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RESEARCH ARTICLE

IN VITRO PROPAGATION AND PRODUCTION OF TEPHROSIA PURPUREA PLANT VIA INTERNODAL EXPLANTS.

[*]Rajender Vadluri, Murali Krishna Thupurani, Epur Manoj Kumar Reddy, B.S Anuradha, G Gayathri and M. Vani.
Department of Biotechnology, Chaitanya Degree and Postgraduate College (Autonomous), Kishanpura, Hanamkonda, Warangal, Telangana-India.

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Abstract

Tephrosia purpurea (Linn.) Pers. (Leguminasae), is a highly suberect, branched and herbaceous perennial herb. According to Ayurveda survey of literature, this plant is extensively used in the treatment of all types of wound healing diseases. The present study was framed out to develop the protocol for the *in vitro* propagation via intermodal cultures of *Tephrosia purpurea*. Initially the nodal explants were inoculated on the MS medium supplemented with various concentrations of BAP and KN (2.0-5.0). The initiation of roots and its elongation was achieved using various concentrations of IAA and NAA in combination. The highest number of shoots and shoot length 22 and 7.15± 1.1was found at 3.4 mg/L of KN. The highest number of roots and root length 28 and 8.09 ±0.1 was observed at 5.0+1.1 mg/L of IAA and NAA. By the current studies we conclude that KN was found more effective in the proliferation of shoot buds comparing to the BAP.

Introduction:-

Tephrosia purpurea (Linn.) Pers. (Leguminasae), is a highly suberect, branched and herbaceous perennial herb. According to Ayurveda survey of literature, this plant is extensively used in the treatment of all types of wound healing diseases. The extraction of this plant is an important ingredient of certain medicine formulations used in the treatment of liver disorders. The various parts of this plant are widely used as remedies in treatment of asthma, impotency, diarrhea, gonorrhea, ulcer and urinary, rheumatism disorders. The plant parts are also extensively used for curing diseases concern to kidney, spleen, liver, heart and blood. The dried herb is effective as tonic, diuretics, laxative, and deobstruents. It is also used in the treatment of bilious febrile attack, boils, bronchitis, bleeding piles and pimples. The crude extracts of root and seeds of this plant are well known to possess insecticidal and pesticidal activities. The roots are known to show high active against leprous wound and its juice, is to the eruption on skin. Extract of pods is used effective against pain, inflammation. The decoction is used as source of anti-vomiting agent. The ethanolic and aqueous extracts of this plant has been proved to possess the anticancer activity anti-hypoglycemic and antibacterial activity. The phytochemical investigations on *Tephrosia purpurea* have revealed for the presence of glycosides, rotenoids, flavanones, chalcones, isoflavones, flavanols, and sterols (Kirtikar and Basu 1999; The Wealth of India 1976; Chopra *et al*., 1956).

Corresponding Author:- Rajender Vadluri. (+91 9959771322, rajenderbio@gmail.com)
Address:-Department of Biotechnology, Chaitanya Degree and Postgraduate College (Autonomous), Kishanpura, Hanamkonda, Warangal, Telangana-India.

Material and Methods:-

Plant material was collected from the plants growing in Kakatiya University sports ground, Warangal, Telangana.

Sterilization of explants:-

Nodal explants were washed under running tap water to remove dust particles for 30 min and treated with liquid detergent (Tween 20) for 10 min, and rinsed with distilled water until the removal of detergent. Bavistin was used as antifungal agent. The explants were treated with Bavistin for about 1 hr and rinsed with distilled water. The explants were disinfected with 0.1% (w/v) mercuric chloride (10-15 sec) under aseptic conditions followed by washing with sterilized double distilled water.

Culture media and culture conditions:-

Murashige and skoog medium with sucrose as carbon source (3% w/v) was used in the study. All phytohormones stock were prepared at a concentration of 1 mg/ml and stored at 4°C. The media pH was adjusted to 5.6-5.8 using 1N HCl and 1 N NaOH. MS media supplemented with different concentration of cytokinins and auxins single and in combination.

Inoculation in culture medium and Shoot Proliferation:-

The Nodal explants were inoculated separately on MS basal medium supplemented with 6-Benzyladenine (2.0-5.0 mg/L) and Kinetin (2.0-5.0 mg/L) for auxiliary bud proliferation and multiplication. All the cultures were maintained at 24±2°C temperature with a photoperiod of 16h light/8h dark under cool white fluorescent lamps (Phillips, India). The number of shoots formed was enumerated after 6 weeks of incubation.

Rooting of shoots and transfer of plantlets to soil:-

Nodal explants approximately with 5-6 number of shoot buds with 2-3 cm in size were selectively chosen for induction of roots. These shoot buds are transferred to MS medium supplemented with IAA in combination with NAA ranging from 3.0-7.0+0.1-2.1 in combination with 200 mg activated charcoal for root induction (See Table 3). The regenerated plant lets were washed transferred to pots containing autoclaved vermiculite soil and sand (1:2:1), and covered with polyethylene bags for one week to maintain high humidity and subsequently exposed to low air humidity for increasing period and finally polyethylene bags were removed. These hardened plants then transferred to the greenhouse.

Statistical analysis:-

The data obtained was analyzed statistically using SAS version 7.0. The significant differences among mean values was calculated using student'*t*' test at $P<0.05$. All experiments were repeated thrice before deriving the final results. Results of shoot and root number and length are expressed as mean ± SD (n=3).

Results and Discussion:-

Inoculation and Shoot bud Proliferation:-

In the present study we screened the efficiency of 6-Benzyladenine (2.0-5.0 mg/L) and Kinetin (2.0-5.0 mg/L) separately for multiple shoot production. The shoot initiation was observed from 3rd week of inoculation. The number of shoots formed was counted after 8 weeks of incubation. In accordance to the data obtained in the present study, among the two plant growth regulators, Benzyladenine was found to be inductive of shoot buds from nodal explants but failed to show the growth of shoot buds in the terms of shoot number and shoot length. On the other hand, comparing to Benzyladenine, Kinetin showed significant shoot initiative response and promoting the growth. The number of shoots 17, 12, 07 and shoot length 6.03±1.1, 3.75±0.3, 4.10±0.1 and 22, 19, 12 and shoot length 7.15± 1.1, 5.88±0.1, 3.23±0.1 was found high at 4.0, 3.8, 3.6mg/L^{-1} of BAP and 3.4, 3.2, 3.6 mg/L of Kinetin respectively (See table 1and 2).

Rooting of shoots and transfer of plantlets to soil:-

The various concentrations of auxins IAA and NAA was tested for root induction and elongation. According to the current study, we observed that root induction and its elongation were effectively found with using both auxins in certain concentrations. Among the concentrations of IAA and NAA in combination 5.0+1.1, 4.8+1.0, 4.6 +.0.9 mg/L^{-1} showed high number of roots with significant root length with root number 28, 22, 20 and the length 8.0 9± 0.1, 4.19 ± 1.1 and 5.26±0.2 were noticed at 3.0+1.5 mg/L^{-1} of IAA and NAA respectively (see table 3).

The current research was framed out to standardize the protocols to develop the complete plant via culturing of nodal explants using various concentrations of auxins and cytokinins. In the earlier research we have published that cytokinins in combination was found more active in proliferation of multiple shoots from callus. We have also included that the root induction and elongation was also found high using auxins in dual concentrations comparing to single.

Owing to bio integrity and ease in isolation of plant derived drugs used in the treatment of various disorders, one needs the raw material of the plants in bulk quantity. Therefore, the mass propagation of plants that are highly medicinal value is in focus. Thus the current studies help the researchers to establish a protocol for *in vitro* propagation of this medicinal plant and produce more number of plants within a short span of time (Patil et al 2011; Sharma et al 2013; Singh et al 2002; Upadhyay et al 2010; William and evans 2006).

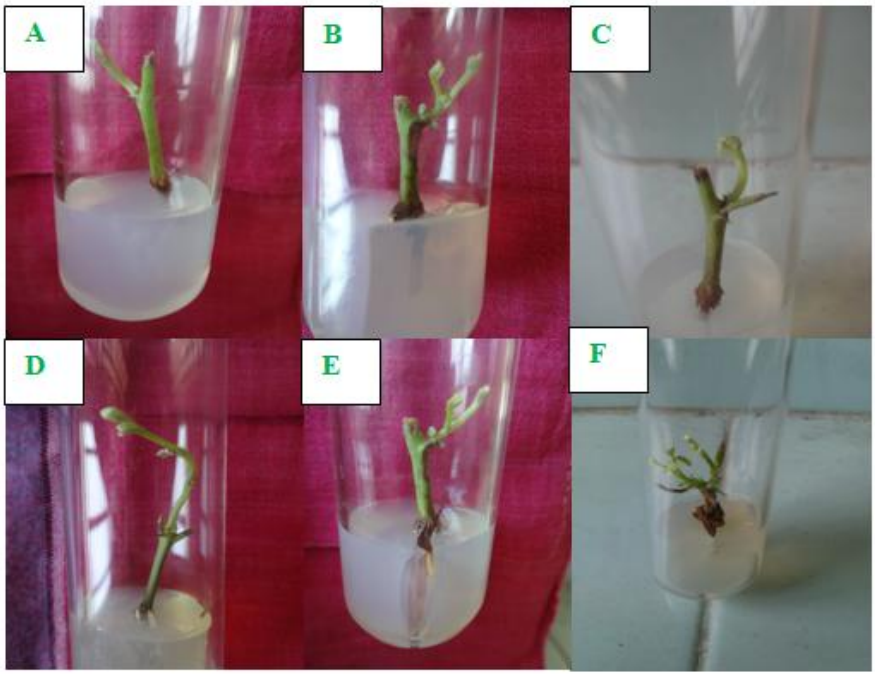

Fig 1. A:--inoculation of nodal explants, B-F- showing the development of auxiliary bud proliferation on MS medium supplemented with various concentrations of BAP and Cytokinins `

Conclusion:-

Basing on the data of current research and past, we conclude that the among the different propagation methods of *Tephrosia purpurea* that we published and presented in the current paper, we suggest that nodal explants were more significant to produce *Tephrosia purpurea* plants comparing to other modes of propagation.

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Table 1:- Shoot regeneration effects of 6-benzylamino purine

MS+Cytokinins/Auxins			
BAP	Shoot regeneration (%)	No. of shoots	Shoot length (cm)
2.0	--	--	--
2.2	--	--	--
2.4	14	03	1.19±0.1
2.6	20	02	2.10±0.8
2.8	28	04	1.13±1.1
3.0	35	03	3.22±1.4
3.2	42	02	0.98±0.5
3.4	65	06	1.35±0.2
3.6	70	07	4.10±0.1
3.8.	87	12	3.75±0.3*
4.0	96	17	6.03±1.1*
4.2	24	03	2.18±1.0
4.4	29	02	3.60±1.1
4.6	21	02	1.56±0.5
4.8	32	02	1.15±0.1
5.0	--	--	--

The significant differences among mean values was calculated using student '*t*' test
n=3* (P<0.05)

Table 2:- Shoot regeneration effects of Kinetin

MS+Cytokinins/Auxins			
KN	Shoot regeneration (%)	No. of shoots	Shoot length (cm)
2.0	14	02	2.55±1.0
2.2	20	04	1.81±1.0
2.4	28	03	2.98±0.1
2.6	30	02	1.81±1.1
2.8	41	02	4.89±1.1
3.0	52	14	6.14±0.8*
3.2	81	19	5.88±0.1*
3.4	94	22	7.15±1.1*
3.6	75	12	3.23±0.3
3.8.	55	02	2.28±0.1
4.0	--	--	--
4.2	--	--	--
4.4	--	--	--
4.6	--	--	--
4.8	--	--	--
5.0			

The significant differences among mean values was calculated using student '*t*' test
n=3* (P<0.05)

Table 3:- Root regeneration effects of 3-indole acetic acid and in combination with Naphthlene acetic acid

IAA	NAA	Root regeneration (%)	No. of Roots	Root length (cm ± SD)
3.0	0.1	12	07	1.47±0.1
3.2	0.2	20	06	1.98±0.3
3.4	0.3	26	07	2.25±1.5
3.6	0.4	30	11	3.96±0.8
3.8	0.5	38	18	2.85±1.1
4.0	0.6	44	08	5.16±0.9
4.2	0.7	51	14	2.28±1.5
4.4	0.8	60	11	2.51±1.0
4.6	0.9	68	20*	5.26±0.2*
4.8	1.0	75	22*	4.19±1.1*
5.0	1.1	88	28*	8.09±0.1*
5.2	1.2	62	08	1.78±2.0
5.4	1.3	53	13	4.55±1.5
5.6	1.4	47	09	1.85±2.0
5.8	1.5	41	12	2.23±0.1
6.0	1.6	35	05	2.80±0.2
6.2	1.7	29	02	1.99±1.0
6.4	1.8	22	02	5.32±1.1
6.6	1.9	16	03	2.19±0.5
6.8	2.0	10	05	4.58±0.1
7.0	2.1	06		

1. The significant differences among mean values was calculated using student '*t*' test
2. n=3* ($P<0.05$)

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