CHAPTER 5

Morphological Variability amongst Populations

5.1 Introduction

Variation in plant morphology, influenced by geography, is a function of phenotypic changes in response to local environmental conditions, genetic variation and evolution among populations, and the biogeographic history of an individual species (Ellison *et al.*, 2004). Morphological variation and geographical separation among populations are equally imperative to the formation of subspecies and species (Losos and Glor, 2003). The environment, viz., light condition, climate, and competition with other species (Tesitel and Stech, 2007) is a governing factor in determining population differentiation affecting morphological features in a species and evolvement of specialized populations adapted to particular environment (Lynn and Waldren, 2001); however, the altitude and seasonal variation are amongst other major responsible factors. Assessment of differences in morphology is the traditional way of determining diversity within and between plant populations (Bayorbor *et al.*, 2010). The existence of localized populations each adapted to the particular environmental conditions of their habitat have been revealed in many plant species (Turesson, 1922; Bradshaw, 1960, 1991; McNeilly, 1968; Kik *et al.*, 1990; Geber et al., 1992; Badola and Pradhan, 2010a, 2010). Such adaptation to specific environmental conditions can occur over a relatively short span of time (Lynn and Waldren, 2001), however, such species preferring specific habitat are much prone to habitat loss and extinction than the species with broad habitat range (Badola and Aitken, 2003; Samant *et al.*, 1996). In addition, the habitat destruction and fragmentation are the major causes restricting the natural populations of important plant species to smaller pockets (Parab and Krishnan, 2008) *vis-à-vis* species extinction (Ehrlich and Ehrlich, 1981). Deleterious effects on the genetic diversity within a species can be resulted due to fragmentation of natural plant communities, because there will be a decrease in levels of gene flow, particularly over longer distances (Ouinsavi and Sokpon, 2010). The subsequent effects of genetic drift in small, isolated populations will be the loss of diversity,

inability of plants to adapt to changes in their environment and ultimately increasing the risk of extinction (Wilson and Provan, 2003). At this juncture, it would be vital to know if such fragmentation leads to inter population variation. In addition increasing human populations and land use intensification (e.g., cultivation, grazing, and urban development) have (i) resulted in the loss and subdivision of native habitats, (ii) increased species extinction rates, and (iii) lowered species diversity (Ouinsavi and Sokpon, 2010). Such circumstances call for the establishment of a strategy to conserve the important species *in-situ* (Karam *et al.*, 2006) which will provide an opportunity for the researchers to understand the evolutionary processes of the plants (Arafeh *et al.*, 2002). In addition, an evaluation of the germplasm in nature generally provides an idea about suitable climatic conditions for domestication and cultivation of an important plant species; and, on the basis of phenotypic traits, the elite germplasm can be identified for crop improvement (Vashistha *et al.*, 2006).

Swertia chirayita, a high value medicinal herb, has huge demand in the national and international market (Badola and Pal, 2002). Its increasing demand has led to unsustainable harvesting from nature resulting in restricting its distribution to small pockets (ecodemos) and specific habitats in the Himalayan belts. These habitats are being destroyed at an alarming rate due to natural as wells as man made calamities resulting in changes in the immediate environment. Also, the species has wide range of altitudinal distribution in Sikkim from 1500m to 3000m and is not unusual to undergo changes within the species to adapt itself to varying climatic conditions. This necessitates evaluating the forgone changes within and amongst the populations of *S. chirayiata* to adapt itself to changing environmental conditions. The present study was undertaken with broad aims to, (1) assess the most discriminating morphological character for evaluating variations amongst the natural populations in *S. chirayita*; (2) based on morphological traits, the identification of superior germplasm for domestication, crop improvement and conservation of the species; (3) determination of appropriate morphological trait to evaluate the below ground biomass; (4) assess the adaptive mechanism to withstand the varying climatic conditions. The outcome will provide helpful clues and suitable approaches for the sustainable use of the herb as medicine in future, as well as for the *in-situ* conservation of the species.

5.2 Materials and Methods

5.2.1 Material

Morphometrical data were collected from 22 natural populations covering all the four districts (east, west, north and south) in Sikkim. Two forms (rosette and reproductive) of *S. chirayita* plants were collected during the end of the growing season (November-December) [Table 5 and Table 6]. From each population, 9 plants each (both form) were randomly selected maintaining at least 2-3 meter distance between each individual plant to avoid sampling clones. For each population, the broad habitat and other topographical features were observed. The altitude, aspect and slope of the site, including latitudinal and longitudinal coordinates and humus depth were recorded (refer chapter 4).

5.2.2 Morphological characters

In the laboratory, the plant materials were thoroughly washed to remove the soil particles and wiped with tissue roll to remove the additional moisture. In the case of rosette form, the morphological characters measured included collar diameter, root diameter, number of leaves, largest leaf length, largest leaf width, root length while in the case of reproductive form, plant height, stem basal diameter and number of branches in addition to the above characters, were taken into account. After recording the fresh weight, the collected materials were oven dried at 70^0C to constant weight in order to obtain the plant biomass.

5.2.3 Statistical analysis

The SPSS 10.0 for windows (SPSS Inc. 1989) was used for statistical interpretations of the data. Multivariate analysis of variance (MANOVA) was conducted to determine the variation and if significant variation existed, Bonferroni ($p<0.05$) test was performed to test the significant difference in means of the recorded growth parameters amongst populations. The coefficient of variation for morphological characters was calculated for each population. Canonical discriminant analysis (CDA) was used to determine the most variable morphological character marking differences amongst populations. Based on the average linkages (between groups) and Pearson's

correlation, a dendrogram was constructed to evaluate the levels of variation amongst populations. Pearson's correlation coefficient analysis was performed to evaluate the correlation amongst the different morphological characters in both rosette and reproductive form; however, here, the populations were not taken into account.

5.3 Results

In the case of the rosette form of plants, the recorded morphological characters in *S. chirayita* except the number of leaves were positively correlated with altitude but showed negative correlation with the humus depth; the growth parameters were positively correlated with soil pH except leaf length (Table 7). Similarly, in reproductive form, all the recorded morphological characters showed positive correlation with the altitude except for the plant height and number of branches; however, the parameters were negatively correlated with the humus depth and positively correlated with soil pH (Table 7). In terms of total plant biomass, altitude and soil pH showed positive correlation while humus depth showed negative correlation in rosette form of *S. chirayita*. In reproductive form, altitude, humus depth and soil pH had positive effect on the total plant biomass (Table 7).

Variability in microhabitat condition has been observed in each of the sites in the present study which resulted in differences in morphological characters amongst different populations in *S. chirayita*. All the recorded morphological characters are presented in Table 5 (rosette form) and Table 6 (reproductive form). One way multivariate analysis of variance (MANOVA) revealed a significant ($p<0.0001$) variation in the recorded morphological characters including total plant dry biomass amongst populations in both rosette and reproductive forms in *S. chirayita* (Table 8). The collar diameter ranged from 2.73 ± 0.4 (Sc5) to 7.99 ± 1.1mm (Sc21) in rosette form; similarly, the value ranged from 4.82 ± 1.1mm (Sc1) to 10.38 ± 2.1mm (Sc17) in reproductive from in *S. chirayita*; however, the difference was significant ($p<0.05$) in both the forms. In terms of root diameter, Sc5 recorded the lowest value (2.3 ± 0.2mm) while Sc21 recorded the highest value (6.64 ± 0.9mm) in rosette form; the value was lowest for Sc22 (4.22 ± 0.8) and highest for Sc17 (10.98 ± 3.6mm) and the difference was significant ($p<0.05$) in both forms. In rosette form, the root length

ranged from 41.66 ± 6.7mm (Sc16) to 539.78 ± 154.7mm (Sc20); while in reproductive form, the root length ranged from 104.44 ± 15.9 (Sc4) to 358.89 ± 103.6mm (Sc1) and the difference was significant ($p<0.05$) in either from of *S. chirayita*. The number of leaves was significantly ($p<0.05$) high in Sc8 (15.56 ± 2.8) and Sc1 (15.0 ± 2.2) compared to lowest record for Sc12 (7.78 ± 2.0) in rosette form; similarly, in reproductive form, Sc6 recorded significantly (246.56 ± 151.2; $p<0.05$) highest number of leaves compared to all the populations except Sc17, for which the value was insignificant. The lowest value for the largest leaf length (31.05 ± 12.7mm) and leaf width (17.68 ± 1.8mm) recorded for Sc2 was significantly ($p<0.05$) lower than majority of the populations in rosette form; nevertheless, in reproductive form, the largest leaf length ranged from 55.19 ± 14.1mm (Sc22) to 189.53 ± 18.9mm (Sc11) and the largest leaf width ranged from 20.89 ± 2.2mm (Sc2) to 50.93 ± 6.8mm (Sc17) and the difference was significant ($p<0.05$) for both leaf length and width. In terms of plant height, Sc17 recorded the maximum plant height (134.1 ± 23.7cm) followed by Sc18 (121.0 ± 24.2cm), Sc5 (120.0 ± 5.7cm), and Sc20 (110.4 ± 17.7cm) and the value was significantly ($p<0.05$) greater compared to Sc11 (20.11 ± 1.7cm) and Sc 4 (39.56 ± 6.6cm), which recorded the minimum plant height than other populations (Table 6). Significant ($p<0.05$) difference in stem basal diameter was recorded between the highest record for Sc11 (7.58 + 1.9mm) and the lowest for Sc7 (2.69 + 0.2mm). In rosette form, in respect of total biomass, the highest value was recorded for Sc20 (4.8 + 1.9) and Sc17 (4.73 + 1.4); however the difference was insignificant compared to other populations (Figure 10). In reproductive form, significantly ($p<0.05$) highest total plant biomass (17.79 + 7.9gm) was recorded for Sc17 compared to all other populations (Figure 10); similarly, the high total plant biomass (10.66 + 12.2) recorded for Sc3 was significant ($p<0.05$) compared to majority of the populations.

In rosette form (Figure 11A, 112B), of the six morphological character recorded, most variations were observed in the root length with coefficient of variation ranging from 14.23% to 69.58% followed by root diameter (4.66% to 48.06%). Largest leaf length (3.62% to 40.89%) and largest leaf width (4.92% to 40.80%) subsequently followed the root diameter. The number of leaves showed lowest coefficient of variation value

(8.75% to 32.18%) followed by collar diameter with coefficient of variation value ranging from 7.07% to 37.37%. In reproductive form (Figure 12A, 12B, 12C), of the nine morphological characters identified, the number of branches recorded the highest coefficient of variation value ranging from 0% to 84.16% followed by number of leaves (8.59% to 83.65%) and root diameter (4.78% to 55.79%). The coefficient of variation value ranged from 3.24% to 43.30% (plant height), 3.45% to 49.95% (largest leaf length), 3.51% to 40.63% (largest leaf width) and 9.31% to 47.14% (root length). The lowest coefficient of variation value was recorded for the stem basal diameter (4.97% to 38.24%), followed by collar diameter (6.63% to 41.17%). In terms of total plant biomass, highest variation was recorded for Sc3 (reproductive) and Sc21 (rosette) while the lowest variation was observed in Sc18 (reproductive) and Sc10 (rosette) [Figure 13].

A cluster analysis, based on average linkage (between group) method and Pearson's correlation, divided the entire populations into three clusters in rosette (Figure 14A) and six clusters in reproductive form (Figure 14B) of *S. chirayita*. All the recorded morphological characters, except few, in both rosette (Table 9) and reproductive form (Table 10) in *S. chirayita* showed positive correlation with each other. In rosette form, the result of canonical discriminant analysis (CDA) indicated that, the morphological characters viz., leaf length; root length and collar diameter was the main discriminating character and were mainly found to be responsible for the total variation amongst the populations in *S. chirayita* (Table 11). The three morphological characters cumulatively accounted for 87.7% of the total variation. Leaf length alone accounted for 50.7% of the total variation; root length resulted for 20.7% variation and collar diameter accounted for 16.3% variation. Similarly, in reproductive form, the plant height, number of leaves, leaf width and stem basal diameter were mainly responsible for the total variations amongst the populations in *S. chirayita*, which cumulatively accounted for 83.6% variation (Table 12). On individual basis, the plant height, number of leaves, leaf width and stem basal diameter accounted for 42.6%, 19.6%, 13.0% and 8.5% variations.

5.4 Discussion

The microhabitat has direct influence on plant growth and vigour; at the same time soil pH and humus depth provides a good indication of the chemical status of soil and can be used to determine the plant growth potential. Increasing soil pH (Martensson, 2010) and altitude (Vare, 1997) decrease the soil nutrient. This might be the reason for positive correlation between the total plant biomass with increasing altitude and soil pH in the present study with *S. chirayita*. Several limiting factors, viz., altitude, competition amongst species for limited resources, disturbance, etc., regulate growth in plant species as observed in the present study. Plant height and altitude are negatively correlated (Kofidis et al., 2007); however, at the same time, the plant height and leaf number provide a measure of photosynthetic and transpiration area and are indicators of the plant quality (Ritchie, 1984). The statement is insufficiently suggestive for the present study because plant species modifies itself to adapt to the changing environment. Even though, in the present study, reduction in plant height and leaf number in *S. chirayita* was observed with increasing altitude; nevertheless, increase in the collar size, root size and leaf size (leaf length and width), which itself are the indicators of healthy plants, with altitude was recorded in both rosette and reproductive form. The positive correlation of the collar diameter and root diameter with below ground biomass further strengthens present findings that the plant quality does not merely depend on plant height and leaf number. Further, the decrease in the photosynthetic and transpiration area caused by reduction in plant height and number of leaves with altitude is nullified by increase in the leaf size (leaf length and width) with altitude.

In rosette form, population Sc5 which occupied bouldery habitat (refer chapter 4) recorded smallest value for collar diameter and root diameter; however, growth in terms of plant height was comparatively high in reproductive form. In adverse microhabitat conditions, plant species are prone to competition for limited available resources; however, the surviving plant species emerges as successful competitor and colonizer as it matures (refer chapter 6). This indicates that the *S. chirayita* is a successful competitor. At the same time, Sc21 which occupied forest-shrubbery

habitat recorded the high value for collar diameter and root diameter in both rosette and reproductive form; but, recorded smaller plant height revealing its sensitivity to habitat condition, as the denseness of the forest prohibited the *S. chirayita* plants from acquiring required amount of sunlight needed for photosynthesis and growth. As an adaptation to high radiation, lesser number of leaves were recorded in Sc12 followed by Sc3, which occupied completely open slope where the plants are directly exposed to the sunlight resulting in high water loss; whereas, Sc8 followed by Sc1 recorded the highest number of leaves, as these two population had occupied forest-shrubbery habitat where the shade of the nurse plant lessens rate of water loss. In reproductive form, the lowest number of leaf in Sc4 is the result of deficiency of the required nutrient and absence of sunlight as the population inhabited streamside and under dense canopy of *Cryptomeria japonica*; similarly, lower number of leaves in Sc13 is the result of disturbance in the form of frequent landslides in the study site.

Disturbance in any form inhibits plant growth and has been experienced in Sc2 with having smallest leaf size (leaf length and width) where fodder collection, trampling, water logging, *S. chirayita* collection (for domestic use) etc., were the major threats observed; similarly Sc22 recorded the smallest leaf size as the population was recovering from the disturbance caused by intense grazing in the past. As an adaptive mechanism to less soil nutrient, Sc20 followed by Sc17 recorded the longest root length because Sc20 occupied the habitat which was dominated by dense *Cryptomeria japonica* trees under which the survival of the plant species is almost impossible, and Sc17 occupied the land-slip naked slope contributing to the high total biomass. The smallest root length in Sc4 could be related to soil nutrient deficiency. In mountainous region, strong altitudinal gradient leads to dramatic variation in environmental conditions (Hong-Li *et al.*, 2009). With the increasing altitude, more environmental stresses, viz., extreme radiation, wind speed, etc., are created which greatly affects the plant growth and leaf size (Li *et al.*, 2008). Similar observations have been made in the present study where plant height decreased with altitude, as observed in other species (Bhadula and Purohit 1994; Bhadula *et al.*, 1996; Kofidis *et al.*, 2007). Nevertheless, the leaf size increased with increasing altitude; Sc11 located at highest altitude (2841m asl) of plant collection site recorded the lowest plant height in *S.*

chirayita appeared as an adaptation to extreme climatic conditions. Cumulatively, the vigorous growth and biomass was observed in Sc17 followed by Sc3 as both the populations occupied open habitat indicating this as the most suitable and preferred habitat by *S. chirayita.*. Nonetheless, less growth and biomass in Sc4 of all the population may be due to the reason that the population was located near to the stream and under dense canopy of *Cryptomeria japonica* forest creating stressful condition for *S. chirayita*. Similarly, the little growth and lower biomass in Sc9 and Sc10 was observed as both the population occupied highly disturbed area in the form of landslide, fuel-fodder collection, etc.

Cluster analysis revealed variability in morphological characters amongst the populations of *S. chirayita* in Sikkim which is further supported by difference in coefficient of variation in morphological characters within populations, indicating the role of topography, climate and microhabitat. The habitat colonized by *S. chirayita* differs from each other with regard to altitude, light, soil humus, soil moisture, slope and aspect as it is assumed that the ecological factors strongly affects genetic variation within and among populations (Nevo *et al.*, 1988; Nevo and Beiles, 1989; Kolliker *et al.*, 1998; Al-Saghir *et al.*, 2009). Location of the site may be responsible for non-clustering of Sc6 (reproductive form) and Sc16 (rosette form) with other populations. For example, Sc6 was located to near the human habitation which always faces the pressure to some extent in one or the other way. Disturbance in the form of forest litter collection for agricultural purpose, fuel and fodder collection results in trampling, which prohibits the plants from growth and development as different types of land-use practices contribute to the variation amongst populations (Poschlod and Jackel, 1993; Poschlod *et al.*, 2000). The altitude could be another factor as Sc6 occurred at the lowest average altitude (1583m) compared to other populations. Similarly, Sc16 was located at the landslide prone area where the plant completing its phenological cycle is merely by chance. In addition, fungal attack due to high moisture (Moles and Westoby, 2004ab) and plant litter (Xiong and Nilsson, 1999) under dense *Quercus lamellose* forest and the continuous flowing stream has negative impact on establishment of *S. chirayita* plants as it prefers the open dry slope, which is clear from the negative correlation of all the morphological characters with the

humus depth in either forms of *S. chirayita*, except for the number of leaves (reproductive form). The smallest root length in Sc16 further strengthens the impact of high soil nutrients on *S. chirayita* plants. Nonetheless, on the basis of cluster analysis, it is clear that when *S. chirayita* is in rosette stage, the plants marks more similarity; but, as when plant attains adult stage, the existing dissimilarity amongst populations becomes more prominent which emphasizes that reproductive stage is more important for exploring the existing morphological variations amongst populations of *S. chirayita*.

The present study revealed that, amongst different populations of *S. chirayita*, discriminating character responsible for total variation was different for the rosette and reproductive forms. The leaf length was the most discriminating character in the rosette form and the plant height emerged as the most discriminating character in reproductive form. However, it is suggested that, for identifying such character in *S. chirayita*, fully developed plant should be considered because in such plants, further development is not possible, however, as in rosette plants the growth process is continued. On the basis of above revelation, plant height emerged as the most appropriate indicator for identifying existing morphological variations amongst populations of *S. chirayita*. Further, the negative correlation between the plant height and the altitude of the site of plant collection revealed that the plant height vary with altitude supporting the above statement.

5.5 Conclusion

The present study concludes the following:

1. Several modifications undergoes in *S. chirayita* to adapt to the varying environmental conditions.
2. For evaluating the morphological variations amongst populations, fully matured plants rather than rosette individuals should be considered in *S. chirayita*.
3. Collar diameter is the good indicator for determining root biomass without undergoing destructive harvesting in *S. chirayita*.

4. Plant height is the most appropriate indicator that can be considered for identifying existing morphological variations amongst populations in *S. chirayita*.
5. Of several studied populations, Sc17 followed by Sc3 can be considered as the healthiest germplasms and can be used for their domestication *vis-a-vis* conservation of the species.
6. *S. chirayita* does not have rigid habitat preferences; however, the species performs well in open slope facing south-east, south-west, east and south direction.
7. The present findings should be considered as baseline for the future studies (recommended), by other researchers, at genetic level to determine extent of genetic variability amongst different populations in *S. chirayita* in Sikkim.

Table 5. Morphological differences amongst populations of *Swertia chirayita* (rosette) in Sikkim (Mean ± SD)

Pop.	Collar diameter (mm)	Number of leaves	Root diameter (mm)	Root length (mm)	Largest leaf length (mm)	Largest leaf width (mm)
Sc1	6.60 ± 1.5	15.00 ± 2.2	5.59 ± 1.4	271.89 ± 104.2	41.45 ± 8.1	19.70 ± 2.9
Sc2	5.28 ± 0.8	11.44 ± 1.9	4.07 ± 0.7	222.00 ± 84.9	31.05 ± 12.7	17.68 ± 1.8
Sc3	4.41 ± 1.2	8.89 ± 2.0	4.03 ± 1.4	226.67 ± 89.3	36.87 ± 9.1	19.72 ± 3.4
Sc4	3.96 ± 0.8	10.78 ± 2.0	3.19 ± 0.6	222.33 ± 154.7	31.66 ± 8.0	20.11 ± 2.8
Sc5	2.73 ± 0.4	11.22 ± 1.6	2.30 ± 0.2	188.56 ± 62.5	110.70 ± 12.0	31.31 ± 2.8
Sc6	4.33 ± 1.3	13.11 ± 2.7	3.25 ± 1.1	165.22 ± 32.5	143.38 ± 33.4	36.85 ± 4.5
Sc7	4.17 ± 1.6	13.67 ± 2.0	3.05 ± 1.0	184.00 ± 48.3	95.87 ± 23.3	31.25 ± 8.5
Sc8	5.22 ± 1.6	15.56 ± 2.8	3.88 ± 0.8	201.44 ± 60.3	136.96 ± 25.5	32.77 ± 11.2
Sc9	4.81 ± 1.3	12.67 ± 3.0	4.33 ± 1.3	205.56 ± 69.6	139.95 ± 28.2	30.50 ± 4.0
Sc10	3.76 ± 0.3	11.11 ± 1.8	3.33 ± 0.3	160.22 ± 28.8	85.26 ± 5.7	17.95 ± 0.9
Sc11	6.17 ± 1.8	12.67 ± 3.6	5.17 ± 2.5	200.67 ± 78.7	147.21 ± 31.6	32.43 ± 12.6
Sc12	3.99 ± 0.6	7.78 ± 2.0	3.23 ± 0.8	179.89 ± 65.1	115.35 ± 21.5	22.76 ± 3.1
Sc13	4.56 ± 1.0	11.11 ± 2.1	3.72 ± 0.8	230.11 ± 56.3	159.34 ± 45.7	30.34 ± 4.9
Sc14	4.42 ± 1.1	13.56 ± 3.0	3.83 ± 1.0	252.89 ± 99.5	168.33 ± 10.0	31.97 ± 4.6
Sc15	4.43 ± 1.3	11.56 ± 3.4	3.91 ± 1.4	223.33 ± 76.0	141.18 ± 28.1	29.31 ± 8.4
Sc16	4.73 ± 0.8	13.56 ± 4.4	4.32 ± 0.8	41.66 ± 6.7	132.97 ± 26.9	29.86 ± 6.8
Sc17	6.30 ± 1.3	14.00 ± 1.2	5.16 ± 1.1	214.00 ± 71.7	155.02 ± 45.3	37.91 ± 3.2
Sc18	5.37 ± 0.4	13.78 ± 2.0	4.18 ± 0.2	189.67 ± 32.8	124.41 ± 4.5	34.81 ± 2.59
Sc19	4.46 ± 1.0	12.67 ± 1.9	3.88 ± 1.0	145.22 ± 34.1	139.37 ± 38.2	35.19 ± 5.4
Sc20	5.93 ± 1.8	14.67 ± 2.5	6.10 ± 2.6	539.78 ± 154.7	175.60 ± 38.0	37.24 ± 6.1
Sc21	7.99 ± 1.1	14.78 ± 2.9	6.64 ± 1.5	274.33 ± 107.4	143.36 ± 48.8	37.58 ± 10.7
Sc22	7.18 ± 1.3	12.56 ± 2.5	5.64 ± 0.9	282.56 ± 40.2	43.43 ± 14.4	22.92 ± 9.4

Table 6. Morphological differences amongst populations of *S. chirayita* (reproductive) in Sikkim (Mean ± SD)

Pop	Plant height (cm)	Stem basal diameter (mm)	Collar diameter (mm)	Number of branches	Number of leaves	Root diameter (mm)	Root length (mm)	Largest leaf length (mm)	Largest leaf width (mm)
Sc1	93.44 ± 23.7	4.49 ± 1.1	4.82 ± 1.2	11.56 ± 5.5	91.56 ± 40.5	4.81 ± 1.3	358.89 ± 103.6	98.13 ± 15.3	23.88 ± 3.1
Sc2	99.56 ± 14.0	4.75 ± 0.7	6.74 ± 0.9	14.78 ± 5.5	56.44 ± 47.0	5.98 ± 0.8	308.89 ± 132.1	108.44 ± 12.4	20.89 ± 2.2
Sc3	99.78 ± 6.3	7.12 ± 2.7	9.65 ± 3.6	21.67 ± 1.9	58.33 ± 48.8	8.28 ± 3.2	225.56 ± 95.7	119.43 ± 24.7	21.98 ± 1.8
Sc4	39.56 ± 6.6	3.54 ± 0.3	4.94 ± 0.6	---	10.33 ± 1.7	4.58 ± 0.5	104.44 ± 15.9	80.04 ± 5.5	24.01 ± 0.8
Sc5	120.00 ± 5.7	4.38 ± 0.9	6.44 ± 0.9	16.22 ± 4.4	141.44 ± 6.3	5.85 ± 1.0	313.33 ± 33.5	127.94 ± 8.0	21.47 ± 1.9
Sc6	92.78 ± 14.9	5.24 ± 0.7	7.78 ± 1.7	17.22 ± 7.1	246.56 ± 151.2	6.39 ± 1.6	251.11 ± 36.6	125.77 ± 7.7	21.06 ± 1.8
Sc7	61.22 ± 5.7	2.69 ± 0.2	5.38 ± 1.5	12.67 ± 6.0	119.67 ± 51.9	5.75 ± 1.6	272.22 ± 49.2	119.70 ± 6.1	20.53 ± 2.0
Sc8	64.33 ± 10.4	3.50 ± 0.8	6.22 ± 2.3	6.78 ± 4.2	71.89 ± 33.8	5.38 ± 1.2	272.22 ± 65.5	124.24 ± 5.1	21.24 ± 2.9
Sc9	79.56 ± 7.7	5.83 ± 0.6	6.53 ± 0.6	5.33 ± 2.3	33.78 ± 10.6	6.17 ± 0.6	173.33 ± 27.4	120.80 ± 6.4	26.77 ± 2.7
Sc10	72.33 ± 2.4	5.06 ± 0.4	5.53 ± 0.4	---	18.67 ± 3.0	5.21 ± 0.3	154.44 ± 26.5	124.46 ± 4.3	26.00 ± 3.0
Sc11	20.11 ± 1.7	7.58 ± 1.9	8.15 ± 2.0	---	15.89 ± 1.4	7.75 ± 3.0	326.67 ± 30.4	189.53 ± 18.9	44.03 ± 4.7
Sc12	95.22 ± 18.0	5.08 ± 1.6	7.51 ± 1.8	11.33 ± 6.0	20.44 ± 5.3	6.11 ± 2.3	218.89 ± 68.6	96.74 ± 22.2	28.06 ± 8.6
Sc13	54.22 ± 3.0	5.33 ± 1.2	7.18 ± 2.0	---	13.78 ± 1.7	6.54 ± 1.7	207.78 ± 98.0	107.31 ± 35.9	27.52 ± 2.8
Sc14	81.44 ± 14.0	4.11 ± 1.2	5.74 ± 2.0	4.00 ± 1.7	31.56 ± 6.2	5.64 ± 1.8	237.78 ± 42.4	83.29 ± 20.1	39.90 ± 7.9
Sc15	80.44 ± 26.6	5.33 ± 1.2	8.34 ± 2.4	5.89 ± 4.38	37.22 ± 17.9	8.72 ± 3.5	241.11 ± 40.5	111.61 ± 34.3	35.31 ± 7.1
Sc16	72.67 ± 31.46	3.87 ± 1.1	5.25 ± 0.6	7.22 ± 6.1	66.44 ± 40.7	4.77 ± 0.7	285.56 ± 76.0	121.18 ± 60.5	32.67 ± 13.3
Sc17	134.10 ± 23.7	6.98 ± 1.2	10.38 ± 2.1	24.78 ± 3.8	172.56 ± 60.1	10.98 ± 3.6	326.67 ± 103.3	157.72 ± 8.1	50.93 ± 6.8
Sc18	121.00 ± 24.2	6.15 ± 2.4	8.28 ± 3.4	19.78 ± 9.1	78.56 ± 31.5	7.45 ± 2.3	327.78 ± 70.3	114.69 ± 23.4	34.05 ± 12.3
Sc19	92.89 ± 16.8	3.97 ± 0.5	5.65 ± 0.6	14.67 ± 6.4	41.67 ± 18.0	5.43 ± 0.7	224.44 ± 65.6	89.98 ± 23.0	27.30 ± 4.3
Sc20	110.44 ± 17.7	4.47 ± 1.2	6.13 ± 3.6	21.22 ± 9.7	140.33 ± 57.0	6.57 ± 3.7	278.89 ± 82.7	112.00 ± 32.9	32.23 ± 8.6
Sc21	89.33 ± 11.6	6.63 ± 0.6	9.69 ± 1.6	17.78 ± 3.0	22.67 ± 6.8	8.29 ± 1.5	332.22 ± 106.5	75.20 ± 10.1	33.79 ± 4.3
Sc22	69.44 ± 8.8	3.32 ± 0.4	5.15 ± 1.1	---	20.22 ± 6.2	4.22 ± 0.8	198.89 ± 47.0	55.19 ± 14.1	22.01 ± 2.6

Table 7. Pearson's correlation coefficient (r) and significance of correlation (P) between the means of observed parameters of *S. chirayita* with altitude and humus depth in Sikkim

	Vegetative (rosette form)						Vegetative (Stock form)					
	Altitude (m)		Humus depth (cm)		Soil pH		Altitude (m)		Humus depth (cm)		Soil pH	
Parameters	***r***	**P**	***r***	**P**	***r***	**P**	***r***	**P**	***r***	**P**	***r***	**P**
Plant height	---	---	---	---	---	---	-0.143	ns	-0.054	ns	0.229	ns
Stem basal diameter	---	---	---	---	---	---	0.275	<0.05	-0.073	ns	0.129	ns
Collar diameter	0.309	<0.05	-0.158	ns	0.178	ns	0.084	ns	-0.001	ns	0.015	ns
Number of branches	---	---	---	---	---	---	-0.005	ns	-0.003	ns	0.172	ns
Number of leaves	-0.033	ns	-0.280	<0.05	0.117	ns	-0.334	<0.01	0.028	ns	-0.154	ns
Root diameter	0.386	<0.01	-0.124	ns	0.066	ns	0.122	ns	-0.017	ns	0.096	ns
Root length	0.192	ns	-0.013	ns	0.133	ns	0.078	ns	-0.087	ns	0.113	ns
Largest leaf length	0.027	ns	-0.390	<0.01	-0.108	ns	0.092	ns	-0.005	ns	0.260	ns
Largest leaf width	0.045	ns	-0.314	<0.01	0.132	ns	0.357	<0.01	-0.155	ns	0.241	ns
Total dry biomass	0.278	<0.05	-0.152	ns	0.152	ns	0.028	ns	0.108	ns	0.245	ns

ns: not significant

Table 8. Result of MANOVA showing morphological variation amongst populations of *S. chirayita* in Sikkim

Dependent Variable	Rosette form			Reproductive form		
	df	F	Sig.	df	F	Sig.
Plant height	---	---	---	21	25.098	.000
Stem basal diameter	---	---	---	21	10.796	.000
Collar diameter	21	10.008	.000	21	6.610	.000
Branch number	---	---	---	21	19.120	.000
Leaf number	21	5.289	.000	21	16.536	.000
Root diameter	21	7.199	.000	21	5.800	.000
Root length	21	10.939	.000	21	7.335	.000
Largest leaf length	21	27.293	.000	21	13.192	.000
Largest leaf width	21	10.434	.000	21	17.127	.000
Total plant dry biomass	21	21.076	.000	21	10.900	.000

Table 9. Pearson's correlation matrix amongst populations of *S. chirayita* (rosette) in Sikkim

	Collar diameter (mm)	No. of leaves	Root diameter (mm)	Root length (mm)	Largest leaf length (mm)	Largest leaf width (mm)	Above ground fresh wt. (g)	Below ground fresh wt. (g)	Above ground dry wt. (g)	Below ground dry wt. (g)	Total plant dry biomass (g)
Collar diameter (mm)	1.000										
Number of leaves	0.382^{c}	1.000									
Root diameter (mm)	0.851^{c}	0.401^{c}	1.000								
Root length (mm)	0.365^{c}	0.167^{a}	0.372c	1.000							
Largest leaf length (mm)	0.092^{ns}	0.249^{c}	0.180a	0.076^{ns}	1.000						
Largest leaf width (mm)	0.229^{b}	0.406^{c}	0.262c	0.121^{ns}	0.722^{c}	1.000					
Above ground fresh wt. (g)	0.643^{c}	0.588^{c}	0.683c	0.355^{c}	0.329^{c}	0.448^{c}	1.000				
Below ground fresh wt. (g)	0.620^{c}	0.380^{c}	0.685c	0.601^{c}	0.190^{c}	0.306^{c}	0.659^{c}	1.000			
Above ground dry wt. (g)	0.470^{c}	0.406^{c}	0.536c	0.405^{c}	0.331^{c}	0.393^{c}	0.684^{c}	0.639^{c}	1.000		
Below ground dry wt. (g)	0.552^{c}	0.329^{c}	0.610c	0.418^{c}	0.300^{c}	0.391^{c}	0.540^{c}	0.765^{c}	0.733^{c}	1.000	
Total plant dry biomass (g)	0.532^{c}	0.404^{c}	0.599c	0.437^{c}	0.342^{c}	0.418^{c}	0.677^{c}	0.728^{c}	0.968^{c}	0.880^{c}	1.000

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$; ns: not significant

Table 10: Pearson's correlation matrix amongst populations of *S. chirayita* (reproductive) in Sikkim

	Plant height (cm)	Stem basal diameter (mm)	Collar diameter (mm)	Number of branches	Number of leaves	Root diameter (mm)	Root length (mm)	Largest leaf length (mm)	Largest leaf width (mm)	Above ground fresh wt. (g)	Below ground fresh wt. (g)	Above ground dry wt. (g)	Below ground dry wt. (g)	Total dry biomass (g)
Plant height (cm)	1.000													
Stem basal diameter (mm)	0.309^{c}	1.000												
Collar diameter (mm)	0.389^{c}	0.830^{c}	1.000											
Number of branches	0.738^{c}	0.384^{c}	0.487^{c}	1.000										
Number of leaves	0.497^{c}	0.143^{a}	0.286^{c}	0.627^{c}	1.000									
Root diameter (mm)	0.398^{c}	0.763^{c}	0.906^{c}	0.474^{c}	0.317^{c}	1.000								
Root length (mm)	0.376^{c}	0.329^{c}	0.379^{c}	0.473^{c}	0.342^{c}	0.384^{c}	1.000							
Largest leaf length (mm)	0.109^{ns}	0.462^{c}	0.329^{c}	0.232^{b}	0.300^{c}	0.369^{c}	0.319^{c}	1.000						
Largest leaf width (mm)	0.167^{a}	0.458^{c}	0.417^{c}	0.119^{ns}	0.011^{ns}	0.480^{c}	0.270^{c}	0.425^{c}	1.000					
Above ground fresh wt. (g)	0.634^{c}	0.550^{c}	0.661^{c}	0.694^{c}	0.517^{c}	0.664^{c}	0.542^{c}	0.300^{c}	0.210^{b}	1.000				
Below ground fresh wt. (g)	0.479^{c}	0.525^{c}	0.699^{c}	0.498^{c}	0.342^{c}	0.734^{c}	0.576^{c}	0.243^{c}	0.418^{c}	0.801^{c}	1.000			
Above ground dry wt. (g)	0.602^{c}	0.600^{c}	0.677^{c}	0.683^{c}	0.381^{c}	0.648^{c}	0.434^{c}	0.249^{c}	0.250^{c}	0.900^{c}	0.747^{c}	1.000		
Below ground dry wt. (g)	0.515^{c}	0.554^{c}	0.669^{c}	0.561^{c}	0.364^{c}	0.726^{c}	0.421^{c}	0.277^{c}	0.456^{c}	0.820^{c}	0.880^{c}	0.852^{c}	1.000	
Total dry biomass (g)	0.599^{c}	0.605^{c}	0.692^{c}	0.675^{c}	0.387^{c}	0.679^{c}	0.442^{c}	0.260^{c}	0.298^{c}	0.905^{c}	0.792^{c}	0.994^{c}	0.903^{c}	1.000

a: $p<0.05$; b: $p<0.01$; c: $p<0.01$; ns: not significant

Table 11. Summary of Canonical Discriminant Function of the growth parameters among populations of *S. chirayita* (rosette) in Sikkim

	Function					
	1	**2**	**3**	**4**	**5**	**6**
Collar diameter	-0.161	-0.084	1.108	-0.210	-0.206	-1.108
Number of leaves	0.091	0.016	0.357	0.487	0.884	0.055
Root diameter	-0.275	0.473	-0.394	-0.449	-0.027	1.409
Root length	0.046	0.862	-0.492	0.273	-0.010	-0.229
Largest leaf length	1.009	0.073	-0.065	-0.510	0.295	-0.213
Largest leaf width	0.057	-0.147	0.225	0.788	-0.831	0.290
Eigen value	3.630	1.483	1.168	0.480	0.276	0.116
% of Variance	50.7	20.7	16.3	6.7	3.9	1.6
Cumulative %	50.7	71.5	87.8	94.5	98.4	100.0
Canonical correlation	0.885	0.773	0.734	0.570	0.465	0.323

Table 12. Summary of Canonical Discriminant Function of the growth parameters among populations of *S. chirayita* (reproductive) in Sikkim

	Function								
	1	2	3	4	5	6	7	8	9
Plant height	1.048	-0.045	-0.161	-0.024	-0.682	0.022	-0.446	0.283	-0.010
Stem basal diameter	-0.825	-0.554	-0.078	0.621	-0.966	0.732	0.626	-0.661	0.300
Collar diameter	0.359	0.260	-0.919	0.318	0.600	-0.608	0.498	1.765	-1.202
Branches number	0.574	-0.342	0.112	0.499	0.870	-0.157	0.132	-0.652	-0.231
Leaf number	-0.130	0.794	0.547	-0.249	-0.430	-0.009	0.669	0.062	0.110
Root diameter	-0.227	-0.148	0.586	-0.403	0.254	-0.529	-0.856	-0.672	1.676
Root length	-0.062	0.068	0.196	-0.130	0.346	0.981	-0.091	0.332	0.201
Largest leaf length	-0.559	0.673	0.075	0.494	0.059	-0.207	-0.689	0.092	-0.273
Largest leaf width	-0.027	-0.703	0.819	-0.359	-0.014	-0.092	0.204	-0.018	-0.336
Eigen value	6.923	3.188	2.112	1.378	0.894	0.755	0.563	0.318	0.134
% Variance	42.6	19.6	13.0	8.5	5.5	4.6	3.5	2.0	0.8
Cumulative %	42.6	62.2	75.1	83.6	89.1	93.8	97.2	99.2	100.0
Canonical correlation	0.935	0.872	0.824	0.761	0.687	0.656	0.600	0.491	0.344

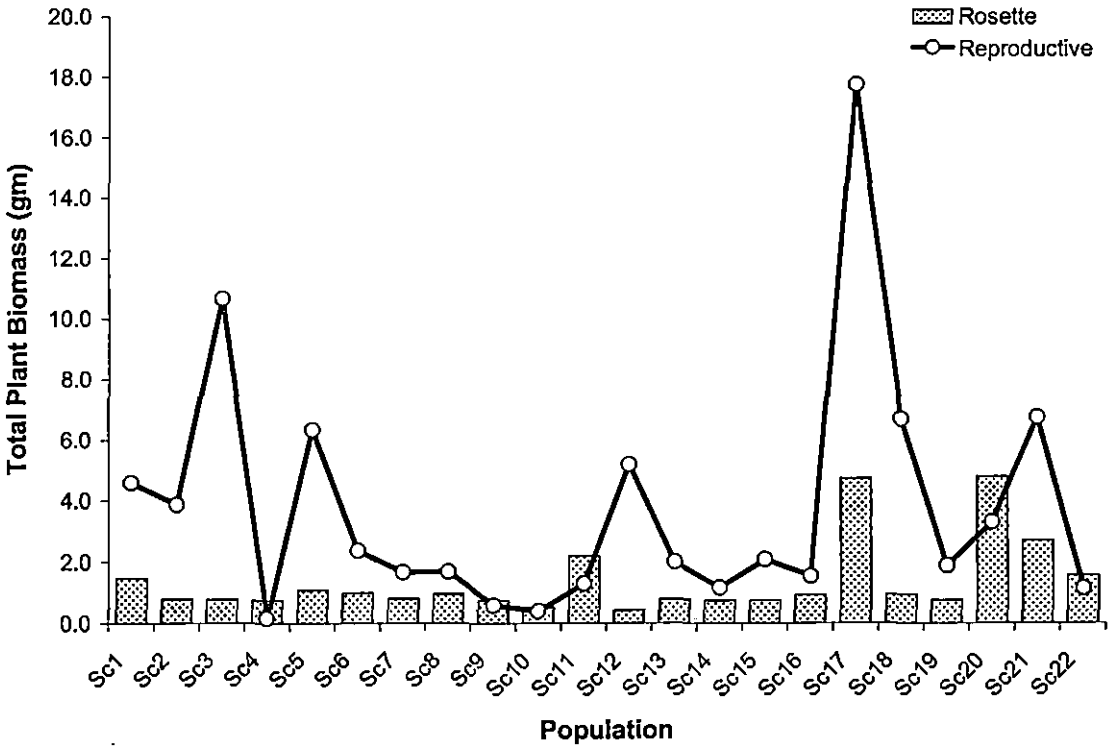

Figure 10. Difference in total plant dry biomass amongst populations of *Swertia chirayita* in Sikkim

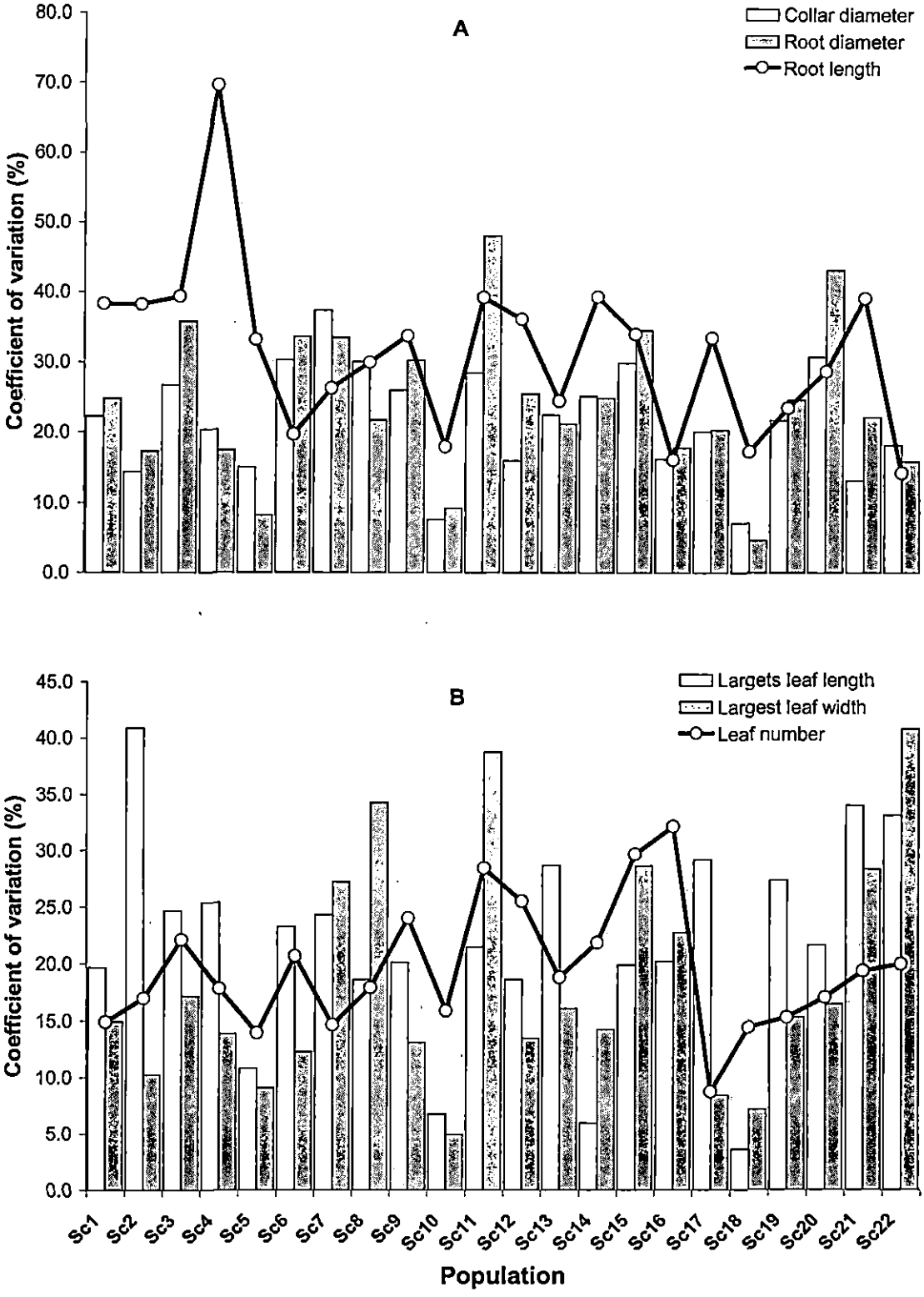

Figure 11. Coefficient of variation in morphological characters in populations of *Swertia chirayita* (rosette) in Sikkim

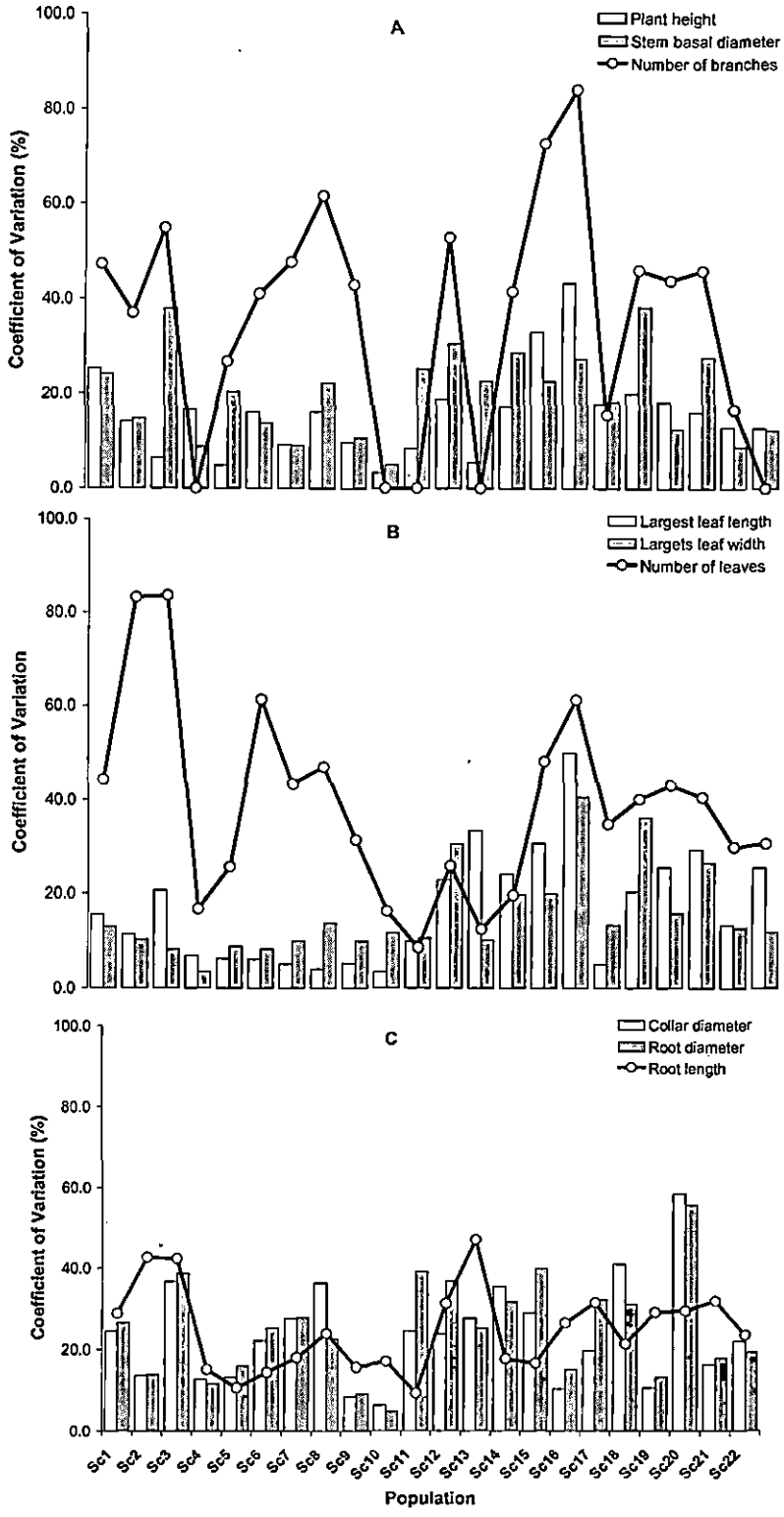

Figure 12. Variation in morphological characters in populations of *Swertia chirayita* (reproductive) in Sikkim

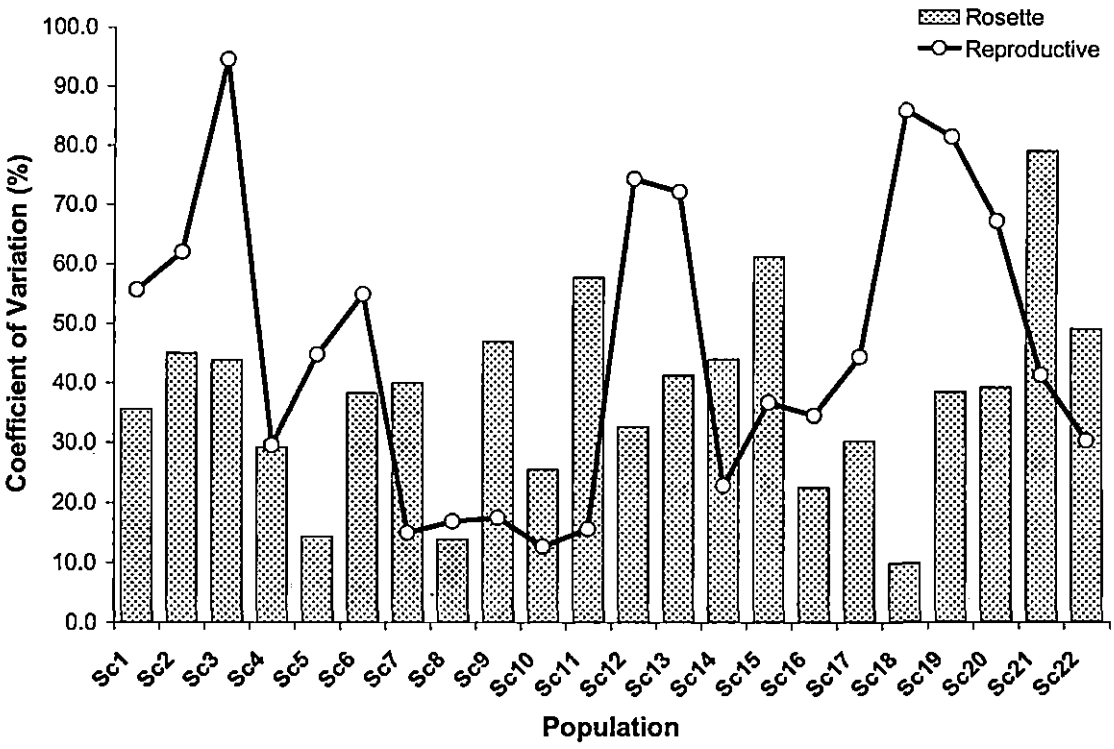

Figure 13. Variation in total plant biomass in population of *Swertia chirayita* in Sikkim

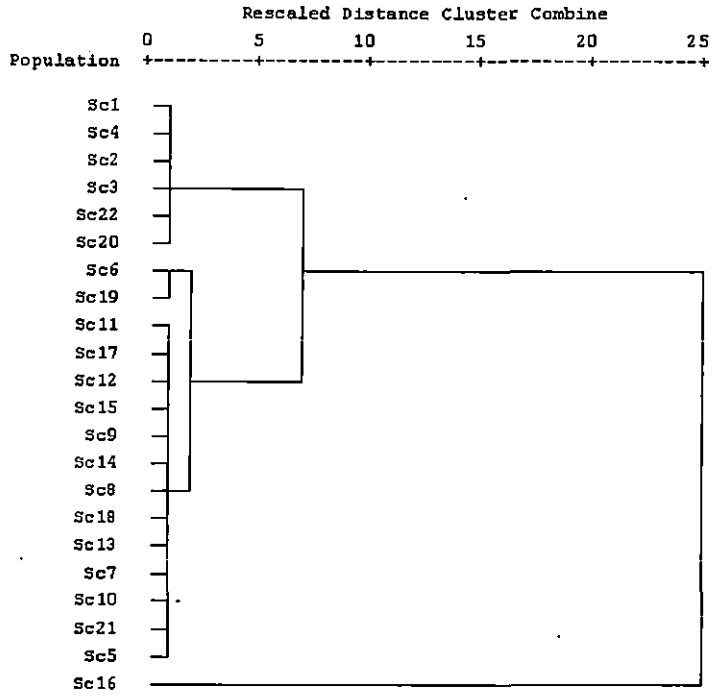

Figure 14A. Dendogram based on average linkage (between groups) method and Pearson correlation in rosette form of *Swertia chirayita* in Sikkim

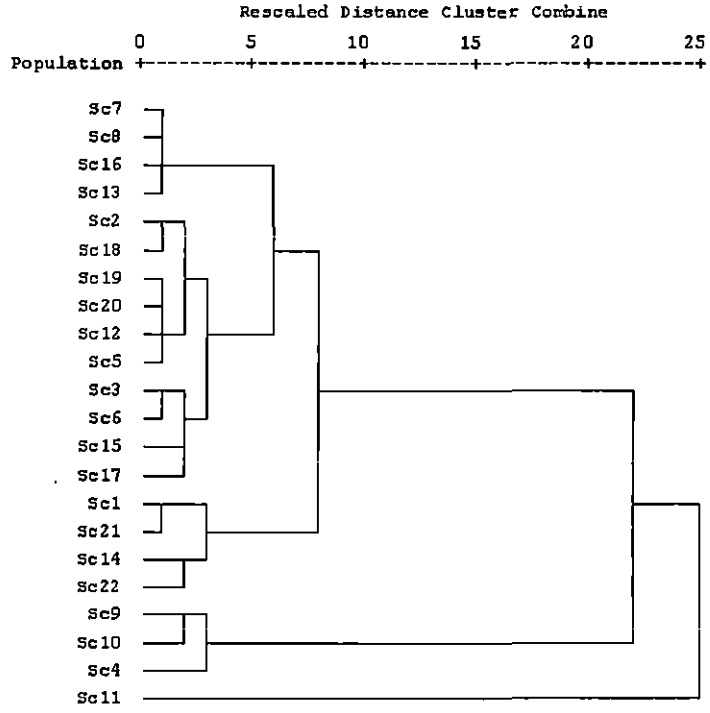

Figure 14B. Dendogram based on average linkage (between group) method and Pearson correlation in reproductive form of *Swertia chirayita* in Sikkim