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Invitro Micropropogation of Nodal Explant of Gymnema Sylvestre R. Br. a Multidimensional Plant

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Abstract: Gymnema sylvestre R. Br. is high on demand yet on verge of extinction as it multipurpose medicinal plant that makes it important to propagate and preserve. The objective of this study was to develop a rapid shoots regeneration technique using growth regulators at varied combinations using mature nodal explants of Gymnema sylvestre, on MS media using combinations of auxin and cytokinins namely 6-Benzylaminopurein, Kinetin and Indole-3-acetic acid Maximum proliferation and elongation of shoot occurred using combination of 1.0K: 0.2 IAA: 0.5BAP.
Key Words-Preserve, Nodal explants, Regeneration, Auxin, Cytokinins

I. INTRODUCTION

Gymnema sylvestre R. Br. (Asclepiadaceae) also known as Gudmar or periploca of the woods is a woody plant found in Indian Forest. The use of gudmar has been prevalent in ayurvedic medicines for a long time but has recently found its place in pharmaceuticals as a miracle drug to treat diabetes mellitus since it aids in secretion of insulin by increasing cellular permeability. It is considered as a medicinal plant due to a unique agent called gymnemic acid that is present in its leaves [1],[2],[7],[8]. The medicinal qualities has made it a much desired plant as it is used as an antidote for snake bites, its extract is used in treatment of eye ailments, it is also used as an anti-allergen, aids in proper bowel movement by curing constipation, used as antiatherosclerotic drug, prevents dental caries, used as a cough medicine, it also aids in controlling obesity, aphrodisiac and used as an anti-viral and antimicrobial agent. Due to its multidimensional uses the Pharmaceutical industries has led to overexploitation in order to meet the commercial demands and has led Gymnema sylvestre into verge of extinction[1],[7],[8],[10] The attempt to reproduction these plants through conventional method is very difficult due to difficulty in producing viable seeds that germinate is very low. Alternative methods of propagation are being devised to accelerate the large scale production in order to meet the demand commercially as well as to prevent the plant from being extinct.

II. MATERIAL AND METHOD

A. Preparation of Explants

Axillary buds were taken from a year old plant of Gymnema sylvestre from home nursery on 16 January 2016. Healthy branches were selected for preparation of explants. The stems were surface sterilized and nodal explants were prepared having nodal section of 10 mm cut at an angle of 45^{o} inclination and keeping the nodal buds intact. This was followed by disinfection using 95% ethanol and then washed with Tween 20 for 10 minutes and rinsing the explants with sterile distilled water. The explants were further treated with 0.1% $HgCl_2$ solution for 1-2 min followed by washing with distilled water [3],[2].

B. Media Preparation

The explants were introduced in pre-autoclaved MS media consisting of microelements and microelements, vitamins 30 g/l, 100 mg l^{-1} inositol, sucrose, 3 g/l having a pH of 6.5± [3],[4]. Growth regulators (Kinetin, 6-Benzylaminopurein and Indole-3-acetic acid) were filtered and added to different autoclaved media tubes at varied combinations (Table. I)[3],[5].

III. RESULTS AND DISCUSSION

Present study on micropropogation using nodal explants was to initiate shoot from explants acquired from field established plants. After surface sterilization using 0.1% HgCl2 for 60 seconds resulted in 80% of sterile culture survival rate and the explants remained healthy and contamination free even after 15 days [7]. The nodal initiation of Gymnema sylvestre was observed in media supplemented with varied combinations of cytokinin i.e. Kinetin (K) and 6-Benzylaminopurein (BAP) supplemented alone as well

as with varying concentrations of auxin i.e. Indole-3-acetic acid(IAA) as well as combination of all the three hormones (Table 1) [2],[3].

The GS-12 nodal explants showed best shoot elongation of 1.8 cm within 20 days and of 2.5 cm in 25 days having hormones combination of 1.0K: 0.2 IAA: 0.5BAP. At higher concentration of IAA with kinetin or IBA did not show as promising elongation and proliferation as compared to the above combination instead on increasing the hormone levels resulted in curbing the capacity of shoot proliferation. Shoot initiation was established from nodal explants that were cultured on Murashige and Skoog media supplemented with various combinations of growth hormones. Similar observations were reported by Reddy [6],[8] were the combination of higher concentration of cytokinin and lower concentration of auxin induced better shooting. However, further increase in auxin and cytokinin levels did not suffice the aim of shoot proliferation growth performance of the shoots [8],[10][11]. 7 days past inoculation, elongation resulted in small newly sprouted buds of primary shoot. In about a month leaf started to grow at a temperature of 25±2. The initiation of Gymnema sylvestre was shown by all media supplemented with different concentration of cytokinin i.e. kinetin with combination of 6-Benzylaminopurein supplemented alone as well as with varying concentrations of auxin i.e. Indole-3-acetic acid though the growth was slow the first week.

TABLE I

INITIATION OF NODAL EXPLANTS INOCULATED ON MS MEDIA SUPPLEMENTED VARIED CONCENTRATION OF AUXIN AND CYTOKININS

SN	Culture code	MS Media concentration Kinetin:IAA	Incubation period and growth in centimeter		
		K:IAA:BAP (mg/l)	10days	20 days	30days
1	GS-1	0.1:0:0	-	-	-
2	GS-2	0.2:0:0	-	-	-
3	GS-3	0.5:0:0	-	-	-
4	GS -4	0.1:0:0.1	-	-	-
5	GS -5	0.2:0:0.1	-	-	-
6	GS -6	0.5:0:0.5	-	-	-
7	GS -7	1.0:0:0.1	-	-	0.1 +
8	GS -8	1.5:0:0.1	- 	0.2 +	0.5 +
9	GS -9	2.0:0:0.2	- 	0.4 +	0.7 +
10	GS -10	2.5:0:0.2	0.3 +	0.7 +	0.9 +
11	GS -11	3.0:0.1: 0.5	0.4 +	1.0 ++	1.3 +++
12	GS -12	1.0:0.2:0.5	0.8 +	1.8 ++++	2 -2.5 +++++
13	GS -13	1.5:0.5:0.5	1.0 +++	1.8 ++++	2.1 ++++
14	GS -14	2.0:1.0:0.5	1.0 +++	1.4 +++	1.8 +++
15	GS -15	2.0:1.0:1.0	0.5 +	0.9 +	1.4 ++

(-) signs indicate no growth, (+) signs indicate the growing speed, elongation and no. of buds in culture.

IV. CONCLUSION

On applying varied combination of Kinetin 6-Benzylaminopurein and Indole-3-acetic acid on explants the best elongation was observed in combination of 1.0K: 0.2 IAA: 0.5BAP in which explants GS-12 showed best shoot elongation and proliferation. At

higher concentration of IAA, 6-Benzylaminopurein with kinetin resulted in curbing of shoot elongation and proliferation. Further different combination of hormones could be optimized for root induction to obtain healthy and disease free plantlets.

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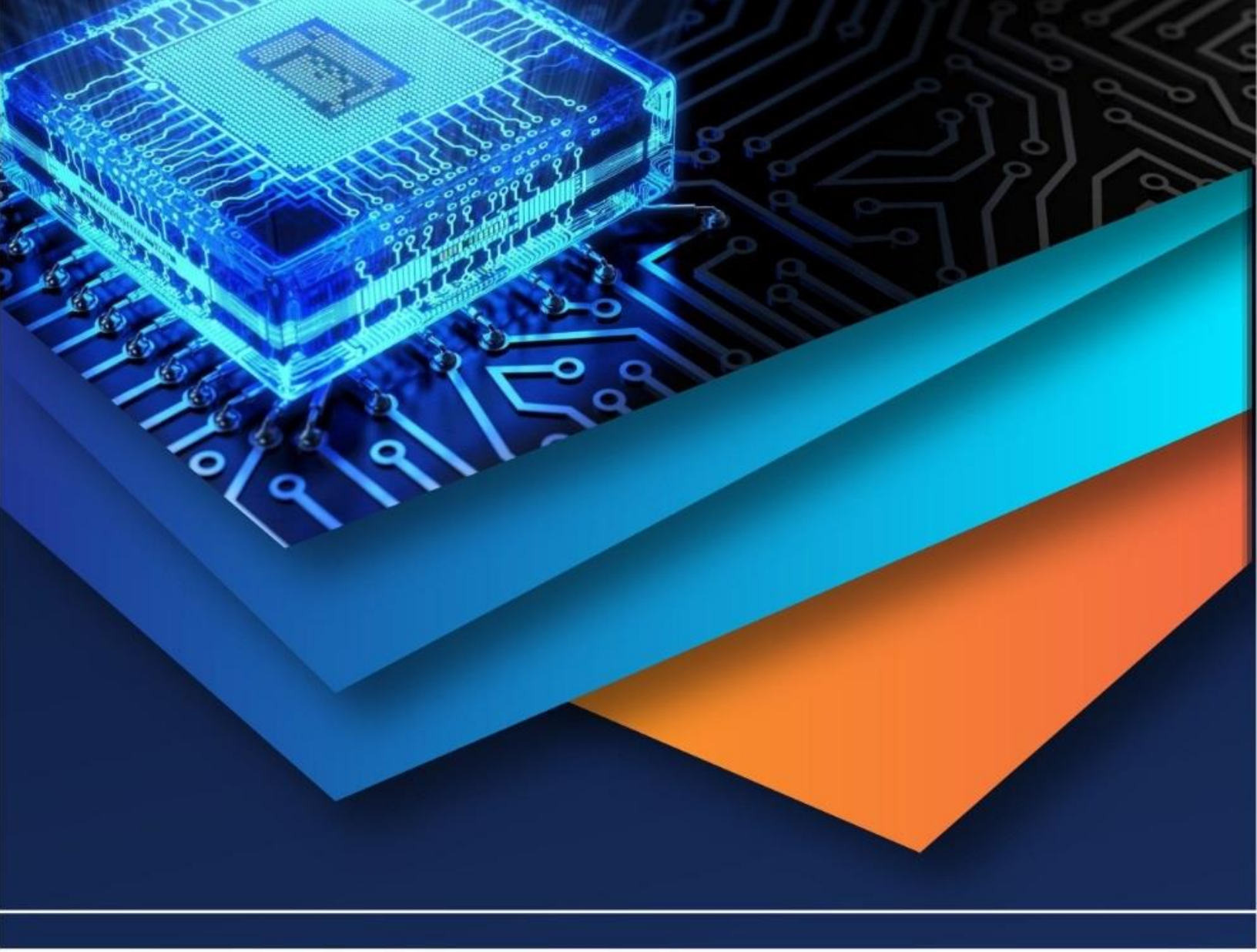

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