

REVIEW OF LITERATURE

Taxonomy

Hibiscus rosa-sinensis L. is one of the most attractive and well-known members of the mallow family Malvaceae. The family Malvaceae Juss. (1789), represented all over the tropics and subtropics by over a 100 genera and 2000 species, is considered to be South American in origin (Fryxell, 1988, 1992). Sivarajan and Pradeep (1996) have reported 19 genera and 78 species from the Southern Peninsular India.

The tribe Hibisceae Reich. (1828) of which *Hibiscus* is the type genus, was delimited from the other tribes in the Malvaceae by the possession of capsular rather than schizocarpic fruits. The features that hold together the Hibisceae include loculicidally dehiscent fruit (capsule), absence of gossypol glands, five toothed staminal column apex, apical branching of the style, terminal stigmata and equality in the number of style branches to the carpel (Bentham and Hooker, 1883; Schuman, 1890; Kearney, 1951; Hochreutiner, 1955; Fryxell, 1988; Paul and Nayar, 1988; Paul 1993; Sivarajan and Pradeep, 1996). Edlin (1935) transferred the tribe Hibisceae together with the genus *Kydia* to Bombacaceae, but Hutchinson (1967) noted that 'Malvaceae without the great genus *Hibiscus* would be like a horse without a tail'.

The genus *Hibiscus* L. stands out in the tribe Hibisceae without unique synapomorphies, and may be considered a paraphyletic assemblage with other

members of the tribe viz. *Fioria* and *Kosteletzkya* C. Presl. with winged capsules, *Abelmoschus* Medik. with asymmetrical deciduous calyx and *Kydia* Roxb. with five antheriferous arms atop the staminal column (Fryxell, 1988; Paul, 1993; Pfeil *et al.*, 2002). The generic name *Hibiscus* derived from the Greek *hibiscus* or *ibiscos* meaning a mallow was first used by Linnaeus (1753, 1754). De Candolle (1824) proposed a sectional classification including 117 species. However, the genus received a comprehensive systematic treatment only in 1900, when Hochreutiner described 197 species in the monograph on *Hibiscus*. Rakshit and Kundu (1972) revised the genus *Hibiscus* in India. *Hibiscus* described as the type genus of Hibisceae has over 300 species distributed all over the world (Willis, 1966; Qian, 1999; Akpan, 2000; Pfeil *et al.*, 2002). The genus is represented by 28 species belonging to 10 sections in India of which 20 species occur in the Southern Peninsula (Sivarajan and Pradeep, 1996).

Hibiscus rosa-sinensis L. commonly referred to as the shoe flower plant or China-rose, is a cultivated horticultural shrubby species comprising over a 1000 cultivars (Singh and Khoshoo, 1970) forming the “*Hibiscus rosa-sinensis* complex”, which are mostly selections derived from crosses involving Hawaiian species like *H. kokio*, *H. arnottianus*, *H. waimae* ($2n=80$) and the East African *H. schizopetalus* ($2n=40$) along with other species (Wilcox and Holt, 1913; Palmer and Palmer, 1954). The multitudinous showy plants comprising the species complex are grouped together as cultivars, and include numerous varieties differing in colour and duplication (Hooker, 1875; Rendle, 1959). *Hibiscus schizopetalus* (Dyer) Hook. has been considered a

separate species distinct from *H. rosa-sinensis* by several taxonomists (Hooker, 1880; Mabberley, 1987; Paul, 1993). Masters (1879) and Dyer (1879) considered it a variety of *H. rosa-sinensis*. This observation was supported by Fryxell (1988) owing to its frequent intergradations with *var.rosa-sinensis*, presumably by hybridization. Sivarajan and Pradeep (1996) have also included this taxon as a variety under *H. rosa-sinensis*.

Origin and distribution

Although the *Hibiscus rosa-sinensis* complex is distributed all over the subtropics and tropics with over 1000 cultivars, there has been much confusion regarding the origin of the species. Loureiro (1788) has held it to be a native of and indigenous to both China and Cochin-China, while Hooker (1880) suggested an African origin as its nearest relatives *H. schizopetalus* and *H. liliflorus* are natives of East Africa and Mascarene islands. Pijl (1937) has suggested an American origin for the species as the pollinators (humming birds) of this group are American. Although *H. rosa-sinensis* has been known and grown very early in China and called 'China- Rose', Hu (1955) observed that the wild form has not yet been discovered in China. But three out of the four genetically compatible species of *H. rosa-sinensis* are native to the South Indian Ocean Islands and African East Coast indicating these as the likely ancestral home of the early forms involved in *H. rosa-sinensis* complex (Hochreutiner, 1900). This was later supported by Bates (1965) who considered the species as a native of continental tropical Asia.

Recently Pfeil *et al.* (2002) have tested the polyphyletic theory of origin of the tribe Hibisceae using two chloroplast DNA sequences, a coding region- *ndh F* and a non-coding region – *rpll 6* intron, and concluded that eastern Gondwana may be the centre of origin of the group.

Economic importance

Hibiscus rosa-sinensis, the China rose is the state flower of Hawaii (Graf, 1982). Cowen (1965) described the species as an ornamental hedge. The flowers are used to blacken shoes (Hooker, 1875) and hence popularly known as shoe flower. In India, the flowers especially those of the red varieties are used for religious ceremonies (Bose and Nair, 1988). Apart from its horticultural value, the species is well known for its medicinal properties. It is a well-known emollient from early days (Hereman, 1868). The leaves are used in fainting fits, intestinal disorders (Nair and Mohanan, 1998) and the curing of ulcers while the buds are used for the treatment of piles and cholera (Rao, 1914). The flowers aid digestion (Graf, 1982), reduce arterial hypertension (Dwivedi *et al.*, 1977) and are used in the preparation of *Annapavala Sindhooram* which reduces hypercholesterolemia in man (Sundaran *et al.*, 1983). Kasture *et al.* (2000) reported anticonvulsive activity of the species in experimental animals. Sachdewa and Khemani (2003) have observed maximal diminution in blood glucose (41-46%), insulin (14%), total cholesterol (22%) and serum triglycerides (30%) levels in diabetic rats after oral administration of an ethanol extract of the leaves. Yamasaki *et al.* (1996) have reported antioxidant activity of the red anthocyanin pigment extracted

from the petals. Johnson (1999) has reported the anti-cancerous efficacy of the species.

The flowers have been reported to possess significant antifertility effect (Tan, 1983; Sethi *et al.*, 1986 and Murthy *et al.*, 1997). It has been proved to be cytotoxic and cytostatic to germ cells and sperms of a non-scrotal bat, *Rhinopoma kinneari wroughton* (Singwi and Lall, 1980). Benzene, chloroform and alcoholic extracts of *H. rosa-sinensis* decreased the spermatogenic elements of the testis and the epididymal sperm count in albino rats (Reddy *et al.*, 1997). Antioestrogenic activity of ethanolic and benzene extracts of *H. rosa-sinensis* has been reported in female rats (Kholkute and Udupa, 1976; Prakash *et al.*, 1985). Prakash *et al.* (1986) observed that oral administration of benzene extract of the flowers at a dose level of 1gm/kg body weight/day from day 5-8 of gestation led to the termination of pregnancy in about 92% of animals.

The mucilage extracted from the leaves is used as “tali” (an oil wash for hair) in India. The infusion of flowers is used to cure baldness (Rao, 1914; Nair and Mohanan, 1998). Leaves and flowers have been observed to be promoters of hair growth (Nadkarni, 1954; Ali and Ansari, 1997) and have anti-graying properties (Kumar *et al.*, 1994). Krishnamurthy and Ranganathan (2000) reported fungicidal activity exhibited by a herbal drug combination of *Hibiscus rosa-sinensis* and *Wrightia tinctoria* against the isolates of the fungus *Pityrosporum ovale* recovered from dandruff. Manikar and Jolly (2001) formulated a natural shampoo with the extract from *H. rosa-sinensis* for conditioning the hair. Adhirajan *et al.* (2003) observed that the petroleum

ether leaf extracts of the species exhibits more potency on hair growth when compared to flower extract by *in vivo* and *in vitro* methods.

Puckaber *et al.* (2002) have highlighted the potential of *Hibiscus* as a new source of edible flowers and natural food colourants, which elevate them to the status as possibly the newest of New Crops. Potential products from these cultivars include fresh food (primarily edible flowers for the restaurant trade) as well as lubricants, mucilage, and complex polysaccharides from fruiting organs, roots, branches etc.

Pollination

Almost all varieties of *H. rosa-sinensis* are scentless, and the flowers open in the morning and wither by 12 hours. The large showy flowers attract insect pollinators for nectar in five pits located at the sides of petal bases. The lower margins of the petals have hairs to protect the nectar from rain (Rendle, 1959). The flowers are popular among sunbirds and tailorbirds that insert their beaks between the petals at the back of the flower in search of nectar (Cowen, 1965). In many cultivars, anthocyanin occurs at the base of the petals forming a deep red eye, which appears to be associated with a relatively long staminal column which may be of biological significance (Lowry, 1976). The evolution of pistil length has been considered as a choice mechanism by Lankinen and Skogsmyr (2002).

Snow *et al.* (2000) have observed that short delays in pollen arrival time altered the success of 'fast' and 'slow' pollen donors, when both types of pollen experienced some delay. Klips (1999) reported that pollen competition

between heteromorphic (foreign) and conspecific pollen might act as a barrier to hybridization. Raghurajan and Namboodiri (1982) have suggested the failure of seed set to be due to inhibitory factors operating at the pre-(placental inhibition) and post-fertilization (embryo abortion) stages.

Cochis (1966) has studied the effect of concentration and type of sugar and agar on the *in vitro* germination of *Hibiscus* pollen. Chandra *et al.* (1980) noted that an attempt to increase the number of anthers did not result in any appreciable increase in pollen production and concluded that the plants of Malvaceae have an adaptation towards constancy in anther number and pollen production.

Cultivation

H. rosa-sinensis described by Graff (1997) as a 'plant for life' has recently increased in popularity as a potted flowering plant due to its profuse, large and coloured flowers and dark green foliage (Miller, 1987). Hence much research has been conducted to increase the marketability of *Hibiscus* by developing techniques to reduce post-production abscission of flower buds and leaves (Gibbs *et al.*, 1989; Kelley and Thaxton, 1987). The plant is sun-loving and should be pruned regularly to avoid becoming straggly and to produce maximum number of blooms (Cowen, 1965; Steinberg *et al.*, 1991). Seed setting though noted in certain cases, is not considered by the horticulturists as an important factor in its reproduction, chiefly due to the rapidity and efficacy of vegetative means of propagation (Sharma and Sharma, 1962). Neumaier *et al.* (1987) have found that when grown under

green house conditions of abundant nutrient supply and reduced light levels, the plants can reach 90 cm in four months, with undesirably long internodes. Daminozide, chlormequat and ancymidol induced shorter internodes, more blooms and earlier flowering in several cultivars (Criley, 1981). Wang and Gregg (1989) observed that uniconazole could be used effectively to control stem length.

Kelty (1984) has observed that stem cuttings of large-flowered types required longer periods for rooting and developed fewer roots. Hess (1962) suggested easy rooting to be due to the presence of certain essential co-factors which may be lacking in difficult to root cultivars. Although Kachecheba (1975) observed variations in the stem anatomy of 27 cultivars of *Hibiscus*, such differences could not be associated with a mechanical impedance to root formation. Kachecheba (1976) has suggested that seasonal differences in rooting responses resulted from differences in auxin contents rather than carbohydrate levels. Higher IBA levels and an optimum temperature (26-30°C) increased rooting in the stem cuttings (Carpenter and Cornell, 1992). Jonathan *et al.* (2001) have reported that adequate potassium nutrition can improve drought resistance and root longevity in *H. rosa-sinensis*.

Although the death of *Hibiscus* flowers appears to be coordinated by ethylene (Woodson *et al.*, 1985), ethylene inhibitors like silver thiosulfate or 1-methyl cyclopropane had only a modest effect in extending flower life (Reid *et al.*, 2002), contrary to the earlier observations made by Serek *et al.* (1995). Selection is currently focused on large-flowered varieties which maintain a large number of blooms at any time, as many tropical varieties are

sensitive to chilling temperature and are adapted to high light intensities (Dole and Wilkins, 1999).

Morphology

Bates (1965) studied the seasonal variation of floral parts in *Hibiscus rosa-sinensis* and reported a large number of forms ranging from single, semi-double to fully double, and in colour from maize yellow to scarlet red. Variations in foliar and floral characters of *H. rosa-sinensis* were studied by Davis and Ghoshal (1966). Other studies include observations on the aestivation (Davis, 1964), left-handed and right-handed twisting of flowers (Satapathy, 1978), the morphology of the androecium and gynoecium (Heel 1966; 1978) and the leaf morphology and venation pattern (Bhatt *et al.*, 1988; Saibaba and Rao, 1990).

There has been much difference of opinion with regard to the terminology of the epicalyx members. Hutchinson (1969) has suggested that the range of floral structures in the Malvaceae is not great and that in large genera such as *Hibiscus*, “the tracing of the gradual evolution of the involucre of bracteoles provides an interesting phylogenetic study”. Rendle (1959), Hooker (1875), Gamble (1935) and Hutchinson (1969) have referred to the epicalyx members as bracteoles, while Abrams (1951) used the term ‘involucel bractlets’. Cowen (1965) and Wight and Walker (1976) considered the epicalyx members to be involucel leaves while Lawrence (1951) suggested that these represent an involucre (epicalyx) of distinct bracts (sometimes interpreted as an involucel of bracteoles). Majority of the later

researchers preferred the term epicalyx segments (Saldanha, 1984; Ramachandran and Nair, 1988; Bose and Nair, 1988 and Vajravelu, 1990). Sivarajan and Pradeep (1996) in the monograph on the taxonomy of the family Malvaceae have referred to the epicalyx members as 'involucellar bracts'.

Palynology

The pollen morphology of *Hibiscus rosa-sinensis* was studied by various researchers (Vilasini *et al.*, 1966; Muller, 1979; Srivastava, 1982 and Christensen, 1986 a ; 1986 b). Nair (1961 a) and Erdtman (1952) described the pollen of the species as pantoporate, spheroidal and spinate, while Saad (1960) observed the interspinal region to be pilate. Dayanadan (1979) conducted SEM studies on the pollen of *H. rosa-sinensis*. Moore and Webb (1978) have reported the exine sculpturing in Malvaceae as echinate with the projecting elements pointed, while Nayar (1990) has suggested that the measurements for size and shape classes and exine should be taken excluding the echinae. Nair (1961a, 1965) traced the evolutionary sequence of pollen variation in *H. rosa-sinensis* at the varietal level. Srivastava (1982) reported various excrescence morphotypes of pollen in the species. Van Campo (1976) has held that the periporate grains of the entire family must have originated from the tricolpate pollen type by successiformy pattern of development and observed that the pore is only a shortened colpus. Chaturvedi and Dutta (1984) have described the pollen of *H. rosa-sinensis* as pantocolpate instead of pantoporate as generally understood.

Heslop-Harrison *et al.* (1973) and Howlett *et al.* (1973) by immunofluorescence methods have established that the exine is the source of much of the antigenic material emitted from moistened baculate pollen. Heslop-Harrison and Heslop-Harrison (1973) have observed that the intine in *Hibiscus* forms a thickening as an annular boss around the aperture margins and that the apertures are occluded by sporopollenin granules, and further sealed by the pollen kit. Further, the apertures closest to the stigmatic surface form the first routes for the ingress of water leading to the hydration of the intine and subsequent emergence of the pollen tubes (Heslop-Harrison, 1979). The function of the enzymic load of the apertural intine might lead to the softening of the intine at the germination site, an essential prelude to the emergence of the pollen tube tip (Stanley and Linskens, 1974). Further, the intine may carry proteins concerned with the recognition of gametophytic incompatibility systems (Heslop-Harrison, 1978).

Epidermal studies

Although Metcalfe and Chalk (1950) had reported only ranunculaceous (anomocytic) stomata in the Malvaceae, Inamdar and Chohan (1969 a) have reported rubiaceous (paracytic) and cruciferous (anisocytic) stomata also in the vegetative and floral organs of *H. rosa-sinensis*. Other studies on the stomata of the group include those by Kidwai (1974), Yuldashev and Gareva (1974) and Inamdar and Chohan (1969 b). Rao and Ramayya (1977 a) investigated the stomatal distribution in the vegetative and floral parts of ten species of *Hibiscus*. This was followed by a more comprehensive study on the distribution of stomata in relation to plant habit in the order Malvales by Rao

and Ramayya (1981) in which the frequency and the distribution of stomata in *H. rosa-sinensis* were studied.

Various workers have reported stellate and tufted hairs in Malvaceae. (Metcalf and Chalk, 1950; Rendle, 1959; Bates, 1967 and Rao and Ramayya, 1976). Inamdar and Chohan (1969 b) have reported glandular and non-glandular hairs in the family. Rao and Ramayya (1977 b) studied the organographic distribution of sixteen trichome types in the Indian species of *Malvastrum*.

Cytology

Although Raven (1975) has suggested $x = 7$ as the original basic chromosome number of the family Malvaceae, Darlington and Wylie (1955) have reported the genus *Hibiscus* to be multibasic with ten different basic numbers $x = 7, 8, 9, 11, 12, 15, 17, 19, 29$ and 39 , out of which $x = 9$ is the most common in *H. rosa-sinensis*. On this basic number a long polyploid series ranging from $2n = 36 - 144$ and a dysploid series ranging from $2n = 46 - 225$ have been known (Goldblatt, 1988). A detailed cytological analysis of 75 cultivars of *H. rosa-sinensis* by Singh and Khoshoo (1970) revealed the species-complex to be a highly polymorphic group of complex hybrids and their derivatives. They grouped the 75 cultivars into twelve cytotypes, which could in turn be divided into two distinct groups – a polyploid group ($2n = 54, 63, 72, 90$ and 144) and a dysploid group ($2n = 46, 68, 77, 84, 96, 112$ and 132). Darlington and Wylie (1955) have suggested that the dysploid series based on $x = 8, 7$ or even 6 might have arisen from the original basic number

$x=9$, by stepwise reduction. Singh and Khoshoo (1970) have observed that despite such high chromosome numbers, the number of quadrivalents is low with most of the chromosomal associations in the form of bivalents, monovalents and a few trivalents. The high levels of pollen fertility on the face of such irregular meioses were considered to be due to the buffering effect of higher levels of polyploidy. They have noticed a distinct correlation between the chromosome number and flower diameter in the polyploid cultivars. However no such correlation was apparent in the dysploid taxa. Sharma and Sharma (1962) advocated aneusomaty as a mechanism of origin of cultivars with dysploid chromosome constitutions, while Singh and Khoshoo (1970) have suggested that repeated intervarietal and interspecific hybridizations involving multibasic series along with loss or gain of chromosomes during the irregular meioses followed by secondary balance might have led to the origin of the present day polyploid-dysploid series of the complex.

Molecular studies

Duke and Doebley (1995) have studied the phylogeny of the Malvaceae based on chloroplast DNA. Bayer *et al.* (1999) have supported the expanded family concept of Malvaceae within the order Malvales using a combined analysis of plastid *atp B* and *rbc L* DNA sequences. Alverson *et al.* (1999) have suggested monophyly of the core Malvales by examining the sequences of chloroplast *ndh F* gene and have held the Malvaceae to be monophyletic, while all the other members of the tribe viz. Bombacaceae, Sterculiaceae and Tiliaceae are polyphyletic.

Jendereck *et al.* (1997) have developed RAPD markers characteristic of *H. rosa-sinensis* and *H. syriacus*. Zhou *et al.* (2002) made comparative studies of genetic diversity in kenaf (*H. cannabinus*) varieties based on agronomic and RAPD data, and achieved clear separation of kenaf varieties based on RAPD variation patterns. Pfeil *et al.* (2002) have traced the phylogeny of *Hibiscus* using chloroplast DNA sequences *ndh F* and *rpl1 6*. Tang *et al.* (2003) have studied the genetic diversity of nine natural populations of *H. tiliaceous* in China using AFLP markers and suggested that long distance dispersal of floating seeds and local environment may play an important role in shaping the genetic diversity of the population and the genetic structure of the species.

Statistical studies

Davis and Ghoshal (1966) have compared the floral morphological features of cultivars of *H. rosa-sinensis* by calculating the mean, standard error etc. and analyzed the significance of such variations employing the chi-square test. Raghurajan and Namboodiri (1982) have made a comparison of the number of ovules fertilized and seeds set in the cultivars of the species by calculating the mean, median and range. Prakash *et al.* (1985) have studied the effect of ethanolic and benzene extracts of *H. rosa-sinensis* on the activity of β -glucuronidase enzyme in the uterus of ovariectomized rats using statistical tools and found significant reduction in oestrogenic activity. Carpenter and Cornell (1992) compared the interaction among IBA concentration, duration of treatment and propagation medium temperature on the rooting of stem cuttings among cultivars of the species using statistical

tools such as correlation coefficient, residual pattern and coefficient of determination. Judd and Manchester (1997) have made a cladistic analysis of the Malvaceae using morphological, anatomical, palynological and chemical characters. Akpan and Hossain (1998) have studied the karyotype and evolutionary relationship of three species of *Hibiscus* using analysis of variance and Neuman-keul's Multiple Range Tests. Reid *et al.* (2002) have employed the statistical t-test and probability analysis to study the role of ethylene in senescence and its inhibition using chemicals to prolong the display life of flowers in *H. rosa-sinensis*.