Seed Biology, Dormancy and Phenophases of Seed Germination of three Rare Himalayan Herbs

B.D. Sharma, Lal Singh and Maninder Jeet Kaur

ABSTRACT : The three of the Astavarga (8) species, *Lilium polyphyllum* (Kshirakakoli), *Polygonatum verticillatum* (Meda) and *P. cirrhifolium* (Mahameda), were included in the study. The seed viability test showed very low viability (22.5%) for *L. polyphyllum*, which was improved by hand pollination. These species possessed seed dormancy from 28 days to over two years. Gibberelic acid treatments of 1000 and 2000 ppm in combination with scarification of soaked seeds gave species specific results for breaking seed dormancy. The species included were described to have deep simple epicotyls morpho-physiological type of dormancy. They were found to have peculiar phenophases of seed germination, which have been discussed in detail along with diagrams.

KEYWORDS: Seed Biology, Dormancy, Phenophases, Meda, Mahameda, Astavarga, Kshirakakoli, *Lilium polyphyllum, Polygonatum verticillatum, Polygonatum cirrhifolium.*

INTRODUCTION

The seed is a miniature form of plant that holds future potential but is constrained with and protected against the risks of damage by various external environmental factors. This is so especially with plants inhabiting the natural habitats of Himalayas where conditions for survival are threatening and sometimes show sudden changes constraining the normal life. In response to this, plants in the temperate Himalayas have developed some inherent mechanisms that allow them to bypass unfavourable conditions. Such mechanisms include reproductive biology of seed, seed dormancy and germination behavior.

The miracle Ashtavarga (*Habenaria intermedia, Habenaria edgeworthii, Malaxis muscifera, Malaxis acuminata, Roscoea procera, Lilium polyphyllum, Polygonatum cirrhifolium,* and *Polygonatum verticillatum*) herbal plants which usually occur in the temperate Himalayan region. Out of these 8 species, three species namely *Lilium polyphyllum* (Kshirakakoli), *Polygonatum cirrhifolium* (Mahameda) and *Polygonatum verticillatum* (Meda) are included in the present study in view of their vulnerable nature and high value in the traditional Indian Medicine system of India as well as being ingredient of famous health food or neutraceutical, Chayavanprash (Shartma and Balkrishan, 2005). The present investigation was undertaken to develop suitable multiplication techniques particularly through the use of sexual seeds, as the multiplication through the use of vegetative propagules is more cost involving being the drug itself of these rare and vulnerable species.

Sharma, B.D. [✉], Singh, L. and Kaur, M.J.
Himalayan Research Group (HRG), Chhota Shimla, Shimla-171002 (Himachal Pradesh).
e-mail: bdsharma.iias@gmail.com

MATERIALS AND METHODS

Collection of Seeds

Seeds of three species, *Polygonatum cirrhifolium, P. verticillatum* and *Lilium polyphylum* were collected on maturity judged by the fruit colour of former two species turning red or orange respectively and capsules of the latter becoming yellow or brown. These were collected from locations between 1600 m and 2500 m altitudes from Chail (2250 m) in district Solan; Hattu (3000 m), Matiana (2500 m) in Shimla district; and Dhangiara (2000 m), Murhala and Surhala (2200 m) in Mandi district. The quantities for each species is shown in the Table-1 below:

Table-1: Showing quantities of sexual seeds and vegetative parts collected for each of the three species

Species	Seeds collected (g)	Rhizome/ Bulb collected (g)
P. cirrhifolium	309.895	6000
P. verticillatum	99.594	5200
L. polyphyllum	72.090	2400

Data recording and Processing of Seeds

Data were recorded on the fruit and seed characters of three species in the laboratory. The mature fruits of two *Polygonatum* species were crushed in water, cleaned of the adhering pulp in water and then thoroughly washed with clean water thrice. The data recorded is presented in the Table-2 below;

Table-2: Data on fruit and seed characteristics of the three species

Species	Fruit colour	Fruits/bunch	Av. no. of seeds/ frt.	Av.100 seed Weight (g)
P. cirrhifolium	Orange, larger	11-14	1.3	10.446
P. verticillatum	Red purple	16-20	1.2	8.549
L. polyphyllum	Yellowish-green	Single capsule	100	1.740

Tetrazolium Test for the Seed Viability of the Three Species

This test is important when it is not known that the cause of non-germination of seed is non-viability or seed dormancy or both, and it takes from days to weeks and even in some cases even months to complete germination. This was noted here as well. In each case 100 seeds were tested in 1% tetrazolium solution with pH at 7.0 with soaking treatment of 21 hours. These seed lots were soaked for over-night prior to the treatment. Before examining the bisected (*Polygonatum*) seeds under the microscope, the seed was removed from the tetrazolium solution, rinsed 2-3-times in water and 2-3 drops of lactophenol clearing solution were added and seeds with stained embryo were counted out of the total number contained in a field and percentage was worked out taking at least three field counts.

Study of Breeding Behavior and Enhancement of Seed Viability Through Hand Pollinations

So, in order to overcome the problem of low viability, it was planned to do hand pollination. But the plants grown in cultivated field did not flower. Therefore, the hand pollination of wild plants was done, though not under control. Somehow a single capsule of Kshirakakoli could be harvested whereas other plants were damaged by grazing animals.

Seed Germination Behavior and Seed Dormancy Breaking

Several experiments were designed on seed germination and breaking seed dormancy. However, satisfactory results were obtained when 100 seeds were first soaked in water for overnight about 12 hours. Then after drying for 2 hours, they were scarified using sand paper. Thereafter, these seeds were given 1 hour soaking treatment in 1000 ppm and 2000 ppm solutions of GA_3. The treated seeds were transferred to autoclaved vermiculite filled polybags, which were moistened and kept in dark. Weekly observations were recorded on seed germination and germinated seeds were later planted in trays filled with 1:1 mixture of vermiculite and vermin-compost. Observations were also taken on seed germination behavior.

RESULTS AND DISCUSSION

The three species under investigation broadly belong to the family Liliaceae but under the revised taxonomy, APG II Classification, the genus *Polygonatum* has been included in family Ruscaceae, which later in 2009 APG II revised and reduced to subfamily and their equivalent subfamilies are mentioned below:

Ruscaceae=Nolinoideae=Asparagaceae (Bot. J. Linnean Soc., 141: 399-436, 2003). KEW has finally accepted Family Asparagaceae and subfamily Nolinoideae for the genus (Tamura *et al.* 1997) and have included in the World Checklist of Selected Families of June, 2014. The genus *Lilium* has also received attention of Horticulturists for classification and accordingly *Lilium* species has been grouped into seven groups and *Lilium polyphyllum* has been placed in group Candidum which includes 8 species. Plant characters of *Polygonatum* species showed slightly higher values for average seeds per fruit and 100-seed weight for *P. cirrhifolium* but the number of fruits/bunch was higher for *P. verticillatum*. The colour of fruits was orange for the former and red-purple for the latter. The capsule of *Lilium polyphyllum* contained 100 seeds / capsule and 100-seed weight was 1.74g where it was 10.45g for *P. cirrhifolium* and 8.55g for *P. verticillatum* (Table-2).

Seed Viability Test

The seeds of three species were subjected to Tetrazolium seed viability test in order to ascertain if there is any seed viability problem. The average per cent is presented below. Tetrazolium seed viability test gave the following results:

P. cirrhifolium	=	54%
P. verticillatum	=	*62.5%*
L. polyphyllum	=	22.5%

Study of Breeding Behavior and Enhancement of Seed Viability Through Hand Pollinations

All the species showed low seed viability but it was very low for seeds of *Lilium polyphyllum* . So, it was planned to try hand pollination for this species. A single capsule was harvested containing about 80 seeds, which showed 40% seed viability against 30% from wild harvest. The low seed viability may be due to washing of pollens from stigmatic surface by wind and heavy rains in these locations. All the three species under study have gametophytic type of self-incompatibility and hence behave as cross-pollinated in the nature, and pollination is done by bees and other insects. The seeds of all the species especially that of *Lilium polyphyllum* show low viability (30%), and small populations, consisting only of few plants, further restrict the seeds availability. This problem is hampering multiplication through the seeds on one hand, and conduct of experimental studies for breaking seed dormancy, on the other. As on today the quickest way of multiplication of the three species would be to use true seeds rather than the vegetative methods. However, seeds pose two problems; first they possess dormancy, and have low viability and secondly, due to heterozygous genetic nature they produce progeny with large variation.

Breaking of Seed Dormancy

Nature has wonderful ways to conserve species in their natural habitats, one such example is illustrated with these three species. Due to predominant cross breeding behavior of these species

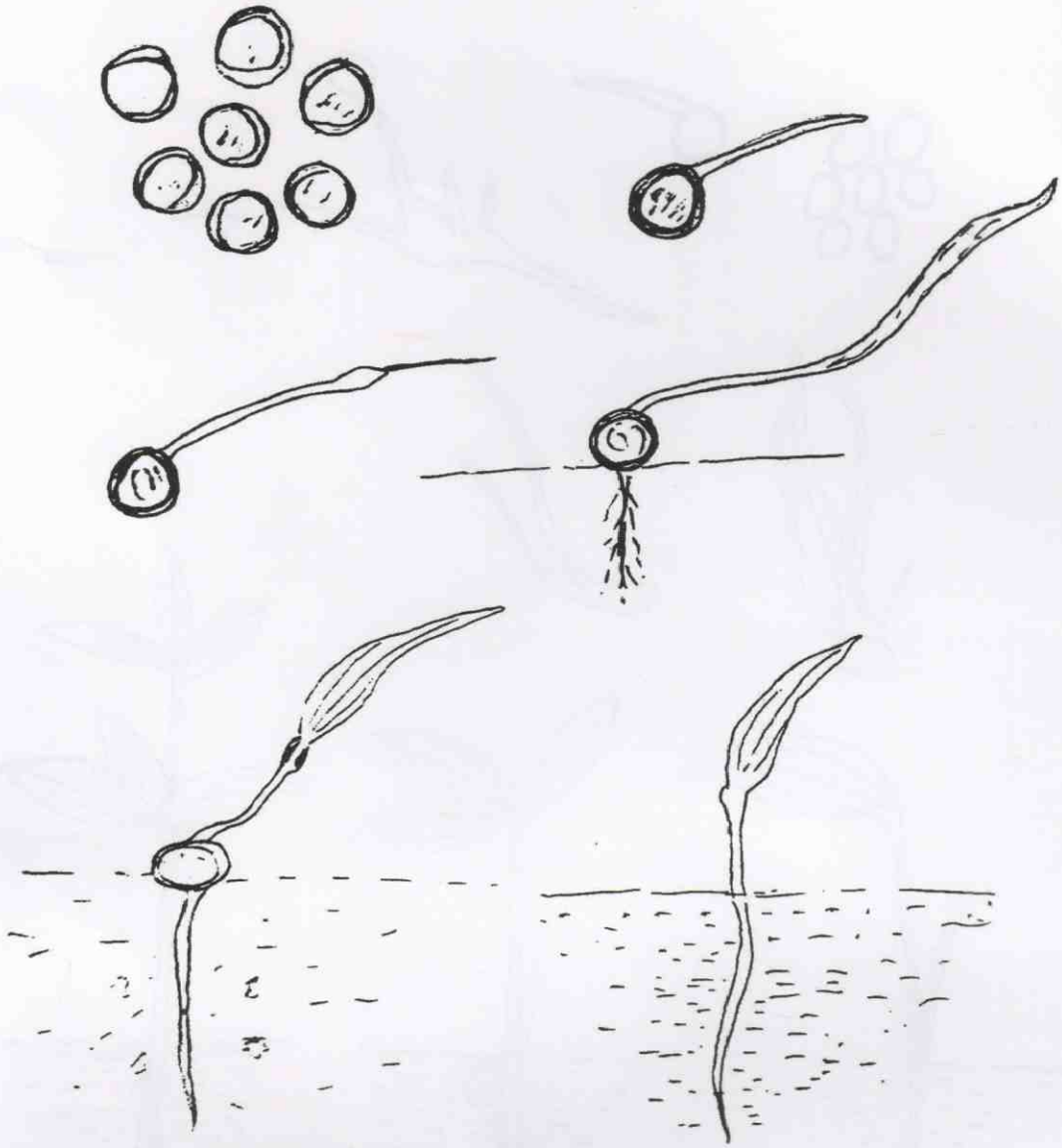

Fig. 1. Seed and Germination Stages of Seed of *Lilium polyphyllum*.

and consequent genetic heterogeneity for dormancy behavior including others, seeds possess dormancy duration ranging from 28 days to over two years and it appears that each seed has its own. About 13 experiments were conducted involving different methods for breaking the seed dormancy, but only one experiment, where gibberelic acid was used in 1000 and 2000ppm doses along with control with overnight water soaked seeds after scarification of seeds using sand paper gave some satisfactory results as shown in the Table given below:

No. of seeds germinated

Species	Duration (days)	1000 ppm	2000 ppm	control
P. cirrhifolium	28-46	33 (14.3%)	68 (27.2%)	1
P. verticillatum	33-53	47 (23.5%)	5 (4%)	3
L. polyphyllum	30-43	23 (46%)	20 (40%)	-

The results obtained, suggest that there is need to design more studies. Earlier studies on seeds of *Polygonatum cirrhifolium* and *Lilium polyphyllum* have reported results, which differ from the results of this study due to the reason that collection of seeds have been from the natural habitats and the storage conditions as well as chemicals and doses also differ (Lattoo *et al.,* 2001; Rana and Samant, 2011). The important fact here is the classification of seed dormancy behavior, which seems to be done on scanty data, as Lattoo *et al.* has described as epicotyls dormancy for *P. cirrhifolium* seeds and for *Lilium polyphyllum* seeds as epicotyls morpho-physiological dormancy (Dhyani *et al.,* 2013). The seed coat of *Polygonatum* species is very hard and is also combined with physiological dormancy involving epicotyls dormancy, whereas the seed coat of seeds of

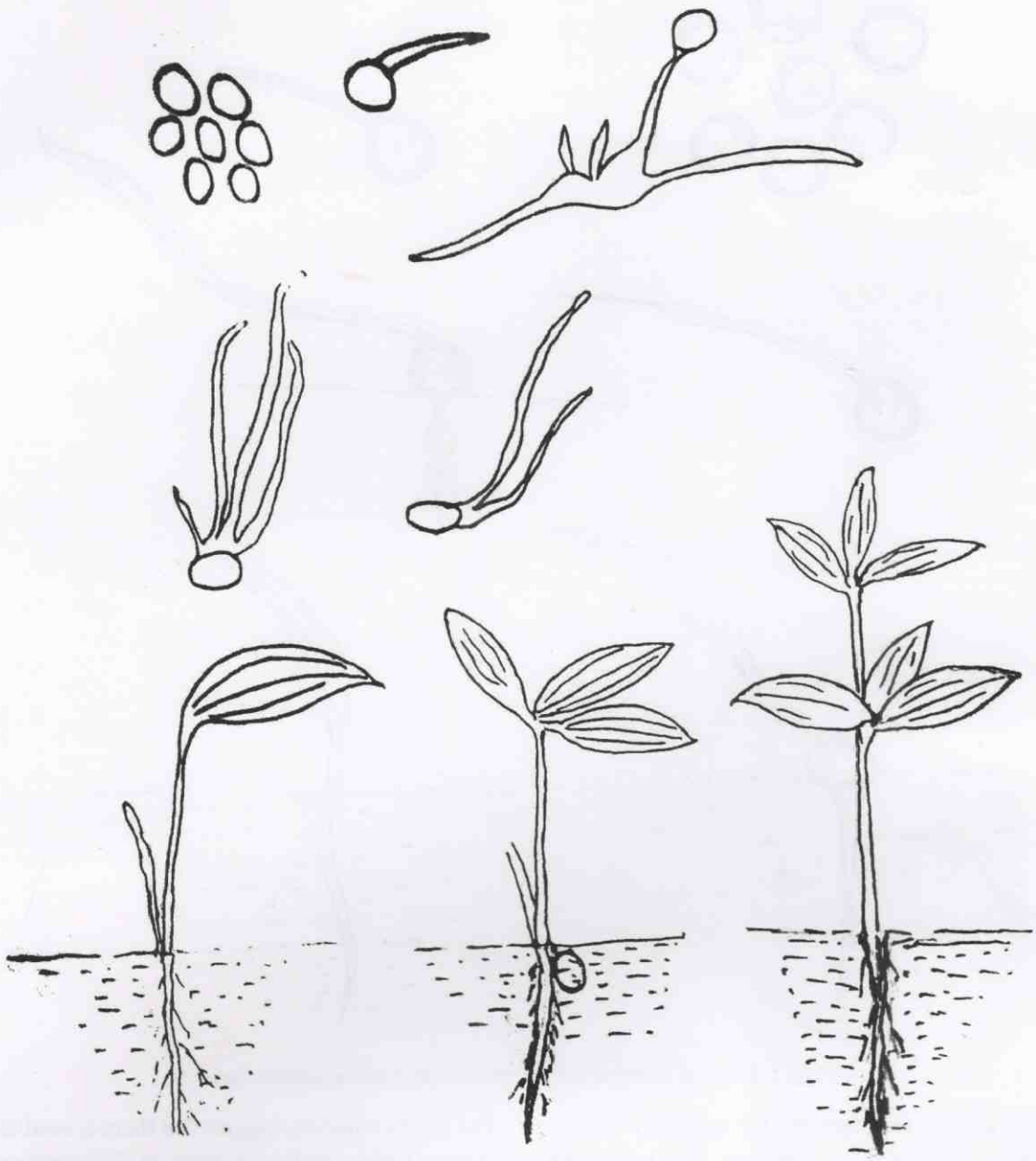

Fig. 2. Seed and Stages of Seed Germination of *Polygonatum* spp.

L. polyphyllum is not hard but seems to have deep physiological dormancy and is combined with epicotyl dormancy, as has been shown in studies by Dhyani *et al.* (2013). According to The Seed Biology Place (website Gerhard Leubner Lab Royal Holloway University of London, 10/20/2014), referring to the dormancy classification system of Nikolaeva (1977) based Baskin and Baskin (1998, 2004) a comprehensive Seed Dormancy classification system which includes five classes of seed dormancy and these five classes further divided into levels and types (Baskin and Baskin, 2004) includes a class, Morpho-physiological dormancy (MPD) in which seeds with undeveloped (in terms of size) embryos have a physiological component to their dormancy and also morphologically seed coat function as block to moisture absorption. Thus, this type of dormancy can be described as deep simple epicotyl morpho-physiological dormancy from amongst the eight known levels of MPD. Seeds with this level of dormancy require longer periods of cold stratification (around 4^0 to 5^0C) embryos require a maturity time combined with epicotyl bulblets cold stratification before the emergence of seedling with a single leaf or sometimes two-leaf bearing structure. Schwienbacher *et al.* (2011) has also observed from their investigation of seed