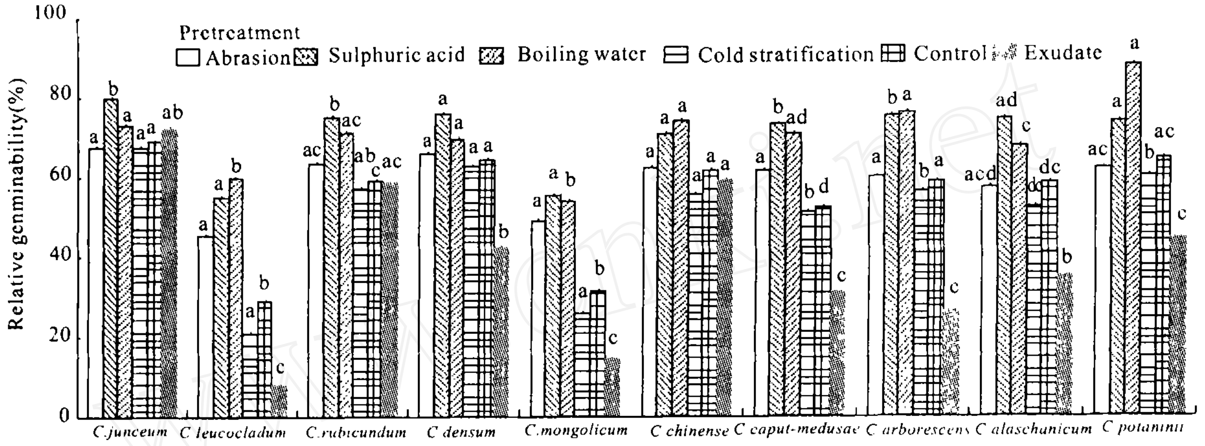

Fig. 2 Seed relative germination percent of 10 *Calligonum* species after five seed pretreatment and one control. Values with the same superscript letters are not significantly different at P < 0.05 according to Duncan's multiple comparison test

The index of germination rate of seeds (IGS) was significantly different between pretreatments in all ten species (one-way ANOVA: $F_{5,24}$ = 5.61, P = 0.002 2 for *C. junceum*; $F_{5,24}$ = 39.56, P < 0.000 1 for *C. leucocladum*; $F_{5,24}$ = 8.07, P = 0.000 3 for *C. rubicundum*; $F_{5,24}$ = 22.49, P < 0.000 1 for *C. densum*; $F_{5,24}$ = 59.6, P < 0.000 1 for *C. mongolicum*; $F_{5,24}$ = 8.8, P = 0.000 2 for *C. chinense*; $F_{5,24}$ = 44.4, P < 0.000 1 for *C. caput-medusae*; $F_{5,24}$ = 57.73, P < 0.000 1 for *C. arborescens*; $F_{5,24}$ = 14.88, P < 0.000 1 for *C. alaschanicum*; $F_{5,24}$ = 21.26, P < 0.000 1 for *C. potaninii*) (Fig. 3).

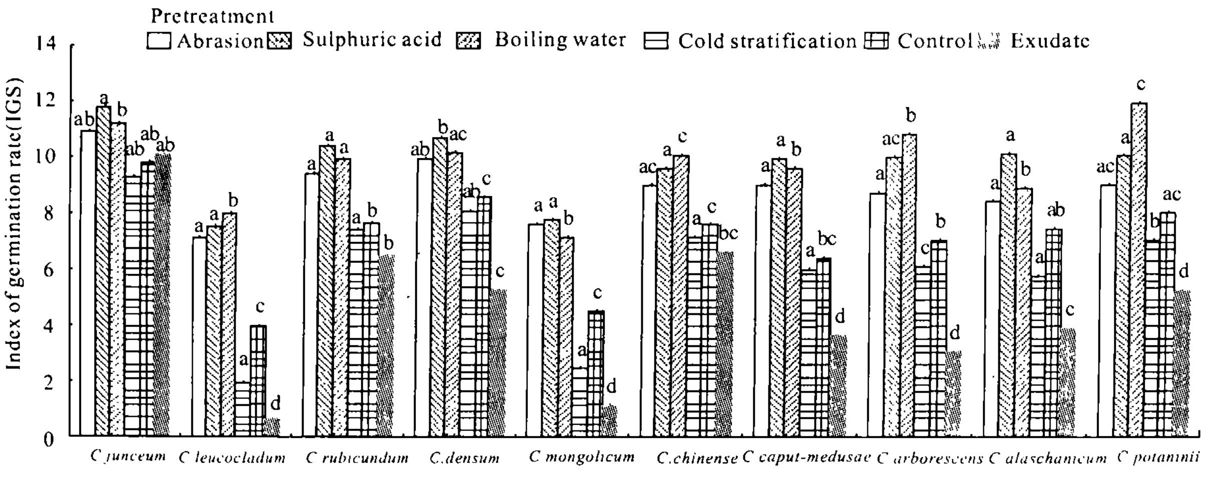

Fig. 3 Index of germination rate of seed (IGS) for 10 *Calligonum* species after five seed pretreatment and one control. Values with the same superscript letters are not significantly different at P < 0.05 according to Duncan's multiple comparison test

For all species, the higher IGS occurred at abrasion, sulphuric acid and boiling treatments. Compared with the control, the cold stratification and exudate treatments significantly decreased the IGS in *C. leucocladum*, *C. mongolicum*, *C. arborescens* and *C. potaninii*, the abrasion, sulphuric acid and boiling water treatments significantly increased the IGS in *C. leucocladum* and *C. mongolicum*. At the control and all pretreatments, *C. junceum* performed highest level of IGS, and *C. leucocladum* performed lowest IGS (Fig. 3).

3 Discussion

The hard seed coat of many desert plant species has evolved to withstand successfully unfavourable conditions as heat caused by sunlight, strong teeth of dispersing animals, severe drought and mechanical damage[16~22]. Therefore, severe treatments are required to make the seed coat permeable to water. Several pre-sowing treatments have been used to overcome hard seed coat imposed dormancy[23~28]. The objective of all these treatments is to make the seed coat permeable to water by acting on specific weak spots of the hard seed coat. Of the various pre-sowing treatments, cold stratification, mechanical, acid and hot water scarification treatments are widely used since they provide good performance on germination within a relatively short period of time.

In the present study, the germination response of seeds to the different pretreatments was more or less similar for all ten *Calligonum* species. Seeds from all species showed an increase in relative germinability after sulphuric acid and boiling water treatments than the control and other treatments. The exudate treatment had different effects on dormancy from different species. Compared with the control, the exudate treatment significantly hampered the percent germination, speed of germination, overall rate of germination and bolstered dormancy for almost all species (Fig. 1, 2, 3). It can be defined that there was the inhibiting material in the yellow water-soluble exudate of *Calligonum* seeds[6,27,29]. The abrasion treatment promoted overall germination of all species. The cold stratification treatment can break the dormancy of viable *Calligonum* seeds and increased the germination, but it has a little lethal effect on viable seeds probably. According to our observation during germination experiments, many seeds treated with boiling water rotted and succumbed to mould attacked, indicating that they are very sensitive and lethal to the seeds.

The positive responses of seeds to the pre-sowing scarification treatments (abrasion and sulphuric acid) indicate that the hard seed coat is responsible for the low per cent germination of untreated seeds used in the control (Fig. 1, 2, 3) by preventing imbibition of water. Prevention of germination by hard seed coat of *Calligonum* species has different ecological advantages[2]. This feature favours the accumulation of persistent seed banks in the soil, spreads germination over time, sustains the extremely environmental condition and increases the chance that some seeds will finish germination, survival and establishment[16,18,20]. Overcoming dormancy, softening of seed coat and water uptake are, therefore, crucial points in the life cycle of hard-seeded species[13,30~32], especially including desert plants adapted to arid regions.

The results from the present study provided evidence that the germination patterns performed significant difference between the pre-sowing treatments for all ten *Calligonum* species (Fig. 1, 2, 3), and the speed and per cent germination of the *Calligonum* species investigated can be greatly increased by subjecting the seeds to either mechanical scarification or acid treatments. The results showed that seeds of *C. junceum* have strong ability of germinating (Fig. 2, 3).

Improper seed pre-sowing treatment can lead to reduced seed germination even viability. The ramifications of a poor seed supply for restoration efforts are substantial. As viability rates are reduced, recommended seeding rates must be increased. Because the availability of *Calligonum* seeds is often limited by climate (rainfall), seasonal and site variability[2] obtaining sufficient seed supply may be difficult and the associated costs prohibitive. The formal standard recommended seed pre-sowing treatments for *Calligonum* species are sulpuric acid and cold stratification[5]. The results of the present study indicate that the abrasion, cold stratification and sulphuric acid all are appropriate to ten *Calligonum* species. These conclusions are very meaningful for propagating *Calligonum* species by seed in the arid desert regions. Further work is required to develop seeding criteria for properly seed pre-sowing treatments to ensure germination in the field.

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