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In vitro Plant Regeneration from the Nodal Explants of *Spermacoce articularis* L. *f.*

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Abstract: ***An in vitro regeneration of plantlets from the nodal explants of Spermacoce articularis L. f., a member of Rubiaceae, has been conducted. Nodal regions with axillary buds were cultured on MS medium supplemented with different concentrations of BAP and KIN ranging from 5 to 25 µM alone or in combination of both. Among the two cytokinins tested 10 µM BAP was found to be the best for shoot proliferation. However, combination of KIN with 10 µM BAP further enhanced the rate of shoot proliferation. Maximum number of 31.8 shoots per node with 100% shoot induction frequency were observed on MS medium containing 10 µM BAP and 4 µM KIN. The regenerated shoots were cultured on half strength MS medium containing different concentration of IAA, IBA and NAA for root induction. Among these three auxins, 4 µM IBA was found to be the best hormone for rooting. The well rooted plantlets were successfully hardened with 80% survival rate.***

Keywords: *Spermacoce articularis*, Nodal Segments, Direct Regeneration, Cytokinins, Rooting, Auxins.

Abbreviations: ANOVA: Analysis of Variance; BAP: Benzylaminopurine; DMRT: Duncan Multiple Range Test; IAA: Indole Acetic Acid; IBA: Indole Butyric Acid; KIN: Kinetin (6-Fufuralaminopurine); NAA: Naphthalene Acetic Acid.

1. Introduction

Spermacoce articularis L. *f.* (=*Borreria articularis* (L. *f.*) F.N.Williams), a member of Rubiaceae, is an important medicinal plant widely used used in folk medicin (Chopra *et al*., 1956). It is a small perennial herb with procumbent or ascending branches characterized by the capsule surrounded by calyx point (*Sperma* - *coce*) and jointed nodes (*arti* - *cularis*) with axillary clusters of 1-6 white or tinged pink flowers. It is commonly called as Shaggy buttonweed in English and Nattaichuri in Tamil. This plant is noted for its immense medicial properties. Each and every part of the plant has an ethno medicinal value. The leaves are used against hemorrhoids, gall stones, jaundice, conjunctivities, roots are used to relieve toothache and the whole herb is used to relieve headache [1]-[4]. The seeds are demulcent in diarrhea and dysentery [5]-[7]. The decoction of leaves, roots and seeds is used in India for dropsy [8]. Experimental studies showed that this plant possesses antibacterial [9],[10], anti-inflammatory [11], hepatoprotective activities [12] and antileukemic activities [13].

Plant tissue culture is one of the mostly rapidly growing areas of biotechnology. With this technique, it is now possible to regenerate any plant in the laboratory. It is also possible to create a new plant or a plant with desired characteristics coupled with rDNA technology. This technique also helps to understand the metabolism, growth, differentiation and morphogenesis of plant cells. Further, it also helps to produce a number of pathogen free plants beside the synthesis of secondary metabolites of pharmaceutical and industrial values.

Recently, *in vitro* regeneration of *S. articularis* has been conducted from the shoot tip explants [14]. So, the present study is aimed to mass propagate this plant from the nodal explants.

2. Materials and Methods

2.1. Source of explants

The plant material, *Spermacoce articularis* L. *f.* was collected from the college campus, Government Arts College (Autonomous), Karur of Tamil Nadu, India. The nodal segments with axillary bud were selected for the source of explants in the present study.

2.2. Preparation of Explants

The nodal explants were excised and washed with running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent (Teepol) for 5 minutes and washed with distilled water for 2-3 times. After thorough washing, the materials were taken in to the Laminar Air Flow Chamber where they were disinfected with 70% alcohol for 30-60 sec followed by 0.1% mercuric chloride for 3-5 min. Finally, the materials were thoroughly rinsed with sterile distilled water for 4-5 times to remove the traces of mercuric chloride.

2.3. Preparation of Culture Media

The basal medium consisted of the mineral salts and organic nutrients of Murashige and Skoog (MS) salts [15], B_5 vitamins [16] with 3% sucrose and 0.8% agar. After adding all the supplements (various concentrations of different hormones) to the basal media, the pH was adjusted to 5.8. The molten media were dispensed in culture tubes or conical flasks and were capped with cotton plugs. The culture media were sterilized by autoclaving at 1.06 kg cm^{-3} and 121°C for 15 min.

2.4. PGR supplements

For direct regeneration from nodal segments, BAP or KIN alone was tested with different concentrations ranging from 5 µM to 25 µM. After fixing the optimum concentration of the best cytokinins, low concentrations of the another cytokinins ranging from 2 - 10 µM were supplemented to enhance the multiplication rate and also to test the synergistic effect of these two cytokinins. For rooting, three auxins, viz., IAA, IBA and NAA ranging from 2 µM to 10 µM were used on half strength MS basal medium.

2.5. Inoculation

The surface sterilized explants were aseptically transferred to the culture tubes containing the basal medium with different concentrations of plant growth regulators in the Laminar Air Flow Chamber.

2.6. Culture Conditions

The cultures were maintained in a culture room at 25±2°C under 16 hr photoperiod with a light intensity of 30-40 µM mm^{-2} s^{-1} supplied by cool white fluorescent tubes. The relative humidity (RH) within culture room was maintained at 55±5%. The media was refreshed at 4 wk intervals.

2.7. Hardening and Acclimatization

Four to six week old plantlets with well established roots were removed from the culture tubes and washed the roots to remove the adherent medium. The plantlets were transferred to foam cups (8 cm diameter) containing a mixture of red soil, sand and farm yard manure (2:1:1) and maintained inside the culture room at 25±2°C under 16 hr photoperiod and 75-80% relative humidity. The potted plants were periodically irrigated with water for a period of two weeks and then they were transferred to the field for acclimatization.

2.8. Experimental design, data collection and statistical analysis

All the experiments were preformed using a randomized complete block design and each experiment consisted of ten tubes with one explant in each and five replicates. The parameters recorded were number of shoots per explant, shoot length, number of roots per shoot, root length and survival rate. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance [17].

3. Results and Discussion

3.1. Shoot induction and multiplication

The nodal explants of *S. articularis* were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-benzyl amino purine (BAP) and kinetin (KIN) ranging from 5 to 25 µM alone or in combination of both the cytokinins for shoot bud induction (Table 1; Fig.1). Among the two cytokinins, BAP at 10 µM was found to be efficient for higher frequency of shoot induction (92 %) and maximum number of shoots (26.58) with the shoot length of 5.75 cm followed by 15 µM KIN with the average number 22.78 and mean shoot length of 5.02 cm. In the present study, 10 µM BAP was found to be the optimum concentration for shoot multiplication from the nodal explants. Optimum shooting response using BAP has been reported in several plants such as *Bacopa monnieri* [18], *Mentha piperita* [19], *Swertia chirayita* [20], *Andrographis paniculata* [21], *Sarcostemma brevistigma* [22] and *Enicostemma littorale* [23].

Table 1: Effect of different concentration of cytokinins on shoot induction and multiplication of *Spermacoce articularis* L. *f.* from nodal explants

Concentration of Plant growth regulators		Shoot induction frequency (%)	No. of shoots	Shoot length (cm)
BAP	KIN			
5 µM	---	86	21.92 ± 1.74^{ef}	4.89 ± 1.09^{jg}
10 µM	---	92	26.58 ± 0.92^{cd}	5.75 ± 1.01^{c}
15 µM	---	84	21.00 ± 1.76^{gh}	4.39 ± 0.95^{j}
20 µM	---	78	15.26 ± 0.95^{kl}	4.69 ± 0.84^{h}
25 µM	---	66	14.44 ± 1.66^{lm}	3.72 ± 1.02^{no}
---	5 µM	72	16.14 ± 1.76^{k}	4.19 ± 1.20^{kl}
---	10 µM	76	20.80 ± 1.42^{hi}	4.63 ± 1.29^{hi}
---	15 µM	86	22.78 ± 1.41^{e}	5.02 ± 1.09^{f}
---	20 µM	80	$14,10 \pm 1.55^{mn}$	3.86 ± 1.27^{m}
---	25 µM	66	12.38 ± 1.96^{o}	3.74 ± 1.09^{mn}
10 µM	2 µM	88	28.56 ± 1.50^{b}	6.03 ± 1.15^{b}
10 µM	4 µM	100	31.80 ± 1.38^{a}	6.31 ± 1.00^{a}
10 µM	6 µM	94	26.80 ± 0.71^{c}	5.60 ± 0.54^{cd}
10 µM	8 µM	86	21.66 ± 1.20^{fg}	5.41 ± 0.94^{e}
10 µM	10 µM	76	20.20 ± 1.66^{ij}	4.32 ± 1.14^{jk}

Values are Mean of 5 replicates recorded after 30 days of culture. Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at $P<0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference)

The optimum level of 10 µM BAP was further tested with different concentrations of KIN ranging from 2 to 10 µM. Among the combinations tested, 10 µM BAP plus 4 µM KIN was found to be more efficient than the other concentrations tested. The highest frequency (100 %) of shoot multiplication with maximum number of 31.80 shoots were noticed. In the present study 10 µM BAP in combination with 4 µM KIN increase the shoot induction frequency and more number of shoots among the various concentrations of KIN used. Our results are in agreement with earlier findings as observed in *Sesamum indicum* [24], *Hybanthus enneaspermus* [25], *Enicostemma littorale* [26] and *E. axillare* [27], where BAP and KIN resulted in marked increase in shoot multiplication.

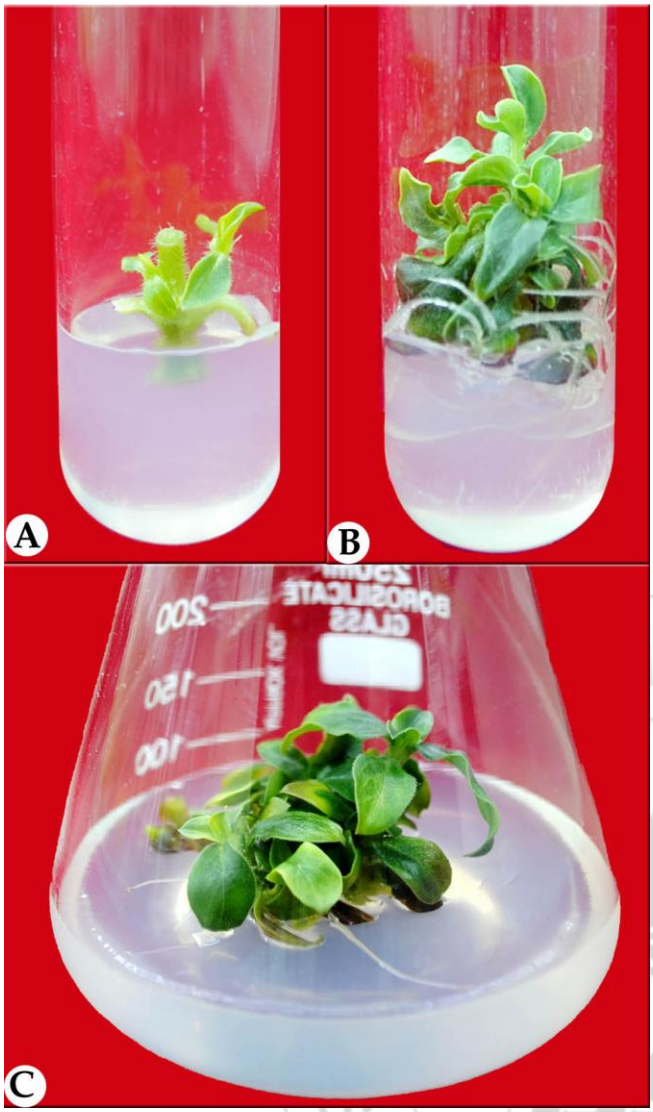

Figure 1: *In vitro* shoot regeneration from the nodal explants of *Spermacoce articularis* L. *f*. A-B. shoot initiation and shoot multiplication from the node; **C.** shoot proliferation.

3.2. Rooting of regenerated shoots

The *in vitro* regenerated shoots of 3-5 cm length were transferred to rooting medium containing half strength MS salts with different concentrations of three auxins, namely, IAA, IBA and NAA (Table 2; Fig.2). Among the various auxins tested, highest frequency of root induction (96%) with maximum number of roots (15.26 roots per shoot) was observed on MS medium containing 4 µM IBA followed by 4 µM NAA. Among the NAA tested, 4 µM NAA was found to be the best for the highest frequency (84 %) of root induction and maximum number of roots (12.54) with root length (5.12 cm). In the case of IAA, the rooting frequency noticed was 78 % with 9.62 roots per shoot on MS medium augmented with 6 µM IAA.

Table 2: Effect of different concentrations of plant growth regulators on rooting of isolated shoots of *Spermacoce articularis* L. *f.*

Concentrations of Plant growth regulators (µM)			Root induction frequency (%)	No. of roots	Root length (cm)
IAA	IBA	NAA			
2	---	---	62	6.36 ± 1.19^{mn}	3.70 ± 0.81^{j}
4	---	---	68	7.90 ± 0.93^{jk}	4.36 ± 0.84^{gh}
6	---	---	78	9.62 ± 1.55^{h}	4.68 ± 1.15^{de}
8	---	---	70	6.70 ± 1.07^{lm}	3.46 ± 1.14^{k}
10	---	---	64	5.70 ± 1.61^{no}	2.68 ± 1.20^{n}
---	2	---	80	13.30 ± 1.79^{b}	5.36 ± 1.11^{b}
---	4	---	96	15.26 ± 1.22^{a}	5.64 ± 1.04^{a}
---	6	---	90	12.74 ± 1.43^{bc}	4.84 ± 1.31^{d}
---	8	---	82	11.54 ± 1.85^{ef}	4.22 ± 0.80^{hi}
---	10	---	74	9.36 ± 1.01^{hi}	3.06 ± 1.29^{lm}
---	---	2	70	11.76 ± 1.76^{e}	4.44 ± 1.34^{fg}
---	---	4	84	12.54 ± 1.47^{cd}	5.12 ± 1.30^{c}
---	---	6	76	10.96 ± 2.24^{fg}	4.56 ± 1.12^{ef}
---	---	8	68	8.28 ± 0.94^{j}	3.46 ± 0.86^{k}
---	---	10	76	7.5 ± 0.53^{j}	3.91 ± 0.94^{k}

Values are Mean of 5 replicates recorded after 30 days of culture. Values in the last two columns are Mean ± SE of Mean followed by the letters within the column indicating the level of significance at $P<0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference

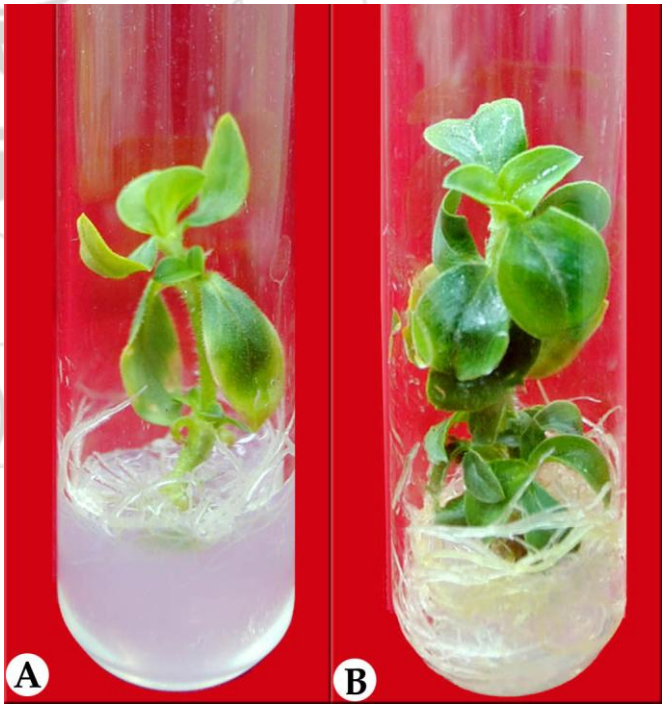

Figure 2: Rooting of isolated shoots of *Spermacoce articularis* L. *f.*

Among the three auxins tested for *in vitro* rooting, IBA was found to be the best auxin for rooting of the cultured shoots of *S. articularis* followed by NAA. Similar results were also reported in *Cichorium intybus* [28], *Passiflora foetida* [29], *Ipomoea sepiaria* [30].

3.3 Hardening and Acclimatization

The *in vitro* rooted plantlets were initially transferred to the paper cups containing red soil, farm yard manures and sand in the ratio of 2:1:1. The hardened plantlets were field transferred successfully with 80 % survival rate [31].

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