Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2014, 4(3):22-25

ISSN : 2249-7412
CODEN (USA): AJPSKY

Direct regeneration of *Agele marmelos (L.)* Corr. from shoot tip explants through *in vitro* studies

C. B. Pradeepa Devi, Gopal R. M. and Settu A.

Medicinal Plants Research Unit, P. G. Research Department of Botany, Govt. Arts and Science College, Ariyalur, Tamilnadu

---

ABSTRACT

An efficient in vitro propagation protocol through shoot tip explants culture was developed for Agele marmelos (L).corr.shoot tip explants of in vitro grown 1 month old seedlings were cultured on MS basal medium supplemented with BAP and combination with IAA were differentiated into shoots directly. The shoots could be easily multiplication on MS Medium supplemented with 2.0mg/l (BAP) along with IAA (0.1-1.0mg/l). The establishment efficiency of in vitro from plantlets in pots containing a mixture of sand: vermiculate soil (1:1) was more than 90%. This rapid regeneration protocol an average of 15 plantlets was able to produce from single set of treatment.

Keywords: *Aegle marmelos* (*L.*) corr, direct regeneration, Shoot tip culture

---

INTRODUCTION

Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds which are commonly used in home remedies against multiple alimets [2],[3].Plant regeneration protocol is a pre-requistate for the application of *in vitro* genetic manipulation techniques, such as variant selection and transformation for economically more desirable characters. *Agele marmelos* (*L*).corr family Rutaceae, is also known as Bale fruit tree. This is generally considered as sacred tree by the Hindus, as its leaves are offered to Lord Shiva during workship. Leaves, fruit, stem and roots of this tree at all stages of maturity are used as ethano medicine against various human alimet [6].

Agele marmelos containing various phyto constituents have been isolated from the various parts of plants such as Aegeline, Lupoel cineol, citral, Eugenol, Marmelide etc. The Bale are used for various treatment of Asthma, Anemia, Fractures, Healing of wounds, swollen joints, high blood pressure ,jaundice, Diarrhea, Brain typhoid troubles during pregnancy**[11]**.

Agele marmelos has been used as an herbal medicine for the management of diabetes mellitus in Ayurvedic, Unanin and Siddha systems of medicine in India. [9]Bangladesh [8] and Srilanka [7].

A high volume of reports elucidating the chemical and pharmacological properties of *Agele marmelos (*L) corr. by [4]**.** However a little attention has been paid with reference *in vitro* regeneration of this highly valuable medicinal plant. This paper describes an efficient method of direct plant regeneration from Shoot tip explants through *in vitro* studies.

MATERIALS AND METHODS

The seeds of the *Agele marmelos (L)* corr. were surface sterilized with 80% alcohol for 10s and followed by 0.1% mercuric chloride to 5 min. then the seeds washed 5 times in sterile distilled water to remove all traces of mercuric chloride.

Each five seeds were inoculated in a tube containing 10ml of MS medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.8% agar (Himedia, India). The pH of the medium was adjusted to 5.8 before autoclaving at 15lbs pressure and 121°c for 20min. All the culture was incubated at 25 ±2°c with 16hrs photoperiod provided by cool while fluorescent tubes.

The fresh Shoot tips were collected from 1 month old seedlings grown *in vitro* of optimum size (15-25mm) was transferred into cultured vials containing sterile MS medium consists of 3%sucrose and 0.8% agar and fortified with various concentrations and combination of BAP (0.5-3.0mg/l) and IAA (0.5mg/l).All plant growth regulators were added prior to autoclaving the medium.

The innoculum was sub cultured at 15 days interval into the same media. After 45days the shoot buds was initiated into the corner of explants. Then the shoots were sub cultured into shoot multiplication medium containing BAP (2.0mg/l) and IAA (0.1-1.0mg/l).After attaining optimum length (above 10cm) the elongated shoots were transferred into freshly prepared sterile rooting medium supplemented with IBA (0.1-1.0mg/l).Then the rooted plantlets were transferred into earthen pots filled with sand: vermiculate soil (1:1) for further establishment.

Each stage of different shoot multiplication, shoot elongation, root induction and acclimatization ten replicates were maintained in each media combination. All experiments were repeated for consistency of results. Observation like number of shoots emerged, root induced were recorded and their average values were tabulated (1-3)

RESULTS AND DISCUSSION

The shoot buds initiation and development were observed on shoot tip after 21 days of culture. The formation of shoot buds occurred on the top of explants. The percentage of shoot buds frequency was varied according to the combination of hormones on the medium (Table-1) (Plate-1). In all concentrations of BAP and combination of IAA a moderate shoot multiplication was observed. But a higher percentage of shoot multiplication was observed in 2.0mg/l of BAP in combination with 0.5mg/l of IAA (Table-2). This report was being confirmatory one in accordance with earlier reports [5] and the number of shoots formed explants at lower concentration of IAA. This may be due to high level of endogenesis auxin with an increase in the auxin concentration beyond a threshold value. After attaining optimum height (10cm) the elongated shoots were transferred into root induction medium supplemented with IBA (0.1mg/l to 1.0mg/l). Root initiations were observed in low frequency during the sub culture in the fresh media with similar hormonal supplementation (IBA) numbers of roots were gradually increased with many laterals. The establishment rate of *in vitro* rooted and acclimatized plant in soil was more than 90%. Rapid *in vitro* regeneration through direct shoot induction from shoot tips explants will be a reliable and less time consuming mode of propagation of the important medicinal plants of this kind in conservation aspect [10],[1].

Table-1 Effect of BAP on direct shoot initiation from Shoot tip Explants in *Agele marmelos (L.)*corr.

S.No	Concentration of Growth Hormone BAP (mg/l)	IAA (mg/l)	Percentage of Shoot Induction Frequency (%)	No. of Shoots in Explants/Tubes
	0.5	0.5	---	-----
	1.0	0.5	43	4.8±2.5
	1.5	0.5	67	6.9±4.2
	2.0	0.5	85	9.5±5.1
	2.5	0.5	71	6.0±4.2
	3.0	0.5	65	5.8±3.4

Concentration of which highest shoot multiplication was observed

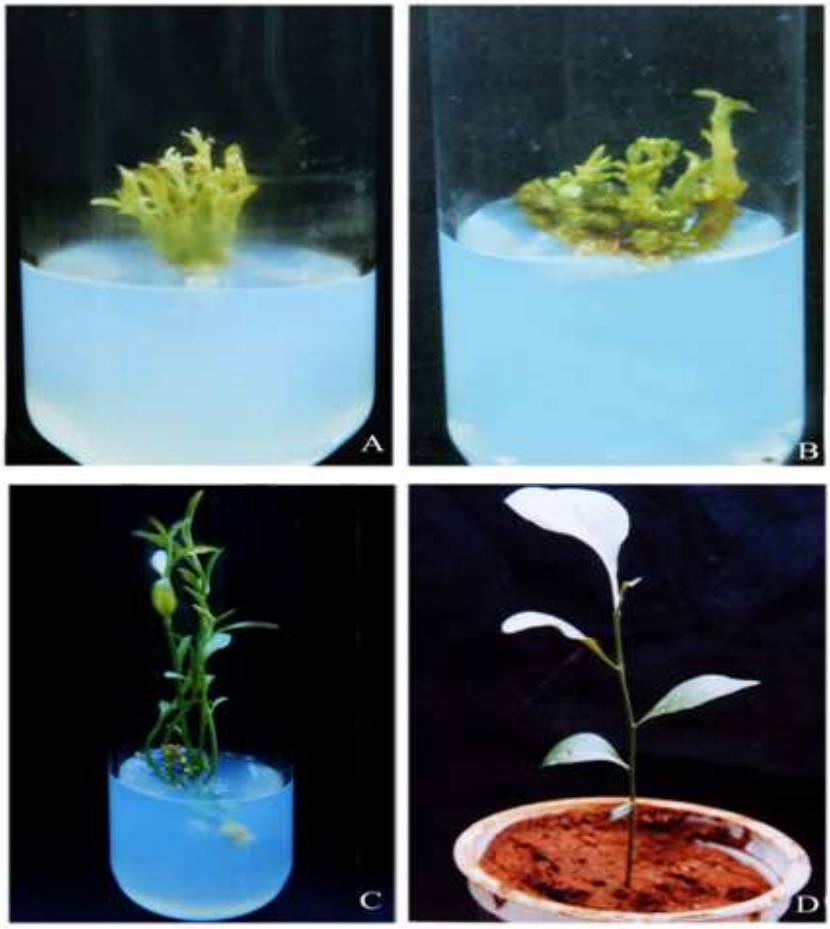

PLATE-1

(A) Initiation of Shoots from Shoot tip Explants (B) Shoot Multiplication(C) Root Induction (D) Completed Plants in plastic Cup

Table-2 Effect of BAP in combination with IAA on direct shoot multiplication in *Agele marmelos(L.)*corr.

S. No	Concentration of Hormone(mg/l)		Percentage of Shoot Multiplication (%)	No. of Shoots in Explants/ Tubes
	BAP	IAA		
	2.0	0.01	80.00	3.0±1.8
	2.0	0.05	85.00	7.3±5.3
	2.0	0.1	90.00	9.0±6.1
	2.0	0.5	95.00	14.0±3.2
	2.0	1.0	93.00	11.0±8.1

Concentration at which highest shoot multiplication was observed.

Table -3 Effect of IBA on root induction frequency of *Agele marmelos (L)*.corr.

S. No	Concentration of Hormone (mg/l)(IBA)	Percentage of Root Induction Frequency (%)	No. of Roots in Explants/ Tubes Mean±SD
1	0.1	-	-
2	0.2	-	-
3	0.3	-	-
4	0.4	5.00	2.8±1.6
5	0.5	10.00	4.5±2.3
6	0.6	20.0	5.2±4.1
7	0.7	40.0	6.7±3.2
8	0.8	60.0	8.5±0.1
9	0.9	80.0	9.0±1.1
10	1.0	70.0	7.5±0.2

Concentration of which highest root induction was observed

REFERENCES

[1] Arumugam A ,M.V.Rao, Advances *in plant science*, **1996,** 9**(**2**):**181-186.
[2] Biswas K, Chatopadhyay.I, Banerjee.R.K ,Bandhopadhyay U, *Cur Sci*, **2002**, 82, 1336
[3] Chatopadhyay I, BiswasK, Bandhopadhyay U ,Baneerjee R.K, Cur *Sci*, **2004,** 87, 44
[4]Ganesh, N.Sharma, susheel.K, Dubay, N, *Int.I.Cur.Pha.Res*, **2011,** (1):13-32.
[5] Islam, R, M.R.Karim, M.Hossain ,O.J.Joarder, Plant *Tissue Culture***. 1992,** 2(2**):**55-59.
[6] Kar A. Choudhry B. K. ,Bandhopadhyay N. G, *J Ethnopharmacol*, **2003,**84, 105-108.
[7] Karunanayake E. H, Welihinda J, Sirimanne S. R, Sinnadorai G,*J Ethnopharmacol* **1984**, 11, 223-231.
[8] Lampronti I, Martello D, Bianchi N, Borgatti M, Lambrtini E, Piva R, Jabbars S, Choudhuri M. S., Khan M. T. , Gambari R, *Phytomedicine*, **2003** 10,300-308.
[9] Murashige .T, F.Skoog ,*Physiologia plantarum*,**1962**, 473-497.
[10] D.P.Premantilake, H.A.S.nilmini ,C.Kudagamage, *Cey.J.sci* ***(****Bio.sci)***, 2006**, 35(1**):**87-90
[11] Saswati Parichha “Bael (Aegle Marmelos): Nature's Most Natural Medicinal Fruit”, **2004.** *Orissa Review.*