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ROLE OF PLANT GROWTH REGULATORS ON *IN VITRO* CULTURES OF *PTEROCARPUS MARSUPIUM* (ROXB.)

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Abstract– Plant growth regulators (PGR's) play an essential role in determining the development pathway of plant cells and tissues in culture medium. The auxins and cytokinins are most commonly used plant growth regulators. Plant growth regulators (PGR) are vital for plant developmental process. *Pterocarpus marsupium* is known as Indian Kino Tree or Malabar Tree, which is exploited due to its medicinal properties such as anti bacterial, anti diabetics, anti analgesic etc. The objective of this research is to study the effect of different growth hormones on *Pterocarpus marsupium* to further standardize the *in vitro* miropropagation protocol. The result of the study conducted showed that cytokinine with low concentration of auxin has optimal for shoot multiplication and maintenance of *in vitro* cultures of *Pterocarpus marsupium*.

INTRODUCTION

Plant growth regulators are chemicals which are naturally biosynthesized and influence physiological processes in plants. Various plant growth regulators and its type and the concentration of hormones used depend mainly on the species of the plant, the tissue or organ cultured and the objective of the experiment (Ting, 1982). Growth and morphogenesis of plant tissue *in vitro* are largely governed by the composition of culture medium. A number of media have been devised for specific tissues and organ but the most notable one, which served as a basic medium for wide spectrum of plant tissue for morphogenetic studies is that formulated by Murashige and Skoog's medium (1962).

Pterocarpus marsupium (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, from the family fabaceae. It is a medium to large sized tree reaching height up to 15-20 meter with dark brown to grey bark having swallow cracks. Fruit is circular, flat, winged pod. Seed is convex and bony (Warrier, 1995). Tree flowers and fruits in the month of March to June (Yadav and Sardesai, 2002). Its bark release a gum called gum kino, which is traditionally used to treat diarrhea, dysentery, leucorrhoea etc. and bark as astringent & for toothache. The Main constituents are pterostilbene and marsupin (Dhayaney and Sibi, 2019).

MATERIALS AND METHODS

Culture Media

MS medium comprises of inorganic salts, organic compounds, amino acids, plant growth regulators, carbohydrates source and gelling agents.

Auxins, cytokinins, GA3, ABA used were dissolved in dilute 1.0N NaOH or 0.1 N HCL. pH of the medium was adjusted to 5.8 by 1.0N HCL or NaOH. Medium was autoclaved and sterilized at 15lb pressure for 20 minutes at 121°C. All media were supplemented with required amount of PGR's that were stored at 4°C.

Plant Material and Surface Sterilization

Explant material were collected with mature tree (10 years and above), growing at nursery at Arid Forest Research Institute, Jodhpur. Nodal segments containing single axillary bud were used as source material for micropropagation. Explants collected from mature plant were cleaned with 70% ethanol swabbed cotton followed by surface disinfection with NaOCl (Sodium Hypochlorite). They were

finally washed thrice with autoclaved distilled water to remove the traces of sterilants that can be harmful for explants.

Culture conditions

All cultures were kept at 25±2°C temperature and relative humidity of 70 (±) 2%, maintained in culture rooms by air conditioners. All cultures were kept under illumination of 16hrs photoperiod with light intensity of 1600 lux, obtained by white cool fluorescent tube of 40 watts (Philips, India).

Statistical analysis

Data collected in Completely Randomized Design (CRD) of experiments (Compton, 1994) was analyzed by using statistical packages viz. SPSS version 17.0. Data was subjected to one way Analysis of Variance (ANOVA) and Multi Variate Analysis. Degree of variations was shown by Standard Error (SE), Duncan's multiple range Test (DMRT) at 5%. The significance of the data as ascertained by F-test and DMRT values computed, were used for comparing differences in means of various treatments.

RESULTS

Shoot Proliferation

Effect of Cytokinin

The effect of cytokinin (BAP and Kn) alone or in combination was studied for axillary bud proliferation. These cytokinins were found to be essential for axillary bud break and development of axillary shoots. The percentage of bud break response was found to be concentration dependent. Basal medium (MS medium) without plant growth regulators did not bring axillary bud break, addition of plant growth regulators markedly influenced the differentiation of explants towards axillary bud proliferation. Axillary bud break was achieved in 15 -20 days in all cultured nodal segments. The number of axillary shoots varied from 1-3 per axillary bud.

Nodal segments containing axillary buds were cultured on MS medium supplemented with different concentration of BAP. A maximum bud break response of 46.66% was obtained at 8.88μM concentration of BAP supplemented MS medium. Number of axillary shoot proliferated per explant varied which was 1.16 at 2.22μM BAP, which increased to1.90 shoots at 8.88μM BAP. Further increase in BAP concentration in MS medium beyond 13.32μM resulted in decreased bud break response.

Amongst cytokinins tried, Kn was found to be better than BAP in inducing multiple shoots. At lower concentration of Kn (2.32-9.30 μM), the bud break response was less. Increased concentration of Kn (13.95μM), increased the response percentage (64.44%) and average number of proliferated shoots were 2.51. Whereas higher concentration of Kn beyond 13.95μM resulted in decreased percentage bud break response of 51.11% at 18.59 Kn μM. MS medium supplemented with 13.95μM Kn, was found to be optimal for maximum bud break response of 64.44% with 2.51±0.10numbers of axillary shoots.

Effect of Cytokinin -Cytokinin Interaction

Effect of cytokinin-cytokinin interaction was also studied for axillary shoot proliferation. MS medium supplemented with combination of cytokinin (Kn + BAP) were tested at various concentrations. Best bud break response of 44.44% was obtained at 4.65 μM Kn and 2.22 μM BAP when supplemented in MS medium.

Effect of Cytokinin –Auxin Interaction

Cytokinin –auxin interaction was also studied for *in vitro* shoot multiplication. For this Kn (4.65, 9.30, 13.95 μM) was used in combination with low doses of NAA (0.27, 0.54, 1.34 μM) in MS medium. Addition of NAA to Kn supplemented medium at all concentration reduced bud break response percentage. Among the Kn and NAA interaction, Maximum 40% bud break response was obtained when medium supplemented with 4.65 μM Kn and 0.54 μM NAA. When compared, in term of number of shoot developed, an average number of 2.51 shoots that developed on 13.95μM Kn alone, reduced to only 1.94 shoots on medium supplemented with NAA (0.54 μM) in combination to 13.95 μM Kn. Any increase in concentration of NAA (1.34 μM) with Kn reduced the number of *in vitro* shoots to (1.18). It was also observed that addition of NAA to MS medium supplemented with Kn also reduced the average shoot length when compared to Kn alone in MS medium.

Kn (4.65, 9.30, 13.95 μM) was also used in combination with IAA (0.29, 0.57, 1.43 μM). A maximum of 35.33% bud break response was obtained on MS medium supplemented with 9.30 μM Kn + 0.57 μM IAA. Whereas when IBA was added with Kn in MS medium gave 37.77% bud break response.

In vitro shoot multiplication

Effect of Cytokinin

The proliferated *in vitro* axillary shoots were excised from mother explants and subcultured on MS medium supplemented with cytokinins for establishment of cultures and multiplication of *in vitro* shoots. These established *in vitro* shoots were carefully dissected into propagule of 2 shoots and were subcultured on MS medium supplemented with cytokinins for further *in vitro* multiplication. Data were recorded after 4 weeks of subcultured.

A propagule of 2 shoots when subcultured on 4.44 μM BAP supplemented medium resulted in 4.82 mean shoot number with 1.58 cm mean shoot length. When BAP concentration increases beyond 4.44μM, mean shoot number decreased with mean shoot length.

On Kinetin supplemented MS medium, 9.30μM Kn gave maximum response for *in vitro* shoot multiplication where 5.22 shoots were developed from a propagule of 2 shoots. Shoot multiplication decreased on lower or higher concentrations of Kn beyond 9.30μM Kn. At 2.32μM Kn in MS medium average 4.69 shoots multiplied whereas only 3.05 shoots developed at 18.59μM Kn. A gradual increase in average shoot length was noticed with the increase of Kn on MS medium. Average length of regenerated shoots on 2.32μM Kn supplemented medium was 1.47 cm which increased to 1.65 cm at 9.30μM Kn. A further increase in Kn in MS medium (18.59μM) drastically reduced the shoot length to 1.38 cm.

Effect of Cytokinin – Auxin interaction

It is reported when small doses of auxins when added with cytokinin, it helped *in vitro* shoot multiplication. It was observed that Kn in combination with NAA increased *in vitro* shoot multiplication response. An average number of 5.22 shoots was obtained at 9.30 μM Kn alone, whereas, with the addition of NAA with Kn in medium it increased to 6.22 shoots on MS medium supplemented with 0.54 μM NAA along with 9.30 μM Kn.

MS medium supplemented with 9.30 μM Kn + 0.57 μM IAA gave 4.19 shoots with shoot length of 1.61 cm. MS medium supplemented with 9.30 μM Kn + 0.49 μM IBA showed 4.58 average number of shoots and shoot length of 1.54 cm. It was observed that MS medium supplemented with 9.30 μM Kn + 0.54 μM NAA showed best *in vitro* shoot multiplication.

DISCUSSION

The inclusion of cytokinin and auxin in the culture MS Medium enhanced *in vitro* shoot regeneration and growth of shoots in several plant species (George, 1993). It was revealed that MS medium consisted of Kn with NAA was most effective with multiplication rate (3.11) with shoot length (1.67cm). The study showed similar results with earlier findings of several workers, where the addition of low level of auxin with cytokinin promoted shoots in *Acacia catechu*, *Eucalyptus grandis* and *Lagerstromia parviflora* (Tiwari *et al.*, 2002). MS medium supplemented with higher concentration of auxin (IAA or NAA) into the cytokinin-rich medium inhibited not only shoot multiplication but also produced some compact callus at the base of the explants. Similar observations were made the in vitro studies of *Acaciaauriculiformis* (Mittal *et al*., 1989); *Pterocarpus santalinus* (Lakshmi Sita *et al*., 1992), and *Acaciamangium* (Nanda *et al*., 2014).

Table 1. Effect of cytokinin (BAP) in MS medium on axillary shoot proliferation of *Pterocarpus marsupium*. Data recorded after 4 weeks.

BAP (μM)	Bud Break response (%)	Mean shoot number	Mean shoot length (cm)
0.00	0.00 ± 0.00^{b}	0.00^{c}	0.00^{c}
2.22	26.66 ± 0.06^{a}	1.16 ± 0.11^{a}	0.71±0.02^{a}
4.44	31.11 ± 0.06^{a}	1.57 ± 0.20^{b}	0.79±0.06^{a}
8.88	46.67 ± 0.07^{a}	1.90 ± 0.19^{b}	1.10±0.03^{b}
13.32	37.78 ± 0.07^{a}	1.29 ± 0.14^{a}	1.08±0.01^{b}
17.76	31.11 ± 0.06^{a}	1.07 ±0.71^{a}	0.77±0.03^{a}
Df	5	5	5
F value	5.92	50.60	410.54
P value	0.00	0.00	0.00

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 2. Effect of cytokinin (Kn) in MS medium on axillary shoot proliferation of *Pterocarpus marsupium*. Data recorded after 4 weeks.

Kn (μM)	Bud Break response (%)	Mean shoot number	Mean shoot length (cm)
0.00	0.00 ± 0.00 ^{c}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
2.32	35.56 ± 0.07^{b}	1.12 ±0.08^{d}	0.90 ± 0.03^{e}
4.65	44.44 ± 0.07^{ab}	1.52±0.11^{c}	1.03 ± 0.03^{d}
9.30	51.11 ± 0.07^{ab}	2.13±0.14^{b}	1.19 ± 0.02^{c}
13.95	64.44 ± 0.07^{a}	2.51±0.10^{a}	1.47 ± 0.02^{a}
18.59	51.11 ± 0.07^{ab}	1.91±0.13^{b}	1.26 ± 0.016^{b}
Df	5	5	5
F value	10.85	1014.92	118.77
P value	0.00	0.00	0.00

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 3. Effect of cytokinin- auxin (Kn+NAA) interaction on axillary shoot proliferation of *Pterocarpus marsupium*. Data recorded after 4 weeks.

Kn (μM)	NAA (μM)	Bud Break response (%)	Mean shoot number	Mean shoot length (cm)
0.00	0.00	0.00 ± 0.00^{b}	0.00±0.00^{d}	0.00±0.00^{e}
4.65	0.27	28.89 ±0.06^{a}	1.30±0.13^{bc}	0.45±0.04^{bc}
4.65	0.54	40.00 ±0.07^{a}	1.94±0.18^{a}	0.59±0.05^{a}
4.65	1.34	35.55 ±0.07^{a}	1.18±0.10^{c}	0.51±0.05^{abc}
9.30	0.27	37.77 ±0.07^{a}	1.58±0.14^{b}	0.53±0.04^{ab}
9.30	0.54	35.55 ±0.07^{a}	1.18±0.10^{c}	0.45±0.04^{bcd}
9.30	1.34	26.66 ±0.07^{a}	1.33±0.14^{bc}	0.45±0.05^{bcd}
13.95	0.27	33.33 ±0.07^{a}	1.33±0.09^{c}	0.45±0.04^{bcd}
13.95	0.54	31.11 ±0.06^{a}	1.21±0.11^{c}	0.40±0.01^{cd}
13.95	1.34	28.88 ±0.06^{a}	1.00±0.00^{c}	0.37±0.03^{d}

Analysis of Variance

	Df	F value	P value	df	F value	P value	df	F value	P value
Kn	2	5.031	0.00	2	4.90	0.00	2	4.69	0.01
NAA	2	212.00	0.00	2	3.23	0.04	2	3.17	0.04
Kn*NAA	4	20.978	0.00	4	0.76	0.54	4	0.75	0.55

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 4. Effect of BAP on *in vitro* shoot multiplication of *Pterocarpus marsupium*. Data recorded after 4 weeks.

BAP (μM)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
0.00	2.22±0.08^{c}	1.27±0.02^{c}	1.11
2.22	4.27±0.09^{b}	1.32±0.02^{c}	2.13
4.44	4.82±0.11^{a}	1.58±0.02^{a}	2.41
8.88	4.62±0.12^{a}	1.50±0.02^{b}	2.31
13.32	4.17±0.07^{b}	1.31±0.01^{c}	2.08
17.76	4.04±0.08^{b}	1.27±0.02^{c}	2.02
df	5	5	
F value	438.01	700.88	
P value	0.00	0.00	

Value within the columm with similar superscrits are not significantly different at $p \leq 0.05$ level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 5. Effect of Kn on *in vitro* shoot multiplication of *Pterocarpus marsupium*. Data recorded after 4 weeks.

Kn (μM)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
0.00	2.27 ± 0.08^{e}	1.44±0.02^{ab}	1.13
2.32	4.69±0.09^{b}	1.47±0.02^{b}	2.34
4.65	4.80±0.11^{b}	1.61±0.02^{a}	2.40
9.30	5.22±0.10^{a}	1.65±0.03^{a}	2.61
13.95	4.33±0.11^{c}	1.48±0.01^{b}	2.16
18.59	3.05 ± 0.14^{d}	1.38±0.02^{c}	1.52
f	5	5	
F value	102.80	13.23	
P value	0.00	0.00	

Value within the columm with similar superscrits are not significantly different at p ≤ 0.05 level as determined using Duncun's multiple range test. A value represents ± standard error.

Table 6. Effect of cytokinin- auxin (Kn+ NAA) interaction on *in vitro* shoot multiplication of *Pterocarpus marsupium*. Data recorded after 4 weeks.

Kn (μM)	NAA (μM)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
0.00	0.00	2.61±0.08^{g}	1.26±0.02^{f}	1.30
4.65	0.27	4.27±0.08^{ef}	1.41±0.02^{cde}	2.13
4.65	0.54	5.00±0.10^{b}	1.59±0.02^{b}	2.50
4.65	1.34	4.66±0.09^{cd}	1.47±0.02^{c}	2.33
9.30	0.27	4.86±0.11^{ab}	1.59±0.03^{b}	2.43
9.30	0.54	6.21±0.11^{a}	1.67±0.01^{a}	3.10
9.30	1.34	4.55±0.12^{de}	1.38±0.02^{de}	2.27
13.95	0.27	3.44±0.09^{de}	1.44±0.02^{cd}	1.72
13.95	0.54	4.27±0.07^{ef}	1.39±0.01^{de}	2.13
13.95	1.34	4.11±0.05^{fc}	1.35±0.01^{e}	2.05

Analysis of Variance

PGR		F value	P value
Kn	Shoot number	72.205	0.00
	Shoot length	33.109	0.00
NAA	Shoot number	54.963	0.00
	Shoot length	31.133	0.00
Kn* NAA	Shoot number	24.700	0.00
	Shoot length	14.594	0.00

Value within the columm with similar superscrits are not significantly different at p ≤ 0.05 level as determined using Duncun's multiple range test. A value represents ± standard error.

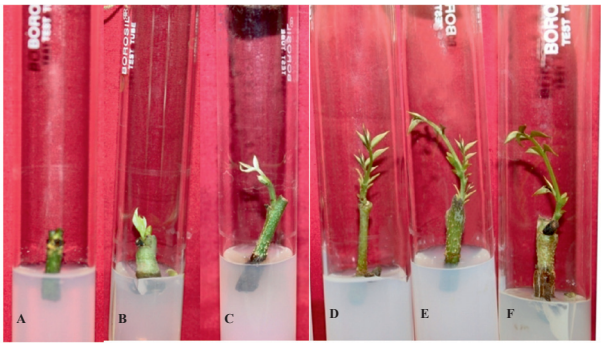

Fig. 1. Effect of Kn on axillary shoot proliferation in *Pterocarpus marsupium*.
(A) Control (B) 2.32 μM (C) 4.65 μM (D) 9.30 μM (E) 13.94 μM (F) 18.95 μM.

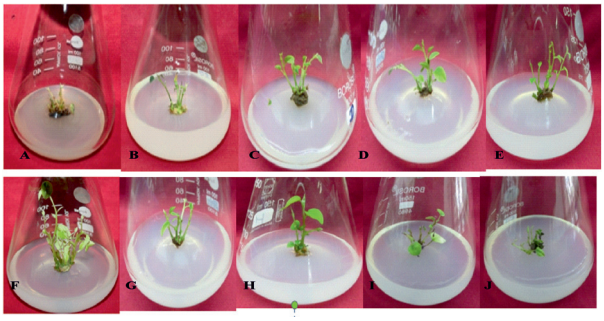

Fig. 2. Effect of (Kn+ NAA) interaction on *in vitro* shoot multiplication of *Pterocarpus marsupium*.
(A) Control (B) 4.65+0.27 μM (C) 4.65+0.54 μMD. 4.65+1.34 μM (E) 9.30+ 0.27 μM (F) 9.30+ 0.54μM (G) 9.30+ 1.34 μM (H) 13.95+ 0.27 μM (I) 13.95+0.54 μM (J) 13.95+1.34 μM.

MS medium supplemented with 9.30 µM Kn + 0.54 µM NAA proved to be best for *in vitro* shoot multiplication.

CONCLUSION

MS medium supplemented with BAP or Kn. Kn at 13.95µM concentration was found best for axillary bud proliferation than other cytokinins, on which 64.44% explants responded. Different cytokinins (BAP, Kn) were tried to select the cytokinin supporting optimal *in vitro* shoot multiplication. MS medium supplemented with 9.30 µMKn + 0.54µM NAA found optimum for shoot multiplication. Of different cytokinins tested, no cytokinin alone was found optimal for shoot multiplication. Cytokinin in combination with auxin enhanced *in vitro* shoot multiplication. In this study reconnaissance study the potential role of plant growth regulator on *Pterocarpus marsupium* were assessed. Thus plant growth regulators and their interactions plays significant role in shoot proliferation of *Pterocarpus marsupium*.

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