

## EFFECTS OF OSMOPRIMING AND STORAGE TEMPERATURE ON THE SEED QUALITY OF *Salvia przewalskii* Maxim.

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### ABSTRACT

*Salvia przewalskii* Maxim. is an important Asian medicinal plant. The aim of the study was to investigate the reaction of seeds of this species to the priming and storage temperature. The germination, vigour and health of seeds were determined for untreated and osmoprimed diaspores, after harvest and 12 months of storage at 4 and 16°C. The seeds were primed in the polyethylene glycol (PEG) water solution with an osmotic potential of –0.8 and –1.1 MPa. It has been found that osmopriming of non-stored and stored seeds strongly reduced the time of germination. One-year storage of seeds both at 4 and 16°C caused statistically significant increase of the total number of germinating seeds, germination energy and capacity. The highest germination rate was found at the seeds stored at 16°C and primed with –0.8 MPa PEG solution.

**Key words:** *Salvia przewalskii*, seed germination, vigour, viability, health, fungi

### INTRODUCTION

*Salvia przewalskii* Maxim. (Gansu sage) from the *Lamiaceae* family is an herbaceous perennial plant endemic to the Chinese provinces of Gansu, Hubei, Sichuan, Tibet, and Yunnan [Du et al. 2019]. As an important medicinal plant, it has been commercially available in the local herbal markets of south-western and north-western China. The dried roots and rhizomes of this species are the herbal raw material which has been widely used as one of over 20 plant substitutes for *Salviae miltiorrhizae radix*, called Danshen in Chinese [Li et al. 2008, 2013]. Main active compounds of *S. przewalskii* constitute tanshinones (abietane diterpenoids) such as tanshinone I, IIA and IIB, cryptotanshinone, dihydrotanshinone as well as phenolic derivatives including rosmarinic, salvianolic, lithospermic and przewalskinic acids [Jiang et al. 2013, Wang et al. 2015, Ożarowski et al. 2017]. It was found that the content of tanshinones in the underground plant parts of this species is higher than in the formal Dan-

shen [Hao et al. 2015]. Triterpenoids, anthraquinones, fatty acids, and volatile oil were also determined in *S. przewalskii* [Wang et al. 2014, Xue et al. 2014, Li et al. 2015, Yang et al. 2017]. This plant has long been used in the traditional Chinese medicine (TCM). It is applied in the treatment of cardio- and cerebrovascular diseases, neurasthenic insomnia, bone loss, hepatitis, hepatocirrhosis, and chronic renal failure. Moreover, it supports blood circulation and shows anti-inflammatory, antibacterial, antioxidant, and cytotoxic properties [Matkowski et al. 2008, Jiang et al. 2013]. The seeds of *S. przewalskii* are rich in oil containing unsaturated fatty acids and showing strong absorbance in the UV range [Wang et al. 2014]. For these reasons, they may be used in pro-health food additives and formulation of UV protectors as well.

Observations conducted in the botanical gardens in Poland [Skała et al. 2007, Matkowski et al. 2008, Buchwald et al. 2014] have shown that Gansu sage

can be successfully cultivated under our climatic/soil conditions. However, establishing plantations requires high-quality seed material and the detailed knowledge of the seed biology of this species. Unfortunately, the literature data in this area are insufficient [Liu et al. 2011, Wu et al. 2013, Buchwald et al. 2014]. *Salvia przewalskii* produces dry fruits of the schizocarp type that, when mature, break up into four mericarps (called seeds). The shape of mericarps is elliptic, while surface verrucose and slightly brilliant. Their back side is convex, longer than abdominal side. Lateral edges are blunt, the abdominal one is sharp. The length of seeds ranges from 3.0 to 3.7 mm, the width from 2.0 to 2.7 mm and the thickness from 1.0 to 1.8 mm. The mass of 1000 seeds is about 5 g [Buchwald et al. 2014].

Previous research has shown that the germination capacity of *S. przewalskii* seeds usually does not exceed 60%, and it varies significantly from year to year, strongly depending on weather conditions during blossoming and ripening of seeds. Additionally, the germination percentage of Gansu sage seeds after one year of storage was only 40% [Buchwald et al. 2014]. Priming is widely used method of improving seed quality, which many a time positively affects their storability [Dorna et al. 2013]. Hence, the aim of the present study was to determine the influence of osmopriming and storage temperature on germination, vigour, and health of *S. przewalskii* seeds.

## MATERIAL AND METHODS

**Plant material.** Seeds of *S. przewalskii* were harvested from the Garden of Medicinal Plants in Plewiska near Poznań, western Poland (Institute of Natural Fibres and Medicinal Plants National Research Institute). Soon after collection, the seeds were air-dried, cleaned, and then the basic biometric features: length, width, thickness and the mass of 1000 seeds [ISTA 2012] were measured. The water content of the seeds was determined after drying at 105°C in a HR73 Halogen Moisture Analyzer (Mettler Toledo, Switzerland).

**Tetrazolium test.** For the seed viability determination, the tetrazolium test was used. The laboratory analyses were carried out in accordance with the ISTA methods [2012]. The sample consisted of 100 randomly selected seeds. The diaspores were soaked in distilled water for 24 hours and then, after the incision of

the seed coat, placed in a 1.0% tetrazolium solution for 24 hours. Afterwards, the staining of tissues including embryos was analysed under the stereo microscope (Stemi 2000-C, Carl Zeiss, Germany) at the magnification of 25–40×.

**Osmopriming.** The seeds were primed in the polyethylene glycol water solution (PEG 8000, Sigma-Aldrich) for 48 hours at 20°C in darkness. The concentration of PEG used was 249 and 299 g · kg<sup>-1</sup> water to give an osmotic potential at 20°C of –0.8 MPa (PEG1) and –1.1 MPa (PEG2), respectively. Afterwards, seeds were washed with water to remove PEG, desiccated with blotting paper, and dried back to the initial moisture content for 48 hours at 20°C [Dorna et al. 2013].

**Seed germination and vigour tests.** To evaluate germination and vigour, the seeds were placed in the Petri dishes with moist filter papers and incubated at 20°C in darkness. It was determined for untreated and osmoprimed diaspores, directly after harvest and after 12 months of storage at 4 and 16°C. For each combination, 300 seeds (6 plates with 50 seeds) were tested. After 7 and 21 days of incubation, the percentages of well-developed seedlings (energy of germination and germination capacity, respectively), and also abnormal seedlings were calculated [Rosińska et al. 2017]. In the vigour test, germinating seeds with a visible radicle were counted every day at the same time. Based on them, we determined the total number of germinating seeds ( $G_{max}$ ), parameters describing the speed of germination:  $T_{10}$  (time to germination of 10% of  $G_{max}$ ),  $T_{50}$  (time to 50% of  $G_{max}$ ), and the germination uniformity:  $U_{75-25}$  (time to germination from 25 to 75% of  $G_{max}$ ),  $U_{90-10}$  (time between 10 and 90% of  $G_{max}$ ). For this evaluation, SeedCalculator 2.1 software [Jalink and van der Schoor 1999] was used.

**Seed health test.** The health of seeds (degree of fungal infection and taxonomic composition) was assessed with the deep-freeze blotter test [Rosińska et al. 2017]. The seeds were placed on the moist filter papers (10 replications of 20 seeds for each treatment) and incubated for 48 hours at 20°C in darkness. Next, seeds were frozen for 24 hours at –20°C, and then incubated for 7 days at 20°C under 12-hour cycles of darkness and UV light with wavelength of 320–360 nm. The fungi were identified based on the growth and sporulation characteristics [Mathur and Kongsdal

2003, Watanabe 2010] using the Stemi 2000-C stereo microscope with the magnification of 50× and the optical microscope (Delta Optical Evolution 300) with the magnifications of 100–400×.

**Statistical analysis.** The results were presented as means and data were analysed by the ANOVA test with  $p < 0.05$  considered significant. The normality of distribution of variables was examined with the Shapiro-Wilk test, while the Levene's and Brown-Forsythe tests were applied to check the homogeneity of variances. The Tukey's multiple range test was used to determine the statistical significance of differences between means at a level of  $\alpha = 0.05$ .

## RESULTS

**Biometric features and viability of seeds.** The average mass of 1000 seeds of *S. przewalskii* was 4.62 g. Their mean length, width and thickness reached 3.2, 2.5 and 1.6 mm, respectively. The tetrazolium test

showed 93% level of viability of non-primed seeds, while 5% of the seeds turned out to be without embryos, and 2% were dead. The moisture content of non-primed seeds ranged from 8.1 to 8.6%, whereas directly after osmopriming it was 37.2–38.0% and 32.6–34.9% for the solution of PEG1 (–0.8 MPa) and PEG2 (–1.1 MPa), respectively.

**Seed germination.** After harvest, *S. przewalskii* seeds showed a low mean level of  $G_{max}$  parameter (total number of germinating seeds with a visible radicle during 21 days of incubation), germination energy (the number of well-developed seedlings after 7 days of incubation), and germination capacity (the number of well-developed seedlings after 21 days). It was 59.3, 41.0 and 45.0%, respectively (Tab. 1). Stored for 12 months seeds germinated at the significantly higher percentage (to about 80%). Additionally, some improvement of germination was observed in the case of osmoprimed seeds (non-stored and stored ones at 16°C). Combination of both investigated treatments

**Table 1.** Effects of 12-month storage and osmopriming on germination and vigour of *Salvia przewalskii* seeds

Seed parameters	Non-stored seeds			Seeds stored at 4°C			Seeds stored at 16°C		
	control <sup>1</sup>	PEG1 <sup>2</sup>	PEG2 <sup>3</sup>	control	PEG1	PEG2	control	PEG1	PEG2
Total number of germinating seeds (%)	59.3 a*	63.7 ab	69.3 a-c	77.0 be	75.7 bd	83.7 de	79.3 c–e	90.0 e	84.3 de
Energy of germination (%)	41.0 a	78.3 c	72.7 bc	71.0 bc	63.0 b	74.0 bc	72.0 bc	82.7 c	73.7 bc
Germination capacity (%)	45.0 a	78.7 c	74.3 bc	81.7 c	63.7 b	81.0 c	74.7 bc	85.0 c	80.0 c
Abnormal seedlings (%)	1.0 a	1.0 a	0 a	0 a	1.0 a	0 a	0.7 a	0 a	0.3 a
Speed of germination: $T_{10}$ <sup>4</sup> (days)	2.37 d	0.67 a	1.63 b	2.45 d	1.59 b	1.98 c	3.32 e	1.40 b	2.32 d
Speed of germination: $T_{50}$ <sup>5</sup> (days)	3.03 c	1.82 a	1.97 a	3.46 d	1.89 a	2.61 b	3.99 e	1.69 a	3.03 c
Germination uniformity: $U_{75-25}$ <sup>6</sup> (days)	0.93 ac	1.61 d	0.47 a	1.40 cd	0.49 a	0.87 ab	0.93 ac	0.54 a	1.13 bc
Germination uniformity: $U_{90-10}$ <sup>7</sup> (days)	2.09 ac	3.12 c	1.11 a	3.10 c	1.36 a	1.91 ab	2.08 ac	1.59 a	2.72 bc

<sup>1</sup> Unprimed seeds. <sup>2</sup> Seeds primed in the polyethylene glycol (PEG) water solution with an osmotic potential of –0.8 MPa. <sup>3</sup> Seeds primed in the –1.1 MPa PEG solution. <sup>4</sup> Time to germination of 10% of the total number of germinating seeds ( $G_{max}$ ). <sup>5</sup> Time to germination of 50% of the  $G_{max}$  parameter. <sup>6</sup> Time to germination from 25 to 75% of the  $G_{max}$ . <sup>7</sup> Time to germination from 10 to 90% of the  $G_{max}$ .

\*Means in the rows with the same letter are not significantly different at  $\alpha = 0.05$  (Tukey's test)

**Table 2.** Effects of 12-month storage and osmopriming on fungal infestation of *Salvia przewalskii* seeds (%)

Fungus taxa	Non-stored seeds			Seeds stored at 4°C			Seeds stored at 16°C		
	control <sup>1</sup>	PEG1 <sup>2</sup>	PEG2 <sup>3</sup>	control	PEG1	PEG2	control	PEG1	PEG2
<i>Alternaria alternata</i> (Fr.) Keissler	93.0 ab*	94.0 ab	91.5 a	94.5 ab	95.0 ab	91.0 a	99.5 b	99.5 b	99.5 b
<i>Cladosporium</i> spp.	40.5 bc	45.0 c	50.5 c	36.0 ac	23.0 ab	19.5 ab	34.5 ac	19.0 a	20.0 ab
<i>Stemphylium</i> spp.	7.5 ab	11.5 ab	17.0 b	7.0 ab	5.0 ab	4.5 ab	2.5 a	0.5 a	12.5 ab
<i>Epicoccum</i> spp.	8.0 b	2.5 ab	7.0 ab	8.0 b	3.5 ab	6.0 ab	4.0 ab	0 a	1.0 ab
<i>Trichothecium</i> spp.	8.0 c	0 a	3.5 ac	2.5 ac	4.0 ac	1.5 ab	7.5 bc	5.0 ac	1.0 a
<i>Ulocladium</i> spp.	7.0 a	4.0 a	2.0 a	0 a	0 a	0.5 a	3.0 a	2.5 a	4.0 a
<i>Cephalosporium</i> spp.	6.0 b	1.5 a	1.5 a	1.0 a	0 a	1.0 a	0 a	0 a	0 a
<i>Acremoniella</i> spp.	9.0 a	5.5 a	0 a	0 a	0 a	0 a	2.0 a	3.5 a	0 a
<i>Gonatobotrys</i> spp.	2.0 a	3.0 a	0 a	1.5 a	1.0 a	2.0 a	0.5 a	1.0 a	0 a
<i>Papulaspora</i> spp.	5.0 a	6.5 a	0 a	0 a	4.5 a	0 a	3.0 a	0 a	0.5 a
<i>Verticillium</i> spp.	0.5 a	2.0 a	0 a	1.0 a	0 a	0 a	0 a	1.0 a	0 a
<i>Phoma</i> spp.	0 a	1.5 a	0 a	0 a	0 a	0 a	0.5 a	0 a	0 a
<i>Melanospora</i> spp.	0 a	0 a	0.5 a	0 a	0 a	0 a	0 a	0 a	0 a
<i>Mortierella</i> spp.	0 a	0 a	1.5 a	0 a	0 a	0 a	1.0 a	0 a	0 a
<i>Bipolaris</i> spp.	0 a	0 a	5.5 a	0 a	0 a	0 a	0 a	0 a	0 a
<i>Botrytis cinerea</i> Pers.	0 a	0 a	10.5 c	9.5 bc	4.0 ac	10.0 bc	1.0 ab	0 a	0 a
<i>Fusarium</i> spp.	3.5 ab	6.5 ac	2.0 a	9.0 ac	22.0 c	19.5 bc	12.5 ac	0 a	11.5 ac
<i>Aspergillus</i> spp.	0 a	0 a	0 a	15.0 b	0.5 a	6.0 a	1.0 a	0.5 a	4.0 a
<i>Mucor</i> spp.	0.5 a	3.0 a	0.5 a	1.0 a	3.5 a	0 a	4.5 a	6.5 a	4.0 a
<i>Penicillium</i> spp.	0 a	0 a	0 a	0 a	0 a	0 a	0.5 a	0.5 a	0 a
<i>Rhizopus</i> spp.	0 a	0 a	0 a	0 a	0 a	0 a	0 a	3.5 a	0 a
Non-sporulating fungi	16.0 c	13.5 bc	7.0 ac	2.5 ab	10.0 ac	8.0 ac	6.0 ac	2.5 ab	2.0 a
Seeds free from fungi	0 a	0 a	0 a	0.5 a	1.0 a	0.5 a	0 a	0 a	0 a
Mean number of identified taxa	7.0 b	7.1 b	5.7 ab	6.8 b	5.8 ab	6.2 ab	6.5 ab	4.5 a	4.9 ab
Total number of identified taxa	13	13	13	12	11	11	16	12	10

<sup>1</sup> Unprimed seeds. <sup>2</sup> Seeds primed in the polyethylene glycol (PEG) water solution with an osmotic potential of –0.8 MPa. <sup>3</sup> Seeds primed in the –1.1 MPa PEG solution

\* Means in the rows with the same letter are not significantly different at  $\alpha = 0.05$  (Tukey's test)

(osmopriming and one-year storage) gave the best results. The highest total number of germinating seeds (90.0%), germination energy (82.7%) and germination capacity (85.0%) were determined for the seed lot primed in the PEG1 (–0.8 MPa) solution and stored at 16°C. Osmopriming of the non-stored seeds resulted in the highest and statistically significant ( $p < 0.001$ ) increase of germination energy and capacity compared

to the control. On the other hand, the osmotic treated seeds showed only slight increase or even drop of these parameters after storage at 4°C. For all tested combinations, the mean number of abnormal seedlings of *S. przewalskii* was very low and ranged between 0 and 1.0%.

**Seed vigour.** Osmopriming of seeds ( $p < 0.001$ ) and storage temperature ( $p < 0.01$ ) had statistically

significant effect on seed vigour (Tab. 1). Priming strongly reduced the time of germination of non-stored and stored seeds. Hence, the  $T_{10}$  and  $T_{50}$  values (time to germination of 10 and 50% of  $G_{max}$ ) varied in the broad range from 0.67 to 3.32 days and from 1.69 to 3.99 days, respectively. The PEG solution with higher osmotic potential (−0.8 MPa) had the strongest positive influence on the time of seed germination. Storage of seeds did not significantly affect uniformity of germination. A strong reduction in the level of  $U_{75-25}$  (time to germination from 25 to 75% of  $G_{max}$ ) and  $U_{90-10}$  (time between 10 and 90% of  $G_{max}$ ) parameters was only observed for the seeds that were primed and stored at the lower temperature (4°C).

**Seed health.** Our investigations showed 21 taxa of fungi developing on *S. przewalskii* seeds, but most of them occurred with a low constancy (Tab. 2). Only 2 taxa: *Alternaria alternata* and *Cladosporium* spp. were very common. Depending on the combination, *A. alternata* infected from 91.0 to 99.5% of seeds, on average. In turn, the level of seed infestation with *Cladosporium* spp. ranged from 19.0 to 50.5% and was the highest after priming of non-stored seeds. A similar relationship was observed in the case of *Stemphylium* spp. that was present on 11.5–17.0% of this seed lot. A slightly higher share of *Acremonium* spp. (9.0%), *Cephalosporium* spp. (6.0%), *Trichothecium* spp. (8.0%), and *Ulocladium* spp. (7.0%) distinguished the control group of non-stored seeds. On the other hand, the occurrence of numerous fungi: *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., and *Rhizopus* spp. was connected with the seeds stored for one year. The highest total number of identified taxa of fungi were found for non-primed seeds stored at 16°C.

## DISCUSSION

Available data regarding the biology of germination of *S. przewalskii* are limited [Liu et al. 2011, Wu et al. 2013, Buchwald et al. 2014]. Investigations of Liu et al. [2011] indicated that Gansu sage seeds germinate poorly in the first year after harvest. Germination percentage of seeds stored dry at 4°C, dry at room temperature (about 15–20°C) and moist (wet substrate) at 3°C were 44.00, 26.67, and 22.67%, respectively. In other studies devoted to the impact of light avail-

ability on germination, the number of germinating seeds, stored for 6 months at 15°C, averaged 35.47% [Wu et al. 2013]. It was also found that light inhibited seed germination of *S. przewalskii*. This regularity was confirmed in our previous research [Buchwald et al. 2014]. The maximum of the germination capacity in the first year after harvest reached over 75%, and it was observed in darkness. After one year of storage in unheated room conditions, germination percentage was only 40%, and after 3 years it decreased to 25%. However, there have been no studies on the effects of seed priming of this species so far.

Priming is a procedure that has long been used to improve seed germination. It increases the germination rate, reduces the germination time and increases germination uniformity. Biochemical processes that occur during priming include increase the synthesis and activity of enzymes responsible for the hydrolysis and mobilization of reserve substances [Kubala et al. 2013, Rosińska et al. 2017]. This treatment causes the acceleration of RNA and protein synthesis. Moreover, priming improves the activity of free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) [Girolamo and Barbanti 2012]. However, it can also lead to undesirable changes, and optimal priming conditions are different for particular species, varieties and even individual seed lots. Literature data indicate that osmopriming belongs to the main seed priming methods, and PEG is one of the most commonly used osmotic active substance [Girolamo and Barbanti 2012, Lechowska et al. 2019].

Effects of priming on germination and seedling growth of several species of *Salvia* genus have been previously documented by Abdollahi et al. [2012]. However, the above-cited work refers mostly to the effect of osmopriming in PEG 6000 on seed germination in connection with tolerance to drought stress. In these studies, within 36 *Salvia* accessions 4 group of species were distinguished: 1) germinating and growing well in stress condition, 2) germinating well, but susceptible at seedling stage, 3) germinating unevenly, but growing well, and 4) susceptible for stress at germination as well as seedling stages.

Chinese researchers conducted experiments on various pre-sowing treatments of *Salvia miltiorrhiza* [Liang and Liu 2015]. It was found, that the priming of



seeds in PEG 4000 had the strongest influence on germination energy in comparison with pre-refrigerating, GA<sub>3</sub> soaking, hydropriming and ultrasonic method of pretreatment. PEG priming of the non-stored seeds caused only the slight increase of germination capacity. However, after one-year storage the germination capacity increased by up to 12%, compared to unprimed seeds. In the case of non-stored primed seeds of *S. przewalskii*, our research demonstrated a strong increase in germination energy and capacity (Tab. 1). In turn, after one-year storage at 16°C primed seeds of Gansu sage germinated better than unprimed ones, but the differences were not statistically significant. On the other hand, priming of both non-stored and stored seeds had a statistically proven impact on reduction the time of germination. It was important that osmopriming removed the negative effect of the seed storage on the speed of germination.

One-year storage of *S. przewalskii* seeds caused statistically significant increase of the  $G_{max}$  parameter, germination energy and capacity. This is probably due to the need to overcome seed dormancy by after-ripening. Similarly, the seeds of *S. officinalis* showed post-harvest maturation. The maximum germination capability for this species occurred 5–7 months after harvest and it was as high as 70 to 90% [Formanowiczowa and Kozłowski 1969]. It is worth mentioning that *S. officinalis* has recently been the subject of priming research. Different methods of organic seed priming, including water soaking, cow urine, cow dung slurry, buttermilk, compost tea, and ash were used [Sharma et al. 2018]. It was shown that using cow dung slurry treatment strongly increased germination percentage, reduced germination time and affected uniformity of germination.

Kruppa and Russomanno [2008] studied occurrence of fungi on medicinal plant species of the *Lamiaceae* family. In total, there were found 24 genera of fungi on seeds of *Rosmarinus officinalis*, *Melissa officinalis*, *Hyssopus officinalis*, *Mentha × piperita*, *Lavandula* sp., *Ocimum basilicum*, *Origanum majorana*, *O. vulgare*, *Salvia officinalis*, *Satureja hortensis*, and *Thymus vulgaris*. Among them, *Alternaria alternata*, *Cladosporium* spp., and *Rhizopus* sp. were the most common fungi. In our research, *A. alternata* and *Cladosporium* spp. also dominated, while *Rhizopus* spp. occurred sporadically. In turn, *Stemphylium* spp. quite

often found in our tests, was recorded rare in the studies of other species of the *Lamiaceae* family. Many authors pay attention to the possibility of seed health deterioration after priming [Szopińska 2007]. The obtained results indicate that osmopriming does not have a significant effect on increasing infection by fungi of *S. przewalskii* seeds.

## CONCLUSIONS

1. One-year storage of *S. przewalskii* seeds caused statistically significant increase of the total number of germinating seeds ( $G_{max}$ ), germination energy and capacity, regardless of the storage temperature (4 and 16°C).

2. Osmopriming of non-stored and stored seeds strongly reduced the time of germination, and the best results were noted for treatment of –0.8 MPa polyethylene glycol (PEG) solution. Generally, primed seeds germinated also in the higher percentage, but the greatest effect of this treatment (compared to the control) was observed for non-stored seeds.

3. The highest values of  $G_{max}$ , germination energy and capacity with a good level of parameters describing the speed and uniformity of germination were determined in the case of seeds stored at 16°C and primed of –0.8 MPa PEG solution.

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