CHAPTER 8

Assessment of Seedling Emergence and Vigour for Quality Planting Material

8.1 Introduction

Indian Himalaya, an immensely rich reservoir of medicinal plant resources, has been targeted to harvest several high value species in meeting out ever-expanding raw material demand of the pharmaceuticals (Badola and Aitken, 2003). However, with growing herbal market in recent few decades, owing to over harvesting and unsustainable extraction practices several medicinal plant species have faced risk of their survival in natural habitats, and many assessed as threatened (Badola and Pal, 2002, 2003). Since, the raw material supply to herbal industry is a societal requirement, and conservation of genetic material is a vital concern towards its safeguard for long-term survival in nature. *Ex-situ* cultivation by developing suitable and easy to handle propagation and agro-technique packages for the farmers are the only answer to meet-out raw material supply of the pharmaceuticals, ensuring lessening harvesting pressures on natural habitats, thus helping *in-situ* conservation of threatened taxa (Nadeem *et al.*, 2000; Badola and Butola, 2005; Badola, 2009). Developing quality planting material is the primary requirement for successful cultivation of wild medicinal plants, especially Himalayan (Badola and Butola, 2003; Butola and Badola, 2006ac). The success of productive cropping will largely depend upon the appropriate choice of growing media or substrate combinations and in many Himalayan herbs, these substrates have proved promising to achieve improved seedling emergence, growth and crop productivity (Nautiyal *et al.*, 2001, 2003b; Pandey *et al.*, 2002; Badola and Butola, 2003; Butola and Badola, 2006b).

Swertia chirayita (Roxb. ex Fleming) H. Karst, a critically endangered herb, indigenous to temperate Himalaya, distributed along 1200 m – 3000 m asl is considered non toxic and safe in ethnomedicinal use (Nadkarni, 1998). The herb is exploited for bitter bioactive compounds including amarogentin, xanthones and iridoid glycosides (Jensen and Schripsema, 2002). The beverage industry has discovered an alternative bitter product from *S. chirayita* (i.e. used in the liquor industries to impart bitter flavour to mouth). *S. chirayita* is valuable Ayurvedic medicinal herb, having high demand in Indian and international markets (Badola and Pal, 2002), and possess multiple use value in local medicine (Pradhan and Badola, 2008). Seeds are the only means of propagation in *S. chirayita* and poor seed germination and low seed viability in its natural environment has been considered as limitation to commercial cultivation of the species (Badola and Pal, 2002; Chaudhari *et al.*, 2007). The reason for poor natural regeneration in *S. chirayita* may be that the seed matures in dry period (November-December) where the availability of moisture in the natural environment is very low. The seed emerges out in late May prior to start of monsoon shower and the species does not get the required moisture for sufficient germination. The present authors field experiences reveals that the immature fruits of *S. chirayita* provides shelter and the necessary nutrients required for the growth and development of insect larvae which prohibits the fruit from further development and could be another factor resulting in poor natural regeneration in *S. chirayita.*

Micro propagation of *S chirayita* has been tried by several workers (Wawrosch *et al.*, 1999; Ahuja *et al.*, 2003; Chaudhari *et al.*, 2007, 2008, 2009; Joshi and Dhawan, 2007; Balaraju *et al.*, 2009b; Wang *et al.*, 2009). There are not many reports on studies relating to germination and viability of *S. chirayita* seeds. Raina *et al.*, (1994) reported 91% seed germination after chilling to 3^0C for 15 days as compared to 3.4% germination in control seeds in Himachal population; Chaudhari *et al* (2007) reported difficulty in germination. However, the present study reports 100% seed germination, initially, under laboratory conditions, without any treatment in majority of the natural populations and also 5 populations retaining above 50% germination after 18 months of storage at 4^0C in *S. chirayita* in Sikkim (refer chapter 7). Nevertheless, physiological dormancy was observed in seeds procured from domesticated plants, and that was broken by suitable chemical treatments (refer chapter 11). In commercial cultivation of the species, besides having slow growth rate (Basnet, 2001), the variability in commencement of seedling emergence has been identified as the major problem resulting in the production of non-uniform seedlings; therefore, the need of the hour was to develop uniform and vigorous seedlings for successful and productive crop cultivation of the species.

In view of this, the present study aimed to (1) standardize effective substrate combination for obtaining uniform and vigorous seedlings, and (2) identify morphological traits for the assessment of healthy seedlings as quality planting material for cultivation in *Swertia chirayita*.

8.2 Materials and methods

8.2.1 Experimental design

The matured fruits of *Swertia chirayita* were collected from thirteen populations (Table 16) distributed in different parts of Sikkim Himalaya (27°04′46″ and 28°07′48″ N lat. and 88°00′58″ and 88°55′25″E. long.) during the month of November – December. The collection of fruits/seeds was made sustainably in such a way that 15-20 robust plants per site were randomly selected from the middle of each population without disturbing the other plants keeping in view that the seeds are the only means of reproduction in endangered species *S. chirayita*; collection from the margins was avoided to prevent the natural spreading of the species and to limit the edge effect, if any. The collected fruits were brought to the laboratory and seeds were segregated by mashing up fruits which followed the thorough cleaning to remove impurities. The seeds were dried in ambient room temperature for ten days, and stored in refrigerator (4^0C) in properly sealed specimen tubes separately for different populations till nursery experiment initiated. Following good result at the initial test in the laboratory (refer chapter 11); the seeds were tested under nursery conditions in the end of February 2006 using garden soil [pH: 6.43±0.05; moisture (%): 42.00±2.00; nitrogen (%): 0.40±0.11; phosphorus (%): 0.41±0.05], forest humus [pH: 5.91±1.00: moisture (%): 42.67±2.31; nitrogen (%): 0.43±0.01; phosphorus (%): 0.29±0.04], farm yard manure [pH: 8.32±0.06; moisture (%): 46.39±0.17; nitrogen (%): 6.57±0.02; phosphorus (%): 7.29±1.25], and sand [pH: 7.17±0.02; moisture (%): 6.40±0.96; nitrogen (%): 0.25±0.03; phosphorus (%): 0.02±0.02]. At first, two substrate combinations of equal volume were prepared, substrate1 (pH: 6.5): Garden soil + Sand + Forest humus (1:1:1) and substrate 2 (pH: 7.08): Garden soil + Sand +

Farm yard manure (1:1:1), and substrate combinations were mixed well. These substrate combinations were filled in earthen trays. Trays filled with sand and Garden soil in 3:1 ratio (pH: 6.7) were considered as control. The seeds of *S. chirayita* were soaked overnight in double distilled water before sowing. Each set of experiment had three replicates of 30 seeds per replicate; the seeds were sown at 0.5cm depth with equal distance between seeds (Photo Plate IV). The experiment was conducted under an automatic temperature controlled green-house ($25^0C \pm 5^0C$). Mist irrigation was done twice a day (0930 hrs and 1630 hrs) till the completion of the seedling emergence which was reduced to once in a day (0930 hrs) after seedling emergence stopped providing optimal moisture to growing plants. Weeding was done at equal interval.

In another set of experiment, 100 seeds per population (Sc1, Sc2, Sc3 and Sc4) were sowed directly in the natural habitat viz., steep forest slope and mild forest slope, and were monitored for seedling emergence.

Seedlings after the completion of emergence (eight weeks) were thinned to mitigate competition, maintaining 8-10cm distances. After 24 weeks, the effect of different substrate combinations on seedling growth, different morphological traits were evaluated by harvesting five seedlings each of uniform size for all populations. During experiment, the seedlings remained in rosette form; the identified traits included collar diameter, largest leaf length, leaf width and number of roots and biomass. To obtain dry biomass, after recording the fresh weight, seedlings were oven dried at 60^0C to constant weight and reweighed and the value obtained was recorded.

8.2.2 Seedling survival

For seedling survival test, fifteen seedlings, of more or less uniform size, per substrate (five each of three replicates), including control, were transplanted in 20 x 20 cm distance under a net shade in the Institute's experimental medicinal plant garden and monitored by the end of growing season. Populations were not taken into consideration for observing survival. Plant survival for each substrate combinations and control set was recorded.

8.2.3 Statistical analysis

SPSS 10.0 for Windows (SPSS Inc. 1989) was used for statistical analysis of the data. Mean emergence rate (*e*) was calculated following Labouriau (1970) as, $e = 1/\sum(xd)/\sum t$; where, x = number of newly emerged seedlings in each day d, and t = total number of seedling emerged at the end of the test. Multivariate analysis of Variance (MANOVA) was carried out to determine the effect of the substrate combinations concerning on the seedling emergence and seedling morphological traits. A Bonferroni test ($p<0.05$) was used to determine differences amongst the treatments. Pearson's correlation analyses were performed to analyze the relationship between different morphological traits for each substrate combinations including control. To identify the best substrate combinations, Seedling Vigour Index (V) was calculated following Butola and Badola (2004) as $V = (d/e)*100$; where, "*d*" is dry weight/seedling; "*e*" is mean emergence rate and 100 is constant.

8.3 Results

Direct sowing in the natural habitat resulted in very low seedling emergence in all the four populations in both the conditions; however, seedling emergence was higher in mild forest slope compared to steep forest slope (Figure 20).

Multivariate analysis of variance (MANOVA) revealed significant effect of populations, treatments and their interaction on various seedling emergence parameters and growth parameters in *S. chirayita* (Table 17 and Table 18).

8.3.1 Effect of substrate combinations on seedling onset, T50 and final emergence

Minimum of four weeks and maximum of five weeks were taken for the onset and completion of 50% seedling emergence (T_{50}), and minimum of five weeks and maximum of seven weeks were taken in completing final emergence in all the populations irrespective of substrate combinations, including control (Figure 21). However, seedling onset, T_{50}, and final emergence was significantly faster in Substrate 1 ($p<0.05$) than Substrate 2 and control.

8.3.2 Effect of different substrate combinations on seedling emergence, mean emergence time and emergence rate

Seedling emergence and altitude of populations (seed collection site) showed negative (non-significant) correlation with all the substrate combinations including control (substrate 1, r = -0.304; substrate 2, r = -0.092; control, r = -0.163). Seedling emergence was significantly higher in substrate 2 ($p<0.05$) over substrate 1 and control. Similarly, Substrate 1 recorded higher seedling emergence over control ($P<0.05$). The lowest (Sc10; 2841m asl) and the highest (Sc11; 2000m asl) seedling emergence in control and substrate 1 and the lowest (Sc6; 1583m asl) and the highest (Sc13; 1867m asl) seedling emergence in substrate 2 were significant at $p<0.05$ level (Figure 22). Mean emergence time was significantly lower in substrate 1 over substrate 2 and control ($p<0.05$); likewise, it was significantly lower in substrate 2 compared to control ($p<0.05$). Comparatively, seedling emergence rate was significantly ($p<0.05$) higher in seeds sown in substrate 1 than substrate 2 and control (Figure 21).

8.3.3 Effect of different substrate combinations on seedling growth, biomass and vigour

Both substrate 1 and substrate 2 combinations were significantly ($p<0.05$) effective in improving all the seedling growth parameters over control in all the populations. However, on comparing between substrate 1 and substrate 2, the latter substrate combination was more effective ($p<0.05$) than former, but in the case of number of leaves the effectiveness was non-significant. Figure 23 and Figure 24 depict the effect of both the substrate combinations including control on various seedling growth parameters in *S. chirayita*. Significant ($p<0.05$) increase in total seedling biomass and SVI was obtained with substrate 1 and substrate 2 over control; however the best result achieved with substrate 2 ($p<0.05$) than substrate 1 (Figure 25). Of 13 populations assessed, seven (Sc1, 2, 3, 8, 9, 11, and 13) exhibited higher total seedling biomass and SVI using substrate 2, of which, Sc2 population followed by Sc8 recorded the highest values. For Sc6, the total seedling biomass and SVI was significantly greater with substrate 1 than substrate 2 ($p<0.05$). All the growth parameters were significantly ($p<0.0001$) correlated with each other (Table 19).

8.3.4 Effect of different substrate combinations on seedling survival

Seedlings developed using substrate 1 and substrate 2 combinations resulted in 77% and 83% survival, respectively, in nursery beds compared to control (63%). During the experimentation period, no insect infestation was observed in the seedlings.

8.5 Discussion

Difference in germination behaviour between the plant populations (Baskin and Baskin, 2001; Cerabolini *et al.*, 2004) has been indicated in earlier studies. Very low seedling emergence in *S. chirayita* sown directly in the natural habitat may be due to washing away of seeds or percolation of seeds to deep under ground due to heavy rain. The present study considered that appropriate substrate combinations are essential for the successful establishment of a species. Higher seedling emergence with substrate 1 and substrate 2 combinations compared to control in the present study further strengthens the above assumption. Of the two substrate combinations, substrate 2 resulted in higher seedling emergence in all the populations than substrate 1, which further significantly enhanced the seedling performance for different growth parameters and resulted in higher percent survival, as well, suggesting substrate 2 as the best substrate combination for *S. chirayita*. The present results are based on a large number of populations and mild use of manure, where all populations exhibited higher performance for both seedling emergence as well as seedling growth, especially greater seedling vigour in majority of populations. That means, the present substrate combination offers wider application for *S. chirayita* in cultivation programme. This is particularly applicable for developing quality planting material, a pre-requisite to successful cultivation of *S. chirayita*, this is also important, as the direct sowing in *S. chirayita* is not suggestive as in the case of *Heracleum candicans* (Badola and Butola, 2003) but the transplantation of one season old seedling performed well (refer chapter 10 and chapter 13). Butola and Badola (2006c) in their study recorded highest seedling emergence with the mixture of sandy loam and vermi-compost and sandy loam and forest humus (mixture in equal proportion, 1:1) in *Angelica glauca* and *Heracleum candicans*, respectively, suggesting that the species responds differently in their nutrient requirements.

The light requirement of the species for germination ensures that the seeds will germinate successfully on or near the soil surface, when other conditions are favourable for seedling emergence. In the present study, the seeds were sown at 0.5cm depth and showed positive result. Similar results have been earlier reported for Himalayan herbs (Butola and Badola, 2005, 2006c, 2007).

Germination of seeds may be inhibited if the amount of water is too low or if it is too much (Baskin and Baskin, 2001). In case of *Swertia chirayita*, healthy and robust form of the species in open slopes compared to flat lands in natural habitats suggested that the water requirement of the species is very low (*personal observation*). Therefore, beside appropriate substrate combinations, proper irrigation in terms of volume and timing is also essential to maximize production of high quality plant material and optimum percent survivability. Warren and Bilderback (2004) suggests an early morning hour (before 10.00 hrs) as suitable time to irrigate plants to reduce potential of wind blowing the irrigation water from the targeted area and to reduce evaporation of irrigation water. Irrigation in the form of mist has resulted in high percent emergence, as the seeds were protected from being swept away by the force of the water due to its minute size, thereby giving high survivability in *S. chirayita* in the present study.

Identifying appropriate morphological traits helps in assessing plant health and productivity (Butola and Badola, 2004b). For example, strong correlation of collar diameter to total seedling biomass suggested that these traits are of great use in pre-assessing crop productivity. The plant height could not be considered for identifying seedling vigour in *S. chirayita* as in the case of *Angelica glauca* (Butola and Badola, 2004b) because, for the first two years the *S. chirayita* plants remains in rosette form and merely exceeds 15 cm height. Instead, collar diameter provides a general idea about the seedling durability and has been considered as one of the best predictors of field survival and healthy growth (Thompson, 1984), which is supported by significantly strong and positive correlation between collar diameter and total seedling dry biomass ($r = 0.864$; $p<0.0001$) and between collar diameter and SVI ($r = 0.841$; $p<0.0001$) in case of *S. chirayita* in present study.

8.6 Conclusion

The present study concludes that substrate 2 (garden soil, sand, farm yard manure in equal proportion) is the most effective substrate combinations resulting in high percent emergence, uniform and vigorous seedlings and high field plant survival after transplantation. In addition, the collar size appeared to be the strongest morphological trait by which the seedling vigorousness could be identified, as an aid to successful *ex-situ* cultivation of high value medicinal crop, *Swertia chirayita.*

Photo Plate IV. A and B: Testing of seedling emergence with substrate combinations in *Swertia chirayita*

Table 16. Site characteristics of the populations of *Swertia chirayita* in Sikkim

Site code	Site Name	Altitude (m)	Aspect	Slope (°)	GPS	
					Latitude (N)	Longitude (E)
Sc1	Luing (ES)	2126	NE	55	27°21'47.60"	88°34'12.17"
Sc2	Railgaon (ES)	1948	NE	40	27°21'52.71"	88°33'47.07"
Sc3	U Pangthang (ES)	2176	SW	45	27°21'13.54"	88°33'53.37"
Sc4	Guransey dara (ES)	2107	SE	60	27°20'51.02"	88°34'07.92"
Sc5	Jaunbari (SS)	1651	SW	50	27°11'47.20"	88°23'33.60"
Sc6	U Chamgaon (SS)	1583	NW	55	27°11'03.80"	88°22'26.20"
Sc7	Tiffin dara1 (SS)	1668	W	60	27°11'14.8"	88°22'46.01"
Sc8	U Changrang (ES)	2055	S	65	27°21'51.97"	88°35'20.31"
Sc9	Phinsyonala (NS)	1761	NE	55	27°37'01.6"	88°37'26.2"
Sc10	Zemathang 1 (NS)	2841	NE	60	27°44'34.7"	88°32'48.0"
Sc11	L. Pangthang (ES)	2000	N	40	27°21'49.52"	88°33'32.15"
Sc12	Kalipokhari (ES)	2450	N	50	27°21'27.31"	88°33'24.18"
Sc13	Khecheopalri (WS)	1867	SW	55	27°21'06.07"	88°11'21.06"

Table 17. Result of MANOVA characteristics by populations, treatments and their interaction on *Swertia chirayita*

Dependent variables	Population (P)		Treatment (T)		P x T	
	F value	Probability	F value	Probability	F value	Probability
Seedling onset	3.407	0.000	38.778	0.000	3.991	0.000
T_{50}	2.111	0.025	19.030	0.000	2.058	0.009
Final emergence	5.086	0.000	13.000	0.000	4.363	0.000
Emergence (%)	8.587	0.000	34.595	0.000	3.566	0.000
Mean emergence time	3.808	0.000	40.388	0.000	4.786	0.000
Emergence rate	3.311	0.000	33.694	0.000	4.460	0.000

Independent variables: Population (P), Treatment (T), P x T

Table 18. Result of MANOVA characteristics by populations, treatments and their interaction on *Swertia chirayita*

	Independent variables					
Dependent variable	**Population (P)**		**Treatment (T)**		**P x T**	
	F value	**Probability**	**F value**	**Probability**	**F value**	**Probability**
Collar diameter	10.115	0.000	119.699	0.000	6.214	0.000
Number of leaves	7.354	0.000	384.623	0.000	8.772	0.000
Largest leaf length	31.524	0.000	880.279	0.000	18.160	0.000
Largest leaf width	9.576	0.000	437.101	0.000	8.233	0.000
Number of roots	10.922	0.000	182.709	0.000	6.502	0.000
Total dry biomass	12.394	0.000	241.057	0.000	0.668	0.000
Seedling vigour index	11.053	0.000	209.046	0.000	8.592	0.000

Table 19. Correlation matrix (Pearson Product-Moment Correlation Coefficient) between different growth parameters in *Swertia chirayita* after 24 weeks of sowing at *ca*. 2000m asl (n = 195; df = 193)

	Collar diameter (mm)	**Number of leaves**	**Largest leaf length (mm)**	**Largest leaf width (mm)**	**Number of roots**	**Total seedling biomass (gm)**	**SVI**
Collar diameter (mm)							
Number of leaves	0.668						
Largest leaf length (mm)	0.824	0.776					
Largest leaf width (mm)	0.773	0.798	0.908				
Number of roots	0.741	0.610	0.832	0.737			
Total seedling biomass (gm)	0.864	0.674	0.916	0.804	0.843		
SVI	0.841	0.695	0.914	0.806	0.815	0.980	

*All seedling growth parameters significant at p< 0.0001

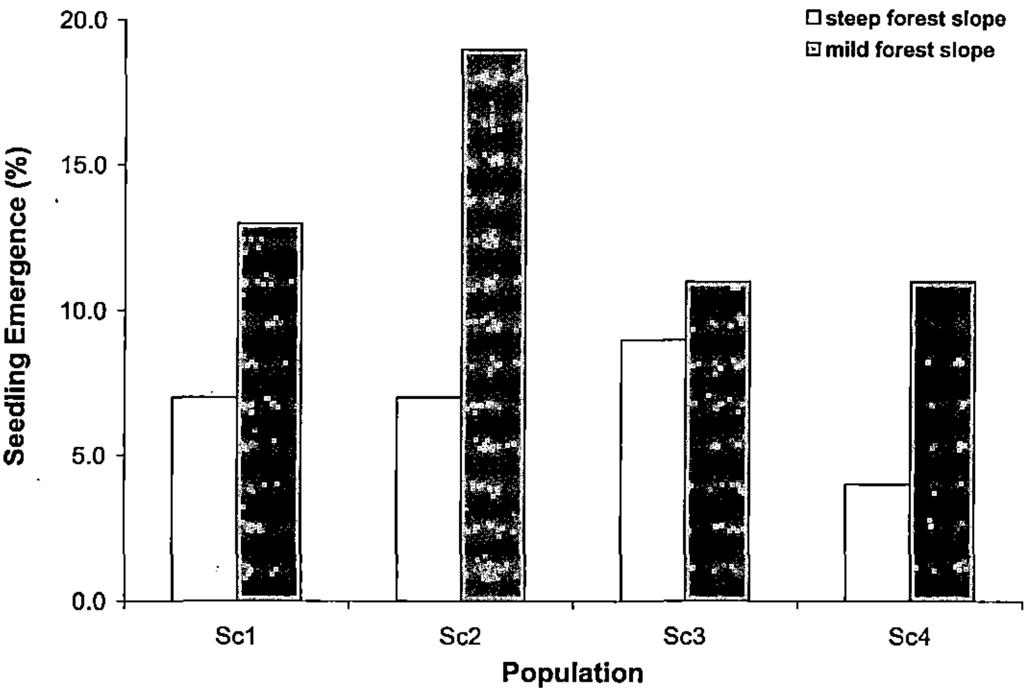

Figure 20. Seedling emergence *Swertia chirayita* as a result of direct sowing in natural habitat

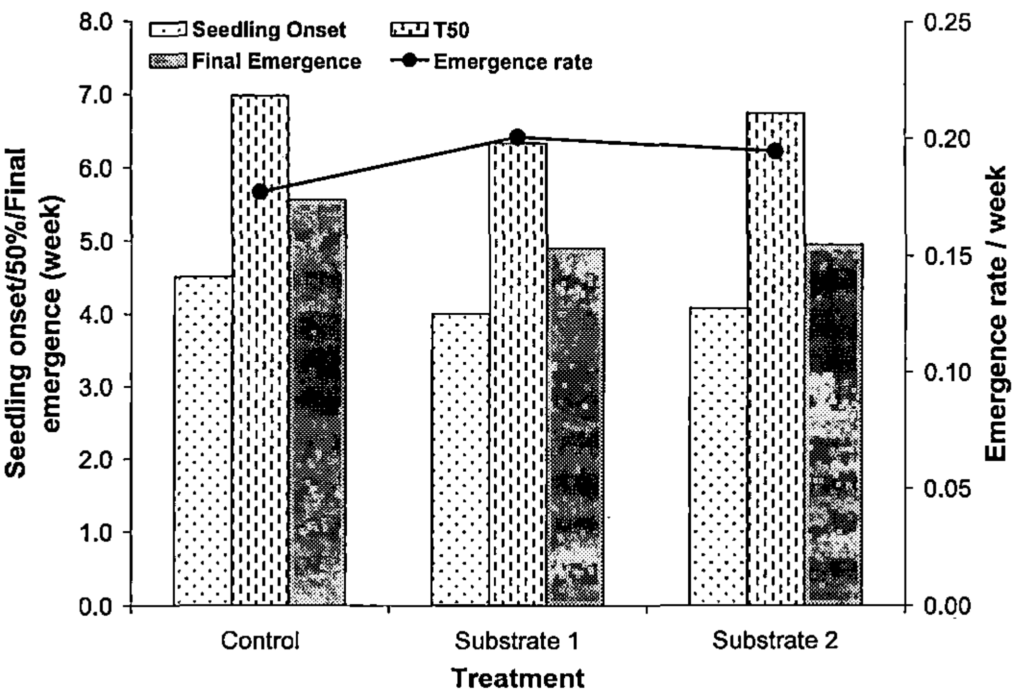

Figure 21. Effect of different substrate combinations on seedling onset, T_{50}, final emergence and emergence rate in *Swertia chirayita*

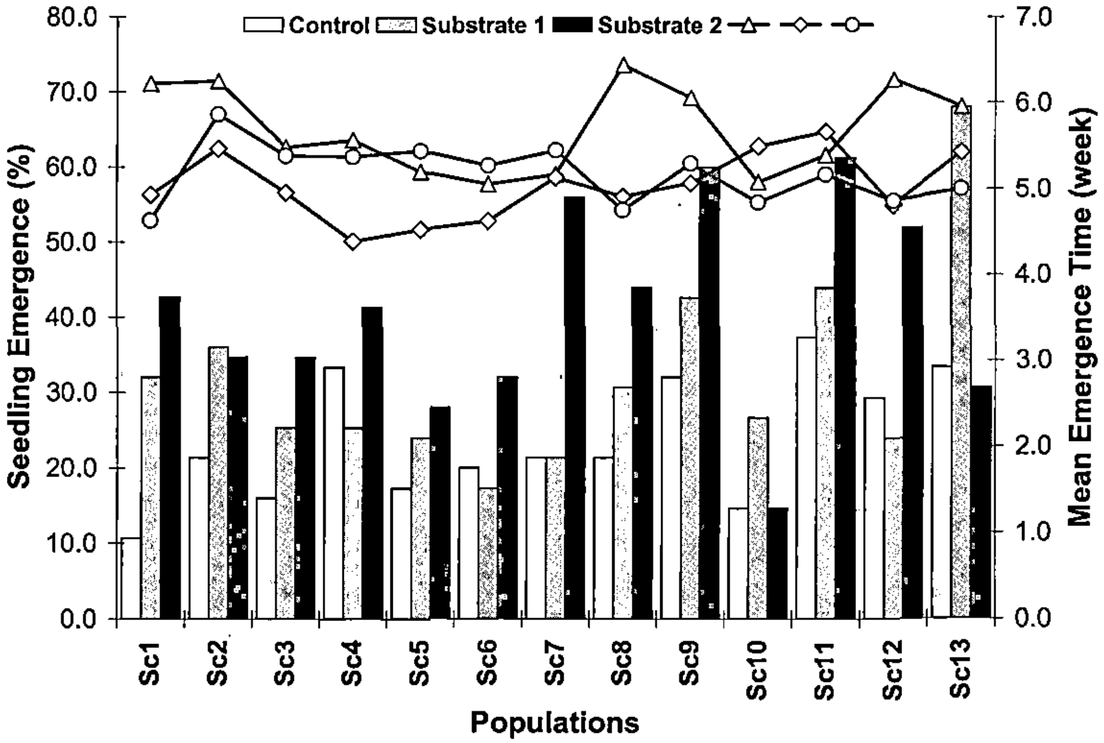

Figure 22. Effect of different substrate combinations on seedling emergence and mean emergence time in populations of *Swertia chirayita*

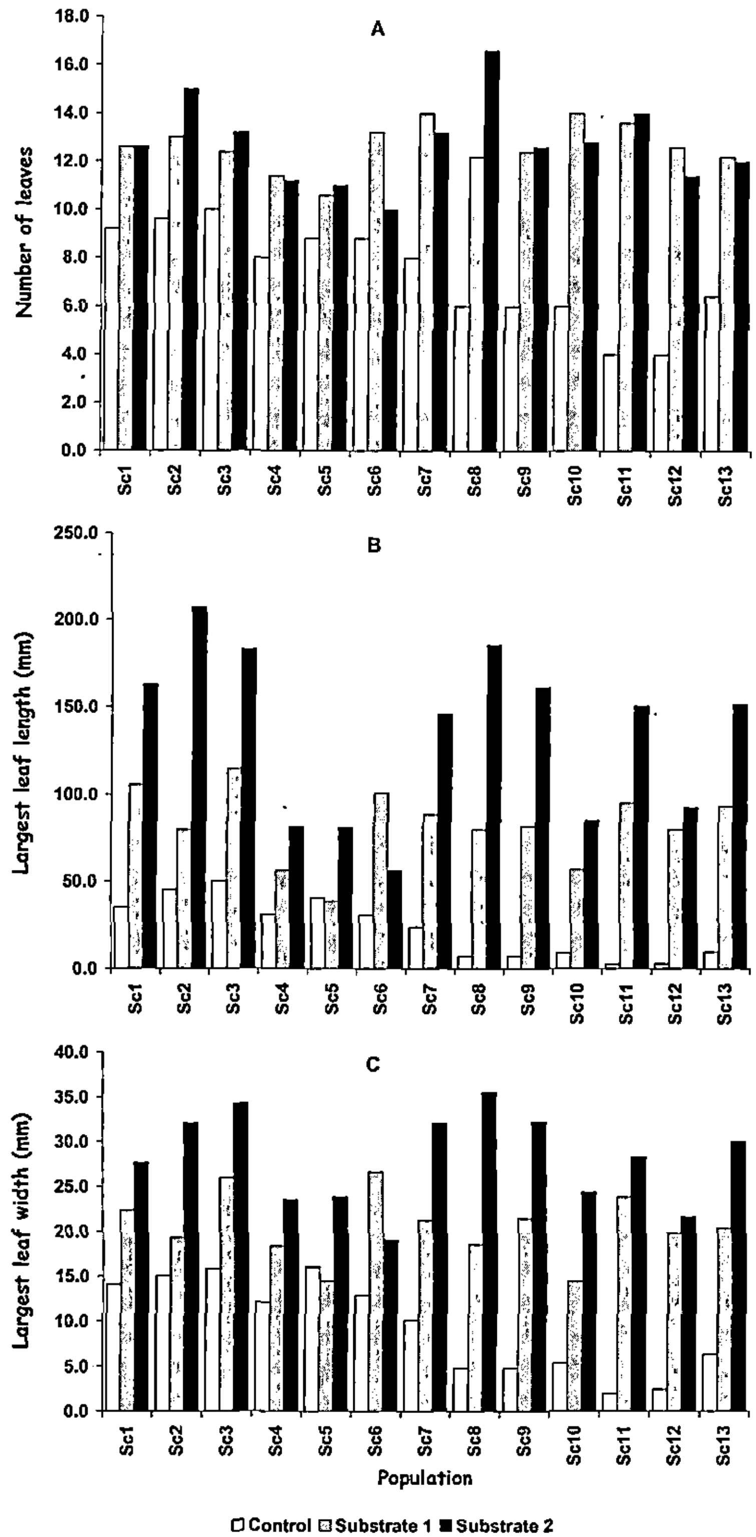

Figure 23. Effect of different substrate combinations on number of leaves, largest leaf length and leaf width in *Swertia chirayita*

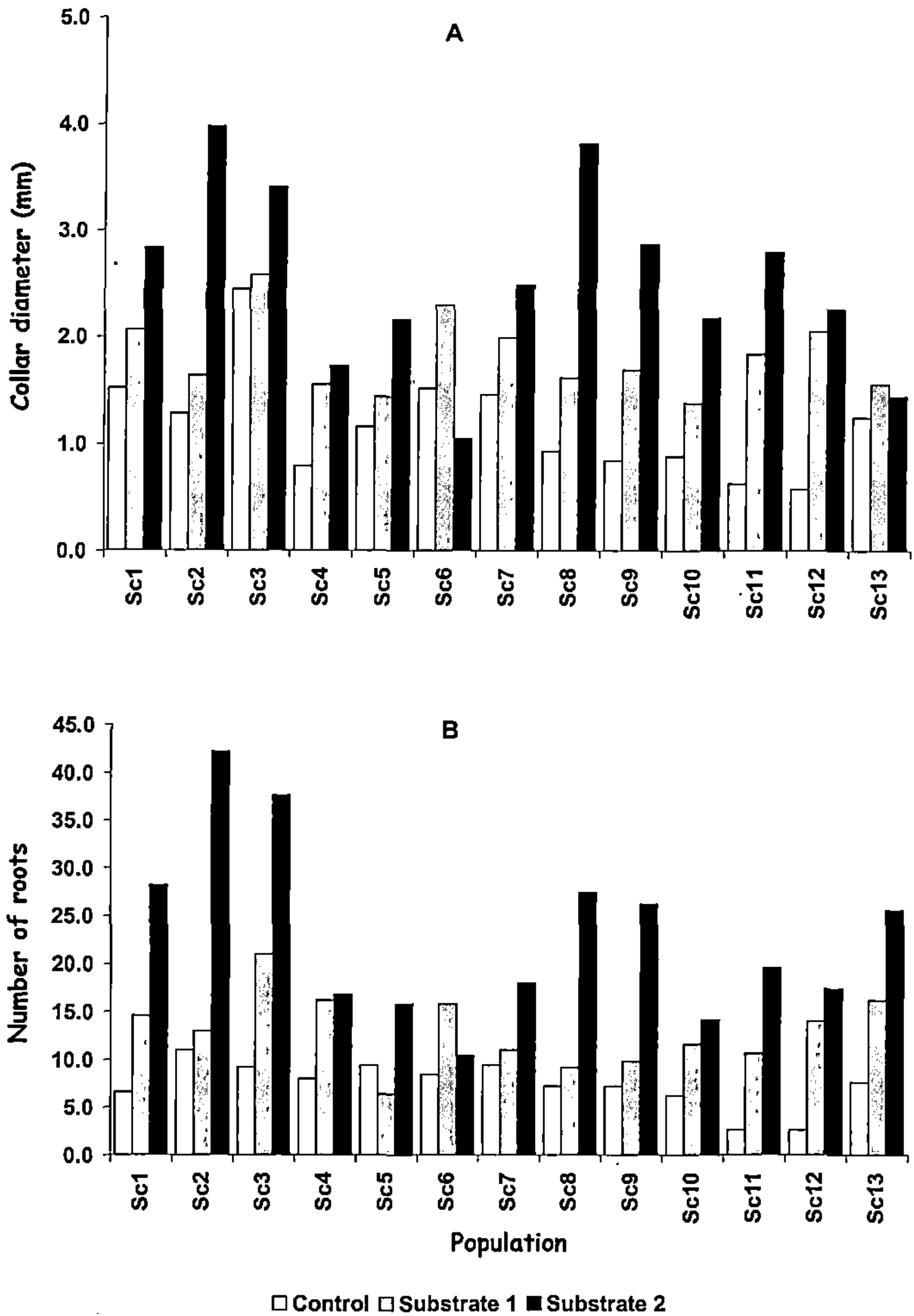

Figure 24. Effect of different substrate combinations on collar diameter and number of roots in *Swertia chirayita*

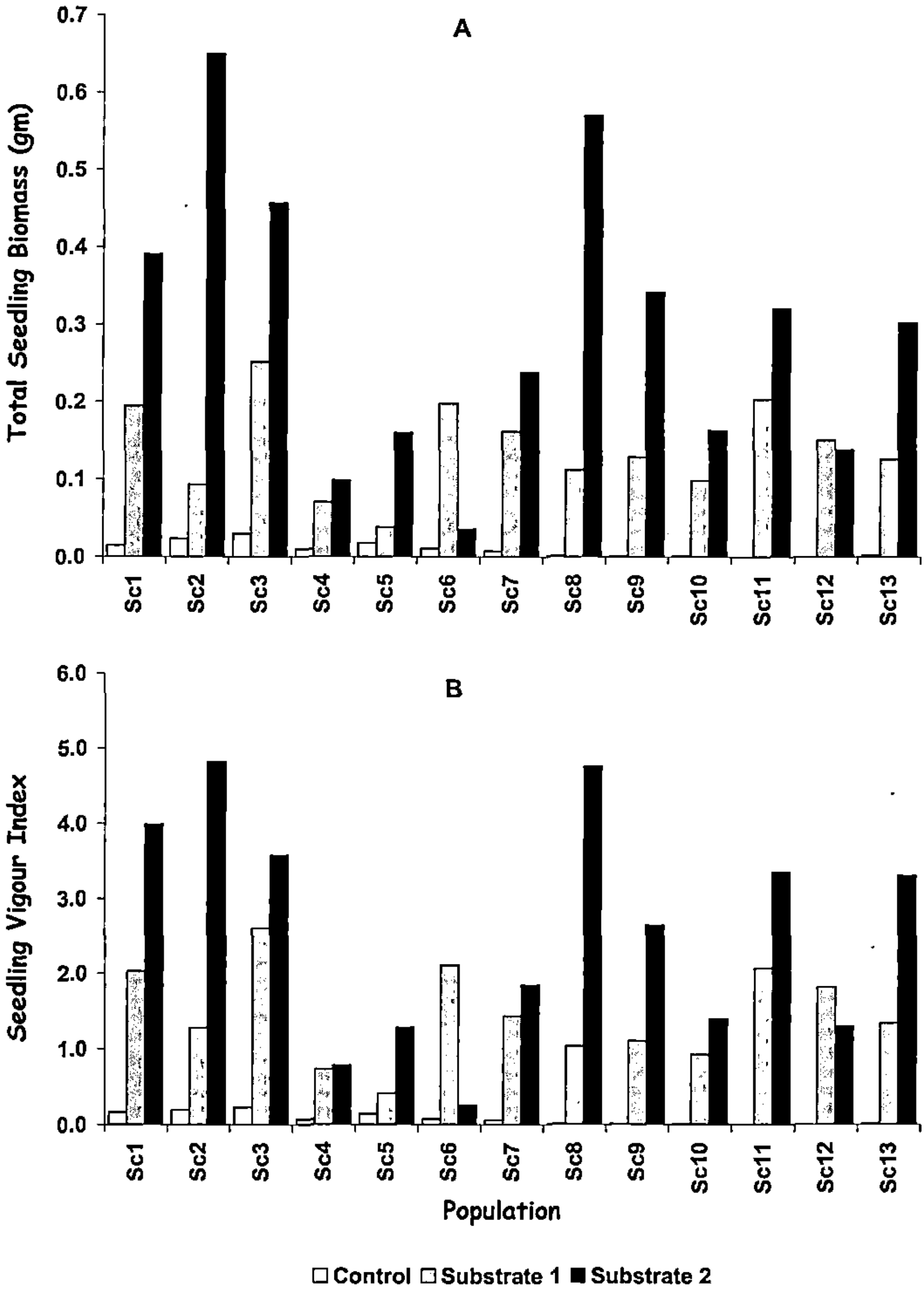

Figure 25. Effect of different substrate combinations on seedling biomass and seedling vigour in *Swertia chirayita*