



## Mass Propagation of *Feronia limonia* L. through Tissue Culture

Shahina Islam\*, Mosfeqa Zahan, Shahina Akter, Tanjina Akhtar Banu, Ahashan Habib, Salim Khan and Miskat Ara Akhter Jahan

*Plant Tissue Culture Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka-1205.*

### Abstract

An efficient mass propagation method for *Feronia limonia* was developed from excised shoot tips and nodal explants of *in vitro* grown seedlings. Explants were cultured on MS medium with different conc. of NAA, Kn, IAA and BAP singly or in combinations. Highest number of micro shoots and better plant growth were obtained from these two explants on MS medium supplemented with 0.2 mg/l BAP alone. The regenerated shoots were successfully rooted on MS medium supplemented with 0.5 mg/l NAA. The *in vitro* raised plantlets were successfully established in soil following the formation of roots with 100% survivability under *ex vitro* condition.

**Key words:** *Feronia limonia*, Mass propagation, Node, Shoot tips, Multiple shoot

### Introduction

*Feronia limonia* L. is one of the most familiar woody fruit trees grown in Bangladesh. It is commonly known in India as wood-apple. It is also known as elephant apple, monkey fruit, curd fruit, kathbel and other dialectal names in India (Islam *et al.*, 2006 and Hossain *et al.*, 1994). The wood-apple is native and common in India and Sri Lanka where it is cultivated along roads and edges of fields and occasionally in orchards. It is also frequently grown throughout Southeast Asia, in northern Malaysia and Penang Island (Islam *et al.*, 2006 and Hossain *et al.*, 1994). *Feronia limonia* L. belonging to the family Rutaceae is a slow-growing tree, with a few upward reaching branches bending out ward near the summit where they are subdivided into slender branchlet drooping at the tips. The fruit is round to oval, the pulp is brown, odorous, resinous, astringent, with numerous small, white seeds scattered through it (Shailendra *et al.*, 2005).

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et al.*, 1997). Among the world's 25 best selling pharmaceutical medicines, 12 are plant derived (O'Neill and Lewis, 1993). *Feronia limonia* L. has economic as well as medicinal value. It contains important medicinal compounds like umbelliferol, dictamine, xanthotoxol, scoparone etc. those could be used in the pharmaceuticals industries. The fruit is used in India as a liver and cardiac tonic,

and when unripe, as an astringent means of halting diarrhoea and dysentery and effective treatment for hiccup, sore throat and diseases of the gums (O'Neill and Lewis, 1993). Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. Oil derived from the crushed leaves is applied on itch and the least decoction is given to children as an aid to digestion (Kirtikar and Basu, 1993). Leaves, bark, roots and fruit pulp are all used against snakebite (Kirtikar and Basu, 1993). Large quantities of citric acid, mucilage and minerals found in its pulp. So the fruits could be good substrates for confectionary products, such as jam, jelly, squash, pickle and so on (Hossain *et al.*, 1994). *Feronia limonia* L. being essentially cross-pollinated and seed propagated shows great variability in natural population and therefore, vegetative propagation of desired genotypes is highly desirable. Tissue culture techniques may be an alternative method for clonal multiplication for this fruit trees. Therefore, the present experiment has been designed to develop an efficient protocol for *in vitro* plant regeneration of kathbel and to select suitable explants for *in vitro* propagation.

### Materials and Methods

Seeds of *Feronia limonia* L. (kathbel) were collected from field grown mature plants. From *in vitro* growing seedlings shoot tips and nodal segments were excised as explant

\* Corresponding author: E-mail: [shahina\\_islam2004@yahoo.com](mailto:shahina_islam2004@yahoo.com)

(Maggon and Singh, 1996; Normah *et al.*, 1997, Paudyal and Haq, 2000, Nagari and Purohit, 2004).

Seeds were first washed with detergent under running tap water for 3 - 5 min before surface sterilization. Floating seeds were considered to be empty and discarded. Later the seeds were dipped in 70% alcohol for 30 s. followed by washing with distilled water. Then the seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 12 - 15 mins. followed by washing five times with sterilized distilled water. About 4-6 surface sterilized seeds were then inoculated into each conical flask containing agar solidified MS (Murashig and Skoog, 1962) medium for germination and development.. Shoot tips and nodal segments were excised from 50 - 65 days old seedlings. These explants were then cultured on agar solidified MS supplemented with various concentrations of NAA, Kn, IAA and BAP in combinations or alone for induction of multiple shoots. All media contained 3% sucrose and 0.65% agar with pH 5.8 adjusted before autoclaving. All *in vitro* grown seedlings and cultures

were maintained under illumination on a 16h photoperiod at  $25 \pm 2^{\circ}\text{C}$ .

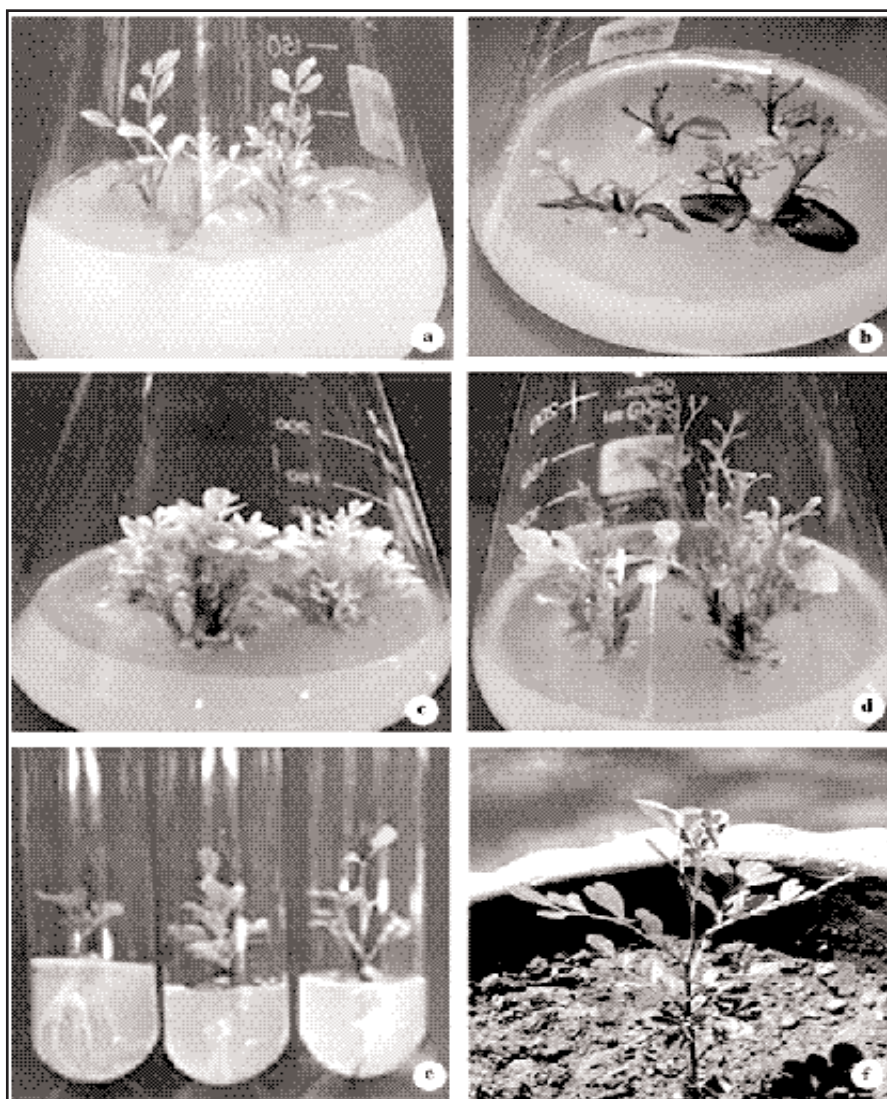
Regenerated shoots (3.5 - 4.5 cm long) were excised and transferred to MS medium supplemented with different concentrations of IAA, IBA and NAA for induction of roots. Rooted plantlets were transferred to small plastic pots containing sterilized soil and covered with polythene bags to maintain high humidity. After 50 days the acclimatized plants were transferred to larger pots.

### Results and Discussion

The *in vitro* experiments of the present study initially involved the establishment of nodal and shoot tip explants in aseptic cultures, which resulted in the induction of multiple shoots, elongation of shoots, development of roots for plantlet formation and finally the establishment of plantlets under *ex vitro* condition. Earlier reports on *Feronia limonia* L. demonstrated that plant regeneration was possible through hypocotyls, node, leaf and cotyledon explants through tissue

**Table I: Effect of different concentrations and combinations of auxins and cytokinins on multiple shoot regeneration from nodal segment and shoot tip explants of *Feronia limonia* L.**

Growth regulators (mg/l)	No. of explants inoculated	% of responsive explants	Days to shoot initiation	Mean no. of shoots/explant	Average length of shoots (cm)
NAA					
0.05	40	-	-	-	-
0.10	40	-	-	-	-
0.50	40	20	25-28	0.65	2.7
0.75	40	22	25-30	1.30	3.0
Kn					
0.05	40	33	22-24	2.85	2.7
0.10	40	30	20-25	2.00	2.8
0.50	40	42	20-25	1.90	2.8
0.75	40	28	22-24	3.62	3.0
BAP					
0.05	40	70	18-22	4.20	2.7
0.10	40	67	20-21	5.50	3.4
0.50	40	80	20-22	7.80	3.0
0.75	40	88	18-20	10.0	3.0
IAA+BAP					
0.02+0.05	40	14	21-24	0.77	2.5
0.02+0.01	40	22	20-25	1.20	2.8
0.02+0.20	40	35	20-25	2.50	3.0
0.05+0.50	40	28	22-24	1.10	2.8



**Fig. 1.** a. *In-vitro* regeneration of *Feronia limonia* shoots from shoot tip culture. b. Regenerated shoots from nodal culture. c. Regenerated multiple shoots (MS+0.75 mg/l BAP). d. Regenerated elongated shoots (MS+0.2 mg/l BAP). e. Root induction in MS+0.5mg/l NAA. f. Transplanted plantlet in soil.

culture. (Hiregoudar *et al.*, 2003; Hossain *et al.*, 1994 and Shailendra *et al.*, 2005). A series of experiments were conducted in this study to induce shoot organogenesis using different auxins and cytokinins individually and in combination, for example, MS+NAA, MS+Kn, MS+BAP and MS+IAA+BAP. Presence of different concentrations of NAA and IAA promoted callus formation and less number of shoots formation from nodal segments and shoot tip explants. Several workers have reported multiple shoot induction with only cytokinins in the growth medium (Hiregoudar *et al.*, 2003; Hossain *et al.*, 2004 and Shailendra *et al.*, 2005). Among the two types of cytokinins (BAP and

Kn) used, BAP is found to be more effective to induce adventitious regeneration. The superior effect of BAP on adventitious shoot regeneration from hypocotyl explants of wood apple has also been reported by Shailendra *et al.* (2005) and from cotyledon explants of apple has been reported by Kouider *et al.* (1985). Kim *et al.* (1988) and Islam *et al.* (2006) have found multiple shoots from the explants of cucumber and bael in presence of BAP. In this study best response towards multiple shoot regeneration of *F. limonia* was obtained on MS medium supplemented with 0.75 mg/l BAP. The shoot tip and nodal segments initially produced two to four shoots within six weeks after inoculation (Fig. 1a

& b). Subculture in the same medium yielded a cluster of five to ten shoots per explant. After fourth subculture the shoot multiplication rate remained constant but the shoots were small in height in the same media composition (Fig. 1c). Best shoot elongation was observed (approximately 4.5 cm) when the regenerating part of the explant was cut into small pieces, each with two to three shoots and cultured on freshly prepared MS media supplemented with 0.2 mg/l BAP (Fig. 1d). No remarkable variation was observed between the shoot tips and the nodal segments towards multiple shoot regeneration.

For root induction, *in vitro* regenerated well developed and elongated shoots were excised and cultured on root induction media. Different concentrations of IAA, IBA and NAA were used with MS media for root induction. Best response was observed on 0.5 mg/l NAA (Fig. 1e), where 80% shoots rooted within seven weeks of culture. Hiregoudar *et al.* (2003) observed 83.3% rooting on half - strength MS medium supplemented with 1mg/l NAA. This might be due to genotypic variation of explants influenced by the cultural and environmental conditions. After sufficient development of roots the plantlets were successfully transplanted into small plastic pots containing sand, soil and cowdung (1:1:1). The survival rate of the transplanted plantlets was found to be about 100%. Following proper acclimatization the plantlets were established in field condition (Fig. 1f). The *in vitro* regeneration protocol described here is easily reproducible, requires minimum hormonal supplements and high multiplication rate of uniform genotype. Moreover, the regeneration of plantlets was achieved without the intervention of callus and this clearly indicates the possibility of obtaining true to type plantlets. The technique described here appears to be readily adaptable for mass propagation of *Feronia limonia* L.

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