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Research Article
Determination of Optimum Germination Temperature and Dormancy Breaking Method for Seeds of Wild Herb

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Abstract

Background and Objective: Low germination and high dormancy are common phenomena in many wild herbs of great value, however, the optimum germination temperatures and dormancy breaking methods have not yet been studied. *Suaeda salsa*, *Suaeda crassifolia*, *Chenopodium glaucum*, *Descurainia sophia*, *Amaranthus retroflexus* and *Alopecurus myosuroides* are common wild herbs in China, which are traditionally used in edible, forage, herbal medicine and chemical industry. Therefore, this study was carried out to determine the optimum germination conditions of the above six wild herb seeds. **Materials and Methods:** Two tests were conducted in this study, the first determining the optimum germination temperature for seeds and the second exploring the dormancy breaking method for dormant species. The effects of four constant temperatures (20, 25, 30 and 35°C), five alternating temperatures (5<=>15, 10<=>20, 15<=>25, 20<=>30 and 25<=>35°C) and four dormancy breaking methods of 0.2% potassium nitrate (KNO_3), 7-days pre-chilling (PC7), 0.2% KNO_3+PC7 and 0.8% sodium hydroxide (NaOH) were studied. **Results:** The results showed that the seeds of *S. salsa*, *S. crassifolia* and *A. retroflexus* had no dormancy and the optimum germination temperatures were 20, 35 and 35°C, respectively. However, the seeds of *C. glaucum*, *D. sophia* and *A. myosuroides* had different levels of dormancy and the optimum germination conditions were all 0.2% KNO_3+PC7 and alternating temperature of 15<=>25°C. In addition, the first and final count time of germination of these six wild seeds was also determined. **Conclusion:** For non-dormant seeds of *S. salsa*, *S. crassifolia* and *A. retroflexus*, the optimum germination temperatures were 20, 35 and 35°C but for dormant seeds of *C. glaucum*, *D. sophia* and *A. myosuroides*, the combination of 0.2% KNO_3+PC7 and 15<=>25°C was the optimum germination condition.

Key words: Temperature, seed germination, dormancy breaking, wild herbs, phenolic acids, Cruciferae, flavonoid glycosides

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wild herbs, the very rich germplasm resources in China, play a vital role in maintaining the biodiversity and ecological balance of grassland. It is a crucial kind of plant germplasm resource and many of them have high value as medicinal materials, vegetable crops, oilseed crops and forage plants. *Suaeda salsa* (L.) Pall., an euhalophyte with high salt tolerance, can be used in the improvement of saline land and restoration of ecological environment[1]; meanwhile, it also can be used as forage due to richening in protein, crude fibre and amino acids[2]. *Suaeda crassifolia* Pall. Illustrator. is an important winter forage in Xinjiang, China, whose seeds also can be extracted oil, edible, or used in the chemical industry and have high medical and health value. *Chenopodium glaucum* L., another annual halophytic herb distributed worldwide, helps to improve the soil texture and its leaf also has feeding value[3]. *Descurainia sophia* (L.) Webb. ex Prantl., an original species of the Cruciferae family, contains multiple bioactive substances such as phenolic acids, flavonoids and flavonoid glycosides and its seeds have been used traditionally in herbal medicine to treat diseases in China[4,5]. *Amaranthus retroflexus* L., an annual C_4 herb that can produce up to 100,000 seeds each plant and provides important resources for the cultivation of grain crops, is sometimes used as a potential nutritious forage[6]. *Alopecurus myosuroides* Huds. is also a common wild grass in farmlands and grasslands in China and its seeds have a lifespan of 6-10 years in the soil seed bank[7]. With the rapid development of grassland livestock husbandry and human health care in China, the demand for suitable cultivated herb species is increasingly prominent but it is difficult to meet the production needs only by introduction and breeding. Thus, vigorously develop and domesticate wild herbs, making them become cultivated species, will be of great significance for the development of grassland agriculture and the research of Traditional Chinese Medicine.

Germination and dormancy, the most important biological characteristics of seeds, determine plant reproductive success. High dormancy and low germination are common phenomena in many wild herbs with important application value, which have become the key factors limiting their development, promotion and utilization[8]. Therefore, in countries with developed animal husbandry, seed testing of herbs used for forage has been paid more attention to and the standardization of quality testing has been realized. In the "International Rules for Seed Testing" of ISTA, the standard testing methods of some kinds of seeds are constantly revised and improved every year, meanwhile, the standard methods of some new types of seeds will be added. However, there are still quite a several seeds that have not been studied and their standard testing methods are not included in the ISTA. With the rapid development of environmental protection and animal husbandry, the herb species involved in the ISTA have been unable to meet the needs of production and scientific research, especially for China that is rich in wild herb resources. Thus, to study seed germination and dormancy characteristics and overcome the limitations caused by the lack of seed testing standards, will be conducive to strengthen the development and utilization of wild herbs.

Seed dormancy, under natural conditions, leads to seed inactivation and germination failure, which may be a factor in the survival of the offspring[9]. Seed dormancy is affected by many factors, including adverse environmental conditions, hard seed coat and immature embryo[10]. However, it is usually undesirable when the production target is plant biomass. Therefore, seed dormancy breaking plays a vital role in the process of germination, growth and reproduction. Various methods have been suggested to break seed dormancy and stimulate germination, such as pre-chilling, chemical reagents and hormones[11]. Especially for seeds of some species with both physical and physiological dormancy, physical treatments [e.g., pre-chilling (PC) and removing coat] along with chemical treatments [e.g., potassium nitrate (KNO_3) and gibberellic acid (GA_3)] were needed[12]. As indicated in the germination of *Bromus auleticus* Trin. ex Nees. seeds that were treated by different dormancy breaking methods, KNO_3+PC and PC were the best treatments to release dormancy, with mean germination time being reduced to half of the control[13]. But for *Leymus chinensis* (Trin.) Tzvel., sodium hydroxide (NaOH) soaking significantly decreased the proportion of dormant seeds and increased germination percentage[8]. In addition, similar studies, in *Brassica tournefortii* Gouan.[14], *Oryza sativa* L.[15] and *Carthamus tinctorius* L.[16] seeds, also demonstrated that KNO_3, pre-chilling or hormone treatment could break dormancy and stimulate germination. However, the dormancy breaking and standard testing methods for seeds of some wild herb species have not yet been studied.

There are many kinds of wild herb resources in China, which is an important basis for forage breeding and utilization. However, studies on seed germination characteristics of the wild herbs, such as *S. salsa*, *S. crassifolia*, *C. glaucum*, *D. sophia*, *A. retroflexus* and *A. myosuroides*, are little. Therefore, to determine the optimum germination temperatures and standard techniques of seed testing, the above six wild herbs were used to study the effects of different constant and alternating temperatures on germination and compare the difference of various dormancy breaking methods, which

would provide a reference for seed quality evaluation and germination standardization.

MATERIALS AND METHODS

Study area: The study was carried out at Grassland Agri-Husbandry Research Center, College of Grassland Science, Qingdao Agricultural University, China, from June, 2020-January, 2021.

Seed material: The seeds of *Suaeda salsa* (L.) Pall. (brown seeds) and *Suaeda crassifolia* Pall. Illustrator were collected at Hongdao Oasis Wetland Demonstration Park, Qingdao, Shandong province (36°13'07"N and 120°08'09"E) in 2019, the seeds of *Chenopodium glaucum* L. were collected at Modern Agricultural Science and Technology Demonstration Park, Jiaozhou, Shandong province (36°26'21"N and 120°04'43"E) in 2019, the seeds of *Descurainia sophia* (L.) Webb. ex Prantl. were collected at Langya Town, Qingdao, Shandong province (35°39'36"N and 119°55'12"E) in 2020, the seeds of *Amaranthus retroflexus* L. were collected at Jimo District, Qingdao, Shandong province (36°34'48"N and 120°27'36"E) in 2018 and the seeds of *Alopecurus myosuroides* Huds. were collected at Pingyu County, Zhumadian, Henan province (32°35'24"N and 114°16'48"E) in 2017. All seeds were collected at the physiological maturity stage, cleaned, dried and stored immediately in hermetic bags at -20°C before the experiments.

Standard germination test: To determine the optimum temperatures for seed germination of these above six wild herbs, standard germination tests were carried out. Nine temperatures were set in this study, including four constant temperatures (20, 25, 30 and 35°C) and five alternating temperatures (5<=>15, 10<=>20, 15<=>25, 20<=>30 and 25<=>35°C) and germination method of "Top of paper (TP)" was adopted. For each wild herb, ripe and plump seeds with uniform sizes were selected and four replicates of 50 seeds each was placed into a petri dish (110×110 mm), in which three layers of filter paper moistened with 10 mL of distilled water were laid. Then, seeds were incubated and germinated in a germination incubator (GXZ-380A, Jiangnan Instrument Company, Ningbo, China) at the preset constant or alternating temperatures, with a photoperiod of eight hrs of light and 16 hrs of darkness. For the germination test under the alternating temperature conditions, the higher temperature should be maintained for eight hrs (with light) and the lower for 16 hrs (with dark). Normal seedlings without defects in morphology were used as a standard to evaluate seed germination. During germination, the germinated seeds were observed and the number of normal seedlings was recorded every day for 21 days. At the end of the 21st day of germination, the number of normal seedlings, abnormal seedlings, fresh seeds and dead seeds were recorded.

Seed dormancy breaking treatment: As for the wild herbs (*C. glaucum*, *D. sophia* and *A. myosuroides*), according to the results of the standard germination test, that did not germinate or whose germination percentage was less than 80% under all the given constant and alternating temperatures, treatments of breaking dormancy were used. In this experiment, Pre-Chilling (PC) treatment was firstly used to study dormancy breaking and the effect of a few days of PC was compared. These three wild herb seeds were pre-chilled for one, two and three days (respectively marked as PC1, PC2 and PC3) at 5°C in the dark, before they were transferred to an incubator for germination at 15<=>25°C, with a photoperiod of eight hrs of light and 16 hrs of darkness. Based on the standard germination test, the germination results under the alternating temperature of 15<=>25°C were used as control, in which seeds were without PC treatment (PC0).

To further explore the ways to break seed dormancy, several other methods were adopted, including 0.2% potassium nitrate (0.2% KNO_3), 7-days pre-chilling (PC7), 0.2% KNO_3+PC7 and 0.8% sodium hydroxide (0.8% NaOH). After breaking dormancy treatments, wild herb seeds were incubated at the temperature of 15<=>25°C, with a photoperiod of eight hrs of light and 16 hrs of darkness. But for *D. sophia*, it was treated and incubated in the darkness:

- 0.2% KNO_3 solution treatment: In the germination test, 0.2% KNO_3 solution was used, instead of distilled water, to saturate the germinating filter papers, until the end of the germination process
- PC7 treatment: The wild herb seeds were placed in contact with the filter papers moistened with distilled water and kept at a low temperature of 5°C (in the darkness) for an initial period of up to seven days before they were moved to the incubator for germination. The number of germination days was calculated when they were transferred into the incubator
- 0.2% KNO_3+PC7 treatment: Similar to PC treatment, the wild herb seeds were placed on filter papers wetted with 0.2% KNO_3 solution, instead of distilled water, kept at 5°C (in the darkness) for an initial period of up to seven days and then transferred into the incubator for germination

- 0.8% NaOH treatment: The wild herb seeds were soaked in 0.8% NaOH solution for 24 hrs, at 20°C in the darkness, thereafter, they were placed on the filter papers moistened with distilled water and germinated in an incubator. The number of germination days was calculated from the day when they were transferred into the incubator

The criterion for evaluating germination was the normal seedlings without defects in morphology and the germinated seeds were observed and recorded every day for 21 days. Germination percentage was calculated, based on which the accumulative germination curve was made. Count time was determined according to the accumulative germination curve under the optimum germination conditions. The first count time was the day when the germination percentage exceeded 50% and the final count time was the day when the germination percentage reached the maximum value.

$$\text{Germination (\%)} = \frac{\text{Number of all normal seedlings at the end of germination}}{\text{Number of tested seeds}} \times 100$$

Statistical analysis: All statistical analyses were performed using one-way analysis of variance (ANOVA), according to Duncan's test by SPSS Statistics software (version 17.0). Differences at the 5% level were considered statistically significant ($p<0.05$). Figures were drawn using Sigma Plot 10.0.

RESULTS

Standard germination test: To investigate the effect of temperature on seed germination of six wild herbs, nine temperature conditions, including four constant temperatures (20, 25, 30 and 35°C) and five alternating temperatures (5<=>15, 10<=>20, 15<=>25, 20<=>30 and 25<=>35°C), were set in this study. The germination percentage of *Suaeda salsa* seeds, under all given constant and alternating temperatures, was over 90% except for 15<=>25°C (Table 1). Although there was no significant difference among the constant temperatures or the alternating temperatures (also except 15<=>25°C), its germination percentage reached the maximum value of 99% at 20°C, without abnormal seedlings and fresh seeds. Therefore, the optimum germination temperature of *S. salsa* seeds was 20°C and there was no need to break dormancy.

Under both constant and alternating temperatures, the germination percentage of *Suaeda crassifolia* seeds increased with temperature increasing, while the proportion of fresh seeds decreased (Table 1). At 30 and 35°C, its germination percentage was 97 and 98%, respectively. Although there was no significant difference between them, they were was no significant difference between them, they were significantly higher than those at other temperatures. Especially at 35°C, the germination percentage reached the maximum, with the least proportion of abnormal seedlings and no fresh seeds. Hence, 35°C was the optimum temperature for germination of *S. crassifolia* seeds.

As for *Chenopodium glaucum* seeds, under constant temperatures, their germination percentage increased with a temperature rising, while the proportion of fresh seeds decreased (Table 1). However, under alternating temperatures, as it rose, the germination percentage first increased and then decreased and the changing trend of fresh seeds was just the opposite. At 15<=>25°C, the germination percentage of 67% was the highest value, which was significantly higher than those at other temperatures, except 20<=>30°C. Although the germination percentage was the highest at 15<=>25°C, there were still 32% of fresh seeds that did not germinate. Therefore, it was necessary to break the dormancy of *C. glaucum* seeds, before they germinated at this optimum temperature.

The seeds of *Descurainia sophia* did not germinate under all given temperatures and 100% of them were fresh and dormant at 20, 25, 35, 15<=>25, 20<=>30 and 25<=>35°C (Table 1). Thus, dormancy breaking treatments needed to be taken for germination of *D. sophia* seeds.

The germination percentage of *Amaranthus retroflexus* seeds increased with temperature increasing, while the proportion of fresh seeds decreased, under both constant and alternating temperatures (Table 1). At 35°C, its germination percentage was 94%, which was the highest value and significantly higher than that at other temperatures. Meanwhile, its proportion of fresh seeds was the least at this temperature, with only 4%. Therefore, for germination of *A. retroflexus* seeds, the temperature of 35°C was the best and there was no need to break dormancy.

Under constant temperatures, the germination percentage of *Alopecurus myosuroides* seeds decreased with temperature increasing, while the proportion of fresh seeds increased (Table 1). However, under alternating temperatures, its germination percentage first increased and then decreased and the changing trend of fresh seeds was just the opposite. At 15<=>25°C, the germination percentage was 73%, which was the highest value, although it was not significantly higher than that at other temperatures, except 30, 35°C and 25<=>35°C. Even

Table 1: Effects of different temperature treatments on seed germination of six wild herbs

Species	Temperature (°C)	Germination (%)	Abnormal seedling (%)	Fresh seed (%)	Dead seed (%)
Suaeda salsa	20	99±1^{a}	0±0^{b}	0±0^{c}	1±1^{a}
	25	97±2^{ab}	0±0^{b}	0±0^{c}	3±2^{a}
	30	95±2^{ab}	0±0^{b}	0±0^{c}	5±2^{a}
	35	98±1^{ab}	1±1^{ab}	0±0^{c}	1±1^{a}
	5<=>15	97±2^{ab}	1±1^{ab}	0±0^{c}	2±2^{a}
	10<=>20	98±2^{ab}	0±0^{ab}	0±0^{c}	2±2^{a}
	15<=>25	89±3^{c}	1±1^{a}	7±2^{a}	3±1^{a}
	20<=>30	98±1^{ab}	0±0^{b}	1±1^{bc}	1±1^{a}
	25<=>35	92±2^{bc}	1±1^{ab}	4±2^{ab}	3±2^{a}
Suaeda crassifolia	20	43±3^{d}	3±1^{bc}	52±3^{b}	2±1^{a}
	25	76±3^{b}	9±1^{ab}	15±3^{d}	0±0^{a}
	30	97±1^{a}	3±1^{bc}	0±0^{e}	0±0^{a}
	35	98±1^{a}	2±1^{c}	0±0^{e}	0±0^{a}
	5<=>15	0±0^{e}	11±3^{a}	88±4^{a}	1±1^{a}
	10<=>20	9±3^{e}	7±3^{abc}	79±2^{a}	5±2^{a}
	15<=>25	55±4^{c}	6±2^{abc}	32±5^{c}	7±6^{a}
	20<=>30	83±4^{b}	2±1^{c}	12±4^{d}	3±1^{a}
	25<=>35	83±4^{b}	3±1^{bc}	7±4de	7±4^{a}
Chenopodium glaucum	20	4±3^{e}	1±1^{a}	95±3^{a}	0±0^{b}
	25	28±4^{d}	1±1^{a}	71±4^{b}	0±0^{b}
	30	40±3^{c}	1±1^{a}	59±3^{c}	0±0^{b}
	35	56±2^{b}	3±2^{a}	38±3^{d}	3±2^{b}
	5<=>15	0±0^{e}	0±0^{a}	100±1^{a}	0±0^{b}
	10<=>20	0±0^{e}	0±0^{a}	100±0^{a}	0±0^{b}
	15<=>25	67±4^{a}	1±1^{a}	32±5de	0±0^{b}
	20<=>30	60±5^{ab}	1±1^{a}	27±5^{e}	12±3^{a}
	25<=>35	55±4^{b}	3±3^{a}	42±4^{d}	0±0^{b}
Descurainia sophia	20	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
	25	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
	30	0±0^{a}	0±0^{b}	97±1^{b}	3±1^{a}
	35	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
	5<=>15	0±0^{a}	4±1^{a}	96±1^{b}	0±0^{b}
	10<=>20	0±0^{a}	5±2^{a}	94±2^{c}	1±1^{b}
	15<=>25	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
	20<=>30	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
	25<=>35	0±0^{a}	0±0^{b}	100±0^{a}	0±0^{b}
Amaranthus retroflexus	20	46±3de	1±1^{bc}	52±3^{b}	1±1^{a}
	25	45±2^{e}	1±1^{abc}	51±3^{b}	3±1^{a}
	30	80±5^{b}	3±1^{abc}	14±3^{e}	3±2^{a}
	35	94±3^{a}	1±1^{abc}	4±2^{f}	1±1^{a}
	5<=>15	0±0^{f}	0±0^{c}	100±1^{a}	0±0^{a}
	10<=>20	6±2^{f}	0±0^{c}	92±3^{a}	2±2^{a}
	15<=>25	54±1^{d}	4±2^{a}	39±3^{c}	3±1^{a}
	20<=>30	71±3^{c}	2±1^{abc}	26±3^{d}	1±1^{a}
	25<=>35	78±3^{bc}	4±2^{ab}	18±4de	0±0^{a}
Alopecurus myosuroides	20	72±3^{a}	1±1^{a}	27±3^{b}	0±0^{b}
	25	71±2^{ab}	1±1^{a}	27±1^{b}	1±1^{b}
	30	63±3^{bc}	1±1^{a}	32±2^{b}	4±1^{a}
	35	50±2^{d}	0±0^{a}	49±2^{a}	1±1^{b}
	5<=>15	67±5^{ab}	1±1^{a}	32±5^{b}	0±0^{b}
	10<=>20	72±2^{a}	0±0^{a}	28±2^{b}	0±0^{b}
	15<=>25	73±2^{a}	0±0^{a}	27±2^{b}	0±0^{b}
	20<=>30	68±4^{ab}	0±0^{a}	31±4^{b}	1±1^{b}
	25<=>35	55±2^{cd}	0±0^{a}	45±2^{a}	0±0^{b}

Values represent Mean±SE (n = 4), One-way ANOVA is adopted to perform the statistical analysis, The values of each parameter for each species followed by different lowercase letters indicate significant differences among different temperature treatments at $p<0.05$

so, there were still 27% of fresh seeds did not germinate at this temperature. Hence, it was necessary to break the dormancy of *A. myosuroides* seeds, before they germinated at 15<=>25°C.

Seed dormancy breaking treatment: For the above three dormant wild herb seeds, *C. glaucum*, *D. sophia* and *A. myosuroides*, several different dormancy breaking methods were used. Firstly, to explore whether a short duration of

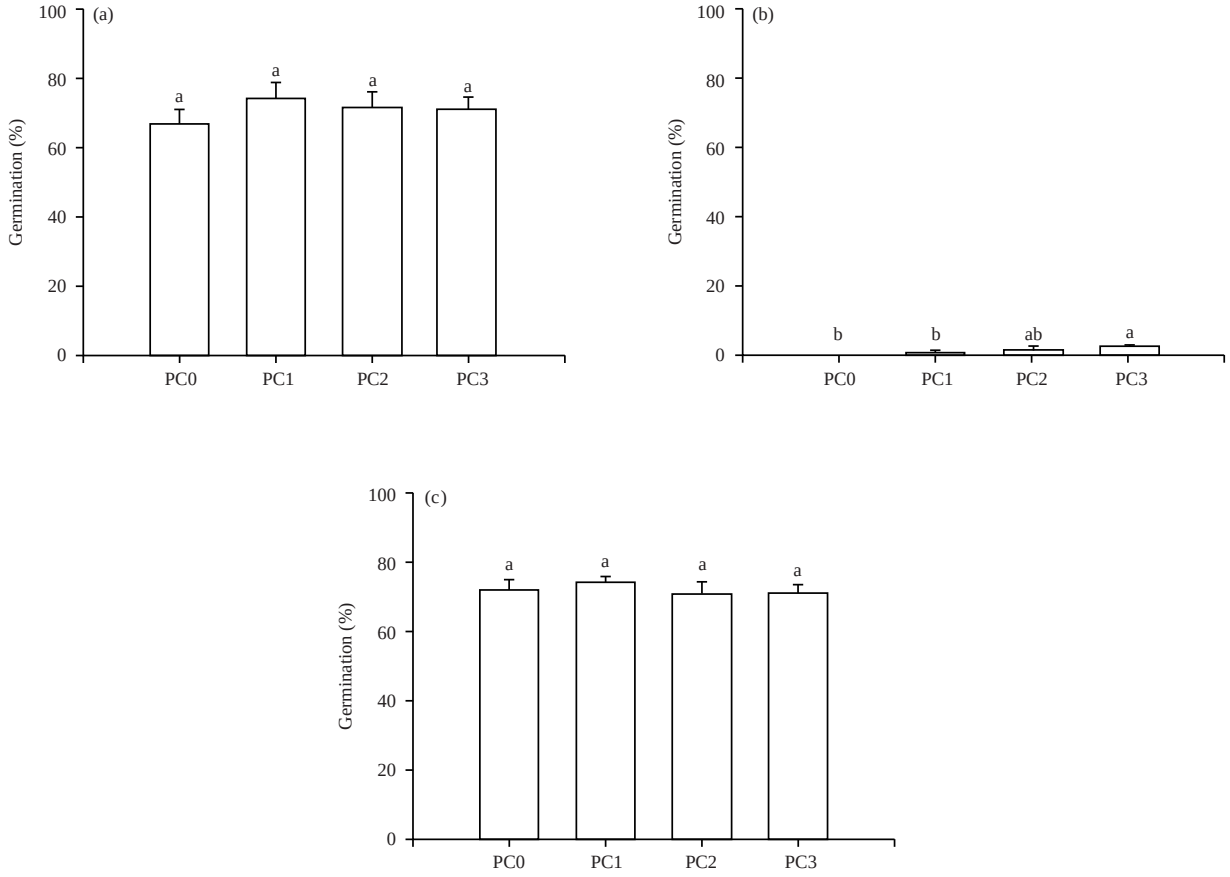

Fig. 1(a-c): Effects of a short-duration pre-chilling on seed germination in three wild herbs of (a) *C. glaucum*, (b) *D. sophia* and (c) *A. myosuroides*

These three wild seeds were without pre-chilling (PC0), pre-chilled for 1 day (PC1), pre-chilled for 2 days (PC2) and pre-chilled for 3 days (PC3), respectively, at 5°C in the dark Data presented are Mean±SE (n = 4), different lowercase letters indicated significant differences among different pre-chilling days at $p<0.05$

pre-chilling treatment could promote germination, the seeds were pre-chilled at 5°C for one, two and three days, respectively, before being transferred to the germination incubator. The results showed that all PC1, PC2 and PC3 could not significantly improve the germination percentage of *C. glaucum* seeds (Fig. 1a). Although pre-chilling for three days significantly promoted germination of *D. sophia* seeds, its germination percentage was only 3%, which was still very low (Fig. 1b). Similar to *C. glaucum*, the germination of *A. myosuroides* seeds was also not significantly improve by PC1, PC2 and PC3 (Fig. 1c). Therefore, a short duration of pre-chilling for three days could not effectively break the dormancy of these three wild herb seeds.

To further explore the methods of dormancy breaking for the above three wild herb seeds, treatments of 0.2% KNO_3, PC7, 0.2% KNO_3+PC7 and 0.8% NaOH were adopted, before seeds were incubated at 15<=>25°C. For *C. glaucum*, compared to control, these four dormancy breaking methods significantly improved germination percentage, which was all higher than 80% (Fig. 2a). Although the germination percentage was not significantly different among treatments of 0.2% KNO_3, PC7 and 0.2% KNO_3+PC7, it was significantly higher than that of 0.8% NaOH treatment. In particular, the germination percentage of seeds treated by 0.2% KNO_3+PC7 was the highest, up to 96%. Therefore, the optimal germination conditions for *C. glaucum* seeds were 0.2% KNO_3+PC7 treatment and alternating temperature of 15<=>25°C.

Compared with the control, except that 0.8% NaOH did not break the dormancy of *D. sophia* seeds, all treatments of 0.2% KNO_3, PC7 and 0.2% KNO_3+PC7 significantly increased the germination percentage. Especially, the effect of 0.2% KNO_3+PC7 treatment on breaking dormancy and promoting germination was the most significant, of which the

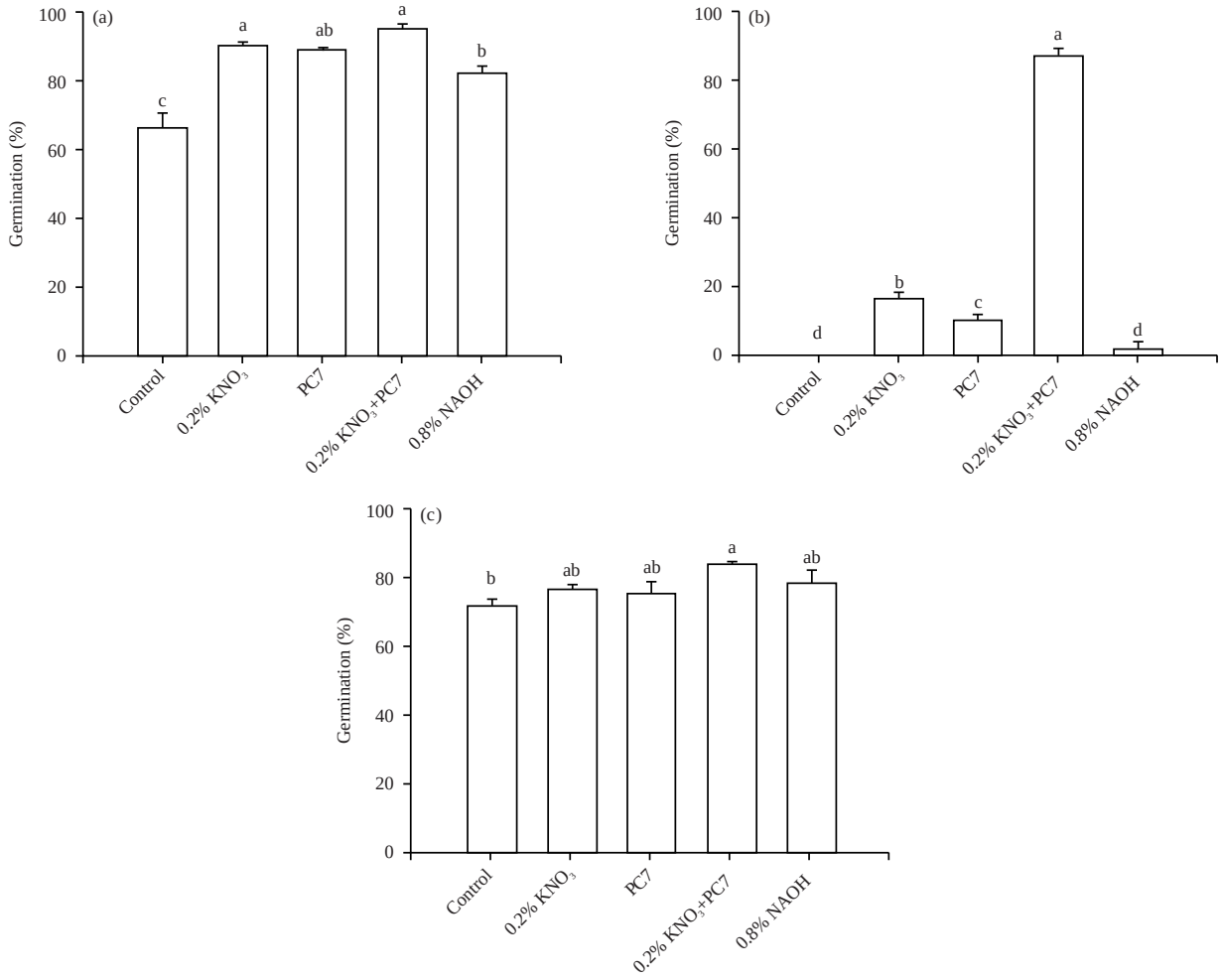

Fig. 2(a-c): Effects of different dormancy breaking methods on seed germination in three wild herbs of (a) *C. glaucum*, (b) *D. sophia* and (c) *A. myosuroides*

Dormancy breaking methods include control, 0.2% potassium nitrate (0.2% KNO_3), 7-days pre-chilling (PC7), 0.2% KNO_3+PC7 and 0.8% sodium hydroxide (0.8% NaOH), data presented are Mean±SE (n = 4), different lowercase letters indicate significant differences among different dormancy breaking methods at $p<0.05$

germination percentage was up to 87% and significantly higher than that of a single 0.2% KNO_3 or single PC7 treatment (Fig. 2b). Therefore, the optimum germination condition for *D. sophia* seeds was the same as *C. glaucum* seeds, that is, 0.2% KNO_3+PC7 treatment and alternating temperature of 15<=>25°C. For *A. myosuroides* seeds, all treatments of 0.2% KNO_3, PC7 and 0.8% NaOH did not break the dormancy significantly but 0.2% KNO_3+PC7 could significantly increase the germination percentage to 84% (Fig. 2c). Therefore, the optimal germination conditions of *A. myosuroides* seeds were also the treatment of 0.2% KNO_3+PC7 and alternating temperature of 15<=>25°C, the same as those of the above two wild herb seeds.

According to the above results, it was found that the germination percentage of the three non-dormant seeds, with no need for dormancy breaking treatment, was 94-99% under 20 or 35°C. However, the germination percentage of *C. glaucum*, *D. sophia* and *A. myosuroides* seeds were the highest after 0.2% KNO_3+PC7 treatment and at 15<=>25°C, which was 96%, 87% and 84%, respectively, increasing by 29, 87 and 11%. Except that there were still 10%-12% fresh seeds in *D. sophia* and *A. myosuroides* the proportion of fresh seeds in other species was less than 5%. Therefore, the dormancy breaking method determined in this study was effective, especially for *D. sophia* (Table 2).

Accumulative germination curve and germination count time: According to the change of accumulative germination percentage under the optimum germination conditions, the first count time and final count time of these six wild herb

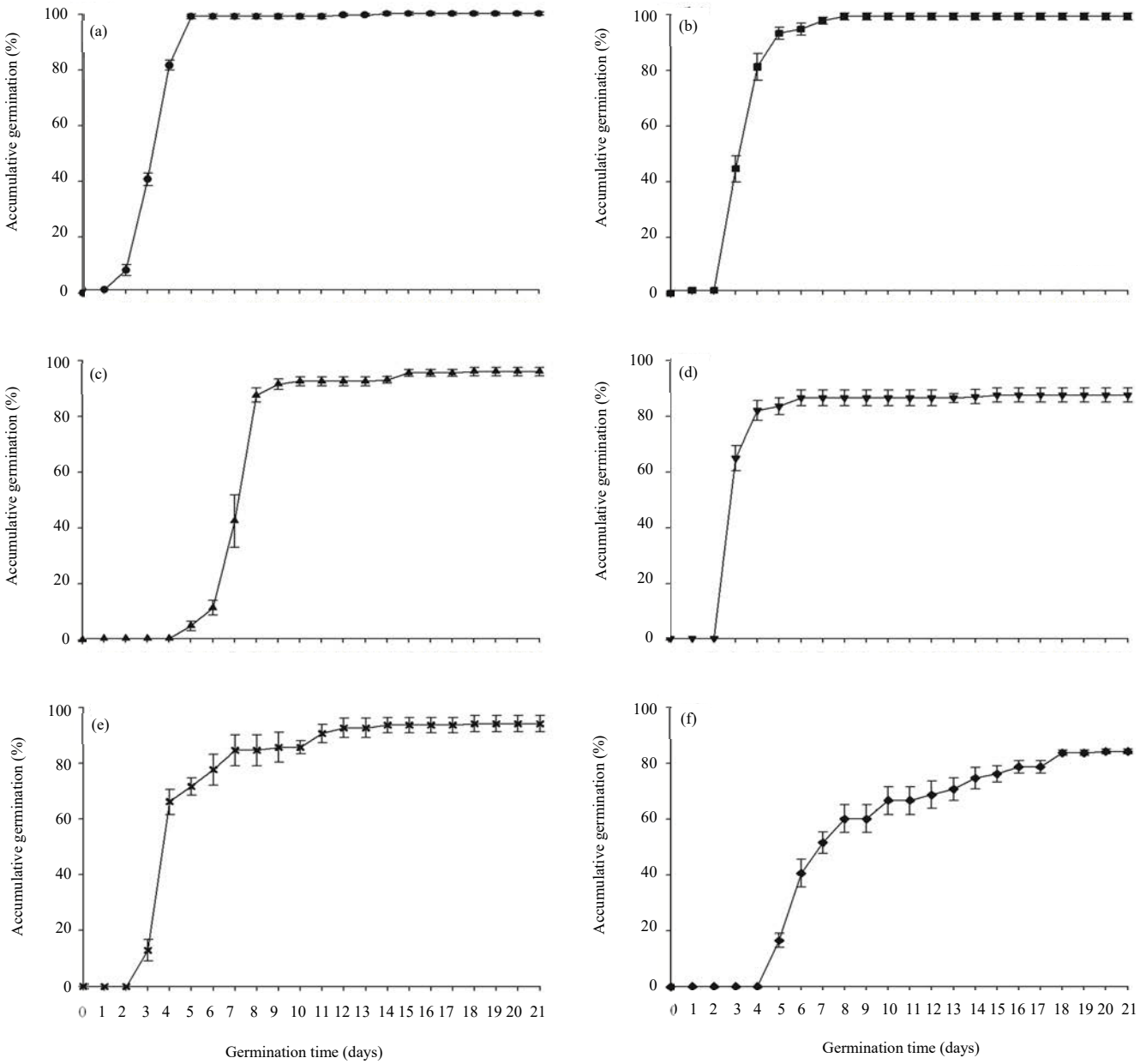

Fig. 3(a-f): Accumulative germination curve of six wild herbs under optimum germination conditions (a) *S. salsa*, (b) *S. crassifolia*, (c) *C. glaucum*, (d) *D. sophia,* (e) *A. retroflexus* seeds germinate, respectively, at 20, 35 and 35°C without dormancy breaking treatment and (f) *A. myosuroides* seeds all germinate at 15<=>25°C, after breaking dormancy with 0.2% potassium nitrate and 7-days pre-chilling (0.2% KNO_3+PC7)

Table 2: Germination information of six wild herb seeds under optimum germination conditions

Species	Optimal germination conditions	Germination (%)	Abnormal seedling (%)	Fresh seed (%)	Dead seed (%)
Suaeda salsa	20°C, /	99±1	0±0	0±0	1±1
Suaeda crassifolia	35°C, /	98±1	2±1	0±0	0±0
Chenopodium glaucum	15<=>25°C, 0.2% KNO_3+PC7	96±2	1±1	3±1	0±0
Descurainia sophia	15<=>25°C, 0.2% KNO_3+PC7	87±2	0±0	10±3	3±1
Amaranthus retroflexus	35°C, /	94±3	1±1	4±2	1±1
Alopecurus myosuroides	15<=>25°C, 0.2% KNO_3+PC7	84±1	0±0	12±1	4±1

"/" represents that seeds do not need to break dormancy

Table 3: Optimum conditions and count time for germination of six wild herb seeds

Species	Temperature (°C)	First count (d)	Final count (d)	Dormancy breaking method
Suaeda salsa	20	4	5	/
Suaeda crassifolia	35	4	8	/
Chenopodium glaucum	15<=>25	8	15	0.2% KNO_3+PC7
Descurainia sophia	15<=>25	3	6	0.2% KNO_3+PC7
Amaranthus retroflexus	35	4	14	/
Alopecurus myosuroides	15<=>25	7	18	0.2% KNO_3+PC7

"/" represents that seeds do not need to break dormancy

seeds were determined respectively. Namely, the first and the final count time were four and five days for *S. salsa* (Fig. 3a, Table 3), four and eight days for *S. crassifolia* (Fig. 3b, Table 3), eight and 15 days for *C. glaucum* (Fig. 3c, Table 3), three and six days for *D. sophia* (Fig. 3d, Table 3), four and 14 days for *A. retroflexus* (Fig. 3e, Table 3) and seven and 18 days for *A. myosuroides* (Fig. 3f, Table 3), respectively.

DISCUSSION

In this study, the germination responses of six wild herb seeds to four constant temperatures and five alternating temperatures were different (Table 1). Seed germination is the beginning of the plant life cycle, which is influenced by environmental factors and controlled by physiological process[17]. Seed germination is also considered the most important stage for the establishment of any species, therefore, it is necessary to determine the optimum range of seeds to various environmental factors during this phase[18]. Light, moisture and temperature are the three factors controlling seed germination, of which temperature is the most critical one varying with seed species and ecotypes[19,20]. There is usually an optimal temperature for each species at which seeds could obtain the best germination[21]. Zhao *et al.*[22] reported on germination behaviour of two types of *S. salsa* seeds under the constant temperatures of 10-5°C that the suitable temperature range for germination of brown seeds was 20-45°C. Moreover, the study of the effect of alternating temperatures on germination found that the highest germination percentage was obtained at 20<=>30°C for brown seeds of *S. salsa*[23]. However, in this study, the germination results of *S. salsa* seeds suggested that the optimum germination temperature was 20°C, with a maximal germination percentage of 99%, which was different from that of previous research of 20<=>30°C[23]. The possible reason was that the criteria used to evaluate seed germination were different and the standard of normal seedlings without defects in morphology based on the ISTA rule was used in this study. Hitherto, very few studies have been done to explore the effect of temperature on seed germination of *S. crassifolia*. In this experiment, its optimum germination temperature was determined to be 35°C, with a germination percentage of 98% and no dormant seeds, which would lay a foundation for the development and utilization of *S. crassifolia*. Previous studies reported in the germination of *A. retroflexus* seeds that the maximum germination occurred between 35 and 40°C and an additive effect on germination speed was observed when temperature increased between 17 and 32°C[24]. The results of *A. retroflexus* in this study showed that the optimum temperature for germination was 35°C, with the highest germination percentage of 94% and a few fresh seeds (< 5%), which was exactly consistent with the results reported above. To sum up, the germination percentage of these three wild herb seeds all exceeded 90% at their optimum temperature, with no or less dormancy, which indicated that these above three species did not need to take dormancy breaking treatment.

The dormancy behaviour of wild herb seeds is a vital factor for their survival under unpredictable climatic and adverse ecological conditions[25]. Seeds are considered dormant when they do not germinate within a certain amount of duration under normal environmental conditions[26]. Seed dormancy also determines the temperature scope of germination[27]. Under the given germination temperatures in this study, the maximum germination percentage of *C. glaucum* and *A. myosuroides* both appeared at 15<=>25°C, with 67 and 73%, respectively, meanwhile, there were still 32 and 27% of fresh non-germinated dormant seeds (Table 1). Henson[28] discovered in *C. glaucum* seeds that, the alternating temperature with a 20°C difference increased germination to more than 40% whereas a variation of 10°C led to only about 5%. Moreover, *A. myosuroides* was reported to generally show a very short primary dormancy[29]. However, *D. sophia* seeds did not germinate under all given temperatures, manifesting as 100% of dormancy. The differences in dormancy levels among species demonstrated that the preset temperatures were not optimal for germination of these three wild herb seeds and the dormancy should be further broken to promote germination.

Normally, seed dormancy, an undesirable characteristic in agricultural production, is needed to be broken by some methods[30]. The most commonly used and effective ones

include treatments of plant hormones[31], pre-chilling, KNO_3 and NaOH[32]. However, different seed species have various responses to dormancy breaking methods and limited studies have focused on wild herbs, such as *C. glaucum*, *D. sophia* and *A. myosuroides*. Previous studies have proven that pre-chilling had a positive effect on germination of *Tridens flavus*[33], *Miscanthus sinensis*[34], *Bromus auleticus*[13], as well as other grasses. Although 7-days pre-chilling treatment significantly increased the germination percentage of both *C. glaucum* and *D. sophia*, they did not reach the maximal value under this condition (Fig. 2a-b). Dormancy of *A. myosuroides* seeds was not effectively broken (Fig. 2c), together with the results of 3-days pre-chilling treatment for these three wild seeds (Fig. 1), suggesting that a broad pre-chilling duration might be needed. KNO_3 treatment is one of the most widely used methods for breaking dormancy[13]. It effectively promoted seed germination of *C. glaucum* and *D. sophia*, however, the germination percentage of *D. sophia* was only increased to 17%, far lower than the expected value of 80%, which indicated that KNO_3 treatment was not the optimal way to break dormancy for *D. sophia*. In addition, NaOH treatment only significantly improved the germination of *C. glaucum* but did not break the dormancy of *D. sophia* and *A. myosuroides*. He *et al*.[8] reported in *L. chinensis* seeds that a combined method of water pre-soaking+30% NaOH+300 μM GA_3 increased germination to 89%, the possible reason was that chemical scarification of seed coat by NaOH softened the pericarp/testa and enhanced the permeability to oxygen. The findings of this study demonstrated that 0.8% NaOH treatment could release dormancy of *C. glaucum* seeds but the detailed methods of NaOH for *D. sophia* and *A. myosuroides* were still needed to be further explored.

Based on single KNO_3 and single pre-chilling treatments, the method of KNO_3+pre-chilling was used to further stimulate germination of tested seeds and determine the optimal germination conditions. The results showed that the effect of 0.2% KNO_3+PC7 on breaking dormancy was better than that of single KNO_3, single pre-chilling and NaOH because the germination percentage of all these three wild seeds was the highest under this condition (Fig. 2). Pre-chilling is an effective method to break the dormancy of wild herb seeds, of which the reason is to change the balance between inhibitor and promoter in seeds[35]. Additionally, it was reported that pre-chilling increased the synthesis of GA_3 in the embryo, thus that it could promote germination[36]. However, KNO_3, one of the most widely used chemicals to accelerate germination, in which K^+ acts as an activator to improve the activity of various enzymes and also participates in the induction and regulation of plant hormone synthesis[37]. Therefore, the combination of KNO_3 and pre-chilling could effectively break dormancy and promote germination of these three wild seeds.

CONCLUSION

In summary, the results of the standard germination test of *S. salsa*, *S. crassifolia*, *C. glaucum*, *D. sophia*, *A. retroflexus* and *A. myosuroides* suggest that there are different levels of dormancy in wild herb seeds. For *S. salsa*, *S. crassifolia* and *A. retroflexus*, the seeds are not dormant, so it is not necessary to take measures to break dormancy and the optimal germination temperature is, respectively, 20, 35 and 35°C. However, for seeds of *C. glaucum*, *D. sophia* and *A. myosuroides*, there are different degrees of dormancy, particularly *D. sophia* seeds having deep dormancy (without germination in standard germination test), therefore, dormancy breaking treatment is needed. The method of 0.2% KNO_3+PC7 and alternating temperature of 15<=>25°C are optimal for these three dormant seeds. Meanwhile, the first and final count time of germination under optimum conditions is also explored. Overall, the optimum germination temperature and dormancy breaking method of the six wild seeds have been determined. Thus, this study would provide an important theoretical reference for seed quality evaluation and germination standardization of these six wild herb species.

SIGNIFICANCE STATEMENT

This study discovered that the optimum germination temperatures of non-dormant seeds of *S. salsa*, *S. crassifolia* and *A. retroflexus* were 20, 35 and 35°C and the combination of 0.2% KNO_3+PC7 and 15<=>25°C was the optimum germination condition of dormant seeds of *C. glaucum*, *D. sophia* and *A. myosuroides*. This study can be beneficial for providing a reference for seed quality evaluation and germination standardization.

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