Effects of Plant Growth Regulators (PGRs) on callus induction from leaf segments explant of *Tecomella undulata* (Sm.) Seem- An Important Medicinal plant

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Abstract- The present study was conducted to investigate the effects of different concentrations and combinations of plant growth regulators (PGRs) on callus induction of *Tecomella undulata* (Sm) Seem. The leaf segments were used as explants and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of auxins such as NAA, 2,4-D, IAA and cytokikinins like BAP and KIN alone and in combinations for callus induction. The maximum callus formation (91.2%) was obtained on MS medium supplemented with in combination BAP with 2,4-D at (3.0+0.5mg/l) and (90.4%) and at (2.5+0.5mg/l) or 2,4-D alone at 3.0 mg/l were the induced calli were whitish green in colour and structurally compact. Remarkable callus biomass of 2.36± 0.06 gm fresh weight and 1.23± 0.08gm dry weight was observed in MS media containing (3.0 mg/l BAP +, 0.5 mg/l 2, 4-D).

Abbreviations: 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

Medicinal plants have long been the subject of human curiosity and need. The use of medicinal plants for health reasons started since ancient times and is still a part of medical practice in all countries of the world. The plant *Tecomella undulata* belonging to family bignoniaceae commonly known as Rohida, is a well-known plant in the Ayurveda system of medicine originated in India and distributed in the drier parts of Arabia and Southern Pakistan.. It is also a very useful species for afforestation of the drier tracts due to its drought and fire resistant and it appears in the list of endangered plants of Rajasthan. (Tripathi and Jaimini, 2002). The whole plant is used as a medicine .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). The bark of plant is used 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 due to secondary metabolites like tecomin, alkenes, alkanols, β-sitosterols, chromone glycosides, undulatoside, A and B, iridoid glucosides,tecomelloside, tecoside,lapachol, veratric acid (Rastogi *et. al.* 2006, and Ambasta *et. al*. 2006). It posses anticancer activity (Ravi *et. al*. 2011), hepatoprotective (Khatri *et. al*. 2008 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.

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 1% bavistin and shake well for 30 mintutes and then wash with sterilized double distilled water after that 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.

III. 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 (1.0-3.0mg/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 with auxins at 0.5mg/l concentration. 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.

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

Different concentrations and combinations of hormones were used in MS medium to observe callus induction using leaf segment as explants. The rate of callus formation varied in different treatments used shown in table 1 and 2. In auxins 2, 4-D and NAA give good response in compared to IAA. The percentage of callus induction increases with the increases the concentration of 2, 4 –D and NAA. 2, 4-D (3.0mg/l) showed the maximum percentage (90.0±0.55) of callus induction shown in table-1. Among cytokiknins both BAP and KIN give moderate growth of callusing compare to auxins 2, 4 –D and NAA. A combined effect of cytokinins and auxins on callus induction from leaf segments was also studied. Remarkable growth of callus was observed when BAP (2.5-3.0mg/l) combined with 2, 4-D (0.5mg/l) and also 2, 4- D (3.0mg/l) alone. The highest amount of callus (91.2±0.80) was produced on MS medium supplemented with BAP (0.3mg/l) combined with auxin 2, 4-D (0.5mg/l). The lowest callusing (34.8 ± 1.16) was observed in 2.0 mg/l IAA. No callus induction was found to in 1.0mg/l and 1.5mg/l of IAA but in combination of IAA with BAP give good response for callusing at lower concentration of BAP but in higher concentration of BAP it should be reduced. (Table 2). The percentage of callusing reduced when KIN was combined with NAA and IAA but in case of 2, 4-D gives positive response. Callus growth in terms of fresh weight and dry weight was also recorded. Maximum fresh weight and dry weight (2.36±0.06 and 1.26±0.08) of callus was obtained from 3.0 mg/l BAP combined with 0.5 mg/l 2, 4-D were the induced callus was structurally compact and whitish green in colour (Table-2). The effect of plant growth regulators (PGRs) on callus induction and growth of different plant species were studied in several research reports. In this respect, Similar response in the callus formation and shoot multiplication of *Oroxylum indicum* was also observed (Gokhale, M. and Bansal, Y. K., 2009). Nandwani D. *et. al.* (1996) reported callus obtained from seedling explants regenerated more profusely than callus obtained from mature stem explants on Murashig and Skoog (MS) medium supplemeted with indole-3-acetic acid (IAA) and benzyladenine (BA). Internodal explants were inoculated in MS (Murashig & Skoog) medium supplemented with various growth hormones like 6-Benzyladenine (BA), Kinetin (Kn), 2, 4-Dichlorophenoxyacetic Acid 2, 4-D) and α-Naphthalene Acetic Acid (NAA) at different concentrations used for regeneration of *T. undulata* reported by Danya U. et al., 2012. The 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 reported by Gang *et al*., 2003.

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 and also in combination. Callus induction was recorded and the maximum callus induction was observed in the 2,4-D 3.0mg/l and 3.0mg/l BAP combined with 0.5mg/l 2, 4-D.

Table 1: Effects of Plant Growth Regulators (PGRs) on callus induction and callus growth of leaf segment 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	55.2 ±1.02	++	Friable	Whitish green	0.45 ± 0.03	0.12 ± 0.03
	1.5	59.8± 0.86	++	Friable	Whitish green	0.50 ± 0.04	0.16 ± 0.03
	2.0	69.4 ± 1.63	+++	Friable	Whitish green	0.76± 0.05	0.28 ± 0.04
	2.5	72.2 ± 1.36	+++	Friable	Whitish green	0.94 ± 0.19	0.35 ± 0.08
	3.0	77.8 ± 0.86	+++	Friable	Whitish green	1.12 ± 0.14	0.65 ± 0.04
MS+KIN	1.0	0.00 ± 0.00	-	-	-	0.00 ± 0.00	0.00 ± 0.00
	1.5	43.1± 0.84	+	compact	Yellowish green	0.37 ± 0.03	0.08 ± 0.01
	2.0	52.0 ± 0.71	+	compact	Yellowish green	0.44 ± 0.03	0.10 ± 0.02

	2.5	63.9 ± 1.19	++	compact	Yellowish green	0.59 ± 0.07	0.16 ± 0.03
	3.0	67.0 ± 0.84	++	compact	Yellowish green	0.73 ± 0.01	0.26 ± 0.04
MS+2,4-D	1.0	75.2 ± 0.49	++	compact	Yellowish green	0.88 ± 0.07	0.48 ± 0.04
	1.5	80.0 ± 0.71	+++	compact	Yellowish green	1.33 ± 0.04	0.94 ± 0.04
	2.0	83.6 ± 0.65	+++	compact	Yellowish green	1.75 ± 0.04	0.89 ± 0.05
	2.5	88.1 ± 0.60	+++	compact	Yellowish green	2.19 ± 0.08	0.92 ± 0.14
	3.0	90.0 ± 0.55	++++	compact	Yellowish green	2.25 ± 0.06	1.16 ± 0.01
MS+NAA	1.0	71.2 ± 0.86	++	Friable	Light green	0.83 ± 0.04	0.52 ± 0.04
	1.5	77.6 ± 1.25	++	Friable	Light green	1.23 ± 0.08	0.63 ±0.04
	2.0	80.6 ± 0.40	+++	Friable	Light green	1.30 ± 0.09	0.68± 0.04
	2.5	85.0 ± 0.89	+++	Frible	Light green	1.51 ± 0.24	0.94 ± 0.07
	3.0	87.4 ± 0.24	+++	Friable	Light green	1.98 ± 0.05	0.98 ± 0.09
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	34.8± 1.16	+	Friable	Whitish green	0.20± 0.02	0.04 ± 0.02
	2.5	41.8 ± 0.58	+	Friable	Whitish green	0.27 ± 0.03	0.08 ± 0.01
	3.0	50.2 ± 0.80	+	Friable	Whitish green	0.33 ± 0.02	0.13 ± 0.02

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

Table 2: Effects of Plant Growth Regulators (PGRs) in combination on callus induction and callus growth of leaf segments 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 +2,4-D	1.0 + 0.5	78.0 ±1.41	++	Friable	Whitish green	1.40 ± 0.04	0.88 ± 0.07
	1.5+ 0.5	84.0 ± 1.41	+++	Friable	Whitish green	1.43± 0.07	0.93 ± 0.09
	2.0+ 0.5	85.6 ± 1.17	+++	Friable	Whitish green	1.66± 0.08	0.88 ± 0.04
	2.5+ 0.5	90.4 ± 0.75	++++	Friable	Whitish green	2.28 ± 0.13	1.21 ± 0.03
	3.0+ 0.5	91.2 ± 0.80	++++	Friable	Whitish green	2.36 ± 0.06	1.23 ± 0.08
MS+ BAP +NAA	1.0 + 0.5	51.5 ± 1.02	+	compact	Yellowish green	0.44 ± 0.08	0.12 ± 0.04
	1.5+ 0.5	64.4± 1.57	++	compact	Yellowish green	0.66 ± 0.08	0.21 ± 0.02
	2.0+ 0.5	72.4 ± 1.17	++	compact	Yellowish green	0.89 ± 0.02	0.27 ± 0.04
	2.5+ 0.5	71.0 ± 0.55	++	compact	Yellowish green	0.88 ± 0.07	0.28 ± 0.02
	3.0+ 0.5	62.2± 0.86	++	compact	Yellowish green	0.57 ± 0.02	0.18 ± 0.08
MS+ BAP +IAA	1.0 + 0.5	84.7 ± 0.66	+++	Friable	Whitish green	1.57 ± 0.24	0.86 ± 0.09
	1.5+ 0.5	81.7 ± 1.24	+++	Friable	Whitish green	1.54 ± 0.06	0.85 ± 0.04
	2.0+ 0.5	76.7 ± 0.70	++	Friable	Whitish green	1.09 ± 0.05	0.79 ± 0.04
	2.5+ 0.5	75.4 ± 0.93	++	Friable	Whitish green	0.93 ± 0.03	0.48 ± 0.03
	3.0+ 0.5	65.4 ± 1.44	++	Friable	Whitish green	0.69 ± 0.07	0.32 ± 0.08
MS+KIN+2,4-D	1.0 + 0.5	69.0 ± 1.18	++	compact	Yellowish green	0.78 ± 0.04	0.31 ± 0.02
	1.5+ 0.5	76.1 ± 1.08	++	compact	Yellowish green	0.95 ± 0.02	0.46 ±0.04
	2.0+ 0.5	83.4 ± 1.21	+++	compact	Yellowish green	1.42 ± 0.05	0.91± 0.04
	2.5+ 0.5	86.2 ± 1.02	+++	compact	Yellowish green	1.76 ± 0.03	0.90 ± 0.08
	3.0+ 0.5	89.0 ± 0.63	+++	compact	Yellowish green	2.22 ± 0.04	1.31 ± 0.10
MS+KIN+NAA	1.0 + 0.5	74.8 ± 1.32	++	compact	Whitish green	0.81 ± 0.05	0.42 ± 0.02
	1.5+ 0.5	67.6 ± 0.60	++	compact	Whitish green	0.73 ± 0.07	0.38 ± 0.03
	2.0+ 0.5	53.4± 1.17	+	compact	Whitish green	0.48 ± 0.03	0.18 ± 0.04
	2.5+ 0.5	44.8 ± 1.01	+	compact	Whitish green	0.33 ± 0.03	0.12 ± 0.02
	3.0+ 0.5	38.2 ± 1.53	+	compact	Whitish green	0.28 ± 0.02	0.09 ± 0.03
MS+KIN+IAA	1.0 + 0.5	0.00 ± 0.00	-	-	-	0.00 ± 0.00	0.00 ± 0.00
	1.5+ 0.5	37.2 ± 2.35	+	Friable	Whitish green	0.24 ± 0.03	0.06 ± 0.00
	2.0+ 0.5	50.4± 1.86	+	Friable	Whitish green	0.36± 0.03	0.08 ± 0.03

	2.5+ 0.5	56.0 ± 0.71	+	Friable	Whitish green	0.53± 0.04	0.11± 0.02
	3.0+ 0.5	60.0 ± 0.63	++	Friable	Whitish green	0.55 ± 0.04	0.23 ± 0.03

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

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