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In vitro Technology for Embryo Rescue and Long-term Stocking of Mangrove Seedlings

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Abstract: The deterioration of mangrove ecosystems worldwide calls for new techniques of propagation to improve tree potentials for seedling production. In the present technique, viable embryos were aseptically excised out of the mature propagules and then cultured on phytohormone-free MS medium. The produced seedlings exhibited relatively slow developmental growth rates which allowed an *in vitro* long-term stocking for these seedlings for more than 90 days with no need for reculturing

Key words: Mangrove, propagules, embryo rescue, stocking, crop protection, shoot running.

Introduction

Mangroves form one of the most extraordinary ecological formations occurring in the Coastal lowlands of the tropics. They have important economic and biological roles for local communities. On a global scale, Mangroves are exposed to many single destructive events, but the cumulative effects of destruction threaten their continued existence (Mastaller, 1996).

For Mangrove restoration in degraded areas, countries of South Asia and many others have planted propagules and seeds (Aksornkoae *et al*., 1993; Chan *et al*., 1993; Soemodihardjo *et al*., 1993; Siddiqi *et al*., 1994; O'Grady *et al*., 1996; Osborne and Berjak, 1997; Osunkoya and Creese, 1997), but their supplies usually depend upon their natural yields. In accordance with planting schedules, it is not possible to obtain enough numbers of good propagules or good seeds (Baba and Onizuka, 1997). The common phenomenon of vivipary among Mangrove plants (Schwamborn and Saint paul, 1996) forms another difficulty for stocking propagules for longer periods, because propagules can not be kept dormant like many seeds of terrestrial tree species. In this report, a new efficient technique could be adopted for long-term preservation and stocking of germinating Mangrove propagules, for further propagation project, for the purposes of restoration of degraded areas and afforestation of shorelines.

Materials and Methods

Mature propagules of *Avicennia marina* were collected from the Mangrove of Nabq protected area (28° 07 N - 28° 18 N), which is allocated on the southern region of the western Coast of the Gulf of Ababa (Southern Sinai Coast area) in November, 1998. Surface sterilization of the collected propagules was conducted using 70% ethanol for 10 minutes, 1% Cl^- concentration of sodium hypochlorite ($NaClO_3$) for 5-10 minutes; and rinsing with sterilized water. These propagules were then aseptically dissected to remove the cotyledonary parts and isolate the viable embryos. These embryos were then put on 10 ml of MS (Murashig and Skoog, 1962) medium (pH= 5.8) with 0.75% agar and 3.5% sucrose in 50 ml sterile glass vials. Cultures were kept in 14-hour photoperiod (4,000 lux) at 28 °C in a growth cabinet (Baba and Onizuka, 1997).

Results

The embryos excised out of mature propagules of *A. marina* were successfully induced to continue germination, root growth, and subsequent seedling development (Fig. 2). These seedlings exhibited relatively slow developmental growth rates on phytohormone-free MS medium. Seedlings were kept alive and healthy for more than 90 days with no need for reculturing.

(a)

(b)

Fig. 1: (a) Intact seed (propagule) load and (b) vanishing fallen mature propagules of *Avicennia marina*

Discussion

The deterioration of Mangrove ecosystems by human activities stress upon the importance of new techniques for micro propagation to improve the potential of seedling production by Mangrove trees. Many techniques have been largely implemented for vegetative propagation. Artificial

Fig. 2: (*a*) Collected mature propagules, (*b*) dissection of propagules under aseptic condition to isolate the viable embryo, © isolated embryos on phytohormone-free MS medium, and (*d*) stocks of *Avicennia marina* seedlings.

developmental techniques such as air-layering (Chinese layering) are forms of asexual reproduction which are only possible due to the ability of certain tissues (undifferentiated meristems) to form nodes and then adventitious roots and accordingly to new individuals if properly stimulated (Hartman and Kaster, 1975; Cordoba, 1976). The present technique deals directly with non-dormant (viviparous), actively dividing embryonic tissue which are able to produce seedlings in few days.

In vitro long-term stocking of Mangrove seedlings provides an endless source of contamination-free explants. The high concentrations of sterilizing solutions used in tissue culture of Mangrove organs severely damaged plant tissues (Baba and Onizuka, 1997).

As an interesting advantageous technique, the direct rescue of both the intact seed load and the fallen mature propagules (Fig. 1) protects them from natural enemies of Mangrove caused by macro climatic variations. Among these changes are abnormal see level fluctuations, salt accumulation in soil or massive sediment deposits after heavy rainfall and flooding. Such conditions disturb the substrate micro topography and prevent seeds from reaching the proper water pocket (wet seed bed) mandatory for the germination of propagules and consequent seedling establishment.

The present investigation introduced a new efficient technique for long-term preserving and stocking of germinating Mangrove propagules. These stocks would provide affordable source of explants of higher potentialities of division activity and growth. Moreover, enough healthy and disease-free seedlings of *A. marina* will be accessible, independent of natural yield or tissue differentiation, for restoration and afforestation purposes of shorelines.

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