

INTRODUCTION

Hibiscus rosa-sinensis L. (Malvaceae) is horticulturally one of the most favoured species on account of its large showy flowers exhibiting great variation in form and shade. This has given rise to a multiplicity of varieties constituting the *H. rosa-sinensis* species-complex. There is profound variability in their leaf characters as well. Innumerable distinct cultivars exist under cultivation as garden plants the world over. The rapidity and efficacy of vegetative means of propagation along with out-breeding has brought about its worldwide establishment and distribution. Immense horticultural importance and interest have gathered momentum over the years, which have led to the constitution of various international organizations associated with this species-complex such as the International Hibiscus Society, the American Hibiscus Society and the Australian Hibiscus Society.

Apart from its ornamental value, the species is well known for its medicinal properties. It is valued for a variety of uses such as an effective emollient (Hereman, 1868), antifertility efficacy (Murthy *et al.*, 1997) with antioestrogenic activity (Prakash *et al.*, 1985), anticonvulsive activity (Kasture *et al.*, 2000), antidiabetic properties (Sachdewa and Khemani, 2003) and anticancerous efficacy (Johnson, 1999). Recently Puckhaber *et al.* (2002) have explored the potential of *Hibiscus* as a source of edible flowers and

natural food colourants, and have elevated it to the status of what is referred to as the “Newest of New Crops”.

The available taxonomic literature on the species is vast, and the informations are mostly on the floral features rather than the vegetative, and so much so most, if not all studies on the morphology of the species have been only on variation of its floral characters (Davis and Ghoshal, 1966; Satapathy, 1978; Heel, 1978). Bhatt *et al.* (1988) and Saibaba and Rao (1990) have devoted attention solely on the leaf morphology associated with the venation patterns of the species. A comprehensive study covering a sizeable number of cultivars on their gross morphology – both vegetative and floral is very much lacking.

The epidermal features such as the epidermal cells, stomata and trichomes are known to yield supplementary clues for solving taxonomic problems. The importance of characters of these components in elucidating the systematic relationships and phylogeny of plants at various taxonomic levels has been emphasized by various experts (Metcalf and Chalk, 1950; Stace, 1984). However, most previous studies on epidermal morphology are restricted to plants of higher taxonomic levels. Studies with emphasis on the epidermal morphology of *H. rosa-sinensis* at the varietal level is virtually meagre or absent.

Pollen morphology has been well recognized as a potential supplementary tool in dealing with taxonomic problems, systematic relationships and phylogeny of angiosperms at all taxonomic levels. The

pollen grains of Malvaceae had attracted attention owing to their large size and spines, but bulk of the known studies have been only on plants at the higher taxonomic levels (Saad, 1960; Christensen, 1986 a, b; Hosni and Araffa, 1999; El Naggar, 2004), while little attention has been paid to the varietal level study despite its potential value in cultivar taxonomy (Nair, 1960; 1961 b; Nair *et al.*, 1964; Bamzai and Randhawa, 1965). Nair and Kapoor (1974) have demonstrated that statistical evaluation of pollen variations is useful for categorizing varieties and for understanding their hybridity status. Scanning Electron Microscopy, in recent years, has become one of the reliable tools for observing the pollen surface architecture with immense precision. Palynologists have used this facility for detailed pollen description concerning the conventional exine characteristics and used the data in unravelling deeper problems of systematic relations at all levels, even down to the microtaxa (Taylor and Levin, 1975; Dayanandan, 1979). Meaningful SEM study and analysis of the intraspecific variations of various pollen features including the delicate variations in the interexcrecence exine ornamentation in the *H. rosa-sinensis* complex is all the more lacking.

Morphometric methods are known to be effective in analyses leading to recognition and discrimination of groups and for evaluation of ontogenetic and phylogenetic changes in plants. (Mc Lellan and Endler, 1998; Jensen *et al.*, 2002). Jensen (2003) has emphasized that morphometric methods of any stripe can be of great value at the species level and below. Previous attempts on statistical analysis of characters of *H. rosa-sinensis* complex are restricted to the analysis of variance and correlation studies of floral features only

(Davis and Ghoshal, 1966; Raghurajan and Namboodiri, 1982). Judd and Manchester (1997) have carried out a multivariate cladistic analysis on the entire Malvaceae. But there has been no attempt to date aimed at clustering of cultivars of this species-complex using metric methods.

Molecular study using Random Amplified Polymorphic DNA (RAPD) technology is known to be a powerful tool for identification of genetic variations in plants by “fingerprinting” them with relatively greater ease (Williams *et al.*, 1990; Caetano-Anolles *et al.*, 1991). This technology is easy to handle and cost-effective as it does not require any expensive equipment or use of radioactive isotopes (Tingey *et al.*, 1992). The technique uses only a single RAPD primer, which enables detection of variation at multiple loci. The RAPD assay has been used for studying the genetic diversity in many crop species such as soya bean, rose and mustard (Fujishiro and Sasakuma, 1994; Debener *et al.*, 1996; Lin *et al.*, 1996) and for clarifying the degree of genetic relationships between taxa. The RAPD profiling is considered to be useful for population and phylogenetic studies as well (Hadrys *et al.*, 1992; Lynch and Milligen, 1994). Stewart and Porter (1995) have demonstrated that RAPD profiling could easily generate seven times more neutral molecular markers compared to the isozyme analysis and that it is accessible to non-molecular biologists as it can be done without prior knowledge of the gene sequence (Henry, 1997), and hence the RAPD based molecular markers are best suited for estimation of genetic diversity leading to varietal identification. Moreover the DNA-based RAPD markers, unlike morphological markers, are stable and little influenced by environmental fluctuations (Gottlieb, 1977).

Many investigators have effectively employed the RAPD markers for identification of cultivars in many crop species like *Brassica* (Hu and Quiros, 1991), pepper (Ballester and Vincente, 1998), *Hibiscus cannabinus* (Zhou *et al.*, 2002), *Capsicum annuum* (Ilbi, 2003), *Sorghum* (Nkongolo and Nsapato, 2003) and coconut (Upadhyay *et al.*, 2004).

The genetic base of the *H. rosa-sinensis* complex has been shrinking down the ages owing to gene erosion by fast disappearance of many potential cultivars. For the conservation and effective utilization of the existing germ plasm, a proper knowledge of the intraspecific variation of the species-complex would be of much avail, and this information in turn would be useful for formulating methods and strategies for their genetic improvement. Great inadequacy prevails concerning morphological characterization of the cultivars of the species-complex, which is due to the extreme range of variability of most of the exomorphological characters. This has apparently made the study of cultivar taxonomy of the species pretty difficult and unamenable. Moreover, great confusion exists regarding authentic identification of the innumerable cultivars on account of the high degree of reticulation of their exomorphic traits, especially of the foliar and floral ones. Although there have been scattered attempts in this direction, a comprehensive study encompassing a fair sample of cultivars is almost lacking. It is expected that an in-depth study of the variability of morphological characters and cultivar-wise morphological characterization would go a long way in circumventing the ambiguity in varietal identification felt by horticulturists and garden people. A large store of such data can also

yield valuable information for assessment of the extent of intraspecific variation, estimation of correlation between various characters, and evaluation of their heritability and also for clustering the cultivars based on character association and isolation. Such an effort would gain added strength if data of characters from other allied areas like epidermal (cell, stomata, trichomes) morphology and palynology are also taken into consideration alongside with conventional exomorphic characters.

The present investigation in the *H. rosa-sinensis* complex covers three aspects of study (A) Morphological, (B) Palynological and (C) Molecular, and accordingly the results are presented in three sections.

The main objectives and lines of work envisaged are

1. Assemble and analyse the variability in respect of various characters of (a) morphology - (i) foliar and floral, (ii) epidermal (epidermal cells, stomata, trichomes) and (b) palynology in 60 popular cultivars, with appropriate illustrations.
2. Work out the correlation between and among various character attributes.
3. Estimate the phenotypic and genotypic correlations.
4. Compute the genotypic and phenotypic correlation coefficients, and heritability of important exomorphic characters.
5. Assess the extent of intraspecific variations in morphological and palynological characters.

6. Grouping of cultivars employing the divergence (D^2) statistics based on exomorphological characters.
7. Clustering of cultivars by metric method (Hierarchical cluster analysis) using the pooled data of characters from exomorphology (foliar and floral), epidermis and palynological features based on association and isolation of various characters.
8. Carry out molecular study making use of the RAPD technology in a representative sample of cultivars for evaluating the genetic diversity within the species-complex at the molecular level. This is expected to generate information on the extent of genetic stability of the species-complex and reveal the underlying genetic mechanism leading to the wide spectrum of intraspecific variation.