

### **Preliminary study on natural hybrids of genus *Rhizophora* in India**

The systemic distinction between *Rhizophora lamarckii* and *Rhizophora annamalayana* remains unclear, and their parental species are not fully understood (Kathiresan, 1995, 1999; Lo, 2003), owing to difficulties in distinguishing *Rhizophora stylosa* and *Rhizophora mucronata*. In India, *Rhizophora* hybrids have been recorded in Pichavaram (Tamil Nadu) and in the Andaman and Nicobar Islands.

In Pichavaram, the hybrid was initially identified as *R. lamarckii* based on morphological features and occurrence of putative parents (Lakshmanan & Rajeswari, 1983; Muniyandi & Natarajan, 1985). However, its parentage was disputed in that *R. stylosa* does not occur in Pichavaram and the morphological features of *R. lamarckii* described by Duke and Bunt (1979) differed from those of the hybrid. Later, the hybrid was identified as *R. annamalayana*, a cross between *Rhizophora apiculata* and *R. mucronata* (Kathiresan, 1995). Although Kathiresan (1995) assigned a new nomenclature in an attempt to distinguish the Indian hybrid from the Australian form, he did not provide detailed taxonomical descriptions of the hybrid, as well as its parents. According to Kathiresan (1995, 1999), the style length of *R. annamalayana* is 2.2 mm. As hybrids are always intermediate in its morphology between its parents, its style length cannot be 2.2 mm, because both parents (*R. apiculata* and *R. mucronata*) have style lengths of less than 1 mm (Duke & Bunt, 1979). Moreover, the style lengths of *Rhizophora* hybrids reported by various authors are very similar (Table 1). Kathiresan (1999) mentioned that style length of *R. apiculata* is 1.8 mm but according to Duke and Bunt (1979) the mean style length of *R. apiculata* is 0.9 mm, varying between 0.5-1.0 mm. Although Kathiresan (1995) compared morphological characters of *R. mucronata* and *Rhizophora* hybrids in Pichavaram with those described by Duke and Bunt (1979), he did not compare the characters of *R. apiculata*. It is understood that Kathiresan (1995) has assigned the new nomenclature based on the presence of dark spots, stamens in two distinct whorls and dark green leaves, but in the absence of *R. stylosa*.

In Andaman and Nicobar, a *Rhizophora* hybrid was first identified by Singh *et al.* (1987) from Havelock. It was named as *R. lamarckii*, and described as occurring with *R. apiculata* and *R. mucronata*, found on rocky substrates, style length 2–3 mm and stamens present in two distinct whorls. As there was no mention of *R. stylosa*, its identity was not reliable.

To resolve the controversies in both locations, a survey was carried out to distinguish *R. mucronata* and *R. stylosa* based on style length with comparison with the two known hybrids of Indo-West Pacific region (Duke, 2006a). To distinguish *R. mucronata* and *R. stylosa*, 50 trees were randomly selected in Havelock, and 20 flowers from different inflorescence were collected from each tree to measure their style length.

Among 50 trees analyzed to delimit the *R. mucronata* and *R. stylosa* based on style length, we found three groups in cluster analysis (Fig. 1). Clade A represents *R. mucronata* (shorter style length of 0.8–1.1 mm), Clade B represents *R. stylosa* (longer style length of 2.4–3.5 mm), and Clade C represents the intermediate forms of *R. mucronata* and *R. stylosa* (medium style length of 1.2–2.2 mm). In addition, sub-groups were also found in each clade, indicating that there is drift in style length between *R. mucronata* and *R. stylosa* (Fig. 2).

Similar kind of drift was observed in *R. mucronata* from Pichavaram (Fig. 3). Out of 11 trees analyzed, we found two groups in the cluster analysis. Clade A represents *R. mucronata* (style length of 0.7–1.0 mm) whereas Clade B represents the intermediate forms (style length of 1.1–2.2 mm). This occurrence of undefined intermediate individuals is indicative of mixed genotypes between variants of one species rather than between genetically distinct, sibling species (Duke, 2006a). This view is supported by the genetic assessment of Lo (2010), showing very close but distinct genetic characteristics between *R. mucronata* and *R. stylosa*.

Similarly, the eight hybrid trees in Havelock were categorized into three groups, based on style length (Table 2). Group A represents hybrids with longer style length (1.9–2.5 mm), Group B represents hybrids with shorter style (0.9–1.2 mm) and Group C represents hybrids with medium style length (1.2–1.6 mm). The observations of all the possible parents of *Rhizophora* hybrids (*R. apiculata*, *R. stylosa*, *R. mucronata*, and intermediate forms of *R. stylosa* and *R. mucronata*) and three groups of hybrids indicate that almost all species of *Rhizophora* could naturally hybridize with one another when their ranges overlap and each of them can serve as the maternal parent in hybridization (Lo, 2010). In addition, all the three groups of hybrids have intermediate characters of their parents. Interestingly, the occurrence of stamens in two distinct whorls was observed in all hybrid individuals.

Singh *et al.* (1987) and Kathiresan (1995, 1999) have described the occurrence of stamens in two distinct whorls in *Rhizophora* hybrids in Andaman and Nicobar, and in Pichavaram, respectively. This suggested that the presence of stamens in two distinct whorls is characteristic of Indian *Rhizophora* hybrids and this kind of stamen arrangement has not been observed in *Rhizophora* hybrids of other regions.

Based on style length it appears that Group A hybrids are products of *R. apiculata* and *R. stylosa* (Fig. 4), Group B hybrids are products of *R. apiculata* and *R. mucronata* (Fig. 5), and Group C hybrids are products of *R. apiculata* and intermediate forms of *R. mucronata* and *R. stylosa* (Fig. 6). Variations in leaf dimensions of individuals 2, 6 and 8 indicates that introgressive hybridization or back-crossing between hybrids and their parental species may have occurred. Particularly, leaf dimensions of individual 6 (L 15 cm; W 9.5 cm and L/W ratio 1.5) are very broad, dark green and the mean values do not come within the range of *R. lamarckii* (L 7.7–14.8 cm, W 3.2–8.2 cm) as described by Duke and Bunt (1979), and of *R. annamalayana* (L 10.8 cm, W 6.0 cm) as described by Kathiresan (1995). It appears that individual 6 is a back-crossing product of *R. mucronata* or *R. stylosa*. Whereas, individual 8 is a back-crossing product of *R. apiculata* because of its long lanceolate leaves (L 17 cm, W 8 cm). The dark green, broad, cordate and acuminate leaves of individual 2 are peculiar among the eight hybrids. It is understood that individuals 2, 6 and 8 are hybrid variants due to introgression or product of *R. apiculata*, and intermediate forms between *R. mucronata* and *R. stylosa*. In putative *Rhizophora* hybrids in Australia, *R. lamarckii* also revealed slight morphological introgression to *R. apiculata*, *R. mucronata* and *R. stylosa* in some of the characters (Duke & Bunt, 1979). Except for individuals 1, 5 and 8, all other individuals exhibited dark green leaves. Comparative leaf morphology of the eight hybrid trees is shown in Fig. 7.

Eleven randomly selected hybrid trees in Pichavaram were also categorized into two groups, based on style length (Table 3). Group A represents hybrids with shorter style length (1.1–1.3 mm) and Group B represents longer style length (1.7–2.4 mm). Although *R. stylosa* is not present in Pichavaram, the presence of intermediate forms of *R. mucronata* and *R. stylosa*, and hybrids with long and short style length indicate that there are two types of hybrids i.e. Group A (shorter style length) are products of *R. apiculata* and *R. mucronata* (Fig. 8), and Group B (longer style length) are products of *R. apiculata* and intermediate forms (Fig. 9). One unique feature of the hybrids in Pichavaram is the presence of broad, dark green leaf with acute tips in all individuals, and leaf size and shape vary widely within the same individual.

Based on style length, we conclude that, among the eight individual hybrids in Havelock, individuals 1 and 3 are products of *R. apiculata* and *R. stylosa* (*R. lamarckii*). Individuals 4, 5 and 7 are products of *R. apiculata* and *R. mucronata* (*R. annamalayana*). Whereas, individuals 2, 6 and 8 are products of *R. apiculata* and intermediate forms of *R. stylosa* and *R. mucronata* or hybrid variants due to introgression or back-crossing with their parents. Among the 11 individual hybrids in Pichavaram, five individuals are products of *R. apiculata* and intermediate forms, and six individuals are products of *R. apiculata* and *R. mucronata*. Drift in style length and the occurrence of intermediate forms of *R. mucronata* and *R. stylosa* indicate that both species are very close morphologically but genetically distinct, and the presence of stamens in two distinct whorls is characteristic of Indian hybrids. Further taxonomical and molecular analyses are needed for confirmation.

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Table 1. Style lengths (mm) of *Rhizophora* hybrids by various authors

Author	<i>R. lamarckii</i>	<i>R. annamalayana</i>
Duke and Bunt (1979)	1.9 (1.5–2.7)	–
Kathiresan (1995)	–	2.2 (Pichavaram)
Parani <i>et al.</i> (1997a,1997b)	–	1.5–2.5 (Pichavaram)
Singh <i>et al.</i> (1987)	2–3 (Andaman & Nicobar)	–
Duke (2010)	3.0–4.5 (New Caledonia)	–
Duke <i>et al.</i> (2002)	2.7 (1.7–3.7) (NW Pacific) 2.0 (1.3–2.9) (Australia)	–
Duke (2006b)	2–3	–
Chan (1996)	2–3 (Malaysia)	–

Table 2. Morphological attributes of eight hybrid individuals of *Rhizophora* in Havelock

Character	Group 1		Group 2			Group 3		Ind 8
	Ind 1	Ind 2	Ind 3	Ind 4	Ind 5	Ind 6	Ind 7	
Leaf length (cm)	12.9 ± 0.4	13.9 ± 0.4	13.0 ± 0.5	12.4 ± 0.3	13.3 ± 0.3	15.3 ± 0.5	13.8 ± 0.3	17.4 ± 0.8
Leaf width (cm)	6.4 ± 0.3	8.0 ± 0.2	7.2 ± 0.3	7.5 ± 0.3	7.1 ± 0.2	9.5 ± 0.3	7.5 ± 0.2	8.3 ± 0.4
Style length (mm)	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	1.4 ± 0.0	1.5 ± 0.0
No. of stamens	OR = 8 IR = 0–7	OR = 8–9* IR = absent	OR = 8–9 IR = 0–4	OR = 8(–11) IR = 0–4	OR = 8(–10) IR = 4–8	OR = 8–13 IR = 0–7	OR = 8(–10) IR = 2–6	OR = 8–11 IR = 2–4*
No. of flowers per inflorescence	2	2–3	2	2–3(4)	2	2	2	2–3(4)

Ind = individual, No. = number, OR = outer row and IR = inner row

\* With 1–3 smaller stamens

• Mostly 3 stamens

Table 3. Morphological attributes of 11 hybrid individuals of *Rhizophora* in Pichavaram

Individual	Style length (mm)	Leaf length (cm)	Leaf width (cm)	No. of stamens	No. of flowers per inflorescence	
1	1.1 ± 0.1	13.1 ± 0.9	6.9 ± 0.6	OR = 8, IR = 1-8	2-4	Group 1
2	1.3 ± 0.3	13.4 ± 0.8	6.7 ± 0.5	OR = 8, IR = 3-8	2-4	
3	1.3 ± 0.1	14.6 ± 1.3	7.8 ± 0.9	OR = 8, IR = 4-8	2-4	
4	1.3 ± 0.2	13.3 ± 1.1	7.4 ± 0.6	OR = 8, IR = 1-8	2-4	
5	1.3 ± 0.1	15.7 ± 1.0	8.5 ± 1.2	OR = 8, IR = 2-8	2-4	
6	1.4 ± 0.1	13.3 ± 0.9	7.2 ± 0.8	OR = 8, IR = 0-8	2-4	
7	1.7 ± 0.2	14.2 ± 0.9	7.7 ± 0.7	OR = 8, IR = 2-8	2-4	Group 2
8	2.1 ± 0.2	15.2 ± 1.8	7.7 ± 1.1	OR = 8, IR = 2-8	2-4	
9	2.1 ± 0.1	12.7 ± 0.9	7.0 ± 0.4	OR = 8, IR = 2-8	2-4	
10	2.1 ± 0.2	14.9 ± 0.7	6.9 ± 0.4	OR = 8, IR = 4-8	2-4	
11	2.4 ± 0.4	13.2 ± 0.5	7.3 ± 0.5	OR = 8, IR = 2-8	2-4	

No. = number, OR = outer row and IR = inner row

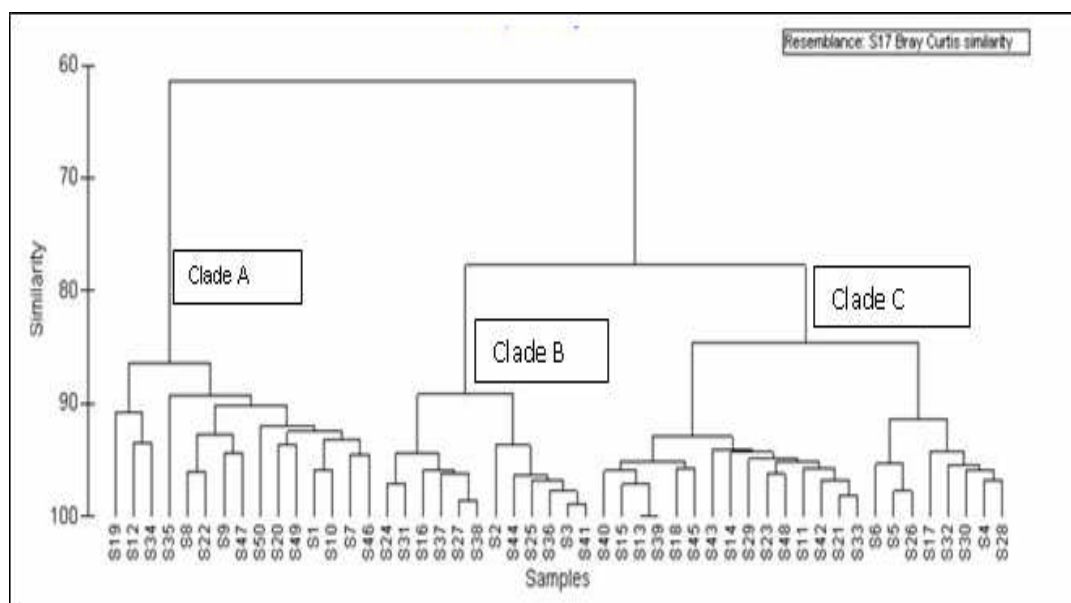


Fig. 1. Cluster analysis based on style length of *Rhizophora mucronata* and *R. stylosa* in Havelock



Fig. 2. Drift in style length between *Rhizophora mucronata* and *R. stylosa* in Havelock

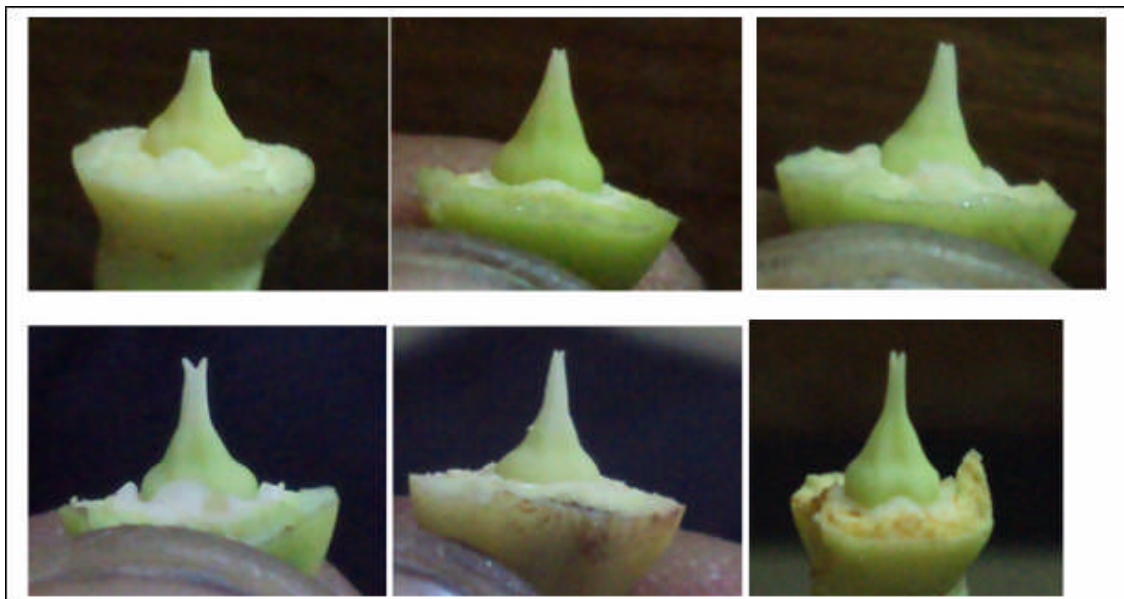


Fig. 3. Drift in style length of *Rhizophora mucronata* in Pichavaram



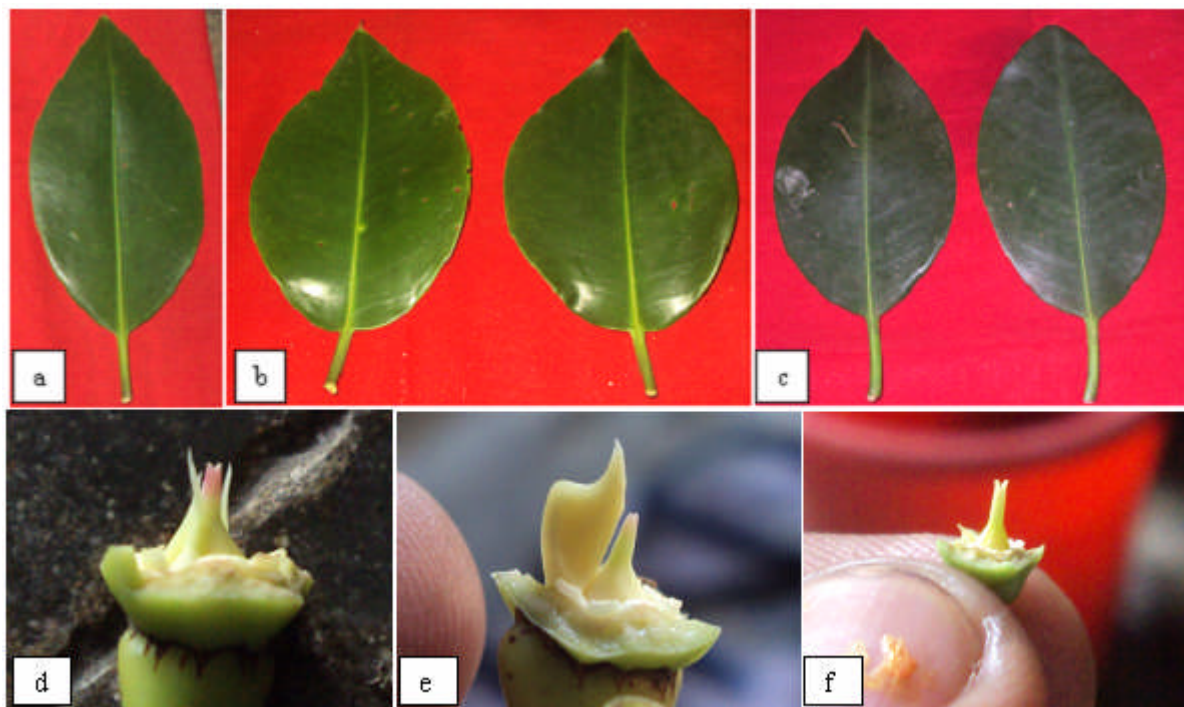


Fig. 4. Leaves and styles of Group A *Rhizophora* hybrids in Havelock

[a & d = individual 1; b & e = individual 2; c & f = individual 3]

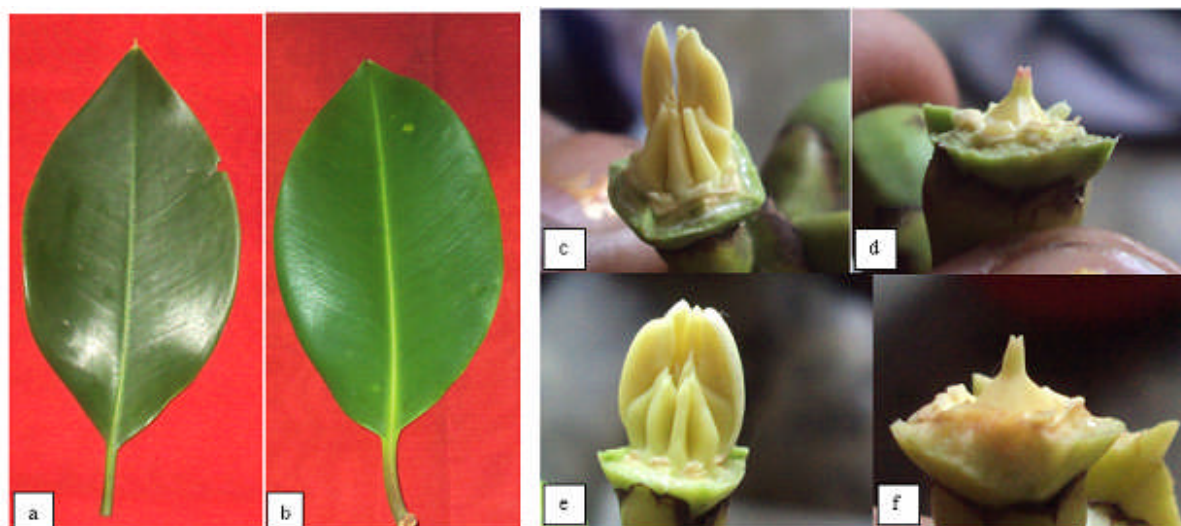


Fig. 5. Leaves, stamens and styles of Group B *Rhizophora* hybrids in Havelock

[a, c & d = individual 4; b, e & f = individual 5]

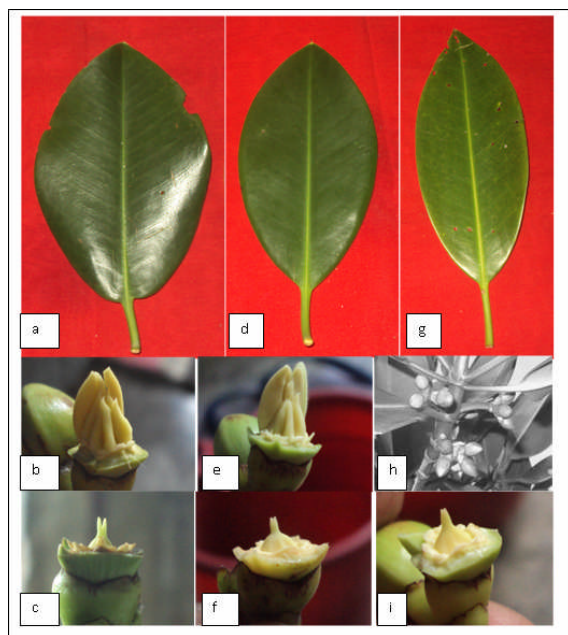


Fig. 6. Leaves, stamens and styles of Group C *Rhizophora* hybrids in Havelock

[a, b & c = individual 6; d, e & f = individual 7; g, h & i = individual 8]

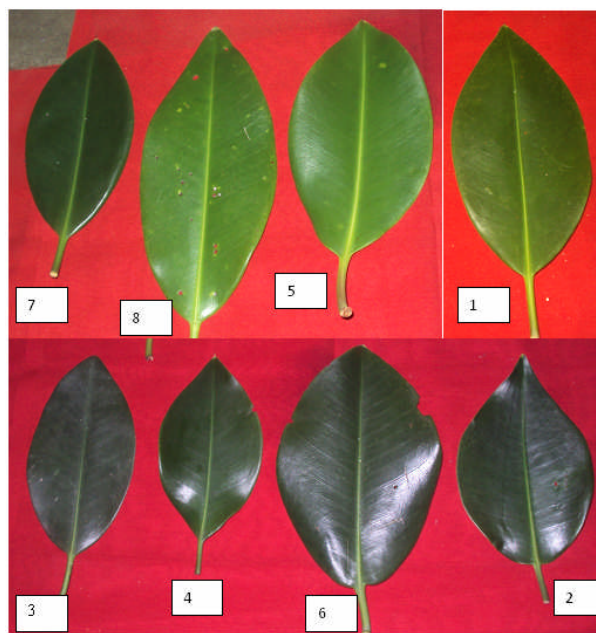


Fig. 7. Comparative leaf morphology of eight hybrid trees of *Rhizophora* in Havelock



Fig. 8. Group A *Rhizophora* hybrids in Pichavaram



Fig. 9. Group B *Rhizophora* hybrids in Pichavaram