virus(es) are not found to be transmitted through seeds[11]; rather almost all the citrus diseases are transmitted through bud-wood. Bud-wood exchange is restricted by many countries due to the hazards of invasion of new and old devastating diseases. This practice ruins the citrus industry in different regions of the world.

The technique developed is novel and can be effectively used for germplasm exchange, especially in those species which are moderately recalcitrant in nature and lose viability in a short time. Furthermore, this protocol with further experiments should be useful in (i) *Agrobacterium*-mediated genetic transformation; (ii) *in vitro* germplasm conservation; (iii) study on the nature of adventitive polyembryony in *Citrus* sp. and; (iv) role of growth regulators and other addenda on adventitive embryony.

The results described here have so far been reproduced three times with the two genotypes.

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ACKNOWLEDGEMENTS. We thank Dr Surinder Kumar and Mr Surinder Pal for providing the technical help and Head, Division of Fruits and Horticultural Technology, IARI, New Delhi for providing the facilities.

Received 27 July 2004; revised accepted 24 December 2004

Somatic embryogenesis and plant regeneration in *Eucalyptus tereticornis* Sm.

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Somatic embryogenesis and plant regeneration were obtained from cultured mature zygotic embryos of *Eucalyptus tereticornis* Sm. through callus. MS and B5 basal media containing different concentrations of NAA and 2,4-D were evaluated for callus induction and different BAP concentrations for somatic embryogenesis. The effect of different light conditions on somatic embryogenesis was also studied. Callus induction and somatic embryogenesis was found to be highest on MS medium compared to B5 medium. Highest frequency of friable callus was obtained on MS medium supplemented with 10.74 μM NAA. When the callus was transferred separately to the respective media on which callus induction occurred, containing various concentrations of BAP, somatic embryos developed after 1–2 weeks with highest frequency (54%) in MS medium containing 2.22 μM BAP. The embryos were germinated on MS basal medium. The rooted plants were successfully transferred to polybags after hardening.

EUCALYPTUS is an economically important multipurpose tree species belonging to the family Myrtaceae. It is widely used for establishing plantations in tropical and subtropical regions of the world[1]. The area under *Eucalyptus* plantations

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is estimated[1] to be around 13 mha. In India, *Eucalyptus tereticornis* is among the most widely cultivated species due to its wide adaptability to varied climatic conditions and economic importance such as fuel wood and raw material for paper pulp[2]. *E. tereticornis* is a fast-growing tree that can reach 30 to 45 m in height and 1 to 2 m in diameter. It has a straight shaft with a big crown that is moderately dense. The trunk has a straight base and cylindrical shaft. The smooth, whitish bark comes loose in thin laminas or long strips, producing whitish, grey, or bluish spots in patches and leaving an accumulation of old bark (dark grey, wrinkled) at the base[2]. *In vitro* micropropagation has been reported for *E. tereticornis* and several other *Eucalyptus* species[3]. As it is well documented, somatic embryogenesis offers many advantages[4,5]. Successful somatic embryogenesis has been reported from *E. leichow*[6], *E. citriodora*[7], *E. grandis*[8,9] and *E. dunni*[10]. However, somatic embryogenesis has not been reported in *E. tereticornis* so far. In the present study, the effect of two basal media, viz. MS[11] and B5[12] with different combinations of growth regulators and cultural conditions, was studied on somatic embryogenesis in *E. tereticornis*.

Seeds were collected from an identified *E. tereticornis* tree growing in the *Eucalyptus* clonal bank of Institute of Forest Genetics and Tree Breeding, Coimbatore, India. The seeds were surface sterilized by immersing in 70% ethanol for 2 min and subsequently in an aqueous solution of 0.1% mercuric chloride (w/v) containing 2–3 drops of Tween-20 for 10 min with constant shaking. The seeds were then rinsed five times with sterile distilled water.

MS and B5 media supplemented with 3% (w/v) sucrose were used for the study, unless otherwise specified. The pH of the media was adjusted to 5.8 with 0.1 N NaOH solution prior to the addition of agar (0.8% (w/v), Himedia, India). All the media were dispensed (25 ml each) in 25 × 150 mm test tubes (Borosil, India), sealed with cotton plugs and sterilized by autoclaving at 121°C (120 psi) for 20 min. Unless otherwise stated, the cultures were maintained under cool white fluorescent tubes under low light condition (20 $\mu Em^{-2}s^{-1}$, 16 hL/8 hD) at 25 ± 2°C.

Seeds were aseptically cultured separately on MS and B5 basal media supplemented with different concentrations of growth regulators at different stages of the cultures. The plant growth regulators used for callus induction include various concentrations of α-naphthaleneacetic acid (NAA; 0–26.85 μM) and 2,4-dichlorophenoxyacetic acid (2,4-D; 0–26.85 μM). The friable callus thus obtained on MS and B5 media containing various concentrations of NAA was separately transferred to the respective medium with different concentrations of 6-benzylaminopurine (BAP; 0–12.22 μM) for somatic embryogenesis. The effect of different light conditions on somatic embryogenesis was studied by transferring the friable callus to MS basal medium containing 2.22 μM BAP and cultured under different light conditions, viz. dark, low light (20 $\mu Em^{-2}s^{-1}$, 16 hL/8 hD) and continuous low light (20 $\mu Em^{-2}s^{-1}$).

The embryogenic callus with 25–30 mature embryos obtained on MS medium containing 2.22 μM BAP was transferred to MS basal medium devoid of growth regulators supplemented with 3% (w/v) sucrose for germination and conversion of embryos.

The cultures were observed periodically under stereo microscope. The embryos were identified based on their morphology. Sections of somatic embryos were taken using a cryomicrotome and stained with safranin. Photographs were taken using Nikon microscopes (NIKON, Japan) attached with camera.

Six different substrates, viz. soil, sand, sand : soil (1 : 3), coir pith, vermiculite and vermiculite : sand (1 : 3) were used to study their suitability for the hardening of regenerated platelets. Well-developed and rooted plantlets of approximately 2 cm height were transferred to disposable plastic trays (15 × 12 × 5 cm) filled with the substrates. Fifteen plants were kept in each tray and three trays were maintained for each substrate. They were kept under polytunnels for 35 days with intermittent misting and transferred to polybags containing potting mixture for further growth. The percentage of survival of plantlets, and length of shoot and root in each substrate were measured after 35 days.

The effect of different treatments was quantified as the percentage of callus induction, calluses producing somatic embryos and the number of somatic embryos per culture under different conditions. The data were analysed statistically and significantly different means were calculated using Duncan's Multiple Range Test.

The surface sterilized seeds cultured on MS and B5 basal media supplemented with different concentrations of NAA and 2,4-D showed callus initiation after 2–3 weeks and the entire explant was converted into callus within 4–5 weeks of culture. However, the callus obtained on 2,4-D medium was white and translucent in nature and was found to be unsuitable for further studies. The analysis of variance revealed that there were significant differences in the callus induction frequency (%) of explants on MS and

Table 1. Effect of basal media (MS and B5) and NAA on friable callus induction from mature zygotic embryos of *Eucalyptus tereticornis* Sm.

NAA (μM)	Percentage of callusing explants (mean ± SD)	
	MS	B5
Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}
0.54	6.33 ± 0.58^{e}	0.00 ± 0.00^{e}
2.69	15.33 ± 2.08^{d}	2.33 ± 0.58^{d}
5.37	26.67 ± 2.08^{b}	11.33 ± 1.53^{c}
10.74	37.00 ± 3.61^{a}	17.33 ± 1.53^{b}
16.31	36.00 ± 2.00^{a}	21.67 ± 1.53^{a}
26.85	22.33 ± 1.53^{c}	18.33 ± 2.52^{b}

Means within the same column followed by same letters are not significantly different by Duncan's Multiple Range Test at 5% probability level. Values are means of four replications with ten cultures and with five explants per treatment. Data were taken after 6 weeks of inoculation.

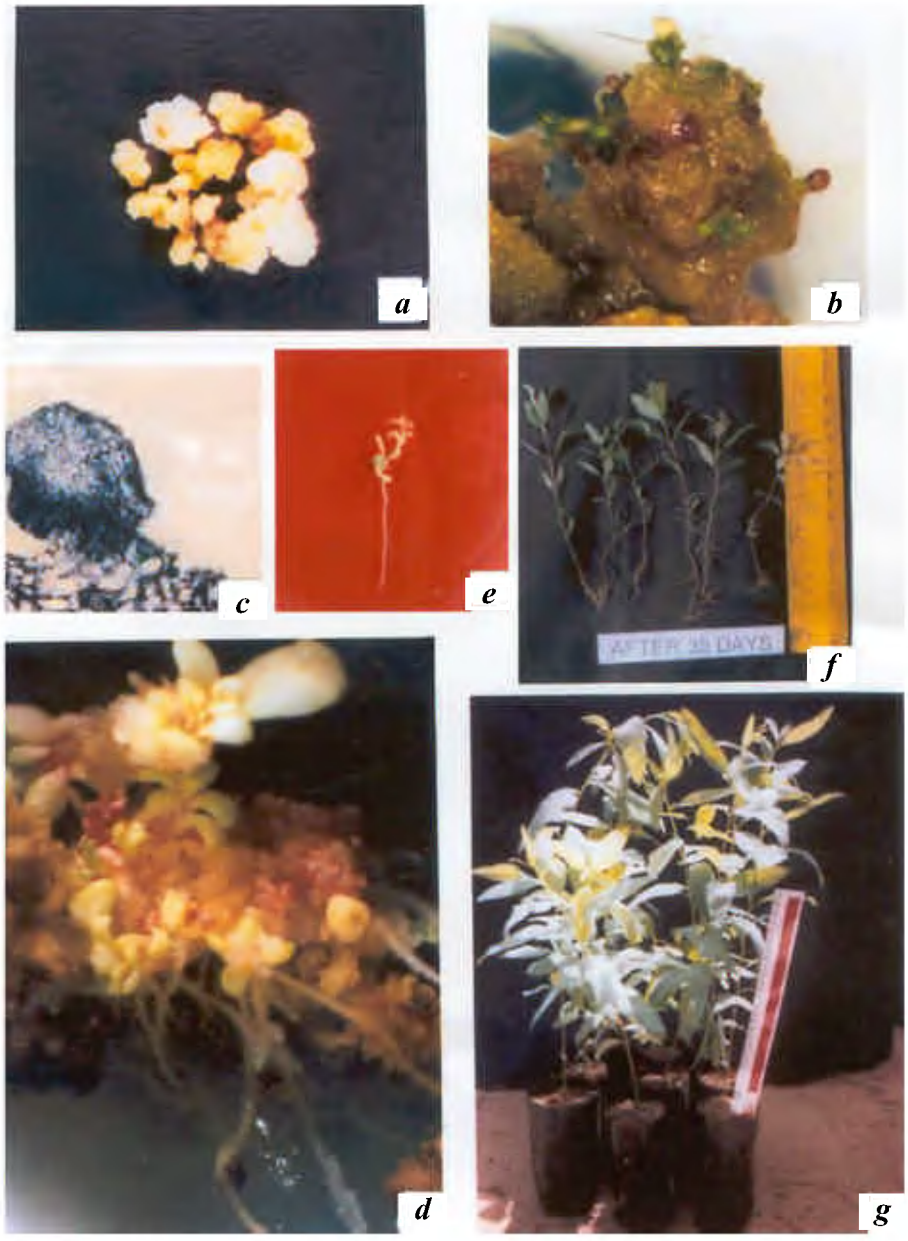

Figure 1. Somatic embryogenesis and plant regeneration in *Eucalyptus tereticornis* Sm. ***a***, Callus derived from mature zygotic embryos of *E. tereticornis* on MS medium containing 10.74 µM NAA and 3% (w/v) sucrose. ***b***, Somatic embryogenesis on MS + 2.22 µM BAP + 3% (w/v) sucrose. ***c***, L. S. of embryogenic callus showing somatic embryogenesis. ***d***, Germination of somatic embryos on growth regulator-free MS basal medium. ***e***, Converted plantlet of *E. tereticornis*. ***f***, *E. tereticornis* plants after 35 days of hardening under polytunnels. ***g***, Hardened *E. tereticornis* plants transferred to polybags.

B5 nutrient media at different NAA concentrations tested. Among the different concentrations of NAA tested, the highest frequency of callus induction was noticed on MS basal medium containing 10.74 µM NAA (Figure 1 *a*). However, the frequency of callus induction on B5 medium was low compared to MS medium and the highest frequency of callusing was obtained in B5 medium containing 16.31 µM NAA (Table 1).

The friable callus obtained on MS and B5 basal medium media was transferred separately to induction medium (MS and B5) containing different levels of BAP alone for somatic embryogenesis. Further proliferation of the callus occurred on this medium and deep pink coloured spots appeared throughout the callus surface after 1–2 weeks of incubation. Somatic embryogenesis was observed after 2–3 weeks of emerging from the callus (Figure 1 *b* and *c*). Among the

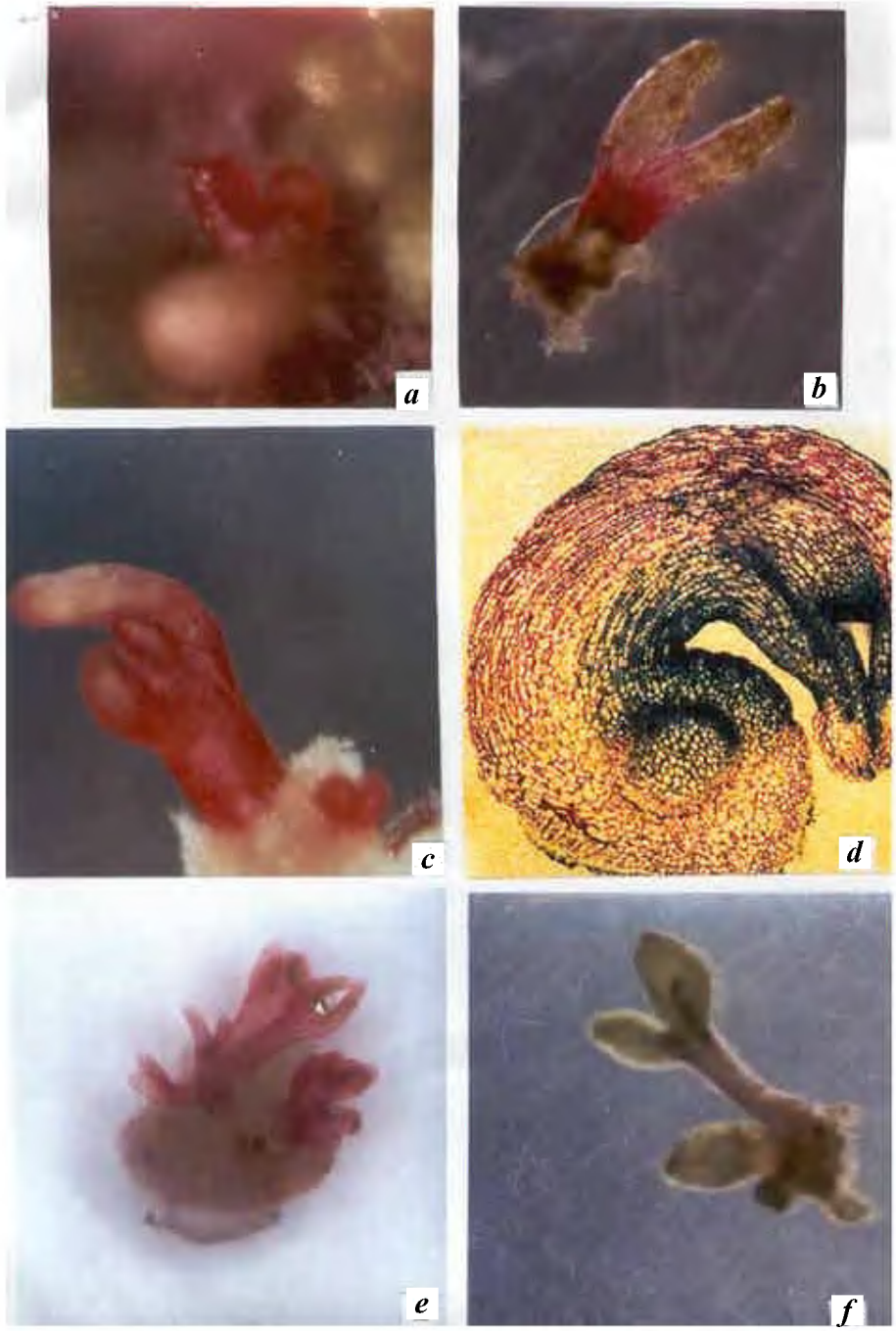

Figure 2. Developmental stages and histology of somatic embryogenesis in *E. tereticornis* Sm. obtained on MS + 3% (w/v) sucrose + 2.22 µM BAP. ***a***, Heart-shaped embryo; ***b***, Torpedo stage; ***c***, Walking stick-like embryo; ***d***, L. S. of somatic embryo; ***e***, Mature embryo showing cotyledon and shoot pimordia (arrow indicating shoot primordium); ***f***, Converted plantlet with leaves and shoot.

two different basal media and BAP concentrations tested, MS medium gave the highest percentage of embryogenesis (54%) with a mean number of 33.33 somatic embryos per culture at 2.22 µM BAP, whereas in B5 medium the maximum embryogenesis (26.67%) was noticed on 4.44 µM BAP, while the highest number of somatic embryos per culture (10 embryos) was obtained at 2.22 µM BAP. When the BAP concentration was increased beyond 4.44 µM, significant reduction in embryogenesis was noticed in both the media (Table 2). A few different developmental stages of somatic embryogenesis were observed in the culture (Figure 2 *a–c*, *e* and *f*). The LS of somatic embryos was taken as mentioned earlier (Figure 2 *d*).

The results showed that the frequency of somatic embryogenesis significantly varied under different light conditions tested. The highest frequency of embryogenesis was observed

Table 2. Effect of BAP on indirect somatic embryogenesis in *E. tereticornis* Sm.

BAP (μM)	MS		B5	
	Percentage of cultures showing embryogenesis (mean ± SD)	Mean number of embryos/culture (mean ± SD)*	Percentage of cultures showing embryogenesis (mean ± SD)	Mean number of embryos/culture (mean ± SD)*
Control	00.00 ± 0.00^{e}	00.00 ± 0.00^{f}	00.00 ± 0.00^{g}	00.00 ± 0.00^{f}
0.44	44.33 ± 2.52^{b}	22.00 ± 1.00^{c}	7.33 ± 1.53^{e}	6.00 ± 1.00^{d}
0.88	48.33 ± 2.52^{b}	28.33 ± 2.52^{b}	12.33 ± 2.52^{d}	9.67 ± 1.53^{b}
2.22	54.00 ± 4.00^{a}	33.33 ± 2.31^{a}	22.33 ± 1.53^{b}	10.00 ± 1.00^{a}
4.44	43.00 ± 3.61^{b}	14.33 ± 1.53^{d}	26.67 ± 1.15^{a}	8.00 ± 1.00^{c}
8.88	28.00 ± 2.65^{c}	12.33 ± 2.08^{d}	14.67 ± 1.53^{c}	6.00 ± 1.00^{d}
12.22	19.00 ± 1.00^{d}	5.33 ± 1.15^{e}	5.00 ± 1.00^{f}	2.67 ± 0.58^{e}

*Abnormal embryos were not counted.

Means within the same column followed by same letters are not significantly different by Duncan's Multiple Range Test at 5% probability level. Values are means of four replications with ten cultures per treatment. Data were taken after 5 weeks of inoculation.

Table 3. Effect of different light conditions on somatic embryogenesis in *E. tereticornis* Sm.

Light condition	Percentage of cultures showing embryogenesis (mean ± SD)	Mean no. of embryos (mean ± SD)
Dark	7.33 ± 1.15^{c}	3.00 ± 0.53^{c}
Low light (20 $\mu Em^{-2}s^{-1}$, 16 hL/8 hD)	54.00 ± 4.00^{a}	33.33 ± 2.31^{a}
Continuous low light (20 $\mu Em^{-2}s^{-1}$)	32.00 ± 0.58^{b}	12.00 ± 2.00^{b}

Means within the same column followed by same letters are not significantly different by Duncan's Multiple Range Test at 5% probability level. Values are means of four replications with ten cultures per treatment. Data were taken after 5 weeks of inoculation.

Table 4. Effect of different substrates on hardening of *E. tereticornis* plantlets

Substrate	Survival (%)	Average shoot length (cm)	Average root length (cm)
Red soil	91.1	5.27	5.17
Sand : red soil (1 : 3)	48.8	4.94	6.13
Coir pith	84.4	4.42	5.08
Sand	40.0	3.78	8.63
Vermiculite	64.8	4.24	9.96
Vermiculite : sand (1 : 3)	60.0	4.12	5.38

*Forty-five plants in each treatment; data taken after 35 days of transplanting on substrates.

under low light conditions (20 $\mu Em^{-2}s^{-1}$, 16 hL/8 hD), while under dark conditions the percentage of embryogenesis was found to be very low (Table 3).

The embryogenic callus with mature embryos obtained on MS basal medium with 2.22 μM BAP was transferred to MS basal medium devoid of growth regulators for germination of embryos. More than 80% of the embryos germinated producing roots into the medium (Figure 1 *d*) and leafy plantlets developed within 2–3 weeks (Figure 1 *e*).

Well-developed plantlets with approximately 2 cm height were selected and carefully separated from each other without damaging the roots. After washing under tap water to remove traces of agar and the medium, they were planted on different substrates for hardening as mentioned earlier. The effects of different substrates were compared. The best survival percentage was noticed on soil (91.1%) followed by coir pith (84.4%). The length of the shoot and root was measured individually for all the seedlings 5 weeks after transplanting (Figure 1*f*). Shoot length was measured from collar region to the shoot tip and root length from the collar region to the tip of the primary root, and their means were calculated. Data on survival percentage, length of shoot and root are summarized in Table 4. Plantlets were transplanted to polybags (20 × 13 cm) containing greenhouse potting medium (red soil : coir pith : sand, 2 : 1 : 1 ratio) for further growth and kept under shade for further field planting (Figure 1 *g*).

Somatic embryogenesis in *Eucalyptus* species depends on the nature of the explant used[6,7,10]. Moreover, basal media and cultural requirements for callus induction and somatic embryogenesis were also found to be varying for different *Eucalyptus* species[6,7,10]. Additional supplements like coconut milk and casein hydrolysate either alone or in combination, and different light conditions in different stages were reported to influence callus induction and somatic embryogenesis in different *Eucalyptus* species[6,7,10]. In the present study, two completely well-defined media (MS and B5) were used and the cultures were incubated under a

low light intensity (20 $\mu Em^{-2}s^{-1}$) with a 16/8 h-light–dark regime at all the stages.

In conclusion, it was found that somatic embryogenesis could be obtained in *E. tereticornis* using a well-defined medium without any additional supplements when cultured under low light conditions at all the stages.

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ACKNOWLEDGEMENTS. We thank the Indian Council of Forestry Research and Education, Dehra Dun for financial support and the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore for providing facilities.

Received 5 November 2003; revised accepted 15 January 2005

Brachyuran crab diversity in natural (Pitchavaram) and artificially developed mangroves (Vellar estuary)

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The brachyuran crab diversity was studied in four stations of Pitchavaram mangroves and three stations of Vellar mangroves. A total of 38 species of brachyuran crabs was recorded in the Pitchavaram mangroves (18 species of grapsids and 8 species of ocypodids besides others), while 8 species were recorded in Vellar mangroves (5 species of grapsids and 3 species of ocypodids). The abundance of crabs also varied between the two mangrove habitats (65–82/m^2 in Pitchavaram mangroves and 27–40/m^2 in Vellar mangroves). The Pitchavaram mangrove forest has been in existence since sixteen to seventeen hundred years. In Vellar estuary, mangrove was established 13 years ago. The mangroves with vast network of roots and trunks offer a good niche for the brachyuran crabs. Due to its age and vast extent, the Pitchavaram mangrove forest has higher brachyuran crab diversity. When the mangroves were established in Vellar estuary, the mangrove-associated crabs were not present. But subsequently due to larval transport from the Pitchavaram mangroves, few species got established. Due to the above process, the remaining species may also get established. But how much time it will take? It is an interesting question worth investigating. Continuous monitoring of brachuran crab diversity may provide the answer.

THE coastal and marine environs have some of the richest biodiversity areas. They include extensive areas of complex and specialized habitats such as enclosed seas and tidal systems, estuaries, salt marshes, coral reefs, sea grass beds and mangroves.

The association of brachyuran crabs with mangrove flora, behaviour, feeding and ecology is of great interest to biologists[1]. The brachyuran crabs are interesting in that they walk on their sides. Another important feature is the much-expanded body in contrast to the elongated one in other decapod crustaceans. Among all the macrofauna inhabiting the mangrove swamps, brachyurans are among the most important taxa with regard to species diversity and total biomass. They make up as much as 80% of the macrofaunal biomass in mangroves[2] and densities reach[3] as much as 80–90 m^2. Among the brachyuran crabs, grapsids, ocypodids, portunids, xanthids and gecarcinids are dominant in the mangroves. In particular, the sesarmids have attained extreme diversity and richness in the Indo-Pacific mangroves.

Crabs also play many important roles in the mangroves. Degradation of mangrove leaf litter by crabs, sesarmids

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