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Research Report

REGENERATION CAPACITIES OF *TEPHROSIA PURPUREA*-A MEDICINALLY IMPORTANT PLANT

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ABSTRACT

Tephrosia purpurea (Linn.) Pers. (Leguminasae), commonly known in Sanskrit as Sharapunkha is a highly branched, suberect, herbaceous perennial herb. The present study was designed to study the effect of various auxins and cytokinins concentrations on *in vitro* morphogenesis of *Tephrosia purpurea* explants. Initially, the seeds of *Tephrosia purpurea* were collected from local areas of Warangal District. The sterilized seeds are inoculated on the MS medium supplemented with various concentrations of GA3. The first and second leaflets are excised and used as effective source of explants and inoculated separately on MS basal medium supplemented, 3-indole acetic acid, NAA (0.1-2.0+1.0-2.0 mg/L) in combination with 6-Benzyladenine (2.5-4.0 mg/L) and 3-indole acetic acid, NAA (0.1-2.0+1.0-2.0 mg/L) in combination Kinetin (2.5-4.0 mg/L) for callus induction and multiple shoot production. Shoots are transferred to MS medium supplemented with IAA in combination with NAA ranging from 1.5-3.5+0.1-2.0 and single IAA 2.5-4.0 mg/L. The number of shoots 34, 30, 28 and shoot length 7.12±0.9, 6.10±0.1, 6.63±1.0 was found high at 3.5+2.0+1.1, 3.4+1.9+1.0, 3.3+1.8+0.9 mg/L^{-1} of Kinetin+IAA+NAA respectively. The highest root number 52 and its length 7.21±0.3 was recorded at 3.0+1.5 mg/L^{-1} of IAA and NAA respectively.

INTRODUCTION

An endangered species is a native species that faces a significant risk of extinction in the near future throughout all or a significant portion of its range. Such species may be declining in number due to threats such as habitat destruction, climate change, or pressure from invasive species. *Tephrosia purpurea* (Linn.) Pers. (Leguminasae), commonly known in Sanskrit as Sharapunkha is a highly branched, suberect, herbaceous perennial herb. According to Ayurveda literature, this plant has also given the name of "Sarwa wranvishapaka" which means that it has the property of healing all types of wounds. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders. In Ayurvedic system of medicine, various parts of this plant are used as a remedy for impotency, asthma, diarrhea, gonorrhea, rheumatism, ulcer and urinary disorders. The plant has been claimed to cure diseases of kidney, liver spleen, heart, and blood. The dried herb is effective as a tonic laxative, diuretics and deobstruents. It is also used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles. The roots and seeds are reported to have insecticidal and piscicidal properties and also used as vermifuge. The roots are also reported to be effective in the leprous wound and their juice, to the eruption on skin. An extract of pods is effective for pain, inflammation and their decoction is used in vomiting. The ethanolic extract of this plant has been reported to have anticancer activity against KB cells in culture. The aqueous extract of seeds has shown significant in vivo hypoglycemic activity in diabetic rabbits. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity. The phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols (Kirtikar and Basu 1999; The Wealth of India 1976; Chopra *et al*., 1956).

MATERIAL AND METHODS

Plant material *Tephrosia purpurea* plants were collected from the plants growing in Kakatiya University sports ground, Warangal, Telangana.

Sterilization of explants

Explants (seeds) were washed under running tap water to remove dust particles for 30 min and treated with liquid detergent (Tween20) for 10 min, and rinsed with distilled water

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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.

Seed germination

Surface sterilized seeds were inoculated on MS basal media alone and in combination with GA_3 concentrations ranging from 1.5-4.0 mg/l. The inoculated cultures were incubated at standard culture conditions (temperature 22±2°C; photoperiod 16h light/8h dark).

Culture media and culture conditions

Murashige and skoog medium with sucrose as a 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 leaves were collected from the germination of seed cultures and inoculated separately on MS basal medium supplemented, 3-indole acetic acid, NAA (0.1-2.0+1.0-2.0 mg/L) in combination with 6-Benzyladenine (2.5-4.0 mg/L) and 3-indole acetic acid, NAA (0.1-2.0+1.0-2.0 mg/L) in combination Kinetin (2.5-4.0 mg/L) for callus induction and multiple shoot production. 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

Shoots of approximately 2-3 cm in height with two to three leaflets were selectively chosen for induction of roots. These shoots are transferred to MS medium supplemented with IAA in combination with NAA ranging from 1.5-3.5+0.1-2.0 and single IAA 2.5-4.0 mg/L in combination with 200 mg activated charcoal for root induction (See Table 3 and 4). The regenerated plantlets 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 were 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

Effect of Gibberellins on germination of Tephrosia purpurea seeds

Seeds inoculated on the MS media supplemented with various concentrations of gibberellins resulted in healthy germination. We noticed that the percentage of germination was directly proportional to the concentration of GA_3 (See Fig 1). Among the concentrations of GA_3 tested 3.0 and 3.5 mg/mL^{-1} resulted in a highest percentage of seed germination with a greater number of leaves and shoots. The percentage of seed germination was shown in graph 1. Young leaves were excised and processed for micropropagation.

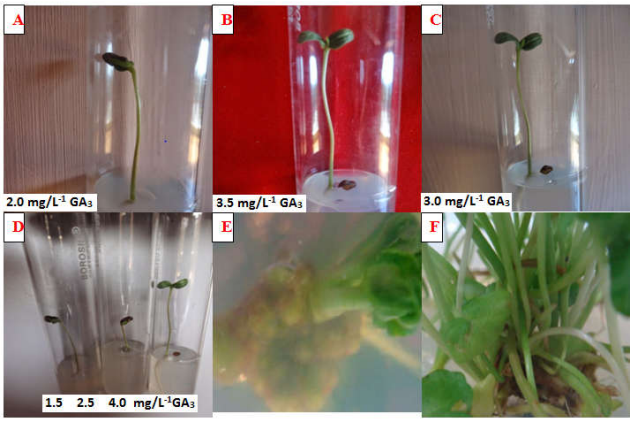
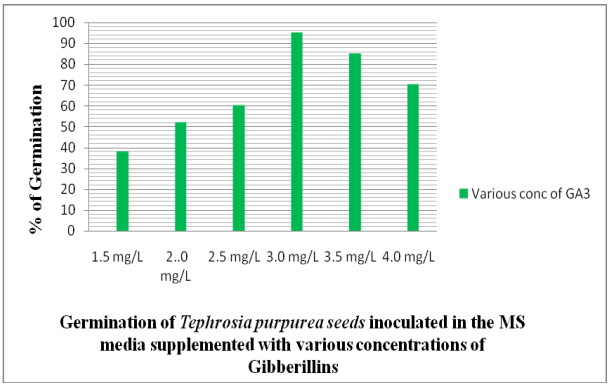


Fig1 A-D Germination of *Tephrosia purpurea* seeds on various concentrations of GA_3, E-F- Callus formation and production of multiple shoots



Graph1 Germination studies of *Tephrosia purpurea* seeds using various concentrations of GA_3 concentrations.

Inoculation in culture medium and Shoot Proliferation

We tested the effect of 3-indole acetic acid and NAA (0.1-2.0+1.0-2.0 mg/L) with 6-Benzyladenine (2.5-4.0 mg/L) and Kinetin (2.5-4.0 mg/L) separately for multiple shoot production. The number of shoots formed was enumerated after 6 weeks of incubation. According to the present study, among the two cytokinins tested Kinetin was found to be effective compared with that from Benzyladenine. The number of shoots 34, 30, 28 and shoot length 7.12±0.9, 6.10±0.1, 6.63±1.0 was found high at 3.5+2.0+1.1, 3.4+1.9+1.0, 3.3+1.8+0.9 mg/L^{-1} of Kinetin+IAA+NAA respectively (See table 1, 2 and Fig 2).

Table 1 Shoot regeneration effects of 6-benzylamino purine in combination with 3-Indole acetic acid and Napthaline acetic acid

MS+Cytokinins/Auxins					
BAP	IAA	NAA	Shoot regeneration (%)	No. of shoots	Shoot length (cm)
2.5	1.0	0.1	25	02	1.27±0.5
2.6	1.1	0.2	32	02	2.05±0.9
2.7	1.2	0.3	40	04	3.23±1.1
2.8	1.3	0.4	48	05	4.57±1.2
2.9	1.4	0.5	55	08	4.97±0.8
3.0	1.5	0.6	59	09	4.61±0.3
3.1	1.6	0.7	64	10	5.32±0.6
3.2	1.7	0.8	68	10	6.27±1.4
3.3	1.8	0.9	75	12	3.98±1.8
3.4.	1.9	1.0	80	15	4.97±1.1
3.5	2.0	1.1	85	17	5.32±1.0
3.6	2.1	1.2	89	17	5.78±1.9
3.7	2.2	1.3	93*	22*	6.65±1.5*
3.8	2.3	1.4	95*	28*	7.65±0.5*
3.9	2.4	1.5	90*	19*	6.15±0.8*
4.0	2.5	1.6	86	16	5.40±1.0

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

Table 2 Shoot regeneration effects of Kinetin in combination with 3-Indole acetic acid and Napthaline acetic acid

MS+Cytokinins/Auxins					
KN	IAA	NAA	Shoot regeneration (%)	No. of shoots	Shoot length (cm)
2.5	1.0	0.1	04	04	1.89±1.0
2.6	1.1	0.2	10	07	4.58±1.0
2.7	1.2	0.3	15	11	2.56±0.4
2.8	1.3	0.4	22	16	5.77±1.2
2.9	1.4	0.5	34	16	4.89±0.1
3.0	1.5	0.6	46	18	3.54±1.5
3.1	1.6	0.7	59	21	6.12±1.7
3.2	1.7	0.8	74	25	5.54±1.1
3.3	1.8	0.9	85*	28*	6.63±1.0*
3.4.	1.9	1.0	92*	30*	6.10±0.1*
3.5	2.0	1.1	98*	34*	7.12±0.9*
3.6	2.1	1.2	85*	25*	6.81±1.1*
3.7	2.2	1.3	79	20	4.59±1.3
3.8	2.3	1.4	70	15	5.13±0.4
3.9	2.4	1.5	62	11	3.59±0.2
4.0	2.5	1.6	49	09	4.44±1.4

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

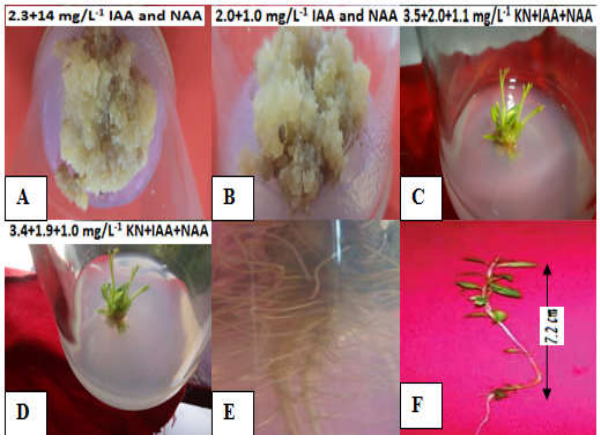


Fig2 A-B formation of callus from leaf explants of *Tephrosia purpurea*using various concentrations of auxins and cytokinins. C-D-Production of multiple shoots using benzyl adenine and kinetin. E-F-Rhizogenesis and production of complete pant

Rooting of shoots and transfer of plantlets to soil

Effect of various auxins IAA and NAA is carried out for root induction and elongation. We found that the root induction was more effectively observed with using dual auxin concentrations. Among the concentrations of IAA and NAA in combination 3.0+1.5, 2.9+1.4, 2.8+.3 mg/L^{-1} of IAA+NAA showed more number of roots and its elongation. The highest root number 52 and its length 7.21±0.3 was recorded at 3.0+1.5 mg/L^{-1} of IAA and NAA respectively (see table 3). Comparing to auxins in combination, auxins in the single was proved as less effective for root induction and elongation (See table 4 and Fig 3).

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)
1.5	0.1	16	07	3.44±0.7
1.6	0.2	24	12	3.61±1.2
1.7	0.3	30	18	3.98±0.1
1.8	0.4	35	21	4.23±0.2
1.9	0.5	43	27	3.94±1.3
2.0	0.6	51	30	5.72±1.8
2.1	0.7	57	33	5.98±1.1
2.2	0.8	62	35	4.88±1.0
2.3	0.9	70	38	6.18±0.6
2.4	1.0	76	40	6.39±1.3
2.5	1.1	82	42	6.66±1.2
2.6	1.2	85*	46*	6.74±2.0*
2.8	1.3	90*	49*	7.14±1.5*
2.9	1.4	94*	49*	7.01±2.1*
3.0	1.5	98*	52*	7.21±0.3*
3.1	1.6	89*	32*	6.77±0.5*
3.2	1.7	83	25	5.96±0.8
3.3	1.8	74	19	4.55±1.1
3.4	1.9	68	12	3.26±0.2
3.5	2.0	60	06	3.05±0.3

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

Table 4 Root regeneration effects of 3-indole acetic acid

IAA	Root regeneration (%)	No. of Roots	Root length (cm)
2.5	28	9	4.57±0.4
2.6	35	11	3.98±0.8
2.7	43	15	4.66±1.2
2.8	52	19	4.85±1.1
2.9	69	24	4.99±0.6
3	78	27	5.18±0.1
3.1	84	31	6.21±0.3
3.2	90*	35*	5.09±1.4
3.3	94*	39*	5.42±1.1
3.4.	97*	44*	5.87±0.9
3.5	85*	39*	5.17±0.5
3.6	77	35	4.55±1.4
3.7	64	31	4.23±0.1
3.8	58	27	4.02±0.5
3.9	46	24	3.19±1.1
4	38	20	3.59±1.7

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

The parts of this plant possess various biological activities and used to cure several types of external wounds and gastroduodenal disorders. The compounds isolated from this plant are majorly used in the treatment of a cough, tightness of chest and enlargement of liver, spleen, and kidney. It is used as a mouth freshener, anti-inflammatory agents etc. (William *et*

al., 2006; Anonymous 2009; Singh *et al*., 2002; Upadhyay *et al*., 2010; Patil *et al* 2011; Sharma *et al*., 2013).

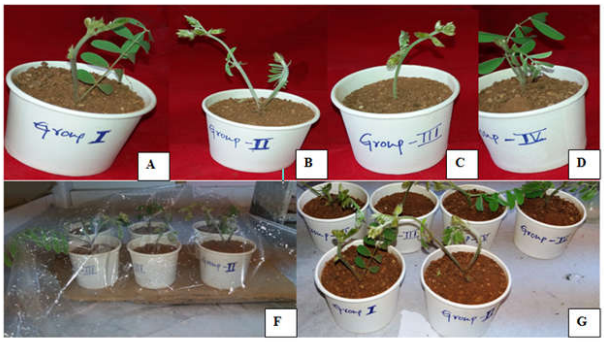


Fig 3. A-Group-I- KN-3.5-IAA-2.0-NAA-1.1, **B-Group-II**- KN-3.3-IAA-1.9-NAA-1.0, **C-Group-III**- KN-3.3-IAA-1.8-NAA-0.9. **D-Group-IV**- KN-3.6-IAA-2.1-NAA-1.2. **F**- Subjecting the plants for heat treatment (covering the plants with polythene cover to maintain the relative humidity 85-90% for prevention of water loss during treatment), **G**- Plants transferred in that contain normal soil and organic manure.

CONCLUSION

The present study concludes that the effect of auxins and cytokinins used in the study are most effective to produce shoot and root system in combination comparing to single.

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