Evaluation of some mangrove species on the nature of their reproduction along the coastal belt of the Indian Sunderbans

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For **Author Contribution** and **Acknowledgement** see end of this article.

जहाँ है हरियाली ।
वहाँ है ख़ुशहाली ॥

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Abstract: Reproductive biology of three dominating mangrove species *Rhizophora mucronata*, *Ceriops decandra* and *Avicennia marina* from the Indian Sunderbans were studied. A comparative account on all floral parts revealed that as the bud progresses to completely open, the length of androecium surpassed the length of gynoecium and the anther matured first thus showing protandrous nature and favouring cross-fertilization. A study of pollen grain viability revealed that all of them produces fairly good amounts of viable pollen grains in their natural condition. The pollens showed their maximum viability late in the morning till early noon. The stigma showed peak receptivity after three days of flower opening in *C. decandra* and four days of flower opening in *R. mucronata* and *A. marina*. All the species showed out-breeding mechanism of pollination. The fruit setting percentage obtained by xenogamy was the highest and autogamy failed to show any result in all the three genera. Although *Ceriops decandra* and *Avicennia marina* showed very limited fruit set with geitonogamy, these two species can be called facultative out-crossers, while *Rhizophora* was obligate outcrosser in nature. The study on floral structure, pollen viability along with stigma receptivity of the investigated taxa guided to maximum exploitation of reproductive behavior for rising artificial and natural plant population in addition to build up a future research strategy in ecosystem conservation.

Keywords: Reproductive biology, stigma receptivity, pollen viability, mangrove, Indian Sunderbans

INTRODUCTION

The Indian Sunderbans maintain an exceptional ecosystem as well as big forests dominated by mangrove plants with a wide range of species diversity in the Indo-Gangetic plain, which exhibit various adaptations to cope with the environment (Datta et al. 2007). The vegetation extends between $21^{0}31'$– $21^{0}31'$ N and $88^{0}10'$–$89^{0}51'$ E within India, covering approximately 2195km^{2} (Sanyal 1996) excluding the network of creeks and backwaters.

The successful vegetation largely depends on the reproductive nature, fruit and seed setting behaviour of the species. Information of floral biology of mangroves is meager particularly in the Indian Sunderbans. However, it is important to study this in respect to its reproductive nature. In general, flowering in mangroves begins in spring and continues throughout the summer in India, whereas in Malaysia most species flower and fruit continuously throughout the year. Mangroves have both self pollinating and cross pollinating mechanisms that vary with species. For example, *Aegiceras corniculatum* and *Lumnitzera racemosa* are self pollinated. *Avicennia officinalis* is self fertile, but can also cross fertilize (Aluri 1990). In *Avicennia marina*, protandry makes self pollination unlikely. Mangroves are pollinated by a diverse group of animals including bats, birds and insects. Pollen is deposited on the animals as they deeply probe the flowers looking for nectar; they subsequently transfer the pollen grains to the stigma of another flower. The mangroves show a wide variety of reproduction, i.e., viviparity, cryptoviviparity, normal germination and vegetative reproduction (Bhoasale & Mulik 1991).

In this paper, we report the reproductive nature of some of the important mangrove species including their floral biology, pollen morphology and breeding behaviour under the Indian Sunderban conditions, and factors that are favourable to sustain them in this ecosystem, as existing information on these aspects is limited (Tomlinson 1986).

Image 1. Study area (shaded) in the Indian Sunderbans.

MATERIALS AND METHODS

Study area

The Patharpratima and Naamkhana blocks (Fig. 1) were selected as the study area out of 19 community blocks in the Indian Sunderbans. Both the islands were surrounded by creeks, channels and rivers which favour luxuriant growth of mangrove flora. The study area was included under the buffer zone of the Sunderbans Tiger Reserve Forest (Image 1). A rough taxonomic survey of both areas revealed that *Avicennia marina* covers approximately 95% of the forest, followed by *Ceriops decandra* and *Rhizophora mucronata*. Other species occur in patches.

Selection of species

Two dominant mangrove families, Rhizophoraceae and Avicenniaceae were selected to find their reproductive nature. Among the flora, Rhizophoraceae was considered a typical mangrove species because it showed viviparous reproduction in addition to special adaptations in saline conditions (Status Report on Mangroves 1987). These species occur in polyhaline zones with salinity ranging from 18–30 % and excluded more than 90% of salt from sea water. Avicenniaceae was known to be the second largest group dominating in the mangrove swamps of the Indian Sunderbans and it showed cryptovivipary. For the above study, one species from the Avicenniaceae family, *Avicennia marina* and two from the Rhizophoraceae family, *Ceriops decandra,* and *Rhizophora mucronata,* were selected as the study material. Since *A. marina* happened to be the most dominant flora and had a high tolerance to salinity (Ghosh & Mandal 1989), it was selected for the present study. *Ceriops decandra* from the Rhizophoraceae family was selected as it was the second dominant taxon in the Indian Sunderbans, its tolerance was lower than *A. marina* and *R. mucronata*, and this species showed localised abundance.

Description of floral parts and experimental studies

The study of *Rhizophora mucronata, Ceriops decandra* and *Avicennia marina* was conducted for a one-year period (2008–09). The observations on floral structure (Dafni 1992) and the comparative length of different floral parts at different stages were recorded. Pollen morphological study, its germinability (%), peak hour of viable pollen on the day of anthesis was studied. The breeding system along with fruit setting percentage was also recorded for the investigated taxa.

A pollen morphological study was carried out with air dried pollen grains using acetolysis method (Faegri & Iversen 1975) so that pollen grains were made clearer to give excellent topographic information. For this, the pollen grains were first heated in a mixture of sulphuric acid and anhydrous acetic acid in order to remove all non sporopollen substances. Six sucrose concentrations (0.2, 0.6, 1.0, 1.2, 1.6 & 2.0 %) were used in a hanging drop test to determine pollen tube elongation (Youmbi et al. 2004). Pollens from 10 different trees of each species were collected on the day of anthesis.

For studying the peak period for viable pollen on the day of anthesis, fresh pollen were collected in two hour intervals starting from 0500 to 1700 hr. The collected pollens were tested through the aceto-orcein test (Muccifora et al. 2003). Anthers were then stained with 3% aceto-orcein solution. Slides were made permanent using Euparol and examined under a microscope. The viable pollen grains appeared bright red in comparison to pale appearance of non visible pollen.

The breeding system was evaluated by hand pollination technique, according to Dafni (1992) in three ways viz. autogamy, geitonogamy and xenogamy. The stigma receptive time was also studied in the same experiment. The flower buds in each inflorescence of three genera were trimmed to retain only 5–6 uniform buds and emasculated a day before flower opening excepting autogamy. Twenty-five mature buds from each genera were used for each set every day from flowers that were completely open (T_1 or 1st day) to withered petal (T_7 or 7th day). They were pollinated manually and bagging was done for recording the number of fruit set in each day of pollination.

RESULTS

In *Rhizophora mucronata,* each branch carried 5–8 axillary cyme inflorescences of 5.0cm long approximately, with 2–4 dichotomously branched, containing four flowers in each peduncle (Image 2a). Flowers were white, perfect, with four sepals and petals in each. Sepals were typically pale yellow at maturity with four lobes. Out of eight free stamens, four were alternating with sepals and four were with petals (Image 2a). Anthers were bilobed, basifixed and introrse. Ovary showed globose and inferior in position (Fig. 1 a-f). Open flowers were located within or below leaf axils at leaf nodes below the apical shoot. Pollen grains were tricolporate, isopolar, radially symmetric, amb circular, peritreme, prolate, PA x ED 27.5 ± 0.13 x 23.7 ± 0.07 µm (Images 3 c,d). The nature of progression of the flower changed the length of the androecium and gynoecium, in the initial stage (bud) the length was the same but later the androecium (1.1cm) surpassed the length of the gynoecium (0.9cm) thus favouring cross pollination (Table. 1).

The flower took 8–10 days to complete its flowering life and it opened in the morning. The pollen tube length was maximum in 1% sucrose solution showing a tube length of 280.9±4.2 µm (Table. 2). As the flower opens, the anthers get exposed and matured fast showing a protandrous nature. Anthesis started in the evening at 1800hr (approx.) and it was completed the next day by 1200hr (approx.) where the calyx lobes were separated to expose the petals. The anthesis started with a slit at the apex and at the end, the epidermal layer of the anther wall droped on the stigma and also to prevent self pollination (Image 2b). The viability of pollen was maximum between 1100 and 1300 hr of the day, showing a viability percentage of 71.13. By hand pollination technique it was found that the *Rhizophora mucronata* was an obligate out-crosser and the stigma attained its receptivity a day after the flower opens and it continued for another four or five days. The maximum receptivity showed on the 4th day (T4) of the flower life with a fruit setting of 93.3%. The stigma was wet and papillate with a distinct groove in the middle that appeared after anthesis. The fruit setting percentage through autogamy and geitonogamy showed nil thus confirming its complete self incompatibility. *Rhizophora* was usually wind pollinated (Tomlinson 1986) but insects like bees, beetles (Image 2c) had been observed visiting flowers.

For *Ceriops decandra* the flowers were borne in condensed cymes inflorescence from dichotomous panicles, which occured in the leaf axils (Image 2e). Flowers were small, white, cup-shaped, bisexual (Image 2f) and took 6–10 days to complete flowering life. Sepals and petals were five, small, with an alternate arrangement. Petals form a short corolla tube crowned by a series of clavate filamentous appendages (Fig. 1 g–i). Out of 10, five antesepalous and five antepetalous stamens were inserted on the rim of the calyx cup. Anthers mature earlier than gynoecium thus confirming its protandry; anthers were longer than filaments. Disc within the stamen ring was well developed and anther lobes enclosed the base of the

Image 2. (a–d) *Rhizophora mucronata*: a - 2–4 dichotomously branched axilary cyme inflorescence; b - L.S of flower showing dehisced anther; c - Beetle pollinating the flower; d - Fruit set with calyx attached to it
(e–h) *Ceriops decandra*: e - Bud arising from the leaf axil; f - Flower with mature anther showing protandry; g - After fruit set-parts of corolla still attached to the fruit; h - Honey Bee pollinating the flower.
(i–l) *Avicennia marina*: i - Bud cluster; j - Complete open flower with mature anther showing protandry; k - *Apis dorsata* pollinating the flower; l - After fruit sets in

Table 1. Comparative account of the floral parts in different maturity stage

Length of different Floral parts	*Rhizophora mucronata*			*Ceriops decandra*			*Avicennia marina*		
	Bud (cm)	Flower about to open (cm)	Completely open flower (cm)	Bud (cm)	Flower about to open (cm)	Completely open flower (cm)	Bud (cm)	Flower about to open (cm)	Completely open flower (cm)
Flower	1.4	1.8	1.7	0.7	0.95	0.9	0.3	0.5	0.45
Sepal	1.2	1.5	1.6	0.35	0.5	0.6	0.15	0.25	0.3
Petal	0.9	1.2	1.4	0.3	0.4	0.4	0.3	0.4	0.45
Androecium	0.7	0.9	1.1	0.2	0.3	0.4	0.3	0.4	0.45
Gynoecium	0.7	0.8	0.9	0.04	0.05	0.05	0.1	0.15	0.15
Style	0.2	0.3	0.4	0.16	0.25	0.35	0.2	0.25	0.3
Stigma	0.1	0.1	0.1	0.2	0.35	0.4	0.15	0.2	0.3
Ovary	0.4	0.4	0.4	0.05	0.05	0.05	0.05	0.09	0.1

Table 2. Growth of pollen tube under different concentrations of sucrose

Name of the genus	Pollen-tube length (µm) under different concentration of sucrose (%)					
	0.2	0.6	1.0	1.2	1.6	2.0
Rhizophora mucronata	-	-	280.9± 4.2	16± 2.3	38.6±2.6	-
Ceriops decandra	-	-	96.2± 2.6	185.5± 4.1	196±1.4	-
Avicennia marina	36±1.83	100±2.8	130.1± 5.2	39.3± 1.06	78±6.3	226±2.15

thick filaments. Ovary was semi-inferior with a total of six ovules. Style is slender and minute separate stigmatic lobes were present. The pollen grains were tricolporate, isopolar, radially symmetric, amb circular, peritreme, subprolate, PA x ED 16.2 ±0.13 x 14.0 ±0.17 µm (Images 3 c,d). As the bud progresses the androecium length (0.4cm) increased to more than the length of the gynoecium (0.05cm) (Table. 1).

The pollen tube length was noted maximum in 1.6% sucrose solution showing a tube length of 196±1.4 µm (Table. 2). The anther dehiscence nature and other activities were the same as in *Rhizophora.* The maximum pollen viability was during 0900–1100 hr showing a percentage of 68.3 (Table. 3). With hand pollination technique, *Ceriops decandra* did not produce fruit/seed through autogamy. The stigma attained its receptivity two days after the flower opens and continued for another four or five days, showing peak receptivity on the 3rd day (T3) of the flower life with a fruit setting percentage of 83.3 through xenogamy (Table. 4). The fruit setting percentage through autogamy showed nil and geitonogamy on the day of peak receptivity (T3) showed a very limited fruit set of 13.3% (Table. 4). Bees (Image 2j), wasps, moths, flies etc. are capable of causing successful pollination in *Ceriops decandra*.

The second dominating family of the mangroves, *Avicennia marina,* was a common species in the Indian Sunderbans. The tree showed flowering in the months of April–August. The hypocotyls did not come out from the fruit due to its cryptoviviparous nature. Tomlinson (1986) described the inflorescence of *Avicennia* as a panicle that ended in a basic unit called flower cluster. Sometimes it was referred to as 'cymose inflorescence' but it was better to call it a flower cluster because the terminal flower did not open first. *A. marina* contained usually three terminal or axillary flower clusters, although it was vary from 1–6 (Image 2i). Each cluster consisted of 1–10 decussately arranged flower buds in a capitate unit (Image 2j). It took 10–30 days for a cluster to complete its flowering, whereas an individual flower retained an open corolla for 2–6 days. In *Avicennia marina* the flowers were small, short filament, minute hairs present on the style.

Table 3. Peak period of viable pollen percentage (0500–1700 hr)

Name of the genus	Percentage of viable pollen in hour of the day (%)					
	5–7 hrs	7–9 hrs	9–11 hrs	11–13 hrs	13–15 hrs	15–17 hrs
Rhizophora mucronata	19.1	20.5	50.9	71.13	9.46	-
Ceriops decandra	3.5	39.6	68.3	29.4	8.04	0.02
Avicennia marina	0.96	25.2	70.1	80.03	17.55	0.67

Table 4. Stigma receptivity by different modes of hand pollination in a flower life (bud - petal dehiscence)

Day of flower life (pollinated)	Seed set percentage (%) through different modes of pollination								
	Rhizophora mucronata			*Ceriops decandra*			*Avicennia marina*		
	Auto gamy	Geiton ogamy	Xenogamy	Auto gamy	Geitonogamy	Xenogamy	Auto gamy	Geitonogamy	Xeno Gamy
T1 (flower just open)	-	-	-	-	-	-	-	-	-
T2	-	-	50	-	-	-	-	-	-
T3	-	-	66.6	-	13.3	83.3	-	-	73.3
T4	-	-	93.3	-	-	50	-	20	90
T5	-	-	33.3	-	-	23.3	-	-	50
T6	-	-	26.6	-	-	16.6	-	-	16.6
T7 (petal withered away)	-	-	-	-	-	-	-	-	-

Flowers were four-lobed, actinomorphic, condensed in terminal or axillary cyme, sessile, 0.5–0.9 cm long and complete; sepal five, polysepalous; petals four, gamopetalous; stamens four, epipetalous; filament 0.2cm long (Image 2j); ovary superior, 0.3cm long (Fig. 1 m–r). The calyx lobes were separated at anthesis and diverged to expose the petals. In a complete flower the length of androecium (0.45cm) surpassed the length of gynoecium (0.15cm), showing a protandrous nature. In acetolysis technique, it was found that pollen grains were tricolporate, isopolar, radially symmetric, amb-circular, peritreme, prolate with PA × ED- 23.7±0.13 × 18.7±0.08 µm (Table. 2) (Images 3 c,d).

The viability of pollen was maximum from 1100–1300 hr showing a percentage of 80.03 (Table 3). The hand pollination technique showed that the stigma attained its receptivity two days after the flower opens and continued for another four or five days, showing peak receptivity on the 4th day (T4) of the flower life with a 90% fruit setting through xenogamy (Table 4). The fruit setting percentage through autogamy and geitonogamy showed nil and 20% respectively on the day of peak receptivity (Table 4). Numerous pollinators like the honey bee (*Apis dorsata*) (Image 2k) were actually attracted to the nectar-like secretion founded at the base of the corolla tube helpful for xenogamy.

DISCUSSION

In the Indian Sunderbans, *Rhizophora mucronata* and *Ceriops decandra* are two prominent species. The peak flowering season for *Rhizophora mucronata* is June–July during the monsoons, again in Nov –Dec (early winter). Table 1 represents the floral characteristics of some dominating taxa, where the flowers are actinomorphic. *R. mucronata* and *C. decandra* of the Rhizophoraceae family are uniformly protected within a comparatively thick and fleshy calyx lobe, its persistent nature seems to be a protective element for successful fruit setting. A number of filiform appendages present at the apex of the petals and the stamens are usually twice as many as the number of petals, but in *Kandelia candel* (a member of the family Rhizophoraceae) the stamens are numerous (Das 1994). According to Tomlinson (1979), the mechanical and biological natures of inflorescence

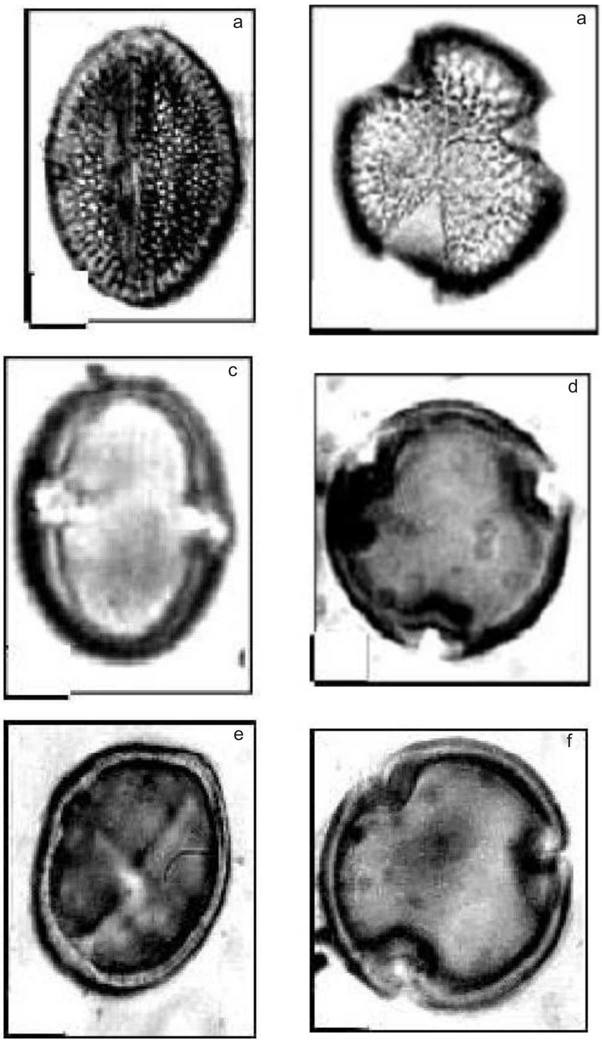

Image 3. (a–f) Photomicrographs of the investigated pollen grains.
(a–b) - *Avicennia marina*: a - equatorial view (x 750); b - polar view (x 750); (c–d) - *Ceriops decandra*: c - equatorial view (x 1000); d - polar view (x1175); (e–f) - *Rhizophora mucronata*: e - Equatorial view (x 870); f - polar view (x 1230).

provide continuous protection to the youngest units by a successive series of bracts, bracteoles and sepal as observed in our three species. In *Avicennia marina* the flowers are small, short filament, minute hairs on the style, same as earlier workers (Ghosh et al. 2008). Tomlinson et al. (1979) observed that a wide range of pollination mechanisms exist in the Rhizophoraceae family. The present work is in conformity with that. The floral characters revealed that cross pollination mechanisms prevail but geitonogamous pollination is reported minimally in the case of *A. marina* & *C. decandra*. Based on the bagging techniques, the fruit setting percentage through autogamy is nil as a result of different anthesis time in a single plant. The fruit setting percentage obtained through xenogamy showed positive which contradicted the results obtained by Sun et al. (1998), where geitonogamous selfing was seen to be high. Setoguchi et al. (1996) worked on *Crossostylis* sp., a species of Rhizophoraceae and found marked differences in floral morphology from that of other mangrove species of the same family. Morphological examinations of pollen grains revealed that all the taxa investigated had tricolporate, prolate or subprolate pollens with surface ornamentation that are reticulate or scabrate. *A. marina* and *R. mucronata* pollens were larger than that of *C. decandra*. Pollen grains viability test at peak hour shows that all the investigated taxa produced more than 50% viable pollens that ultimately lead to successful participation in pollination mechanisms and seed production. Farkas & Orosz (2004) obtained above 50% viable pollens in their experiment on pear (*Pyrus betulifolia*) and it is sufficient for successful pollination as well as enough to attract bees. Any results in mangrove pollen viability has not been reported so far. Bernal et al. (2005) said that *in vitro* pollen germination is a suitable method for studying male fertility, and probably a reliable process of estimation for seed production.

The rate of pollen germination and pollen tube elongation varies with sucrose concentrations as well as species specific. So, a selection pressure must be involved to determine the accurate germination procedure and it may be extended to the atmospheric interaction.

CONCLUSION

From the above study, it is clear that these plants adapted to stresses like salinity by an out-crossing method of pollination, strongly supported by a protandrous nature, to combat stressful substrata. The out-breeding mechanism modifies the gene pool with wider adaptability especially in stress. Generally, for establishment of a wider genetic background, a selection pressure must be involved in the process. The plant adapted continuum of life without having any dormancy by exhibiting its reproductive nature. Therefore, we can conclude that the reproductive nature is considered an adaptive strategy for seedling development to overcome the harsh conditions and a

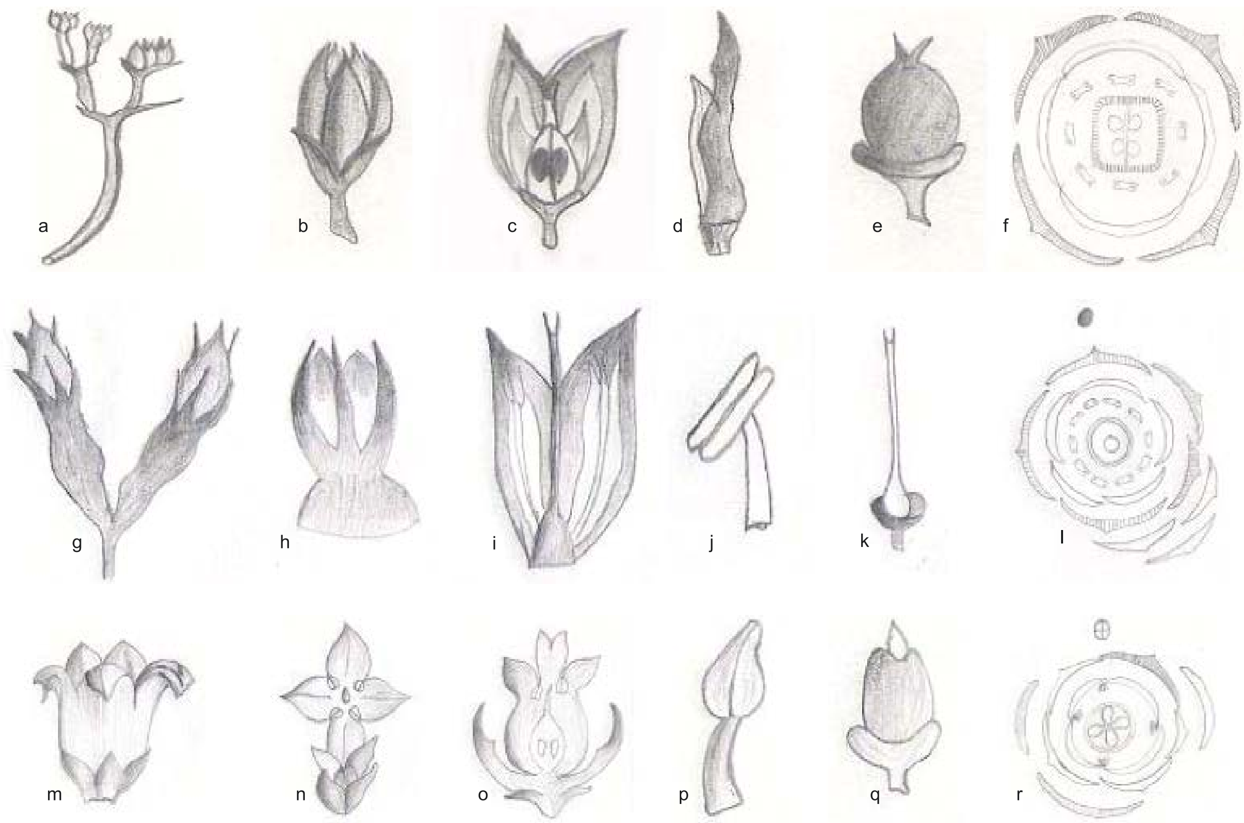

Figure 1. Floral parts and floral diagram of (a–f) *R. mucronata*, (g–l) *C. decandra* and (m-r) *A. marina*

meaningful conservation strategy can be adopted for preservation of these endangered species.

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