CHAPTER 7

Effect of Storage Period on Seed Germination

7.1 Introduction

Understanding response of different populations on seed germination provides helpful clues on genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 2001), essential to select elite seeds (Jayshanker *et al.*, 1999) for *ex-situ* conservation of gene resource. For the same, seed storage and its testing to monitor losses in germination rate, which might adversely affect nursery recovery rates, is considered as one of the most efficient methods (Gonzalez-Benito *et al.*, 1998, Williams *et al.*, 2007). However, poor seed germination in several cases is one of the limiting factors (Butola and Badola, 2004b, 2006a; Badola and Butola, 2005). Due to inappropriate storage, germination capacity of a species may decline during first few months after collection (Romanas, 1991); whereas, proper storage may be effective over a considerable period (Butola and Badola, 2004ab; Chen *et al.*, 2007).

Swertia chirayita (Roxb. ex Fleming) H. Karst, (Gentianaceae) grows mostly in temperate Himalaya (1200-3000m asl) from Afghanistan to Bhutan (Kirtikar and Basu, 1984), and used ethno-medicinally since centuries (Wawrosch *et al.*, 2005). Plant has numerous medicinal properties (Joshi and Dhawan, 2005), and is used as a liver stimulant and in bronchial asthma, dyspepsia, debility, fever, gastrointestinal infections, curing various skin problems and scorpion bite (Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Nadkarni, 1976). For *Swertia chirayita,* the presently targeted species, very meager efforts are reported, such as, micro-propagation (Wawrosch *et al.*, 1999, Joshi and Dhawan, 2007) in the absence of successful domestication (Joshi and Dhawan, 2005). The current literature lacks any populations' based assessment of seed germination, especially related to storage in *S. chirayita*, a critically endangered species (Anonymous, 1997). The species has very high pharmaceutical value in national and international markets and is prioritized at top for ex-situ cultivation in an international experts' exercise (Badola and Pal, 2002). As the species regenerates only through seeds, understanding response of different populations on seed germination behaviour, especially seed tolerance to periodical storage would be crucial for *ex-situ* conservation of the same. Low density of species in nature (Bhatt *et al.*, 2006) further necessitates such investigation.

The present study aimed to investigates *S. chirayita* for, (i) variability amongst populations for their seed characteristics and response on germination, (ii) trends of seed germination responses to storage periods amongst populations and individually for each population, to identify elite seed resources.

7.2 Materials and Methods

7.2.1 Seed collection

Matured seeds of *S. chirayita* were collected from 13 populations (Table 14) of Sikkim Himalaya ($27^0$04'46" and $28^0$07'48" N lat. and $88^0$00'58" and $88^0$55'25" E. long.) during November-December 2005. At each population site, seeds were collected sustainably from 15 to 20 individuals randomly selected form the mid of the population without disturbing their natural spreading. The seeds for each population were mixed thoroughly to minimize effects of single parental plant on germination. The seeds were brought to the laboratory and cleaned thoroughly for impurities and dried for 10 days at room temperature.

7.2.2 Seed morphology

To determine the moisture content, 50 seeds/replicate (3 replicates/population) were weighed and oven dried (60^0C; 48 hrs). To evaluate the number of seeds per fruit, seeds were counted using 10 healthy fruits per population. Seed size was measured using 30 seeds per population under microscope (10 seeds each of 3 replicates).

7.2.3 Seed germination

Owing to minute seeds and finding difficulty in detaching embryo, the viability test using 2,3,5, Triphenyle tetrazolium chloride solution could not be executed. Seeds after room drying were tested for initial germination and remaining seeds were stored population wise in proper sealed specimen tubes at 4^0C, for periodical re-testing (6 months interval). For each test, 30 seeds/replicate (3 replicates/population) were disinfected (5 sec.) with sodium hypochlorite solution (4% w/v available Chlorine), washed thoroughly with double distilled water (DDW), and separately soaked in DDW for 24 hours, before placing in Petri-plates (90mm dia.) lined with single filter paper (Whattman No. 1) under incubator (average temperature $25^0C \pm 2.00$; 14/10 hrs light/dark periods), maintaining randomized design and monitored daily (Photo Plate III). The filter papers were moistened daily using DDW. Seeds were considered germinated upon radical emergence and counted daily.

7.2.4 Statistical analysis

Mean germination time (MGT) was calculated using equation, MGT = $\Sigma(nd)/ \Sigma N$; where n = number of newly germinated seeds after each incubation period in days d, and N = total number of seeds emerged at the end of the test (HartMann and Kester, 1989).Univariate and Multivariate ANOVA in general linear model (GLM) using SPSS 10.0 for Windows were used to determine the effects of population and storage period on seed germination and mean germination time. Bonferroni test was employed to determine the variation in means of seed germination and MGT.

7.3 Results

A variety of habitats supports populations of *Swertia chirayita*, which differed significantly for various seed characteristics (Table 14). Multivariate ANOVA revealed significant variation in mean germination percentage and mean germination time in *S. chirayita* amongst populations, increasing storage period and their interaction (Table 15).

During initial testing, 100% seed germination was recorded in majority of the populations (Figure 17). Testing of seeds of *S. chirayita* for their germination potential at initial (r = -0.724; $p<0.05$), after 6 month (r = -0.384) and 12 months (r = -0.004) revealed negative correlation with altitude of the seed collection sites, while testing after 18 months and 24 months of storage showed positive correlation between seed germination and altitude. The duration of storage had significant effect on seed

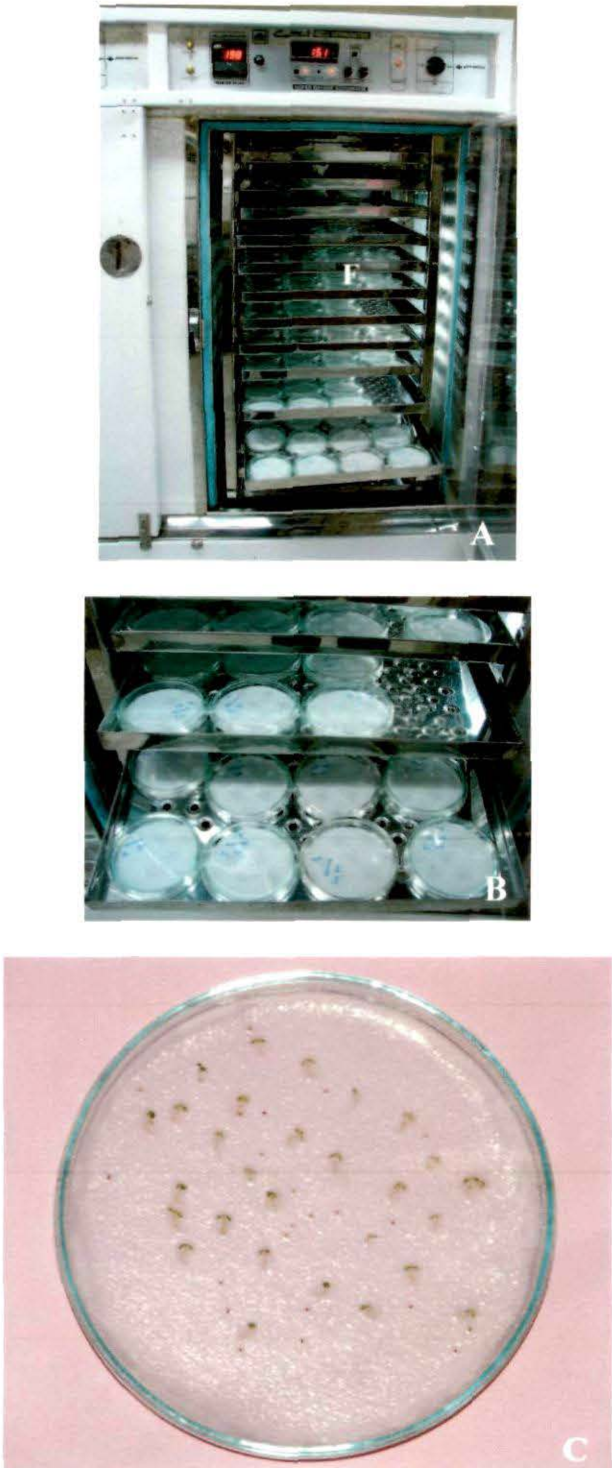

Photo Plate III. Laboratory testing of seed germination potential in *Swertia chirayita*
A and B: Incubation of seeds in the germination chamber;
C: Petriplate containing germinated seeds

germination in *S. chirayita*; nevertheless, there was not much variation in seed germination percentage between initial and the 6 months storage in populations of *S. chirayita*. Across plant populations, mean seed germination in all the samples was significantly ($p<0.001$) higher than Sc10 during initial testing ($p<0.001$), and was significant at $p<0.05$ level after six month of storage except Sc11. However, Sc10 resulted in lower seed germination (70%) compared to 6 months (86.67%) and 12 months (81.11%). Further increasing the storage period to 12 months, Sc8 and Sc9 resulted in above 90% seed germination while other population retained above 60% germination; however, seed germination in P2 (52%) was significantly ($p<0.05$) low compared to other populations. Seed germination fell drastically in seeds collected from Sc4, Sc6, Sc7 and Sc11 after 18 months of storage; however populations viz., Sc1, Sc3, Sc5, Sc8 and Sc9 recorded above 50% seed germination (Figure 17). The seed germination remained maximum in P9 (79%), followed by P1 (74%) over other populations. Some of the populations such as Sc1, Sc8 and Sc9 resulted in above 50% seed germination even after 24 months of storage. The maximum seed germination percent was showed by P1 (59%) and P9 (58%). Of all the populations, Sc4 recorded fastest fall in seed germination percentage from initial (100%) to 24 months (8.89%) of storage (Figure 17). Collectively, for all the populations, seed germination percentage reduced significantly with increase in duration of storage (Figure 18).

The highest mean germination time (MGT) was recorded during initial test ($p<0.0001$) which ranged from 15.17 days (Sc3) to 59 days (Sc10) [Figure 19]. Cumulatively, six month storage recorded significantly ($p<0.0001$) lowest mean germination time in all the populations which showed increasing trend with increase in storage period upto 12 and 18 months but showed irregularity in MGT after 24 months storage (Figure 18). One way and Two way ANOVA revealed the main effect on seed germination in *S. chirayita* was the storage period followed by altitude of the seed collection site and their interaction (Table 15).

7.4 Discussion

The present experiment clearly indicates that 16% to 43% moisture content in seeds can retain seed viability for longer period in *S. chirayita* which is in conjunction with our another study where 19% to 44% seed moisture resulted in longer seed viability in some of the populations in *S. chirayta* (refer chapter 10). Hampton and Hill (2002) also suggested 10% to 40% seed moisture content as a requirement for maintaining seed viability for longer period.

The variation in seed germination percentage amongst different populations in *S. chirayita* indicates the existence of species genetic diversity. Similar differences in germination response amongst plant population have been reported (Perez-Garcia *et al.*, 1995; Baskin and Baskin, 2001; Cerabolini *et al.*, 2004), which will provide valuable clues for selection of elite populations for seeds (Jayshanker *et al.*, 1999). 100% seed germination in majority of population during initial test, reveal the availability of potential gene bank of *S. chirayita* in Sikkim Himalàya. However, steady decline in germination from initial to final test (24 month storage) in *S. chirayita* indicates the seeds are destined to deteriorate over time, even under appropriate conventional storage. The increase in seed germination percentage from initial upto 12 months storage in Sc10 reveals that seeds of *S. chirayita* from high altitude (2841m asl) attains physiological dormancy, and dry storage at 4^{0}C upto 6 to 12 months helps in breaking seed dormancy.

In *S. chirayita*, storage (4^{0}C) proved quite effective up to 12 months, suggesting valuable genetic material for propagation, retaining high seed longevity and providing an environment for minimum physiological and pathological deterioration (Williams, 2000). However, using of seeds after 6 month storage helps in reducing the germination time. Positive response of seeds after storage at 4^{0}C responds after 6 months (Butola and Badola, 2004ab) to 12 months (Chen *et al.*, 2007) have been reported. This is important for scheduling spring/summer sowing in field, an appropriate time for many Himalayan herbs (Badola and Singh, 2003; Badola and Butola, 2003, 2005). In addition, the result of our study has broad implications for people who stores seed for longer period. In order to understand the germination potential of seeds of important plant species, it is imperative to test seed within a few days after the collection.

A majority of present populations of *S. chirayita* showed high seed germination, in general, irrespective of altitude or habitat differences indicating that a majority of sites in Sikkim offer suitable growing conditions for *S. chirayita*, which is an encouraging finding useful to restoration of this critically endangered species (Anonymous, 1997). Still, slow disappearance of this species from nature apart from anthropogenic pressure is a matter of concern, which may require further field studies on seedling growth and survival.

7.5 Conclusion

This study concludes that Sikkim Himalaya has high potential gene bank of *S. chirayita*. The seed of *S. chirayita* responds best if sown within 12 months after storage at 4^0C and more suitably after 6 months and scheduling seed sowing in pre-monsoon for best results, instead of immediate sowing after collection.

Table 14. Seed characteristics of different populations of *Swertia chirayita* in Sikkim

Name of Population	Altitude (m asl)	No. seeds/fruit	Seed weight (mg/50 seeds)	Seed length (µm)	Seed width (µm)	Moisture content (%)
P1-Luing (ES)	2126	212	1.6	487.33	355.67	23.47
P2 - Railgaon (ES)	1948	302	1.5	534.83	381.83	20.54
P3 – U Pangthang (ES)	2176	316	1.6	503.00	381.50	27.15
P4 - Guransey dara (ES)	2107	230	1.1	457.30	356.17	24.39
P5 - Jaunbari (SS)	1651	221	1.3	452.33	376.67	35.16
P6 - U Chamgaon (SS)	1583	233	1.3	432.17	343.33	20.60
P7 – Tiffin dara (SS)	1667	227	1.2	435.83	335.83	16.24
P8 – U Changrang (ES)	2055	275	1.7	497.33	370.83	17.32
P9 – Phinsyonala (NS)	1761	233	1.2	446.00	347.00	38.23
P10 – Zemathang-1 (NS)	2841	249	1.3	461.67	333.17	42.61
P11 – Khecheopalri (WS)	1867	200	1.4	429.00	221.17	25.47
(P<0.05)		**81.84**	**0.16**	**29.53**	**38.25**	**7.01**
F		**5.36**	**15.08**	**11.07**	**20.86**	**12.60**

Table 15. Result of Multivariate ANOVA depicting the effect of population, storage period and their interaction on seed germination and mean germination time in *Swertia chirayita*

	Population (P)		Storage Period (SP)		P x SP	
	F value	**Probability**	**F value**	**Probability**	**F value**	**Probability**
Germination (%)	77.705	.000	1169.434	.000	18.802	.000
MGT	22.367	.000	218.493	.000	21.231	.000

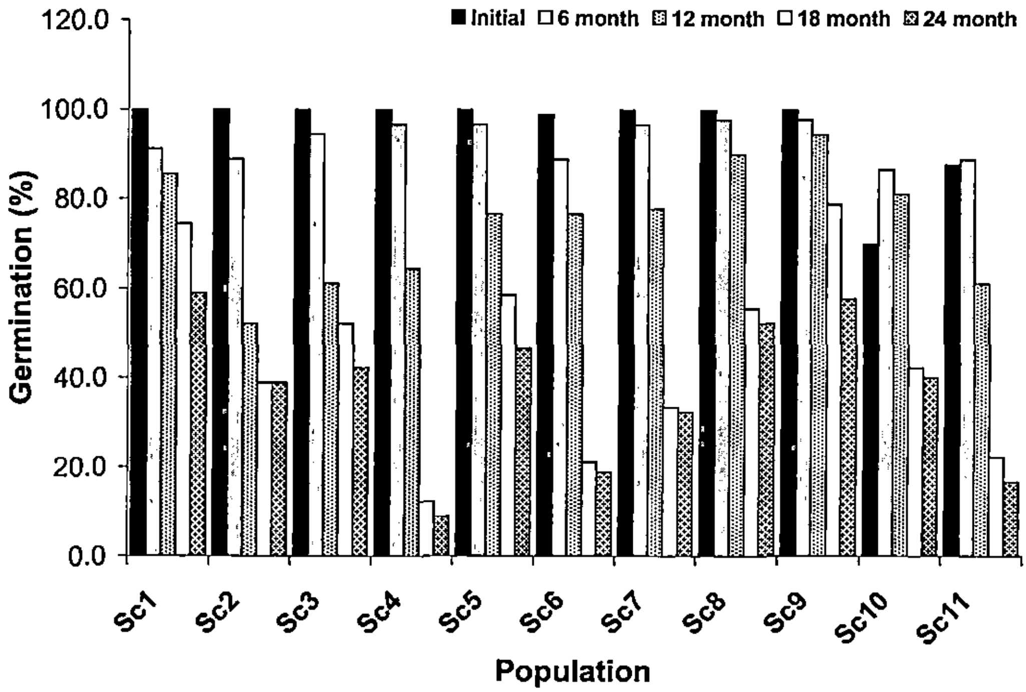

Figure 27. Effect of storage period on seed germination in populations of *Swertia chirayita*

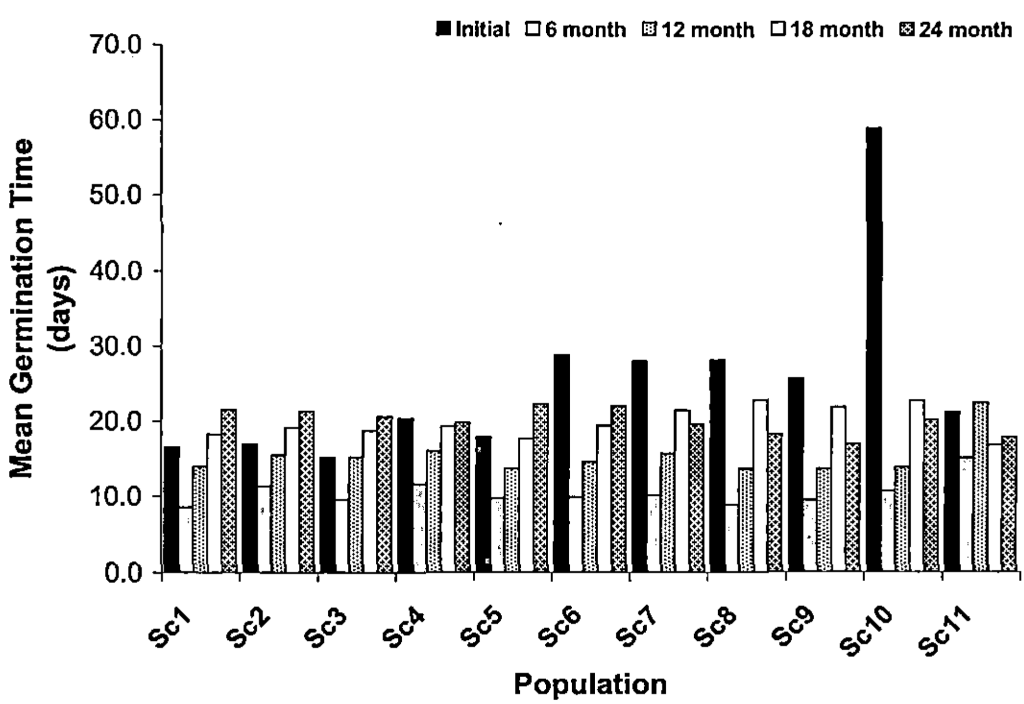

Figure 18. Effect of storage period on mean germination time in populations of *Swertia chirayita*

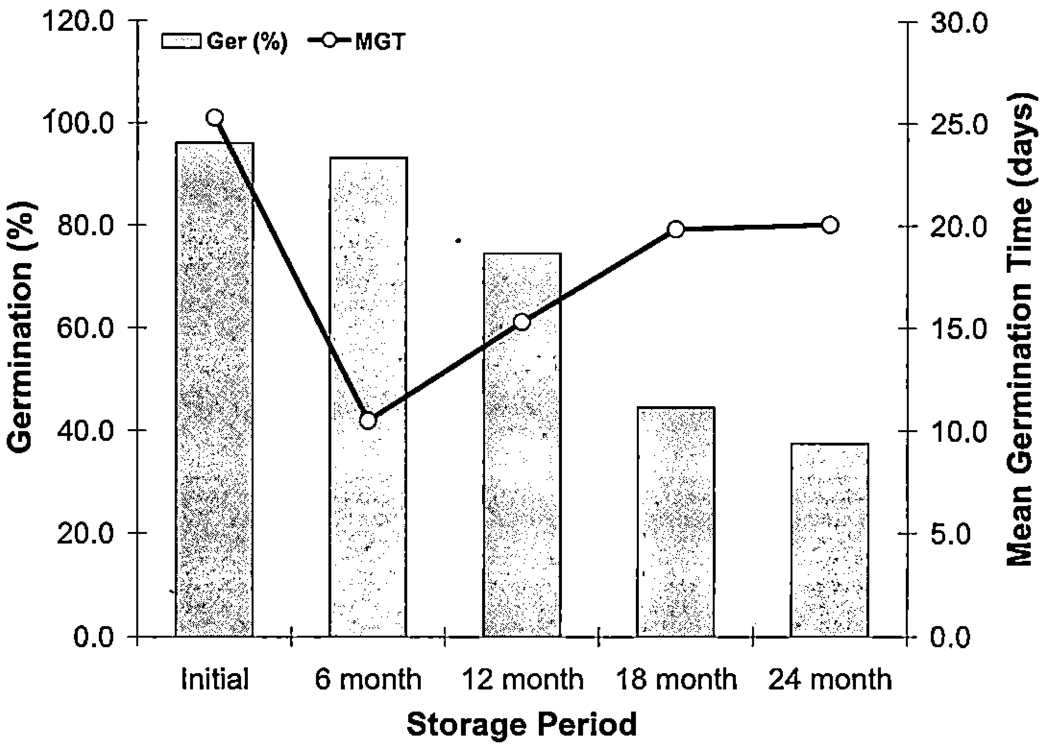

Figure 19. Effect of storage period on seed germination and mean germination Time in *Swertia chirayita*