

SUMMARY

The work embodied in the thesis concerns three aspects of study carried out in the *Hibiscus rosa-sinensis* complex such as Morphological, Palynological and Molecular.

A. Morphological study

(i) Foliar and floral

1. Detailed morphological description of 60 cultivars of *H. rosa-sinensis* based on field observations are provided, duly illustrated.
2. A total of 34 characters were subjected to detailed study of which 18 are quantitative and 16 qualitative. An exhaustive cultivar-wise characterization using appropriate abbreviations has been provided.
3. The quantitative characters studied refer to the various parameters of the leaf (petiole, lamina), stipule, epicalyx segments and flower (pedicel, calyx, petal, staminal column, stamen, style and stigma).
4. All the eighteen quantitative characters studied came under the medium category of character state in >90% of the cases. However, within the medium range, the cultivars exhibited much variation with regard to most characters.
5. The single-flowered cultivars showed greater numbers of stamens, longer staminal columns, longer filaments, longer styles and

consistently five stigmata. The cultivars with pendulous flowers (C-2 and C-7) showed the longest pedicels and filaments. The double-flowered varieties showed increased incidence of sterility, and had lesser number of stamens on shorter staminal columns, as majority of the stamens were transformed into petaloid appendages.

6. The genotypic and phenotypic coefficients of variation (GCV and PCV) were above 30 for majority of the quantitative morphological characters suggesting their high intraspecific variability. The epicalyx segment number, petal length and calyx length showed values <30%.
7. Seventy percent of the cultivars were single-flowered, with the remaining 30% possessing double-flowers. The cultivar C-3 possessed variegated leaves. The leaves were ovate (50%), elliptic, oblong, orbiculate or lobed. The leaves were chartaceous (76 %) or coriaceous in texture, with serrate (90%) , lobed or entire leaf margins. The leaf apices were acute (85%), obtuse, acuminate or rounded and bases acute, obtuse, rounded or cordate .The leaf venation ranged from simple craspedodromous to palinactinodromous types.
8. The various floral members such as the petals, petal bases, staminal columns, filaments, anthers and stigmata exhibited much variation in their colourations ranging from red to orange, pink, magenta, white, cream, mustard, yellow or saffron.

9. Great bulk of the cultivars - 83.33% were fertile (majority single-flowered) while 8.33% were female-sterile (all double-flowered varieties). The cultivar C-6 was male-sterile while C-57 fully sterile.
10. The analysis of variance showed highly significant variation at 1% level for all the 18 quantitative characters, suggesting high degree of variation among the 60 cultivars for these characters.
11. All the 18 quantitative characters showed high values of heritability reaching up to 99% for stamen and stigma numbers, suggesting the major role played by the genetic component in generating the wide spectrum of phenotypic variability, in contrast to the environmental component which has little effect on the phenotype.
12. The genotypic correlation coefficient was higher than the phenotypic correlation coefficient for most of the quantitative characters suggesting the interplay of genetic mechanisms like pleiotropism and linkage disequilibrium stabilizing the gene pool of the cultivars.
13. Significant positive correlation was observed between all the leaf characters ($r = 0.93-0.81$) at 1% level. This might be suggestive of similar quantitative processes involved in leaf growth. The floral characters were also correlated highly with each other ($r = 0.77-0.60$). Many of the floral characters were correlated to the leaf characters as well.
14. The epicalyx segment number, pedicel length and filament length showed no correlation to many of the foliar and floral characters.

The calyx length showed no association with most of the leaf characters, while the stigma number was correlated with a few of the floral characters. Significant negative correlations were observed between the characters pertaining to the epicalyx segments and the essential whorls.

15. The complex patterns of variations in the morphology within the species-complex might be considered to reflect evolutionary patterns at higher taxonomic levels in the family.
16. The Divergence (D^2) analysis of the quantitative characters grouped the 60 cultivars into 6 clusters, among which cluster I was the largest with 42 cultivars followed by cluster II with 9. The clusters III and IV included 5 and 2 cultivars respectively, while the remaining two clusters (V and VI) consisted of only one cultivar each.
17. The intracluster distances were greater for cluster I (562) and cluster III (560), while for clusters V and VI, it was negligible. Clusters I and III, II and IV and IV and VI were very distant (>2900) from one another. Cluster I was the closest to cluster IV (1082) while clusters III and IV were also very close to each other (1065).
18. Such an elaborate intraspecific morphological characterization and analytical studies on the cultivars of *H. rosa-sinensis* at the lower taxonomic level has been attempted for the first time.

(ii) Epidermal study

1. The epidermal characters pertaining to the epidermal cells, stomata and trichomes of the 60 cultivars were studied.
2. The epidermal cells were irregular and highly, moderately or less sinuous, with more undulations in the lower epidermis. The small size and lesser undulations on the upper epidermis might be attributed to the increased water stress experienced by the upper epidermal cells owing to exposure to the tropical sun.
3. Almost all the cultivars except C-29 were hypostomatic which is considered to be yet another xeric adaptation to limit transpiration to the lower surface of the leaf.
4. The stomata were anisocytic in 73.33% of the cultivars, with the remaining cultivars exhibiting a mixture of both anisocytic and anomocytic stomata. Stomatal diversity on the same surface of leaf may be considered to reflect the ongoing process of evolution within the species-complex.
5. The stomatal complexes showed wide variation in size (length and breadth) ranging from 41.43 μ m x 42.9 μ m in C-1 to 101.57 μ m x 85.43 μ m in C-29. Although the stomatal frequency was as high as 34.11 in the cultivar C-1 with the smallest stomata, the remaining cultivars possessed lower stomatal frequencies (<18.5). The stomatal indices ranged from 7.80 in C-8 to 24.21 in C-1.

6. The values of GCV and PCV were very low (<30) for all the stomatal characters except the stomatal index, revealing more variation among the 60 cultivars for the stomatal index than the remaining characters.
7. The analysis of variance showed highly significant variance at 1% level for all the 4 quantitative stomatal characteristics suggesting notable variation among the 60 cultivars for these characters.
8. The values of heritability were also high ($>75\%$) for all the 4 quantitative stomatal characters suggesting that the stomatal variations within the species-complex are mainly due to the genotypic effect and the environmental component has played little role in this regard.
9. The genotypic correlation coefficient was slightly higher than the phenotypic correlation coefficient in all the 4 cases, suggesting the influence of the genotype in generating the intraspecific variations rather than the environmental component. The length of the stomatal complex showed highly significant positive correlation to both breadth of the stomatal complex and the stomatal frequency. The latter two were highly correlated to the stomatal index, while the length of the stomatal complex showed negative correlation to the stomatal index. Moreover the breadth of the stomatal complex was negatively correlated to the stomatal frequency apparently revealing

that it is more crucial than the length in reducing the stomatal frequency.

10. Single guard cells and degenerating guard cells were observed in a few cultivars, reflecting stomatal ontogenic aberrations due to various factors.
11. The trichomes showed the maximum diversity, ranging from unicellular conical hairs to biradiate, stellate (triradiate, tetraradiate, H-shaped and multiradiate) and tufted (triradiate, tetraradiate and multiradiate) hairs.
12. Eight cultivars were glabrous, and the remaining cultivars exhibited various degrees of diversity, possessing 1, 2 or more types of trichomes. The cultivar C-50 was the most diverse with 5 different trichome types, highlighting the evolutionary progress achieved by the species-complex.

B. Palynological study

1. The various pollen morphological characters in the 60 cultivars of *H. rosa-sinensis* were studied by LM and /or SEM observations.
2. The pollen in general were large, globose or spherical, radiosymmetrical, pantoporate and echinate in all the 58 male fertile cultivars. But the individual pollen exhibited wide range of intraspecific variation for both the quantitative and qualitative characters.

3. The aperture number varied from 8 in the cultivar C-3 to 34 in C-34. The aperture diameter ranged from 4.95 μm in C-54 to 13.57 μm in C-1. Majority of the cultivars possessed thin apertural margins, while a few showed distinctly thickened margins. In 5 cultivars the apertures were encircled by croton patterns formed by sporopollenin thickenings. Further, the apertures were fully open, or partially or fully plugged with sporopollenin granules.
4. Earlier studies had suggested the pores in *H. rosa-sinensis* as pantocolporate and not pantoporate as is generally understood. This suggestion is supported by the present SEM observations also. In the cultivars with fully open apertures (C-1 and C-5) the ectoapertures are clearly visible as narrow colpi.
5. Although the thickness of exine was constant in 25 cultivars (4.95 μm), much variation was observed in the remaining cultivars (4.03 μm in C-19 to 9.35 μm in C-46).
6. The columns were spinate (56%), baculate (40%), capitate (C-46) or verrucate (C-51). Divisibaculate spines were observed in 3 cultivars (C-18, C-23 and C-42) in low frequencies. The occurrence of divisibaculate and verrucate types of column reflect the ongoing process of evolution along the lines of both multiplication and reduction respectively.
7. The spine length varied from 9.9 μm in C-51 to 31.53 μm in C-59. The width of the spine base also varied considerably (6.6 μm in C-47

and C-48 to $16.5\mu\text{m}$ in C-4). The interspinal distances were more apically ($23.83\mu\text{m}$ in C-1 to $45.83\mu\text{m}$ in C-24) than basally ($9.9\mu\text{m}$ in C-2 to $36.3\mu\text{m}$ in C-52). The spine number per unit area ranged from 26.89 in C-15 to 53.89 in C-50.

8. Spine dimorphism was observed in 3 cultivars (C-18, C-46 and C-47) with the spines showing differences in length and/or shape in the same pollen.
9. The spine bases were with or without a basal collar. Polymorphism with regard to spine base was more evident in the SEM. The spine bases showed presence or absence of micropores. In two cases (C-23 and C-48) the microreticulum at the spine base extended into the adjacent interspinal area, while two other cultivars showed a ring of large sporopollenin globules at their spine bases (C-4 and C-53). The cultivar C-30 possessed a well-developed basal cushion with a ring of sporopollenin at the spine base.
10. The interspinal area was mostly pilate and less frequently pilate-scabrate, punctate-pilate, scabrate-foveolate, scabrate-punctate or scabrate-reticulate.
11. The cultivars were polymorphic with regard to pollen size with 51% dimorphic, 40% monomorphic and the remaining trimorphic. Trimorphic pollen were observed in the dark coloured flower groups, while the yellow flower colour group was predominantly monomorphic.

12. Ten cultivars possessed gigantic pollen while the remaining ones possessed very large grains showing the evolutionary advancement in the species-complex. The range in pollen size was from 128.33 μm in C-2 to 228.07 μm in C-28.
13. The values of genotypic and phenotypic coefficients of variation were high (>30) for only two characters (aperture number and distance between spine tips). The remaining characters showed values >25 suggesting wide variation among the different cultivars with regard to these characters.
14. The analysis of variance showed highly significant values at 1% level for all the quantitative palynological characters indicating wide variation among the 60 cultivars with regard to these characters.
15. Very high degree of positive correlation was observed between majority of the pollen characters at 1% level with no negative correlation, revealing that the major pollen characteristics are highly conserved within the species-complex, permitting adaptive modifications along the lines of least resistance via gene mutations.

Hierarchical cluster analysis

1. The pooled data from the morphological, epidermal and palynological studies on 60 cultivars of *H. rosa-sinensis* were standardized and subjected to Hierarchical cluster analysis using the Average linkage study.

2. In the proximity matrix, the minimum squared euclidean distance was observed between the cultivars C-9 and C-15 (1.9) followed by C-4 and C-7 (2.1), showing that these cultivar pairs are very closely linked to each other. The cultivar C-29 was very divergent from both C-57 (26.7) and C-6 (24.4).
3. The dendrogram depicting the results obtained from the Hierarchical cluster analysis grouped the 60 cultivars into 15 clusters at a rescaled distance of 10. Cluster II was the largest with 21 members.
4. The cultivars C-6 (male-sterile) and C-57 (fully fertile) are the most divergent followed by C-29 as they stand apart at a rescaled distance of 25 itself. The cultivars C-1 and C-5 stand together forming a separate cluster, revealing tight linkage between them.
5. The female sterile cultivars form a separate cluster at a rescaled distance of <15.
6. The Hierarchical cluster analysis reveals that the species-complex is a very closely knit group of cultivars with only 5 of the 60 cultivars showing divergence at a rescaled distance of >20.

C. Molecular study

1. A modest attempt of molecular study using RAPD-based markers was done in 16 representative samples from the 15 clusters obtained from the Hierarchical cluster analysis.

2. The number of bands produced by the 17 primers varied from 3-18, the least for OPX 10 and the most for OPP 06. The various band numbers produced could be arbitrarily grouped into 3 band classes such as 3-7, 8-13 and 15-18.
3. The 17 primers together generated a total of 183 products of which 180 were polymorphic. The high level of polymorphism could be attributed to the cross pollinating nature of the species. The high mean value of the number of polymorphic bands produced per primer (10.5) might reveal small divergences due to insertional, deletional or substitutional mutations.
4. The cluster analysis based on RAPD results grouped 12 of the 16 cultivars studied under a major cluster I with 3 subgroups – I a (C-1 and C-5), I b (6 cultivars) and I c (4 cultivars). The remaining 4 cultivars viz. C-6, C-33, C-38, and C-43 formed separate loose groups.
5. The findings of the RAPD cluster analysis were found to corroborate with those of the D^2 and Hierarchical cluster analysis in many respects. The cultivars C-6 and C-57 stood apart as the most divergent in all the three analyses. The cultivars C-1, C-5 and C-29 grouped together into a single cluster along with a large number of cultivars by the D^2 analysis were found to be divergent in the multivariate Hierarchical clustering. However, these 3 cultivars are much similar genetically as evidenced from the RAPD results.

6. The three analyses also highlight the close affinity between C-1 (*H. rosa-sinensis*) and C-5 (*H. rosa-sinensis* var. *schizopetalus*). The very high value of genetic identity between C-1 and C-5 (0.98) in the RAPD assay supports the inclusion of *H. rosa-sinensis* var. *schizopetalus* as a variety under the *H. rosa-sinensis* species-complex.
7. The coefficient of similarity in terms of Nei's measure of genetic identity ranged from 0.87 to 0.99, with an overall mean value of 0.95 suggesting a narrow gene pool for the species-complex. Even C-43, the most divergent among the 16 cultivars studied, showed a genetic similarity mean value as high as 0.92.
8. The wide spectrum of intraspecific variability within the species-complex is multidimensional and could be traced back to its polyphyletic origin from distant crosses at higher taxonomic levels. Human interferences hindering the segregation of such genetic variations narrow the gene pool of the species. However, it is apparent that further insertional, deletional or substitutional mutations occurring at the molecular level along with recombination via out-breeding serve to enlarge the dimensions of such variations within the species-complex, leading to the evolution of newer forms.