

## Research article

## Development of propagation protocol for the endangered species *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm.

Amber Srivastava

Botanical Survey of India, Northern Regional Centre, Dehradun-248195, Uttarakhand, India

Corresponding Author: [ambersri108@gmail.com](mailto:ambersri108@gmail.com)

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**Abstract:** The low regeneration potential is one of the main causes of the depleting population of threatened species. *Phlomoides superba* an endangered species is facing depletion in its natural habitats due to various causes including habitat destruction, low regeneration and exploitation. The ornamental potential of this species makes it suitable for cultivation in gardens for sake of both *ex-situ* conservation and beautification as well. Because of this, a suitable mass scale propagation protocol is required to prevent wild exploitation of this species for commercial use and also for its reintroduction in suitable habitats.

**Keywords:** Threatened - Propagation - *Eremostachys superba* - Conservation - Endangered.

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### INTRODUCTION

*Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. syn. *Eremostachys superba* Royle ex Benth. is an endangered tuberous herb, which is endemic to lower hills of Western Himalaya (Srivastava *et al.* 2017). The species naturally grows in moist, loamy soil on forest edges. Since many of these habitats are occupied by local farmers that has restricted the species to grow on edge of crop fields. It mainly propagates through seeds and due to scarcity of natural pollinators and insect infestation the seed production is affected. The low regeneration potential of this species was described as one of the major cause of its declining population by Garg & Rao (1997).

Since this species is having high ornamental potential due to its beautiful flowers (Pundir 2015) which has resulted in its exploitation from its type locality. Therefore it has been realized that a good propagation protocol is of prime importance for its successful conservation and cultivation. Though trials have been made on the *in-vitro* propagation of this species but these techniques lack easy accessibility (Sunnichan & Shivanna 1998, Panwar *et al.* 2015). In the present study, various conventional propagation techniques were experimented with to develop a suitable propagation protocol for this species.

### MATERIAL AND METHODS

The propagating material *viz.*, seeds were collected from the plants growing in the garden of Botanical Survey of India, Dehradun. Some amount of seeds were also collected from the wild population of Kangra district. The following propagation techniques were experimented with for the propagation of *Phlomoides superba*.

#### Through seeds

Sexual propagation or propagation through seeds is the most common method for propagation of this species in its natural condition. Though different studies have been already done on the seed germination of the species but every study revealed different results. Seeds were collected from various sources (cultivated and wild populations) in May and June to test their viability and germination rate.

During the study it has been observed that the length of inflorescence and number of flowers is more in cultivated plants whereas it was comparatively less in wild population. Similarly the number of healthy seeds produced per inflorescence were also higher in cultivated plants than wild plants. However, the percentage of insect infestation was maximum in case of wild ones and sometimes nearly 85% of the seeds were found

infected which was comparatively less in cultivated plants mainly due to proper care and timely spray of insecticides (Fig. 1).



**Figure 1.** *Phlomis superba* (Royle ex Benth.) Kamelin & Makhm. propagation through seeds: **A**, Seed Collection; **B**, Sorted seeds (i. Healthy, ii. Non-viable, iii. Infected); **C**, Seed germination stages; **D**, Germinated seedlings; **E**, 1st year seedlings; **F**, 2<sup>nd</sup> year seedlings; **G**, Propagated plants shifted to polybags.

#### Seed selection

The collected seeds were sorted out into healthy, non-viable and infected seeds. The heavy, darker and turgid



seeds were selected as healthy ones and dull colored, thin and apparently empty seeds were categorized as non-viable ones. For the present experiment only healthy seeds were selected for germination trial.

### Dormancy

The seeds possess internal dormancy due to which they do not germinate immediately after sowing. In nature, seeds take about 2–3 months to overcome the dormancy. Most of the seeds get infected by pests and fungal infection in the field during their dormancy phase. Also many of the seeds are eaten by birds during their dormant phase.

### Seed germination

According to some of the earlier workers, the seeds of this species are not viable and do not germinate in natural condition and even *in vitro* (Sunnichan & Shivanna 1998) which was not found true during the present study. In nature, the seeds of *Phlomoidea superba* mature during April–May, and the germination of the seeds starts during August–September after the commencement of rains. Due to some internal dormancy the sown seeds do not germinate readily and take nearly one month for germination.

Different methods were experimented for breaking the dormancy and increasing the germination rate of the seeds. The treated seeds were sown in washed, sterilized coco-peat medium and monitored every 3 days to determine the germination percentage in response to each treatment. The pre-treatment methods applied are explained as follows:

- i. Hot Stratification: The seeds were soaked overnight in warm water (70–80°C) before sowing. The pre-treated seeds were sown in washed and sterilized cocopeat medium in three replicates of 50 seeds each.
- ii. Cold Stratification: Seeds were first soaked in mild fungicide solution, mixed with sterilized moist coco peat and packed in plastic zip bags, these zipbags were kept in refrigerator under 4–5°C for 15 days. The seeds were then sown in the growing medium for germination. This treatment is found to be very effective for the germination of this species and it has been observed that the seeds started germinating within the zipped polybags inside the refrigerator after 10–12 days of cold stratification.  
Precaution: Since in this process, the seeds are kept in moist medium for a longer period of time, the mixture should be properly sterilized and treated with fungicide otherwise the seeds get infected resulting in lowering of germination rate.
- iii. Acid Treatment: Healthy seeds were soaked in dilute H<sub>2</sub>SO<sub>4</sub> solution for a period of 30 minutes. The treated seeds were washed with water, treated with fungicide and then sown in vermiculite medium.
- iv. Phyto-hormone treatment: The seeds were also treated with Gibbrellic acid for increasing the germination percentage (Panwar & Srivastava 2015). For this the seeds were soaked in 400 ppm solution of GA<sub>3</sub> for a period of 12 hours and then washed with distilled water. The washed seeds were then sown in cocopeat medium for germination. This treatment is quite effective in increasing the germination percentage of the species with a mean germination percentage of 82%.
- v. Control: Seeds treated with fungicide and soaked overnight in cold water were used as control.

## RESULT AND DISCUSSION

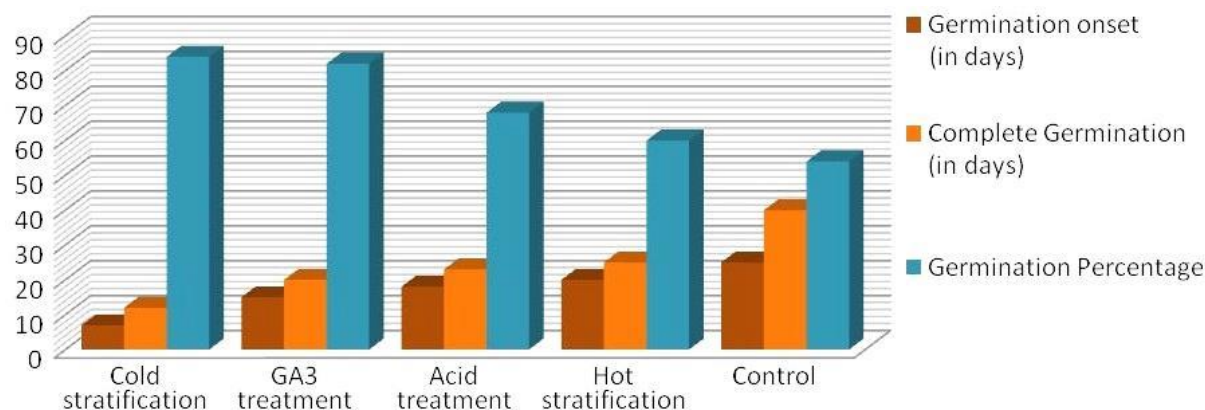
On the basis of above experiments following results have been obtained which showed variable germination rates in response to different treatment (Table 1).

**Table 1.** Showing seed germination rate of *Phlomoidea superba* (Royle ex Benth.) Kamelin & Makhm. in response to different pre-treatment.

S.N.	Number of seeds	Treatment	Germination onset	Complete germination	Total seeds germinated	Germination %
1.	50	Cold stratification	7 days	12 days	47.00±2.16	84%
2.	50	GA <sub>3</sub> treatment	15 days	20 days	41.00±1.70	82%
3.	50	Acid treatment	18 days	23 days	34.34±2.86	68%
4.	50	Hot stratification	20 days	25 days	30.07±2.05	60%
5.	50	Control	25 days	40 days	27.00±2.44	54%

From the above data it is evident that the germination rate was maximum in cold stratification and also the germination time was least in this treatment. Whereas the control method showed maximum germination time

and least germination rate (Fig. 2).

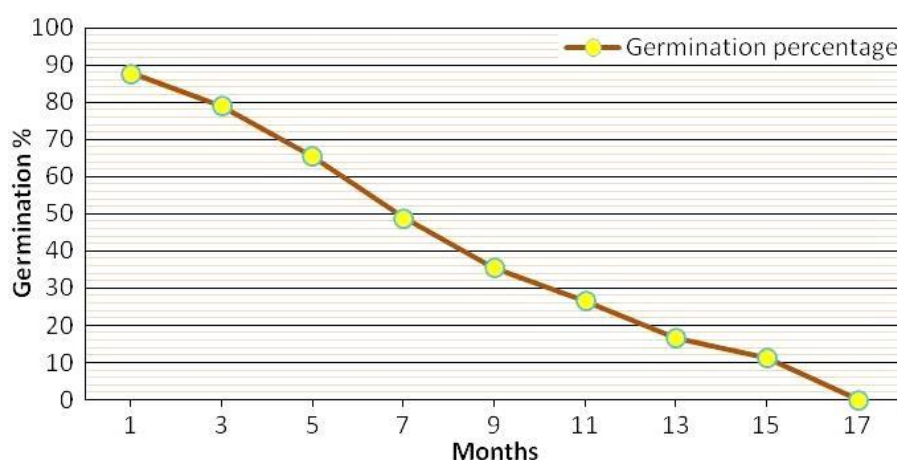


**Figure 2.** Effect of different pre-treatment on seed germination rate of *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm.

#### Seed viability

The seed viability of *Phlomoides superba* always remained a controversial topic. The earlier workers reported very less or no viability which does not seem true seeing the regeneration of this species in wild. According to Garg & Rao (1997) the seeds are viable for only one month, however the viability period is reported as five months and eight months by Sunnichan & Shivanna (1998) and Verma (2001) respectively. Increase in viability period of seeds stored in low temperature is also reported to last for 12 months (Panwar & Srivastava 2015).

In the present study, the seeds were found viable for 15 months when stored under room temperature. For this, the seeds were sown in triplicates of 30 at regular interval of one months to analyze the germination percentage and viability rate with time (Table 2). Thus the seeds of *Phlomoides superba* are viable for a longer period than it was mentioned by the previous workers (Fig. 3).



**Figure 3.** Seed germination percentage of *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. with response to storage period.

**Table 2.** Showing variation in seed germination of *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. with storage duration.

	Duration (months)								
	One	Three	Five	Seven	Nine	Eleven	Thirteen	Fifteen	Seventeen
Number of seeds sown	30	30	30	30	30	30	30	30	30
Total germinated seeds (number)	26.34±1.25	23.67±1.25	19.67±2.35	14.67±1.89	10.67±0.94	8±1.42	5±0.81	3.34±1.24	0
Germination percentage (%)	87.8	78.9	65.57	48.9	35.57	26.67	16.67	11.13	0

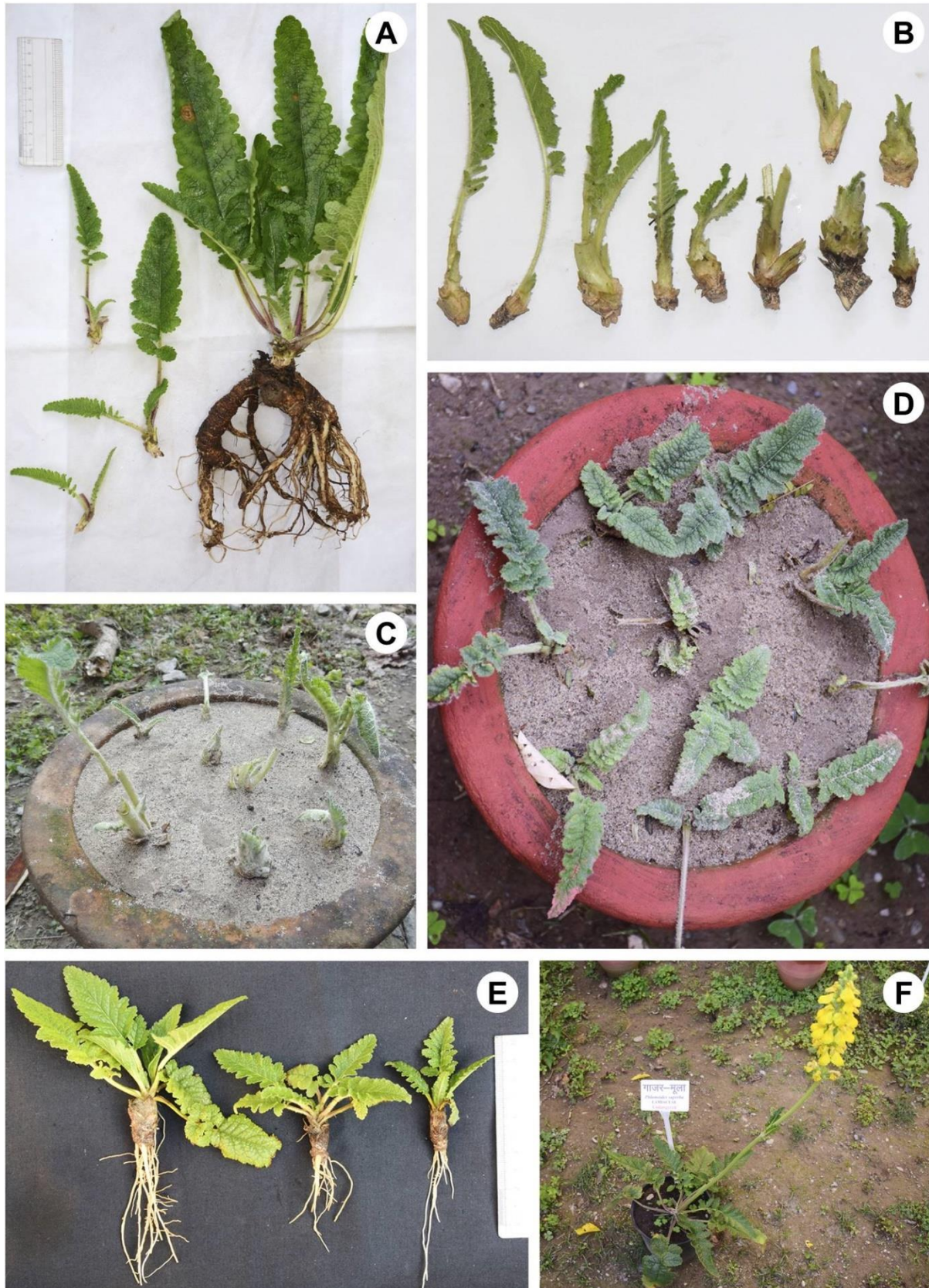
#### Survival percentage

The seedling survival rate in *Phlomoides superba* is quite satisfactory and more than 95% seedling survival has been observed. The seedling survival rate in *Phlomoides superba* is quite high which may be due to the



presence of tuberous roots and non-habitat specific nature of the species. After 3 months the saplings were transferred to small polybags for further growth and development. The saplings were kept in these polybags for 1 year before plantation in suitable sites and distribution for *ex-situ* conservation purpose.

#### Vegetative propagation



**Figure 4.** Vegetative propagation of *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm.: A, Crown cuttings taken from plant; B, Different types of crown cuttings; C, Cuttings planted in sand; D, Growth initiation in cuttings; E, Rooted crown cuttings; F, Vegetatively propagated plant flowering in pot.

In mature plants of *Phlomoides superba* the stem becomes branched from the base to form dense basal rosette or crown. The vegetative propagation was carried out through division of the foliage crown. The buds arising from the sides of the main plant were separated carefully with the help of a sharp knife and used for propagation purpose. These cuttings were planted in the well-drained medium for rooting after treatment with rooting hormones.

Cuttings from basal rosette were taken from the well-developed mature plants in September. The cut ends were treated with three concentrations of IBA 100 ppm, 200 ppm and 500 ppm and planted in the pure sand medium for root initiation. After 25 days the planted cuttings were inspected and it was found that 200 ppm IBA showed maximum root initiation (95%) followed by 70% in 500 ppm IBA and 55% in 100 ppm IBA. These rooted cuttings were placed in the same medium for further growth of roots. After 60 days these cuttings were shifted to plastic polybags in normal planting medium. Some of these vegetatively propagated plants flowered after 6 months (Fig. 4).

This method is best suited for taking early flowering as this species takes nearly 3–4 years to come to flower when propagated through seeds. This method can be used for mass scale propagation of this species, especially for the horticultural purpose.

## CONCLUSION

The analysis of the results obtained through different propagation methods used in the present study revealed that the species can be propagated through both vegetative and sexual means. For mass propagation of this species, propagation through seeds is the advisable way whereas for horticultural purpose vegetative propagation is best suited which will produce early forming as the plants propagated through seed take nearly three years to come to flower.

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