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Impact of Plant Growth regulators (PGRs) on callus induction from internodal explants of Tecomella undulata (Sm.) Seem- A Multipurpose Medicinal plants

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Abstract- A study of callus induction of *Tecomella undulata* was conducted using a various concentrations of PGRs namely cytokinins such as BAP and KIN and auxins such as 2, 4-D, NAA and IAA. Internodal explants were aseptically cultured on MS medium supplemented with different concentrations (1.0- 3.0 mg/l) of BAP, KIN, 2, 4-D, IAA and NAA. After two weeks callus was formed at the cut surface of the explants. The highest number of callus forming was obtained from explants cultured on the medium supplemented with 2.0 mg/l in 2.5mg/l 2,4-D alone were the induced calli were ceramish white in colour and structurally friable.

Abbreviations s- MS - Murashige and Skoog (1962) basal medium; BAP - Benzyl amino purine; KIN- Kinetin 2,4-D - 2,4-Dichlorophenoxyacetic acid; NAA - a-naphthalene acetic acid; IAA - Indole acetic acid.

I. Introduction

Tecomella undulata (Sm.) Seem (Bignoniaceae) is a medicinally and economically important plant that originated in India and distributed in the drier parts of Arabia and Southern Pakistan. It is one of the co-dominant species of Drier parts of India in the desert of western Rajasthan and Gujarat. It is an important agro-forestry (Anonymous 2003), deciduous or nearly evergreen tree grows under natural conditions in wild, unprotected and highly exploited. It appears in the list of endangered plants of Rajasthan. (Tripathi and Jaimini, 2002). The plant has been extensively used since ancient times for the treatment of human ailments. It posses anticancer activity (Ravi *et. al*. 2011), hepatoprotective (Khatri *et. al.* 2008 and Gupta 2011 and Goyal *et. al*. 2012), analgesic activity (Ahmed *et. al.* 1994), antibacterial activity (Gehlot *et. al.* 2007), mild relaxant, cardiotonic and chloretic activities (Khare *et. al.*2007) etc. The bark obtained from the stem is contains certain secondary metabolites like tecomin, alkenes, alkanols, β-sitosterols, chromone glycosides, undulatoside, A and B, iridoid glucosides,tecomelloside, tecoside,lapachol, veratric acid (Nandkarni *et. al*. 2000, Rastogi *et. al.* 2006, and Ambasta *et. al*. 2006) and is employed for the treatment of various diseases of skin, central nerves system, urinary disorders, enlargement of spleen, gonorrhoea, leucoderma, liver diseases, jaundice, diabetes, cancer and swellings. Leaves shows significant antimicrobial activity and contains certain chemical constituents like triacontanol, betulinic acid, oleanolic acid and ursolic acid. Triacontanol is an effective plant growth regulator while both betulinic acid and ursolic acid is potent antihuman immunodeficiency virus (HIV) and are used in treatment of AIDS (Azam, M. M., 1999 and 2000). Due to overexploitation and considerable demand of this medicinal plants, we are faced with the problem of losing our precious plant resource in the future need to conserve this plants.

II. Material and Methods

Collection and Sterilization of Explants

Explants were collected from Botanical Garden of H. N. G. University, Patan (Gujarat). The explants were washed thoroughly in running tap water and then surface sterilized with surfactant Tween-20 for 10 minutes followed by repeated rinsing with sterile double distilled water. The surface-sterilized explants were treated with 0.5% (4% sodium hypochloride) for 5 minutes and finally rinsed with sterilized double distilled water for 3-4 times to remove the traces of sterilants. They were further sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes under aseptic conditions in a Laminar Air flow Chamber and finally, the explants were washed thoroughly with autoclaved double distilled water for several times to remove traces of $HgCl_2$. Explants were cut into 1 cm segments and carefully cultured on the MS culture medium (Murashige and Skoog, 1962) consisting of different concentrations and combinations of auxin and cytokinin.

Media Preparation and culture conditions

The MS medium was used for callus induction containing 3% (w/v) sucrose was solidified with 0.8% (w/v) agar (Hi-Media, India). The MS medium is supplemented with various concentrations (0.5-3.0mg/l) of growth regulators namely cytokinins such as BAP and KIN and auxins such as 2, 4-D, NAA and IAA and also in combination. The pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCl solutions prior to autoclaving at 121°C at 15 psi pressure for 15 to 30 minutes. The cultures were incubated at were incubated at 25± 2°C and light intensity (3500- 4000 lux) under 16 hours photoperiod with cool-white fluorescent tubes and 55± 5% relative humidity.

III. Statistical analysis

The experiments of callus culture were conducted with a minimum of five replicates. All experiments were repeated three times. The data were analyzed by mean ± standard error.

IV. Results and Discussion

Internodal explants of *Tecomella undulata* were cultured on MS media supplemented with BAP, KIN, 2,4-D, NAA and IAA alone for callus induction. The effect of PGRs on callus formation is shown in Table 1. The maximum callus induction to be found (94.00 ± 0.00) was observed in 2.5 mg/l 2,4-D were the induced calli were ceramish white in colour and structurally friable followed by (92.00 ± 0.00) was observed in 3.0 mg/l BAP were the induced calli were brownish white in colour and structurally compact. The callusing response increases with increases the concentration of BAP. The lowest callusing (22.7 ± 0.72) was observed in 3.0 mg/l IAA. No callus induction was found to in 1.0mg/l and 1.5mg/l of IAA and also the callusing response decreases with increases the concentration of IAA. The highest callus growth in terms of fresh and dry weight (2.58 ± 0.8 g and 1.12 ± 0.14 g) 2.5mg/l 2,4-D followed by 2.47 ± 0.02 g fresh weight and 0.76 ± 0.04 g dry weight was obtained in in 3.0 mg/l BAP (Table.1). The lowest growth rate of 0.12 ± 0.02g fresh weight and 0.04 ± 0.01 g dry weight was obtained in 3.0 mg/l IAA. The effect of growth regulators on callus growth of different plant species were studied in several research reports. In this respect,

Similar response was also observed in the callus formation and shoot multiplication of *Oroxylum indicum (*Gokhale, M. and Bansal, Y. K., 2009). In vitro regeneration of *Tecomella undulata* (Sm.) Seem- an endangered medicinal plant reported by Danya U. *et al*.,2012. Ragavendra singh et al (2009) achieved *in vitro* adventitious shoot regeneration in *T. undulata*. Gang *et al*., (2003) reported that auxins and cytokinins are the most widely used plant growth regulators in plant tissue culture and auxins play an important role in the callus induction and different types of auxins had various effects (Baskaran *et al*., 2006). Rao *et al*., (2006) reported the cytokinins facilitated the effect of auxin in callus induction.

V. Conclusion

The MS medium contains various plant growth regulators like 2,4-D, BAP, KIN, NAA and IAA using the range between 1.0-3.0mg/l. Callus induction was recorded and the maximum callus induction was observed in the 2,4-D 2.5mg/l.

Table 1: Effects of Plant Growth Regulators (PGRs) on callus induction and callus growth of internodal explant of *Tecomella undulata (Sm.) Seem* (Mean± S.E)

Plant Growth Regulators (PGRs)	Concentration of (PGRs) (mg/l)	Percentage (%) for callus induction (Mean±SE)	Response intensity of callus	Texure of callus	Callus colour	Fresh weight of callus (g) (Mean±SE)	Dry weight of callus (g) (Mean±SE)
Control	-	-	-	-	-	-	-
MS+ BAP	1.0	83.4 ±1.36	+++	Friable	Brown	1.25 ± 0.02	0.14 ± 0.04
	1.5	86.8 ± 0.58	+++	Friable	Whitish brown	1.28 ± 0.01	0.12 ± 0.03
	2.0	89.2 ± 0.49	+++	Friable	Whitish brown	1.2 4± 0.02	0.11 ± 0.03
	2.5	90.0 ± 0.54	++++	compact	Creamish brown	2.09 ± 0.02	0.52 ± 0.04
	3.0	92.0 ± 0.00	++++	compact	Brownish white	2.47 ± 0.02	0.76 ± 0.04
MS+KIN	1.0	47.2 ± 1.28	+	compact	Brownish white	0.44 ± 0.02	0.12 ± 0.02
	1.5	55.2 ± 1.59	+	compact	Brownish white	0.48 ± 0.06	0.11 ± 0.01
	2.0	67.4 ± 1.20	++	compact	Brownish white	0.52 ± 0.05	0.14 ± 0.02
	2.5	73.4 ± 1.36	++	Friable	Creamish brown	1.11 ± 0.07	0.28 ± 0.09
	3.0	89.6 ± 0.81	+++	Friable	Creamish brown	1.22 ± 0.07	0.90 ± 0.08
MS+2,4-D	1.0	65.0 ± 0.44	++	Friable	Creamish white	0.92 ± 0.03	0.14 ± 0.09
	1.5	74.4 ± 1.20	++	Friable	Creamish white	0.84 ± 0.03	0.52 ± 0.04
	2.0	89.5 ± 0.50	+++	Friable	Creamish white	1.23 ± 0.03	0.28 ± 0.04
	2.5	94.0 ± 0.00	++++	Friable	Creamish white	2.50 ± 0.80	1.12 ± 0.14
	3.0	85.8 ± 0.73	+++	Friable	Creamish white	1.96 ± 0.16	0.81 ± 0.01
MS+NAA	1.0	60.0 ± 0.70	++	Friable	Light green	0.69 ± 0.07	0.14 ± 0.03
	1.5	71.0 ± 0.44	++	Friable	Light green	0.78 ± 0.03	0.16 ±0.02
	2.0	77.6 ± 0.67	++	Friable	Light green	0.93 ± 0.09	0.49± 0.07
	2.5	88.0 ± 1.30	+++	compact	Brownish white	1.37 ± 0.09	0.86 ± 0.07
	3.0	88.8 ± 0.86	+++	Friable	Light green	1.05 ± 0.09	0.18 ± 0.03
MS+IAA	1.0	0.00 ± 0.00	-	-	-	0.00 ± 0.00	0.00 ± 0.00
	1.5	0 .00 ± 0.00	-	-	-	0.00 ± 0.00	0.00 ± 0.00
	2.0	40.0 ± 0.54	+	Friable	Whitish green	0.30 ± 0.01	0.11 ± 0.02

	2.5	37.6 ± 0.87	+	Friable	Whitish green	0.28 ± 0.01	0.13 ± 0.04
	3.0	22.7 ± 0.72	+	Friable	Whitish green	0.12 ± 0.02	0.04 ± 0.01

Note: (-) No response, (+) poor growth, (++) modrate growth, (+++) good growth, (++++) very good growth

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