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Micropropagation of Karanj (*Pongamia pinnata pierre*) through Shoot Apex Segments-A Medicinal and Bio-Fuel Plant

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ABSTRACT: The Study was undertaken with an objective to develop a protocol for micropropagation of *Pongamia pinnata pierre* through shoot apex segments shoot of 0.5 to 1.0 cm were collected and used as a explant. The treatment of 1.0 NaOCl (Sodium hypochloride) (W/v) solution 1 minute to 10 minute time duration. These treated explant washed trice with double distilled water and cultured in MS (Murashige and skoog) medium. In this experiment auxin 2, 4-D, NAA and cytokinin BAP, Kinetin were used for optimization of maximum callus induction.

Shoot apex explant culturing callus induction maximum callus is produced when MS medium with 3.0 mg/l, 2, 4-D and BAP 0.5 mg/l, the optimized physical condition has to be maintain throughout the experiment. In this study about 30 to 35% mature sotmatic embryos germinated after sub culture from shoot apex. Different concentration and combination of NAA, IAA, IBA and BAP were used to inducted rooting on MS based medium. When the hight *in vitro* shoot, were reached up to 8 cm with healthy shooted roots, the plants were ready for hardening. The complete protocol for somatic embryogenesis, shoot induction, root induction up to hardening.

Keywords : Karanj, micropropogation, shoot apex segments, MS

INTRODUCTION

Pongamia a small genus, medium size tree, seed yield oil biofuel like diesel. Natural propagation is through seeds when remain viable for one year. Artificial regeneration is carried out through direct sowing or transplanting one year old seedling raised in nursery. There are very few reports in literature on reproductive biology of *Pongamia*. In nature germination of seeds in low and seedling mortality is very high. Due to these reasons the growth of new plant is difficult through seeds in nursery also.

Advantages in micro-propagation through tissue culture for rapid multiplication in a shoot cycle results in increased number of seedlings. *In vitro* propagation appears to have permanent advantage in cases where serious problems of disease occur. This is because of the fact that *in vitro* method given more pathogen free plants and can be maintained economically.

Micro propagation techniques have wide scope in producing uniform germplasm. The present study was conducted with an objective to develop a protocol for *Pongamia* micro propagation through shoot apex.

MATERIAL AND METHOD

Explant of *Pongamia* shoot apex segments 0.5 to 1.0 cm were collected from young and healthy plants and experiment was conducted at tissue culture laboratory, Saifia Science College, Bhopal. The explants were thoroughly washed under running tap water for 15-20 minutes then washed with double distilled water (DDW). The explants subjected to surface sterilant NaOCl 1.0% for 2 to 8 minutes. Now explants were dipped in 70% ethyl alcohol for 30 sec. and finally rinsed thrice with double distilled water. The explants inculcated aseptically on MS medium supplemented with various concentration of auxin & cytokinin. The temperature maintained at 27 ± 2°C. The culture was kept in light 16 hours (1000 to 4000 lux) and 8 hours dark period. The average number of shoots were recorded and analyzed statistically when hight of multiple shoots attained 5-7 cm. The shoots were isolated and transfered on rooting medium. The rooted plantlets were removed from agar medium and washed in shallow tray containing sterile water to remove adhering agar. They were then transfered to pot containing soil: sand: cowdung: Bio fertilizer (*Azatobactor*).

The temperature, humidity and photoperiod were maintained during hardening *in vitro* grow plantlets. Then pots were kept under poly house conditions for nearly two weeks for hardening and then transferred to the field. The experimental data were statistically analyzed.

RESULT AND DISCUSSION

Highest of percentage of aseptic culture was found 98.3% for shoot apex segment. Similarly highest percentage of survival was 90.0% with the treatment of 1% NaOCl for 10 minutes while 67.4% acetic culture and 62.0% survival under the treatment of 1% $HgCl_2$ for 6 minutes and under 1% KCl 63.0% aseptic culture and 61.0% survival for 10 minutes (Table 1).

$HgCl_2$ and NaOCl have beneficial effect reported by several workers; Syamal *et al*., (2007); Rana and Singh, (2002) used surface sterilant NaOCl and $HgCl_2$ for sterilization of citrus explant. Increase of exposure of surface sterilant effect the explant survival. It may due to exposure of explant at longer duration, contaminants of heavy metals, in present study NaOCl is best surface sterilant 1% for 8 minutes exposure.

.**Table 1. Effect of exposure time of surface sterilization, and present of survival of explants.**

S.No.	Exposure Time (minute)			Percent %	
1	$Hgcl_2$ 0.1%	Naocl 1%	kcl 1%	Aseptic culture	Shoot apex segment survival
1	0.0	0.0	0.0	-	30.0
2	2	-	-	42.3	40.0
3	4	-	-	53.3	48.0
4	6	-	-	67.4	62.0
5	8	-	-	59.2	56.0
6	10	-	-	55.2	59.0
7	-	2	-	62.0	58.0
8	-	4	-	75.0	68.0
9	-	6	-	83.4	78.0
10	-	8	-	98.3	90.0
11	-	10	-	89.7	82.0
12	-	-	2	38.0	30.0
13	-	-	4	43.0	41.0
14	-	-	6	48.3	45.0
15	-	-	8	59.1	56.0
16	-	-	10	63.3	61.0

CD at 5%

Table 2. Effect of 2, 4-D and BAP on induction of callus in *Pongmia pinnata* Pierre. after 25 days.

Explant	Concentration 2,4-D mg/l	Concentration of BAP mg/l	Average fresh weight of callus mg ± SE	Average dry weight of callus mg±S.E.	Physical nature of callus based on 25 observation
Sprout of *Pongamia pinnata invitro*	0.0	0.0	-	-	-
	0.3	0.5	273±3.8	38.1±1.8	Fc, yw
	0.6	0.5	270±4.7	37.3±2.0	Fc, yw
	0.9	0.5	281±5.1	40.1±2.3	F
	1.2	0.5	273±4.9	39.1±1.9	F
	1.5	0.5	279±4.6	39.2±2.0	F
	1.8	0.5	282±5.6	40.7±2.3	F
	2.1	0.5	287±6.1	43.2±2.1	F
	2.4	0.5	290±6.0	45.9±1.9	F
	2.7	0.5	291±5.8	46.1±1.7	F
	3.0	0.5	298±6.2	46.3±2.0	Fc, yw, yG

Observation based on mean of 20 explants ± SE, x = visually observed, F = Friable, C = Compact, YW = yellowish white, YG = yellowish Green

Table 2 shows that the treatment of 2, 4-D 3.0 mg/l and BAP 0.5 mg/l was found to be bast for callus fresh weight 298±6.2 and callus dry weight 46.2±2.0, This callus fuiable yellowish white and yellowish green of shoot apex sprowtly, 2, 4-D and BAP help in shoot proliferation and multiple shoots induction and early sprowting of explant shoot apex, Kour and Khee (2000) also reported best proliferation resulted in greatest no. 5.34 shoots.

Table 3 showed that the treatment of 3.0 mg/l 2,4-D, 0.5 mg/l BAP with 5.0% sucrose gave maximum percentage 67% embroynic callus and 30±0.3 average somatic embryo per callus in *Pongamia pinnata* after 20 days of sub culture. Cytokinins promote induction of cell division, cell organ enlargement and BAP is the best cytokinins. BAP with 2, 4-D and sucrose efficacy also increase. cytokinins seem to act either by removing free redicals or by preventing their formation. In *pongamia pinnata* 2, 4-D and BAP with sucrose concentration enhanced the percentage of embryonic callus.

Table 3. Effect of sucrose concentration on somatic embryognesis with Ms-4 medium, 2,4-D and BAP combination in *Pongamia pinnata,* after 20 days of subculture.

Explant	2,4-D mg/l	BAP mg/l	Sucrose %	% of Embryozenic callus	Average of somatic embryo (per callus)
M. S-4	-	-	-	-	-
In invitro* calus of *Pongamia pinnata	0.3	0.5	0.5	22%	19±0.2
	0.6	0.5	1.0	28%	20±0.3
	0.9	0.5	1.5	30%	21±0.1
	1.2	0.5	2.0	38%	22±0.2
	1.5	0.5	2.5	42%	24±0.4
	1.8	0.5	3.0	48%	27±0.3
	2.1	0.5	3.5	49%	24±0.2
	2.4	0.5	4.0	52%	27±0.1
	2.7	0.5	4.5	61%	28±0.2
	3.0	0.5	5.0	67%	30±0.3

Observation based on 20 explant ± SE

Table 4. Effect of 2, 4-D and BAP in MS on induction of shoot after 30 & 60 days in *Pongamia pinnata.*

Explant	Concentration 2,4-D mg/l	Concentration of BAP mg/l	Mean number of shoots per explants after 30 days ±S.E.	Mean number of shoots per explants after 60 days±S..
Sprout of *Pongamia pinnata in vitro*	0.0	0.0	-	-
	0.2	0.4	-	2.2±0.2
	0.2	0.8	2.1±0.3	2.9±0.3
	0.2	1.2	2.4±0.2	3.2±0.4
	0.2	1.6	2.8±0.1	3.9±0.3
	0.2	2.0	3.1±0.2	4.0±0.2
	0.2	2.4	3.7±0.3	4.6±0.3
	0.2	2.8	4.2±0.1	5.1±0.4
	0.2	3.2	4.9±0.2	6.8±0.5
	0.2	3.6	4.6±0.3	5.7±0.4
	0.2	4.0	3.4±0.2	4.0±0.3

Observation based on mean of 20 explants ± SE

Effect of 2, 4-D and BAP in MS medium for induction shoot after 30 and 60 days of inoculcation is given in Table 4. Maximum mean number of shoots per explant after 30 days and 60 days were observed under 0.2 mg/l 2, 4-D and 3.2 mg/l BAP *in vitro* callus of *Pongamia pinnata* L. These results are in agreement of finding of Rana and Singh (2002). Treatment of 2.5 mg/l of IAA 0.5 mg/l IBA combination was found to be significantly superior over all the treatment with respect to maximum root initiation of 6.2±0.4 after 30 days and 8.2±0.2 after 60 days. The above results have same resemblance with earlier finding of Kumar *et al*; 2001.

IAA promote adventitious roots development and IBA is the most effective over than any other growth regulator in most of the cases apparently because it is not destroyed by IAA oxidase of other enzymes and there for persist longer IAA stimulate cell expansion by cell wall losing after a short time following exposures, in addition auxin promote protein synthesis by controlling gene expression as a responses after along time interval. The well developed plant lets with roots and shoots were transfered to plastic bags filled with mixture of soil + sand + cow dung + biofertilizers (*Azatobactor*) (1:1:1:1) for hardening of plants before transfer to field 56.6% survival after 30 days in soil + sand + cow dung + vermiculture. 60.0% survival after 30 days.

In soil + sand + cow dung + bio fertilizer (1:1:1:1) for hardening of plants before transfer to field.

Table 5. Effect of IAA and IBA combination on induction of root after 30 days and 60 days of inoculation of shoot in half strenth MS medium in *Pongamia pinnata.*

Explant	Concentration of IAA mg/l	Concentration of IBA mg/l	Average no of root/ flask ± SE after 30 days	Average no. of root/flask ±SE after 60 days.
Callus of *Pongamia piannata in vitro*	0.0	0.0	-	-
	0.5	0.5	4.1±0.3	6.3±0.2
	1.0	0.5	4.2±0.2	6.9±0.1
	1.5	0.5	4.7±0.2	7.2±0.2
	2.0	0.5	5.9±0.4	7.8±0.3
	2.5	0.5	6.2±0.4	8.2±0.2
	3.0	0.5	5.4±0.5	7.6±0.3
	3.5	0.5	4.6±0.3	7.0±0.2
	4.0	0.5	4.1±0.3	6.8±0.3
	4.5	0.5	3.9±0.2	6.1±0.2
	5.0	0.5	3.2±0.2	6.0±0.2

Table 6. *In vitro* hardening of plants of *Pongamia pinnata* in different soil condition after 15 and 30 days of transfer in green house and their survival percentage.

Plant	Soil Mixture	In vitro plants transfer in green house	Mean number of plant survival after 15 days and 30 days transfer in green house		% of survival of plants after 15 days and 30 days transfer in green house	
Pongamia pinnata	Soil + Sand + Cowdung + Vermi Culture 1:1:1:1	30	22	17	73%	56.6%
	Soil + Sand + Cow dung biofertilizers (Azalobaeter) 1:1:1:1	30	21	18	70%	60.0%

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