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Callus Induction of *Erinocarpus Nimonii* Grah. Critical Endangered Species of Western Ghats

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Abstract -

Callus cultures of Erinocarpus nimonii Grah. (Tiliaceae) were successfully established from cotyledon explants obtained from green mature seeds. Benzyladeninepurine (BAP) alone (2.5 mg L⁻¹) significantly increased callus formation but was not significantly different when combined with increasing levels of 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5–2.5 mg L⁻¹) and further supplemented with 1-Naphthaleneacetic acid (NAA). Loose crystalline calli were observed in media containing 2,4-D and BA singly or in combination, while compact, nodular and loose calli were obtained when the culture media were further enriched with NAA. BAP and 2,4-D singly or in combination did not significantly enhance degree of callus formation but BAP in combination with 1.0 and 2.5 mg L⁻¹ 2,4-D and NAA significantly increased degree of callusing. A significant increase in callus weight was observed in BAP at different levels combined with 2,4-D (0.5 and 2.5 mg L⁻¹). Addition of NAA to BAP and 2,4-D containing media further increased callus weight.

Key words: Callus Induction, *Erinocarpus nimonii*.

Introduction

Erinocarpus nimonii Grah. (Tiliaceae), a tree species of Western Ghats is a critical endangered plant most important the regeneration of this plant conventionally the plant can be propagated through seed. However the seed germination is poor (Vinaya, 1997). The present study was conducted to establish a protocol for large scale propagation through in callus induction using cotyledonary explant from elite tree of *Erinocarpus nimonii*.

Material And Methods

Fruits of *Erinocarpus nimonii* were collected from the Thane district Khodala forest. For sterilization, fresh fruits were washed thoroughly with distilled water and also surface sterilized by 70 % ethanol for 5 min. and 0.1 % HgCl₂ for 5 min. followed by washing with sterile water for three times. Excised seed were then aseptically incubated in culture test tube containing agar gelled MS medium. After 30 to 40 days root tip and shoot tip of seedling were dissected out and culture on MS medium supplemented with various concentration and combination of 2,4-D BAP & NAA. The culture vials were transferred to growth room and were allowed to grow in controlled environment. The temperature of the growth room was maintained with in 2C by an air conditioner. A 10 hours light period was maintained with light intensity of 2000 lux of the growth and development of culture.

Results And Discussion

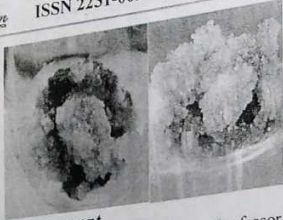
Callus induction occurred in about 20 days. Callus

induction and growth started with the swelling of the margin cells of the leaf explant. callus inductions was observed in MS medium supplemented with 2,4-D or NAA (1.0mg/l) alone. Best callus initiation and growth were observed in combination of 2,4-D (0.1 mg/l) + BAP (1-2.5 mg/l) + NAA (0.1 mg/l) in comparison to all other media used for the same purpose. This callus was further transferred on medium with various concentration of Auxin and cytokinin, separately or in combination for further analysis callus induction was poor on MS medium supplemented with auxin alone, though NAA supplementation resulted in fragile green to brown callus. With cytokinin alone in MS medium compact yellow to brown callus was formed. Interestingly, auxin (NAA) induced browning of the callus induction without further growth in *Erinocarpus nimonii* but with NAA and increasing concentration of BAP (1-2.5 mg/l) compact white best responding callus developed in about 5 weeks time. With 0.1 + 2.5 mg/l BAP added to 0.1 mg/l NAA and 0.1 mg/l 2,4-D the response was rapid (30 days) and good, but the callus became fragile white from compact white with Kn alone the callus was yellow to dark yellow exhibiting poor growth in 5 weeks but with addition of NAA, an improved response was observed within 10 days through the colour of callus turned brown. NAA with BAP also resulted in changed colour from brown to dark brown to dark yellow and the response delayed a little from 20 to 25 days (Table-1)

Table - 1: Influence of various PGRs on callus induction and growth *Erinocarpus nimonii* explants.

Sr. No.	MS + 3% Sucrose + 2% Clarigel + (mg/l) PGR	Color of Callus	Type of Callus	Day of Response	Degree of Response
1	0.5 NAA + 0.5 BAP	White	Compact	30	--
2	1.0 NAA + 1.0 BAP	White	Compact	30	--
3	1.5 NAA + 1.5 BAP	White	Compact	30	---
4	2.0 NAA + 2.0 BAP	White Green	Compact	25	----
5	2.5 NAA + 2.5 BAP	White Green	Compact	25	----
6	0.5 2,4-D + 0.5 BAP	Brown	Compact	30	--
7	1.0 2,4-D + 1.0 BAP	Brown	Fragile	30	--
8	1.5 2,4-D + 1.5 BAP	Brown	Fragile	30	--
9	2.0 2,4-D + 2.0 BAP	Brown	Fragile	30	--
10	2.5 2,4-D + 2.5 BAP	Brown	Fragile	30	--
11	0.5 NAA + 0.5 2,4-D + 0.5 BAP	White	Compact	30	--
12	1.0 NAA + 1.0 2,4-D + 1.0 BAP	White	Compact	30	--
13	1.5 NAA + 1.5 2,4-D + 1.5 BAP	White	Compact	30	+
14	2.0 NAA + 2.0 2,4-D + 2.0 BAP	White	Compact	25	--
15	2.5 NAA + 2.5 2,4-D + 2.5 BAP	White	Compact	30	---
16	0.1 NAA + 0.1 2,4-D + 0.5 BAP	White	Compact	30	----
17	0.1 NAA + 0.1 2,4-D + 1.0 BAP	White	Compact	30	----
18	0.1 NAA + 0.1 2,4-D + 1.5 BAP	White	Compact	30	--
19	0.1 NAA + 0.1 2,4-D + 2.0 BAP	White	Compact	30	--
20	0.1 NAA + 0.1 2,4-D + 2.5 BAP	White	Compact	30	---

+++ Good Response; ++ Medium Response; + Low Response



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