International Journal of General Medicine
and Pharmacy (IJGMP)
ISSN(P): 2319-3999; ISSN(E): 2319-4006
Vol. 3, Issue 5, Sep 2014, 111-116

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

IN VITRO MULTIPLE SHOOT REGENERATION FROM SHOOT TIP EXPLANTS OF *SPERMACOCE ARTICULARIS L. F.*

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ABSTRACT

An efficient in vitro propagation protocol through shoot tip explants culture was developed for *Spermacoce articularis* L. f. shoot tip explants of in vitro grown 1 month old seedlings were cultured on MS basal medium supplemented with BAP and combination with KIN were differentiated into shoots directly. The shoots could be easily multiplication on MS Medium supplemented with 5-25 µM (BAP) along with KIN (5-25 µM). The establishment efficiency of in vitro from plantlets in pots containing a mixture of sand: vermiculate soil (1:1) was more than 90%. This rapid regeneration protocol an average of 15 plantlets was able to produce from single set of treatment.

KEYWORDS: *Spermacoce articularis L. f.* Direct Regeneration, Shoot Tip Culture

INTRODUCTION

Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds which are commonly used in home remedies against multiple alimets. Plant regeneration protocol is a prerequistate for the application of *in vitro* genetic manipulation techniques, such as variant selection and transformation for economically more desirable characters. *Spermacoce articularis* L.*f.* (Rubiaceae) is also known Nathaisuri(Tamil).

Advanced biotechnological methods for culturing plant cells and tissues would provide new means of propagating rapidly and conserving the valuable, rare and endangered plant species. Uniformity in the plantlets obtained through micropropagation technique and the year round availability of the plantlets surpassing field dormancy make tissue culture technique as attractive alternative to the conventional methods of propagation. The *in vitro* morphogenic processes are usually affected by physical environment, growth regulators carbon source, gelling agent and explant type and origin. It is well known that cytokinins suppress the growth of apical meristems and instead induce excess formation of lateral meristems, resulting in multiple shoots. The cytokinin–auxin combination has also been used widely for shoot regeneration in various protocols.

Therefore specific objectives of the present study to produce optimize protocol in vitro propagation of *Spermacoce articularis* L. *f.* Plant regeneration by tissue culture technique would be a feasible alternative for improving the quality and production of *Spermacoce articularis* L.*f.* plant. So in vitro plant regeneration is the best method available for the production of high quality plants which are free of any disease and pest ensuring the maximum production potential of varieties that are genetically identical to parent plant as well as to one another (Raven et al., 1999). So the objective of the present study was to investigate the effect of different growth regulators on number of shoots, lengths of shoot/leaf and

to develop an effective in vitro method for plant propagation of *Spermacoce articularis* L.*f.* and the propagated plants were successfully established in field conditions.

MATERIALS AND METHODS

Plant Material

Shoot tip explants of *Spermacoce articularis* L. *f.* were collected from earthen pot grown plant of author's roof top garden in early spring. Explants were excised from 4 months old plant were surface sterilized by cleaning thoroughly under running tap water for 20 minutes and washed with commercial detergent followed by running tap water and rinse with distilled water.

Sterilization of Explant

The plant materials were washed with 70% ethanol, 0.1% Hgcl2 (W/V) for 3 minutes and rinse with sterile distilled water for 3 to four times under aseptic condition to remove traces of Hgcl2. The explants were cultured on MS containing 3.0% sucrose (W/V), 0.8% agar and BA (2.22 to 0. 44 µM/L) an IBA (4.9 to o.44 µM/L) for multiplication of plants (Table 1). The PH of all the media was adjusted to 5.8 before autoclaving at 121o centigrade, 15lb pressure for 20 minutes. Cultures were incubated in a culture room at 25o centigrade under 16/8 hr photoperiod by cool white fluorescent tubes (Phillips, India). The shoot numbers, lengths including were measured.

Rooting and Hardening

Well-developed shoots were transferred for root induction on half strength MS medium supplemented with IBA (0.5µM). For ex vitro establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual nutrient from the plant body. The regenerated plantlets were then transferred to plastic cups containing sterile soil, sand, compost (1:1:1) and covered with polythene and maintained in tissue culture conditions. The well-developed plantlets were transferred to cup kept in greenhouse and finally transferred to the field.

Each stage of different shoot multiplication, shoot elongation, root induction and acclimatization ten replicates were maintained in each media combination. All experiments were repeated for consistency of results. Observation like number of shoots emerged, root induced were recorded and their average values were measured (Table 1).

RESULTS AND DISCUSSIONS

The shoot buds initiation and development were observed on shoot tip after 21 days of culture. The formation of shoot buds occurred on the top of explants. The percentage of shoot buds frequency was varied according to the combination of hormones on the medium (Table 1) (Figure 1). In all concentrations of BAP alone, KIN alone and combination with BAP + KIN a moderate shoot multiplication was observed. But a higher percentage of shoot multiplication was observed in 10 µM of BAP in combination with 10 µM of KIN . This report was being confirmatory was first reports and the number of shoots formed explants at lower concentration of KIN. This may be due to high level of endogenesis auxin with an increase in the auxin concentration beyond a threshold value.

The isolated shoots were cultured on MS medium supplemented with different concentrations of IAA, IBA, NAA and BAP ranging from 2µM. Roots were initiated and well established in all the concentrations of the auxins studied. The basal medium supplemented with 9µM IBA and combination of 2µM(BAP) showed 100% root sprouting frequency,

with 27.42 number of roots with a root length of 9.86 cm. The basal medium supplemented with 3µM, 6µM, 9µM, 12µM and 15µM IBA showed 100% root sprouting frequency. 9µM IBA produced maximum number of roots and a maximum root length . The basal medium supplemented with 6µM and 9µM NAA showed 100% root sprouting frequency. The basal medium with 9µM NAA produced 23.84 number of roots with a root length of 8.82 cm. The basal medium supplemented with 12µM IAA showed 95% root sprouting frequency.

Among auxins IBA was found to be more effective on rooting. The basal medium supplemented with 3µM, 6µM, 9µM, 12µM and 15µM IBA showed 100% root sprouting frequency. 9µM IBA produced 27.42 numbers of roots and a maximum root length of 9.86 cm was produced in 2 µM BAP in basal medium. In many studies, IAA, IBA and NAA were used to induce rooting. Higher frequency of rooting was achieved by IBA in Jatropha curcas (Kalimuthu et.al, 2007).

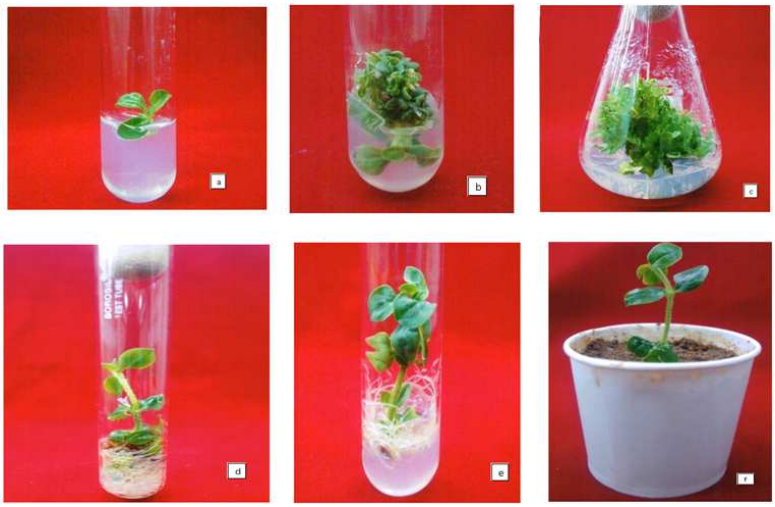

Figure 1: Multiple Regeneration *Spermacoce articularis* by Using Shoot Tip Explants (a) Shoot Tip Explant, (b) Shoot Induction in the Medium Fortified with BAP (15 µM), (c) Multiple Shoot Induction after 15-20 days, (d) Regeneration (e) Rooting (f) Hardening

Table 1: Effect of Different Concentrations of Cytokinins on Shoot Induction Multiplication and Rooting from Shoot Tip of *Spermacoce articularis L. f*

Shoot Induction and Multiplication from Shoot Tip					Rooting						
Concentration of Hormones		Shoot Induction Frequency (%)	Number of Shoots	Shoot Length (cm)	Concentration of Hormones				Root Induction Frequency (%)	Number of Roots	Root Length (cm)
BAP	KIN				IAA	IBA	NAA	BAP			
5 µM		86	23.45 ± 1.82^{i}	5.35 ± 0.69^{h}	3 µM			2 µM	66	7.28 ± 1.11^{no}	3.44 ± 0.85^{no}
10 µM		100	32.78 ± 2.06^{bc}	7.56 ± 0.73^{bc}	6 µM			2 µM	74	12.14 ± 1.52^{j}	4.60 ± 0.74^{kl}
15 µM		90	26.30 ± 1.60^{fg}	6.15 ± 0.81^{ef}	9 µM			2 µM	86	14.30 ± 1.78^{hi}	5.34 ± 1.16^{i}
20 µM		78	22.16 ± 1.89^{ijk}	4.74 ± 0.76^{j}	12 µM			2 µM	92	18.64 ± 1.65^{d}	7.34 ± 1.09^{d}
25 µM		70	19.62 ± 1.26^{lm}	3.97 ± 0.84^{mn}	15 µM			2 µM	80	9.78 ± 1.47^{kl}	4.18 ± 1.17^{lm}
	5 µM	70	20.84 ± 1.55^{kl}	4.47 ± 0.63^{jk}		3 µM		2 µM	76	14.32 ± 1.60^{gh}	5.82 ± 1.21^{gh}
	10 µM	82	26.66 ± 2.45^{ef}	5.25 ± 1.06^{hi}		6 µM		2 µM	92	21.24 ± 1.30^{c}	8.56 ± 1.48^{bc}
	15 µM	94	29.86 ± 2.65^{d}	6.96 ± 1.06^{d}		9 µM		2 µM	100	27.42 ± 1.85^{a}	9.86 ± 1.61^{a}
	20 µM	84	18.74 ± 1.40^{mn}	4.24 ± 0.94^{kl}		12 µM		2 µM	82	16.60 ± 2.05^{ef}	6.36 ± 1.14^{ef}
	25 µM	68	16.12 ± 1.02^{o}	3.61 ± 0.75^{o}		15 µM		2 µM	70	10.22 ± 1.53^{k}	4.70 ± 1.09^{ijk}
5 µM	10 µM	88	27.46 ± 1.27^{e}	6.26 ± 0.29^{e}			3 µM	2 µM	68	8.94 ± 0.81^{lm}	3.68 ± 0.55^{mn}
10 µM	10 µM	100	35.76 ± 1.44^{a}	8.07 ± 0.71^{a}			6 µM	2 µM	84	15.72 ± 0.95^{fg}	6.66 ± 1.26^{e}
15 µM	10 µM	100	33.10 ± 1.99^{b}	7.69 ± 1.21^{b}			9 µM	2 µM	94	23.84 ± 2.31^{b}	8.82 ± 1.35^{b}
20 µM	10 µM	82	25.30 ± 2.07^{gh}	5.88 ± 0.41^{fg}			12 µM	2 µM	88	17.4 ± 0.86^{de}	5.88 ± 1.13^{g}
25 µM	10 µM	76	22.62 ± 2.00^{ij}	4.18 ± 1.06^{im}			15 µM	2 µM	72	8.68 ± 1.44^{mn}	4.72 ± 0.90^{j}

The establishment rate of *in vitro* rooted and acclimatized plant in soil was more than 90%. Rapid *in vitro* regeneration through direct shoot induction from shoot tips explants will be a reliable and less time consuming mode of propagation of the important medicinal plants of this kind in conservation aspect (Arumugam A et. al, 1996; D. P. Premantilake et. al, 2006).

In the present study, development of shootlets from shoot tip explants were observed on MS medium containing BAP10 μM and 10 μM NAA after 35 days in culture. In earlier study, the production of shoot buds from nodal explants of *Spermacoce articularis* L. *f.* showed the development of shoots (25. 5, 26. 9 and 15. 8 and 17. 1) both in second, third and fifth subcultures on MS supplemented with two different hormones BAP (13.32 μM) and Kn (13.8 μM) (Elangomathavan et al. 2006). The use of BAP and NAA for shoot development from shoot tip explants observed in this study is in agreement with earlier reports on organogenesis of dicotyledonous plants (Asamenew and Narayanaswamy 2000, Dhar and Joshi 2005). Individual shoots from a multiple shoot complex were separated after 28 days of culture and transferred to half strength of MS supplemented with IBA (0.49 - 12.30 μM). The root induction was initiated after two weeks of culture, and after four weeks, the root system was well developed (Figure 1: F, G). The maximum rooting response (80%) was achieved on medium supplemented with IBA (2.46 μM) with an average of 5.3 roots per shoot explant (Table 1).

In the present study root induction was obtained with lower concentration of IBA. About 80 per cent of plantlets survival were observed after hardening of the regenerated *Spermacoce articularis* L. *f.* for three weeks. Seventy per cent of the plants transferred to pots survived and resumed growth (Figure 1: H). There was no detectable phenotypic variation among the acclimatized plants.

CONCLUSIONS

In conclusion, the results showed the ability of the shoot tip explants to produce 29 shootlets without any intervening callus phase, where all the plantlets were uniform in height and growth. The present paper reports an efficient micropropagation system for generating a large number of plants through high frequency axillary shoot multiplication in *Spermacoce articularis* L. *f.*

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