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重要藏药川西獐牙菜种子萌发的研究*

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摘要：比较了温度、生长素 (赤霉素 GA_3) 和储藏条件对川西獐牙菜 (*Swertia mussotii* Franch) 高海拔野生自然种群和低海拔栽培后种子发芽率的影响。未经任何处理的高海拔野生自然种群种子的发芽率明显高于低海拔栽培种群。无论是赤霉素处理还是低温处理对种子的发芽率都有显著提高。经过处理后，两种来源的种子最终发芽率没有明显的变化。结果表明：(i) 野生的或栽培的川西獐牙菜种子都存在休眠现象；(ii) 通过引种栽培不能打破川西獐牙菜种子的休眠，该机制可能是受遗传因素的控制；(iii) 赤霉素处理和 4 ℃低温冷藏对打破种子的休眠具有重要作用。

关键词：川西獐牙菜；休眠机制；种子发芽率
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Seed Germination of *Swertia mussotii*, an Important Application in Tibetan Folk Medicine

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Abstract: In this study, the effects of temperature, the growth regulator GA_3 and storage conditions on seed germination of the biennial *Swertia mussotii* Franch were compared in seeds from a natural high altitude alpine site and after one-cycle of artificial cultivation at a low altitude. The untreated seeds from high altitude displayed higher germination than those from low altitude. Both GA_3 and storage conditions enhanced germination from all sources. After treatment, the final germination of seeds from different sources shows no distinct difference. These results suggest that: (i) all seeds of this species, irrespective of their sources, have similar kinds of dormancy; (ii) the dormancy of this species can not been broken through one-cycle adaptation in *ex situ* cultivation and thus might be genetically controlled; (iii) both GA_3 and 4 ℃ cold storage are effective for dormancy breaking in *ex situ* cultivation of this important medicinal species.

Key words: *Swertia mussotii*; Dormancy mechanism; Seed germination

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Swertia mussotii Franch, a biennial herb in the family Gentianaceae, has long been used as an extremely important and famous Tibetan folk medicine, "ZangYinChen", to cure gall and liver disorders as well as other diseases (Yang, 1991). Xue *et al* (2002 a, b) reported the embryology and morphology of some species in *Swertia*. Recent investigations on the active constituents of this drug revealed that it contains a large quantity of oleanolic acid, mangiferin, swertiamarin, swertisin, and other xanthones (Ding *et al*, 1980; Sun *et al*, 1991). *S. mussotii* is distributed in southern Qinghai, western Sichuan and eastern Tibet, rigidly restricted to the high altitude area of the Tibet Plateau ranging from 3 200 m to 4 000 m (Ho, 1988). Typical habitats of this species are south-facing and nutrient-poor grasslands. More than 10 types of complex Tibetan folk medicine include it as a necessary crude drug. A medicine to cure hepatitis, using its aqueous extracts, has been produced by a Qinghai local factory for about 10 years (Yang, 1991). Two other new medicines, based on the ethanolic extracts of this species, have been developed for curing gall and gastric disorders, but await production when enough resource is available (Liu *et al*, 1999). However, the natural resource of this species is less than 50 t every year while the crude drug market needs more than 500 t (Yang, 1991). The natural resource base is decreasing annually because of increased harvesting over its whole distribution range. Harvesting the flowering herbs leaves no seeds to provide offspring (Yang, 1991). The harvesting of this species has usually resulted in deteriorating alpine meadows or other local ecosystems. This species has been listed as an endangered species by some local governments and harvesting is now being prohibited in some areas of its natural distribution. Most plant species for Tibetan folk medicines grow in alpine mountains of the Tibet Plateau where agriculture is not practiced. However, *ex situ* cultivation of this species seems to be the only way for its effective conservation and application in folk medicine.

Most alpine species have similar dormancy responding to the low temperature (Thomson, 1970). For some such species, dormancy can be broken by GA_3 or cold storage (Sulaiman, 1993; Negi and Todaria, 1993; Airi *et al*, 1998; Pandey *et al*, 2000). Many studies have reported changes in seed germination of vegetable cultivars in response to changed habitats (e.g. Ramin, 1997), and furthermore, variation in the temperature requirement or pretreatment for germination in different populations of the same species (McWilliams *et al*, 1968; Manjkhola *et al*, 2003). Little is known on the seed germination flexibility of alpine annuals or biennials when transferred to different siter under the changed habitats. The present investigation was, therefore, designed to examine the seed dormancy variation of biennial *S. mussotii* between when grown *ex situ* and from natural distribution, in addition to a study of seed dormancy and methods to overcome it.

Materials and Methods

Seeds of *Swertia mussotii* were firstly collected from Yushu, Qinghai (altitude, 3 520 m) in October 2000. The seeds were stored at 4 ℃ for one month and then sown at two low altitude agriculture areas, Nanshan (altitude, 2 400 m) and Huangzhong (altitude, 2 620 m), Qinghai. In summer 2001, the seeds of *Swertia mussotii* planted at the low altitude germinated, formed rosettes and died back above ground in winter. In April of 2002, individuals re-emerged, formed buds in July, flowered in early August and began to set fruits in middle August. The final *ex situ* cultivated seeds for the present experiments from these two sites were collected in late August in 2002. Seeds of *S. mussotii* were also collected from nature population at Yushu, Qinghai (altitude, 3 520 m) and Jiangda, Tibet (altitude, 3 580 m), both in the natural distribution range of this species in late September in 2002. The seeds from cultivation and natural distribution in 2002 were used for the following experiments.

In the initial experiments, we compared the germination rate using 100 seeds and 20 seeds as replicates under the same treatment condition and found no difference between these two arrangements. Because we hoped to place all different treatments in the same germination box at the same time, we only used replicates of 20 seeds for all comparisons of two different origins. The mature seeds of each locality were divided into 7 replicates of 20 seeds for each experiment. The experimental seeds were placed on a layer of moist gauze, which was extended on the top of a petri dish (6 ×6 cm). This small dish was further placed in a larger petri dish (20 ×20 cm). The distilled water was regularly added to both dishes to keep the gauze moist. The germination experiments were conducted in an incubator (model: SPX-250-BG, YUEJIN INC, Shanghai, China) set to give 12 h-light and 12 h dark every day for all experiments. In the first experiment, seeds without any treatment, but stored at the room temperature (20 ℃) were set to germinate at 10 ℃, 15 ℃, 20 ℃, 25 ℃ or 30 ℃. This test was designed to investigate final germination from different sources without any pretreatment. In the second experiment, the seeds from different sources were pretreated by storage at 4 ℃ in a refrigerator for one month. In the third experiment, control treatment seeds were pretreated with 800 ppm GA_3 for 24 hours, which was demonstrated to be best in our previous treatments of seeds from the same origin when treated with 200 ppm to 1200 ppm.

Most of the experimental seeds started to germinate on the ninth day after being placed in the incubator, and continued to the sixteen day. The remaining not-germinated seeds were kept for another 10 days. The germination for each replicate during the total period was monitored and counted. The mean germination was calculated and compared by means of the SPSS 10.0 for Windows software package. The mean values obtained in the different groups were compared by one-way ANOVA, post hoc-LSD and *t*-test, assuming that there were significant differences between mean values when statistical comparison gave $p < 0.05$.

Results

The change of phenology in *ex situ* cultivation at the low altitude

The artificially cultivated individuals of *S. mussotii* from the low altitude flowered and set seeds almost one month earlier than plants those in the natural distribution in the high altitude. Bumblebees were observed to pollinate flowers in the natural distribution. However, in the two *ex situ* sites, bees were found to pollinate flowers instead of bumblebees.

The seed germination without pretreatment

No seed germinated at 10 ℃ or 30 ℃. Seeds from all four sites reached highest germination at 20 ℃ (Table 1) ($p < 0.05$ for all tests). But the final germination increases with altitude of site at all three temperatures (Table 1) ($p < 0.05$ for all tests). Seed germination started earlier at higher temperature and the duration of germination was greater: at 15 ℃, the seed germination began on the 10th day and ended on the 16th day; however, the germination began on the 7th day and ended on 12th day at 25 ℃.

The effects of storage at 4 ℃ on seed germination

No seed germinated at 10 ℃ or 30 ℃. Compared to seeds from other locations, the seeds from Nanshan had the lowest germination ($p < 0.05$ for all tests). The highest germination at all temperatures of seeds from Nanshan was at 20 ℃. In contrast, for seeds cultivated at Huangzhong, there was no significant difference between germination at 15 ℃, 20 ℃ or 25 ℃ ($p > 0.05$). There was no common germination pattern with altitude between the four sources at 15 ℃, 20 ℃ and 25 ℃ ($p > 0.05$) although seeds from Huangzhong always had the highest germination at all temperatures (Table 2) ($p < 0.05$ for all tests).

Table 1 Mean germination (mean ±SE) of seeds without being pretreated at constant temperatures of 15 ℃, 20 ℃ and 25 ℃ in the first experiment (n = 7)

Altitude (m)	Sources of seeds	15 ℃ Germination (%)	20 ℃ Germination (%)	25 ℃ Germination (%)
2620	Huangzhong (cultivated)	20.00 ±4.08	25.00 ±5.77	22.14 ±9.94
2400	Nanshan (cultivated)	22.86 ±4.88	37.86 ±8.59	30.71 ±10.97
3520	Yushu (naturally distributed)	33.57 ±8.99	51.43 ±11.44	40.00 ±7.07
3580	Jiangda (naturally distributed)	52.14 ±14.96	64.29 ±6.73	50.00 ±15.81

Table 2 Mean germination (mean ±SE) of seeds with 4 ℃ cold storage at constant temperatures of 15 ℃, 20 ℃ and 25 ℃ in the second experiment (n = 7)

Altitude (m)	Sources of seeds	15 ℃ Germination (%)	20 ℃ Germination (%)	25 ℃ Germination (%)
2620	Huangzhong (cultivated)	76.43 ±10.69	84.29 ±3.45	78.75 ±10.61
2400	Nanshan (cultivated)	30.00 ±7.07	50.71 ±11.34	37.14 ±12.86
3520	Yushu (naturally distributed)	43.57 ±11.44	72.14 ±11.85	72.86 ±13.18
3580	Jiangda (naturally distributed)	54.29 ±9.32	70.00 ±9.57	75.00 ±8.16

The effects of GA_3 regulator on the seed germination

Application of GA_3 had similar effects to 4 ℃ cold storage. No seed germinated at 10 ℃ or 30 ℃. At 15 ℃, the seeds from all sources had lower germination than at 20 ℃ and 25 ℃ ($p < 0.05$), but no distinct difference was found between seeds germinated at 20 ℃ or 25 ℃ for seeds from different sites ($p > 0.05$, Table 3). There was no obvious trend of germination with altitude at which the seed matured. ($p > 0.05$, Table 3).

Table 3 Mean germination (mean ±SE) of seeds with being pretreated by 800 ppm GA_3 at constant temperatures of 15 ℃, 20 ℃ and 25 ℃ in the third experiment (n = 7)

Altitude (m)	Sources of seeds	15 ℃ Germination (%)	20 ℃ Germination (%)	25 ℃ Germination (%)
2620	Huangzhong (cultivated)	70.71 ±5.35	90.71 ±7.87	87.14 ±3.93
2400	Nanshan (cultivated)	59.29 ±13.36	83.57 ±10.69	70.00 ±11.55
3520	Yushu (naturally distributed)	45.71 ±10.97	75.71 ±7.32	74.29 ±8.86
3580	Jiangda (naturally distributed)	60.00 ±15.55	85.71 ±7.32	78.57 ±9.45

Discussion

The changes in phenology of *Swertia mussotii* in *ex situ* cultivation are mainly due to flexible adaptation of this species to the high average temperature of the low altitude and changed ecological environments. In most cases, annuals or biennials show flexible adaptations to different habitats (Stebbins, 1950). Such phenotypic plasticity has been recognized as an ecological adaptation, or a result from a combination of physiological and genetic controls (Dobzhansky, 1970).

S. mussotii is threatened by increasing exploitation for medicine. *Ex situ* cultivation requires good seed germination. In most species within *Swertia* and Gentianaceae, the embryos are often immature (Akhalkats and Wagner, 1997; Liu and Ho, 1996; Liu *et al*, 1999), and incapable of germination without further development of the embryo, but this is often prevented by embryo dormancy. Such dormancy exists in other alpine perennial species, e.g., *Trollius* and *Aconitum* (Ranunculaceae; Hichmough *et al*, 2000; Pandey *et al*, 2000) and *Meconopsis* (Papaveraceae; Sulaiman, 1993). These species normally grow at high altitudes and seeds are exposed to subzero temperatures and snowfall during the winter. Our results revealed that chilling or GA_3 application increases effectively germination of biennial *S. mussotii* from both origins. These results agree with reports of increased germination percentage for other alpine species resulting from GA_3 and low temperature stratification (Nickell, 1982; Bradbeer, 1988).

In *Amaranthus retoflexus*, seed germination varied from different populations, and the germination trend was found to be related to the habitats in which the seeds matured, with seeds from the harsher habitats showing lower germination (McWilliams *et al*, 1968). Seeds of *Trollius* species collected from wild had the most dormancy, but cultivated types were less dormant (Hitchmough *et al*, 2000). In the present experiments of *S. mussotii*, the seeds cultivated at low altitude had a higher dormancy. This result suggests that the germination increased with altitude, which might reflect that the seeds in the high altitude received a natural chill prior to collection. The north cultivation of *S. mussotii* with decreased temperature did not balance the decreased altitude, which was expected to have similar chilling roles as in the high altitude areas. Another possibility is that the short time of artificial domestication, only one cycle of cultivation, might allow dormancy to persist at low altitude. Furthermore, the dormancy of most species is genetically controlled, particularly in tree and perennial species (Nickell, 1982; Bradbeer, 1988).

The response of seeds of *S. mussotii* pretreated with GA_3 or cold storage at 4 ℃, did not differ between the different sites. This indicates that both GA_3 and 4 ℃ cold storage are likely to be effective methods to overcome the dormancy of *S. mussotii* from a range of locations.

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