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ORIGINAL ARTICLE **OPEN ACCESS**

Influence of phytohormones and additives on *in vitro* shoot initiation from nodal explants of *Elaeocarpus ganitrus* and *Adansonia digitata* in liquid medium.

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ABSTRACT

Elaeocarpus ganitrus Roxb. and Adansonia digitata are two precious trees in nature. The present investigation deals with the effects of cytokinins and several other additives for in vitro shoot initiation. The study was carried out using nodal segments as explant source. The (Murashige and Skoog Medium) MS, (Woody Plant Medium) WP and Anderson Medium without addition of agar were used in the studies. The media were fortified with cytokinins, activated charcoal, silver nitrate, ascorbic acid, polyvinylpyrrolidone (PVP), citric acid and caesin hydrolysate. Initiations of shoots were observed on specific concentrations of cytokinins. MS medium was found best in shoot initiations for both E.ganitrus and A.digitata.

Key words: Silver nitrate, Coconut coir, Liquid medium

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INTRODUCTION

Elaeocarpus ganitrus and *Adansonia digitata* are important and precious trees in the world. In India, these trees are having religious importance for several groups of people. Both the trees are generally multipurpose, so the conservation of these trees through various methods is necessary. *E.ganitrus* is well known for Rudraksh beads which come under use for prayers. Rudraksh beads can also be obtained from other species of Elaeocarpus.

Bodhi beads are religiously precious and very important for Buddhists. Modern bodhi bead culture is an outcome of mixture of Buddhist and Chinese culture of collection [1]. *E.ganitrus* seeds from the trees of Ilam district, Nepal shown high anti-oxidant and anti-microbial character [2]. The *in vitro* based study of *E. blascoi* an endangered tree species of southern part of Western Ghats (Tamil Nadu) was carried out for micropropagation, in which Thidiazuron (TDZ), Kinetin (Kn), 6-Benzylaminopurine (BAP), $AgNO_3$ (Silver nitrate) and activated charcoal were used [3]. During the study, gelling agent was used in the growth medium for *in vitro* culturing of *E. sphaericus* nodal parts, which were taken as explant material [4,5]. The *in vitro* tissue culture approach was implemented for *E. robustus* [6,7,8]. The micropropagation investigations on *E. sphaericus* [9] and on *E. tuberculatus* [10] reported. The agar based media were used for investigating the effects of plant growth regulators on *in vitro* studies of *E. ganitrus* [11].

A.digitata is also well known for its multiple uses. It is indigenous in Africa and commonly known as Baobab tree. A study was performed to select important bio-active components to analyze antimicrobial activities and to standardize *in vitro* propagation protocol for *A. digitata*. The plant growth regulators like BAP, NAA and TDZ were used for the study of callus induction and somatic embryogenesis [12]. Shoot multiplication was obtained from bud multiplication and breaking of bud was cytokinin dependent. The combination of 1.0/10.0mM zeatin riboside and 10.0mM indole-3-butyric acid after 8 weeks enhanced microshoot development [13]. Another study was performed using mature and immature seeds of *A.digitata* as explants source from the mother tree and used for *in vitro* plant regeneration [14].Through different types of explants sources like cotyledonary nodes, axillary nodes and terminal apex used from

twenty day old sterile seedlings for *in vitro* propagation of African Baobab (*A.digitata* L.) [15]. African baobab tree is an important nutritional and medicinal source. As baobab products are gaining interest, it is necessary to concentrate on research studies for development of new cultivar that must have short maturation time because the plants have slow growth [16].The combination of 3mgL^{-1} BAP and 0.2 mgL^{-1} of NAA along with 100mgL^{-1} of Ascorbic acid and citric acid was observed best for the *in vitro* experiment of *A. digitata*. For the investigation explants were collected from the potted plantlets of *A.digitata* [17]. *A. digitata* is not indigenous as plant regeneration from seed is a restricting factor in India. The carbohydrate source optimization is very important and in an investigation it was observed that the rate of germination was higher with sucrose [18]. The seeds of *A.digitata* were pre-treated in full and half strength sulfuric, hydrochloric and nitric acid. The acid treatment helped in breaking seed dormancy and it also helped in seed germination. The treatment was given for 1 and/or 2 h [19]. Using embryos of *A.digitata* as starting experimental material the investigation was performed for the development of multiple shoots and buds. The MS and WPM media were used for the inoculation of mature embryos [20]. The *in vitro* micropropagation investigations on *A. digitata* were reported [21].

Micropropagation procedures using shoot tips and nodal segment were studied to produce seedlings of high quality. Using plant growth regulators (PGRs) in studies, micropropagation was done for *Quercus aliena* Blume by adventitious shoot proliferation [22]. In a study, tissue culture technique used for *Calendua officinalis* and in one experiment liquid ½ MS medium was taken. The beach sand was used to provide support [23]. *In vitro* rooting in liquid medium was reported for *Malus domestica*, *Betula lenta* and *Musa sp*. [24].

In the present study Murashige and Skoog Medium (1962) [27], Woody Plant Medium (1981) [28] and Anderson Medium (1984) [29], Plant Growth Regulators **(**PGRs) and several other additives were used for the *in vitro* shoot initiation from nodal segments of *E. ganitrus* and *A. digitata*. The research may be helpful for the *in vitro* propagation experiments of endangered tree species related with other genus too.

MATERIAL AND METHODS

Explants Material and Sterilization

The nodal explants used for the study were collected from 6 years old trees of *E. ganitrus* and 3-4years old tree of *A. digitata* at SIET, Modipuram, Meerut, UP, India [Fig.1]. After proper washing the explants of 4cm ± 0.5cm in length were treated for 30 m with 1.5% bavistin [11] (*w/v*). Under laminar air flow surface sterilization of explants was performed using 70% ethanol for 30 seconds and 0.1% (*w/v*) $HgCl_2$ for 4 min. The explants were rinsed with autoclaved double distilled water for 4 min (5 times) [11].

Liquid medium and *in vitro* cultures

BAP (6-Benzyl aminopurine), Kinetin (Kn) were taken in the range of 1.0 to 2.0 mgL^{-1} and added to the medium. Antioxidants (Ascorbic acid130 mgL^{-1}, PVP130 mgL^{-1} and Citric acid 8 mgL^{-1}) were added to the medium [11]. Activated charcoal 20mgL^{-1}, Silver Nitrate ($AgNO_3$) 7mg L^{-1} and Caesin hydrolysate (CH) 5mg L^{-1}. Sucrose 2% in WP medium and 3% was added in MS and Anderson medium. The pH was adjusted at 5.8±0.5. Coconut coir was properly dipped under double distilled water for 48h in a beaker of 500 ml and then after 48h coir was rinsed by dipping under fresh double distilled water and then kept for drying. Small portion of dried coconut coir was fitted under the test tubes. Medium was autoclaved for 20 min at 121 ºC. The explants were cultured under laminar flow. The cultures were maintained in the culture room at 25ºC ± 2ºC under 16/8 h conditions. Cultures with no cytokinins were taken as control.

Statistical analysis

The experimental works were carried out with three biological and ten technical replicates. One way analysis of variance (ANOVA) was implemented to evaluate significant differences between control and treated experiments ($p \leq 0.05$). Asterisk mark was used to assign the significant difference.

RESULTS AND DISCUSSION

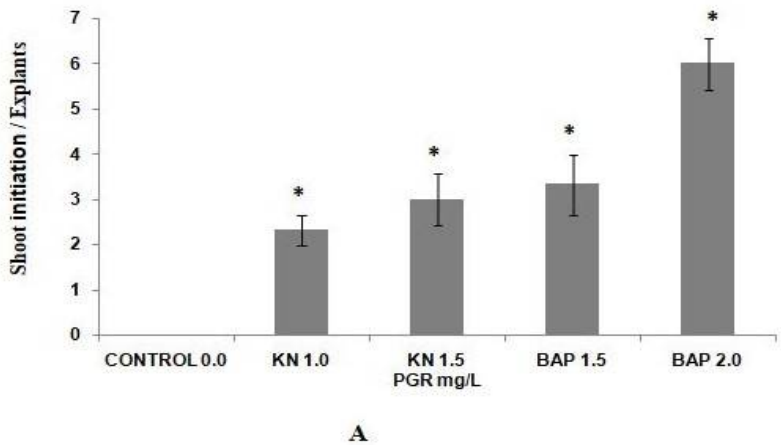
Mercuric chloride with 0.1% (*w/v*) [11] was found much effective for the surface sterilization of nodal explants material of *E.ganitrus* and *A.digitata*. Initiations of shoots were observed after 5-6 weeks in liquid MS medium for both *E.ganitrus* and *A.digitata* [Fig.2]. No shoot initiation was observed in WP and Anderson medium. It was analysed that BAP at 2mgL^{-1} concentration played crucial role in shoot initiation [Fig.3]. First sub-culturing was performed after 1-2 weeks of shoot initiation on MS medium containing similar concentrations of additives. No shoot induction was observed in the cultures which were taken as control. However, on several concentrations of kinetin and BAP also, response was not observed in the cultures of *E.ganitrus* and *A.digitata* [Fig.4]. Ascorbic acid, polyvinylpyrollidone, citric acid helped in lowering the problem of phenolic exudation.

The tissue culture investigations on *A.digitata* were reported previously by Ishii and Kambou, 2007; Singh, 2015; Singh *et al*., 2010; Rolli *et al*., 2014; N'Doye *et al*., 2012; Kumari, 2020; Aldin, 2015; Fasola and Okerenkporo, 2019.The liquid medium was implemented during *in vitro* investigations of *A.digitata* [12].

The coconut coir and liquid media were used for *in vitro* production of *Gladiolus* (cv. 'White Friendship'). In the study higher frequency of *in vitro* corms observed under liquid MS medium with 0.5mgL^{-1} NAA and with 6% sucrose. 2mgL^{-1} BAP was combined with 0.2mgL^{-1} NAA and supplemented in MS media [25]. Due to high water retention capability, coir was picked out among jute and paddy straw matrices for rooting in liquid medium [26]. Earlier studies by the researchers had shown the importance of coir on the experimental outcome.

Fig1: *E. ganitrus* [A] and *A.digitata* [B] tree at SIET (Deemed to-be University), Modipuram, Meerut (UP) India

Fig 2: Initiation of shoots from nodal explants of *E.ganitrus* [A,B]and *A.digitata* [C,D]at BAP 2mgL^{-1} concentration in liquid MS medium.



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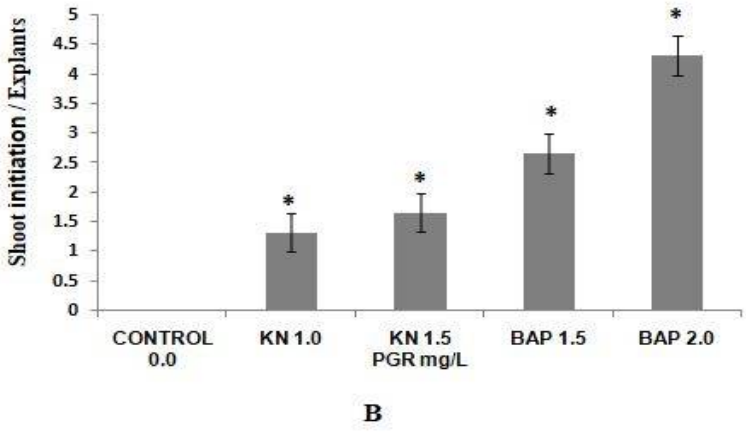

Fig 3: Graph showing the specific concentrations of PGR at which the shoot initiations were observed from the nodal segments of **[A]** *E.ganitrus* and **[B]** *A.digitata*. The mark of Asterisk (*) used to indicate $p \leq 0.05$ (n=10) for significant difference.

Explant	Treatment	Concentration (mg/L)	Response (Shoot initiation)
E.ganitrus Nodal segment	Kn	0	-
	Kn	1	++
	Kn	1.1	+
	Kn	1.2	-
	Kn	1.3	-
	Kn	1.4	+
	Kn	1.5	++
	Kn	1.6	-
	Kn	1.7	-
	Kn	1.8	-
	Kn	1.9	-
	Kn	2	-
	BAP	0	-
	BAP	1	-
	BAP	1.1	-
	BAP	1.2	-
	BAP	1.3	-
	BAP	1.4	-
	BAP	1.5	++
	BAP	1.6	-
	BAP	1.7	-
	BAP	1.8	-
A	BAP	1.9	-
	BAP	2	+++*

Explant	Treatment	Concentration (mg/L)	Response (Shoot initiation)
A. digitata Nodal segment	Kn	0	-
	Kn	1	++
	Kn	1.1	-
	Kn	1.2	-
	Kn	1.3	-
	Kn	1.4	-
	Kn	1.5	++
	Kn	1.6	-
	Kn	1.7	-
	Kn	1.8	-
	Kn	1.9	-
	Kn	2	-
	BAP	0	-
	BAP	1	-
	BAP	1.1	-
	BAP	1.2	-
	BAP	1.3	-
	BAP	1.4	-
	BAP	1.5	++
	BAP	1.6	-
	BAP	1.7	-
	BAP	1.8	-
B	BAP	1.9	-
	BAP	2	+++*

Fig 4: Showing the specific concentrations of cytokinins at which shoot initiations were observed from the nodal segment explants of [A] *E.ganitrus* and [B] *A.digitata*. The mark of **-** negative is denoting no response, **+** positive is denoting very less response, **++** less response, **+++*** denoting better response as compared to the other concentrations.

CONCLUSION

The experimental investigations were performed on media without agar. The coconut coir may be useful for tissue culture experiments based on liquid MS medium. The combination of growth regulators and

several kinds of additives which helped in shoot initiation from nodal segments of *E.ganitrus* also worked for *A.digitata*. Due to rising importance of *A.digitata* and *E.ganitrus* several researchers worked and working on various kinds of investigations related to these trees.

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CONFLICT OF INTEREST
Conflict of interest does not exist.

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